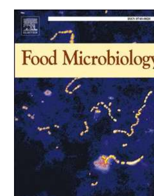
Food Microbiology 132 (2025) 104819

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



# Association of antimicrobial resistant *Vibrio* and species pathogenic to humans with aquacultured seafood

Samuel J. Bloomfield[a,b], Raphaëlle Palau[a,b,1], Nicol Janecko[a,b], Craig Baker-Austin[c], Alison E. Mather[a,b,d,*]

[a] Quadram Institute Bioscience, Rosalind Franklin Road, Norwich Research Park, Norwich, NR4 7UQ, United Kingdom
[b] Centre for Microbial Interactions, Norwich Research Park, Norwich, NR4 7UG, United Kingdom
[c] Centre for Environment, Fisheries and Aquaculture Science, Weymouth, DT4 8UB, United Kingdom
[d] University of East Anglia, Norwich, NR4 7TJ, United Kingdom

ARTICLE INFO

*Keywords:*
*Vibrio*
Genomics
Antimicrobial resistance
Aquaculture
Seafood
Plasmids

ABSTRACT

*Vibrio* is a genus of bacteria commonly found on seafood products and includes many important human pathogens. Most seafood is produced using aquaculture systems, which frequently use antimicrobial agents. Here we aimed to determine if method of seafood production was associated with *Vibrio* pathogenic to humans and/or antimicrobial resistant (AMR) *Vibrio*. Retail prawn and salmon samples that were produced using aquaculture or were wild-caught were cultured for *Vibrio* spp. Isolates were sequenced to identify the species and AMR genes (ARGs) present, followed by long-read sequencing of a subset of genomes to identify mobile genetic elements (MGEs). *Vibrio* was cultured from 136/279 of prawn and 4/157 of salmon samples, and ARG-containing *Vibrio* and *Vibrio* pathogenic to humans were associated with aquacultured prawn samples. A quarter of ARGs were found on plasmids, mostly in close vicinity to the insertion sequence type IS6/IS26. Most intrinsic chromosomal ARGs were not associated with an MGE, but most acquired chromosome ARGs were associated with a MGE, most commonly IS91. *Vibrio* isolates belonging to different species contained ARGs associated with similar MGEs. *Vibrio* has an arsenal of MGEs that can facilitate the spread of ARGs. Aquaculture practices may need to be adjusted in order to prevent the spread of AMR *Vibrio* and *Vibrio* pathogenic to humans.

## 1. Introduction

*Vibrio* is a genus of Gram-negative bacteria associated with aquatic environments and the animals that inhabit those environments. Some *Vibrio* species are pathogenic to humans, causing a range of diseases from gastroenteritis and wound infections to life-threatening conditions such as sepsis. The most relevant *Vibrio* species to human public health are *Vibrio parahaemolyticus*, *V. vulnificus* and *V. cholerae*, including non-O1/non-O139 *V. cholerae* (Koutsoumanis et al., 2024). *Vibrio* can also be pathogenic to other animals, especially aquatic animals (Goulden et al., 2012; Talpur et al., 2011). A wider range of *Vibrio* species are known to cause disease in aquatic animals (Lavilla-Pitogo et al., 1990; Zhang et al., 2014). Although this study is focused on *Vibrio* from seafood, it focuses on those species that are dangerous to humans and the food-borne infections they may cause.

Treating *Vibrio*-associated diseases often requires antimicrobial treatment (Leibovici-Weissman et al., 2014; Yun and Kim, 2018); antimicrobial resistant (AMR) *Vibrio* are frequently isolated from retail seafood samples (Tan et al. 2020; Janecko et al., 2021) and are a growing concern for public health agencies. These strains are harder to treat and have the mechanisms to transmit resistance to other bacteria (Nonaka et al., 2022). Preventing human morbidity and mortality associated with *Vibrio* infections relies in part on understanding food production systems that may promote the acquisition of resistance and transmission of *Vibrio* pathogenic to humans.

Aquaculture (seafood farming) has increased dramatically since the 1960s, replacing capture fishery as the largest type of seafood production in 2013 (Ritchie, 2019). Aquaculture intensification and increasing pathogen incidence within the production system drives antimicrobial usage, with an expected 33 % increase in antimicrobial usage from 2017 to 2030 (Schar et al., 2020). Antimicrobial usage in aquaculture farms is selecting for AMR bacteria, including human pathogens such as *Vibrio* (Cabello et al., 2016; Jeamsripong et al., 2022). However, the genetic

* Corresponding author. Quadram Institute Bioscience, Rosalind Franklin Road, Norwich Research Park, Norwich, NR4 7UQ, United Kingdom.
*E-mail address:* alison.mather@quadram.ac.uk (A.E. Mather).
[1] Current address: Institute for Bioengineering of Catalonia, Carrer de Baldiri Reixac, 10, 12, Les Corts, 08028 Barcelona, Spain.

https://doi.org/10.1016/j.fm.2025.104819
Received 23 March 2025; Received in revised form 16 May 2025; Accepted 18 May 2025
Available online 30 May 2025
0740-0020/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

**Abbreviations**

| | |
|---|---|
| AMR | Antimicrobial resistance |
| ARG | Antimicrobial resistance gene |
| bp | Base-pair |
| DNA | Deoxyribonucleic acid |
| ICE | Integrative and conjugative elements |
| IME | Integrative and mobilisable elements |
| IS | Insertion sequence |
| MDR | Multi-drug resistance |
| NCBI | National Center for Biotechnology Information |
| MGE | Mobile genetic element |
| SCI | Sedentary chromosomal integron |
| SRA | Sequence Read Archive |
| ST | Sequence type |
| T3SS | Type 3 secretory system |
| VFDB | Virulence finder database |

mechanisms that facilitate the spread of AMR in *Vibrio* on food have not been widely studied.

*Vibrio* has a plastic genome, able to gain, lose and rearrange genetic material using mobile genetic elements (MGEs) such as plasmids (Rivard et al., 2020), integrons (Vit et al., 2021), integrative and conjugative elements (ICE) (Sarkar et al., 2019) and integrative and mobilisable elements (IME) (Pérez-Duque et al., 2021). We previously tested 214 prawn samples at retail in the United Kingdom for *Vibrio*, analysed 148 *Vibrio* genomes collected from the 100 samples that tested positive for this bacterium, and found a wide range of *Vibrio* species and strains which contained AMR genes (ARGs) (Janecko et al., 2021). This was a part of a wider food study (Janecko et al., 2023) and in this current study we investigate the remaining 65 prawn and 157 salmon samples for *Vibrio* to investigate factors associated with AMR *Vibrio* and *Vibrio* pathogenic to humans, and additionally used long-read sequencing to identify genetic elements allowing AMR to spread amongst *Vibrio*.

## 2. Materials and methods

### 2.1. Vibrio isolation and metadata collection

A population- and market share-weighted longitudinal repeated cross-sectional study, previously described, was used to collect prawn (n = 279) and salmon (n = 157) products at retail in Norfolk, UK, between May 2018 and November 2019 (Janecko et al., 2023). Prawns and salmon were investigated as they are the most commonly produced and imported seafood types in the UK (Löfstedt et al., 2025). Samples were cultured for *Vibrio* using an adapted ISO21872–1:2017 method, and up to two typical presumptive *Vibrio* isolates from thiosulfate citrate bile salt (TCBS) agar were selected from each sample and analysed further. The exception was three prawn samples where six isolates were previously selected and analysed to measure within sample *Vibrio* diversity (Janecko et al., 2021). Metadata collected included, where available, how the sample was produced, sample origin and the species of prawn or salmon present (Janecko et al., 2023).

### 2.2. Short-read genome analysis

*Vibrio* genomes were sequenced using short read technology as described previously (Janecko et al., 2021). DNA was extracted using the Maxwell RSC Cultured Cells DNA Kit (Promega, Madison, Wisconsin, USA), according to the manufacturer's instructions. Libraries were created using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, California, USA) and sequenced on a NextSeq 550 System (Illumina) as 150 bp paired-end reads.

Genomic analyses were performed on the Cloud Infrastructure for Microbial Bioinformatics (CLIMB) server (Connor et al., 2016). Illumina reads were trimmed using Trimmomatic v0.36 (Bolger et al., 2014) (Supplementary material). Trimmed reads were assembled using Spades v3.11.1 (Bankevich et al., 2012) in "careful" mode. The quality of the assemblies was assessed using QUAST v4.6.3 (Gurevich et al., 2013), CheckM v1.1.2 (Parks et al., 2015) and by aligning reads to assemblies using the Burrows-Wheeler aligner (BWA) v0.7.17 (Li and Durbin, 2009). Assemblies were accepted if they consisted of less than 500 contigs that were over 500 bp, less than 50 duplicate genes and had a mean read depth of the four largest contigs above 30. *Vibrio* species were predicted using the Genome Taxonomy Database Toolkit (GTDB-Tk) v2.2.2 (Chaumeil et al., 2022).

ARG determinants, virulence genes and plasmid replicons (genetic elements of interest) were identified using ARIBA v2.14.4 (Hunt et al., 2017) and the NCBI AMR (Feldgarden et al., 2019), virulence finder database (VFDB) (Chen et al., 2016) and PlasmidFinder (Carattoli et al., 2014) databases, respectively. Isolates were classified as multi-drug resistant (MDR) strains if the genomes contained ARGs that encoded resistance to three or more antimicrobial agent classes.

### 2.3. Vibrio parahaemolyticus analysis

Enterobase (Zhou et al., 2020) was interrogated for all *V. parahaemolyticus* genomes to compare food-derived isolates with isolates collected from human and other sources. For all genomes downloaded, paired reads were processed and assembled using the QC parameters previously described. MLST v2.16.1 (https://github.com/tseemann/mlst) was used to predict the sequence types (STs), and ARIBA was used to identify the genetic elements of interest. Virulence gene analysis targeted those encoding toxins and type 3 secretory system (T3SS) effectors (Wang et al., 2015).

*V. parahaemolyticus* genome SAMD00058707 was used as the reference genome. Phaster (Arndt et al., 2016) was used to identify phage regions that were then blocked out. Snippy v3.2 (https://github.com/tseemann/snippy) was used to align reads to this reference genome. Gubbins v2.3.1 (Croucher et al., 2015) was used to remove single nucleotide polymorphisms (SNPs) putatively associated with recombination before the number of SNPs between isolates was quantified.

### 2.4. Vibrio cholerae analysis

All *V. cholerae* genomes were uploaded to PathogenWatch (https://pathogen.watch/) to predict the ST and compare genomes with similar strains. ARIBA was used to identify the genetic elements of interest.

### 2.5. Long-read genome analysis

A subset of 68 *Vibrio* isolates were selected for long-read sequencing. For the most commonly isolated *Vibrio* species in this study, *V. parahaemolyticus*, 57 isolates were chosen to be representative of the *V. parahaemolyticus* STs isolated in this study. An additional three isolates of *V. cholerae* and eight additional isolates representing eight other *Vibrio* species were selected to represent the other *Vibrio* isolated in this study. Libraries for the selected isolates were formed from genome extractions using the Native barcoding kit and were sequenced on a MinION (Oxford Nanopore Technologies, Oxford, UK). Filtlong v0.2.0 (https://github.com/rrwick/Filtlong) was used to trim long reads using a 1000 bp cut-off. Long reads were assembled using Canu v2.2 (Koren et al., 2017), Flye v2.9 (Kolmogorov et al., 2019) and Raven v1.1.10 (Vaser and Šikić, 2021). To create hybrid assemblies utilising the trimmed long and short reads, Unicycler v0.4.8 (Wick et al., 2017) was used. To correct for mismatches, short reads were aligned to the assemblies using BWA and corrected using five rounds of Pilon v1.22 (Walker et al., 2014).

ART v2.5.8 (Huang et al., 2012) was used to simulate paired Illumina

reads from the assemblies. ARIBA was used to search the Illumina and simulated reads against the NCBI AMR and PlasmidFinder databases. Abricate v0.9.9 (https://github.com/tseemann/abricate) was used to help investigate any discrepant ARIBA results between the long/hybrid assemblies and the short reads. Assemblies that consisted of circular contigs and were not missing any ARGs or plasmid replicons found using short reads were taken forward for further investigation.

Abricate with the NCBI AMR, PlasmidFinder and VFDB were used to identify the location of ARGs, plasmid replicons and virulence genes, respectively. Abricate matches with identity and coverage values over 90 % were accepted. tBLASTn v2.9.0 (Gertz et al., 2006) was used with the BacMet database (Pal et al., 2014) to determine the location of metal-tolerance genes using 90 % identity and coverage cut-offs. ISEScan v1.7.1 (Xie and Tang, 2017) was used to determine the location of "complete" insertion sequences (ISs). Genes of interest within 10,000 bp of an IS were classified as "in close proximity". IntegronFinder v2.0.3 (Néron et al., 2022) was used to identify integrons. ICEfinder v1.0 was used to identify ICEs and IMEs.

Plasmid sequences were annotated using BAKTA v1.8.2 (Schwengers et al., 2021) and genes were clustered using Roary v3.10.2 (Page et al., 2015) using an identity cut-off of 90 %. EggNOG v5 (Huerta-Cepas et al., 2019) was used to predict the function of the plasmid-located genes. MOB-Typer v3.0.3 (Robertson and Nash, 2018) was used to predict the mobility of the plasmids. The Jaccard distance amongst plasmid sequences was estimated based on the presence and absence of genes, with a dendrogram formed based on the Jaccard distances. Plasmids were clustered using pling v1.1 (Frolova et al., 2024); Easyfig v2.1 (Sullivan et al., 2011) was used to align plasmid sequences that clustered together and IQ-TREE v2.1.2 (Nguyen et al., 2015) was used to form a maximum likelihood tree of ARG-containing plasmid communities based on core gene alignments produced using Roary.

For groups of ARGs found on chromosomes (ARGs within 10 kb of each other), the sequences were extracted along with 10 kb margins on each side of the ARGs. For each species, the sequences had the ARGs blocked out before the margins were annotated, then the sequences were clustered based on the presence of genes and a dendrogram formed for each species as was performed with the plasmid sequences. For ARG regions belonging to *V. cholerae* and *V. parahaemolyticus*, their closest location on the reference genomes SAMN12572458 and SAMD00058707, respectively, were determined. This was achieved using BLASTn v2.6.0 and by extending the margins of the ARG regions to 50 kb as using 10 kb margins returned no matches for some ARG regions. A representative of each chromosomal ARG sequence cluster from each species was compared using Roary and Easyfig to determine if these regions were similar amongst *Vibrio* species.

### 2.6. Statistical analyses

Statistical analyses were performed in R v4.1.2 (R core team, 2019). Outside of sequence alignments, figures were created using the GenomicRanges v1.46.1 (Lawrence et al., 2013), ggbio v1.42.0 (Yin et al., 2012), ggplot2 v3.4.4 (Wickham, 2016) and ggtree v3.2.1 (Yu et al., 2017) packages.

### 2.7. Data availability

Raw Illumina reads were uploaded to the Sequence Read Archive (SRA) under bioprojects: PRJNA699735, PRJNA1107692 and PRJNA1155317. Raw MinION reads were uploaded to SRA under project PRJNA1155659.

## 3. Results

### 3.1. Vibrio species

A total of 279 prawn and 157 salmon samples were collected from retail outlets in Norfolk and cultured for *Vibrio*, as part of a population- and market share-weighted longitudinal repeated cross-sectional study (Janecko et al., 2023). In total, 111 stores were visited: 269 prawn and 153 salmon samples were collected from supermarket chains, and ten prawn and four salmon samples were collected from independent stores such as independent grocers and fishmongers.

Salmon and prawn samples were randomly chosen within retail stores to keep the samples representative of those available to consumers. Of those, 136/279 (49 %) prawn and 4/157 (2.5 %) salmon samples were confirmed positive for *Vibrio* (Table 1). Up to two morphologically typical isolates from TCBS agar were collected from these samples, preserved and tested further. The exception was three prawn samples where six isolates were previously collected to measure diversity of *Vibrio* present. Analysing more than two TCBS colonies did not identify more species of *Vibrio* but did identify more sequence types (STs) belonging to the same species (Janecko et al., 2021). In total, 202 *Vibrio* isolates were short-read sequenced for genome analysis.

Of the total prawns collected, 224 were derived from aquaculture-type production systems, 41 were labelled as wild-caught, and 14 samples did not contain production type information. *Vibrio* was identified in 127 (57 %) aquacultured samples, whilst four (9.8 %) wild-caught samples contained *Vibrio*, a statistically significantly difference (two proportion *t*-test: $p = 7.8 \times 10^{-8}$) (Fig. 1). *Vibrio* was identified in five (36 %) samples from unknown production systems. *V. anguillarum* and *V. diabolicus* were cultured from both aquacultured and wild-caught prawns. *V. aestuarianus, V. alginolyticus, V. brasiliensis*, *V. campbellii*, *V. cholerae*, *V. harveyi*, *V. hepatarius*, *V. metschnikovii*, *V. mimicus*, *V. owensii*, *V. parahaemolyticus*, *V.* sp006124995*, V. vulnificus* and a putative novel *V.* species were only isolated from aquacultured prawn samples or samples whose production system was unknown (Fig. 2). The potential novel species was isolated from two prawn samples and could only be classified to the *Vibrio* genus level by GTDB-Tk, however, the isolates contained 99.96 % average nucleotide identity to each other. No *Vibrio* species were solely isolated from wild-caught prawns.

Of the total salmon samples collected, 96 were derived from aquaculture production systems, 58 were derived from wild-caught production systems and three samples did not have production type information. *Vibrio* was identified in three (3.1 %) aquaculture samples, whilst one (1.7 %) wild-caught sample contained *Vibrio* (Fig. 1). *Vibrio* was not recovered from the unknown production type samples. *V. anguillarum* were isolated from both aquacultured and wild-caught salmon. *V. alginolyticus* was only isolated from aquacultured salmon. No *Vibrio* species were solely isolated from wild-caught salmon.

*Vibrio* was associated with specific prawn species (Fisher's exact test: p-value $<1 \times 10^{-7}$) but not salmon species (Fisher's exact test: p-value $= 0.19$), although only four salmon samples were positive for *Vibrio*. However, food production system was associated with prawn (Fisher's exact test: p-value $<1 \times 10^{-7}$) and salmon (Fisher's exact test: p-value $<1 \times 10^{-7}$) species. (Fig. S1). For prawns, *Litopenaeus vannamei* (whiteleg shrimp) and *Penaeus monodon* (giant tiger prawn) were the most commonly sampled species and all *Vibrio* species identified in this study were found on one or both of these prawn species. In addition,

**Table 1**
Number of seafood samples investigated.

| Commodity | Samples investigated | *Vibrio*-positive samples | *Vibrio* isolates investigated[a] |
|---|---|---|---|
| Prawns | 279[b] | 136 (49 %) | 198[b] |
| Salmon | 157 | 4 (2.5 %) | 4 |

[a] Up to two *Vibrio* isolates were isolated from each positive sample apart from three prawn samples where six samples were investigated.

[b] 214 prawn samples were previously investigated in Janecko et al. (2021) yielding 148 *Vibrio* isolates; an additional 65 prawn samples yielding 50 additional *Vibrio* genomes as well as the 157 salmon samples were examined in this study.

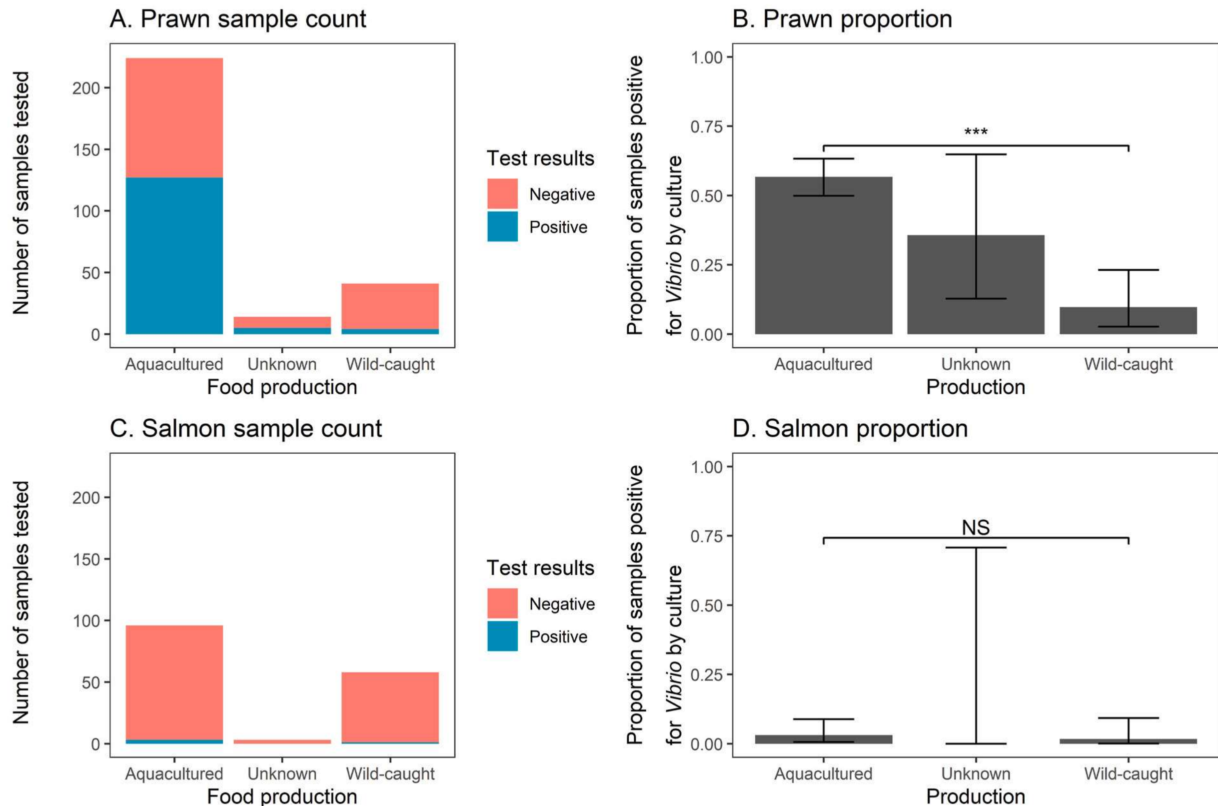



**Fig. 1.** Number (A and C) and proportion (B and D) of prawn (A and B) and salmon (C and D) that were positive for *Vibrio* by culture, separated by their method of production. The error bars represent the 95 % confidence intervals using a binomial distribution, and the brackets indicate if the proportions are significantly different.

*V. anguillarum* was also isolated from *Pleoticus muelleri* (Argentina red shrimp), and *V. diabolicus* was also isolated from *Crangon crangon* (brown shrimp), *Pandalus borealis* (caridean shrimp) and *P. muelleri*. For salmon, *V. anguillarum* was isolated from *Oncorhynchus nerka* (sockeye salmon) and *Salmo salar* (Atlantic salmon), and *V. alginolyticus* was isolated from *S. salar*.

The origin of seafood samples varied amongst food commodities. For prawn samples, four (1.4 %) were produced domestically, 232 (83 %) were imported and the origin was unknown for 43 samples (15 %) (Fig. S2). *Vibrio* was isolated from 3/4 (75 %) of domestic prawn samples and 129/232 (56 %) of imported prawn samples, but these percentages were not significantly different (Fisher's exact test: p-value = 0.63). For salmon samples, 53 (34 %) were produced domestically, 22 (14 %) were imported, 23 (15 %) were associated with a mixture of imported and domestic sources, and the origin was unknown for 59 samples (38 %). *Vibrio* was isolated from 3/53 (5.7 %) of domestic salmon samples and none of the imported salmon samples, but these percentages were not significantly different (Fisher's exact test: p-value = 0.55).

### 3.2. *Vibrio* ARG determinants

*Vibrio* isolates contained 0–16 different ARGs using ARIBA (Fig. 2). No ARGs were identified in *V. aestuarianus*, *V. brasiliensis*, *V. diabolicus*, *V. harveyi*, *V. hepatarius* or *V. mimicus*. ARGs were identified in *V. alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. cholerae*, *V. metschnikovii*, *V. owensii*, *V. parahaemolyticus*, *V.* sp006124995, *V. vulnificus* and the putative novel *Vibrio* species. A larger percentage of *Vibrio* from aquacultured prawns (82 %) contained ARGs compared to wild-caught prawns (0 %) and these percentages were significantly different (two proportion *t*-test: p-value = $7.3 \times 10^{-5}$). A larger percentage of *Vibrio* from salmon (67 %) contained ARGs compared to wild-caught salmon (0 %), but these percentages did not significantly differ (two proportion *t*-test: p-value = 0.37), likely due to the small number of *Vibrio* isolates from salmon. MDR was identified in *V. cholerae* (3/10), *V. owensii* (1/12), *V. parahaemolyticus* (40/125), *V.* sp006124995 (4/17) and *V. vulnificus* (1/3), all derived from aquacultured prawn samples or prawn samples whose production system was unknown.

### 3.3. *V. parahaemolyticus* comparison with publicly available genomes

*V. parahaemolyticus* was the most common *Vibrio* species isolated in this study (125/202) and was exclusively from aquacultured prawns or prawns whose production type was unknown. This set of isolates was compared with publicly available *V. parahaemolyticus* genomes for context (n = 1447) (Fig. S3). Publicly available *V. parahaemolyticus* genomes were attributed to birds, clams, crabs, deer, environment, fish, humans, molluscs, otters, planktons, prawns or an unknown source. Genomic comparisons between *V. parahaemolyticus* genomes sequenced in this study and publicly available genomes identified two ARGs considered core ARGs (>95 % of isolates): *blaCARB* (99.9 % of isolates) and *tet(35)* (99.4 % of isolates) genes. *V. parahaemolyticus* genomes that contained more than these core ARGs were isolated from environmental, fish, human, mollusc, plankton, prawn (those collected in this study and those that are publicly available) or unknown sources (Fig. S3).

Virulence gene analysis of *V. parahaemolyticus* isolates found 38 core and 35 accessory virulence genes. The distribution of the number of virulence genes was bimodal, with 1400 *V. parahaemolyticus* genomes containing 27–43 virulence genes, and 172 containing 55–72 virulence genes (Fig. S3). The *V. parahaemolyticus* isolates collected in this study contained 30–41 virulence genes. All prawn-derived isolates contained the *tlh* toxin gene and genes encoding for T3SS1 effectors, but these virulence genes are core to *V. parahaemolyticus* (Table S1). None of the prawn *V. parahaemolyticus* isolates collected from this study or publicly available isolates from prawns contained genes associated with

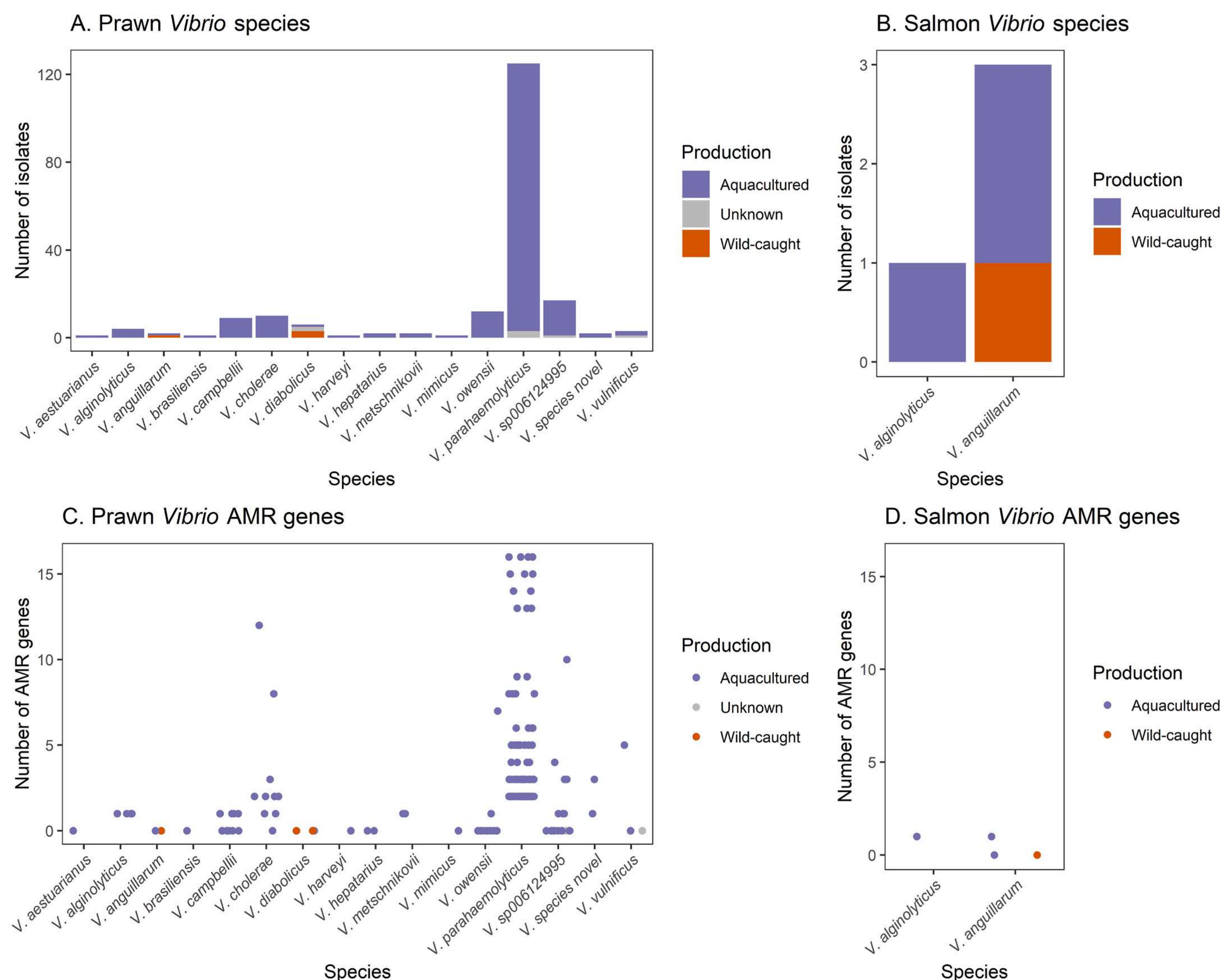



**Fig. 2.** Number of *Vibrio* isolates by species from prawns (A) and salmon (B), and the number of antimicrobial resistant genes found in *Vibrio* from prawns (C) and salmon (D), coloured by method of production.

gastrointestinal disease (*tdh* or *trh* toxin genes, or genes encoding T3SS2 effector proteins), nor did 32 % of the analysed publicly available *V. parahaemolyticus* isolates from humans.

Analysis of known plasmid replicons found IncA/C2 in 4 % of *V. parahaemolyticus* isolates from prawns in this study and 1 % of prawn isolates from other studies. IncQ1 was identified in 8 % of *V. parahaemolyticus* isolates from prawns in this study, 3 % of publicly available isolates from prawns, and 0.2 % of publicly available isolates from molluscs. Six other plasmid replicons were identified in the publicly available dataset of *V. parahaemolyticus* genomes, but none of these six were found in prawns from this study.

The *V. parahaemolyticus* genomes sequenced in this study and in the publicly available dataset belonged to 577 STs, 353 of which had previously been described, leaving 224 undescribed STs (Table S2). *V. parahaemolyticus* cultured from prawns in this study belonged to 72 STs. However, only two of these STs were also identified from human sources: ST-674 and a novel ST (FY). The most closely related human-derived *V. parahaemolyticus* ST-674 isolate to a seafood-derived isolate from this study differed by 324 SNPs and was isolated from the United States in 2006 (Fig. S4). For novel ST-FY, the most closely related human isolate differed by 81 SNPs and was isolated from China in 2010 (Fig. S5).

### 3.4. Vibrio cholerae comparison

*V. cholerae* was isolated from ten aquacultured prawn samples and a single isolate from each sample was analysed. These ten *V. cholerae* isolates contained 139–147 virulence genes, but none contained genes encoding for the cholera toxin genes *ctxA* or *ctxB*, and no isolates were identified as belonging to the pandemic *V. cholerae* lineages O1 or O139. Of the *V. cholerae* isolates, 0–12 ARGs were identified with one isolate containing the carbapenemase encoding gene, *blaNDM-1*. This isolate also contained the IncA/C2 plasmid replicon. No other plasmid replicons were found in the remaining *V. cholerae* isolates. Two of the *V. cholerae* isolates belonged to ST-833, and the rest belonged to novel STs. PathogenWatch had no other isolates belonging to these STs, therefore no further comparison was possible.

### 3.5. Long-read analysis

Long-read genomics were used to determine what MGEs were associated with ARGs in *Vibrio*, which may enable AMR to spread in this genus. The 125 *V. parahaemolyticus* isolates collected from prawns in this study belonged to 72 STs. ST-722 was the most common ST, with 30 ST-722 isolated from prawns. For the remaining 71 STs, 1–4 isolates were

cultured from prawns. Three ST-722 *V. parahaemolyticus* genomes were long-read sequenced along with 54 other *V. parahaemolyticus* genomes representing 54 additional STs. In addition, three *V. cholerae*, one *V. diabolicus*, one *V. harveyi*, one *V. hepatarius*, one *V. metschnikovii*, one *V. mimicus*, one *V. owensii*, one *V.* sp006124995 and one *V. vulnificus* genomes were long-read sequenced to represent the other *Vibrio* species isolated from seafood.

Of the 68 *Vibrio* genomes that were long-read sequenced, 64 contained ARGs identified using Abricate. Abricate identified additional ARGs to ARIBA in one *V. cholerae*, one *V. diabolicus* and two *V. parahaemolyticus* isolates, likely because these ARGs were found in duplicate in the assemblies, or were close to the 90 % identify coverage cut-off, preventing their detection by ARIBA.

Collectively, the 68 *Vibrio* long-read assemblies contained 261 ARGs using Abricate, 64 (25 %) of which were found on plasmids and 197 (75 %) on chromosomes. However, only eight of these isolates contained ARG-containing plasmids: seven *V. parahaemolyticus* and one *V. cholerae* genome (Fig. S6). Of the ARGs found on plasmids, 98 % were in close proximity to an IS, whilst 37 % of those on chromosomes were in close proximity to an IS (Fig. 3). On plasmids, ARGs were predominantly in close proximity to IS6/IS26 (64 %) (Fig. 3).

Chromosomal *Vibrio* ARGs could be intrinsic or acquired. *V. parahaemolyticus* has two intrinsic ARGs on its chromosomes: *blaCARB* and *tet(35)*. The *blaCARB* ARG found on the *V. diabolicus* genome is also likely intrinsic (Sebastian et al., 2025). These intrinsic ARGs comprised 58 % of the chromosomal ARGs and only 8.7 % were associated with an IS (Fig. S7). The remaining 42 % of chromosomal ARGs we presumed to be acquired and 76 % of which were in close proximity to an IS, most commonly to IS91 (41 %).

Metal-tolerance genes were investigated as they can be co-selected for with ARGs (Kang et al., 2018). A total of 1329 metal-tolerance genes were identified in the 68 isolate subset that were long-read sequenced, 62 (4.7 %) of which were found on plasmids and 1257 (95 %) were found on chromosomes. Of those found on plasmids, all were in close proximity to an IS, whilst 6 % of those on chromosomes were in close proximity to an IS (Fig. 3). Metal-tolerance genes on plasmids were predominantly in close proximity to IS6/IS26 (77 %) and IS110 (37 %), whilst on the chromosomes there was no IS type predominantly in close proximity to metal-tolerance genes. Collectively, 2413 virulence genes were identified in the 68 isolate long-read subset, all of which were found on chromosomes, and 2 % were in close proximity to an IS. There was no IS type predominantly in close proximity to virulence genes.

### 3.6. Plasmid analysis

Eighty-one plasmids were identified in the 68 isolate long-read subset and clustered into 34 plasmid communities (Fig. S8). One of

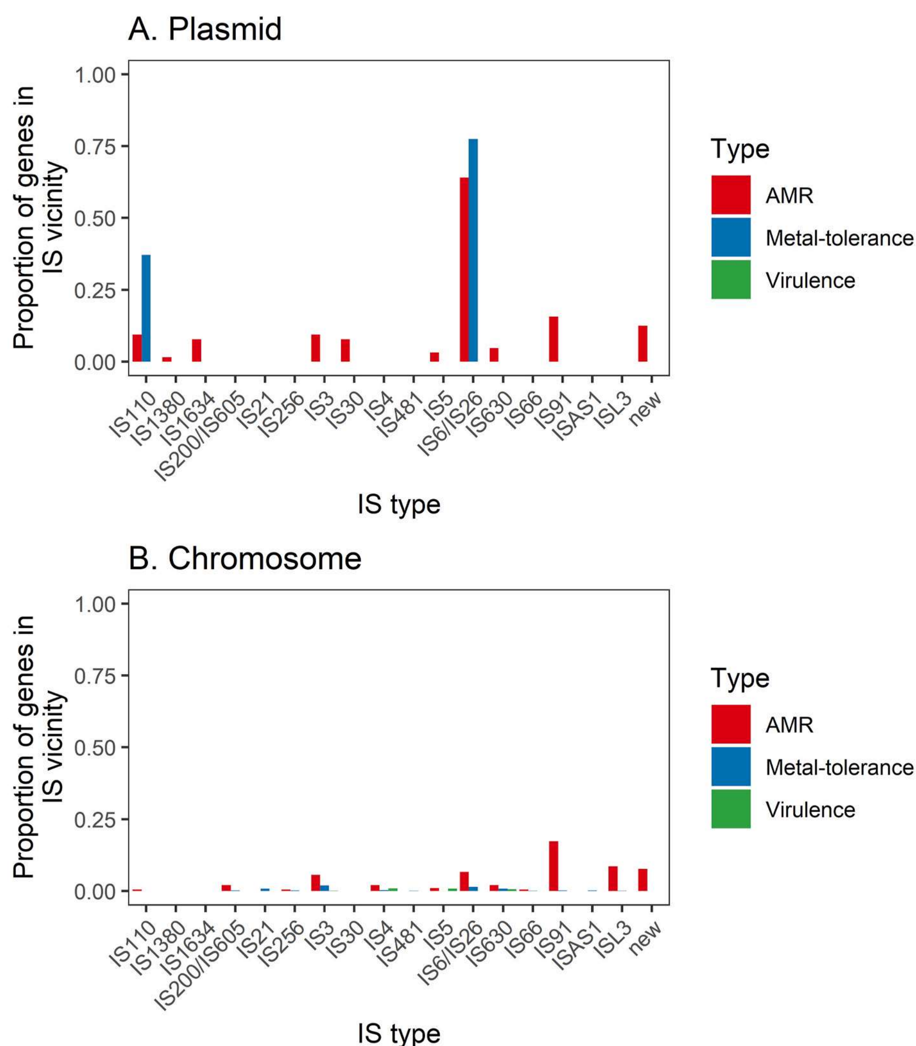



**Fig. 3.** Number of antimicrobial resistance (red), metal-tolerance (blue) and virulence (genes) associated with different insertion sequences (ISs) on plasmids (A) and chromosomes (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the plasmid communities consisted of three plasmid sequences that contained the IncA/C2 plasmid replicon and ARGs, another plasmid community consisted of five plasmid sequences that contained ARGs, and one plasmid community consisted of a single plasmid sequence that contained an ARG. The remaining 31 plasmid communities consisted of plasmid sequences without any known ARGs.

Alignments of the plasmid communities that contained the IncA/C2 replicon (Fig. 4A) and the other plasmid community that consisted of multiple plasmid sequences that contained ARGs (Fig. 4B) demonstrated that they had conserved backbones with variable regions that contained ARGs and ISs. *Vibrio* isolates with ARG-containing plasmids were collected from different *Litopenaeus vannamei* prawn samples aquacultured in Thailand or Vietnam. Plasmids belonging to these communities also contained metal-tolerance genes. The function of the 72 non-ARG-containing plasmids is unclear as there was variation in the presence of genes attributed to different functional groups (Fig. S9).

### 3.7. Chromosome ARG analysis

*Vibrio* has two chromosomes, and long-read analysis enabled determination of the genetic content of the *Vibrio* chromosomes, the location of ARGs and whether or not the ARGs were associated with any MGEs. The location of the 167 chromosomal ARGs amongst the 68 isolate subset were compared amongst the *Vibrio* species.

Analysis of the ARGs on the chromosomes of the 57 *V. parahaemolyticus* genomes that were long-read sequenced identified 12 regions that contained ARGs (Fig. S10). All genomes contained a region on chromosome 1 that contained *tet(35)* (Region 1) and a region on chromosome 2 that contained a *blaCARB* gene (Region 2). There was variation in the *blaCARB* gene with Abricate identifying different *blaCARB* genes at this position for different isolates. This was supported by ARIBA short-read analysis that also found different *blaCARB* gene variants in different isolates but found sufficient sequence similarity to cluster them together: 4–20 SNPs difference (97.7–99.5 % identity). The remaining ten ARG-containing regions were variably found in *V. parahaemolyticus* genomes throughout chromosome 1 (Regions 3–12) (Fig. S11).

Analysis of ARGs on the chromosomes of the three *V. cholerae* genomes identified four regions that contained ARGs (Fig. S12). All three *V. cholerae* genomes contained *varG* on chromosome 1 (Region 1). However, one *V. cholerae* isolate that was not long-read sequenced contained no ARGs, so this ARG region is not common to all *V. cholerae* genomes. Chromosome 1 also contained two other regions that contained ARGs, but these were variably found in the three *V. cholerae* isolates analysed (Regions 2–3) (Fig. S13). ARGs were identified within chromosome 2 of all three isolates, where the Sedentary Chromosomal Integron (SCI) is located (Region 4). Due to the repetitive nature of this SCI, some *V. cholerae* isolates contained multiple copies of the same ARG in this region.

In the remaining *Vibrio* species, *V. diabolicus*, *V. metschnikovii*, *V.* sp006124995 and *V. vulnificus* genomes, each contained one ARG-containing region. The *V. harveyi*, *V. hepatarius*, *V. mimicus* and *V. owensii* genomes examined contained no ARG-containing regions. Comparisons of chromosomal ARG regions amongst *Vibrio* species identified four chromosomal ARG regions that were similar amongst different *Vibrio* species (Fig. 5): two contained similar ARGs, ISs and margins, indicating a potentially transmissible element with a predilection for certain regions of the *Vibrio* genome (A and C); one contained similar margins and ARGs but no ISs to move them, indicative of an ARG region conserved amongst different *Vibrio* species (B); and one consisted of similar ARG regions and ISs but the margins were different, indicative of an ARG region found in different sections of the genome (D). Cluster D was found in a *V. metschnikovii* and two *V. parahaemolyticus* genomes, but in separate genome regions for these isolates. Clusters A, C and D consisted of isolates obtained from *L. vannamei* prawns produced in Vietnam or multiple countries including Vietnam (Table S3). Cluster B was found in all *V. parahaemolyticus* isolates, preventing further comparisons with sample metadata.

Amongst the 68 *Vibrio* isolates, 106 ICEs and 46 IMEs were identified. ICEs and IMEs were found in 63/68 of the long-read *Vibrio* subset; they were not found in the *V. harveyi* genome, one of the *V. cholerae* genomes and three of the *V. parahaemolyticus* genomes. Amongst *V. cholerae* and

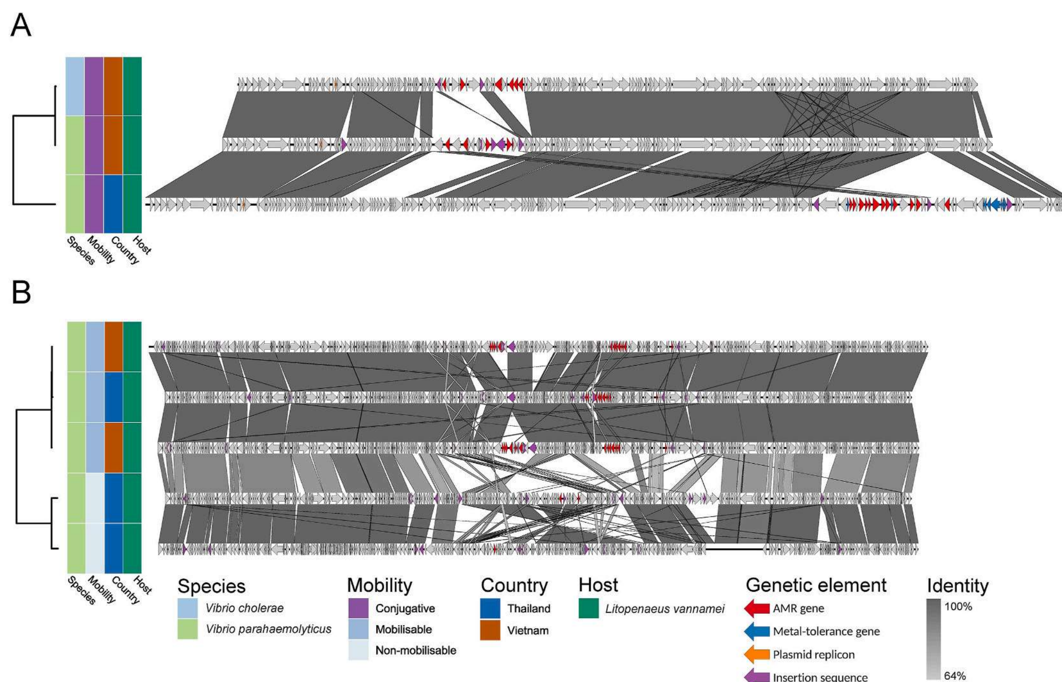



**Fig. 4.** Alignments of plasmids with the IncA/C2 replicon (A) and the other plasmid community consisting of multiple ARG-containing plasmids (B), with dendrograms based on core gene alignments, metadata on the *Vibrio* species from which they originate, their putative mobility, the country of origin and host species, and genetic elements highlighted. Bars between plasmid sequences represent sequence similarity.

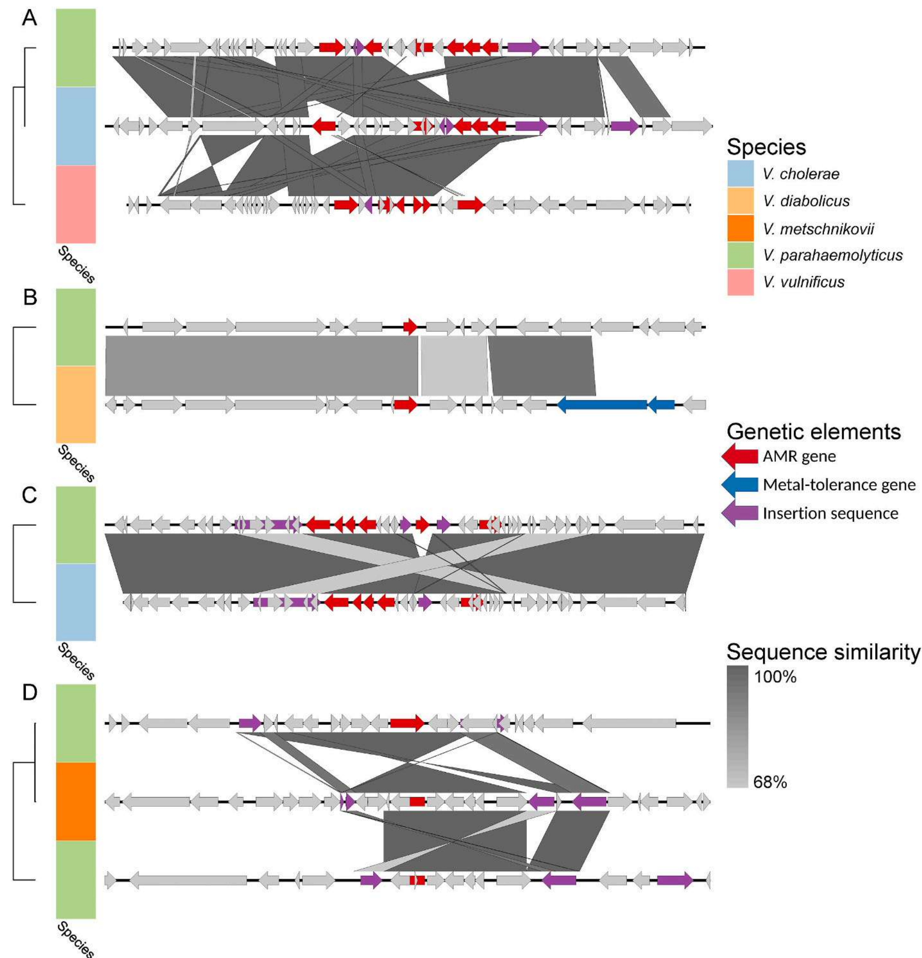



**Fig. 5.** Alignments of representative chromosomal ARG-containing regions found in different *Vibrio* species (Clusters A-D), with dendrograms based on the presence and absence of genes, metadata on the *Vibrio* species from which they originate and genetic elements highlighted. Bars between plasmid sequences represent sequence similarity.

*V. parahaemolyticus* genomes, ICEs/IMEs were found on chromosomes 1 and 2. Five chromosomal ARG regions co-located with ICEs/IMEs: *V. parahaemolyticus* ARG regions 1 (3/57), 3 (1/1) and 9 (1/1). *V. parahaemolyticus* ARG region 9 is part of the cluster D ARG region cluster with *V. parahaemolyticus* ARG region 7 and the ARG region identified in *V. metschnikovii*, but these other ARG regions were not co-located with ICEs/IMEs.

Amongst the 68 *Vibrio* isolates, 6135 integrons were identified: 4183 were complete, 1898 were clusters of *attC*s lacking an associated integron-integrase (CALIN), and 54 were missing gene cassettes (In0 element). These were identified in all *Vibrio* species. Amongst *V. cholerae* and *V. parahaemolyticus* genomes, integrons were found on chromosome 1 and 2. Four chromosomal ARG regions were co-located with integrons: *V. cholerae* ARG regions 3 (1/1) and 4 (2/5), and *V. parahaemolyticus* ARG region 11 (1/1). These ARG regions were not shared between *Vibrio* species.

## 4. Discussion

*Vibrio* is the most important genus of human bacterial pathogens associated with marine and coastal waters, and seafoods associated with these environments. Human *Vibrio* infections are increasing in incidence worldwide in part due to climate change increasing ocean temperatures and the regions in which *Vibrio* can thrive (Archer et al., 2023; Vezzulli et al., 2016), a lack of regulations regarding traded seafood, and more frequent use of coastal waters for recreation (Baker-Austin et al., 2024). This study took a molecular approach to characterise the potential transmissibility of ARGs in *Vibrio* by using genomics to investigate the potential health risk from consuming contaminated seafood.

Aquaculture is the cultivation of aquatic organisms in a controlled environment. This often involves a large density of these organisms, where risk of infection and disease is high, including disease caused by *Vibrio* (Sony et al., 2021). In response, antimicrobials are often used in aquaculture to prevent and treat disease (Chowdhury et al., 2022). Prawns produced through aquaculture systems are associated with increased contamination with *Vibrio*, as we have previously shown (Janecko et al., 2023). In this study, we demonstrated that species pathogenic to humans such as *V. cholerae*, *V parahaemolyticus* and *V. vulnificus* were only isolated from aquacultured prawns or prawns whose production could not be determined. *Vibrio* species *V. alginolyticus* and *V. anguillarum* were isolated from aquacultured and wild-caught salmon but were rarely identified (2.5 %) and rarely cause human infections (Sinatra and Colby, 2018; Uh et al., 2001). A larger percentage of *Vibrio* isolates from aquacultured prawns were associated with AMR *Vibrio* compared to isolates from wild-caught prawns, but insufficient *Vibrio* isolates from salmon were recovered to determine if this is the case for salmon. The increase in aquaculture-produced seafood and associated AMR may be contributing to the increased detection of AMR *Vibrio* from patients (Koutsoumanis et al., 2024). It is possible that if more wild-caught prawn samples were cultured for *Vibrio* then we may isolate AMR or *Vibrio* pathogenic to humans. However, the proportion of wild-caught prawn samples that were positive for AMR or *Vibrio*

pathogenic to humans were lower than that of aquacultured prawns.

*Vibrio* species vary in their tolerance to different salinities (Naughton et al., 2009). The wild-caught prawn were likely caught at sea and exposed to salinity of 33–37 parts per million (ppm) (NOAA, 2023). Aquaculture environments for prawns vary between prawn species but are usually lower in salinity compared to the ocean (Li et al., 2017; Tantulo and Fotedar, 2006). This may explain why *Vibrio* species not tolerant to high salt concentrations such as *V. cholerae* (Miller et al., 1984) were not found in wild-caught prawns. However, *V. cholerae* has been detected in coastal and transitional regions of the ocean associated with reduced salinity (Goh et al., 2017; Gyraite et al., 2019).

The *V. parahaemolyticus* species includes strains that are and are not pathogenic to humans, and this species was associated with aquacultured prawn samples. We previously demonstrated how diverse the population of *Vibrio* on prawns can be, including the presence of multiple *V. parahaemolyticus* STs on individual samples (Janecko et al., 2021). *V. parahaemolyticus* strains that are and are not pathogenic to humans have been isolated from seafood sources (Lopez-Joven et al., 2015). *V. parahaemolyticus* isolates from human clinical cases are often associated with thermostable direct hemolysin (TDH) or TDH-related hemolysin (TRH), which form pores in host cells, altering the intracellular concentration of various ions (Honda et al., 1992; Takahashi et al., 2000), and the T3SS2 that injects many effector proteins into host cells (Meador et al., 2007). We did not identify any *V. parahaemolyticus* isolates from prawn samples in this study that contained genes encoding for these toxins or TS332, nor were these genes found in any of the publicly available *V. parahaemolyticus* genomes from prawns compared in this study, suggesting that *V. parahaemolyticus* from prawns may be non-pathogenic to humans. However, similar to other studies (Jones et al., 2012), we found that 32 % of the publicly available *V. parahaemolyticus* isolates from humans, which would predominantly derive from clinical cases, also did not contain these genes. In addition, two *V. parahaemolyticus* isolates collected from prawns in this study belonged to the same ST as isolates collected from humans, despite the small number of publicly available human isolates for comparison (n = 340). Furthermore, Lynch et al. (2005) found that *V. parahaemolyticus* can damage host cells using other mechanisms in the absence of these toxins. This suggests that prawns do have the potential to contribute to human *V. parahaemolyticus* infection cases, and *V. parahaemolyticus* pathogenicity must be examined outside of the *tdh, trh* and T3SS2 virulence genes.

Long-read sequencing enabled the determination of which MGEs were potentially responsible for the transmission of AMR. We identified multiple communities of plasmids, but only three contained ARGs. The ARGs on plasmids were usually in close proximity to IS6/IS26 that facilitate the movements of ARGs in other Gram-negative bacteria (Bloomfield et al., 2022). One of the plasmid types, IncA/C2, was found in *V. cholerae* and *V. parahaemolyticus* and has previously been described as an important vehicle of ARG transmission for these species (Li et al., 2015), including epidemic cholera strains (Folster et al., 2014). In this study the carbapenemase resistant gene, *blaNDM-1*, was located on this plasmid. The presence of this plasmid type in different *Vibrio* species suggests it can spread ARGs across the species barrier. However, most of the plasmids we identified in this study had no plasmid type marker or ARGs, but did contain a range of genes of various functions; therefore, their overall effect on their *Vibrio* host remains to be determined.

ARGs can be found throughout the genome, not only on plasmids, and in *Vibrio* we found that three quarters of the ARGs were found on the chromosome. These may be more stable than plasmid-encoded ARGs and allow for ARG distribution in the absence of an antimicrobial selective pressure (Hirai et al., 2013). However, they were not evenly distributed across the two *Vibrio* chromosomes, with most chromosomal ARG regions on *V. cholerae* and *V. parahaemolyticus* associated with chromosome 1. However, one ARG region was found on chromosome 2 of *V. cholerae*, associated with its SCI that consists of a promoter followed by a cassette of genes that can be re-arranged and those closest to the promoter are expressed (Vit et al., 2021). Transcriptomics and phenotypic testing would be required to determine if ARGs in this SCI are expressed.

*Vibrio* chromosomes can carry both intrinsic and acquired ARGs. *V. parahaemolyticus* comprised most of the genomes selected for long-read sequencing, and all isolates contained two ARGs, *tet(35)* gene on chromosome 1 and *blaCARB* on chromosome 2. *blaCARB* has been described as intrinsic to *V. parahaemolyticus*, responsible for resistance to ampicillin (Jiachi et al., 2015). *tet(35)* has also been described to be intrinsic to *V. parahaemolyticus* (Vandeputte et al., 2024), but many *V. parahaemolyticus* isolates with the *tet(35)* gene are susceptible to tetracycline (Wang et al., 2025). *V. diabolicus* is also predicted to have an intrinsic *blaCARB* gene (Sebastian et al., 2025). This ARG was missed by ARIBA likely due to its low identity compared to the closest reference but was identified using Abricate to be in a similar chromosomal region to the intrinsic *blaCARB* gene in *V. parahaemolyticus*, supporting it being classified as intrinsic to *V. diabolicus*. All the *V. metschnikovii* isolates collected in this study had the *tet(B)* gene. However, all *V. metschnikovii* isolates in this study were recovered from one sample and tetracycline-sensitive *V. metschnikovii* isolates have been isolated (Håkonsholm et al., 2020) so we did not classify *tet(B)* as intrinsic to this species. Few intrinsic ARGs were associated with an MGE; 8.7 % were associated with an IS, 2.6 % were associated with an ICE/IME and none were associated with integrons. Excluding these, we were left with the presumptive acquired chromosomal ARGs, 76 % of which were associated with an IS, most commonly IS91, 2.4 % of which were associated with an ICE/IME and 12 % of which were associated with an integron.

Comparisons of chromosomal ARG regions amongst different *Vibrio* species identified three ARG regions that were similar, associated with MGEs and found in different *Vibrio* species, indicating that chromosomal ARG regions could potentially be transmitted amongst different *Vibrio* species. This highlights the plasticity of the *Vibrio* genome and how this might allow the genus to transmit ARGs through a variety of mechanisms (Verma et al., 2019).

*Vibrio* isolates containing ARG plasmids or chromosomal ARG regions similar amongst different *Vibrio* species were isolated from *L. vannamei* from Thailand, Vietnam or attributed to multiple countries including these two countries. However, 67 % of prawn samples investigated were *L. vannamei*, and 2.9 % were from Thailand, 33 % were from Vietnam, and 14 % were attributed to multiple countries. Therefore, it is difficult to determine if this species of prawn or these countries are associated with increased ARG spread in *Vibrio* or if they are simply the most commonly imported prawn food type to the United Kingdom. In addition, during the time that these seafood samples were investigated and since then, Thailand and Vietnam have introduced regulations and monitoring to decrease antimicrobial usage in the aquaculture sector (Carrique-Mas et al., 2023; Lekagul et al., 2023). Further research is required to determine if this has altered the prevalence of AMR *Vibrio* on prawns from these countries.

One of the limitations of this study is that we only investigated genotypic evidence of AMR not phenotypic evidence. This is important as many studies have found discrepancies between the presence of ARGs in *Vibrio* and AMR phenotypes (Lepuschitz et al., 2019; Sebastian et al., 2025). *Vibrio* AMR is further complicated by how culture conditions can influence the AMR phenotype, e.g., aerobic versus anaerobic environments (Creasy-Marrazzo et al., 2022). Despite this, through the use of long-read sequencing this study was able to identify ARGs associated with MGEs that have the potential to be spread amongst *Vibrio* and identify other factors that may influence the AMR phenotype, e.g., the location of ARGs on SCIs. This approach may be used to identify the mobility potential of other genetic risk factors.

Aquaculture practices vary worldwide but could be adjusted to prevent the spread of AMR *Vibrio*. This could include reducing the use of antimicrobials (Tendencia and de la Peña, 2001), preventing fish and prawns being exposed to pathogenic and AMR bacteria by stopping livestock waste being used as a nutrient source (Petersen and Dalsgaard,

2003), and sterilising recirculated water (Wang et al., 2019). The development of vaccines (Lin et al., 2013) and maintaining aquatic environments that encourage strong immune systems (Lin et al., 2012) could also decrease the risk of infectious diseases and therefore the need for antimicrobials.

## 5. Conclusions

Aquacultured prawns were associated with AMR *Vibrio* and *Vibrio* pathogenic to humans. The high level of AMR is made possible by the large arsenal of MGEs found in the *Vibrio* genome that enable ARGs to be spread amongst *Vibrio* belonging to the same and different species. Aquaculture practices may need to be adjusted in order to prevent the spread of pathogenic and AMR *Vibrio*.

## CRediT authorship contribution statement

**Samuel J. Bloomfield:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raphaëlle Palau:** Writing – review & editing, Methodology, Investigation, Data curation. **Nicol Janecko:** Writing – review & editing, Methodology, Investigation, Data curation. **Craig Baker-Austin:** Writing – review & editing. **Alison E. Mather:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Funding

This project was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) Institute Strategic Programme Microbes in the Food Chain BB/R012504/1 and its constituent project BBS/E/F/000PR10348 (Theme 1, Epidemiology and Evolution of Pathogens in the Food Chain) and BBSRC Institute Strategic Programme Microbes and Food Safety BB/X011011/1 and its constituent project BBS/E/QU/230002A (Theme 1, Microbial threats from foods in established and evolving food systems). It was also supported by Food Standards Agency (FSA) project FS101185 through an FSA Fellowship to AEM. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alison Mather reports financial support and article publishing charges were provided by Biotechnology and Biological Sciences Research Council. Alison Mather reports financial support was provided by Food Standards Agency. Alison Mather reports a relationship with Biotechnology and Biological Sciences Research Council that includes: consulting or advisory, funding grants, and travel reimbursement. Alison Mather reports a relationship with Food Standards Agency that includes: consulting or advisory and funding grants. Alison Mather reports a relationship with University of East Anglia that includes: employment. Nicol Janecko reports a relationship with Food Standards Agency that includes: consulting or advisory and travel reimbursement. Alison Mather reports a relationship with UK Research and Innovation Medical Research Council that includes: funding grants. Alison Mather, Samuel Bloomfield have a patent pending to Quadram Institute Bioscience. All 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

We thank the Quadram Institute Bioscience core facilities sequencing and bioinformatics teams, laboratory technicians, media preparation and laboratory managers for their assistance in the project, and the CLIMB-BIG-DATA-computing servers, an infrastructure supported by the UKRI Medical Research Council grant MR/T030062/1.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2025.104819.

## Data availability

Raw Illumina reads were uploaded to the Sequence Read Archive (SRA) under bioprojects: PRJNA699735, PRJNA1107692 and PRJNA1155317. Raw MinION reads were uploaded to SRA under project PRJNA1155659.

## References

Archer, E.J., Baker-Austin, C., Osborn, T.J., Jones, N.R., Martínez-Urtaza, J., Trinanes, J., Oliver, J.D., González, F.J.C., Lake, I.R., 2023. Climate warming and increasing *Vibrio vulnificus* infections in North America. Sci. Rep. 13, 1–11. https://doi.org/10.1038/s41598-023-28247-2.

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. Nucleic Acids Res. 44, W16–W21. https://doi.org/10.1093/nar/gkw387.

Baker-Austin, C., Lake, I., Archer, E., Hartnell, R., Trinanes, J., Martinez-Urtaza, J., 2024. Stemming the rising tide of *Vibrio* disease. Lancet Planet. Health 8, e515–e520. https://doi.org/10.1016/S2542-5196(24)00124-4.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Compulational Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.

Bloomfield, S., Duong, V.T., Tuyen, H.T., Campbell, J.I., Thomson, N.R., Parkhill, J., Le Phuc, H., Chau, T.T.H., Maskell, D.J., Perron, G.G., Ngoc, N.M., Vi, L.L., Adriaenssens, E.M., Baker, S., Mather, A.E., 2022. Mobility of antimicrobial resistance across serovars and disease presentations in non-typhoidal *Salmonella* from animals and humans in Vietnam. Microb. Genom. 8, 1–13. https://doi.org/10.1099/mgen.0.000798.

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.

Cabello, F.C., Godfrey, H.P., Buschmann, A.H., Dolz, H.J., 2016. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. Lancet Infect. Dis. 16, E127–E133. https://doi.org/10.1016/S1473-3099(16)00100-6.

Carattoli, A., Zankari, E., Garcia-Fernandez, A., Larsen, M.V., Lund, O., Villa, L., Aarestrup, F.M., Hasman, H., 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob. Agents Chemother. 58, 3895–3903. https://doi.org/10.1128/AAC.02412-14.

Carrique-Mas, J.J., Hue, L.T., Dung, L.T., Thuy, N.T., Padungtod, P., 2023. Restrictions on antimicrobial use in aquaculture and livestock, Viet Nam. Bull. World Health Organ. 101, 223–225. https://doi.org/10.2471/BLT.22.289187.

Chaumeil, P.-A., Mussig, A.J., Hugenholtz, P., Parks, D.H., 2022. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinformatics 38, 5315–5316. https://doi.org/10.1093/bioinformatics/btac672.

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

Chowdhury, S., Rheman, S., Debnath, N., Delamare-Deboutteville, J., Akhtar, Z., Ghosh, S., Parveen, S., Islam, K., Islam, M.A., Rashid, M.M., Khan, Z.H., Rahman, M., Chadag, V.M., Chowdhury, F., 2022. Antibiotics usage practices in aquaculture in Bangladesh and their associated factors. One Health 15, 1–8. https://doi.org/10.1016/j.onehlt.2022.100445.

Connor, T.R., Loman, N.J., Thompson, S., Smith, A., Southgate, J., Poplawski, R., Bull, M. J., Richardson, E., Ismail, M., Elwood-Thompson, S., Kitchen, C., Guest, M., Bakke, M., Sheppard, S.K., Pallen, M.J., 2016. CLIMB (the cloud infrastructure for microbial bioinformatics): an online resource for the medical microbiology community. Microb. Genom. 2, 1–6. https://doi.org/10.1099/mgen.0.000086.

Creasy-Marrazzo, A., Saber, M.M., Kamat, M., Bailey, L.S., Brinkley, L., Cato, E., Begum, Y., Rashid, M.M., Khan, A.I., Qadri, F., Basso, K.B., Shapiro, B.J., Nelson, E. J., 2022. Genome-wide association studies reveal distinct genetic correlates and increased heritability of antimicrobial resistance in *Vibrio cholerae* under anaerobic conditions. Microb. Genom. 8, 1–14. https://doi.org/10.1099/mgen.0.000905.

Croucher, N.J., Page, A.J., Connor, T.R., Delaney, A.J., Keane, J.A., Bentley, S.D., Parkhill, J., Harris, S.R., 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 43, 1–13. https://doi.org/10.1093/nar/gku1196.

Feldgarden, M., Brover, V., Haft, D.H., Prasad, A.B., Slotta, D.J., Tolstoy, I., Tyson, G.H., Zhao, S., Hsu, C.-H., McDermott, P.F., Tadesse, D.A., Morales, C., Simmons, M., Tillman, G., Wasilenko, J., Folster, J.P., Klimke, W., 2019. Validating the AMRFinder

tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob. Agents Chemother. 63, 1–19. https://doi.org/10.1128/AAC.00483-19.

Folster, J.P., Katz, L., McCullough, A., Parsons, M.B., Knipe, K., Sammons, S.A., Boncy, J., Tarr, C.L., Whichard, J.M., 2014. Multidrug-resistant IncA/C plasmid in *Vibrio cholerae* from Haiti. Emerg. Infect. Dis. 20, 1951–1953. https://doi.org/10.3201/eid2011.140889.

Frolova, D., Lima, L., Roberts, L., Bohnenkämper, L., Wittler, R., Stoye, J., Iqbal, Z., 2024. Applying rearrangement distances to enable plasmid epidemiology with pling. Microb. Genom. 10, 1–13. https://doi.org/10.1101/2024.06.12.598623.

Gertz, E.M., Yu, Y.-K., Agarwala, R., Schaffer, A.A., Altschul, S.F., 2006. Composition-based statistics and translated nucleotide searches: improving the tBLASTn module of BLAST. BMC Biol. 4, 1–14. https://doi.org/10.1186/1741-7007-4-41.

Goh, S.G., Bayen, S., Burger, D., Kelly, B.C., Han, P., Babovic, V., Gin, K.Y.-H., 2017. Occurrence and distribution of bacteria indicators, chemical tracers and pathogenic vibrios in Singapore coastal waters. Mar. Pollut. Bull. 114, 627–634. https://doi.org/10.1016/j.marpolbul.2016.09.036.

Goulden, E.F., Hal, M.R., Bourne, D.G., Pereg, L.L., Lone, H., 2012. Pathogenicity and infection cycle of *Vibrio owensii* in larviculture of the ornate spiny lobster (*Panulirus ornatus*). Appl. Environ. Microbiol. 78, 2841–2849. https://doi.org/10.1128/AEM.07274-11.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

Gyraite, G., Katarzyte, M., Schernewski, G., 2019. First findings of potentially human pathogenic bacteria *Vibrio* in the south-eastern Baltic Sea coastal and transitional bathing waters. Mar. Pollut. Bull. 149, 1–9. https://doi.org/10.1016/j.marpolbul.2019.110546.

Håkonsholm, F., Lunestad, B.T., Aguirre Sánchez, J.R., Martinez-Urtaza, J., Marathe, N. P., Svanevik, C.S., 2020. Vibrios from the Norwegian marine environment: characterization of associated antibiotic resistance and virulence genes. MicrobiologyOpen 9, 1–19. https://doi.org/10.1002/mbo3.1093.

Hirai, I., Fukui, N., Taguchi, M., Yamauchi, K., Nakamura, T., Okano, S., Yamamoto, Y., 2013. Detection of chromosomal blaCTX-M-15 in *Escherichia coli* O25b-B2-ST131 isolates from the Kinki region of Japan. Int. J. Antimicrob. Agents 42, 500–506. https://doi.org/10.1016/j.ijantimicag.2013.08.005.

Honda, T., Ni, Y., Miwatani, T., Adachi, T., Kim, J., 1992. The thermostable direct hemolysin of *Vibrio parahaemolyticus* is a pore-forming toxin. Can. J. Microbiol. 38, 1175–1180. https://doi.org/10.1139/m92-192.

Huang, W., Li, L., Myers, J.R., Marth, G.T., 2012. ART: a next-generation sequencing read simulator. Bioinformatics 28, 593–594. https://doi.org/10.1093/bioinformatics/btr708.

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernandez-Plaza, A., Forslund, S.K., Cook, H., Mende, D.R., Letunic, I., Rattei, T., Jensen, L.J., von Mering, C., Bork, P., 2019. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 47, D309–D314. https://doi.org/10.1093/nar/gky1085.

Hunt, M., Mather, A.E., Sanchez-Buso, L., Page, A.J., Parkhill, J., Keane, J.A., Harris, S. R., 2017. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb. Genom. 3, 1–11. https://doi.org/10.1099/mgen.0.000131.

Janecko, N., Bloomfield, S.J., Palau, R., Mather, A.E., 2021. Whole genome sequencing reveals great diversity of *Vibrio* spp in prawns at retail. Microb. Genom. 7, 1–14. https://doi.org/10.1099/mgen.0.000647.

Janecko, N., Zamudio, R., Palau, R., Bloomfield, S.J., Mather, A.E., 2023. Repeated cross-sectional study identifies differing risk factors associated with microbial contamination in common food products in the United Kingdom. Food Microbiol. 111, 1–12. https://doi.org/10.1016/j.fm.2022.104196.

Jeamsripong, S., Thaotumpitak, V., Anuntawirun, S., Roongrojmongkhon, N., Atwill, E. R., Hinthong, W., 2022. Molecular epidemiology of antimicrobial resistance and virulence profiles of *Escherichia coli, Salmonella* spp., and *Vibrio* spp. isolated from coastal seawater for aquaculture. Antibiotics 11, 1–17. https://doi.org/10.3390/antibiotics11121688.

Jiachi, C., Ruichao, L., Sheng, C., 2015. CARB-17 family of β-lactamases mediates intrinsic resistance to penicillins in *Vibrio parahaemolyticus*. Antimicrob. Agents Chemother. 59, 3593–3595. https://doi.org/10.1128/aac.00047-15.

Jones, J.L., Lüdeke, C.H.M., Bowers, J.C., Garrett, N., Fischer, M., Parsons, M.B., Bopp, C. A., DePaola, A., 2012. Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. J. Clin. Microbiol. 50, 2343–2352. https://doi.org/10.1128/jcm.00196-12.

Kang, C.-H., Shin, Y., Yu, H., Kim, S., So, J.-S., 2018. Antibiotic and heavy-metal resistance of *Vibrio parahaemolyticus* isolated from oysters in Korea. Mar. Pollut. Bull. 135, 69–74. https://doi.org/10.1016/j.marpolbul.2018.07.007.

Kolmogorov, M., Yuan, J., Lin, Y., Pevzner, P.A., 2019. Assembly of long, error-prone reads using repeat graphs. Nat. Biotechnol. 37, 540–546. https://doi.org/10.1038/s41587-019-0072-8.

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H., Phillippy, A.M., 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res. 27, 722–736. https://doi.org/10.1101/gr.215087.116.

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Baker-Austin, C., Hervio-Heath, D., Martinez-Urtaza, J., Caro, E.S., Strauch, E., Thébault, A., Guerra, B., Messens, W., Simon, A.C., Barcia-Cruz, R., Suffredini, E., 2024. Public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU. EFSA J. 22, 1–92. https://doi.org/10.2903/j.efsa.2024.8896.

Lavilla-Pitogo, C.R., Baticados, M.C.L., Cruz-Lacierda, E.R., de la Pena, L.D., 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture 91, 1–13. https://doi.org/10.1016/0044-8486(90)90173-K.

Lawrence, M., Huber, W., Pagès, H., Aboyoun, P., Carlson, M., Gentleman, R., Morgan, M.T., Carey, V.J., 2013. Software for computing and annotating genomic ranges. PLoS Comput. Biol. 9, 1–10. https://doi.org/10.1371/journal.pcbi.1003118.

Leibovici-Weissman, Y., Neuberger, A., Bitterman, R., Sinclair, D., Salam, M.A., Paul, M., 2014. Antimicrobial drugs for treating cholera. Cochrane Database Syst. Rev. 1–139. https://doi.org/10.1002/14651858.CD008625.pub2.

Lekagul, A., Kirivan, S., Tansakul, N., Krisanaphan, C., Srinha, J., Laoprasert, T., Kaewkhankhaeng, W., Tangcharoensathien, V., 2023. Antimicrobial consumption in food-producing animals in Thailand between 2017 and 2019: the analysis of national importation and production data. PLoS One 18, 1–16. https://doi.org/10.1371/journal.pone.0283819.

Lepuschitz, S., Baron, S., Larvor, E., Granier, S.A., Pretzer, C., Mach, R.L., Farnleitner, A. H., Ruppitsch, W., Pleininger, S., Indra, A., Kirschner, A.K.T., 2019. Phenotypic and genotypic antimicrobial resistance traits of *Vibrio cholerae* non-O1/non-O139 isolated from a large Austrian lake frequently associated with cases of human infection. Front. Microbiol. 10, 1–9. https://doi.org/10.3389/fmicb.2019.02600.

Li, E., Wang, X., Chen, K., Xu, C., Qin, J.G., Chen, L., 2017. Physiological change and nutritional requirement of Pacific white shrimp *Litopenaeus vannamei* at low salinity. Rev. Aquacult. 9, 57–75. https://doi.org/10.1111/raq.12104.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

Li, R., Lin, D., Chen, K., Wong, M.H.Y., Chen, S., 2015. First detection of AmpC β-lactamase bla(CMY-2) on a conjugative IncA/C plasmid in a *Vibrio parahaemolyticus* isolate of food origin. Antimicrob. Agents Chemother. 59, 4106–4111. https://doi.org/10.1128/AAC.05008-14.

Lin, Y.-C., Chen, J.-C., Li, C.-C., Morni, W.Z.W., Suhaili, A.S.N.A., Kuo, Y.-H., Chang, Y.-H., Chen, L.-L., Tsui, W.-C., Chen, Y.-Y., Huang, C.-L., 2012. Modulation of the innate immune system in white shrimp *Litopenaeus vannamei* following long-term low salinity exposure. Fish Shellfish Immunol. 33, 324–331. https://doi.org/10.1016/j.fsi.2012.05.006.

Lin, Y.-C., Chen, J.-C., Morni, W.Z.W., Putra, D.F., Huang, C.-L., Li, C.-C., Hsieh, J.-F., 2013. Vaccination enhances early immune responses in white shrimp *Litopenaeus vannamei* after secondary exposure to *Vibrio alginolyticus*. PLoS One 8, 1–15. https://doi.org/10.1371/journal.pone.0069722.

Löfstedt, A., Scheliga, B., Aceves-Martins, M., de Roos, B., 2025. Seafood supply mapping reveals production and consumption mismatches and large dietary nutrient losses through exports in the United Kingdom. Nat. Food 6, 244–252. https://doi.org/10.1038/s43016-024-01102-x.

Lopez-Joven, C., de Blas, I., Furones, M.D., Roque, A., 2015. Prevalences of pathogenic and non-pathogenic *Vibrio parahaemolyticus* in mollusks from the Spanish Mediterranean Coast. Front. Microbiol. 6, 1–10. https://doi.org/10.3389/fmicb.2015.00736.

Lynch, T., Livingstone, S., Buenaventura, E., Lutter, E., Fedwick, J., Buret, A.G., Graham, D., DeVinney, R., 2005. *Vibrio parahaemolyticus* disruption of epithelial cell tight junctions occurs independently of toxin production. Infect. Immun. 73, 1275–1283. https://doi.org/10.1128/IAI.73.3.1275-1283.2005.

Meador, C.E., Parsons, M.M., Bopp, C.A., Peter, G.-S., Painter, J.A., Vora, G.J., 2007. Virulence gene- and pandemic group-specific marker profiling of clinical *Vibrio parahaemolyticus* isolates. J. Clin. Microbiol. 45, 1133–1139. https://doi.org/10.1128/jcm.00042-07.

Miller, C.J., Drasar, B.S., Feachem, R.G., 1984. Response of toxigenic *Vibrio cholerae* 01 to physico-chemical stresses in aquatic environments. J. Hyg. 93, 475–495. https://doi.org/10.1017/s0022172400065074.

Naughton, L.M., Blumerman, S.L., Carlberg, M., Fidelma Boyd, E., 2009. Osmoadaptation among *Vibrio* species and unique genomic features and physiological responses of *Vibrio parahaemolyticus*. Appl. Environ. Microbiol. 75, 2802–2810. https://doi.org/10.1128/AEM.01698-08.

Néron, B., Littner, E., Haudiquet, M., Perrin, A., Cury, J., Rocha, E.P.C., 2022. IntegronFinder 2.0: identification and analysis of integrons across bacteria, with a focus on antibiotic resistance in *Klebsiella*. Microorganisms 10, 1–14. https://doi.org/10.3390/microorganisms10040700.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300.

NOAA, 2023. Sea Water [WWW Document]. Natl. Ocean. Atmos. Adm. https://www.noaa.gov/jetstream/ocean/sea-water. accessed 2.19.25.

Nonaka, L., Masuda, M., Yano, H., 2022. Atypical integrative element with strand-biased circularization activity assists interspecies antimicrobial resistance gene transfer from *Vibrio alfacsensis*. PLoS One 17, 1–24. https://doi.org/10.1371/journal.pone.0271627.

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A., Parkhill, J., 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31, 3691–3693. https://doi.org/10.1093/bioinformatics/btv421.

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E., Larsson, D.G.J., 2014. BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Res. 42, D737–D743. https://doi.org/10.1093/nar/gkt1252.

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25, 1043–1055. https://doi.org/10.1101/gr.186072.114.

Pérez-Duque, A., Gonzalez-Muñoz, A., Arboleda-Valencia, J., Vivas-Aguas, L.J., Córdoba-Meza, T., Rodriguez-Rey, G.T., Díaz-Guevara, P., Martinez-Urtaza, J., Wiesner-Reyes, M., 2021. Comparative genomics of clinical and environmental isolates of *Vibrio* spp. of Colombia: implications of traits associated with virulence and resistance. Pathogens 10, 1–22. https://doi.org/10.3390/pathogens10121605.

Petersen, A., Dalsgaard, A., 2003. Species composition and antimicrobial resistance genes of *Enterococcus* spp., isolated from integrated and traditional fish farms in Thailand. Environ. Microbiol. 5, 395–402. https://doi.org/10.1046/j.1462-2920.2003.00430.x.

R core team, 2019. R: a Language and Environment for Statistical Computing [WWW Document]. R Found. Stat. Comput. http://www.r-project.org/.

Ritchie, H., 2019. The World now Produces More Seafood from Fish Farms than Wild Catch. Our World Data [WWW Document]. https://ourworldindata.org/rise-of-aquaculture. accessed 12.4.24.

Rivard, N., Colwell, R.R., Burrus, V., 2020. Antibiotic resistance in *Vibrio cholerae*: mechanistic insights from IncC plasmid-mediated dissemination of a novel family of genomic islands inserted at *trmE*. mSphere 5, 1–18. https://doi.org/10.1128/mSphere.00748-20.

Robertson, J., Nash, J.H.E., 2018. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb. Genom. 4, 1–7. https://doi.org/10.1099/mgen.0.000206.

Sarkar, A., Morita, D., Ghosh, A., Chowdhury, G., Mukhopadhyay, A.K., Okamoto, K., Ramamurthy, T., 2019. Altered integrative and conjugative elements (ICEs) in recent *Vibrio cholerae* O1 isolated from cholera cases, Kolkata, India. Front. Microbiol. 10, 1–13. https://doi.org/10.3389/fmicb.2019.02072.

Schar, D., Klein, E.Y., Laxminarayan, R., Gilbert, M., Van Boeckel, T.P., 2020. Global trends in antimicrobial use in aquaculture. Sci. Rep. 10, 1–9. https://doi.org/10.1038/s41598-020-78849-3.

Schwengers, O., Jelonek, L., Dieckmann, M.A., Beyvers, S., Blom, J., Goesmann, A., 2021. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microb. Genom. 7, 1–13. https://doi.org/10.1099/mgen.0.000685.

Sebastian, P.J., Schlesener, C., Byrne, B.A., Miller, M., Smith, W., Batac, F., Goertz, C.E. C., Weimer, B.C., Johnson, C.K., 2025. Antimicrobial resistance of *Vibrio* spp. from the coastal California system: discordance between genotypic and phenotypic patterns. Appl. Environ. Microbiol. 1–24. https://doi.org/10.1128/aem.01808-24.

Sinatra, J.A., Colby, K., 2018. Fatal *Vibrio anguillarum* infection in an immunocompromised patient - Maine, 2017. Morb. Mortal. Wkly. Rep 67, 962–963. https://doi.org/10.15585/mmwr.mm6734a5.

Sony, M., Sumithra, T.G., Anusree, V.N., Amala, P.V., Reshma, K.J., Alex, S., Sanil, N.K., 2021. Antimicrobial resistance and virulence characteristics of *Vibrio vulnificus, Vibrio parahaemolyticus* and *Vibrio harveyi* from natural disease outbreaks of marine/estuarine fishes. Aquaculture 539, 1–14. https://doi.org/10.1016/j.aquaculture.2021.736608.

Sullivan, M.J., Petty, N.K., Beatson, S.A., 2011. Easyfig: a genome comparison visualizer. Bioinformatics 27, 1009–1010. https://doi.org/10.1093/bioinformatics/btr039.

Takahashi, A., Kenjyo, N., Imura, K., Myonsun, Y., Honda, T., 2000. Cl(-) secretion in colonic epithelial cells induced by the *Vibrio parahaemolyticus* hemolytic toxin related to thermostable direct hemolysin. Infect. Immun. 68, 5435–5438. https://doi.org/10.1128/IAI.68.9.5435-5438.2000.

Talpur, A.D., Memon, A.J., Khan, M.I., Ikhwanuddi, M., Daniel, M.M., Abol-Munaf, A.B., 2011. A novel of gut pathogenic bacteria of blue swimming crab *Portunus pelagicus* (Linneaus, 1758) and pathogenicity of *Vibrio harveyi* a transmission agent in larval culture under hatchery conditions. Res. J. Appl. Sci. 6, 116–127. https://doi.org/10.3923/rjasci.2011.116.127.

Tan, C.W., Rukayadi, Y., Hasan, H., Thung, T.Y., Lee, E., Rollon, W.D., Hara, H., Kayali, A.Y., Nishibuchi, M., Radu, S., 2020. Prevalence and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor, Malaysia. Saudi J. Biol. Sci. 27, 1602–1608. https://doi.org/10.1016/j.sjbs.2020.01.002.

Tantulo, U., Fotedar, R., 2006. Comparison of growth, osmoregulatory capacity, ionic regulation and organosomatic indices of black tiger prawn (*Penaeus monodon* Fabricius, 1798) juveniles reared in potassium fortified inland saline water and ocean water at different salinities. Aquaculture 258, 594–605. https://doi.org/10.1016/j.aquaculture.2006.04.038.

Tendencia, E.A., de la Peña, L.D., 2001. Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195, 193–204. https://doi.org/10.1016/S0044-8486(00)00570-6.

Uh, Y., Park, J.-S., Hwang, G.-Y., Jang, I.-H., Yoon, K.-J., Park, H.-C., Hwang, S.-O., 2001. *Vibrio alginolyticus* acute gastroenteritis: report of two cases. Clin. Microbiol. Infect. 7, 104–106. https://doi.org/10.1046/j.1469-0691.2001.00207.x.

Vandeputte, M., Coppens, S., Bossier, P., Vereecke, N., Vanrompay, D., 2024. Genomic mining of *Vibrio parahaemolyticus* highlights prevalence of antimicrobial resistance genes and new genetic markers associated with AHPND and tdh +/trh + genotypes. BMC Genom. 25, 1–14. https://doi.org/10.1186/s12864-024-10093-9.

Vaser, R., Šikić, M., 2021. Time- and memory-efficient genome assembly with Raven. Nat. Comput. Sci. 1, 332–336. https://doi.org/10.1038/s43588-021-00073-4.

Verma, J., Bag, S., Saha, B., Kumar, P., Ghosh, T.S., Dayal, M., Senapati, T., Mehra, S., Dey, P., Desigamani, A., Kumar, D., Rana, P., Kumar, B., Maiti, T.K., Sharma, N.C., Bhadra, R.K., Mutreja, A., Nair, G.B., Ramamurthy, T., Das, B., 2019. Genomic plasticity associated with antimicrobial resistance in *Vibrio cholerae*. Proc. Natl. Acad. Sci. 116, 6226–6231. https://doi.org/10.1073/pnas.1900141116.

Vezzulli, L., Grande, C., Reid, P.C., Hélaouët, P., Edwards, M., Höfle, M.G., Brettar, I., Colwell, R.R., Pruzzo, C., 2016. Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic. Proc. Natl. Acad. Sci. 113, E5062–E5071. https://doi.org/10.1073/pnas.1609157113.

Vit, C., Richard, E., Fournes, F., Whiteway, C., Eyer, X., Lapaillerie, D., Parissi, V., Mazel, D., Loot, C., 2021. Cassette recruitment in the chromosomal integron of *Vibrio cholerae*. Nucleic Acids Res. 49, 5654–5670. https://doi.org/10.1093/nar/gkab412.

Walker, B.J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C.A., Zeng, Q., Wortman, J., Young, S.K., Earl, A.M., 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9, 1–14. https://doi.org/10.1371/journal.pone.0112963.

Wang, J.-H., Lu, J., Wu, J., Zhang, Y., Zhang, C., 2019. Proliferation of antibiotic resistance genes in coastal recirculating mariculture system. Environ. Pollut. 248, 462–470. https://doi.org/10.1016/j.envpol.2019.02.062.

Wang, Q., Li, Q., Ouyang, F., Ke, B., Jiang, S., Liu, J., Yan, J., Li, B., Tan, W., He, D., 2025. Molecular epidemiology and antimicrobial resistance of *Vibrio parahaemolyticus* isolates from the Pearl River Delta region, China. Int. J. Food Microbiol. 429, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2024.111025.

Wang, R., Zhong, Y., Gu, X., Yuan, J., Saeed, A.F., Wang, S., 2015. The pathogenesis, detection, and prevention of *Vibrio parahaemolyticus*. Front. Microbiol. 6, 1–13. https://doi.org/10.3389/fmicb.2015.00144.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, 1–22. https://doi.org/10.1371/journal.pcbi.1005595.

Wickham, H., 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Xie, Z., Tang, H., 2017. ISEScan: automated identification of insertion sequence elements in prokaryotic genomes. Bioinformatics 33, 3340–3347. https://doi.org/10.1093/bioinformatics/btx433.

Yin, T., Cook, D., Lawrence, M., 2012. Ggbio: an R package for extending the grammar of graphics for genomic data. Genome Biol. 13, 1–14. https://doi.org/10.1186/gb-2012-13-8-r77.

Yu, G., Smith, D.K., Zhu, H., Guan, Y., Lam, T.T., 2017. Ggtree : an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol. Evol. 8, 28–36. https://doi.org/10.1111/2041-210X.12628.

Yun, N.R., Kim, D.-M., 2018. *Vibrio vulnificus* infection: a persistent threat to public health. Korean J. Intern. Med. 33, 1070–1078. https://doi.org/10.3904/kjim.2018.159.

Zhang, X.-J., Bai, X.-S., Yan, B.-L., Bi, K.-R., Qin, L., 2014. *Vibrio harveyi* as a causative agent of mass mortalities of megalopa in the seed production of swimming crab *Portunus trituberculatus*. Aquac. Int. 22, 661–672. https://doi.org/10.1007/s10499-013-9695-9.

Zhou, Z., Alikhan, N.-F., Mohamed, K., Fan, Y., Achtman, M., Brown, D., Chattaway, M., Dallman, T., Delahay, R., Kornschober, C., Pietzka, A., Malorny, B., Petrovska, L., Davies, R., Robertson, A., Tyne, W., Weill, F.-X., Accou-Demartin, M., Williams, N., Grp, A.S., 2020. The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. Genome Res. 30, 138–152. https://doi.org/10.1101/gr.251678.119.