



Vibrio fluvialis: an emerging human pathogen

Thandavarayan Ramamurthy^{1*}, Goutam Chowdhury¹, Gururaja P. Pazhani¹ and Sumio Shinoda²

¹ National Institute of Cholera and Enteric Diseases, Kolkata, India

² National Institute of Cholera and Enteric Diseases, Collaborative Research Center of Okayama University for Infectious Diseases in India, Kolkata, India

Edited by:

Rita R. Colwell, University of Maryland, USA

Reviewed by:

Carlos R. Osorio, University of Santiago de Compostela, Spain
Brian Austin, University of Stirling, UK

*Correspondence:

Thandavarayan Ramamurthy, National Institute of Cholera and Enteric Diseases, P-33, CIT Road, Scheme-XM, Beliaghata, Kolkata-700010, India
e-mail: tramu@vsnl.net

Vibrio fluvialis is a pathogen commonly found in coastal environs. Considering recent increase in numbers of diarrheal outbreaks and sporadic extraintestinal cases, *V. fluvialis* has been considered as an emerging pathogen. Though this pathogen can be easily isolated by existing culture methods, its identification is still a challenging problem due to close phenotypic resemblance either with *Vibrio cholerae* or *Aeromonas* spp. However, using molecular tools, it is easy to identify *V. fluvialis* from clinical and different environmental samples. Many putative virulence factors have been reported, but its mechanisms of pathogenesis and survival fitness in the environment are yet to be explored. This chapter covers some of the major discoveries that have been made to understand the importance of *V. fluvialis*.

Keywords: *V. fluvialis*, diarrhea, virulence factors, antimicrobial resistance, molecular typing

INTRODUCTION

Vibrio fluvialis is a halophilic Gram-negative bacterium, which has a curved cell morphology and polar flagella for motility. The important biochemical features of this organism include conversion of nitrate to nitrite, do not cleave L-lysine or ornithine, activate arginine dihydrolase, produce indole but not acetoin, ferment sucrose, D-mannitol, L-arabinose, maltose, trehalose, D-galactose, and D-galacturonate. Most of the vibrios, including *V. fluvialis* occur widely in the aquatic milieu, mostly in the seas, estuaries and brackish waters. Even though more than 100 species have been reported in the Genus *Vibrio* (<http://www.bacterio.net/uw/vibrio.html>), about 13 of them have been reported to cause several human diseases. Among the pathogenic vibrios, *V. alginolyticus*, *V. cholerae*, *V. costicola*, *V. mimicus*, *V. cincinnatiensis*, *V. hollisae*, *V. furnissii*, *V. parahaemolyticus*, *V. vulnificus*, *V. carchariae* (a junior synonym of *V. harveyi*) and *V. metschnikovii* are clinically important as they cause different types of vibriosis. One of the *Vibrio* spp., *V. damsela* has now been renamed as “*Photobacterium damsela* subsp. *damsela*.” The toxigenic *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are associated with well-known cholera and diarrhea and extraintestinal infections, respectively. Prevalence of *V. cholerae* in developing countries is mostly related to the breakdown of sanitary conditions and/or due to scarcity of drinking water. On the other hand, infections caused by *V. parahaemolyticus* and other vibrios denote contamination of seafood in many countries, irrespective of their economic conditions.

V. fluvialis is one of the emerging foodborne pathogens all over the world. The distribution of virulence factors and molecular epidemiological features of this pathogen remain mostly unknown. Among the foodborne infections in the United States, there has been a considerable increase (43%) in the *Vibrio*-mediated infections till 2012 compared with the rates reported during 2006–2008 (Centers for Disease Control and Prevention (CDC), 2013). Several recent publications indicate the epidemiological

importance of *V. fluvialis* (Chowdhury et al., 2012; Liang et al., 2013).

IDENTIFICATION AND TAXONOMY

Thiosulfate-citrate-bile salts-sucrose agar (TCBS) has been conventionally used as a selective medium for the isolation of clinically important vibrios. The colony morphology of *V. fluvialis* in this medium remains indistinguishable from *V. cholerae*, i.e., it grows as sucrose fermenting yellow color colonies after direct plating of clinical specimens or after enrichment in alkaline peptone water (pH 8.0). After preliminary screening in the TCBS, a battery of biochemical testes is essential for the species-specific identification of *V. fluvialis*. Minimal biochemical tests such as lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, and L-arabinose are mandatory for the identification of *V. fluvialis*. Without these minimal tests, the identification is incomplete and the isolate will be improperly classified as *V. cholerae* or *Aeromonas* spp. In most resource-poor countries, these tests are not methodically performed, which may lead to labeling of *V. fluvialis* as *V. cholerae*. Considering such situation, there is a high possibility that the *V. fluvialis* could be reported as *V. cholerae* non-O1, non-O139 or non-agglutinable vibrios (NAGs). It is worth to mention here that *V. cholerae* O1 and O139 serogroups can be easily confirmed by slide agglutination with corresponding antiserum.

For the identification of *V. fluvialis* and other vibrios, rapid identification kits must be used with caution as they need additional tests for the final confirmation. While testing the commercially available identification kits, *V. fluvialis* remain as a major challenge with API 20E and Vitek GNI+ systems (Israil et al., 2003; O'Hara et al., 2003). Biochemically, *V. furnissii* expresses fibrin and mucin hydrolysis but no phosphate or esculin hydrolysis, for which *V. fluvialis* varied. *V. fluvialis*, *V. furnissii*, and *V. mimicus* are distinctive from *V. cholerae*, as the later exhibit strong mannose-sensitive hemagglutination. These test results may have a strong influence in the confirmation of strains.

Molecular tools such as PCR are useful in the identification of many uncommon vibrios and most of these assays are comparable to the conventional identification methods. The sequence of amplified 16S–23S intergenic spacers (IGSs) has demonstrated 37 ribosomal RNA (*rnm*) operons representing seven different IGS types in different *Vibrio* spp. with IGS(0), IGS(IA), and IGS(Glu) as major ones. The sequence difference in these IGS types was used to design species-specific primers for PCR for *V. fluvialis* and other vibrios (Lee et al., 2002). In some of the reports, a universal primer PCR that covers conserved regions of bacterial 16S rRNA genes followed by denaturing gradient gel electrophoresis (DGGE) was found to be useful in the identification of *V. fluvialis* either as axenic bacteria or mixed with other pathogens (Ji et al., 2004).

Initially, *V. furnissii* was taxonomically assigned with *V. fluvialis* and named as aerogenic biogroup of *V. fluvialis*. Based on DNA relatedness and several biochemical tests, *V. furnissii* has been separated as a new species (Lee et al., 1981; Brenner et al., 1983). In the phylogenetic analysis with several housekeeping genes, *V. furnissii* and *V. fluvialis* have been linked as close species. The nucleotide comparison of 16S-rRNA, *recA*, and *toxR* sequences showed that *V. furnissii* and *V. fluvialis* had 100% similarity. The gene *toxR* of *V. fluvialis* had 84% similarity with *V. harveyi* (Franco and Hedreya, 2006). With the *gyrB*, *V. cholerae*, *V. mimicus*, *V. furnissii*, and *V. fluvialis* shared 93% sequence similarity.

Toxigenic vibrios have a homolog of the *toxRS* operon, which regulates the virulence expression. The gene *toxR* encodes a transcriptional activation domain (TAD), a transmembrane domain (TMD), and a periplasmic domain (PD). Among the vibrios, there is essentially no homology within the region between TAD and TMD. Hence, this region has been used in designing of primers for the species-specific identification of many vibrios. Chakraborty et al. (2006) described a species-specific identification of *V. fluvialis* by PCR targeted to the conserved transcriptional activation and variable membrane tether regions of the *toxR* gene. The functional virulence genes encoding hemolysin (*vfh*), heme-utilization (*hupO*), and central regulation (*vfpA*) have been used as targets in a multiplex PCR for the identification of *V. cholerae*, *V. parahaemolyticus*, and *V. fluvialis*, respectively (Vinothkumar et al., 2013). For the detection of clinical vibrios in seafood samples, a multiplex primer-extension reaction (PER) assay targeting the *rpoA* gene has also been reported (Dalmaso et al., 2009).

Pyrolysis-mass spectrometry with metastable atom bombardment and pattern recognition seemed to be suitable for the identification of *V. fluvialis* and other vibrios (Wilkes et al., 2005). The mass spectra have been generated via an alternative ionization method, metastable atom bombardment followed by component-discriminant analysis. Since the outer membrane protein K (OmpK) of *V. fluvialis*, *V. alginolyticus*, *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus* is highly similar, the antibodies against these proteins have been proposed in the diagnosis (Li et al., 2010). The whole cell protein profile using SDS-PAGE was also considered in the identification of clinically important vibrios including *V. fluvialis* (Lee et al., 2012).

Since simple phenotypic diagnostic tests are not available, Chen et al. (1995) used species-specific bacteriophages as a tool

for the identification of *V. fluvialis* and with a set of phages, the diagnostic probability of human isolates was more than 84%. At least in one study, the importance of phage-typing of *V. fluvialis* has been demonstrated using six specific bacteriophages with 73% typability (Suthienkul, 1993). However, availability of these bacteriophages makes this assay technique less popular.

PHENOTYPIC AND GENETIC CHARACTERISTICS OF *V. fluvialis*

Based on the somatic antigen variation, several serotypes of *V. fluvialis* have been identified. Though Shimada et al. (1999) identified more than 50 somatic antigens, the serological based typing of *V. fluvialis* remains non-customary. *V. fluvialis* strains belonging to serogroup O19 possessed the C (Inaba) antigen of *V. cholerae* O1, but not the B (Ogawa) or A (common) antigens (Shimada et al., 1987; Kondo et al., 2000). In the crossed immuno-electrophoresis, antibodies against the oral cholera vaccines containing killed whole cells (WC) of *V. cholerae* O1 Inaba El Tor reacted with a few strains of *V. fluvialis* (Ciznār et al., 1989). Presence of shared WC antigens indicates that the oral cholera vaccine could stimulate immunity effectively against other vibrios also. It is known that the antigenic nature of flagella of vibrios is highly homologous. Tassin et al. (1983) and Shinoda et al. (1984) demonstrated independently that anti-L-flagella antisera of *V. fluvialis* did not agglutinate other *Vibrio* species in the H-agglutination tests. Further studies placed *V. fluvialis* and *V. furnissii* in the same lateral flagellar serogroup-HL8 (Shinoda et al., 1992). However, in practice, serotyping based on H-flagella is also not in use.

A chemotaxonomic study based on sugar composition of the polysaccharide portion of their lipopolysaccharide (LPS) has divided 35 O-antigen groups of *V. fluvialis* into 21 chemotypes (Iguchi et al., 1993). This seems to be a unique finding since the D-glycero-D-manno-heptose, and two kinds of uronic acids, i.e., galacturonic and glucuronic acids are rare in Gram-negative bacteria. In addition, 2-keto-3-deoxyoctonate, which is a typical sugar component of Gram-negative bacterial LPS was not detectable in any of the chemotypes.

Of all the molecular typing methods, the pulsed-field gel electrophoresis (PFGE) has proven to be highly useful in tying the bacterial isolates. Unlike *V. cholerae* O1 and pandemic *V. parahaemolyticus*, the isolates of *V. fluvialis* from acute diarrheal patients exhibited large genetic diversity (Chowdhury et al., 2012, 2013).

PREVALENCE OF *V. fluvialis* IN THE AQUATIC REALM

Even though the presence of vibrios is mostly documented from coastal environs, the domination of a particular species depends on many physico-chemical and biological factors. In warmer regions like Florida, USA, *V. fluvialis* was predominantly detected in sediments during winter months (Williams and Larock, 1985). Due to rise in seawater temperature, the identification rate of *V. fluvialis* has increased considerably (29%) in several niches at the Toulon harbor, France (Martin and Bonnefont, 1990). However, in Chesapeake Bay, *V. fluvialis* infections are always less during winter months, indirectly reflecting its minimal occurrence in

this season (Hoge et al., 1989). *V. fluvialis* along with *V. vulnificus* and *V. cholerae* non-O1 unusually existed in the Seto Inland Sea of Japan, which is a eutrophic zone with riverine influence (Venkateswaran et al., 1989a). In South East Queensland, Australia, next to *V. cholerae* (10.2%), *V. fluvialis* (8.2%) has been isolated more frequently from river waters, sediments, and plants (Myatt and Davis, 1989).

Due to high load of pollution in the upstream of the river Ganges, presence of *V. fluvialis* (0.74%) with other potential pathogens have been detected in several points of Varanasi, India (De et al., 1993). *V. fluvialis* has also been isolated from natural waters in Myanmar (Oo et al., 1993) and in a wide range of coastal environments of Japan (Uchiyama, 2000). Compared to other vibrios, the recovery of *V. fluvialis* has been high (41.4%) from suburban community effluents in South Africa. However, their occurrence was not associated with any season or plankton blooms, but positively correlated with temperature, salinity, and dissolved oxygen (Igbinsosa et al., 2011a).

In many investigations, the detection frequency of *V. fluvialis* was very high in marine mollusks, mostly in bivalves, as they accumulate large number of pathogens during the process of filter-feeding. Findings of Kelly and Stroh (1988) from Pacific Northwest showed that oysters are the main source of *V. fluvialis* and other vibrios especially during warmer seasons. In Hong Kong, *V. fluvialis* was one of the important pathogenic vibrios identified in coastal waters and seafood sold in the markets (Chan et al., 1986, 1989). *V. fluvialis* has been isolated from mussels from Senegal (Schandevyl et al., 1984), Brazil (Matté et al., 1994), bivalves and mud from Costa Rica (García and Antillón, 1990) and cultured fishes from Denmark (Pedersen et al., 1999), copepods from Southern Italy (Dumontet et al., 2000) and cockles of Malaysia (Elhadi et al., 2004). In Turkey, next to *V. alginolyticus* (>30%), *V. fluvialis* was the most common *Vibrio* in blue crabs and retail fishes (>10%; Yalcinkaya et al., 2003; Yücel and Balci, 2010).

Generally, fecal pollution has been monitored in aquaculture areas to forecast human pathogens in the products. In Italy, about 11–27% of the mollusks and shrimps contained *V. fluvialis* without any association between presence of this pathogen and conventional fecal pollution indicators (Ripabelli et al., 2004). The micro fauna and flora occasionally support the occurrence human pathogens. *V. fluvialis* (36.5%) was significantly associated with plankton in the effluents of a rural wastewater treatment facility in the Eastern Cape Province of South Africa (Igbinsosa et al., 2009). In the Atlantic coast of France, Deter et al. (2010) showed that chlorophyll-A had a significant influence on pathogenic vibrios including *V. fluvialis* in mussels.

There are few reports about identification of *V. fluvialis* from wound infections that took place in recreational areas. Since *V. fluvialis* has been cultured from the teeth of a great white shark (*Carcharodon carcharias*), there may be an association of this pathogen with wound infections caused by sharks in humans (Buck et al., 1984). Fibropapillomatosis (FP) is a mutilating disease among turtles that cause tumors on the skin and other internal organs. In a study conducted by Aguirre et al. (1994) showed the presence of *V. fluvialis* (47%) in green turtles with FP.

In the marine environment, *V. fluvialis* plays a major role in the production of hydrogen from starch acquired from the algal mass in the presence of *Rhodobium marinum*. In co-culture experiments, *V. fluvialis* degrade starch leading to the formation of acetic acid and ethanol, which are subsequently utilized for hydrogen production by *R. marinum* (Ike et al., 1999).

SPORADIC CASES AND OUTBREAKS OF DIARRHEA DUE TO *V. fluvialis*

Early reports from the US indicated involvement of *V. fluvialis* with gastroenteritis among infants (Hickman-Brenner et al., 1984; Bellet et al., 1989; Kolb et al., 1997). Since 1979, *V. fluvialis* was isolated as one of the important pathogens in Tenri Hospital, Japan (Aihara et al., 1991). Prevalence of *V. fluvialis* among children with diarrhea was very less during 1988 (0.6%) in Calcutta (now, Kolkata), India (Chatterjee et al., 1989). In the same region, progressive increase in the prevalence of *V. fluvialis* (>2%) among hospitalized acute diarrheal patients has been reported in the following years (Chowdhury et al., 2012). During 1996–1998, prevalence of *V. fluvialis* was 9.4% among hospitalized diarrheal patients in North Jakarta (Lesmana et al., 2002). In Zhejiang Province, China, *V. fluvialis* was identified as the second most pathogen (12%) among acute diarrheal cases but next to *V. parahaemolyticus* (64%; Jiang, 1991). Investigations carried out after the 1998 floods in Bangladesh showed involvement of *V. fluvialis* in a diarrhea outbreak (Tanabe et al., 1999). However, the number of cases was less compared to *V. cholerae* O1 and O139 infections.

Vibrio-mediated infections frequently occur in countries where the raw seafood is largely consumed. In many instances, *V. fluvialis* was found to be associated with cholera-like diarrhea (Allton et al., 2006). Between 1982 and 1988, 10 gastroenteritis cases of *V. fluvialis* have been reported in Florida due to consumption of contaminated seafood (Klontz and Desenclos, 1990). In the Gulf coast, the majority of the *Vibrio*-mediated gastroenteritis has been associated with intake of raw oysters and in about 6% of the cases *V. fluvialis* was the causative pathogen (Levine and Griffin, 1993). Foodborne outbreaks were reported in several communities implicating *V. fluvialis* alone or with either *V. parahaemolyticus*/*Salmonella* spp. (Tokoro et al., 1984; Chowdhury et al., 2013).

Foodborne diarrheal outbreaks caused by *V. fluvialis* have been reported during 1981 in Maharashtra (Thekdi et al., 1990) and 2012 in Kolkata (Chowdhury et al., 2013). In Brazil, and USSR, the first report on the association of *V. fluvialis* with diarrhea was reported during 1990 and 1991, respectively (Magalhães et al., 1990; Libinzon et al., 1991). Though the incidence of cholera among high socioeconomic population in Brazil was very low (0.07%), but the other vibrios including *V. fluvialis* comparatively prevailed more (1.2%; Magalhães et al., 1993). In Volga delta, Russia, acute enteric infections caused by *V. fluvialis* reaches about 30% during the summer months, mainly due to consumption of water than sea/fresh water fishes (Boïko, 2000). Among travelers with diarrheal symptoms, the incidence of *V. fluvialis* seems to be low compared to other enteric pathogens. Early studies conducted with US Peace Corps volunteers in Thailand identified *V. fluvialis* in about 3% of the cases (Taylor et al., 1985).

OTHER INFECTIONS

Vibrio fluvialis causes a variety of infections in immune-competent/HIV patients, including bacteremia, biliary tract infection and acute diarrhea (Albert et al., 1991; Usó et al., 2010; Liu et al., 2011). The other rarely reported infections caused by this pathogen include suppurative cholangitis (Yoshii et al., 1987), peritonitis (Lee et al., 2008), acute otitis (Cabrera et al., 2005; Chen et al., 2012) and endophthalmitis (Penland et al., 2000). Large numbers of (29%) endophthalmitis patients were reported to have mixed infection with *V. fluvialis* (Hassan et al., 1992). A report from Cuba showed that *V. fluvialis* was one of the predominantly identified pathogens from different extraintestinal samples (Cabrera et al., 2007). Cases of bacteremia with diarrhea (Lai et al., 2006) hemorrhagic cellulitis and cerebritis (Huang and Hsu, 2005), peritonitis (Ratnaraja et al., 2005) have also been reported.

QUORUM SENSING

Quorum sensing (QS) is a process in which bacterial cells in a population are able to crosstalk with one another, thereby supporting them as a unit to synchronize gene regulation and consequent phenotypic changes. The importance of QS in pathogenic *V. cholerae* has been well established. Wang et al. (2013) have shown that QS in *V. fluvialis* regulates two potential virulence factors, including an extracellular protease and hemolysin. In addition, QS also regulates *in vitro* cytotoxic activity against epithelial cell lines.

VIRULENCE FACTORS

The clinical as well as environmental *V. fluvialis* strains express many putative virulence factors. The common virulence factor in *V. fluvialis* reported in several investigations is the expression of hemolysin that can be easily identified in sheep-blood agar plates. In majority of the toxin detection assays, eukaryotic cell lines are being used *in vitro*. In cell-free extracts, *V. fluvialis* has expressed Chinese hamster ovary (CHO) cell elongation factor, CHO cell-killing factors, cytolysins against erythrocytes and proteases active against azocasein (Lockwood et al., 1982). Various putative virulence factors of *V. fluvialis* are presented in **Table 1**. However, the ability to produce these factors is not uniform in all the isolates (Liang et al., 2013).

Purification of cytotoxin produced by *V. fluvialis* showed that the protein was heat-labile, and deactivated by proteases. The

culture supernatant retained hemolytic and phospholipase A2 activities and were coeluted in the gel filtration (Wall et al., 1984). The purified extracellular hemolysin produced by *V. fluvialis* showed virulence features including lyses of erythrocytes of different animal origin and activation of fluid accumulation in suckling mice (Han et al., 2002; Kothary et al., 2003).

The transmembrane regulatory protein (ToxR) is essential for the expression of virulence factors in pathogenic vibrios. Similar to *V. cholerae*, the ToxR plays a major role in bile resistance of *V. fluvialis*, which is an initial phase in the progression of vibrios as potential intestinal pathogens (Provenzano et al., 2000). Adaptability of vibrios to the intestinal environment, especially the bile salts favors colonization and expression of virulence factors. After initial adaptation to the bile salts under *in vitro* conditions, the *V. fluvialis* exhibited swarming mobility, biofilm formation and adherence (Di Pietro et al., 2004). In the animal models, *V. fluvialis* and the cholera toxin (CT) produced by *V. cholerae* O1 strains confirmed skin permeability factor (SPF). However, the antibodies against CT did not neutralize the SPF of *V. fluvialis* (Rodrigues et al., 1993; Ahsan et al., 1988).

The exocellular metalloprotease produced by *V. fluvialis* (VFP) was found to be similar to the one produced by *V. vulnificus*, which has also been used for the hemagglutination activity (Miyoshi et al., 2002). In addition, the amino acid sequence of VFP was found to be a member of the thermolysin family. It is interesting to note that most of the *V. fluvialis* isolated from the diarrheal patients harbored genes encoding hemolysin and metalloprotease (Chowdhury et al., 2012).

SURVIVAL

Vibrio fluvialis has the capacity to survive in the seawater microcosm for more than 15 days at ambient temperature regardless of carbonated substrate uptake (Munro et al., 1994). In microcosms, *V. fluvialis* has been shown culturally viable for a year without losing its virulence and in sediments this organism was recovered from viable but non-culturable stage, even after 6 years (Amel et al., 2008).

ANTIMICROBIAL RESISTANCE

Compared to other clinical vibrios, antimicrobial resistance (AMR) is largely reported in *V. fluvialis*. In Mediterranean fish farms, many of the vibrios including *V. fluvialis* were resistant to ampicillin, carbenicillin, kanamycin, cefalotin, and sulfadiazine-trimethoprim (Laganà et al., 2011). In South Africa, treated effluent system was found to be the reservoir for *V. fluvialis* strains, which are resistant to ampicillin, penicillin-G, streptomycin, sulfamethoxazole, trimethoprim, chloramphenicol, erythromycin, ciprofloxacin, and polymyxin B (Igbinosa et al., 2011b). In China, majority of the *V. fluvialis* strains were resistant for β -lactams, azithromycin, and sulfamethoxazole (Liang et al., 2013).

Several mobile genetic elements carrying AMR have been found in *V. fluvialis*. The integrative and conjugative element (ICE) is a conjugative transposon commonly detected in *V. cholerae*, which carries resistance genes for sulfamethoxazole-trimethoprim (SXT), chloramphenicol and streptomycin (Srinivasan et al., 2006; Taviani et al., 2008). This SXT element has also been reported

Table 1 | Different putative virulence factors described in *V. fluvialis*.

Factor	Reference
Cytolysin	Lockwood et al. (1982)
Heat-labile cytotoxin	Wall et al. (1984)
Cytotoxic	Venkateswaran et al. (1989b)
Hemolysin	Wong et al. (1992)
Mucinase	Janda (1986)
Mannose sensitive hemaagglutination	Rahman et al. (1992)
Cell adherence	Carvalho et al. (1994), Scoglio et al. (2001)
Cell vaculation	Chakraborty et al. (2005)

in *V. fluvialis* that has integrase gene similar to that of *V. cholerae* (Ahmed et al., 2005). The aminoglycoside acetyltransferase encoding gene *aac(3)-Id* was identified in class 1 integron from a clinical *V. fluvialis* strains (Ahmed et al., 2004).

Transfer of large plasmids carrying AMR genes is rarely detected in *V. fluvialis* (Rajpara et al., 2009). Efflux systems responsible for nalidixic acid and ciprofloxacin resistance have been reported in several clinical *V. fluvialis* strains (Srinivasan et al., 2006). Two putative multi antimicrobial extrusion (MATE) protein family efflux pumps viz., H- and D-type were found to be responsible for fluoroquinolones resistance in *V. fluvialis*. The sequences of these MATE encoding genes were found to be ~99% identical to *V. cholerae* (Mohanty et al., 2012). In addition, many *V. fluvialis* strains had mutation (serine to isoleucine) at position 83 of the quinolone resistance-determining region (QRDR) of *gyrA*. Apart from this mutation, presence of plasmid-borne *qnrVC*-like genes have been reported for quinolone resistance in some of the *V. fluvialis* strains (Singh et al., 2012). *V. fluvialis* isolated from diarrheal patients in Kolkata were resistant to fluoroquinolones and β -lactam antimicrobials had mutations in the QRDR of *GyrA* at position 83 and of *ParC* at position 85 (Chowdhury et al., 2011). In addition, these strains carried a transferrable 150-kb plasmid that harbored the quinolone resistance *qnrA1* in a complex *sul1*-type integron, the ciprofloxacin-modifying enzyme-encoding gene *aac(6)-Ib-cr* and genes encoding for extended-spectrum β -lactamases such as *bla_{SHV}* and *bla_{CTX-M-3}*.

CONCLUSION

Though the pathogen *V. fluvialis* has known for quite some time, its clinical importance is realized now, as the prevalence of diarrhea cases is reportedly increasing. In depth studies on the pathogenesis of *V. fluvialis* has to be established as there are many descriptions about the putative virulence factor.

ACKNOWLEDGMENTS

This work was supported by the Japan Initiative for Global Research Network for Infectious Diseases, Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

- Aguirre, A. A., Balazs, G. H., Zimmerman, B., and Spraker, T. R. (1994). Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. *J. Wildl. Dis.* 30, 8–15. doi: 10.7589/0090-3558-30.1.8
- Ahmed, A. M., Nakagawa, T., Arakawa, E., Ramamurthy, T., Shinoda, S., and Shimamoto, T. (2004). New aminoglycoside acetyltransferase gene, *aac(3)-Id*, in a class 1 integron from a multiresistant strain of *Vibrio fluvialis* isolated from an infant aged 6 months. *J. Antimicrob. Chemother.* 53, 947–951. doi: 10.1093/jac/dkh221
- Ahmed, A. M., Shinoda, S., and Shimamoto, T. (2005). A variant type of *Vibrio cholerae* SXT element in a multidrug-resistant strain of *Vibrio fluvialis*. *FEMS Microbiol. Lett.* 242, 241–247. doi: 10.1016/j.femsle.2004.11.012
- Ahsan, C. R., Sanyal, S. C., Zaman, A., Neogy, P. K., and Huq, M. I. (1988). Immunobiological relationships between *Vibrio fluvialis* and *Vibrio cholerae* enterotoxins. *Immunol. Cell Biol.* 66, 251–252. doi: 10.1038/icb.1988.32
- Aihara, M., Sakai, M., Iwasaki, M., Shimakawa, K., Kozaki, S., Kubo, M., et al. (1991). Isolation of enteropathogenic microorganism from patients with infection of the digestive tract during 1976 to 1988 in Tenri Hospital. *Kansenshogaku Zasshi* 65, 864–874. [In Japanese].
- Albert, M. J., Hossain, M. A., Alam, K., Kabir, I., Neogi, P. K., and Tzipori, S. (1991). A fatal case associated with shigellosis and *Vibrio fluvialis* bacteremia. *Diagn. Microbiol. Infect. Dis.* 14, 509–510. doi: 10.1016/0732-8893(91)90008-4
- Allton, D. R., Forgione, M. A. Jr., and Gros, S. P. (2006). Cholera-like presentation in *Vibrio fluvialis* enteritis. *South Med. J.* 99, 765–767. doi: 10.1097/01.smj.0000223657.22296.e6
- Amel, B. K., Amine, B., and Amina, B. (2008). Survival of *Vibrio fluvialis* in seawater under starvation conditions. *Microbiol. Res.* 163, 323–328. doi: 10.1016/j.micres.2006.06.006
- Bellet, J., Klein, B., Altieri, M., and Ochsenschlager, D. (1989). *Vibrio fluvialis*, an unusual pediatric enteric pathogen. *Pediatr. Emerg. Care* 5, 27–28. doi: 10.1097/00006565-198903000-00008
- Boiko, A. V. (2000). The etiological structure of acute intestinal infections caused by noncholera vibrios in the Volga delta. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 1, 15–17. [In Russian].
- Brenner, D. J., Hickman-Brenner, F. W., Lee, J. V., Steigerwalt, A. G., Fanning, G. R., Hollis, D. G., et al. (1983). *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. *J. Clin. Microbiol.* 18, 816–824.
- Buck, J. D., Spotte, S., and Gadbow, J. J. Jr. (1984). Bacteriology of the teeth from a great white shark: potential medical implications for shark bite victims. *J. Clin. Microbiol.* 20, 849–851.
- Cabrera, R. L. E., Castro, E. G., Ramírez, A. M. M., Llop, H. A., Llanes, C. R., Castañeda, E. N., et al. (2007). Isolation and identification of species from the genera *Aeromonas*, *Vibrio*, and *Plesiomonas* from extraintestinal samples in Cuba. *Rev. Chilena Infectol.* 24, 204–208. [In Spanish]. doi: 10.4067/S0716-10182007000300005
- Cabrera, R. L. E., Monroy, S. P., Morier, L., Ramírez, A. M. M., Fernández, A. A., Castro, E. G., et al. (2005). Severe otitis due to *Vibrio fluvialis* in a patient with AIDS: first report in the world. *Rev. Cubana Med. Trop.* 57, 154–155.
- Carvalho, I. T., Magalhães, V., Leal, N. C., Melo, V., and Magalhães, M. (1994). *Vibrio fluvialis* attaches to but does not enter HeLa cell monolayers. *Mem. Inst. Oswaldo Cruz* 89, 221–223. doi: 10.1590/S0074-02761994000200019
- Centers for Disease Control and Prevention (CDC). (2013). Incidence, and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. sites, 1996–2012. *MMWR Morb. Mortal Wkly. Rep.* 62, 283–287.
- Chakraborty, R., Chakraborty, S., De, K., Sinha, S., Mukhopadhyay, A. K., Khanam, J., et al. (2005). Cytotoxic and cell vacuolating activity of *Vibrio fluvialis* isolated from paediatric patients with diarrhoea. *J. Med. Microbiol.* 54(Pt 8), 707–716. doi: 10.1099/jmm.0.45820-0
- Chakraborty, R., Sinha, S., Mukhopadhyay, A. K., Asakura, M., Yamasaki, S., Bhattacharya, S. K., et al. (2006). Species-specific identification of *Vibrio fluvialis* by PCR targeted to the conserved transcriptional activation and variable membrane tether regions of the *toxR* gene. *J. Med. Microbiol.* 55, 805–808. doi: 10.1099/jmm.0.46395-0
- Chan, K. Y., Woo, M. L., Lam, L. Y., and French, G. L. (1989). *Vibrio parahaemolyticus* and other halophilic vibrios associated with seafood in Hong Kong. *J. Appl. Bacteriol.* 66, 57–64. doi: 10.1111/j.1365-2672.1989.tb02454.x
- Chan, K. Y., Woo, M. L., Lo, K. W., and French, G. L. (1986). Occurrence and distribution of halophilic vibrios in subtropical coastal waters of Hong Kong. *Appl. Environ. Microbiol.* 52, 1407–1411.
- Chatterjee, B. D., Thawani, G., and Sanyal, S. N. (1989). Etiology of acute childhood diarrhoea in Calcutta. *Trop. Gastroenterol.* 10, 158–166.
- Chen, K., Lin, Y., and Chen, G. (1995). Studies on diagnostic bacteriophage of *Vibrio fluvialis*. *Zhonghua Yu Fang Yi Xue Za Zhi* 29, 138–140.
- Chen, P. J., Tseng, C. C., Chan, H. T., and Chao, C. M. (2012). Acute otitis due to *Vibrio fluvialis* after swimming. *Case Rep. Emerg. Med.* 2012, 838904. doi: 10.1155/2012/838904
- Chowdhury, G., Pazhani, G. P., Dutta, D., Guin, S., Dutta, S., Ghosh, S., et al. (2012). *Vibrio fluvialis* in patients with diarrhea, Kolkata, India. *Emerg. Infect. Dis.* 18, 1868–1871. doi: 10.3201/eid1811.120520
- Chowdhury, G., Pazhani, G. P., Nair, G. B., Ghosh, A., and Ramamurthy, T. (2011). Transferable plasmid-mediated quinolone resistance in association with extended-spectrum β -lactamases and fluoroquinolone-acetylating aminoglycoside-6'-N-acetyltransferase in clinical isolates of *Vibrio fluvialis*. *Int. J. Antimicrob. Agents* 38, 169–173. doi: 10.1016/j.ijantimicag.2011.04.013
- Chowdhury, G., Sarkar, A., Pazhani, G. P., Mukhopadhyay, A. K., Bhattacharya, M. K., and Ramamurthy, T. (2013). An outbreak of foodborne gastroenteritis

- caused by dual pathogens, *Salmonella enterica* serovar Weltevreden and *Vibrio fluvialis* in Kolkata, India. *Foodborne Pathog. Dis.* 10, 904–906. doi: 10.1089/fpd.2013.1491
- Ciznár, I., Hussain, N., Ahsan, C. R., Kay, B. A., Clemens, J. D., and Sack, D. A. (1989). Oral cholera vaccines containing B-subunit-killed whole cells and killed whole cells only. I. Cross-reacting antigens of members of family Vibrionaceae and the vaccines. *Vaccine* 7, 111–116. doi: 10.1016/0264-410X(89)90047-9
- Dalmasso, A., Civera, T., and Bottero, M. T. (2009). Multiplex primer-extension assay for identification of six pathogenic vibrios. *Int. J. Food Microbiol.* 129, 21–25. doi: 10.1016/j.ijfoodmicro.2008.10.029
- De, A., Sen, P. C., and Tewari, I. C. (1993). Enteropathogenic bacteria in river Ganges in Varanasi. *Indian J. Pathol. Microbiol.* 36, 425–432.
- Deter, J., Lozach, S., Derrien, A., Véron, A., Chollet, J., and Hervio-Heath, D. (2010). Chlorophyll a might structure a community of potentially pathogenic culturable Vibrionaceae. Insights from a one-year study of water and mussels surveyed on the French Atlantic coast. *Environ. Microbiol. Rep.* 2, 185–191. doi: 10.1111/j.1758-2229.2010.00133.x
- Di Pietro, A., Picerno, I., Visalli, G., Chirico, C., and Scoglio, M. E. (2004). Effects of “host factor” bile on adaptability and virulence of vibrios, foodborne potential pathogenic agents. *Ann. Ig.* 16, 615–625. [In Italian].
- Dumontet, S., Krovacek, K., Svenson, S. B., Pasquale, V., Baloda, S. B., and Figliuolo, G. (2000). Prevalence and diversity of *Aeromonas* and *Vibrio* spp. in coastal waters of Southern Italy. *Comp. Immunol. Microbiol. Infect. Dis.* 23, 53–72. doi: 10.1016/S0147-9571(99)00059-4
- Elhadi, N., Radu, S., Chen, C. H., and Nishibuchi, M. (2004). Prevalence of potentially pathogenic *Vibrio* species in the seafood marketed in Malaysia. *J. Food Prot.* 67, 1469–1475.
- Franco, P. F., and Hedreya, C. T. (2006). Amplification and sequence analysis of the full length *toxR* gene in *Vibrio harveyi*. *J. Gen. Appl. Microbiol.* 52, 281–287. doi: 10.2323/jgam.52.281
- García, C. V., and Antillón, F. (1990). Isolation of enteropathogenic *Vibrio* in bivalves and mud from the Nicoya Gulf, Costa Rica. *Rev. Biol. Trop.* 138, 437–440. [In Spanish].
- Han, J. H., Lee, J. H., Choi, Y. H., Park, J. H., Choi, T. J., and Kong, I. S. (2002). Purification, characterization and molecular cloning of *Vibrio fluvialis* hemolysin. *Biochim. Biophys. Acta* 1599, 106–114. doi: 10.1016/S1570-9639(02)00407-7
- Hassan, I. J., MacGowan, A. P., and Cook, S. D. (1992). Endophthalmitis at the Bristol Eye Hospital: an 11-year review of 47 patients. *J. Hosp. Infect.* 22, 271–278. doi: 10.1016/0195-6701(92)90012-B
- Hickman-Brenner, F. W., Brenner, D. J., Steigerwalt, A. G., Schreiber, M., Holmberg, S. D., Baldy, L. M., et al. (1984). *Vibrio fluvialis* and *Vibrio furnissii* isolated from a stool sample of one patient. *J. Clin. Microbiol.* 20, 125–127.
- Hoge, C. W., Watsky, D., Peeler, R. N., Libonati, J. P., Israel, E., and Morris, J. G. Jr. (1989). Epidemiology and spectrum of *Vibrio* infections in a Chesapeake Bay community. *J. Infect. Dis.* 160, 985–993. doi: 10.1093/infdis/160.6.985
- Huang, K. C., and Hsu, R. W. (2005). *Vibrio fluvialis* hemorrhagic cellulitis and cerebritis. *Clin. Infect. Dis.* 40, 75–77. doi: 10.1086/429328
- Igbinosa, E. O., Obi, L. C., and Okoh, A. I. (2009). Occurrence of potentially pathogenic vibrios in final effluents of a wastewater treatment facility in a rural community of the Eastern Cape Province of South Africa. *Res. Microbiol.* 160, 531–537. doi: 10.1016/j.resmic.2009.08.007
- Igbinosa, E. O., Obi, C. L., and Okoh, A. I. (2011a). Seasonal abundance and distribution of *Vibrio* species in the treated effluent of wastewater treatment facilities in suburban and urban communities of Eastern Cape Province, South Africa. *J. Microbiol.* 49, 224–232. doi: 10.1007/s12275-011-0227-x
- Igbinosa, E. O., Obi, L. C., Tom, M., and Okoh, A. I. (2011b). Detection of potential risk of wastewater effluents for transmission of antibiotic resistance from *Vibrio* species as a reservoir in a peri-urban community in South Africa. *Int. J. Environ. Health Res.* 21, 402–414. doi: 10.1080/09603123.2011.572278
- Iguchi, T., Kondo, S., and Hisatsune, K. (1993). A chemotaxonomic study of *Vibrio fluvialis* based on the sugar composition of the polysaccharide portion of the lipopolysaccharides. *Microbiol. Immunol.* 37, 153–157. doi: 10.1111/j.1348-0421.1993.tb03193.x
- Ike, A., Murakawa, T., Kawaguchi, H., Hirata, K., and Miyamoto, K. (1999). Photo-production of hydrogen from raw starch using a halophilic bacterial community. *J. Biosci. Bioeng.* 88, 72–77. doi: 10.1016/S1389-1723(99)80179-0
- Israil, A. M., Balotescu, M. C., Alexandru, I., and Dobre, G. (2003). Discordancies between classical and API 20E microtest biochemical identification of *Vibrio* and *Aeromonas* strains. *Bacteriol. Virusol. Parazitol. Epidemiol.* 48, 141–143.
- Janda, J. M. (1986). Mucinase activity among selected members of the family Vibrionaceae. *Microb. Lett.* 33, 19–22.
- Ji, N., Peng, B., Wang, G., Wang, S., and Peng, X. (2004). Universal primer PCR with DGGE for rapid detection of bacterial pathogens. *J. Microbiol. Methods* 57, 409–413. doi: 10.1016/j.mimet.2004.02.010
- Jiang, W. P. (1991). A study on diarrhoea disease caused by Vibrionaceae along coast the east of Zhejiang Province. *Zhonghua Yu Fang Yi Xue Za Zhi* 25, 335–337. [In Chinese].
- Kelly, M. T., and Stroth, E. M. (1988). Occurrence of Vibrionaceae in natural and cultivated oyster populations in the Pacific Northwest. *Diagn. Microbiol. Infect. Dis.* 9, 1–5. doi: 10.1016/0732-8893(88)90054-5
- Klontz, K. C., and Descenclos, J. C. (1990). Clinical and epidemiological features of sporadic infections with *Vibrio fluvialis* in Florida, USA. *J. Diarrhoeal Dis. Res.* 8, 24–26.
- Kolb, E. A., Eppes, S. C., and Klein, J. D. (1997). *Vibrio fluvialis*: an underrecognized enteric pathogen in infants? *South Med. J.* 90, 544–545. doi: 10.1097/00007611-199705000-00017
- Kondo, S., Haishima, Y., Ishida, K., Isshiki, Y., and Hisatsune, K. (2000). The O-polysaccharide of lipopolysaccharide isolated from *Vibrio fluvialis* O19 is identical to that of *Vibrio* bioserogroup 1875 variant. *Microbiol. Immunol.* 44, 941–944. doi: 10.1111/j.1348-0421.2000.tb02586.x
- Kothary, M. H., Lowman, H., McCardell, B. A., and Tall, B. D. (2003). Purification and characterization of enterotoxigenic El Tor-like hemolysin produced by *Vibrio fluvialis*. *Infect. Immun.* 71, 3213–3220. doi: 10.1128/IAI.71.6.3213-3220.2003
- Laganà, P., Caruso, G., Minutoli, E., Zaccone, R., and Santi, D. (2011). Susceptibility to antibiotics of *Vibrio* spp. and *Photobacterium damsela* ssp. piscicida strains isolated from Italian aquaculture farms. *New Microbiol.* 34, 53–63.
- Lai, C. H., Hwang, C. K., Chin, C., Lin, H. H., Wong, W. W., and Liu, C. Y. (2006). Severe watery diarrhoea and bacteraemia caused by *Vibrio fluvialis*. *J. Infect.* 52, e95–e98. doi: 10.1016/j.jinf.2005.05.023
- Lee, C. Y., Hong, Y., Ryu, J., Kim, Y. R., Oh, S. S., Lee, S. H., et al. (2012). Detection and identification of *Vibrio* species using whole-cell protein pattern analysis. *J. Microbiol. Biotechnol.* 22, 1107–1112. doi: 10.4014/jmb.1201.01001
- Lee, J. V., Shread, P., Furniss, A. L., and Bryant, T. N. (1981). Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). *J. Appl. Bacteriol.* 50, 73–94. doi: 10.1111/j.1365-2672.1981.tb00873.x
- Lee, J. Y., Park, J. S., Oh, S. H., Kim, H. R., Lee, J. N., and Shin, J. H. (2008). Acute infectious peritonitis caused by *Vibrio fluvialis*. *Diagn. Microbiol. Infect. Dis.* 62, 216–218. doi: 10.1016/j.diagmicrobio.2008.05.012
- Lee, S. K., Wang, H. Z., Law, S. H., Wu, R. S., and Kong, R. Y. (2002). Analysis of the 16S-23S rDNA intergenic spacers (IGSs) of marine vibrios for species-specific signature DNA sequences. *Mar. Pollut. Bull.* 44, 412–420. doi: 10.1016/S0025-326X(01)00256-9
- Lesmana, M., Subekti, D. S., Tjaniadi, P., Simanjuntak, C. H., Punjabi, N. H., Campbell, J. R., et al. (2002). Spectrum of *Vibrio* species associated with acute diarrhea in North Jakarta, Indonesia. *Diagn. Microbiol. Infect. Dis.* 43, 91–97. doi: 10.1016/S0732-8893(02)00373-5
- Levine, W. C., and Griffin, P. M. (1993). *Vibrio* infections on the Gulf Coast: results of first year of regional surveillance. Gulf Coast *Vibrio* Working Group. *J. Infect. Dis.* 167, 479–483.
- Li, Y. D., Ren, H. L., Lu, S. Y., Zhou, Y., Han, X., Gong, B. B., et al. (2010). Cloning, expression, and genus-specificity analysis of 28-kDa OmpK from *Vibrio alginolyticus*. *J. Food Sci.* 75, 198–203. doi: 10.1111/j.1750-3841.2010.01565.x
- Liang, P., Cui, X., Du, X., Kan, B., and Liang, W. (2013). The virulence phenotypes and molecular epidemiological characteristics of *Vibrio fluvialis* in China. *Gut Pathog.* 5, 6. doi: 10.1186/1757-4749-5-6
- Libinzo, A. E., Levanova, G. F., and Gal'tseva, G. V. (1991). The isolation of *Vibrio fluvialis* on the territory of the USSR. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 2, 20–23. [In Russian].
- Liu, W. L., Chiu, Y. H., Chao, C. M., Hou, C. C., and Lai, C. C. (2011). Biliary tract infection caused by *Vibrio fluvialis* in an immunocompromised patient. *Infection* 39, 495–496. doi: 10.1007/s15010-011-0146-0
- Lockwood, D. E., Kreger, A. S., and Richardson, S. H. (1982). Detection of toxins produced by *Vibrio fluvialis*. *Infect. Immun.* 35, 702–708.

- Magalhães, V., Lima, R. A., Magalhães, E., and Magalhães, M. (1990). Human gastroenteritis associated with *Vibrio fluvialis* in Recife. *Arq. Gastroenterol.* 27, 141–143. [In Portuguese].
- Magalhães, V., Magalhães, M., and Marques, L. R. (1993). Vibrios among patients of good socioeconomic conditions during the cholera epidemic in Recife, Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 35, 345–346. doi: 10.1590/S0036-46651993000400007
- Martin, Y. P., and Bonnefont, J. L. (1990). Annual variations and identification of Vibrios growing at 37 degrees C in urban sewage, in mussels and in seawater at Toulon harbour (Mediterranean, France). *Can. J. Microbiol.* 36, 47–52. doi: 10.1139/m90-008
- Matté, G. R., Matté, M. H., Sato, M. I., Sanchez, P. S., Rivera, I. G., and Martins, M. T. (1994). Potentially pathogenic vibrios associated with mussels from a tropical region on the Atlantic coast of Brazil. *J. Appl. Bacteriol.* 77, 281–287. doi: 10.1111/j.1365-2672.1994.tb03075.x
- Miyoshi, S., Sonoda, Y., Wakiyama, H., Rahman, M. M., Tomochika, K., Shinoda, S., et al. (2002). An exocellular thermolysin-like metalloprotease produced by *Vibrio fluvialis*: purification, characterization, and gene cloning. *Microb. Pathog.* 33, 127–134. doi: 10.1006/mpat.2002.0520
- Mohanty, P., Patel, A., and Bhardwaj, A. K. (2012). Role of H- and D-MATE-type transporters from multidrug resistant clinical isolates of *Vibrio fluvialis* in conferring fluoroquinolone resistance. *PLoS ONE* 7:e35752. doi: 10.1371/journal.pone.0035752
- Munro, P. M., Brahic, G., and Clément, R. L. (1994). Seawater effects on various *Vibrio* species. *Microbios* 77, 191–198.
- Myatt, D. C., and Davis, G. H. (1989). Isolation of medically significant *Vibrio* species from riverine sources in south east Queensland. *Microbios* 60, 111–123.
- O'Hara, C. M., Sowers, E. G., Bopp, C. A., Duda, S. B., and Strockbine, N. A. (2003). Accuracy of six commercially available systems for identification of members of the family Vibrionaceae. *J. Clin. Microbiol.* 41, 5654–5659. doi: 10.1128/JCM.41.12.5654-5659.2003
- Oo, K. N., Myint, T., New, Y. Y., and Aye, T. (1993). *Vibrio* spp. isolated from natural waters of the city of Yangon, Myanmar. *J. Diarrhoeal Dis. Res.* 11, 105–107.
- Pedersen, K., Austin, B., Austin, D. A., and Larsen, J. L. (1999). Vibrios associated with mortality in cultured plaice *Pleuronectes platessa* fry. *Acta Vet. Scand.* 40, 263–270.
- Penland, R. L., Boniuk, M., and Wilhelmus, K. R. (2000). *Vibrio* ocular infections on the U.S. Gulf Coast. *Cornea* 19, 26–29. doi: 10.1097/00003226-200001000-00006
- Provenzano, D., Schuhmacher, D. A., Barker, J. L., and Klose, K. E. (2000). The virulence regulatory protein ToxR mediates enhanced bile resistance in *Vibrio cholerae* and other pathogenic *Vibrio* species. *Infect. Immun.* 68, 1491–1497. doi: 10.1128/IAI.68.3.1491-1497.2000
- Rahman, M. M., Qadri, F., Albert, M. J., Hossain, A., and Mosihuzzaman, M. (1992). Lipopolysaccharide composition and virulence properties of clinical and environmental strains of *Vibrio fluvialis* and *Vibrio mimicus*. *Microbiol. Immunol.* 36, 327–338. doi: 10.1111/j.1348-0421.1992.tb02032.x
- Rajpara, N., Patel, A., Tiwari, N., Bahuguna, J., Antony, A., Choudhury, I., et al. (2009). Mechanism of drug resistance in a clinical isolate of *Vibrio fluvialis*: involvement of multiple plasmids and integrons. *Int. J. Antimicrob. Agents* 34, 220–225. doi: 10.1016/j.ijantimicag.2009.03.020
- Ratnaraja, N., Blackmore, T., Byrne, J., and Shi, S. (2005). *Vibrio fluvialis* peritonitis in a patient receiving continuous ambulatory peritoneal dialysis. *J. Clin. Microbiol.* 43, 514–515. doi: 10.1128/JCM.43.1.514-515.2005
- Ripabelli, G., Sammarco, M. L., Fanelli, I., and Grasso, G. M. (2004). Detection of *Salmonella*, *Listeria* spp., *Vibrio* spp., and *Yersinia enterocolitica* in frozen seafood and comparison with enumeration for faecal indicators: implication for public health. *Ann. Ig.* 16, 531–539. [In Italian].
- Rodrigues, D. P., Ribeiro, R. V., Alves, R. M., and Hofer, E. (1993). Evaluation of virulence factors in environmental isolates of *Vibrio* species. *Mem. Inst. Oswaldo Cruz* 88, 589–592. doi: 10.1590/S0074-02761993000400016
- Schandevyl, P., Van, D. E., and Piot, P. (1984). Halophilic *Vibrio* species from seafish in Senegal. *Appl. Environ. Microbiol.* 48, 236–238.
- Scoglio, M. E., Di Pietro, A., Picerno, I., Delia, S., Mauro, A., and Lagana, P. (2001). Virulence factors in vibrios and aeromonads isolated from seafood. *New Microbiol.* 24, 273–280.
- Shimada, T., Arakawa, E., Okitsu, T., Yamai, S., Matsushita, S., Kudoh, Y., et al. (1999). Additional O antigens of *Vibrio fluvialis* and *Vibrio furnissii*. *Jpn. J. Infect. Dis.* 52, 124–126.
- Shimada, T., Sakazaki, R., and Tobita, K. (1987). *Vibrio fluvialis*: a new serogroup (19) possessing the Inaba factor antigen of *Vibrio cholerae* O1. *Jpn. J. Med. Sci. Biol.* 40, 153–157.
- Shinoda, S., Nakahara, N., and Kane, H. (1984). Lateral flagellum of *Vibrio fluvialis*: a species-specific antigen. *Can. J. Microbiol.* 30, 1525–1529. doi: 10.1139/m84-242
- Shinoda, S., Yakiyama, I., Yasui, S., Kim, Y. M., Ono, B., and Nakagami, S. (1992). Lateral flagella of vibrios: serological classification and genetical similarity. *Microbiol. Immunol.* 36, 303–309. doi: 10.1111/j.1348-0421.1992.tb01667.x
- Singh, R., Rajpara, N., Tak, J., Patel, A., Mohanty, P., Vinothkumar, K., et al. (2012). Clinical isolates of *Vibrio fluvialis* from Kolkata, India, obtained during 2006: plasmids, the *qnr* gene and a mutation in gyrase A as mechanisms of multidrug resistance. *J. Med. Microbiol.* 61(Pt 3), 369–374. doi: 10.1099/jmm.0.037226-0
- Srinivasan, V. B., Virk, R. K., Kaundal, A., Chakraborty, R., Datta, B., Ramamurthy, T., et al. (2006). Mechanism of drug resistance in clonally related clinical isolates of *Vibrio fluvialis* isolated in Kolkata, India. *Antimicrob. Agents Chemother.* 50, 2428–2432. doi: 10.1128/AAC.01561-05
- Suthienkul, O. (1993). Bacteriophage typing of *Vibrio fluvialis*. *Southeast Asian J. Trop. Med. Public Health* 24, 449–454.
- Tanabe, K., Nakamura, S., and Kunii, O. (1999). Bacteriological survey of diarrheal epidemics in the 1998 Bangladesh floods. *Kansenshogaku Zasshi* 73, 918–919. [In Japanese].
- Tassin, M. G., Siebeling, R. J., Roberts, N. C., and Larson, A. D. (1983). Presumptive identification of *Vibrio* species with H antiserum. *J. Clin. Microbiol.* 18, 400–407.
- Taviani, E., Ceccarelli, D., Lazaro, N., Bani, S., Cappuccinelli, P., Colwell, R. R., et al. (2008). Environmental *Vibrio* spp., isolated in Mozambique, contain a polymorphic group of integrative conjugative elements and class 1 integrons. *FEMS Microbiol. Ecol.* 64, 45–54. doi: 10.1111/j.1574-6941.2008.00455.x
- Taylor, D. N., Echeverria, P., Blaser, M. J., Pitarangsi, C., Blacklow, N., Cross, J., et al. (1985). Polymicrobial aetiology of travellers' diarrhoea. *Lancet* 1, 381–383. doi: 10.1016/S0140-6736(85)91397-2
- Thekdi, R. J., Lakhani, A. G., Rale, V. B., and Panse, M. V. (1990). An outbreak of food poisoning suspected to be caused by *Vibrio fluvialis*. *J. Diarrhoeal Dis. Res.* 8, 163–165.
- Tokoro, M., Kato, M., Goto, K., Watanabe, M., Yamada, F., Sako, T., et al. (1984). Community outbreaks of mixed food-borne infection with *Vibrio parahaemolyticus* and *Vibrio fluvialis*. *Kansenshogaku Zasshi* 58, 1038–1045. [In Japanese]
- Uchiyama, H. (2000). Distribution of *Vibrio* species isolated from aquatic environments with TCBS agar. *Environ. Health Prev. Med.* 4, 199–204. doi: 10.1007/BF02931258
- Usó, J., Tirado, M. D., Moreno, R., and Campos, A. (2010). *Vibrio fluvialis* diarrhoea in an HIV infected patient. *Enferm. Infecc. Microbiol. Clin.* 28, 748–749. doi: 10.1016/j.eimc.2010.02.020
- Venkateswaran, K., Nakano, H., Okabe, T., Takayama, K., Matsuda, O., and Hashimoto, H. (1989a). Occurrence and distribution of *Vibrio* spp., *Listonella* spp., and *Clostridium botulinum* in the Seto Inland Sea of Japan. *Appl. Environ. Microbiol.* 55, 559–567.
- Venkateswaran, K., Kiiyukia, C., Takaki, M., Nakano, H., Matsuda, H., Kawakami, H., et al. (1989b). Characterization of toxigenic vibrios isolated from the freshwater environment of Hiroshima, Japan. *Appl. Environ. Microbiol.* 55, 2613–2618.
- Vinothkumar, K., Bhardwaj, A. K., Ramamurthy, T., and Niyogi, S. K. (2013). Triplex PCR assay for the rapid identification of 3 major *Vibrio* species, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio fluvialis*. *Diagn. Microbiol. Infect. Dis.* 76, 526–528. doi: 10.1016/j.diagmicrobio.2013.04.005
- Wall, V. W., Kreger, A. S., and Richardson, S. H. (1984). Production and partial characterization of a *Vibrio fluvialis* cytotoxin. *Infect. Immun.* 46, 773–777.
- Wang, Y., Wang, H., Liang, W., Hay, A. J., Zhong, Z., Kan, B., et al. (2013). Quorum sensing regulatory cascades control *Vibrio fluvialis* pathogenesis. *J. Bacteriol.* 195, 3583–3589. doi: 10.1128/JB.00508-13
- Wilkes, J. G., Rushing, L. G., Gagnon, J. F., McCarthy, S. A., Rafii, F., Khan, A. A., et al. (2005). Rapid phenotypic characterization of *Vibrio* isolates by pyrolysis metastable atom bombardment mass spectrometry. *Antonie Van Leeuwenhoek* 88, 151–161. doi: 10.1007/s10482-005-3990-z

- Williams, L. A., and Larock, P. A. (1985). Temporal occurrence of *Vibrio* species and *Aeromonas hydrophila* in estuarine sediments. *Appl. Environ. Microbiol.* 50, 1490–1495.
- Wong, H. C., Ting, S. H., and Shieh, W. R. (1992). Incidence of toxigenic vibrios in foods available in Taiwan. *J. Appl. Bacteriol.* 73, 197–202. doi: 10.1111/j.1365-2672.1992.tb02978.x
- Yalcinkaya, F., Ergin, C., Agalar, C., Kaya, S., and Aksoylar, M. Y. (2003). The presence and antimicrobial susceptibilities of human-pathogen *Vibrio* spp. isolated from blue crab (*Callinectes sapidus*) in Belek tourism coast, Turkey. *Int. J. Environ. Health Res.* 13, 95–98. doi: 10.1080/0960312021000063304
- Yoshii, Y., Nishino, H., Satake, K., and Umeyama, K. (1987). Isolation of *Vibrio fluvialis*, and unusual pathogen in acute suppurative cholangitis. *Am. J. Gastroenterol.* 82, 903–905.
- Yücel, N., and Balci, S. (2010). Prevalence of *Listeria*, *Aeromonas*, and *Vibrio* species in fish used for human consumption in Turkey. *J. Food Prot.* 73, 380–384.
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 17 December 2013; accepted: 19 February 2014; published online: 07 March 2014.
- Citation: Ramamurthy T, Chowdhury G, Pazhani GP and Shinoda S (2014) *Vibrio fluvialis*: an emerging human pathogen. *Front. Microbiol.* 5:91. doi: 10.3389/fmicb.2014.00091
- This article was submitted to *Aquatic Microbiology*, a section of the journal *Frontiers in Microbiology*.
- Copyright © 2014 Ramamurthy, Chowdhury, Pazhani and Shinoda. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.