



What drives changes in the virulence and antibiotic resistance of *Vibrio harveyi* in the South China Sea?

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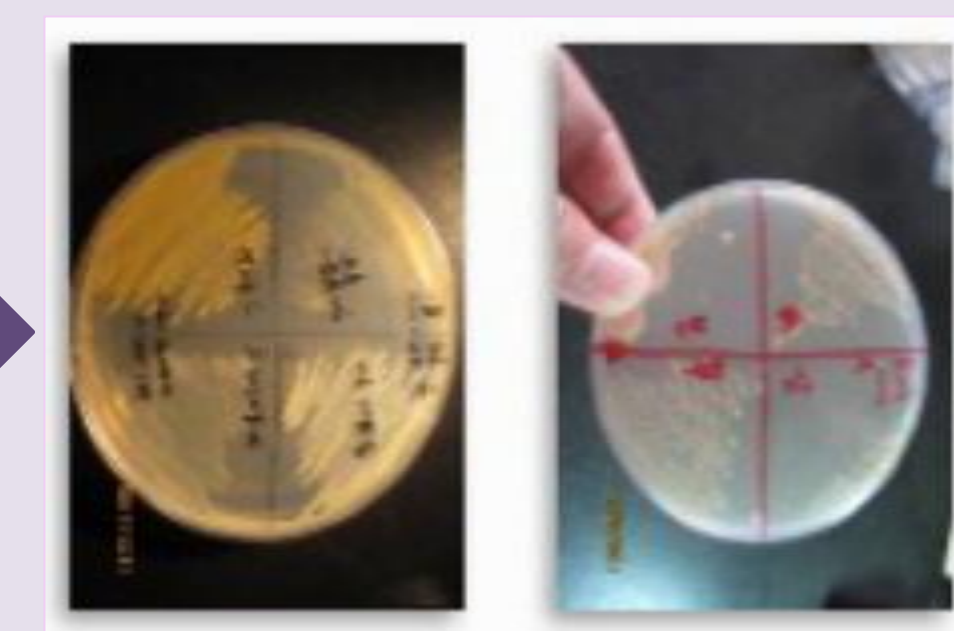
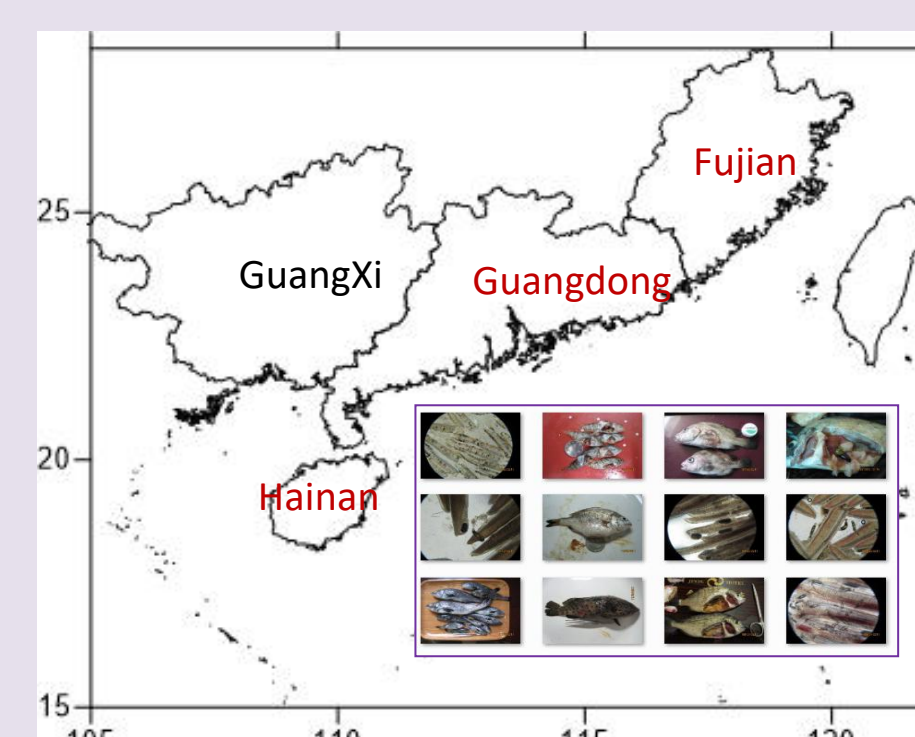
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Introduction

Vibrio harveyi was found to be the dominant species that cause serious infection and mortality of marine fishes in southern China. Antibiotics are widely used to prevent or treat bacterial diseases in aquaculture. The intense and increasing use of antibiotics has caused a large amount of antibiotic residues in the environment, leading to the selection of antimicrobial-resistant bacteria (AMRB) and promote the evolution of antibiotic resistance mechanisms. Virulence and antibiotic-resistant genes are key to the fundamental cause of pathogenicity and drug resistance in *V. harveyi*. The unreasonable use of antibiotics, high nutritional load by overfeeding, pollution by biocides and heavy metals, and high temperature by global warming can not only promote the expression of virulence and antibiotic resistance genes, but also enhance horizontal gene transfer (HGT) of virulence and antibiotic resistance genes, thus to regulating bacterial resistance and pathogenicity. In this study, we have isolated and identified potential *V. harveyi* pathogens from diseased marine fish in the breeding area of South China, and analyzed their virulence and drug resistance pattern with the objective to compare the virulence and antibiotic resistance among different cities and estimate the potential factors that drive the virulence and antibiotic resistance changes of *V. harveyi* in the South China Sea.

Materials and method



➤ Diseased marine fishes from Guangdong, Hainan, and Fujian.

➤ Isolate and identified potential pathogens.



➤ PCR amplification of: typical *V. harveyi* virulence genes; atypical *V. harveyi* virulence genes; antibiotic resistance genes (ARGs); mobile genetic elements (MGEs).

➤ Antibiotic resistance analysis by K-B method.

Results

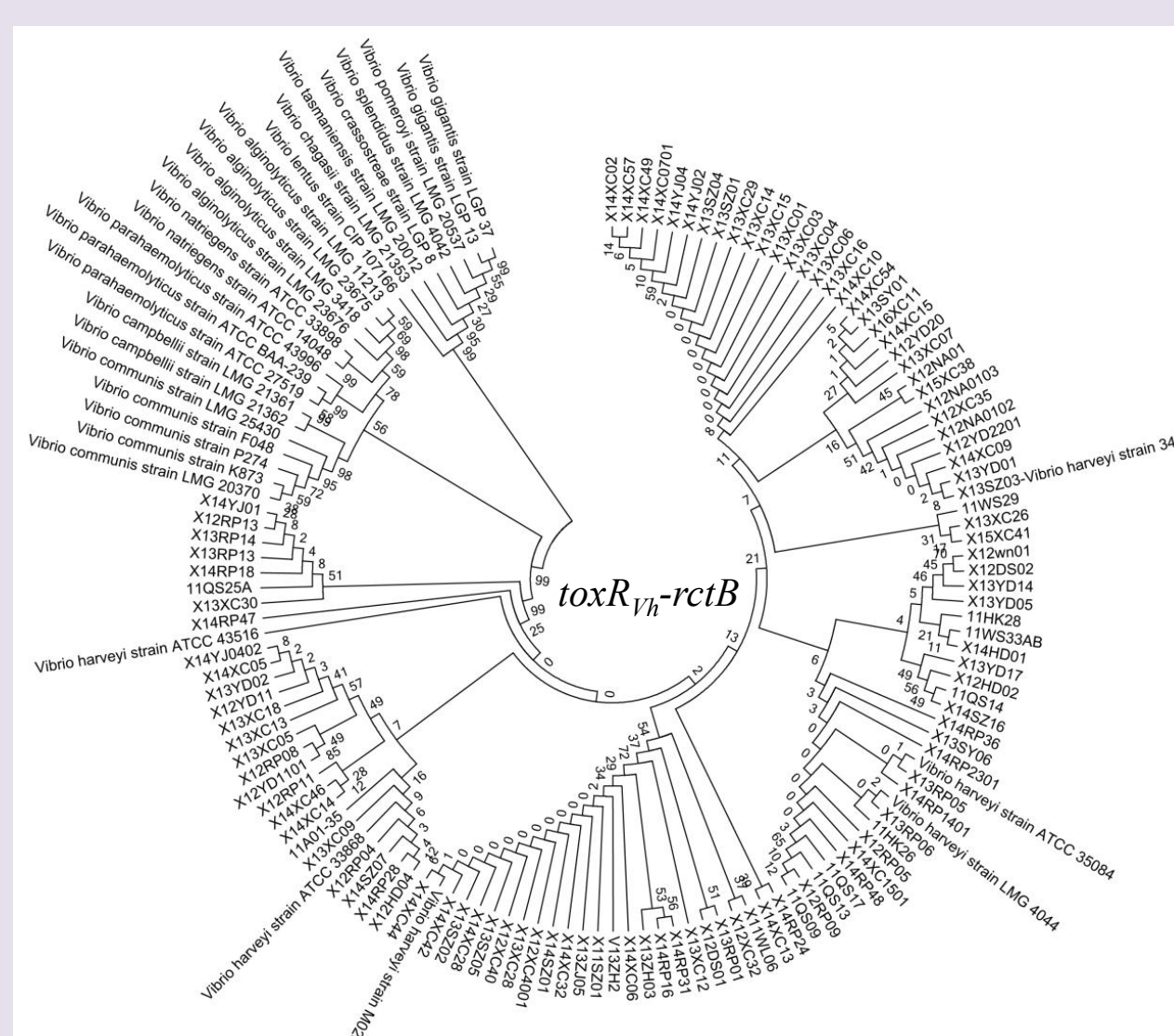


FIG 1 The phylogenetic tree of *toxR_{vh}*-*rctB* concatenated sequences with representative microbes aligned by using ClustalW.

TABLE 3 Distribution of ARGs and MGEs in *V. harveyi* isolates

ARGs	% (n)	ARGs	% (n)
<i>bla</i> TEM	5.61 (6)	<i>sul</i> 3	0 (0)
<i>bla</i> SHV	8.41 (9)	<i>qnr</i> Sm	4.67 (5)
<i>erm</i> C	0.93 (1)	<i>qnr</i> Am	0 (0)
<i>erm</i> B	2.8 (3)	<i>qnr</i> Bm	9.35 (10)
<i>aac</i> (6')-Ib	14.02 (15)	<i>qep</i> A	0 (0)
<i>tet</i> O	0 (0)	<i>oxq</i> A	4.67 (5)
<i>tet</i> S	0 (0)	<i>oxq</i> B	2.8 (3)
<i>tet</i> W	20.56 (22)	ARG (n) ^a	0.93 ± 0.09
<i>tet</i> Q	0 (0)	ARG (n)/HN	1.11 ± 0.15
<i>tet</i> B	4.67 (5)	ARG (n)/GD	0.75 ± 0.10
<i>tet</i> X	0 (0)	<i>P</i> _{AVG} (GD vs. HN)	0.075
<i>sul</i> 1	1.87 (2)	<i>int</i>	14.95 (16)
<i>sul</i> 2	12.15 (13)	<i>int</i> 1	24.3 (26)

^aAverage number of antibiotic resistance genes.

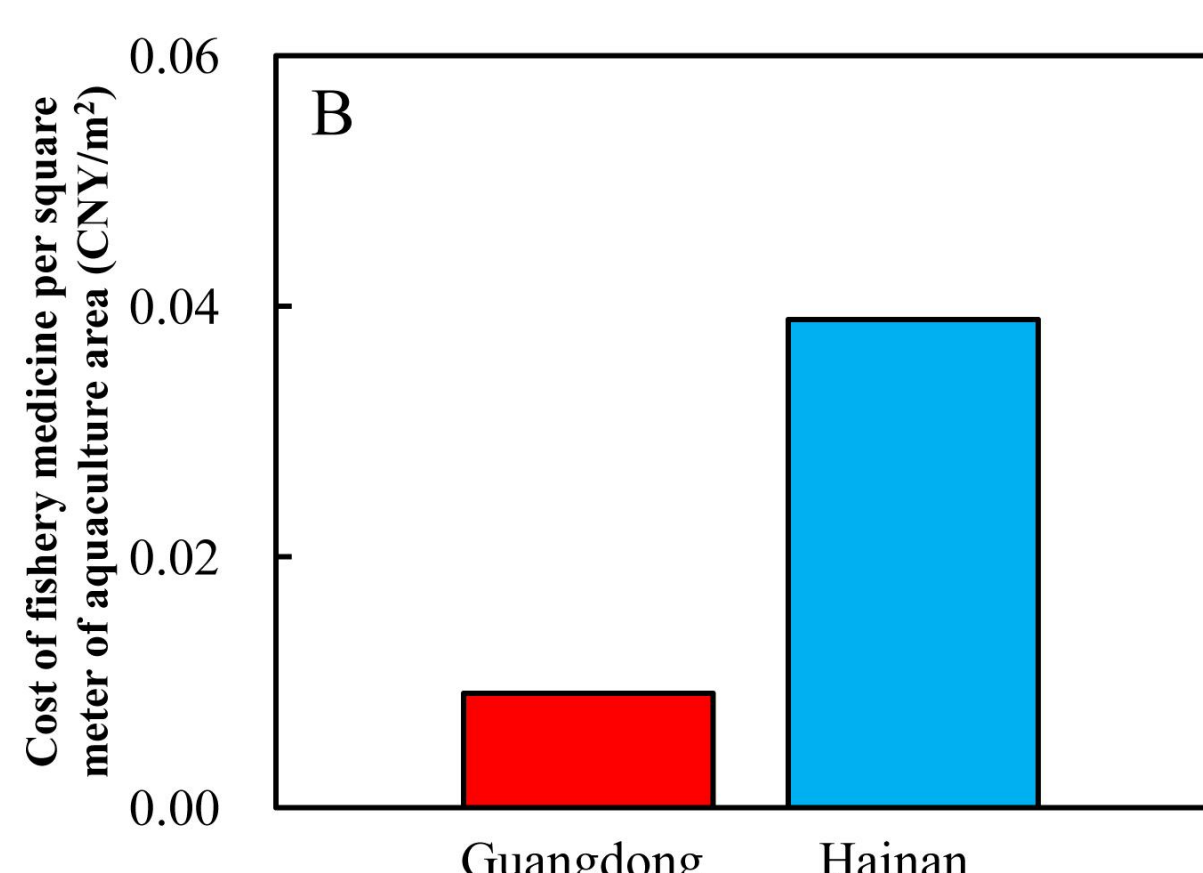
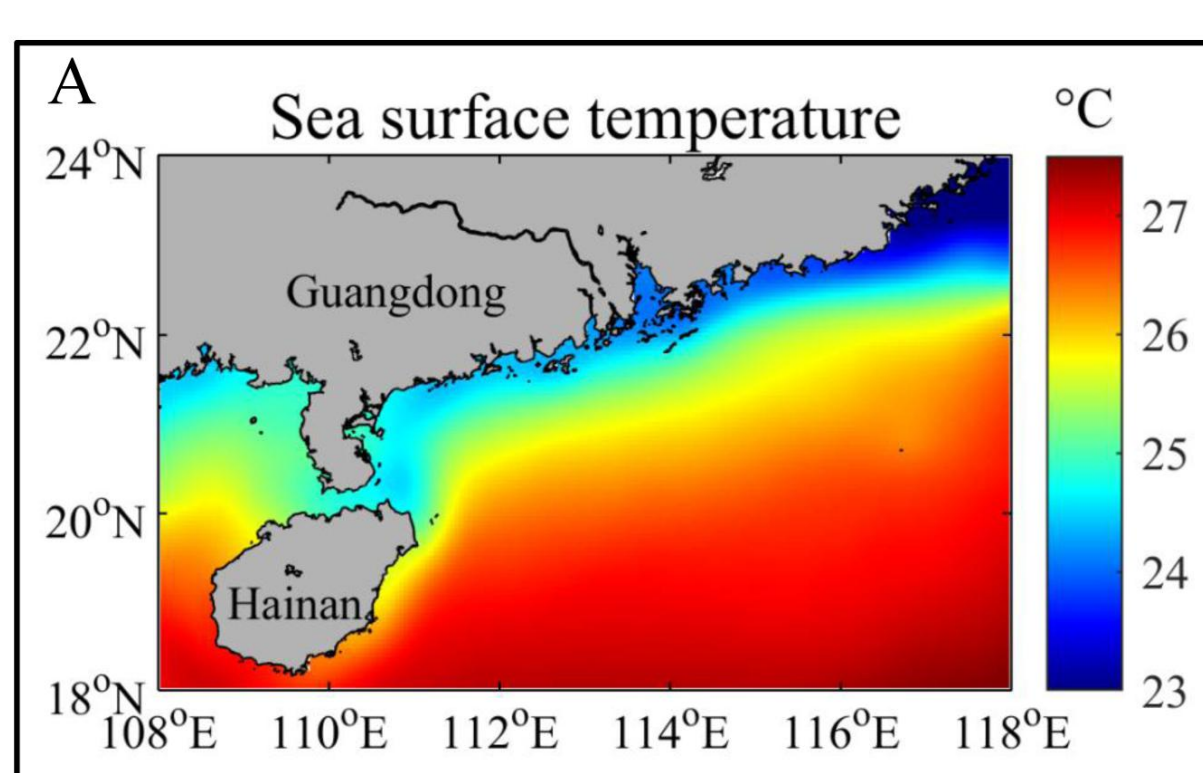


FIG 2 The average sea surface temperature (SST) (A) and cost of fishery medicine per square meter of aquaculture area (B) during sampling period in Guangdong and Hainan.

TABLE 1 Distribution of virulence factor genes

Typical virulence genes	% (n)	Atypical virulence genes	% (n)
<i>cqs</i> S	38.32 (41)	<i>toxR_{vc}</i>	28.04 (30)
<i>lux</i> N	54.21 (58)	<i>hly</i> A	15.89 (17)
<i>lux</i> P	71.96 (77)	<i>fla</i> C	83.18 (89)
<i>ahp</i> A	85.98 (92)	<i>tdh</i>	8.41 (9)
<i>Vih</i>	90.65 (97)	<i>trh</i>	0 (0)
<i>Hflk</i>	84.11 (90)	<i>vvh</i>	63.55 (68)
<i>lux</i> R	94.39 (101)	AtyAVG (n) ^a	1.99 ± 0.10
<i>chi</i> A	95.33 (102)	AtyAVG (n)/HN	2.30 ± 0.20
TyAVG (n) ^b	7.15 ± 0.19	AtyAVG (n)/GD	1.70 ± 0.10
TyAVG (n)/HN	7.39 ± 0.24	<i>P</i> _{AtyAVG} (GD vs. HN)	0.002
TyAVG (n)/GD	6.91 ± 0.28		
<i>P</i> _{TyAVG} (GD vs. HN)	0.137		

^aAverage number of atypical virulence genes in each strain.

^bAverage number of typical virulence genes in each strain.

TABLE 2 Resistance to antibiotics by *V. harveyi* isolates

Antibiotics	% (n)	Antibiotics	% (n)
Vancomycin	97.22 (104)	Trimethoprim-sulfamethoxazole	16.47 (17)
Amoxicillin	74.3 (80)	Norfloracin	2.49 (3)
Midecamycin	79.41 (85)	Ciprofloxacin	2.49 (3)
Erythromycin	4.11 (4)	Chloramphenicol	6.65 (3)
Tobramycin	43.75 (47)	Florfenicol	0 (0)
Gentamicin	19.89 (21)	TAR (n) ^a	4.57 ± 0.18
Furazolidone	31.78(34)	TAR (n)/HN	5.25 ± 0.27
Rifampicin	30.8 (33)	TAR (n)/GD	3.87 ± 0.21
Tetracycline	15.12 (16)	<i>P</i> _{TAR} (GD vs. HN)	0.0001
Doxycycline	7.75 (8)		

^aAverage number of antibiotic-resistant isolates.

Conclusions

- 2, 52 and 53 *V. harveyi* strains were isolated from Fujian, Hainan and Guangdong, respectively, and identified them by multilocus sequence analysis of 16S rRNA-*toxR_{vh}*-*rctB*.
- Nine typical virulence genes were represented at a higher average in Hainan (7.39 ± 0.24) than in Guangdong (6.91 ± 0.28).
- Five atypical virulence genes were detected in some isolates. In particular, *flaC* and *vvh* (>60%). Their average number was significantly higher in Hainan (2.30 ± 0.20) than in Guangdong (1.70 ± 0.10).
- Both the average number of antibiotic resistance and antibiotic resistance genes were higher in Hainan (5.25 ± 0.27 and 1.11 ± 0.15 , respectively) than in Guangdong (3.87 ± 0.21 and 0.75 ± 0.10 , respectively).

Our results showed that the QS system, hemolysin, and extracellular bacterial proteases were relatively conserved virulence factors in *V. harveyi*. Atypical virulence genes were present in *V. harveyi* that promoted bacterial virulence and broadened their host range. Higher temperatures and longer use of antibiotics might have led to more enhanced drug resistance and bacterial virulence in Hainan than in Guangdong. These results will help evaluate isolate pathogenicity and guide the effective use of antibiotics in marine fish farming. It also highlights the necessity for more detailed investigations for improving consumer protection and public health safety. However, further research should focus on: (1) the influence of warming temperature and antibiotic pollutants on virulence and antibiotic resistance; (2) the correlation between virulence and antibiotic resistance.