Draft genome sequence of a multidrug-resistant emerging pathogenic isolate of *Vibrio alginolyticus* from the Red Sea

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Abstract

The marine ecosystem is a growing reservoir of antimicrobial-resistant bacteria, and thus an emerging risk to human health. In this study, we report the first draft genome sequence of multidrug-resistant *Vibrio alginolyticus* strain OS1T-47, isolated from an offshore site in the Red Sea. The draft genome of *V. alginolyticus* OS1T-47 is 5 157 150 bp in length and has DNA G + C content of 44.83%. Strain OS1T-47 possesses 22 antimicrobial resistance genes, including those associated with multidrug-resistant efflux pumps.

Keywords: β-lactamase, genome, multidrug-resistance, Red sea, *Vibrio alginolyticus*
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Vibrio alginolyticus is a Gram-negative halophilic bacterium commonly found in the marine environment. It is pathogenic in sea animals, and causes wound and ear infections in humans [1,2]. In immunocompromised patients, it can develop into necrotizing soft-tissue infections, and bacteraemia that could lead to septic shock [2]. Antimicrobial resistance in *V. alginolyticus* has increased in the last decade, representing a risk to human health and food security [2].

The sediment sample was collected from a relatively pristine offshore site (22°08′50.6″ N, 38°58′35.6″ E) from a depth of 9 m, as described previously [3]. Strain OS1T-47 of *V. alginolyticus* was isolated following serial dilution using marine agar medium (HiMedia, Mumbai, India) supplemented with 66% sterilized Red Sea water [3]. The media plates were incubated at 28°C for 72 hours, then colonies were sub-cultured to obtain pure isolates. The taxonomic classification of the strain was performed through comparative analyses of the 16S rRNA gene sequence and genome assembly using the EzBioCloud database (https://www.ezbiocloud.net) and SpeciesFinder 2.0 (https://cge.cbs.dtu.dk/services/SpeciesFinder/), respectively. Antimicrobial susceptibility was measured using the agar dilution method [3]. A paired-end library was prepared from the genomic DNA, and sequencing was performed using a V3 kit on a MiSeq platform (Illumina, Inc., San Diego, CA, USA), as described previously [4]. *De novo* assembly of the OS1T-47 genome was prepared using a SPAdes 3.9 algorithm from high-quality sequence reads. Annotation of the OS1T-47 genome was performed using PATRIC, a bacterial bioinformatics resource centre (https://www.patricbrc.org/) and Rapid Annotation using Subsystem Technology (RAST; http://rast.theseed.org/FIG/rast.cgi). Antimicrobial-resistance genes were identified using the Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/analyze/rgi), ResFinder 3.1, RAST and PATRIC. The sequence reads were deposited into the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under Accession number SAMEA6169146.

The draft genome of *V. alginolyticus* OS1T-47 is 5 157 150 bp long and has a DNA G + C content of 44.83%. A total of 4818 coding sequences were identified in the genome, consisting of 3787 proteins with functional assignments and 1031 hypothetical proteins (Table 1). Moreover, 142 RNA genes were detected, consisting of 131 transfer RNA and 11 ribosomal RNA genes.

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TABLE 1. Genome features, and antimicrobial resistance genes found in the draft genome of *Vibrio alginolyticus* strain OS1T-47

Genome features	Attribute	Number
	DNA G + C content	44.83%
	Draft genome length	5 157 150 bp
	Coding region	4818
	Transfer RNA	131
	Ribosomal RNA	11
	Hypothetical proteins	1031
	Proteins with functional assignments	3787
	Proteins with EC number assignments	1113
	Proteins with GO assignments	932
	Proteins with Pathway assignments	816
Antimicrobial resistance genes		
Antibiotic class	**Attribute**	**Genes**
Multidrug-resistance	RND efflux pump	*cme*A
	RND efflux pump	*cme*B
	RND efflux pump	*ade*F
	MATE	*ydh*E/*nor*M
	RND efflux pump	*crp*
	MFS efflux pump	*mdt*L
	RND efflux pump	*tol*C
	RND efflux pump	*cpx*R
	Transcriptional regulator	*omp*R
	RND efflux pump	*acr*B
	ABC efflux pump	*mac*B
β-lactam	Class C β-lactamase	bla_{AmpC}
	CARB β-lactamase	$bla_{CARB-42}$
	Peptidoglycan DD-transpeptidase	*mrd*A
	Penicillin-binding protein	*pbp*1a
Fluoroquinolone	DNA topoisomerase IV	*par*C
	DNA topoisomerase IV	*par*E
	DNA gyrase	*gyr*A
	DNA gyrase	*gyr*B
Elfamycin	Elongation factor	*EF-Tu*
Tetracycline	Tetracycline efflux pump	*tet*35
	Tetracycline inactivation enzyme	*tet*34
Peptide antibiotics	Phosphoethanolamine transferase	*ugd*

Strain OS1T-47 was phenotypically resistant to ampicillin, cefepime and tetracycline, but was susceptible to co-amoxiclav, cefotaxime, meropenem, streptomycin, ciprofloxacin and azithromycin. Also, the US CDC show that *V. alginolyticus* isolates are mainly resistant to ampicillin and cephem antibiotics [2,5]. Annotation revealed several antimicrobial resistance genes linked to fluoroquinolones (*par*C, *par*E, *gyr*A and *gyr*B), tetracycline (*tet*34 and *tet*35), elfamycin (*EF-Tu*) and β-lactamase (bla_{AmpC} and $bla_{CARB-42}$) (Table 1). It identified multidrug-resistant efflux pumps and genes potentially providing resistance to the heavy metals copper, cobalt, zinc, cadmium and arsenic. Moreover, several multidrug-resistance-associated genes, such as *mdt*A, *mdt*B, *mdt*E, *mdt*G, *mdt*L, *mdt*N, *mex*A, *mex*B, *norm*, *mar*A and *mar*R, were specifically found in the OS1T-47 in common with the 37 public genomes, using the application *Compare Genomes from Pangenome* in the KBase platform (http://www.kbase.us/). The isolate is predicted to be a human pathogen with a probability of 0.65, and 54 families matched with pathogenic families using the PATHOGENFINDER 1.1 tool (https://cge.cbs.dtu.dk/services/PathogenFinder/).

In conclusion, we found that the genome of OS1T-47 carries clinically significant genes associated with pathogenicity and antimicrobial resistance. The possibility of dissemination of these genes in marine environments is considered to be a risk to seafood safety and could impact human health.

Conflict of interest

The authors have declared that there are no conflicts of interest in relation to this article.

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