

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



#### Yiqin Deng<sup>1,2</sup>, Liwen Xu<sup>1</sup>, Songlin Liu<sup>3</sup>, Qian Wang<sup>1</sup>, Zhixun Guo<sup>1</sup>, Chang Chen<sup>4</sup>, Juan Feng<sup>1</sup>\*

1 South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China 2 Tropical Aquaculture Research and Development Centre, SCSFRI, CAFS, Hainan, 572426, China 3 South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China 4 Xisha/Nansha Ocean Observation and Research Station, SCSIO, CAS, Guangzhou 510301, China

\*Corresponding author: juanfeng@scsfri.ac.cn

#### Introduction

### Materials and method

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.

Results







FIG 1 The phylogenetic tree of *toxR<sub>Vh</sub>-rctB* concatenated sequences with representative microbes aligned by using ClustalW.

**TABLE 1** Distribution of virulence factor genes

S       38.32 (41)       tox $R_{Vc}$ 28.04 (30)         N       54.21 (58)       hlyA       15.89 (17)         P       71.96 (77)       flaC       83.18 (89)         AA       85.98 (92)       tdh       8.41 (9)         AA       95.93 (101)       AtyAVG (n) <sup>4</sup> 1.99 ± 0.10         AA       95.33 (102)       AtyAVG (n)/HN       2.30 ± 0.20         AVG (n) <sup>b</sup> 7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         AVG (n)/GD       6.91 ± 0.28	oical ulence genes	s % (n)	Atypical virulence genes	% (n)	20 <sup>0</sup> N	· ·
th       54.21 (58)       hlyA       15.89 (17)         th       71.96 (77)       flaC       83.18 (89)         pA       85.98 (92)       tdh       8.41 (9)         th       90.65 (97)       trh       0 (0)         lk       84.11 (90)       vvh       63.55 (68)         cR       94.39 (101)       AtyAVG (n)/HN       2.30 ± 0.20         AVG (n) <sup>b</sup> 7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         AVG (n)/GD       6.91 ± 0.28	sS	38.32 (41)	toxR <sub>Vc</sub>	28.04 (30)	20 N	5
$x_{P}$ 71.96 (77) $flaC$ 83.18 (89) $hpA$ 85.98 (92) $tdh$ 8.41 (9) $hhh$ 90.65 (97) $trh$ 0 (0) $flk$ 84.11 (90) $vvh$ 63.55 (68) $hxR$ 94.39 (101)       AtyAVG (n)'HN       2.30 $\pm$ 0.20 $yAVG$ (n)'B       7.15 $\pm$ 0.19       AtyAVG (n)/GD       1.70 $\pm$ 0.10 $hhi$ 95.33 (102)       AtyAVG (n)/GD       0.002 $yAVG$ (n)/HN       7.39 $\pm$ 0.24 $PA_{tyAVG}$ (GD vs.       0.002 $yAVG$ (n)/GD $6.91 \pm$ 0.28	uxN	54.21 (58)	hlyA	15.89 (17)		Ha
hpA       85.98 (92)       tdh       8.41 (9)         hh       90.65 (97)       trh       0 (0)         flk       84.11 (90)       vvh       63.55 (68)         xR       94.39 (101)       AtyAVG (n) <sup>a</sup> 1.99 ± 0.10         niA       95.33 (102)       AtyAVG (n)/HN       2.30 ± 0.20         vAVG (n)/b       7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         vAVG (n)/GD       6.91 ± 0.28       0.002         TyAVG (GD vs.       0.137       0.137         TyAVG (GD vs.       0.137       0.002         erage number of typical virulence genes in each strain.       0.02         erage number of typical virulence genes in each strain.       0.02         erage number of typical virulence genes in each strain.       0.02         moxicillin       74.3 (80)       Norfloxacin       2.49 (3)         idecamycin       79.41 (85)       Ciprofloxacin       2.49 (3)         ythromycin       4.11 (4)       Chloramphenicol       6.65 (3)         obramycin       17.8(34)       TAR (n)/HN       5.25 ± 0.27         fampicin       30.8 (33)       TAR (n)/GD       3.87 ± 0.21         etaracycline       15.12 (16)       P tar (GD vs. HN)       0.0001    <	хP	71.96 (77)	flaC	83.18 (89)	$18^{\circ}N$	
fnh       90.65 (97)       trh       0 (0)         flk       84.11 (90)       vvh       63.55 (68)         ixR       94.39 (101)       AtyAVG (n) <sup>a</sup> 1.99 ± 0.10         hiA       95.33 (102)       AtyAVG (n)/HN       2.30 ± 0.20         yAVG (n) <sup>b</sup> 7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         yAVG (n)/HN       7.39 ± 0.24       P A <sub>tyAVG</sub> (GD vs.       0.002         yAVG (n)/GD       6.91 ± 0.28	hpA	85.98 (92)	tdh	8.41 (9)	108	Έ
f/k       84.11 (90)       vvh       63.55 (68)         xR       94.39 (101)       AtyAVG (n) <sup>a</sup> 1.99 ± 0.10         niA       95.33 (102)       AtyAVG (n)/HN       2.30 ± 0.20         vAVG (n) <sup>b</sup> 7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         vAVG (n)/HN       7.39 ± 0.24       P A <sub>tyAVG</sub> (GD vs. HN)       0.002         vAVG (n)/GD       6.91 ± 0.28	hh	90.65 (97)	trh	O (O)		
xxR       94.39 (101)       AtyAVG (n) <sup>a</sup> 1.99 ± 0.10         hiA       95.33 (102)       AtyAVG (n)/HN       2.30 ± 0.20         yAVG (n) <sup>b</sup> 7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         yAVG (n)/HN       7.39 ± 0.24       P A <sub>tyAVG</sub> (GD vs.       0.002         yAVG (n)/GD       6.91 ± 0.28	lflk	84.11 (90)	vvh	63.55 (68)	0.0	)6
hiA 95.33 (102) AtyAVG (n)/HN 2.30 ± 0.20 yAVG (n) <sup>b</sup> 7.15 ± 0.19 AtyAVG (n)/GD 1.70 ± 0.10 yAVG (n)/HN 7.39 ± 0.24 PA <sub>tyAVG</sub> (GD vs. 0.002 HN) yAVG (n)/GD 6.91 ± 0.28 TyAVG (GD vs. 0.137 TyAVG (Antibiotics by V. harveyi isolates BLE 2 Resistance to antibiotics by V. harveyi isolates ntibiotics % (n) Antibiotics % (n) ancomycin 97.22 (104) Trimethoprim- sulfamethoxazole 16.47 (17) sulfamethoxazole 16.47 (17) sulfameth	ıxR	94.39 (101)	AtyAVG (n) <sup>a</sup>	$1.99 \pm 0.10$	re ;;	10
vAVG (n) <sup>b</sup> 7.15 ± 0.19       AtyAVG (n)/GD       1.70 ± 0.10         vAVG (n)/HN       7.39 ± 0.24       P A <sub>tyAVG</sub> (GD vs. HN)       0.002         vAVG (n)/GD       6.91 ± 0.28	niA	95.33 (102)	AtyAVG (n)/HN	$2.30 \pm 0.20$	lua Y/n	
yAVG (n)/HN $7.39 \pm 0.24$ $PA_{tyAVG}$ (GD vs. HN) $0.002$ yAVG (n)/GD $6.91 \pm 0.28$ $1.137$ TyAVG (GD vs. PANNO $0.137$ $0.137$ treage number of atypical virulence genes in each strain. treage number of typical virulence genes in each strain. $0.02$ BLE 2 Resistance to antibiotics by V. harveyi isolatesntibiotics $\%$ (n)Antibiotics $\%$ (n)Antibiotics $\%$ (n)ancomycin $77.22$ (104)Trimethoprim- sulfamethoxazolemoxicillin $74.3$ (80)Norfloxacin $2.49$ (3)ythromycin $4.11$ (4)Chloramphenicol $6.65$ (3)obramycin $97.921$ TAR (n)^a $4.57 \pm 0.18$ urazolidone $31.78(34)$ TAR (n)/HN $5.25 \pm 0.27$ fampicin $30.8$ (33)TAR (n)/GD $3.87 \pm 0.21$ tracycline $15.12$ (16) $P_{TAR}$ (GD vs. HN) $0.0001$ oxycycline $7.75$ (8) $0.001$	yAVG (n) <sup>b</sup>	7.15 ± 0.19	AtyAVG (n)/GD	$1.70 \pm 0.10$	r sc	
waves yaves (GD vs. TAAVG (GD vs. (GD vs. (GD vs. TAAVG (GD vs. (GD vs.)0.137	īyAVG (n)∕HN	7.39 ± 0.24	P A <sub>tyAVG</sub> (GD vs. HN)	0.002	cine pe area (	)4
Trank (GD vs. $0.137$ Verage number of atypical virulence genes in each strain. verage number of typical virulence genes in each strain.BLE 2Resistance to antibiotics by V. harveyi isolates $0.02$ ntibiotics $\%$ (n)Antibiotics $\%$ (n)ancomycin $97.22$ (104)Trimethoprim- sulfamethoxazole $16.47$ (17) sulfamethoxazole $FIG 2$ moxicillin $74.3$ (80)Norfloxacin $2.49$ (3)rythromycin $4.11$ (4)Chloramphenicol $6.65$ (3)obramycin $43.75$ (47)Florfenicol $0$ (0)entamicin $19.89$ (21)TAR (n) <sup>a</sup> $4.57 \pm 0.18$ urazolidone $31.78(34)$ TAR (n)/HN $5.25 \pm 0.27$ ifampicin $30.8$ (33)TAR (n)/GD $3.87 \pm 0.21$ moxycycline $7.75$ (8) $7.75$ (8) $1000$	yAVG (n)/GD	6.91 ± 0.28			edic	
erage number of atypical virulence genes in each strain.erage number of typical virulence genes in each strain.BLE 2 Resistance to antibiotics by V. harveyi isolatesntibiotics $\%$ (n)Antibiotics $\%$ (n)ancomycin $97.22$ (104)Trimethoprim- sulfamethoxazole16.47 (17) sulfamethoxazoleFIG 2 temper fishery of a que stracyclinemoxicillin $74.3$ (80)Norfloxacin2.49 (3) cliptofloxacin2.49 (3) cliptofloxacinFIG 2 temper fishery of a que sampling fishery of a que sampling fampicin19.89 (21)TAR (n)^a4.57 ± 0.18 sampling tracyclineTAR (n)/GD3.87 ± 0.21attracycline15.12 (16) $P_{TAR}$ (GD vs. HN)0.0001Hainan cliptoflowHainan cliptoflow	<sub>TyAVG</sub> (GD vs. HN)	0.137			ery m uacult	<b>)</b> 7
while% (n)Antibiotics% (n)ancomycin $97.22 (104)$ Trimethoprim- sulfamethoxazole $16.47 (17)$ moxicillin $74.3 (80)$ Norfloxacin $2.49 (3)$ idecamycin $79.41 (85)$ Ciprofloxacin $2.49 (3)$ rythromycin $4.11 (4)$ Chloramphenicol $6.65 (3)$ obramycin $43.75 (47)$ Florfenicol $0 (0)$ entamicin $19.89 (21)$ TAR (n) <sup>a</sup> $4.57 \pm 0.18$ urazolidone $31.78(34)$ TAR (n)/HN $5.25 \pm 0.27$ afampicin $30.8 (33)$ TAR (n)/GD $3.87 \pm 0.21$ etracycline $15.12 (16)$ $P_{TAR}$ (GD vs. HN) $0.0001$	erage number BLE 2 Res	r of typical virule istance to antibi	nce genes in each stra iotics by V. harveyi is	iin. olates	Cost of meter o	00
ancomycin $97.22 (104)$ Trimethoprim- sulfamethoxazole $16.47 (17)$ moxicillin $74.3 (80)$ Norfloxacin $2.49 (3)$ fidecamycin $79.41 (85)$ Ciprofloxacin $2.49 (3)$ rythromycin $4.11 (4)$ Chloramphenicol $6.65 (3)$ obramycin $43.75 (47)$ Florfenicol $0 (0)$ entamicin $19.89 (21)$ TAR $(n)^a$ $4.57 \pm 0.18$ urazolidone $31.78(34)$ TAR $(n)/HN$ $5.25 \pm 0.27$ ifampicin $30.8 (33)$ TAR $(n)/GD$ $3.87 \pm 0.21$ etracycline $15.12 (16)$ $P_{TAR} (GD vs. HN)$ $0.0001$	ntibiotics	% (n)	Antibiotics	% (n)	0.0	
moxicillin $74.3 (80)$ Norfloxacin $2.49 (3)$ lidecamycin $79.41 (85)$ Ciprofloxacin $2.49 (3)$ cythromycin $4.11 (4)$ Chloramphenicol $6.65 (3)$ obramycin $43.75 (47)$ Florfenicol $0 (0)$ entamicin $19.89 (21)$ TAR $(n)^a$ $4.57 \pm 0.18$ urazolidone $31.78 (34)$ TAR $(n)/HN$ $5.25 \pm 0.27$ ifampicin $30.8 (33)$ TAR $(n)/GD$ $3.87 \pm 0.21$ etracycline $15.12 (16)$ $P_{TAR} (GD vs. HN)$ $0.0001$	ancomycin	97.22 (104)	Trimethoprim– sulfamethoxazole	16.47 (17)	FIG	2 -
Iidecamycin       79.41 (85)       Ciprofloxacin       2.49 (3)         rythromycin       4.11 (4)       Chloramphenicol       6.65 (3)         obramycin       43.75 (47)       Florfenicol       0 (0)         entamicin       19.89 (21)       TAR (n) <sup>a</sup> 4.57 ± 0.18         urazolidone       31.78(34)       TAR (n)/HN       5.25 ± 0.27         ifampicin       30.8 (33)       TAR (n)/GD       3.87 ± 0.21         etracycline       15.12 (16)       P TAR (GD vs. HN)       0.0001	moxicillin	74.3 (80)	Norfloxacin	2.49 (3)	temr	יסר
ythromycin       4.11 (4)       Chloramphenicol       6.65 (3)         obramycin       43.75 (47)       Florfenicol       0 (0)         entamicin       19.89 (21)       TAR (n) <sup>a</sup> 4.57 ± 0.18         urazolidone       31.78(34)       TAR (n)/HN       5.25 ± 0.27         fampicin       30.8 (33)       TAR (n)/GD       3.87 ± 0.21         tracycline       15.12 (16)       P TAR (GD vs. HN)       0.0001	idecamycin	79.41 (85)	Ciprofloxacin	2.49 (3)		
bbramycin       43.75 (47)       Florfenicol       0 (0)         entamicin       19.89 (21)       TAR (n) <sup>a</sup> 4.57 ± 0.18         urazolidone       31.78(34)       TAR (n)/HN       5.25 ± 0.27         ifampicin       30.8 (33)       TAR (n)/GD       3.87 ± 0.21         etracycline       15.12 (16)       P TAR (GD vs. HN)       0.0001	rythromycin	4.11 (4)	Chloramphenicol	6.65 (3)	tishe	ry
entamicin       19.89 (21)       TAR ( $n$ ) <sup>a</sup> 4.57 ± 0.18         urazolidone       31.78(34)       TAR ( $n$ )/HN       5.25 ± 0.27         fampicin       30.8 (33)       TAR ( $n$ )/GD       3.87 ± 0.21         etracycline       15.12 (16) $P_{TAR}$ (GD vs. HN)       0.0001         oxycycline       7.75 (8)       Image: State of the second seco	bramycin	43.75 (47)	Florfenicol	0 (0)	of a	an
urazolidone       31.78(34)       TAR (n)/HN       5.25 ± 0.27         fampicin       30.8 (33)       TAR (n)/GD       3.87 ± 0.21         etracycline       15.12 (16)       P TAR (GD vs. HN)       0.0001         oxycycline       7.75 (8)       Image: Sampling the second secon	entamicin	19.89 (21)	TAR (n) <sup>a</sup>	4.57 ± 0.18		ч ч 1 ч
fampicin       30.8 (33)       TAR (n)/GD       3.87 ± 0.21         etracycline       15.12 (16)       P TAR (GD vs. HN)       0.0001         oxycycline       7.75 (8)	urazolidone	31.78(34)	TAR (n)/HN	5.25 ± 0.27	samp	JII
etracycline 15.12 (16) P <sub>TAR</sub> (GD vs. HN) 0.0001 oxycycline 7.75 (8)	ifampicin	30.8 (33)	TAR (n)/GD	3.87 ± 0.21	Haina	an.
oxycycline 7.75 (8)	etracycline	15.12 (16)	P <sub>TAR</sub> (GD vs. HN)	0.0001		
	oxycycline	7.75 (8)				

ARGs	% (n)	ARGs	% (n)
blaTEM	5.61 (6)	sul 3	0 (0)
blaSHV	8.41 (9)	qnrSm	4.67 (5)
ermC	0.93 (1)	qnrAm	0 (0)
ermB	2.8 (3)	qnrBm	9.35 (10)
aac(6′)-Ib	14.02 (15)	qepA	0 (0)
tetO	O (O)	oqxA	4.67 (5)
tetS	O (O)	oqxB	2.8 (3)
tetW	20.56 (22)	ARG (n) <sup>a</sup>	0.93 ± 0.09
tetQ	O (O)	ARG (n)/HN	$1.11 \pm 0.15$
tetB	4.67 (5)	ARG (n)/GD	0.75 ± 0.10
tetX	O (O)	P <sub>AVG</sub> (GD vs. HN)	0.075
sul 1	1.87 (2)	int	14.95 (16)
sul 2	12.15 (13)	intl1	24.3 (26)

<sup>a</sup>Average number of antibiotic resistance genes



> 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<sub>Vh</sub>-rctB*. > Nine typical virulence genes were represented at a higher average in Hainan  $(7.39 \pm 0.24)$ than in Guangdong ( $6.91 \pm 0.28$ ). Five atypical virulence genes were detected in some isolates. In particular, *flaC* and *vvh* (>60%). Their average number was significantly higher in Hainan  $(2.30 \pm 0.20)$  than in Guangdong ( $1.70 \pm 0.10$ ). Both the average number of antibiotic resistance and antibiotic resistance genes were higher in Hainan (5.25  $\pm$  0.27 and  $1.11 \pm 0.15$ , respectively) than in Guangdong  $(3.87 \pm 0.21)$ and  $0.75 \pm 0.10$ , respectively).

## Conclusions

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.

<sup>a</sup>Average number of antibiotic-resistant isolate

Hainan Guangdong verage sea surface

(SST) (A) and cost of cine per square meter ure area (B) during riod in Guangdong and