

Supplementary Materials

Antibiotic and heavy metal susceptibility of non-cholera *Vibrio* isolated from marine sponges and sea urchins: could they pose a potential risk to public health?

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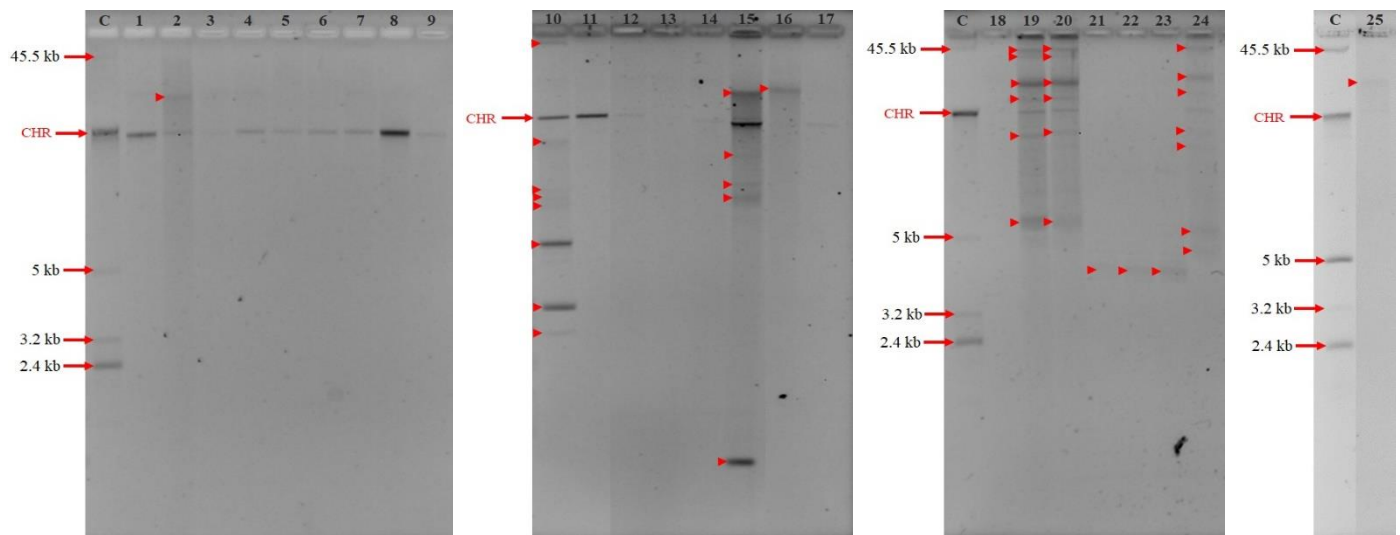


Figure S1. Plasmid profile of the *Vibrio* spp. strains. Plasmid DNA analyzed by 0.8% agarose gel electrophoresis. Lanes C: *K. pneumoniae* Kp13 (molecular size plasmids control); 1: 51BHI25; 2: 51BHI28; 3: 84BHI7b; 4: 51BHI31; 5: 51BHI31a; 6: 64BHI6; 7: 64BHI7; 8: 84BHI4; 9: 84BHI5; 10: 51BHI30; 11: 84BHI6; 12: 84BHI10; 13: 84B1:104; 14: 84MA3; 15: 84MA4; 16: HIM15.10⁻³; 17: HEM9.10⁻³; 18: ME7; 19: P1I-2; 20: P1I-8; 21: P3I-9; 22: P3I-12; 23: P3I-23; 24: P5I-6; 25: 84BHI7a. Fragmented chromosomal (CHR) and plasmid DNAs are indicated by arrows and arrow heads, on the left side.

Table S1. Identification, antimicrobial resistance profile, antimicrobial and heavy metal resistance genes, virulence genes and the plasmid profile of *Vibrio* spp. isolates from marine sponges (Brazil) and sea urchins (France).

Host	Strain	Accession Number ^a	BLASTn (%; Accession Number) ^b	MALDI-TOF identification (score)	Phenotypic Resistance	Resistance genes	Virulence genes	Plasmids ^c
<i>Darwinella</i> sp. 1 (marine sponge)	<i>Vibrio</i> sp. 51BHI25	KX148503	<i>Vibrio alginolyticus</i> (99.34; MH643615.1)	<i>Vibrio alginolyticus</i> (2.169)	CFE	-	-	-
	<i>Vibrio</i> sp. 51BHI28 ^d	KX148504	<i>Vibrio alginolyticus</i> (99.10; MK988029.1)	<i>Vibrio alginolyticus</i> (1.758)	AMI, CFE, SXT ⁱ	-	-	1
	<i>Vibrio</i> sp. 51BHI30	KX148505	<i>Vibrio alginolyticus</i> (99.87; AP022865.1)	<i>Vibrio alginolyticus</i> (2.068)	-	-	-	8

	<i>Vibrio</i> sp. 51BHI31	KX148506	<i>Vibrio</i> <i>alginolyticus</i> (99.60; MK988029.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.046)	AMC ⁱ , CFE, SXT ⁱ	-	-	-
	<i>Vibrio</i> sp. 51BHI31a	KX148507	<i>Vibrio</i> <i>alginolyticus</i> (99.60; MK988029.1)	<i>Vibrio</i> <i>alginolyticus</i> (1.803)	CFE, ERY	-	-	-
<i>Plakina</i> <i>cyanorosa</i> (marine sponge)	<i>Vibrio</i> sp. 64BHI6	KX148484	<i>Vibrio</i> <i>alginolyticus</i> (97.09; MK988029.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.169)	-	-	-	-
	<i>Vibrio</i> sp. 64BHI7	KX148485	<i>Vibrio</i> <i>alginolyticus</i> (98.94; MK102585.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.054)	CFE	-	-	-
<i>Darwinella</i> sp. 2 (marine sponge)	<i>Vibrio</i> sp. 84BHI4	KX148486	<i>Vibrio</i> <i>alginolyticus</i> (98.50; MK452731.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.165)	CFO ⁱ , OFX ⁱ	-	-	-
	<i>Vibrio</i> sp. 84BHI5	KX148487	<i>Vibrio</i> <i>alginolyticus</i> (98.62; MH368400.1)	<i>Vibrio</i> <i>alginolyticus</i> (1.744)	CFE	-	-	-
	<i>Vibrio</i> sp. 84BHI6 ^d	KX148488	<i>Vibrio</i> <i>alginolyticus</i> (97.73; MH368400.1)	<i>Vibrio</i> <i>alginolyticus</i> (1.803)	APS ⁱ , CIP ⁱ , ERY	-	-	-
	<i>Vibrio</i> sp. 84BHI7a	MH404096	<i>Vibrio</i> <i>proteolyticus</i> (94.39; GQ131641.1)	<i>Vibrio</i> <i>parahaemolyt</i> <i>icus</i> (2.012)	CFE	-	-	1
	<i>Vibrio</i> sp. 84BHI7b	MH404095	<i>Vibrio azureus</i> (99.42; JQ665317.1)	<i>Vibrio mytili</i> (1.95)	AMI ⁱ , CFE	-	-	-
	<i>Vibrio</i> sp. 84BHI10	KX148490	<i>Vibrio</i> <i>alginolyticus</i> (98.90; MN733131.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.283)	APS ⁱ , ERY	<i>bla</i> ^{SHV}	-	-
	<i>Vibrio</i> sp. 84B1:104	KX148492	<i>Vibrio</i> <i>alginolyticus</i> (98.17; MH368400.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.061)	ERY	-	-	-
	<i>Vibrio</i> sp. 84MA3	MF919340	<i>Vibrio</i> <i>alginolyticus</i> (99.17; MK988029.1)	<i>Vibrio</i> <i>alginolyticus</i> (2.133)	-	-	-	-
	<i>Vibrio</i> sp. 84MA4	MF919341	<i>Vibrio</i> <i>alginolyticus</i> (99.32; MN733127.1)	<i>Vibrio</i> <i>alginolyticus</i> (1.968)	AMI ^h , APS ⁱ , CFE, GEN ^h , TOB ^h	-	-	5

	<i>Vibrio</i> sp. HIM 15.10 ⁻³	MG561916	<i>Vibrio harveyi</i> (100; CP035693.1)	<i>Vibrio harveyi</i> (2.254)	ERY	-	-	1
<i>Haliclona</i> sp. (marine sponge)	<i>Vibrio</i> sp. HEM 9.10 ⁻³	MH404093	<i>Vibrio neocaledonicus</i> (94.71; KX037098.1)	<i>Vibrio parahaemolyticus</i> (1.910)	ERY	-	-	-
	<i>Vibrio</i> sp. ME7	MH404094	<i>Vibrio coralliilyticus</i> (94.45; MH613238.1)	<i>Vibrio harveyi</i> (2.179)	ERY	-	<i>vvh</i>	-
	<i>Vibrio</i> sp. P1I-2	KX898819	<i>Vibrio oceanisediminis</i> (98.29; NR_137371.1)	not reliable identification (1.438)	-	<i>cusB</i>	-	6
	Vibrionales P1I-8	KX898821	<i>Vibrio oceanisediminis</i> (99.26; NR_137371.1)	not reliable identification (1.425)	-	-	-	6
<i>Paracentrotus lividus</i> (sea urchin)	<i>Vibrio</i> sp. P3I-9	KX898836	<i>Vibrio cyclitrophicus</i> (92.37; MK102602.1)	not reliable identification (1.323)	-	-	-	1
	Vibrionaceae P3I-12	KX898830	<i>Vibrio gigantis</i> (98.47; MH712999.1)	not reliable identification (1.242)	-	-	-	1
	<i>Vibrio</i> sp. P3I-23	KX898833	<i>Vibrio ichthyenteri</i> (97.04; NR_117888.1)	not reliable identification (1.37)	-	-	-	1
	Vibrionales P5I-6	KX898861	<i>Vibrio oceanisediminis</i> (99.26; NR_137371.1)	not reliable identification (1.55)	-	-	-	7

(^a) GenBank accession numbers of *Vibrio* spp.' 16S rRNA sequences; (^b) Strains isolation and 16S rRNA identification performed by Freitas-Silva et al. (2020) and Laport et al. (2018) studies; (^c) number of plasmid forms; (^d) multidrug-resistant *Vibrio* spp. strain; (^e) intermediate; (^f) heteroresistance; (-) negative test; AMC: amoxicillin-clavulanic acid; AMI: amikacin; APS: ampicillin-sulbactam; CFE: cephalixin; CFO: cefoxitin; CIP: ciprofloxacin; ERY: erythromycin; GEN: gentamicin; OFX: ofloxacin; SXT: trimethoprim/sulfamethoxazole; TOB: tobramycin.

Table S2. Primers used in this study.

Primer	Target	Sequence of Primers (5'-3')	Size (bp)	References
	<u>β-lactams</u>			
MTEMF	<i>bla</i> _{TEM}	5'-CCCTTATTCCCTTTTGTGCGG-3'*	650	[36]
MTEMR		5'-AACCAGCCAGCCWGAAGG-3'*		
MSHVF	<i>bla</i> _{SHV}	5'-CTTGACCGCTGGGAAACGG-3'	200	
MSHVR		5'-AGCACGGAGCGGATCAACGG-3'		
MGESF	<i>bla</i> _{GES}	5'-AGCAGCTCAGATCGGTGTTG-3'	750	
MGESR		5'-CCGTGCTCAGGATGAGTTG-3'		
	<u>aminoglycosi</u>			
	<u>des</u>			
aac6-aph2a fw		5'-GCCAGAACATGAATTACACGAG-3'	611	[37]

aac6-aph2a rev	<i>aac(6')-Ie-aph(2')-Ia</i>	5'-CTGTTGTTGCATTTAGTCTTTCC-3'		
maac6-IbF		5'-TTGCGATGCTCTATGAGTGGCTA-3'	482	[38]
maac6-IbR	<i>aac(6')-Ib</i>	5'-CTCGAATGCCTGGCGTGTTC-3'		
Fant		5'-TGGGCGATCGATGCACGGCTRG-3'*		
Rant	<i>ant(2'')-I</i>	5'-AAAGCGGCACGCAAGACCTCMAC-3'*	428	[39]
<u>macrolides</u>				
MefC-F		5'-ATGGAAAACCGTAAATGGTT-3'	885	[40]
MefC-R	<i>mef(C)</i>	5'-TTAAATATTTTGTATTTTAC-3'		
MphG-F		5'-ATGAAAAATAGAGATATTCA-3'	1224	[40]
MphG-R	<i>mph(G)</i>	5'-CTACTCAACACCTAACTGTA-3'		
ermB-1		5'-CATTTAACGACGAAACTGGC-3'	425	[41]
ermB-2	<i>ermB</i>	5'-GGAACATCTGTGGTATGGCG-3'		
ereA-1		5'-AACACCCTGAACCCAAGGGACG-3'	420	[42]
ereA-2	<i>ereA</i>	5'-CTTCACATCCGGATTTCGCTCGA-3'		
mphA-1		5'-AACTGTACGCACTTGC-3'	837	[42]
mphA-2	<i>mphA</i>	5'-GGTACTCTTCGTTACC-3'		
MF4a		5'-ACCGATTCTATCAGCAAAG-3'	940	[43]
MF6	<i>mefA</i>	5'-GGACCTGCCATTGGTGTG-3'		
<u>quinolones</u>				
QnrAm-F		5'-AGAGGATTTCTCACGCCAGG-3'	580	
QnrAm-R	<i>qnrA</i>	5'-TGCCAGGCACAGATCTTGAC-3'		
QnrBm-F		5'-GGMATHGAAATTCGCCACTG-3'*	264	
QnrBm-R	<i>qnrB</i>	5'-TTTGCGYGYCGCCAGTCGAA-3'*		
QnrCm-F		5'-GCGAATTTCGAAGGGGCAAA-3'	135	
QnrCm-R	<i>qnrC</i>	5'-ACCCGTAATGTAAGCAGAGCAA-3'		[44]
QnrDm-F		5'-AGGTGTAGCATGTATGGAAAAGC-3'	691	
QnrDm-R	<i>qnrD</i>	5'-ACATTGGGGCATTAGGCGTT-3'		
QnrSm-F		5'-GCAAGTTCATTGAACAGGGT-3'	428	
QnrSm-R	<i>qnrS</i>	5'-TCTAAACCGTCGAGTTCGGCG-3'		
QnrVCm-F		5'-GAGYTKTATGGTTTAYCCTCG-3'*	71	
QnrVCm-R	<i>qnrVC</i>	5'-TGTTCTGTGTGCCACGARCA-3'*		
<u>heavy metals</u>				
A1F		5'-ACCATCGGCGGCACCTGCGT-3'	1226	[45]
A5R		5'-ACCATCGTCAGGTAGGGGACCAA-3'		
merA-F	<i>merA</i>	5'-GTGCCGTCCAAGATCATGAT-3'	933	[46]
merA-R		5'-TAGCCYACRGTSGCSACYTG-3'*		
czcB-I		5'-ATGAAAAAAAATCGCGCTT-3'	1200	[47]
czcB-II	<i>czcB and cusB</i>	5'-ATGCGCATGGGTAGCACT-3'		
cad1		5'-CAAAYTGYGCRGGHAARTTYGA-3'*	1058	[48]
cad2	<i>cadA</i>	5'-AACTAATGCACAAGGACA-3'		
pbrA-N		5'-ATGAGCGAATGTGGCTCGAAG-3'	2400	[49]
pbrA-C	<i>pbrA</i>	5'-TCATCGACGCAACAGCCTCAA-3'		
<u>Virulence genes</u>				
toxR-F		5'-GTCTTCTGACGCAATCGTTG-3'	399	[50]
toxR-R	<i>toxR</i>	5'-ATACGAGTGGTTGCTGTCATG-3'		
tdh-F	<i>tdh</i>	5'-CCAAATACATTTTACTTGG-3'	251	

tdh-R		5'-GGTACTAAATGGCTGACATC-3'		
trh-F		5'-GGCTCAAAATGGTTAAGCG-3'		
trh-R	<i>trh</i>	5'-CATTTCCGCTCTCATATGC-3'	250	
		5'-		
VHF1	<i>vvh</i>	ATCATGAATAAACTATTACGTTACT	1300	[25]
		-3'		
VHR1		5'-GAAAGGATGGTTTGACAAT-3'		
VP1409-F		5'-TGTTGCTTTCTATTGCGAC-3'		
VP1409-R	<i>T6SS</i>	5'-CCATAACGACTTTTCTTTC-3'	869	[51]
	<u>Integrase</u>			
	<u>Class 1</u>			
intI1F		5'-GGCTTCGTGATGCCTGCTT-3'		
intI1R	<i>intI1</i>	5'-CATTCCTGGCCGTGGTTCT-3'	146	[52]

*: M = A or C; H = A or C or T; Y = C or T; K = T or G; R = A or G; S = G or C; W = A or T.

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