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Vibrio floridensis sp. nov., a novel species closely related to the human pathogen *Vibrio vulnificus* isolated from a cyanobacterial bloom

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Abstract

A Gram-stain-negative, rod-shaped bacterial strain, designated *Vibrio floridensis* IRLE0018 (=NRRL B-65642=NCTC 14661), was isolated from a cyanobacterial bloom along the Indian River Lagoon (IRL), a large and highly biodiverse estuary in eastern Florida (USA). The results of phylogenetic, biochemical, and phenotypic analyses indicate that this isolate is distinct from species of the genus *Vibrio* with validly published names and is the closest relative to the emergent human pathogen, *Vibrio vulnificus*. Here, we present the complete genome sequence of *V. floridensis* strain IRLE0018 (4535135 bp). On the basis of the established average nucleotide identity (ANI) values for the determination of different species (ANI <95%), strain IRLE0018, with an ANI of approximately 92% compared with its closest relative, *V. vulnificus*, represents a novel species within the genus *Vibrio*. To our knowledge, this represents the first time this species has been described. The results of genomic analyses of *V. floridensis* IRLE0018 indicate the presence of antibiotic resistance genes and several known virulence factors, however, its pathogenicity profile (e.g. survival in serum, phagocytosis avoidance) reveals limited virulence potential of this species in contrast to *V. vulnificus*.

INTRODUCTION

The family *Vibrionaceae* encompasses a highly diverse group of aquatic Gram-negative bacteria [1, 2]. The family includes some important human pathogens such as *Vibrio cholerae*, the etiological agent of the severe diarrheal disease cholera [3–5], *Vibrio vulnificus,* an emergent pathogen source of a fulminant septicemia [6–8], and *Vibrio parahaemolyticus*, which causes seafood-associated gastrointestinal infections [9, 10]. We recently surveyed a large estuary in Eastern Florida, the Indian River Lagoon, in search of potentially pathogenic *Vibrios* [11]. We sampled different fractions and reservoirs during our survey including oysters, copepods and cyanobacteria as, besides inhabiting the water column, species of the genus *Vibrio* are known to be associated with several dwellers in their natural environment [12–15]. In one sample associated with a cyanobacterial bloom in Shepard's Park (27° 11′ 48.864″ N; 80° 15′ 33.172″ W), we identified a strain representing a novel species of the genus *Vibrio*, which we term *Vibrio floridensis* IRLE0018, and sequenced its genome. The results of bioinformatic analyses indicate that *V. floridensis* is the closest known relative to *V. vulnificus*, which is endemic to this area, yet does not appear to possess virulence-associated traits (e.g. it is serum-sensitive and does not exhibit phagocytosis resistance)

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Keywords: cyanobacterial bloom; *Vibrionaceae*; *Vibrio floridensis*; *Vibrio vulnificus*.
Abbreviations: AMP, ampicillin; ANI, average nucleotide identity; CAV, CHROMagar vibrio; CFU, colony-forming unit; CM, chloramphenicol; CPS, capsular polysaccharide; DDH, DNA-DNA hybridization; DNA, deoxyribonucleic acid; FOS, fosfomycin; GM, gentamycin; IPM, imipenem; IRL, Indian river lagoon; KAN, kanamycin; LB, luria bertani; MOI, multiplicity of infection; NA, nalidixic acid; OCT, oxytetracycline; OD, optical density; ONPG, ortho-nitrophenyl-ß-galactoside; PB, polymyxin B; PCR, polymerase chain reaction; PM, phenotypic microarrays; SD, standard deviation; SM, streptomycin; SZ, sulfadiazine; TCBS, thiosulfate-citrate-bile salts-sucrose agar; TMP, trimethoprim.
The whole genome and 16s rRNA sequences have been deposited at GenBank under the accession numbers JAKGCY000000000 and OM310777, respectively.
Two supplementary figures and five supplementary tables are available with the online version of this article.

Table 1. Sequencing and annotation data for *V. floridensis* IRLE0018^T

Genomic features	**IRLE0018**
Genome length (bp)	4535135
Number of chromosomes	2
Genome coverage (×)	50
Average DNA G+C content (%)	46.6
Coding density (%)	88
Number of contigs	61
Number of coding sequences	4044
Number of tRNAs	45
Number of rRNAs	6
GenBank accession number	JAKGCY000000000
Number of plasmids	0
BioProject number	PRJNA794138
BioSample number	SAMN24611338
16 s rRNA accession number	OM310777

ISOLATION AND ECOLOGY

Cyanobacterial samples from Shepard Park were collected in September 2018 in triplicates and transported on ice until arrival at the laboratory. Enrichment and screening was performed based on modified protocols for isolation of species of the genus *Vibrio* [16, 17]. Briefly, cyanobacterial samples were pelleted, supernatant removed, and pellets were homogenized in phosphate buffered saline (Gibco) by vortexing. Samples from each replicate were cultured in alkaline peptone water (1:5 v/v) overnight at 37 °C for enrichment. Enriched cultures were then serially diluted and plated on CaV (CHROMagar), a selective agar for species of the genus *Vibrio*. Turquoise blue colonies (potential *V. cholerae* or *V. vulnificus*) were further screened on thiosulfate citrate bile salts sucrose (TCBS; Sigma) agar plates. Colonies that appeared turquoise blue on CaV and green on TCBS were considered potential isolates of *V. vulnificus* and were further confirmed by PCR using *thiF* as a gene marker [11]. The gene encodes a sulphur carrier protein adenylyltransferase and was recently determined to be a highly reliable gene marker to identify isolates of *V. vulnificus* and distinguish them from isolates of other species of the genus *Vibrio* [11]. This initial screening indicated that strain IRLE0018 was potentially an isolate of *V. vulnificus*.

GENOME FEATURES

To determine more precisely the phylogeny of the strain, genomic DNA of IRLE0018 was extracted using the Gentra Puregene Yeast/Bact. Kit (Qiagen). DNA libraries were prepared using the Nextera DNA Flex Library Prep Kit from Illumina following the manufacturer's instructions and sequenced using the iSeq100 Sequencing System (Illumina). Sequenced genomes were analysed using the BaseSpace Sequence Hub (Illumina). The generated reads were trimmed and assembled using Trimmomatic v0.36 [18] and SPAdes v3.11.1 [19], respectively. The draft genome of IRLE0018 consisted of 61 contigs (4535135 bp) with a DNA G+C content of 46.6% and 4044 annotated genes (Table 1). All genome-sequence-related data are summarized in Table 1.

The analysis of divergence with respect to the reference strain of *V. vulnificus* CMCP6 showed an average nucleotide identity (ANI) [20] value of 90.73% (coverage 77.58%; Table 2). Considering the established values for the determination of different

Table 2. Average nucleotide identity of *V. floridensis* IRLE0018^T compared with *V. vulnificus*

Comparison to *Vibrio vulnificus*	**IRLE0018**
ANI chromosome I (%, SD)	92.42±0.61
Coverage chromosome I (%, SD)	76.04±2.63
ANI chromosome II (%, SD)	92.30±1.13
Coverage chromosome II (%, SD)	52.25±1.99

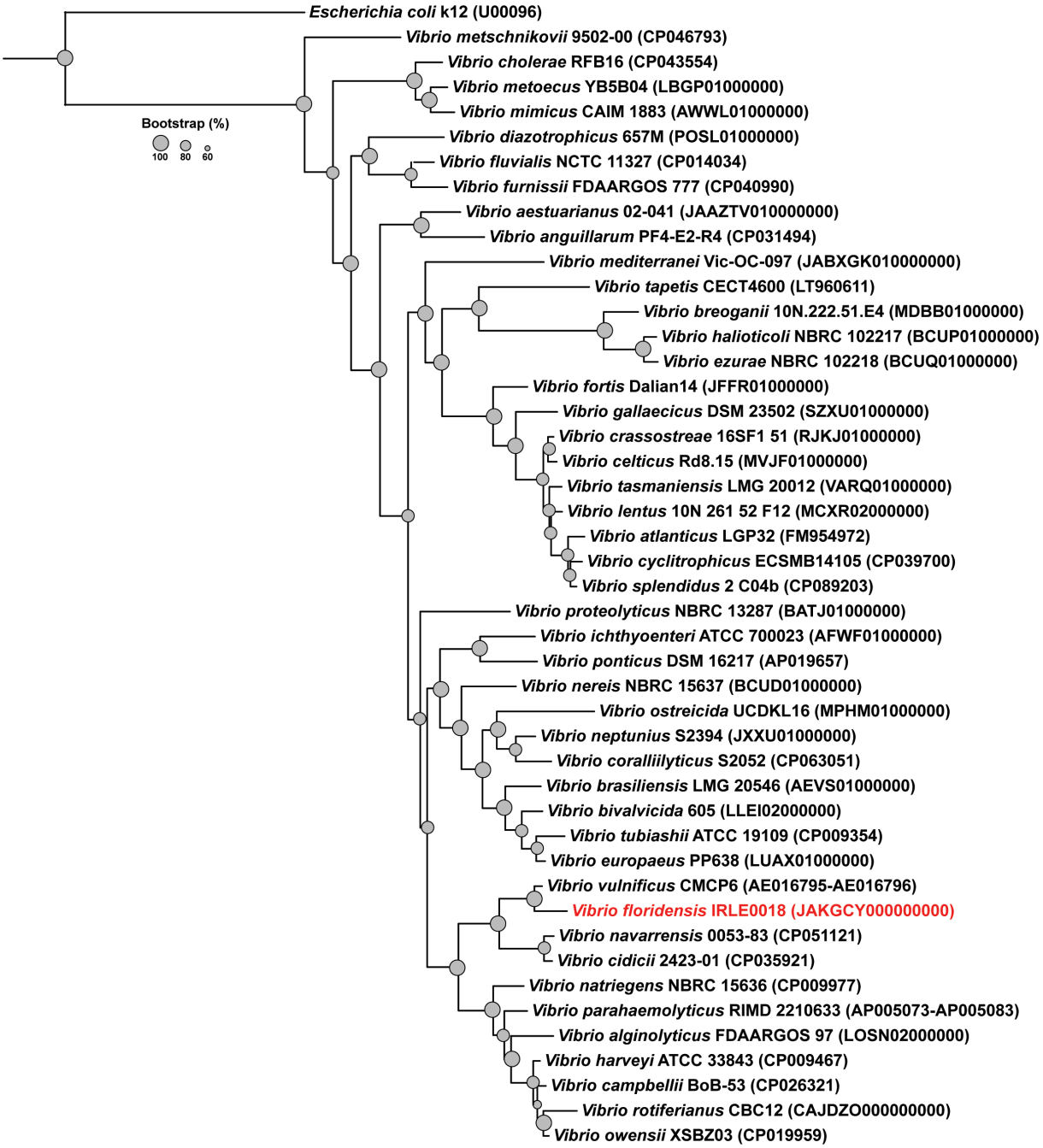

Fig. 1. Phylogenetic tree *V. floridensis* IRLE0018^T based on protein coding genes. The phylogenetic tree was reconstructed using 374 protein coding genes common to 44 species within the family *Vibrionaceae*. The phylogenetic tree was reconstructed using Phylophlan3 with an ultrafast bootstrap of 1000 replicates. *Escherichia coli* K12 was used as an outgroup.

species (ANI less than 95%), these values indicate that this genome represents a novel species closely related to *V. vulnificus*. The *in-silico* comparison of DNA–DNA hybridization (DDH) also showed consistent values with genomes of different species (63.5%, confidence interval 59.7–67.1). Subsequently, the location within the family *Vibrionaceae* was analysed through a phylogenomic analysis with reference genomes (based on NCBI) of 44 species. Using Phylophlan3 [21], a total of 374 genes were used to classify the sequences phylogenomically (Fig. 1) using the following parameters: -d phylophlan -t a –diversity medium –accurate -f supermatrix_aa.cfg. *Escherichia coli* K12 was used as an outgroup. IQ-TREE [22] with Le and Gascuel plus amino acid frequencies plus four gamma categories (LG+F+G4) amino acid model and an ultrafast bootstrap of 1000 replicates were used [23]. While ANI indicated that IRLE0018 represents a different species from *V. vulnificus*, phylogeny indicated that it is also the most closely

related species to *V. vulfinicus* within the Vulnificus clade consisting of three species, *Vibrio cidicii*, *Vibrio navarrensis* and *V. vulnificus*. Therefore, on the basis of these considerations, we have determined that IRLE0018 is a novel species which we refer to as *Vibrio floridensis* (derived from 'Floridian': native of Florida or that inhabits Florida).

PHYSIOLOGY

V. floridensis IRLE0018 was routinely cultured in Luria–Bertani (LB) media overnight. On LB agar, cells appeared as translucent, smooth, and circular colonies with a diameter of 2–3 mm. Gram-negative staining was confirmed using standard Gram's reaction. Cells exhibited a rod-shaped morphology typical of strains of members of the family *Vibrionaceae*, as observed using Zeiss Axio Observer inverted microscope (Carl Zeiss) (Fig. S1, available in the online version of this article). The biochemical profile of *V. floridensis* IRLE0018 was determined using the API 20E Microbial Identification kit and reagents (BioMerieux) in accordance with the manufacturer's protocol, with 0.85% NaCl for cell suspensions [24, 25]. The oxidase test was performed by adding oxidase reagent (Oxidrop, Hardy Diagnostics) to a patch of colonies. Biochemical profiles of *V. floridensis* IRLE0018 were compared with those of type strains from its closest phylogenetic neighbours, *Vibrio cidicii* LMG 29267^T [26], *Vibrio navarrensis* LMG 15976^T [26] and *Vibrio vulnificus* B324^T [27]. The effect of salinity and pH on the growth of IRLE0018 was studied using Biolog Phenotypic Microarrays PM9 and PM10 (Biolog) following the manufacturer's protocol. Plates were incubated at 37 °C using the Tecan Sunrise microplate reader (Tecan) and optical density (OD) was measured at 595 nm using the Magellan plate reader software (Tecan). Effects of temperature were examined by incubating 1:100 dilution of cells overnight in LB or LB supplemented with 2% NaCl (LB2%) at 25, 30, 37 or 42 °C with shaking, using the Tecan system. The biochemical and physiological characteristics are summarized in Table S1. All experiments were performed in duplicates (Biolog) or triplicates (all others) and compared with results for *V. vulnificus* CMCP6 and *V. cholerae* N16961, commonly used representatives of these pathogenic species of the genus *Vibrio*.

Phenotypic and biochemical profiles that differ among the three species related closely to *V. floridensis* IRLE0018 were also examined and compared to identify features unique to IRLE0018. Traits examined are listed in Table 3 and phenotypic data for each type strain were extracted from previously published sources. Briefly, to examine swarming, marine broth was prepared according to the manufacturer's instructions and supplemented with 0.3% agar. Inoculated plates were incubated at 25 °C overnight. Effects of temperature were examined by inoculating individual colonies in LB and incubating them overnight under aerobic conditions at 4, 20 and 25 °C. All other biochemical data for IRLE0018 were extracted from data collected from PM1, PM2a and API20E (Tables S1–S3). Comparisons of biochemical and phenotypic characteristics of IRLE0018 with type strains of the closest phylogenetic neighbours (*V. cidicii* LMG 29267^T, *V. navarrensis* LMG 15976^T, *V. vulnificus* B324^T) reveal that all are positive for oxidase, indole, glucose fermentation and growth at 30 and 37 °C and negative for arginine dihydrolase, urease, *myo*-inositol, growth at 4 °C and growth in nutrient broth supplemented with 0 and 10% NaCl (Table 3). The inability of IRLE0018 to utilize citrate distinguishes it from *V. navarrensis* and *V. vulnificus* and subsequent test for lysine decarboxylase can distinguish it from *V. cidicii* that lacks this enzyme.

Given that the closest relative of the novel species *V. floridensis* is the emergent human pathogen *V. vulnificus*, we sought to further examine differences between the two species at the genome level. The common part of the IRLE0018 genome with the commonly used *V. vulnificus* reference strain, CMCP6, was excluded using CD-HIT-2D [28] and leaving for functional analysis using the SEED Subsystems database [29] those sequences that were unique (Fig. S2). The highest differences between the two strains were observed for genes associated with cell wall and capsule as well as carbohydrate utilization. Carbohydrates are critical sources of nutrients for microbes and fuel a number of important biochemical and metabolic pathways [30, 31]. The ability to utilize a diverse set of carbon sources not only ensures survival of the bacteria in the environment, particularly when faced with competition for limited nutrients [31], but is also crucial for virulence and survival of pathogens within the host [32–35]. Since carbohydrate utilization had one of the highest differences in gene content between the two strains, we reasoned that *V. floridensis* may utilize unique carbon sources. We examined the ability of *V. floridensis* IRLE0018 to metabolize a diverse range of carbon sources and compared it to the carbon source utilization profile of *V. vulnificus* CMCP6 using Biolog phenotypic microarrays PM1 and PM2a (Biolog) [36] following the manufacturers protocol. Our analyses indicate that the novel species *V. floridensis* IRLE0018 can utilize several carbon sources to a higher extent (fold change greater than 1.5) than *V. vulnificus* CMCP6 (Fig. 2, Tables S2 and S3). From PM1, these include l-proline, d-glucosaminic acid, myo-inositol, tricarballylic acid, mso-tartaric acid, 1,2-propanediol and monomethyl succinate and from PM2a, α-cyclodextrin, butyric acid, hydroxy-l-proline, glycine, l-histidine and caproic acid. Interestingly some of these sources are essential for metabolism and virulence in other bacteria such as -proline [34], myo-inositol [37], tricarballylic acid [38], glycine [39] and l-histidine [40].

V. floridensis IRLE0018 was isolated from a region endemic to *V. vulnificus* outbreaks [11]. Given the close relatedness between the two species, we speculated that IRLE0018 may harbour pathogenic characteristics, similar to those of *V. vulnificus*. To elucidate the virulence potential of IRLE0018, first, we analysed all protein-coding genes for signatures of antibiotic resistance or the presence of known virulence genes using MegaRES [41] and Virulence Factors Database [42] respectively. We found genes related to efflux pumps and target alterations for several drug classes such as fluoroquinolones, macrolides, tetracyclines, nitroimidazoles and

Table 3. Phenotypic characteristics differentiating *V. floridensis* IRLE0018 from closely related species. Characteristics of closely related type strains were obtained from previous reports unless otherwise indicated. All strains are positive for oxidase, indole, glucose fermentation, assimilation of cellobiose, growth in nutrient broth supplemented with 1.5% NaCl (30 °C) and growth at 30 and 37 °C and negative for Voges–Proskauer test, arginine dihydrolase, urease, *myo*-inositol, swarming (marine agar, 25 °C), assimilation of melbiose, growth at 4 °C and growth in nutrient broth supplemented with 0 and 10% NaCl. The differential characteristics distinguishing IRLE0018 from *V. vulnificus* are indicated in bold type

Strains: 1,*V. floridensis* IRLE0018^T; 2, *V. cidicii* LMG 29267^T; 3, *V. navarrensis* LMG 15976^T; 4, *V. vulnificus* B324^T. +, Positive; –, Negative; w, weakly positive, v, variable, ND, no data available.

Citrate utilization	–	–	+	+
Lysine decarboxylase	+	–	–	+
Ornithine decarboxylase	–	–	–	+
Ortho-nitrophenyl-β-galactoside (ONPG)	+	–	+	+
Acid production from:				
α-Methyl-d-glucoside	–	+	–	–
-Galactose	+	–	–	+
l-Rhamnose	–	+	–	–
d-Sorbitol	–	+	–	–
Assimilation of:				
l-**Arabinose**	w	v	v	–
d-Mannose	+	+	v	+
l-Rhamnose	–	+	+	–
l-Serine	+	+	+	–
d-Trehalose	+	+	+	–
Growth in nutrient broth (at 30 °C) supplemented with:				
6.5 % NaCl	+	+	+	–
8 % NaCl	–	+	–	–
Growth at:				
20 °C	+	ND	ND	+
25 °C	+	ND	ND	+

Phenotypes for *V. cidicii* LMG 29267^T and *V. navarrensis* LMG 15976^T were obtained from [26].
Phenotypes for *V. vulnificus* B324^T were obtained from [27].

peptide antibiotics (Table S4). To functionally validate the genomic resistance profile, we exposed IRLE0018 to several antibiotics (Fig. 3a) typically recommended by the Centres for Disease Control and Prevention for the treatment of various species of the genus *Vibrio* [43] using a protocol previously described [11]. Resistance was defined as growth of at least 2 mm in the respective antibiotics, while strains exhibiting no growth were classed as sensitive, and any intermediate growth diameter was considered as intermediate resistance. Validating the antibiotic resistance signatures observed in its genome, IRLE0018 exhibited resistance or intermediate resistance patterns similar to those of *V. vulnificus* CMCP6 for multiple antibiotics such as oxytetracycline, imipenem, fosfomycin, sulfadiazine and polymyxin B (Fig. 3a). Interestingly, while oxytetracycline is one of few antibiotics that render almost all *V. vulnificus* strains susceptible [11, 44], IRLE0018 remained moderately resistant to it. On the other hand, like most strains of *V. vulnificus*, IRLE0018 was found to be susceptible to nucleic acid and protein synthesis inhibitors like nalidixic acid, chloramphenicol, kanamycin and trimethoprim and the cell wall synthesis inhibitor ampicillin.

Among the virulence factors detected in the genome of *V. floridensis* IRLE0018 (Table S5), the capsular polysaccharide (CPS) is of particular interest, since it is the most critical virulence factor associated with *V. vulnificus* pathogenicity. The CPS of *V. vulnificus* is responsible for resistance to complement-mediated killing in the serum as well as evasion of the host innate immune system by conferring antiphagocytic abilities [45–49]. Furthermore, serum resistance has often been correlated with the ability of bacteria to utilize and transport sialic acid [11, 50], a carbohydrate important for host–pathogen interactions [32, 33, 35]. Additionally, clinical strains of *V. vulnificus* have been shown to induce macrophage apoptosis during host

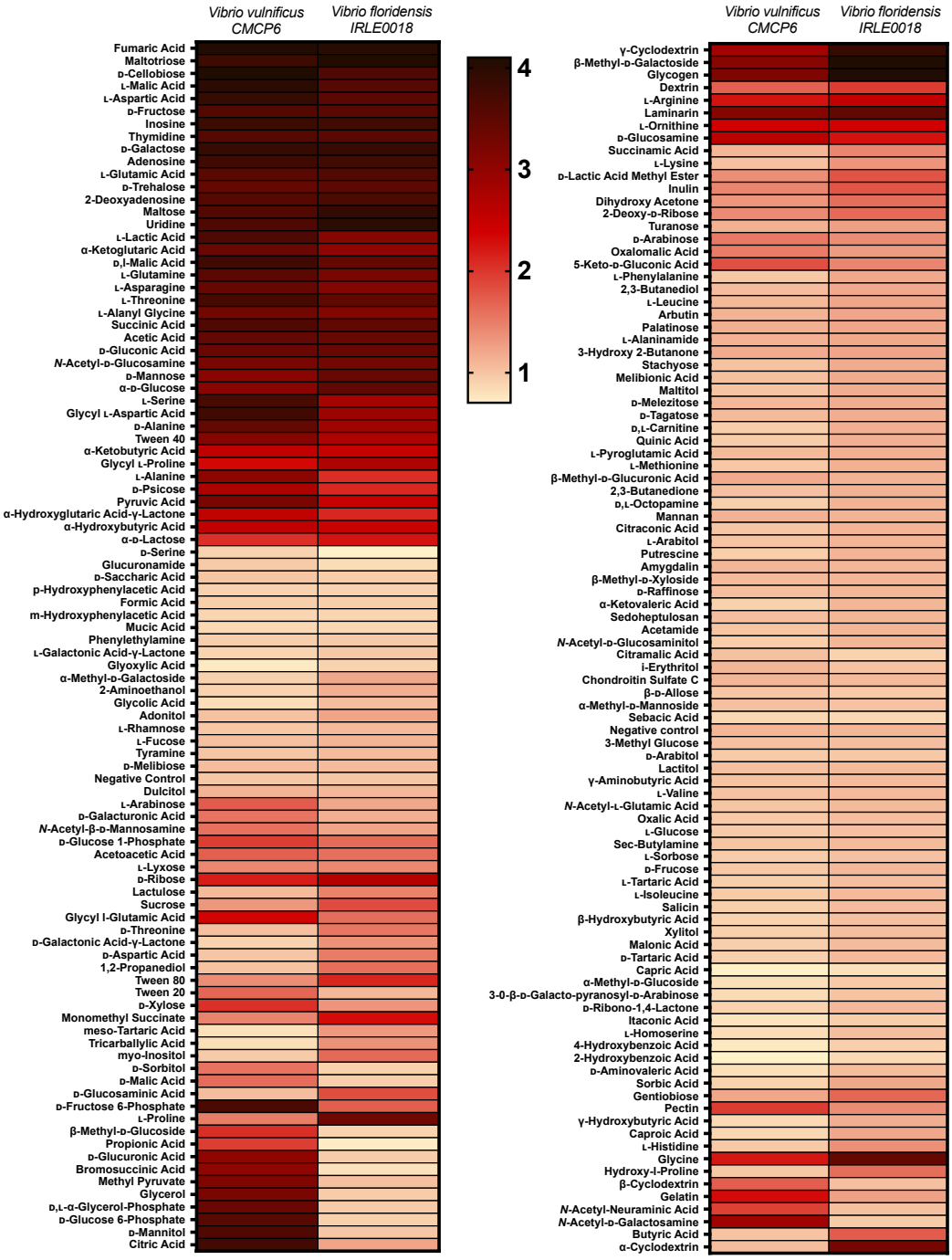

Fig. 2. Carbon source utilization of *V. floridensis* IRLE0018. Phenotypic microarrays of *V. floridensis* IRLE0018 were produced using Biolog plates PM1 and PM2a, which contain a wide variety of carbon sources, and compared with those for its close relative *V. vulnificus* CMCP6. The heat map represents the fold change of the area under the curve for each carbon source and shows the average levels of carbon utilization of each strain relative to the negative control.

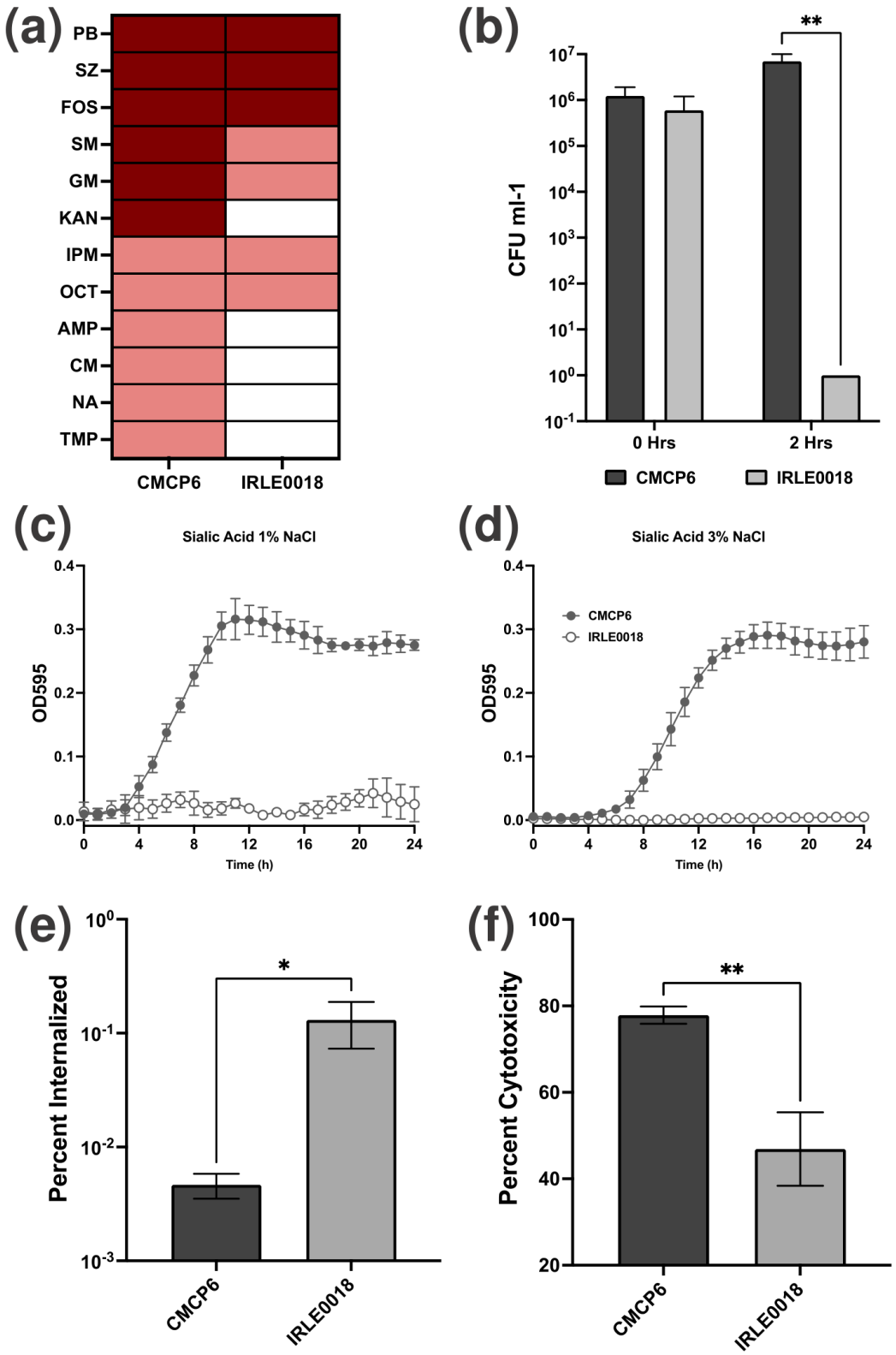

Fig. 3. Virulence potential of *V. floridensis* IRLE0018. a) Antibiotic resistance profile. Red: resistant; light red: intermediate resistance; white: sensitive. b) Resistance to normal pooled human serum. c) Utilization of sialic acid as a sole carbon source in the presence of 1% NaCl and d) 3% NaCl. e) Evasion of phagocytosis by THP-1 monocytes f) Induction of apoptosis of THP-1 monocytes. All experiments were performed in triplicate. Statistical comparisons were made using the Student's *t*-test. ***P* <0.01. **P* <0.05. PB, polymyxin B; SZ, sulfadiazine; FOS, Fosfomycin; SM, streptomycin; GM, gentamycin; KAN, kanamycin; IPM, imipenem; OCT, oxytetracycline; AMP, ampicillin; CM, chloramphenicol; NA,nalidixic acid; TMP, trimethoprim.

colonization [51, 52]. We therefore investigated the ability of *V. floridensis* IRLE0018 to a) resist serum, b) utilize sialic acid as the sole carbon source, c) evade phagocytosis and d) induce macrophage apoptosis. Serum resistance was examined by exposing exponential-phase cells at an optical density of 0.15 to 0.25 to normal pooled human serum (Fisher Bioreagents) for 2 h and measuring the colony-forming units (CFUs) before and after exposure [11]. Unlike *V. vulnificus* CMCP6, which was not only resistant to but also able to grow in human serum, *V. floridensis* IRLE0018 was extremely sensitive (Fig. 3b). Furthermore, *V. floridensis* IRLE0018 was unable to utilize *N*-acetylneuraminic acid (Chem-Impex International), the predominant form of sialic acid in human cells, as the sole carbon source when grown for up to 24 h (Fig. 3c, d). From these results, it appears that, despite harbouring genes associated with CPS, *V. floridensis* IRLE0018 is serum-sensitive, indicating that this bacterium may not possess pathogenic potential unlike its closest relative *V. vulnificus*. Resistance to phagocytosis was evaluated using THP-1 human monocytic cells according to a modified protocol [53]; THP-1 monocytes were infected with bacterial cells (OD 0.5) at a multiplicity of infection (MOI) of 10 for 1 h. Extracellular bacteria were killed by gentamicin (100 $\mu g\,ml^{-1}$) treatment for 30 min and internalized bacteria were enumerated by plate count (CFU ml^{-1}). Apoptosis of THP-1 monocytes exposed to bacterial cells (MOI 10) for 4 h was measured using a CyQUANT LDH Cytotoxicity Assay kit (Invitrogen). IRLE0018 was internalized to a significantly greater extent (> 1 log) than *V. vulnificus* CMCP6 (Fig. 3e), further confirming the reduced virulence of this strain. On the other hand, *V. floridensis* IRLE0018 induced macrophage apoptosis (Fig. 3f), albeit to a lesser extent than *V. vulnificus* CMCP6, upon infection of THP-1 monocytes, indicating a partially virulent phenotype.

Overall, on the basis of the ANI and the results of sequence analysis, *V. floridensis* IRLE0018 is a novel species closely related to *V. vulnificus*. The results of phylogenetic, morphological, chemotaxonomic and physiological analyses indicate that this cyanobacterial isolate is distinct from other species of the genus *Vibrio* and its pathogenic profile exhibits a reduced virulence potential compared with *V. vulnificus*.

DESCRIPTION OF *VIBRIO FLORIDENSIS* SP. NOV.

Vibrio floridensis (flo.ri.dien'sis. N.L. masc. adj. floridensis, pertaining to Florida).

The bacterium is Gram-stain-negative, rod-shaped and non-sucrose fermenting, forming green colonies on TCBS. Colonies grown on LB agar appear as translucent, smooth and circular with a diameter of 2–3 mm. Biochemical characteristics of IRLE0018 include positive for oxidase, galactosidase, lysine decarboxylase, indole, galactose fermentation, glucose fermentation, amygdalin fermentation, assimilation of cellobiose, d-mannose, l-serine, and d-trehalose, and able to weakly assimilate l-arabinose. The type strain is able to grow at temperatures ranging from 20-37˚C in LB supplemented with 0.5 and 2% NaCl, and grow in LB supplemented with 1.5 and 6.5% NaCl at 30˚C. Phenotypic screening of the bacterium showed optimal growth at salinities ranging from 1-3% and 5.5%. The strain was also able to tolerate pH ranging from 5.5-10. l-proline, myo-inositol, tricarballylic acid, glycine, and l-histidine can be used as sole carbon sources. On the other hand, *V. floridensis* IRLE0018 is negative for citrate utilization, ornithine decarboxylase, arginine dihydrolase, Voges-Proskauer test, and urease and unable to ferment α-methyl-d-glucoside, l-rhamnose, d-sorbitol, and myo-inositol, assimilate melbiose and l-rhamnose, grow at 4°C and grow in nutrient broth supplemented with 0 and 10% NaCl (Table 3). IRLE0018 exhibits resistance to antibiotics such as oxytetracycline, imipenem, fosfomycin, sulfadiazine and polymyxin B. In addition, is sensitive to serum and unable to evade phagocytosis by ThP-1 monocytes.

The type strain, *V. floridensis* IRLE0018 (=NRRL B-65642=NCTC 14661) was isolated from a cyanobacterial bloom along the Indian River Lagoon (IRL), a large and highly biodiverse estuary in eastern Florida (USA). The genome size of the type strain is 4.5 Mb with an average DNA G+C content of 46.6%. The genome of the type strain *V. floridensis* ILRE0018 has a median ANI value of approximately 92% to the closely related *V. vulnificus* CMCP6.

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Author contributions
S.A.M. and M.L.P. designed research. T.A.G., J.M.J. and M.L.P. performed experiments and analyzed data. T.A.G., J.M.J., M.L.P. and S.A.M. wrote the manuscript.

Conflicts of interest
The authors declare no conflict of interest.

References

1. Reen FJ, Almagro-Moreno S, Ussery D, Boyd EF. The genomic code: inferring *Vibrionaceae* niche specialization. *Nat Rev Microbiol* 2006;4:697–704.
2. Vezzulli L, Colwell RR, Pruzzo C. Ocean warming and spread of pathogenic vibrios in the aquatic environment. *Microb Ecol* 2013;65:817–825.

3. Colwell RR. Global climate and infectious disease: the cholera paradigm. *Science* 1996;274:2025–2031.
4. Almagro-Moreno S, Taylor RK. Cholera: environmental reservoirs and impact on disease ransmissiont. *Microbiol Spectr* 2013;1:OH-0003-2012.
5. Sakib SN, Reddi G, Almagro-Moreno S. Environmental role of pathogenic traits in *Vibrio cholerae J Bacteriol* 2018;200:e00795–17.
6. Gulig PA, Bourdage KL, Starks AM. Molecular pathogenesis of *Vibrio vulnificus*. *J Microbiol* 2005;43 Spec No:118–131.
7. Oliver JD. *Vibrio vulnificus*: death on the half shell. a personal journey with the pathogen and its ecology. *Microb Ecol* 2013;65:793–799.
8. Phillips KE, Satchell KJF. *Vibrio vulnificus*: from oyster colonist to human pathogen. *PLoS Pathog* 2017;13:e1006053.
9. Han D, Yu F, Tang H, Ren C, Wu C, *et al*. Spreading of pandemic *Vibrio parahaemolyticus* O3:K6 and its serovariants: a re-analysis of strains isolated from multiple studies. *Front Cell Infect Microbiol* 2017;7:188.
10. Li L, Meng H, Gu D, Li Y, Jia M. Molecular mechanisms of *Vibrio parahaemolyticus* pathogenesis. *Microbiol Res* 2019;222:43–51.
11. López-Pérez M, Jayakumar JM, Grant T-A, Zaragoza-Solas A, Cabello-Yeves PJ, *et al*. Ecological diversification reveals routes of pathogen emergence in endemic *Vibrio vulnificus* populations. *Proc Natl Acad Sci U S A* 2021;118:40.
12. Reddi G, Pruss K, Cottingham KL, Taylor RK, Almagro-Moreno S. Catabolism of mucus components influences motility of *Vibrio cholerae* in the presence of environmental reservoirs. *PLoS One* 2018;13:e0201383.
13. Groubert TN, Oliver JD. Interaction of *Vibrio vulnificus* and the Eastern Oyster, *Crassostrea virginica*. *J Food Prot* 1994;57:224–228.
14. Islam MS, Mahmuda S, Morshed MG, Bakht HBM, Khan MNH, *et al*. Role of cyanobacteria in the persistence of *Vibrio cholerae* O139 in saline microcosms. *Can J Microbiol* 2004;50:127–131.
15. Warner E, Oliver JD. Population structures of two genotypes of *Vibrio vulnificus* in oysters (*Crassostrea virginica*) and seawater. *Appl Environ Microbiol* 2008;74:80–85.
16. Huq A, Haley BJ, Taviani E, Chen A, Hasan NA, *et al*. Detection, isolation, and identification of *Vibrio cholerae* from the environment. *Curr Protoc Microbiol* 2012;Chapter 6:Unit6A.5.
17. Schuster BM, Tyzik AL, Donner RA, Striplin MJ, Almagro-Moreno S, *et al*. Ecology and genetic structure of a northern temperate *Vibrio cholerae* population related to toxigenic isolates. *Appl Environ Microbiol* 2011;77:7568–7575.
18. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
19. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
20. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Anal Methods* 2016;8:12–24.
21. Segata N, Börnigen D, Morgan XC, Huttenhower C. PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes. *Nat Commun* 2013;4:2304.
22. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;32:268–274.
23. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 2018;35:518–522.
24. Lydon KA, Kinsey T, Le C, Gulig PA, Jones JL. Biochemical and virulence characterization of *Vibrio vulnificus* isolates from clinical and environmental sources. *Front Cell Infect Microbiol* 2021;11:637019.
25. DePaola C a. A. Bacteriological analytical manual chapter 9. *vibrio* 2004.
26. Orata FD, Xu Y, Gladney LM, Rishishwar L, Case RJ, *et al*. Characterization of clinical and environmental isolates of *Vibrio cidicii* sp. nov., a close relative of *Vibrio navarrensis*. *Int J Syst Evol Microbiol* 2016;66:4148–4155.
27. Farmer J. J III JM, Brenner FW, Cameron DN, Birkhead KM. *Bergey's Manual® of Systematic Bacteriology–Volume Two: The Proteobacteria, Part B: The Gammaproteobacteria*, ed. N.R.K. Don J. Brenner, James T. Staley (Chairman), George M. Garrity, David R. Boone (Vice Chairman), Paul Vos, Michael Goodfellow, Fred A. Rainey, Karl-Heinz Schleifer. Vol. 2. New York, NY: Springer; 2005.
28. Huang Y, Niu B, Gao Y, Fu L, Li W. CD-HIT Suite: a web server for clustering and comparing biological sequences. *Bioinformatics* 2010;26:680–682.
29. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang H-Y, *et al*. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Res* 2005;33:5691–5702.
30. Grondin JM, Tamura K, Déjean G, Abbott DW, Brumer H. *Polysaccharide* utilization loci: fueling microbial communities. *J Bacteriol* 2017;199:15.
31. Durica-Mitic S, Göpel Y, Görke B. Carbohydrate utilization in bacteria: making the most out of sugars with the help of small regulatory RNAs. *Microbiol Spectr* 2018;6.
32. Almagro-Moreno S, Boyd EF. Sialic acid catabolism confers a competitive advantage to pathogenic *Vibrio cholerae* in the mouse intestine. *Infect Immun* 2009;77:3807–3816.
33. Lubin J-B, Kingston JJ, Chowdhury N, Boyd EF. Sialic acid catabolism and transport gene clusters are lineage specific in *Vibrio vulnificus*. *Appl Environ Microbiol* 2012;78:3407–3415.
34. Christgen SL, Becker DF. Role of Proline in Pathogen and Host Interactions. *Antioxid Redox Signal* 2019;30:683–709.
35. Lubin J-B, Lewis WG, Gilbert NM, Weimer CM, Almagro-Moreno S, *et al*. Host-like carbohydrates promote bloodstream survival of *Vibrio vulnificus in vivo*. *Infect Immun* 2015;83:3126–3136.
36. Bochner BR, Gadzinski P, Panomitros E. Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. *Genome Res* 2001;11:1246–1255.
37. Dong Y, Li S, Zhao D, Liu J, Ma S, *et al*. IolR, a negative regulator of the *myo*-inositol metabolic pathway, inhibits cell autoaggregation and biofilm formation by downregulating RpmA in *Aeromonas hydrophila*. *NPJ Biofilms Microbiomes* 2020;6:22.
38. Lewis JA, Horswill AR, Schwem BE, Escalante-Semerena JC. The tricarballylate utilization (*tcuRABC*) genes of *Salmonella enterica* serovar Typhimurium LT2. *J Bacteriol* 2004;186:1629–1637.
39. Vanhove AS, Hang S, Vijayakumar V, Wong AC, Asara JM, *et al*. *Vibrio cholerae* ensures function of host proteins required for virulence through consumption of luminal methionine sulfoxide. *PLoS Pathog* 2017;13:e1006428.
40. Bender RA. Regulation of the histidine utilization (Hut) system in bacteria. *Microbiol Mol Biol Rev* 2012;76:565–584.
41. Lakin SM, Dean C, Noyes NR, Dettenwanger A, Ross AS, *et al*. MEGARes: an antimicrobial resistance database for high throughput sequencing. *Nucleic Acids Res* 2017;45:D574–D580.
42. Chen L, Zheng D, Liu B, Yang J, Jin Q. VFDB 2016: hierarchical and refined dataset for big data analysis--10 years on. *Nucleic Acids Res* 2016;44:D694–7.
43. Shaw KS, Rosenberg Goldstein RE, He X, Jacobs JM, Crump BC, *et al*. Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. *PLoS One* 2014;9:e89616.
44. Baker-Austin C, McArthur JV, Lindell AH, Wright MS, Tuckfield RC, *et al*. Multi-site analysis reveals widespread antibiotic resistance in the marine pathogen *Vibrio vulnificus*. *Microb Ecol* 2009;57:151–159.
45. Kreger A, DeChatelet L, Shirley P. Interaction of *Vibrio vulnificus* with human polymorphonuclear leukocytes: association of virulence with resistance to phagocytosis. *J Infect Dis* 1981;144:244–248.

46. Campos MA, Vargas MA, Regueiro V, Llompart CM, Albertí S, *et al*. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. *Infect Immun* 2004;72:7107–7114.
47. Pettis GS, Mukerji AS. Structure, function, and regulation of the essential virulence factor capsular polysaccharide of *Vibrio vulnificus*. *Int J Mol Sci* 2020;21:E3259.
48. Kim H, Ayrapetyan M, Oliver JD. Survival of *Vibrio vulnificus* genotypes in male and female serum, and production of siderophores in human serum and seawater. *Foodborne Pathog Dis* 2014;11:119–125.
49. Williams T, Ayrapetyan M, Ryan H, Oliver J. Serum Survival of *Vibrio vulnificus*: Role of Genotype, Capsule, Complement, Clinical Origin, and *in Situ* Incubation. *Pathogens* 2014;3:822–832.
50. Severi E, Randle G, Kivlin P, Whitfield K, Young R, *et al*. Sialic acid transport in *Haemophilus influenzae* is essential for lipopolysaccharide sialylation and serum resistance and is dependent on a novel tripartite ATP-independent periplasmic transporter. *Mol Microbiol* 2005;58:1173–1185.
51. Kashimoto T, Ueno S, Hanajima M, Hayashi H, Akeda Y, *et al*. *Vibrio vulnificus* induces macrophage apoptosis in vitro and in vivo. *Infect Immun* 2003;71:533–535.
52. Kashimoto T, Ueno S, Hayashi H, Hanajima M, Yoshioka K, *et al*. Depletion of lymphocytes, but not neutrophils, via apoptosis in a murine model of *Vibrio vulnificus* infection. *J Med Microbiol* 2005;54:15–22.
53. Lo H-R, Lin J-H, Chen Y-H, Chen C-L, Shao C-P, *et al*. RTX toxin enhances the survival of *Vibrio vulnificus* during infection by protecting the organism from phagocytosis. *J Infect Dis* 2011;203:1866–1874.