

Supplementary material

**Exploring the Pathogenic Potential of *Vibrio vulnificus* Isolated from Seafood Harvested Along the
Mangaluru Coast, India**

**Caroline D'Souza, Kattapuni Suresh Prithvisagar, Vijay Kumar Deekshit, Indrani Karunasagar,
Iddy Karunasagar, Ballamoole Krishna Kumar***

Nitte University Centre for Science Education and Research, Nitte (Deemed to be University),
Deralakatte, Mangaluru-575018, Karnataka, India.

Table S1: Biochemical and physiological characteristics of *V. vulnificus*

| Biochemical trait | Result (n=21) |
|-------------------------------|---------------|
| Indole test | + |
| Salt tolerance 0% | - |
| Salt tolerance 3% | + |
| Salt tolerance 8% | - |
| Salt tolerance 11% | - |
| Citrate test | + |
| Arginine decarboxylase | - |
| Lysine decarboxylase | + |
| Ornithine decarboxylase | Variable |
| Mannitol fermentation | + |
| Lactose fermentation | Variable |
| Oxidase test | + |
| Catalase test | - |
| O/F | +/- |
| Lecithinase activity | - |
| Starch hydrolysis | + |
| Caseinase activity | + |
| Hemolysis (human erythrocyte) | + |
| Gelatinase activity | + |
| Siderophore | + |

Table S2: Genotypic attributes of *Vibrio vulnificus* analysed in this study

| Genotypic attributes | Result (n=21) |
|---|---------------|
| <i>viuB</i> (vulnibactin siderophore) | + |
| <i>vcgC</i> (Virulence correlated gene, Clinical type) | + |
| <i>vcgE</i> (Virulence correlated gene, Environmental type) | - |
| HP1 (CPS Operon allele 1) | + |
| HP2 (CPS Operon allele 2) | - |
| <i>vvhA</i> (<i>Vibrio vulnificus</i> hemolysin A) | + |
| <i>rtxA1</i> (Repeats in toxin) | + |

Table S3: Details of isolation of *V. vulnificus* from seafood

| Type of sample | Total no of sample | No of samples positive for <i>V. vulnificus</i> |
|---|--------------------|---|
| Bivalves (clams, oyster & mussel) | 44 | 19 |
| Crustaceans (crab, shrimp) | 16 | 2 |
| Fin fishes (sardine, mackerel, sole fish) | 6 | - |
| Marine sediment | 4 | - |
| Total | 70 | 21 |

Table S4: Details of sampling location and coordinates

| Sampling station | Coordinates |
|--------------------|--------------------------------------|
| Mulki | 13°5'18.6936" N 74°46'57.396" E |
| Mangalore old port | 12°51'37.2024" N 74°49'54.9336" E |
| Sasihithlu | 13°3'58.1724" N 74°46'47.5176" E |
| Ullala | 12°49'53.4972" N 74°50'14.0208" E |
| Manjeshwara | 12°42'21.1896" N 74°53'16.4832" E |
| Bengre | 12°52'58.5192" N 74°49'5.0808" E |

Table S5: Details of primers used for species specific confirmation, genotyping and virulence gene detection

| Primer code | Primer sequence (5'-3') | Product size (bp) | Annealing temperature (°C) | Reference |
|--------------|--|-------------------|----------------------------|-------------------------|
| <i>gyrB</i> | (F): GTCCGAAGTGGAATCCTTCA (R): TGGTTCTTACGGTTACGGCC | 285 | 63 | Kumar et al. 2006 |
| <i>vvhA</i> | (F): GACTATCGCATCAACAACCG (R): AGGTAGCGAGTATTACTGCC | 704 | 55 | Lee et al. 1998 |
| <i>rtxA1</i> | (F): TGAAGGTGTCGTGGTTACA (R): GTCAGGTCTTCTCGACAGC | 510 | 60 | Acharya et al. 2013 |
| <i>viuB</i> | (F): GGTTGGGCACTAAAGGCAGAT (R): TCGCTTCTCCGGGGCGG | 316 | 60 | Panicker et al. 2004 |
| <i>vcgC</i> | (F): AGCTGCCGATAGCGATCT (R): CGCTTAGGATGATCGGTG | 278 | 55 | Rosche et al., 2005 |
| <i>vcgE</i> | (F): CTCAATTGACAATGATCT (R): CGCTTAGGATGATCGGTG | 278 | 55 | |
| HP1 | (F): TTTGGGTTGAAAGGCTTG (R): GTGCCTTGCAGAATTGAT | 342 | 50 | Han et al. 2009 |
| HP2 | (F): TTCCATCAAACATCGCAGAA (R): CTTTGTCCGGCTTCTATGC | 152 | 50 | |

Table S6: Details of primers used for real time PCR

| Primer code | 5'-3' | Product size (bp) | Reference |
|--------------|-------------------------|-------------------|------------|
| <i>rtxA1</i> | CCGCATGGTCAAGTAAGGTT | 175 | This study |
| | TGTCAGGCAATGAGAAGCAC | | |
| <i>vvhA</i> | CTATCGTGCACGCTTGGTA | 213 | This study |
| | ACCGTTTGTCAACGTTCTC | | |
| <i>vvpE</i> | AGCATGGAGCAAAGAACAT | 183 | This study |
| | CCTTGCAATGCTCTGTCGTA | | |
| <i>Flp</i> | TGACAAAAGCGATTGAGTGG | 114 | This study |
| | CAGACATAGCAACGGCAATAATC | | |
| <i>ompU</i> | ACACCGTATAAGGCCGTCTTG | 166 | This study |
| | CTGGTGTTAACGCTGCTGAA | | |
| <i>pilA</i> | GAGTTAATGATAGTGGTGGCG | 225 | This study |
| | CAGTGTATGGTACCAAGAGC | | |