Complete Whole Genome Sequence of Vibrio parahaemolyticus RP0132 Strain Isolated from Shrimp in Malaysia

Ke-Yan Loo1,7, Loh Teng-Hern Tan1,2, Jodi Woan-Fei Law1,3, Kah-Ooi Chua4, Priyia Pusparajah5, Learn-Han Lee1,6*, Vengadesh Letchumanan1,7*

Abstract: Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium that is ubiquitous in marine environments. Its perilous co-existence with aquatic animals increases the risk of infections and diseases, especially those in aquaculture systems, thus resulting in reduced production and economic losses in the aquaculture industry. Moreover, V. parahaemolyticus can be easily transmitted to humans via consumption of contaminated seafood, resulting in gastroenteritis outbreaks. However, the rise in multidrug resistance...
within the species has challenged the efficacy of antimicrobial treatments against *V. parahaemolyticus* infections. Therefore, we report the genome sequence of *V. parahaemolyticus* RP0132 isolated from shrimp to gain insight into its antimicrobial resistance traits and potential resistance mechanisms. The findings will facilitate the development of effective anti-*Vibrio* agents to manage *V. parahaemolyticus* infections.

**Keywords:** *Vibrio parahaemolyticus*; multi-drug resistance; genome; antibiotic; next generation sequencing

1. Introduction

*Vibrio parahaemolyticus* is a Gram-negative, halophilic bacterium that is abundant in aquatic environments such as estuaries, rivers, and oceans [1-3]. The cohabitation of this pathogen and marine animals increases the risk of infections and diseases, particularly in the cultivated animals in aquaculture systems [4-6]. This reduces seafood yield and causes massive economic losses within the aquaculture industry. Furthermore, *V. parahaemolyticus* can be easily transmitted to humans by consumption of contaminated seafood, resulting in gastroenteritis [7-11]. This seafood-borne pathogen has been associated with gastroenteritis outbreaks across various countries worldwide [12-14]. Treatment of *V. parahaemolyticus* typically focuses on rehydration and antimicrobial therapy [15], but the emergence of antibiotic resistance within the species has reduced the efficacy of the antibiotics [16]. The rise in antibiotic resistance ( AMR) in *V. parahaemolyticus* can be attributed to the uncontrolled use of antibiotics in aquaculture, where antimicrobial agents are used as prophylaxis and treatment for infected cultivated animals [17-20]. Antibiotic residues from the aquaculture systems create environmental pressures that result in the development of AMR in *V. parahaemolyticus* isolates [21]. Multiple studies have also reported on multidrug resistant (MDR) *V. parahaemolyticus* strains [22-27]. Moreover, AMR can be spread inter- or intra-species via horizontal gene transfer, producing new generations of MDR bacteria in our surrounding environment [28-30]. Consequently, bacterial infections by MDR bacteria will become more prevalent, thereby increasing the difficulty of providing effective treatment, resulting in increased fatality rates [31, 32]. As an alternative to antibiotics, researchers have been exploring the use of probiotics as a means of preventing bacterial infections via gut microbiome modulation [33-35]. However, additional clinical research is still needed to produce conclusive evidence on the effectiveness of probiotics [34, 36, 37]. Therefore, it is crucial to maintain vigilant, ongoing surveillance of the AMR patterns of *V. parahaemolyticus* strains in our surrounding environment [38, 39]. These efforts are vital for safeguarding the aquaculture industry's viability and preserving public health.

With the increasing availability and accessibility of next-generation sequencing technologies [40, 41], whole genome analysis has provided extensive genomic information on various organisms [42-45]. To better understand the MDR patterns of *V. parahaemolyticus*, we examined the whole genome sequence (WGS) of *V. parahaemolyticus* RP0132, a strain obtained from our previous study [46]. The strain, RP0132 was isolated from speckled shrimp
(Metapenaeus monoceros), which originated from a wet market in Malaysia. This strain was resistant to five out of fourteen types of antibiotics tested with a multiple antibiotic resistance (MAR) index of 0.36. The antibiogram of RP0132 showed resistance towards ampicillin, third-generation cephalosporins (ceftazidime and cefotaxime), gentamicin, and the combination antibiotic, ampicillin/sublactam. The AMR patterns of RP0132 raise concern as they depict resistance against the recommended antimicrobial agents in V. parahaemolyticus infections, such as third-generation cephalosporins and aminoglycosides [47, 48]. As AMR spreads, the antibiotic choice will become very limited when treating these bacterial infections. Their AMR and corresponding genes can be identified by examining the whole genome sequence of V. parahaemolyticus isolates [49, 50]. This can potentially help develop effective treatment for MDR V. parahaemolyticus. Therefore, the whole genome sequence of the MDR V. parahaemolyticus RP0132 strain was studied to gain insight into the potential mechanisms driving its resistance and enhance the understanding of AMR within the species.

2. Data description

The genomic DNA of RP0132 was extracted using MasterPure Complete DNA and RNA Purification Kit (LGC Biosearch Technologies) according to the manufacturer’s instructions with slight modifications. The DNA quality and quantity were checked using agarose gel electrophoresis and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). SMRTbell DNA libraries were generated according to standard protocols and checked with Qubit for quantification and bioanalyzer for size distribution detection. The whole genome of RP0132 was sequenced on PacBio Sequel II/IIe systems, yielding a genome coverage of 102.3-fold. After sequencing, the raw reads were assembled using Falcon which is based on the hierarchical genome assembly process (HGAP). BUSCO assessment was also done to assess the genome assembly and annotation completeness with single-copy orthologs. Ribosome RNA (rRNA) genes were predicted by the RNAmmer and transfer RNA (tRNA) genes were predicted by the tRNAscan-SE. Annotation was performed using PATRIC (PathoSystems Resources Integration Center) for antibiotic-resistant genes. Further, the PATRIC-annotated antibiotic genes were BLAST against the National Center for Biotechnology Information (NCBI) database to confirm the gene identities.

The whole genome of RP0132 comprised of 2 contigs, and the assembled complete genome size of V. parahaemolyticus RP0132 contains 5,165,100 bp, with an average genome coverage of 102.3-fold with a GC content of 45%. The BUSCO score of the whole genome of RP0132 was 100%, indicating a reliable data output [51]. A total of 132 tRNA genes and 37 rRNA genes were predicted in the RP0132 genome (Table 1).
Table 1. Genomic features of Vibrio parahaemolyticus RP0132.

<table>
<thead>
<tr>
<th></th>
<th>Vibrio parahaemolyticus RP0132</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of contigs</td>
<td>2</td>
</tr>
<tr>
<td>Genome size (bp)</td>
<td>5,165,100</td>
</tr>
<tr>
<td>GC content (%)</td>
<td>45</td>
</tr>
<tr>
<td>Genome coverage</td>
<td>102.3x</td>
</tr>
<tr>
<td>DNA scaffold</td>
<td>2</td>
</tr>
<tr>
<td>Number of chromosomes</td>
<td>2</td>
</tr>
<tr>
<td>Total number of predicted genes</td>
<td>4797</td>
</tr>
<tr>
<td>Total number of protein-coding genes</td>
<td>4523</td>
</tr>
<tr>
<td>Total number of RNA-coding genes</td>
<td>165 (tRNA-coding-genes: 132, rRNA-coding genes: 33)</td>
</tr>
<tr>
<td>Total number of NCRNA-coding genes</td>
<td>4</td>
</tr>
<tr>
<td>Total number of pseudogenes</td>
<td>105</td>
</tr>
</tbody>
</table>

From the WGS of V. parahaemolyticus RP0132, multiple antibiotic resistance genes (ARG) were detected, and the findings corroborated the phenotype of the strain. For instance, the gene encoding for the detected aminoglycoside phosphotransferase is associated with the antibiotic resistance of RP0132 towards gentamicin, and the strain exhibited intermediate resistance towards kanamycin. Tian et al. identified a possible mechanistic explanation for the resistance towards aminoglycoside in V. parahaemolyticus whereby there is an upregulation of antibiotic resistance genes involved in phosphotransferase systems such as aminoglycoside phosphotransferases [52]. In addition, resistance towards the beta-lactams, including ampicillin, ampicillin/sulbactam, cefazidime, and cefotaxime is closely related to the presence of ARGs encoding for metallo-beta-lactamase and carbenicillin-hydrolyzing class A beta-lactamase. Metallo beta-lactamases are enzymes that catalyze the hydrolysis of beta-lactam antibiotics by binding the negatively charged carboxylate or similarly charged group on beta-lactams with hydrogen bonds [53, 54]. Subsequent reactions involve the acylation of the beta-lactamase and deacylation of the beta-lactam-beta-lactamase complex with a water molecule. This will result in the inactivation of the antibiotic and the regeneration of an active beta-lactamase [55]. Currently, no metallo-beta-lactamase inhibitors are available, making resistance conferred via metallo-beta-lactamases a difficult challenge to overcome [56]. A study by Coutinho et al. determined the presence of CARB-18 gene, which encodes for a beta-lactamase in the studied V. parahaemolyticus strain JPA1 [57]. The presence of the beta lactamase was associated with the strain's resistance towards the beta-lactams such as ampicillin, ampicillin/sulbactam, and cefotaxime [57]. These findings are similar to those from the phenotype and genome of RP0132.

Besides, the presence of ARGs encoding for chloramphenicol acetyltransferase (CAT) conferred its intermediate resistance towards chloramphenicol. CAT is the most encountered resistance mechanism of bacteria towards chloramphenicol. The CAT enzymes inactivate the drug via acetylation to ensure bacteria survival [58, 59]. ARGs encoding for CAT have been found in V. parahaemolyticus and members of the Vibrio family, such as V. cholerae and V. vulnificus are known to cause human diseases [57, 60]. Moreover, multiple genes encoding for efflux pumps were also found within the genome, indicating other possible mechanisms of drug
efflux contributing to its antibiotic resistance (Table 2). These efflux pumps are active solute transport systems that pump the antibiotics into the extracellular space, allowing *V. parahaemolyticus* isolates to survive and proliferate even under highly distinct antimicrobial agents \[61\]. The ARGs in the genome of RP0132 encode efflux pumps that can be further categorized into resistance-nodulation-cell division (RND) and multidrug and toxic compound extrusion (MATE) families of efflux pumps. The expression of these efflux pumps can confer resistance towards aminoglycosides, beta-lactams, chloramphenicol, fluoroquinolones, novobiocin, rifampin, erythromycin, tetracyclines, and trimethoprim \[62-64\].

**Table 2.** Antibiotic resistance genes and their corresponding proteins detected from WGS of *V. parahaemolyticus* RP0132.

<table>
<thead>
<tr>
<th>Antibiotic resistance genes</th>
<th>BLAST result</th>
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<tbody>
<tr>
<td>vmeA</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeA</td>
</tr>
<tr>
<td>vmeC</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeC</td>
</tr>
<tr>
<td>vmeE</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeE</td>
</tr>
<tr>
<td>vmeJ</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeJ</td>
</tr>
<tr>
<td>vmeT</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeT</td>
</tr>
<tr>
<td>vmeU</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeU</td>
</tr>
<tr>
<td>vmeY</td>
<td>Multidrug efflux RND transporter periplasmic adaptor subunit VmeY</td>
</tr>
<tr>
<td>vmeB</td>
<td>Multidrug efflux RND transporter permease subunit VmeB</td>
</tr>
<tr>
<td>vmeD</td>
<td>Multidrug efflux RND transporter permease subunit VmeD</td>
</tr>
<tr>
<td>vmeF</td>
<td>Multidrug efflux RND transporter permease subunit VmeF</td>
</tr>
<tr>
<td>vmeK</td>
<td>Multidrug efflux RND transporter permease subunit VmeK</td>
</tr>
<tr>
<td>vmeV</td>
<td>Multidrug efflux RND transporter permease subunit VmeV</td>
</tr>
<tr>
<td>vmeZ</td>
<td>Multidrug efflux RND transporter permease subunit VmeZ</td>
</tr>
<tr>
<td>vmeI</td>
<td>Efflux RND transporter permease subunit VmeI</td>
</tr>
<tr>
<td>vmeQ</td>
<td>Efflux RND transporter permease subunit VmeQ</td>
</tr>
<tr>
<td>vmeG</td>
<td>Efflux RND transporter periplasmic adaptor subunit VmeG</td>
</tr>
<tr>
<td>vmeH</td>
<td>Efflux RND transporter periplasmic adaptor subunit VmeH</td>
</tr>
<tr>
<td>abgT</td>
<td>AbgT family transporter</td>
</tr>
<tr>
<td>bcr/cflA</td>
<td>Bcr/CflA family multidrug efflux MFS transporter</td>
</tr>
<tr>
<td>dinF</td>
<td>MATE family efflux transporter DinF</td>
</tr>
</tbody>
</table>

The MDR patterns of *V. parahaemolyticus* RP0132 observed through both phenotype and genotype provide insight into the extent of antibiotic contamination in the environment. The MAR index of RP0132 is greater than 0.2, indicating the strain originated from a high-risk source of contamination where antibiotics are frequently used \[65, 66\]. This could be attributed to the extensive use of antibiotics in aquaculture, which remains a major contributor to AMR in *V. parahaemolyticus* \[67\]. Moreover, the effects of climate change, such as the increase in seawater temperatures, have driven the growth of *V. parahaemolyticus* populations in the environment \[13, 68\]. With the rise in global temperatures, infectious diseases are becoming
more apparent, jeopardizing the livelihoods of global citizens [68-70]. Therefore, there is a dire need to search for solutions to resolve this public health issue to protect the public from AMR infections. Recent research has been looking into alternative treatment methods to manage the populations of *V. parahaemolyticus* and reduce the spread of AMR in our surrounding environments [71-74]. A compelling candidate is *Streptomyces* sp., a group of Gram-positive, filamentous bacteria belonging to the phylum *Actinobacteria* [75]. They are abundant in nature and have been frequently isolated from various sources such as soils, mangroves, and marine environments [76-81]. The use of streptomycetes as a probiotic has been widely studied due to their ability to produce bioactive secondary metabolites during their life cycle [82-88]. Moreover, *Streptomyces* sp. has shown various bioactivities, including antioxidative, anticancer, cytotoxic, antibacterial, and more specifically anti-*Vibrio* properties [46, 89-91].

Research has shown the various potential mechanisms behind the anti-*Vibrio* properties of *Streptomyces* sp [92-95]. For instance, the production of siderophores or enzymes by streptomycetes has been shown to inhibit the growth of *Vibrios* in aquatic environments [96, 97]. In addition, the production of melain compounds from marine *Streptomyces* has exhibited good activity against *Vibrios* including *V. parahaemolyticus* [98-100]. Furthermore, the supplementation of *Streptomyces* sp. in aquatic animals has increased the population of antimicrobial producers in the host, thereby increasing the host immunity and defense against *Vibrios* [101, 102]. Moreover, the supplementation of *Streptomyces* sp. in marine animals stimulated the release of growth hormones in the host, thereby increasing the growth rates of the marine animals [103, 104]. Hence, using *Streptomyces* as a probiotic can help control *Vibrio* populations while simultaneously increasing host immunity against *Vibrios* and increasing the growth rates of the cultivated animals. However, the developmental process of new antibiotics or alternatives is rigorous and time-consuming, and the rapid emergence of AMR may outpace the innovation timeline [105-108]. Therefore, it is crucial to establish and implement stringent regulations and policies governing the use of antibiotics in aquaculture systems [109, 110]. This is vital in safeguarding and preserving antibiotics’ efficacy, ultimately upholding food safety and public health. In summary, the whole genome sequence of *V. parahaemolyticus* RP0132 provides a better understanding of the AMR patterns and the underlying resistance mechanisms within *V. parahaemolyticus*.

The whole genome sequence of *Vibrio parahaemolyticus* R0132 has been deposited at DDBJ/EMBL/GenBank under accession numbers CP131930.1 and CP131931.1. The version described in this genome report is the first version. The genome data is publicly available at NCBI GenBank under the BioProject accession number PRJNA1000768, and the BioSample accession number SAMN36780134.

**Author Contributions:** K-YL conducted the laboratory research work, data analysis and manuscript writing. K-OC provided support on the data analysis and proofreading. LT-HT, JW-FL, PP, L-HL and VL provided resources, supervision, proofreading and technical support. VL and L-HL conceptualized and founded this writing project. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.
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