MINI-REVIEW

Non-serogroup O1/O139 agglutinable Vibrio cholerae: a phylogenetically and genealogically neglected yet emerging potential pathogen of clinical relevance

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Abstract
Somatic antigen agglutinable type-1/139 Vibrio cholerae (SAAT-1/139-Vc) members or O1/O139 V. cholerae have been described by various investigators as pathogenic due to their increasing virulence potential and production of choleragen. Reported cholera outbreak cases around the world have been associated with these choleragenic V. cholerae with high case fatality affecting various human and animals. These virulent Vibrio members have shown genealogical and phylogenetic relationship with the avirulent somatic antigen non-agglutinable strains of 1/139 V. cholerae (SANAS-1/139- Vc) or O1/O139 non-agglutinating V. cholerae (O1/O139-NAG-Vc). Reports on implication of O1/O139-NAGVc members in most sporadic cholera/cholera-like cases of diarrhea, production of cholera toxin and transmission via consumption and/or contact with contaminated water/seafood are currently on the rise. Some reported sporadic cases of cholera outbreaks and observed change in nature has also been traceable to these non-agglutinable Vibrio members (O1/O139-NAGVc) yet there is a sustained paucity of research interest on the non-agglutinable Vibrio cholerae members. The emergence of fulminating extraintestinal and systemic vibriosis is another aspect of SANAS-1/139- Vc implication which has received low attention in terms of research driven interest. This review addresses the need to appraise and continually expand research based studies on the somatic antigen non-serogroup agglutinable type-1/139 V. cholerae members which are currently prevalent in studies of water bodies, fruits/vegetables, foods and terrestrial environment. Our opinion is amassed from interest in integrated surveillance studies, management/control of cholera outbreaks as well as diarrhea and other disease-related cases both in the rural, suburban and urban metropolis.

Keywords Choleragenic · Agglutinable · Somatic antigen agglutinable type-1/139 of Vibrio cholerae (SAAT-1/139- Vc) · Somatic antigen non-agglutinable strains-1/139 of Vibrio cholerae (SANAS-1/139- Vc) · Vibriosis · Surveillance

Introduction
Cholera has been listed amongst tropical disease which occurs from infection by pathogenic microorganisms whose members are grouped as enterobacteriaceae. This family houses pathogens which are implicated in systemic Vibriosis, diarrhea and diarrhea-like symptoms as it infects both intestinal tissue and enterocytes. The tissue/enterocyte infecting pathogenic family of organism is grouped taxonomically as Vibrionacea members. According to the Bergeys manual of Systematic Bacteriology (Garrity et al. 2005; George et al. 2005), the family of Vibrionacea is made up of 44 known species. Amongst them, 12 are implicated in human diseases including neonatal meningitis, septicaemia, diarrhea and/or wound infections (Igere et al. 2022; Lu et al. 2014; Farmer et al. 2003). This family (Vibrionacea) is classified under the eleventh Vibrionales

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order and their members are known opportunistic pathogens of animals. Other characteristics common to its members includes free living chemohetrotrophs, marine commensal which play the role of nutrient regeneration, biogeochemical cycling, and biodegradation, etc. (Peng et al. 2021; Wang et al. 2021). *V. cholerae* is a member of the family *Vibrionaceae* which has shown high variability especially amongst successive outbreaks or occurrence. Its existence and endemic/pandemic status in habitats depend on the presence of environmental reservoir strain especially if such environment is employing high sanitary and public hygienic procedures (Ramirez et al. 2021; Brehem et al. 2021). In poor sanitary environment, their endemic/pandemic level depends on a transient strain or transit type which may have been carried to such environment (Wang et al. 2021). It has a high global distribution and density in Coastal water bodies (Morris et al. 2018). Some of its members do not carry the gene type for cholera disease (choleragen) while others do. Based on the cell wall polysaccharide component type of these pathogens, they are categorized into two serogroups which are the somatic antigen agglutinating type (SAAT) or O1/O139 *V. cholerae* while the second group is the somatic antigen non-agglutinating strains type-1/139 *V. cholerae* (SANAS-1/139- Vc). This division into two sero-group members are based on some characteristic features possess by some members. Whereas some strains harbour in their cell surface polysaccharide, a particular antigenic determinant called the somatic antigen, others somatic antigen differs due to mutation on their somatic antigenic gene locus (Aydanian et al. 2011). These antigenic determinants are defined on the basics of bacterial surface antigens categorized as, somatic (O), flagellar (F) and capsular (K), which are associated with the *Vibrio* lipo-polysaccharide extracellular or surface capsule and flagellum, respectively (Aydanian et al. 2011; Farmer et al. 2011). The somatic antigen is represented by the alphabet ‘O’ while the numbers preceding the alphabets indicates the case period of isolation or detection (from first to the last). The numbers preceding the alphabets may also be associated with the pathogenic potential and the relative implication of the potential pathogen in outbreak cases. Clinically, the O1/O139 *V. cholerae* has been reported as the pathogen of increased relevance as it is involve in most documented cholera cases in the world today (Momba and Azab El-Liethy 2017; Rao and Surendran 2010). The World Health Organisation further affirmed that major cholera reported outbreak cases are implicated by the sero-group O1, and O139 members (WHO 2014; 2017). However, the other members (O2, O6, O10, O12, O14, O37, O75, O141 etc.) are referred to as somatic antigen type-1/139 non-agglutinative strains (SANAS-1/139-Vc or non-serogroup agglutinating O1/O139 *V. cholerae* (NSA-O1/139Vc) (Chatterjee et al. 2011; Sack et al. 2003). The SANAS-1/139-Vc member does not possess cell surface polysaccharide and antigenic determinant for agglutinating somatic antigen type-1/139 as the name specifies, yet its members (*V. cholerae* strains) (Shan et al. 2022; Devi et al. 2022; Malayil et al. 2011) may be implicated in cholera outbreak case, wound, septisemia, gastro-enteritis and diarrhea. These *Vibrio* members have been in existence in the environment (water, plankton/sediment and fishes) since the discovery of the family *Vibrionaceae* in 1890 (Huq et al. 2012). According to various investigators and researchers in the area of vibriology, the non-serogroup O1/O139 agglutinating *V. cholerae* members existed prior to reports on pathogenic or choleragenic *Vibrio* strains (Faruque et al. 2003). The NAG members were first reported at Germany in 1896 (Huq et al. 2012). According to the study, somatic antigen non-agglutinable strains (SANAS) were isolated from fishes in the Elbe river of Germany which necessitated their biotype name as “Albensis” (Huq et al. 2012). By 1983, a similar isolate of somatic antigen non-agglutinable strain of type-1/139-*V. cholerae* was isolated from the Santa Cruz Pacific Ocean indicating that the pathogen exist in the environment as a residential microbial flora (Huq et al. 2012). *V. cholerae* Non-O1/Non-O139 strains have been reported in Norway to be implicated in food borne Vibriosis (Rehulka et al. 2015; Bauer et al. 2006). The European food related disease report data base reveals that in 2005, the consumers of ready-to-eat headless shell fish and black tiger shrimps were observed to pass out diarrhea stool and express other choleragenic Vibriosis symptom (Rehulka et al. 2015; Bauer et al. 2006). The observation was further confirmed to be associated and implicated with SANAS-type-1/139-*V. cholerae*. A similar incidence was reported at Denmark in 2008 as the culture of diarrhea stool from consumers of shell fish and shrimps show growth of the NAG Vibrio members (Collin and Rehnstam-Holm, 2011; Halpern et al. 2008). This occurrence of SANAS-type-1/139-Vc lasted for almost the same year resulting increased concern amongst clinicians until it was eliminated toward the end of 2008 (ec.europa.eu/food/food/rapid-alert/risff_portal-database_en.htm). Halpern and his group in 2008 also isolated and detected SANAS-type-1/139-Vc amongst some fishes (Tilapia specie) in Northern Israel fresh water bodies (Halpern et al. 2008). Other SANAS-type-1/139-Vc strains have also been recovered from fecal specimen of some apparently healthy *Tursiops truncates*, an Atlantic bottlenose dolphin which was collected from both Florida and Texas (Alphonsa 2013; Buck et al. 2006; Buck and McCarthy, 1994). It is imperative to note that strains of the NAGVc (generally known as "non-O1/non-O139 *V. cholerae"”) are shown to be: non-choleragenic, non-pathogenic, they are asymptomatic colonizers of human subjects, they are associated with mild and/or sporadic illness (such a gastroenteritis), they are implicated in abrasive tissue infections (wound or ear infections) in apparently healthy individuals (Octavie et al. 2013; Cariri et al. 2010; Morris, et al. 1981). In immuno-compromised hosts (or those with underlying disease), NAG (non-O1/ non-O139) *V. cholerae* members are capable of causing exfoliated wound infections, tissue necrosis or sepsis, and high
mortality (Zmeter et al. 2018, 2017). Notable example of such members of the NAG Vibrio are O2, O6, O10, O12, O14, O37, O75, O141 etc. as depicted in Table 1 (Crowe et al. 2016; Haley et al. 2014; Aydanian et al. 2011; Tobin-D’Angelo et al. 2008). It is worthy to note that recent documents from CDC-COVIS-WHO revealed that members of O75 and O141 which are NAG-O1/O139 have been observed in outbreak cases (WHO 2017; CDC-COVIS 2012). These reports reveal the need for research based interest/focus/expansion on the non-agglutinating O1/O139 V. cholerae (SANAS-1/139-Vc) pathogen and necessitates an integrated surveillance, control and management strategy for the potential pathogen Table 2. It is imperative especially in areas/regions of non-documented history of outbreaks or cholera non-endemic regions as previous reports have shown that outbreak of new strains does occur in regions were there are no previous history Fig. 1. In addition, following the trends of previously reported outbreaks, it was shown that only the causal strains are managed/controlled during outbreaks (choleragenic V. cholerae O1/O139) due to its pathogenic relevance in the environment. There is a potential possibility that the source pathogen to an outbreak is masked or a possibility of non-O1/non-O139 pathogen causing a reported outbreak is masked.

A global improved surveillance scheme, epidemiology and comparative genomics of the pathogen must be initiated and inclusive when considering the management of cholera and cholera-related diseases in both rural, urban, province, country and continent. There have also been a notable under-reporting of vibrios cases globally (Ali et al. 2015), which may be tracable to discouragement from social, economical, political and societal policy due to the norm that non-O1/non-O139 are non-pathogenic. Low reporting may also be attributable to limited capacity for epidemiological surveillance and poor work incentives (Ali et al. 2015; WHO 2014; CDC 2010a, b, 2011a, b, 2013). It is to this end the review was designed to appraise such neglected concern. It also addresses the level of neglect that has greeted research on NAG V. cholerae occurrence, the present situation of cholera in both endemic and non-endemic regions and the involvement of these neglected pathogens in recently observed cases of cholera.

**Origin and genesis of non-serogroup O1/O139 agglutinating (NSAG) V. cholerae**

Cholera epidemic has been associated with somatic antigen type-1 (O1) V. cholerae of the classical biotype since the emergence of the disease in 1817. Amongst the 206 and more documented members of the V. cholerae, the Classical biotype of O1 dominated in all cases of cholera until a new biotype was observed as the El Tor. This El Tor type was later displaced in 1992 by a non-O1 serogroup of the somatic antigen which has similar character as the O1 but different in pathotype. Its genes were compared with other somatic antigenic types known (which are O2–O138) at then but it shows remarkably difference from all existing sero-group types (Faruque et al. 2003). It was later referred to as O139 V. cholerae which continues to cause epidemic, spreading from India through the entire Asia continent to other continents (Aydanian et al. 2011; Mooi and Bik, 1997; Manning et al. 1995; Nair et al. 1994). These novel characters observed amongst the O139 members actually change the myth of the Non-O1 involvement in epidemic and choleragenic potential. Some of such characters are acquisition of enterotoxin (CT) and other epidermic associated genes (tcp-gene) (Shan et al. 2022; Faruque et al. 2003; Basu et al. 2000). From the forgoing, there is a likelihood that the Non-O1 members originated from the O1 V. cholerae members. Early genomic studies observed that the O139 arise from the O1 El Tor member in 1992 which is today classified as the seventh pandemic El Tor Vibrio (7PET) (WHO 2017; Faruque et al. 2003). According to various investigators, the classification was not based on the detection of the CT gene and tcp gene but other virulence-related genes (WHO 2017; Faruque et al. 2000). The study also shows similarity in ribotyping analysis (Faruque et al. 1994, 2000). In the study of Aydanain and his group, one difference between the O1 and O139 V. cholerae members is the deletion on the gene responsible for the biosynthesis of the somatic antigen in both pathogens (Takahashi et al. 2021; Aydanian et al. 2011). This genetic mutation type allows the organism to effect increased virulence and pathogenesis on individual who are previously immune to O1 V. cholerae, hence adults are it’s major host. Suffix to say that the somatic antigen carries the epitope for attachment and pathogenicity, hence similar pathogenic effects are expressed amongst O1/O139 members that are agglutinating and other non-agglutinating types (also known as non-O1/non-O139) eg; O5, O2, O4, O27, O37, O53, O65, O75, O141, etc. (Takahashi et al. 2021; Aydanian et al. 2011; Tobin-D’Angelo et al. 2008; Nesper et al. 2002; Stine et al. 2000; Yamai et al. 1997). From the study of Aydanain and his group, it was hypothesized that pathogenic O1/O139 V. cholerae members have the tendency to mutate or change or exchange their somatic antigenic genes which confirms their genetic heterogeneity or plasticity (Aydanian et al. 2011). This is so, as it ensures escape from preexisting immunity in any reemerging situation of cholera in an environment. This mutation-based activity of O-antigen genes also influence phage lysogeny since somatic antigenic determinants serves as receptor to phage. Other genetic dynamics influenced by the mutation potential of V. cholerae somatic antigenic genes are uptake of
Table 1  | Isolated and identified O1/O139-NAGVc members so far

| Identified O1/O139-NAGVc | Source | Diseases | Country/place of isolation | Clonal complexity | Year isolated | Ref |
|-------------------------|--------|----------|-----------------------------|-------------------|---------------|-----|
| O2                      | C/E    | Acute diarrhea | Bangladesh | S | 1997 | Filetici et al. 1997 |
| O4                      | C      | Intestinal diarrhea | Bangladesh/Peru | S | 1994/2005 | Dziejman et al. 2005 |
| O5                      | C      | Acute diarrhea | Bangladesh | S | 1995–2002 | Dziejman et al. 2005; Rudra et al. 1996 |
| O6                      | E (Water and Shellfish) | Acute gastroenteritis | Italy | S | 1997 | Filetici et al. 1997 |
| O7                      | C      | Acute gastroenteritis | India | S | 1962 | Octavia et al. 2013 |
| O8                      | E (Water and Shellfish) | Acute gastroenteritis | Italy | S | 1997 | Filetici et al. 1997 |
| O9                      | C      | Acute gastroenteritis | India | S | 1995 | Li et al. 2002 |
| O10                     | E (Water and Shellfish)/C | Acute gastroenteritis | India | S | 1995/996 | Dalsgaard et al. 1995; Rudra et al. 1996; Dziejman et al. 2005 |
| O11                     | C      | Acute gastroenteritis | Bangladesh/Kolkata | S | 1977/2013 | Dutta et al. 2013 |
| O12                     | C (fecal) | Gastroenteritis | Bangladesh | CC3 | 1994 and 2005 | Dziejman et al. 2005; Rudra et al. 1996; Octavia et al. 2013 |
| O14                     | C/E    | Acute gastroenteritis | New Zealand/Bangladesh | S | 2001/2005 | Dziejman et al. 2005 |
| O15                     | C      | Acute gastroenteritis | Bangladesh | S | 2001 | Octavia et al. 2013 |
| O16                     | E      | Acute gastroenteritis | Bangladesh | S | 2002 | Dziejman et al. 2005 |
| O18                     | E      | Acute gastroenteritis | Bangladesh | S | 2002 | Dziejman et al. 2005 |
| O22                     | C      | Acute gastroenteritis | Philippines | S | 1968 | Octavia et al. 2013 |
| O23                     | E      | Acute gastroenteritis | Bangladesh | S | 2002 | Dziejman et al. 2005 |
| O24                     | C      | Acute gastroenteritis | Bangladesh | S | 2001 | Octavia et al. 2013 |
| O25                     | E      | Acute gastroenteritis | Germany | S | 1993 | Octavia et al. 2013 |
| O26                     | C      | Acute gastroenteritis | Philippines | S | 1972 | Octavia et al. 2013 |
| O27                     | E/Prawn | Intestinal diarrhea | Japan/Maryland/Thailand | S | 1992/2002 | Li et al. 2002 |
| O31                     | C      | Acute gastroenteritis | Japan | S | 1990 | Li et al. 2002 |
| O35                     | C      | Acute gastroenteritis | Bangladesh/Kolkata | S | 1977/2013 | Dutta et al. 2013 |
| O37                     | E/C    | Acute gastroenteritis | Bangladesh, Sudan, Northern Italy | S | 1992/2002/2008 | Li et al. 2002; Farina et al. 2010 |
| O39                     | C      | Intestinal diarrhea | Bangladesh | S | 2001 | Dziejman et al. 2005 |
| O40                     | E/Shell fish | Intestinal diarrhea | Italy | S | 1997 | Filetici et al. 1997 |
| O41                     | E/Shell fish | Intestinal diarrhea | Italy | S | 1997 | Filetici et al. 1997 |
| O44                     | C      | Acute gastroenteritis | Bangladesh | S | 1998 | Octavia et al. 2013 |
| O49                     | C      | Intestinal diarrhea | Bangladesh | S/CC3 | 2000/2001 | Octavia et al. 2013 |
| O52                     | E      | Intestinal diarrhea | Denmark | S | 1994 | Octavia et al. 2013 |
| O53                     | E/C    | Intestinal diarrhea | Iraq/Bangladesh/Sudan | S | 1966/2002 | Li et al. 2002 |
| O57                     | C      | Acute gastroenteritis | India | S | 1976 | Octavia et al. 2013 |
| O59                     | C      | Acute gastroenteritis | Bangladesh/Kolkata | S | 1977/2013 | Dutta et al. 2013 |
| O64                     | E      | Intestinal diarrhea | Italy | S | 1997 | Filetici et al. 1997 |
| O65                     | E/C    | Intestinal diarrhea | Japan/India | S | 1975/2002 | Li et al. 2002 |
| O66                     | E      | Intestinal diarrhea | Argentina | S | 1993 | Octavia et al. 2013 |
| O70                     | E      | Intestinal diarrhea | Germany | S | 1994 | Octavia et al. 2013 |
| O73                     | C      | Intestinal diarrhea | India | S | 1979 | Octavia et al. 2013 |
| O74                     | E      | Intestinal diarrhea | Korea/Argentina | S | 1993/1994 | Octavia et al. 2013 |
| O75                     | E/Shell fish | Sporadic cholera | US Gulf Coast | S | 2010/2011 | Ceccarelli et al. 2015 |
Table 1 (continued)

| Identified O1/O139-NAGVc Source | Diseases               | Country/place of isolation | Clonal complexity | Year isolated | Ref       |
|--------------------------------|------------------------|-----------------------------|-------------------|---------------|-----------|
| O77                            | Intestinal diarrhea    | India                        | S                 | 1976          | Octavia et al. 2013 |
| O79                            | Intestinal diarrhea    | Bangladesh                   | S                 | 2002          | Dziejman et al. 2005 |
| O80                            | Acute gastroenteritis  | India                        | S                 | 1977          | Octavia et al. 2013 |
| O83                            | Intestinal diarrhea    | Germany                      | S                 | 1994          | Octavia et al. 2013 |
| O89                            | Acute gastroenteritis  | India                        | S                 | 1981          | Octavia et al. 2013 |
| O94                            | Acute gastroenteritis  | Bangladesh                   | CC2               | 2001          | Octavia et al. 2013 |
| O97                            | Acute gastroenteritis  | Bangladesh/Kolkata/Argentina | S                 | 1993/1997/2013| Dutta et al. 2013  |
| O99                            | Intestinal diarrhea    | Germany                      | S                 | 1993          | Octavia et al. 2013 |
| O100                           | Otitis media           | Israel/ Haifa,Australia/Murray River Near Mildura VIC | S | 2017     | Kechker et al. 2017 |
|                                |                       |                              |                   |               |           |
| O101                           | Soft tissue infection  | Japan                        | S                 | Not reported  | Octavia et al. 2013 |
| O103                           | Soft tissue infection  | Japan                        | S                 | Not reported  | Octavia et al. 2013 |
| O105                           | Intestinal diarrhea    | China                        | S                 | 1988          | Octavia et al. 2013 |
| O107                           | Intestinal diarrhea    | Italy                        | S                 | 1997          | Filetici et al. 1997 |
| O108                           | Intestinal diarrhea    | Philippine/Japan             | S                 | 1964/1990     | Octavia et al. 2013 |
| O110                           | Intestinal diarrhea    | Japan                        | S                 | 1992          | Octavia et al. 2013 |
| O111                           | Intestinal diarrhea    | Japan                        | S                 | 1993          | Octavia et al. 2013 |
| O113                           | Intestinal diarrhea    | Japan                        | S                 | 1995          | Octavia et al. 2013 |
| O114                           | Soft tissue infection  | Japan                        | S                 | 1996          | Octavia et al. 2013 |
| O115                           | Soft tissue infection  | Japan                        | S                 | Not reported  | Octavia et al. 2013 |
| O116                           | Soft tissue infection  | Japan                        | S                 | Not reported  | Octavia et al. 2013 |
| O117                           | Soft tissue infection  | Japan                        | S                 | Not reported  | Octavia et al. 2013 |
| O123                           | Intestinal diarrhea    | India                        | S                 | 1981          | Octavia et al. 2013 |
| O124                           | Acute gastroenteritis  | India                        | S                 | 1981          | Octavia et al. 2013 |
| O135                           | Intestinal diarrhea    | Chesapeake Bay               | S                 | 1998          | Octavia et al. 2013 |
| O141                           | Extra-intestinal diarrhea | New Jersey and Arizona     | S                 | 2011–2012    | CDC, 2012 |
| O144                           | Intestinal diarrhea    | India                        | S                 | 1996          | Li et al. 2002 |
| O145                           | Acute gastroenteritis  | Bangladesh                   | S                 | 2001          | Octavia et al. 2013 |
| O158                           | Intestinal diarrhea    | Italy                        | S                 | 1997          | Filetici et al. 1997 |
| O333                           | Acute gastroenteritis  | Bangladesh                   | S                 | 1975          | Li et al. 2002 |
| O340                           | Intestinal diarrhea    | Japan/India                  | S                 | 1966/2002     | Li et al. 2002 |
| O-Untypeable                   | Acute gastroenteritis  | Bangladesh/Kolkata           | S                 | 1977/2013     | Dutta et al. 2013  |

E environmental, C clinical, CC clonal complexity, S singleton

Table 2  Case report to CDC-COVIS between 1984 and 2014

| Continent | Numbers of detected O1/O139-NAG-Vc Cases | Range of risk factors associated | References |
|-----------|------------------------------------------|----------------------------------|------------|
| Globally  | 154                                      | Contaminated fresh water, sea food, travel, occupation, natural disaster and hobbies | Engel et al. 2016 |
| America   | 52                                       | Contaminated fresh water, sea food, travel and immuno-compromised clinical cases | Crowe et al. 2016 |
| Europe    | 23                                       | Contaminated fresh water, sea food, travel and immuno-compromised clinical cases | Engel et al. 2016 |
| Asia      | ND                                       | Contaminated fresh water, sea food, travel, occupation, natural disaster and hobbies | CDC-COVIS 2017 |
| Africa    | ND                                       | Contaminated fresh water, sea food, travel, occupation, natural disaster and hobbies | CDC-COVIS 2017 |
free mobile DNA as well as mobile genetic elements (Xu et al. 2013; Blokesch and Schoolnik 2007; Faruque et al. 1998a, b). The studies of Yamasaki et al. (1999), Blokesch and Schoolnik (2007) on *V. cholerae* growth on chitin substrate media also further substantiate serogroup conversion potential. The various genes responsible for somatic antigen were further studied amongst *V. cholerae* (Samuel and Reeves 2003; Yamasaki et al. 1999) which ensures their classification into different serogroups. These somatic antigenic genes in *V. cholerae* O1 consists of 18–56 varying units of open reading frames (ORFs) at the lipopolysaccharide subunit (LPS), and consist of sugars and aminoglycosides. Some of these are glucose, fructose and 2,4-Diacetyl-amido-2,4,6-trideoxyglucose (QuiNAc4NAc) which are found in O5 and O8 (Kocharova et al. 2001; Hermansson et al. 1993), fucosamine and quinovosamine found in O139 and O108. Other additional subunits are fucosamine (O108), galactosamine (O108), glycerol-D-manno-heptose (O5, O8, and O108) and glucosamine (O108) (Kondo et al. 1997). Hence, the classification of *V. cholerae* to various serogroup was informed by the subunit sugars and aminoglycosides.

The wbf gene regions of these serogroup members also possess high-molecular weight capsules eg O31 and O139 serogroups.

The somatic gene sequence study of *V. cholerae* by Aydanian et al. (2011) shows that there is 96–100 percent similarity amongst polysaccharide biosynthetic genes. Present also in *V. cholerae* members LPS is the tendency for gene duplication which suggest a possible future antigenic lineage associated change during epidemic occurrence (Aydanian et al. 2011).

### The genetics of NSAG/NAG-O1/O139 *V. cholerae*: an overview

One of the concerns of non-reporting and negligence in observed disease cases of the Non-O1/Non-O139 *V. cholerae*, is accessing the genetics of the potential pathogen. Although very few vibriology investigators have reported genetic basics of the potential pathogen, there is paucity of documents that addresses the genetic basis of NSAG/NAG-O1/O139-Vc. The poor research interest/attention given to studies on the *V. cholerae* non-agglutination subgroup after many years of its prevalence reports till the middle of 1980 further emphasized such neglect. Furthermore, following the previous reports from the study of Octavia et al. (2013), the genomic data of O1 *V. cholerae* isolates was published which describes some genetic dynamics. They observed about 66 unique sequence types (STs) in their study, three clonal complexities and four subpopulations. The subpopulation I–III are predominantly clinical strains while the subpopulation IV is environmental based. All strains of NSAG/NAG-O1/O139-Vc were described to belong to four clonal complexity (CC) which are CC1, CC2, CC3 and singleton as interpreted by the eBURST analysis (Octavia et al. 2013). Amongst all identified NSAG/NAG-O1/O139 *V. cholerae* members, only the O94 (CC2), O49 (CC3) and O12 (CC3) have different clonal complexity, other members have singleton as their clonal dynamics (Octavia et al. 2013). The genetics of the Lipopolysaccharide somatic antigen biosynthetic gene of Non-O1 or O139 *V. cholerae* also shows that it does not possess any side chain in the core LPS while its O1 counterpart possess about 17 side chains of smaller repeated units of 4-NH2-4,6-dideoxymannose. The sub-unit of each O1
member is substituted with the tert-butyl esterified 3-deoxy-L-glycero-tetronic acid, while their major functional gene is wbf which consist of four other genes (gmhD, rjg, orf and manC) (Octavia et al. 2013).

In 2003, the studies of Chatterjeea and Chaudhuri reported that *V. cholerae* transfer genes by a P-factor which is not the case with other Gram-negative pathogens (F-factor in other organism). A growing multiple drug resistance was also observed which is associated with R-factor and exclusively traced to plasmid acquisition (Chatterjeea and Chaudhuri 2003). Other genetic basis of the O1/O139 *V. cholerae* pathogen were also reported by various investigators (Okoh et al. 2014; Octavia et al. 2013; Igbinosa et al. 2009), yet there is dearth of research or low documention in reports for non-agglutinating O1-type or O139-type *V. cholerae* strains. We are of the view that this negligence or under-reporting of these emerging potential pathogens may pose a negative public health impact, affecting management and control negatively especially in disease distribution. It is worthy to note that management/control is said to be plausible when an uncompromised and unused attention is given to any suspected source of a problem, hence the need for an unbiased research attention on the SANAS-Vc is reasonable.

Genealogy and phylogenetic relationships

Comparative genomic studies, basic genetic relatedness studies and various online data base analysis have shown that all *V. cholerae* members are closely related as they cluster together indicating that they have a common ancestor/origin. The phylogenetic study of Rahman et al. (2008); Katz et al. (2013) and Aydanian et al. (2015) have shown that all cholera-associated *V. cholerae* and members that express choleraigen tend to cluster closely together. This is related to the associative-concept of the *V. cholerae* members as having similar "epidemic genotype" which arise from multiple genes implicated in any reported epidemic (Faruque et al. 2004). A continuous phylogenetic study by other investigators shows that the virulence genes from some non-epidemic associated strain does not cluster with that of *V. cholerae* members which are usually reported and associated in outbreak cases (Chen et al. 2007). However recent studies show lineage similarity (Aydanian et al. 2015) between SANAS-Vc and the SAAT-Vc. Other atypical cholera cases have been reported to show similar occurrence of diverse unrelated virulent genes among *V. cholerae* strains, bringing about a debate in the relativity/relationship of the *V. cholerae* members.

The study of Hasan and his group (Hasan et al. 2012a, b, c) observed a different phylogenetic report after their study on the prevalence and genetic relatedness of Non-O1/Non-O139 or type-O1/O139 non-agglutinating *V. cholerae* on patients assessed after the Haiti epidemic. A report written to Mekalanos and his group on their observation of differences in phylogenetic deviation was addressed/discussed. Suffix to say that Mekalanos and his group are regular investigators of vibriology. This report sharing was to harmonize the dichotomy in the results generated from various genomic diversity studies (Hasan et al. 2012a, b, c; Mekalanos et al. 2012), and to interprete the subjects appropriately.

The debate on the relatedness of O1/O139 and Non-O1/Non-O139 especially as it concerns pathogenic dynamics in outbreak association was then addressed by Hasan and his group. Their report after a reinforced high through-put phylogenetic analysis showed that all clinical isolates of Non-O1/Non-O139 *V. cholerae* members formed a closely related cluster with clinical isolates of O1/O139 *V. cholerae* as depicted in a monophyletic clade data which is interpreted as epidemic genotype related (Hasan et al. 2012a, b, c).

In a comparative study on the genomic island (GI) and pathogenicity island (PI) of NSAG/NAG-O1/O139 *V. cholerae* and the O1/O139-choleraigenic *V. cholerae*, isolated from Haitian outbreak, it was reported that 18 GIs and PIs were related. A similar study on clinical and environmental isolates revealed 40 similar GIs and PIs genes. This also confirms that both cholera members arise from a single origin. Although there were high GIs and PIs amongst the clinical and environmental isolates, it only indicates possession of additional unidentified fitness factors which may be involve in other virulence indices (Hasan et al. 2012a, b, c). Other studies also corroborates these reports (Yamasaki et al. 1999; Comstock et al. 1996; Bik et al. 1995; Stroheder et al. 1995; Aydanian et al. 2015). These reports confirm the genealogical and phylogenetic relationship of both SAAT-1/139-Vc and SANAS/NSAG/NAG-Vc as members of the family Vibrionaceae.

Case document of NSAG/NAG-O1/O139 *Vibrio cholerae* and negligence

Following the definition of cholera by the Unites State Centre for Disease Control and Prevention (CDCs) in 1996, the clause on “only confirmed cases of cholera be notified” has contributed to the negligence associated with the report on the incidence and/or prevalence of O1/O139-NAG *V. cholerae*. A confirmed case is such which has received a reproducible and state-of-the-art laboratory analysis for differentiating non-choleragenic situation, cholera-like and choleragenic vibriosis (CDC-COVIS 2011, 2012). Other investigators prefer cholera to be a case if a diarrhea disease can be reproducible by oral ingestion of purified cholera-toxin (Mekalanos et al. 2012). The correctness of these definitions can never be over emphasized, yet it has contributed and welcomed the low-report and/or negligence in reporting.
all prevalence and incidence results of Non-O1/Non-O139 V. cholerae. It has also necessitated the paucity and research driven interest on the study of 1/139-NSAGVc or Non-O1/Non-O139 V. cholerae. The Tables 3 and 4 below shows some of the case documents from both environmental and clinical sources but may not have been reported as cases to the appropriate regulatory body eg COVIS.

Vibriosis and O1/O139-NAGVc cases definition/classification

According to the 2017 document of Centre for Disease Control-Cholera and Other Vibrio Illness Surveillance System (CDC-COVIS 2017) and the National Notifiable Disease Surveillance System (NNDSS), defining/classifying any observed vibriosis case depends on some basic criterials. The COVIS document indicated that probable vibriosis cases must be based on laboratory detection of any specie member of Vibrionaceae (other than the O1/O139 V. cholerae) from both clinical specimen using culture dependent and culture independent test methods. The document also emphasize that any clinical case which is epidemiologically associated with a basic/supportive laboratory diagnosis indicates a confirmed case criteria and it is also inclusive (CDC-COVIS 2012a, b. In addition, the confirmed vibriosis cases must be such that have shown positive culture dependent diagnosis (using TCBS or VCA or TTGA), serology and/or rapid culture independent diagnostic test (CIDTs) from vomitus or fecal samples. Such case must include also the non-choleragenic (non-toxigenic) and the choleragenic (toxigenic) V. cholerae genotypes irrespective of their serogroup, although special attention must be given to the O1 and O139 members. This is so, as the O1 and O139 V. cholerae members are known for epidemic and pandemic situations. Today other members of the previously known non-epidemic and non-pandemic related have shown potential for pandemic and epidemic cases eg O75 and O141 V. cholerae (CDC-COVIS 2014, 2017), hence, studies on Vibrio is case dependent. Case studies of such should separate the SAAT from the SANAS V. cholerae members. Case reports should also include existing cases or new case reports. Vibriosis detection or observation following the basic laboratory detection criteria (as described by CDC) of any non-O1/non-O139 V. cholerae is tagged an existing case when it is always found in two or more specimen collected while a new case is that which has shown polymorphic gene potential from previously isolated and detected strains. Such detected polymorphism should be based on the new strains virulence determinants, plasmid or extra-chromosomal DNA acquisition, integrative conjugative elements (ICE), antibiotic resistant genes, genomic island (GI), phage acquisition, serogroup diversity etc. The new strain or case associated potential pathogen must be isolated from one or more specimens collected which must also be reported to COVIS. Vibriosis implicated by toxigenic or choleragenic O1/O139-NAGVc continues to pose high public health concern to individual in various areas yet there is a sustained neglect in studying the potential pathogens infectivity and control.

Table 3 Cases of O1/O139-NAGVc in the environment

| Specimen source          | Country/place of isolation       | Specimen type                        | Reference          |
|--------------------------|----------------------------------|--------------------------------------|--------------------|
| River                    | Nir David, Israel                 | Jordan St. Peter’s fish (Oreochromis aureus) | Senderovich et al. 2010 |
| Water from marine inshore| Peru                             | Lorna fish (Sciaena deliciosa)       | Carvajal et al. 1998 |
| Lake                     | Sea of Galilee Israel            | Longhead barbel (Barbus longiceps)   | Senderovich et al. 2010 |
| Fish pond                | Kfar Rupin, Israel               | Galilee St. Peter’s fish (Sarotherodon galilaeus) | Senderovich et al. 2010 |
| Fish pond                | Atlit, Israel                    | Grass carp, white-amur (Ctenopharyngodon idella) | Senderovich et al. 2010 |
| Mediterranean Sea (Marine water) | Akko, Israel                  | Blotcheye soldierfish (Myripristis mordjan) | Senderovich et al. 2010 |
| Royapuram coast (Marine water) | Chennai, Tamil Nadu, India      | Bulls eye (Priacanthus hamrur)       | Sujatha et al. 2011  |
| Adult zebrafish cultured in tanks | Auckland, New Zealand          | Zebrafish (Danio reio)               | Lan and Love 2012   |
| Marine aquaculture       | Qingdao, China                   | Turbot fish (Scophthalmus maximus)   | Xing et al. 2013    |
| Tanghin freshwater reservoir | Ouagadougou, Burkina Faso (Africa) | Tilapia (Oreochromis niloticus)       | Traoré et al. 2014  |
| Fowl river (estuarine)   | Gulf of Mexico                   | Sheephead (Archosargus probatocephalus) | Jones et al. 2013   |
| Food industry            | Vietnam                          | Frozen tra fish (Pangasius hypophthalmus) fillet | Thi et al. 2014     |
| Year of occurrence | Disease/specimen | Country/place                                      | Age of patient | References                  |
|--------------------|------------------|---------------------------------------------------|----------------|-----------------------------|
| 2006               | Pleural effusion  | Korea                                             | 62 yrs         | Kim et al. 2007             |
| 2007               | Inguinal skin and skin lesion | Spain/Canary Island                              | 36 yrs         | Aguinaga et al. 2009        |
| 2002               | Septicemia/blood  | Korea/Hospital                                     | 54 yrs         | Choi et al. 2003            |
| 1988–1998          | Necrotised skin lesion | Taiwan/NCKU Hospital                              | 75 yrs         | Ko et al. 1998              |
| 2009–2014          | Acute gastroenteritis stool | Taiwan/Hospital                                    | 45.3 ± 17.0    | Chen et al. 2015            |
| 2009–2014          | Billary tract infection/exudates | Taiwan/Hospital                                    | 66.5 ± 16.7    | Chen et al. 2015            |
| 2009–2014          | Bacteremia/blood   | Taiwan/Hospital                                     | 61.0 ± 14.1    | Chen et al. 2015            |
| 2012               | Liver cirrhosis biopsized tissue, acute gastroenteritis/blood, stool | Taiwan/NTU Hospital, College of Medicine           | 61 yrs         | Lai et al. 2012             |
| 2010               | Liver, gastroenteritis/blood, stool | France/Brest Hospital                             | 63 yrs         | Petsaris et al. 2010        |
| 2005               | Gastroenteritis, septicemia/stool, blood | Kuwait                                              | 9 yrs          | Dhar et al. 2004            |
| 2008               | Brain disease/tissue | Kuwait                                             | 12 yrs         | Arnett et al. 2008          |
| 2002               | Septicemia/blood   | India/Vellore                                      | 10 day         | Kertetta et al. 2002        |
| 1993               | Septicemia/blood   | United Kingdom/Kettering                           | 12 month       | Naidu et al. 1993           |
| 2001               | Septicemia/blood   | Kuwait                                             | Preterm baby   | Ismail et al. 2001          |
| 2015               | Gastroenteritis, septicemia/stool, blood | Not reported                                       |                | Ali et al. 2015             |
| 2015               | Otitis media, gastroenteritis, water/ear exudate, stool | Israel/Haifa and Australia/Murray River near Mildura VIC | 27 yrs | Kechker et al. 2017        |
| 2006               | Otitis media, gastroenteritis/ear exudate, stool | Austria                                            | 22 yrs         | Huhulescu et al. 2007       |
| 2002               | Otitis media/ear exudates | Austria                                            | 14 yrs         | Huhulescu et al. 2007       |
| 2005               | Otitis media/ear exudates | Austria                                            | 9 yrs          | Huhulescu et al. 2007       |
| Unknown            | Otitis media/ear exudates | United Kingdom                                    | Not reported   | Marek et al. 2013           |
| 2015               | Otitis media/ear exudates | United Kingdom                                      | 21 yrs         | Hirk et al. 2016            |
| 2012               | Gastroenteritis, septicemia/stool, blood | China/Beijing                                      | 70 yrs         | Lu et al. 2014              |
| 2012               | Gastroenteritis, septicemia/stool, blood | Lebanon                                            | 54 yrs         | Feghali and Adib 2011       |
| 2006 and 2009      | Liver cirrhosis biopsized tissue, acute gastroenteritis/blood, stool food material | Italy/Apulian region                               | 49 yrs         | Ottaviani et al. 2009, 2011 |
| 2000–2013          | Septicemia/blood   | Lebanon                                            | Preterm baby   | Feghali and Adib, 2011      |
| 2008               | Gastroenteritis, septicemia/stool, blood | Australian population                              | 40–86 yrs      | Trubiano et al. 2014        |
| 1982               | Gastroenteritis, septicemia/stool, blood | Italy and Egypt                                    | 54 yrs         | Farina et al. 2010          |
| 2011               | Liver cirrhosis biopsized tissue, septicemia/blood | Japan (Osaka)/ Kindai University                   | Not reported   | Inoue et al. 2012           |
| 2008               | Septicemia/blood   | Malaysia                                           | 28 yrs         | Deris et al. 2009           |
| 2012               | Liver cirrhosis biopsized tissue, septicemia/blood, tissue | India/Kerala                                       | 49 yrs         | Khan et al. 2013            |
| 2011               | Septicemia/blood   | Portuguese                                         | Not reported   | Albuquerque et al. 2013     |
| 2015               | Liver cirrhosis biopsized tissue, septicemia/blood, tissue | Taiwan/Taipei                                      |                | Hsieh et al. 2016           |
| 2014               | Septicemia/blood   | China/Jinan                                        | 11 day         | Hao et al. 2015             |
| 2009               | Gastroenteritis, septicemia/stool, blood | India/Gujarat                                      | Not reported   | Rajpara et al. 2013         |
| 2017               | Gastroenteritis, septicemia/stool, blood | Pakistan/Karachi                                   | 2 months       | Baig et al. 2018            |
| 2015               | Gastroenteritis, septicemia/stool, blood | Lebanon/AUB medical Centre                         | 74 yrs         | Zmeter et al. 2018          |
| 2016               | Acute gastroenteritis stool | Delhi,Ghaziabad, Loni,Ashek,Vihar,Kachi colony/ AAA Hospital | 5 month | Kumar et al. 2017          |
| 2014               | Gastroenteritis, septicemia/stool, blood | China/ Qingdao Maternal and Child Health Care Hospital | 11 day | Hao et al. 2015             |
Epidemiology of cholera and risk factors

Risk associated with cholera cases are based on seven factorial subjects; Food, Occupation, recreational activities, trauma, natural disaster, international or foreign travel and Hobbies. Table 5 shows the risk factors associated with Non-O1/Non-O139 *V. cholerae*. The Kansas Department of Health (KDHE) and Environment in conjunction with the Bureau of Epidemiology and Public Health Informatics (BEPHI) documented in 2016 that Non-01/Non-0139 V. *cholerae* has been associated with over 2–3% of reported diarrhea cases/illnesses in all developing tropical countries were everyday activities of man are implicated (KDHE 2015).

It is imperative to note that these risk determinants as depicted in Table 5 above are specific to the everyday activities of man, animals, plants and the environment. Some reported cases of O1/O139-NAGVc have implicated migration/travel and consumption of contaminated seafood as vehicles of transmission of these emerging pathogens, yet low attention has been given to studies of these *Vibrio* members (Diez-Quinonez et al. 2014; Newton et al. 2011). Other recent study from the REP-PCR, RAPD-PCR, BOX-PCR and ERIC-PCR study of various investigators (Dalusi et al. 2015a, b; Fooladi et al. 2013; Ateba and Mbewe, 2013; Dutta et al. 2013; Waturangi et al. 2012; Chokesajjawatee et al. 2008). A significant multiplicity of virulent genes has also been reported by various investigators (Gupta et al. 2016; CDC-COVIS 2013, 2014). This is a clear indication that the pathogenesis of the 1/139-SANAS *V. cholerae* members does not possess a mono-pathogenesis mechanism since their virulent dynamics are multi-facets. As reported by Morris et al. (2018), Madhusudana and Surendran, (2013) and Lu et al. (2014), other factors may also enhance their pathogenesis and development to illness in an infected person. Some of such factors include nearness-to or contact with an infected or carrier agent, poor hygienic practice, immune-stability of hosts, and environmental factors. Other investigators have reported the association/relationship of human blood group type with the infectivity and/or pathogenesis of NSAG/NAG-O1/O139 *V. cholerae*. (Bennett et al. 2015; Janda et al. 2015). In human, infection and pathogenesis is initiated only after contact with an infected agent or consumption of contaminated food or water. The food or water passes through the stomach acid barrier allowing the organism to colonize the small intestinal epithelium. With the aid of the tepgene (toxin coregulated pili), it releases its various virulent factors (including enterotoxin CT) through the bacterial outer membrane into the extracellular milieu of the small intestine to disrupt active ionic transport in ilia cells. This

| Year of occurence | Disease/specimen | Country/place | Age of patient | References |
|-------------------|------------------|---------------|----------------|------------|
| 2010              | Bacteremia,liver cirrhosis/blood and liver exudates | Senegal       | 70 yrs         | Deshayes et al. 2015 |
| 2006–2007         | Gastroenteritis, septicemia/stool, blood             | Netherlands   | 50–70 yrs      | Engel et al. 2016 |
disruption later results subsequent loss of electrolytes and water which is called diarrhea. Continuous structural analysis on the enterotoxin CT, lead to the identification of its subunits (A and B), after appropriate verification using Robert Koch’s (1876) observation as a template scheme. Subunit A consist specific enzymatic function which acts by changing the cellular level of cAMP and disrupting the intestinal absorption tendency. While subunit B acts enzymatically with neuraminidase to bind the toxin to intestinal epithelial cell receptor (ganglioside GM1). Although the CT has been associated with disease situation, it’s now clear that the mutation which occurred at the somatic antigen polysaccharide gene locus amongst the SANAS-1/139-Vc or Non-O1/Non-O139-Vc ensures the acquisition of the CT (Aydanian et al. 2011; Chatterjeea and Chaudhuri 2004). This further depict that other non-O1 and non-O139 members may acquire the potential to cause epidemic in a place where they had not been any report of previous epidemic. The emergence of the O139 V. cholera as reported by various investigators has further substantiate

| Table 5 | Possible risk factors associated with the spread of O1/O139-NAG-Vc |
|---------|--------------------------------------------------------------------------------------------------|
| Risk factors | Activity/example |
| Recreational activities | Boating, parasailing, Skimboarding, Skurfing, Marco polo, Sharks and Minnows Water Surfing, Swimming, waboba, Synchronised swimming4 Water Skiing, Water polo, Triathlon, Picigin Shore Walking, Pentathlon, Aquajogging Diving (Scuba, Springboard, synchronised etc.) Water rugby, Aquathlon, other recreational diving |
| Natural disaster | Flood Sinkholes Lumnic eruptions Tsunamis Blizzards Avalanches Cyclones Typhoons Mudslides Earthquakes Hurricanes |
| Occupation | Seafood Handlers Fish farming Water analyst and technologist Transportation in water Benthic ecologist and hydrologist Fishing |
| Food | Mollusc/Shell fishes: Oyster, Clams (Cockies) Water: Freshwater, contaminated or adulterated water, well water Crustacean Shell fish: Shrimp, Crabs, Crayfish, Prawns Others: Eel (raw), fishes (Raw unspecified type), turtle eggs |
| Hobbies | Ornamental aquariums Kayaking Sailing |
| International travel | China Japan Haiti Africa Asia Europe Latin America |
| Trauma | Lacerating and Penetrating injuries caused by Fish fin, Coral, Harpoon, Fish hook, river/sea/Ocean accident and fish net clearing |
our aforementioned statement as the potential pathogens emergence shows that it possess the CT and other virulent genes which may be associated with epidemic tendency. Some of the virulence factors are ctxAB (encoding the A and B subunits of CT), zot (encoding zonula occludens toxin), cep (encoding core-encoded pilin), ace (encoding accessory cholera enterotoxin), hap (haemagglutinin protease), orfU (encoding a product of unknown function) etc. The CT also consists of the repetitive sequence (RS) insertion element which directs integration of virulent genes. The core region of the toxin and the RS element makes up the cholera enterotoxin (Mekalanos et al. 2010; Li et al. 2003; Hlady and Klontz, 1996; Mekalanos, 1983). In addition, the classical and El Tor members of the V. cholerae possess diverse alleles within the rstR gene. A similar gene profile amongst the SAAT-1/139 members isolated from Calcutta shows two variants type of the rstR allele amongst the O139 strain from Calcutta and environmental rstR allele in environmental Non-O1/Non-O139 strains. Because of the variants found within the rstR allele and tendency to possess or harbor prophages (CTXφ), they are classified/biotyped as classical, Calcutta, ElTor, Bengal and/or environmental phage (Grim et al. 2010; Hsieh et al. 2008; Kimsey et al. 2004). Other classical virulence genes found in Non-O1/Non-O139 includes HA/P, HlyA, type III secretion system T3SS, type VI secretion system T6SS, NAG-stn and RTX toxin etc. Analysis of a purified HA/P gene isolated from SAAT-1/139 V. cholerae members shows its enterotoxigenic potential in rabbit ileal loop test (RILT). In addition, a heat stable enterotoxin gene (stn/sto) was also detected in the Non-O1/Non-O139 V. cholerae members called NAG-st which also mediate their enterotoxigenic potential (Makri et al. 2007; Purdy et al. 2005).

**Infections of NSAG/NAG-O1/O139**

**Gastroenteritis**

This is a disease condition which is associated with passage of watery stool and loss of fluid resulting from the ingestion of a cholera or cholera-like protein/toxin expressing bacterial. Patients with cholera exhibit symptoms of profuse watery diarrhea, vomiting and mild to severe dehydration (Mekalanos et al. 2012). This is characteristically seen in epidemic cholera cases implicated by O1 or O139 V. cholerae. Non-O1/Non-O139 V. cholerae which harbours this CT-toxin gene and/or produces similar cholerae seen in most diarrhea cases (Bi et al. 2001), but does not possess the epidemic cholera capabilities. It has been reported that some of the O1/O139-NAGVc members tend to harbour the epidermic associated genes of V. cholerae O1/O139 in the environment (Zmeter et al. 2018; Baig et al. 2018; Keckker et al. 2017; Engel et al. 2016).

**Septicaemia or bacteraemia and meningitis in neonate**

This is a bacterial in blood infection which is associated with NSAG/NAG-O1/O139 V. cholerae members, where the infected person does not present any diarrhea or vomiting symptoms as in the case of cholera (Hao et al. 2015). The pathogen infects blood and meninges of patients resulting remarkable weight loss, increased pulse (about 150 beats/mins), a temperature of 38–40 °C, abnormal white blood cell count (6.65 × 10⁹ L⁻¹), 67.1% neutrophils and an elevated level of C-reactive protein (CRP, 88.9 mg/L). It will also show a cerebrospinal fluid (CSF) WBC value ranging from 16.65-18.06 × 10⁹ with a protein level of 218.6 mg/dl (Schirmeister et al. 2014). Infants that express such symptoms appear to develop convulsion within 24 h, coagulopathy and metabolic acidosis. A cerebral CT scan of an infected neonate shows that the frontal and temporal lobe possess hemorrhagic focus with symmetric lesions (Hao et al. 2015; Namdari et al. 2000). Various reported cases of bacteraemia which are implicated by non-O1 V. cholerae infections results increased fatality especially amongst immune-compromised patients (Safrin et al. 1988; Hughes et al. 1978).

**Wound and soft tissue infection**

Members of the NSAG/NAG-O1/O139 are said to be associated with variety of extra-intestinal and soft tissue infections, including wounds, necrotizing fasciitis, skin, ear, urine, sputum and cerebrospinal fluid (CSF) (Hao et al. 2015; Feghali and Adib 2011; Aguinaga et al. 2009; Restrepo et al. 2006; Morris and Acheson 2003; Sharma et al. 1998). The symptoms produced by the potential pathogen may in most cases be very severe such that it mimics cholera (Piergentili et al. 1984). In some cases, the pathogen may be detected in Otitis media (bacterial infection of the ear) as observed by Keckker and his team (Keckker et al. 2017). In other related cases it is detected in skin lesion or infected liver disease and brain tissue (Hsieh et al. 2016; Deshayes et al. 2015; Khan et al. 2013; Inoue et al. 2012; Lai et al. 2012; Petsaris et al. 2010; Arnett et al. 2008).

**Other associated vibriosis**

Other related vibrioses caused by members of the Non-O1/Non-O139 V. cholerae are associated with the consumption of contaminated food materials (Kansas Disease Investigation Guidelines 2016). The V. cholerae non O1/O139 strains are occasionally isolated from cases of diarrhea (usually associated with consumption of fish, vegetables, Crab, shellfish...
etc.). In the environment, members of the O1/O139-NAGVc has been reported to be involved as contaminants of water bodies such as final effluents of wastewater treatment plants/systems (feWWTPs), rivers, recreational water bodies, irrigation canals, dams, as well as related water systems (Traore et al. 2014; Xing et al. 2013; Jones et al. 2013; Senderovich et al. 2010). Various investigators have at diverse study area and countries also associated clinical reports to contact with contaminated water systems (Zmeter et al. 2018; Keckker et al. 2017; Kumar et al. 2017; Thi et al. 2014; Lan and Love 2012; Ottaviani et al. 2009, 2011; Sujatha et al. 2011; Patel et al. 2009; Carvajal et al. 1998). Suffice to say that some of the investigators have isolated the potential pathogen from hospitalized patients whose disease case is traceable to contact with various receiving water sheds, creeks as well as other domestic water sources. Table 1 below shows some of the isolated strains/types by various investigators.

**Occurrence and persistence of NAG-O1/O139 Vibrio cholerae in the environment**

Members of the NSAG/NAG-O1/O139 have been shown to thrive mainly in the fresh water environment. The studies of Igbinosa et al. (2009) and Huq et al. (2012) has demonstrated that these Vibrio members are found attached to plankton, plankton associated water bodies, copepod and other zooplankton. Some of them exist in association with sediment, crab, shellfish etc. (Huq et al. 2012). This continuous persistence of the potential pathogen in water bodies as well as domestic water systems may nonetheless generate a negative impact on the management and control of the potential pathogen. It may also have a link with the observed difficulty in the eradication of cholera disease (Okoh et al. 2013; Valdespino and García-García 2011) in most affected regions. Ceccarelli and his group have observed the prevalence of SANAS-1/139 in environment with multiple pathogenic dynamics of either pandemic or epidemic relevance (Ceccarelli et al. 2015). Other vibriologists reported incidence of Non-O1/Non-O139 in plankton, copepod, rotifers, cladocerans and zooplankton (De Magny et al. 2011). It is envisioned that apart from the incidence of Vibrios/cholera outbreaks, there is paucity of experimental data/information to verify prevalence of the SANAS V. cholerae in estuaries and wastewater final effluents. The association of Vibrios/cholera with flood and other natural disaster is also an epidemiological concern (Bhuyan et al. 2016).

**Detection methods**

The detection of NSAG/NAG-O1/O139 V. cholerae members are subdivided into pathogen differential enumeration, presumptive identification from the environmental and/or clinical specimens using cultural characteristics, biochemical reaction, sugar fermentation, serological delineation and nucleic acid based confirmation using Polymerase Chain Reaction. These combined detection strategies are then divided in four protocols: Culture-based protocol, Serology protocol, biotype protocol and Nucleic acid based protocol which have been standardized overtime (CDC 2015; APHA 2012; ISO 2007). In outbreak situations, most of these tests should be avoided since a result needs to be confirmed to initiate treatment and report. However, according to Karaolis et al. (2001) and APHA (2012), the serology and biotype protocols are combined in commercial immunoassay test kits, somatic antigen serology of O1/O139 and hemolysis of sheep red cells which have been applied as diagnostic strategies of proven clinical relevance. The classical biotype members are negative to sheep cell hemolysis while the El Tor biotype members are positive to the sheep red cell hemolysis. Currently, there are other methods of detection which are not culture dependent. This is the Culture Indipendent Diagnostic tests (CIDTs).

**Immune-assay of the SANAS-1/139 V. cholerae pathogen**

Immuno-diagnostic detection of the SANAS-1/139 V. cholerae members in disease situation remains an unattended area of immediate concern in public health system. This is so, as there is only an applied and borrowed protocol from the parent pathogen V. cholerae O1/O139 due to limited technique, resources and neglect in report of observed cases both in rural, urban and continent alike. Most applied methods are based on reverse or negative detection of O1/O139 members. Other related detection methods are measurement of vibriocidal antibodies, antibody/antigen agglutination, indirect hemagglutination, reverse passive latex agglutination (RPLA), immunochromatographic test strips and enzyme-linked immunosorbent assay (ELISA) (Yamasaki et al. 2013; Keddy et al. 2013). Interests are on the rise on rapid detection of the SANAS V. cholerae with a view to determine point-of-occurrence cases of the potential pathogen and environmental monitoring/surveillance of V. cholerae. It is to this end that monoclonal fluorescent antibody technique and immunochromatographic dipsticks technique were applied for somatic antigen (LPS subunit) detection and serotyping (Chen et al. 2014; Goel et al. 2005; Nato et al. 2003). Further immunoassay techniques such as immunofluorescent-aggregation assay (IFAGA) were also designed for the detection of O1 and O139 V. cholerae in water bodies and the environment (Wang et al. 2010) but not the non-O1/non-O139 V. cholerae members.
**Culture-based protocol**

It employs the isolation in a selective media and standard bacteriological determination procedures.

**Isolation and detection of environmental SAAT/ SANAS type 1/139-Vc**

Standard Microbiological methods are being employed to a great extent in the isolation of the SANAS-1/139 members. As documented by Huq et al. (2012), standard must be ensured when isolating *Vibrio cholerae* from environmental and domestic water samples. This standard includes collection of water (source), plankton and plankton associated specimen in other to isolate a representative organism in nature from its immediate environment. These water specimen are processed by culturing onto any of Thiosulfate Citrate Bile-salts sucrose (TCBS) agar plates or *Vibrio CHROMagar* (VCA) or Tellurite taurocholate gelatin agar (TTGA) plates and incubate at 37 °C (Eddabra et al. 2011). It was also reported that there is need to subject specimen to varying culture techniques and incubation temperature. To enumerate presumptive pathogens, specimen is subjected to membrane filtration technique and 0.20 or 0.45 μm pore size filter membrane is cultured onto freshly prepared TCBS agar or orderwise and incubated at 37 °C for 24–48 h. Observed yellow culture growth are then purified and inoculated onto 20% glycerol stock and stored at -80 °C. Other post detection study may include subjection to a battery of biochemical reaction and sugar fermentation (Cowan and Steel 1974). Afterwards, the pure colonies are then subjected to an agglutination or serological test. The SANAS does not agglutinate with the somatic antisera (type-1 or type-139), hence it is selected as non-O1 and non-O139. The CDC (2014) has pointed out that some of these strains are not accessed and/or reported during outbreak related studies or updated surveillance studies. The relevance of such SANAS *V. cholerae* members which are neglected due to their presumed non-possession of virulent dynamics should also be assessed both those strains of clinical relevance, environmental relevance and industrial relevance alike.

**Isolation and detection of clinical SAAT and SANAS type 1/139**

Specimen of clinical sources should be analyzed after an appropriate observation and cross-examination of some suspected/expected diarrheogenic symptoms. Although, according to Blake and Weaver (1980), members of NSAG/NAG-O1/O139 *V. cholerae* and other related organisms, which do not have any epidemic/pandemic-related potential, should not be analyzed. Sufficient to say that such symptoms are peculiar with NAG-O1/O139 *V. cholerae* infections. Others may include mucus stools formation, abdominal cramps initiation, severe to mild fever depending on the immune-competence of the individual, blood type, etc. (Luo et al. 2013). Unlike the agglutinative strains of O1/O139 *Vibrio* members, the NSAG/NAG-O1/O139 *Vibrio* strains are regularly implicated in most invasive diseases (eg: septicemia) especially amongst immune-compromised patients (Luo et al. 2013; Rao and Surendran 2010; Lin et al. 1996). The specimen (fecal/stool, wound swab, exudates) are culture onto specialized selective media and cultures of positive growth are purified and stored as described earlier. According to Luo et al. (2013), No. 4 Agar may be used for isolation of clinical pathogens since it consists of additional inhibitory agents of clinical diagnostic relevance eg 0.003% rivano powder, 1% sodium citrate, 500 μg/L gentamicin, 0.2% sodium sulphite, 0.5% pig gall powder, 0.1% sodium lauryl sulphate, 0.001% potassium tellurite. It is also of specific note that not all clinical specimen habours choleragenic/toxigenic strains of *V. cholerae* and/or the toxigenic strains of NSAG/NAG-O1/O139 *V. cholerae*. Some of such clinical specimen may also harbour non-toxigenic strains. In a similar manner, the environment may also harbour both toxigenic/choleragenic and non-toxigenic/non-choleragenic strains. Most reported human clinical cases of Vibriosis associated with NSAG/NAG-O1/O139 clearly show that disease is initiated from ingestion of contaminated food material as well as sea food (Madhusudana and Surendran 2013). Other sources of contamination include domesticated use of contaminated environmental water, contact with carrier agents and consumption of contaminated shell fish (Rao 2015; Rao and Surendran 2010). In both clinical and environment sources, O1/O139 *V. cholerae* implication in infection cases has far been reported than cases of NSAG/NAG-O1/O139 *V. cholerae* members which belongs to higher numerical serogroups apart from O1 and O139 does not habour the cholera-related toxin gene (*ctx*) and the toxin co-regulated pilli (*tcp*). Hence some of these strains are not accessed and/or reported during outbreak related studies or updated surveillance studies.
cholerae members (CDC-COVIS 2017; Kaysner et al. 1987; Baumann et al. 1984). The study of various early investigators from countries such as Italy has shown high prevalence of other Non-O1/Non-O139 Vibrio strains (eg: O2, O6 O8, O40, O41, O64, and O107 and O158), with negative cholera toxin gene (Filetici et al. 1997). The situation today appears to differ as current reports show that Non-O1/Non-O139 Vibrio members are positive to cholera toxin (Kechker et al. 2017; Ceccarelli et al. 2015; Dutta et al. 2013; CDC, 2012; Hasan et al. 2012a,b). In addition, various strains have also been characterized as NSAG/NAG-O1/O139 V. cholerae, as some either lack the classical virulence determinants or possess the specific virulence factors. These strains include AM-19226, AM-15622, MZO-2, MZO-3, MO10 etc. (Dziejmen et al. 2005). Following these aforementioned evolving natures of V. cholerae members, various investigators in vibriology are currently generating research interest. Such interest is directed toward classifying V. cholerae as a subparent group in the family Vibrionaceae, while other members in the subgroup become sub-species.

Nucleic acid-based protocol

Till date, there are no specific Nucleic acid-based techniques applied for the detection of any member of the Non-O1/Non-O139 V. cholerae. Known procedures depend on the negative results from the O1 and O139 detection in both clinical and environmental samples. Although such procedures have survived the taste of time in terms of high sensitivity, specificity, and speed, it is based only on the detection of V. cholerae and the O1/O139 serogroups which use the conventional polymerase chain reaction (PCR) and/or quantitative real-time PCR (Wang et al. 2007; Tebbs et al. 2011; Teklehamanot et al. 2014; Rao 2015). The technique detects O1/O139 V. cholerae strains using 16S rRNA, specific gene for detection of the pathogen using the sob gene or the OmpW gene region and agarose electrophoresis of the amplified gene. Other nucleic acid based protocols are nested PCR, duplex PCR and multiplex PCR. Another protocol applied in the separation/detection and diversity study of the cholera toxin is pulsed-field gel electrophoresis (PFGE) (Kong et al. 2002; Tarr et al. 2007; Mendes et al. 2008; Keshav et al. 2010). However, nucleic acid based detection and characterization of pathogenic indices including the cholera toxin genes (ctxs) of V. cholerae are being evaluated by numerous investigators (Ceccarelli et al. 2015; Luo et al. 2013; Son et al. 2011; Lipp et al. 2003; Blackstone et al. 2007). In the environment, some of these investigators are interested in developing rapid detection methods for the detection of the pathogen. Currently, a developed nucleic acid-sensing dais for choleragenic O1/O139 V. cholerae detection of high specificity and sensitivity is being applied. Such method applies the use of an electrochemical gene sensor and dry reagent or in-silico-based nucleic acid amplification to detect specifically multiple targeted gene of interest (Yu et al. 2015). This nucleic acid based dais protocol can be harnessed in the detection of other Non-O1 and Non-O139 members of the V. cholerae. Other related hetero-genetic detection of NSAG/NAG-O1/O139 V. cholerae involves virulence or toxigenic/choleragenic genes and in the expression of somatic antigen unidentifiable types or O-untypeable strains (Octavia et al. 2013; Dutta et al. 2013). It is important to note that some strains of NSAG/NAG-O1/O139 V. cholerae recently isolated and identified from fecal specimen in Kolkata, Argentina and Bangladesh shows non-relation with the identity of already known 230 V. cholerae strains, hence they are called O-untypeable strains. Typing and fingerprinting the current heterogeneity relies on molecular typing protocols which includes Restriction Fragment Length Polymorphism (RFLP), Repetitive Element Primed PCR (rep-PCR), Repetitive Extragenic Palindromic-PCR (REP-PCR), Randomly Amplified Polymorphic DNA, Enterobacterial Repetitive Intergenic Consensus sequence PCR (ERIC-PCR), Ribotyping of heterogenic genes, BOX-PCR (the Box-A derived element PCR), Pulsed Field Gel Electrophoresis (PFGE), Amplified Fragment Lenght Polymorphism (AFLP) and the application of Next Generation Sequencing (NGS) for Whole Organismal Genome Sequence Typing (WOGST). Results generated from such fingerprinting analysis would provide needed information of epidemiological, environmental, clinical and sub-specie relevance. It will also help in determining the distribution of virulent genes amongst the NSAG/NAG-O1/O139 V. cholerae members (Tindall et al. 2010).

Restriction fragment length polymorphism (RFLP)-polymerase chain reaction PCR

Restriction Fragment Length Polymorphism which is pronounce riflip or RFLP is a nucleic acid based protocol which detect differences in homologous DNA sequences. It is a technique that employs the digestion of a specimen amplified DNA using known endonuclease or DNA scissor to cut at specific regions of the sampled DNA which eventually produce varying fragments of differential sizes. The cut/fragmented or restricted DNA specimen is then separated by agarose gel electrophoresis. This method has been applied in various Vibrio studies to delineate closely related biotypes, serogroups and phage-types (Chowdhury et al. 2010; Saha et al. 2006). It involves the PCR amplification of a specific gene of interest (eg gyrB, tcp, ctxA etc.) in a specimen extracted DNA and treatment with the endonuclease to fragment into varying/polymporphic sizes. Its banding profile is thereafter detected by electrophoresis and ultra-violet visual system (Urakawa et al. 1997). This method has been applied...
in differentiating *Vibrionaceae* (Ura kawa et al. 1997) members especially those of high clinical relevance.

**Random amplified polymorphic DNA (RAPD) with PCR**

Random amplified polymorphic DNA is generally called RAPD which is a nucleic acid based technique that involve amplification of multiple target sites using a short arbitrary single oligonucleotide primer. The primer is synthesized to amplify multiple non-allelic regions of a sampled DNA (Williams et al. 1990). The band size profile and differences in amplified region sizes were used to delineate various organisms both at intra or inter specie level and also determine their polymorphism. It has been applied in the polymorphic studies of *Vibrio* (Sadok et al. 2013; Marhual et al. 2012; Maiti et al. 2009; Welsh and McClelland 1993).

**Repetitive extragenic palindromic PCR (REP-PCR)**

The Repetitive Extragenic Palindromic- PCR (REP-PCR) was initially described as regulatory palindromic sequences of untranslated operon regions which is use in epidemiological relationship study. Its applicability is based on the protocol and primers ability to form structures of stable stem-loop in any transcribed or extracted RNA (Higgins et al. 1982). It consists of about 38 bp consensus sequence which has six degenerate regions and 5 bp variable loop between the palindromes which are associated with transcription, termination, chromosome organization and mRNA stability (Versalovic et al. 1991; Stern et al. 1984). This technique has be applied in typing various members of the *Vibrionaceae* eg *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* etc. (Balcazar et al. 2014; Sarita 2014; Sellek et al. 2012; Tulatorn et al. 2018) after specific amplicon size of 59 bp, 45 bp and 50 bp (Linda and C). Each of the BOX AIR repeat sequence produces specific allele of enterobacterial repetitive intergenic consensus (ERIC) sequences which produce multiple targets of enterobacterial during PCR amplification. (Bachellier et al. 1999; Hult on et al. 1991). ERIC-PCR would produce a genetic profile of sampled genomic DNA with regions around ERIC elements and other repeated short DNA sequences. The profile of genetic composition which is produced after electrophoresis may be used to determine relationship and polymorphism amongst sampled DNA. The profile thus produced consists of short base pair regions (126 bp) that appear to be either intergenic regions of polycistronic operons. It’s sequences may also appear to be untranslated regions within the open reading frames (Rodriguez et al. 2006). Some of these regions are novel, homologous and highly conserved non-specie related hemolysin gene (Hulton et al. 1991). ERIC PCR has been successfully applied in the typing of *Vibrio* species and other bacterial strain (Sawabe et al. 2002; Rivera et al. 1995). ERIC studies on *Vibrio* members have shown evident polymorphism in genotypes which is associated with the level of rain fall (Goel and Jiang, 2011). Other recent reports on the application of ERIC-PCR on *V. cholerae* also show great diversity in genomic expression. It also indicates a discriminating potential and lesser complexity of ERIC. (Waturangi et al. 2012).

**Amplified fragment length polymorphism (AFLP)**

Amplified fragment length polymorphism also called (AFLP) is referred to as another highly sensitive nucleic acid based technique used to identify differences in DNA after treatment with endonuclease. Its basic steps include the use of adaptor complementary primers, extraction of specimen DNA, amplification of DNA, ligation of adaptors to fragmented sticky/blunt ends, amplification of fragments and sequencing of amplified fragments. These fragments are then visualized in a UV-transilluminator or gel documentation system after separation on an agarose gel electrophoresis. Polymorphic genetic elements are then detected from the AFLP profile as relationship and deviation are observed by size of specimen amplified region. The application of this technique in vibriology by various researchers and the discriminating potential of the method has made various investigator to suggest its use in *Vibrio* studies especially amongst the 7PETs (Mishra et al. 2011; Gomez-Gil et al. 2004; Lan and Reeves 2002; Jiang et al. 2000).

**Enterobacterial repetitive intergenic consensus (ERIC) sequences**

This is a molecular typing protocol which is based on the application of enteric-bacterial repetitive intergenic consensus oligonucleotide primers for detection of related genes and associated gene patterns. These short primer (126 bp) families are highly conserved sets of oligonucleotide sequences which produce multiple target allele of enterobacterial during PCR amplification. (Bachellier et al. 1999; Hulton et al. 1991). ERIC-PCR would produce a genetic profile of sampled genomic DNA with regions around ERIC elements and other repeated short DNA sequences. The profile of genetic composition which is produced after electrophoresis may be used to determine relationship and polymorphism amongst sampled DNA. The profile thus produced consists of short base pair regions (126 bp) that appear to be either intergenic regions of polycistronic operons. It’s sequences may also appear to be untranslated regions within the open reading frames (Rodriguez et al. 2006). Some of these regions are novel, homologous and highly conserved non-specie related hemolysin gene (Hulton et al. 1991). ERIC PCR has been successfully applied in the typing of *Vibrio* species and other bacterial strain (Sawabe et al. 2002; Rivera et al. 1995). ERIC studies on *Vibrio* members have shown evident polymorphism in genotypes which is associated with the level of rain fall (Goel and Jiang, 2011). Other recent reports on the application of ERIC-PCR on *V. cholerae* also show great diversity in genomic expression. It also indicates a discriminating potential and lesser complexity of ERIC. (Waturangi et al. 2012).

**BOX-PCR**

BOX AIR elements are multiple, conserved and repetitive DNA sequences which are present in bacterial genome (Gram positive and Gram Negative). There are sometimes referred to as variable number of tandem repeat with sequences that are both specie specific, virulent related and regulatory DNA regions. Earlier reports shows that they were first observed in Gram positive organism (*Streptococcus* specie) with three specific boxes (Box A, Box B, Box C). Each of the BOX AIR repeat sequence produces specific amplicon size of 59 bp, 45 bp and 50 bp (Linda and Sarita 2014; Sellek et al. 2012; Tulatorn et al. 2018) after PCR amplification. These amplicons are produced from the region around BOX AIR element as well as inverted repeat sequences (154 bp) that occur between 12 and 25 amplicons (Tulatorn et al. 2018). The profile produced by the primer
sequences can be interpreted and applied in the classification of sampled organism and used as a tool in the study of pathogenic dynamism (Ateba and Mbewe 2013). It is used in inter-specie and intra-specie clustering and recently in pathogen source tracking (PST) (Yang and Yen 2012; Johnson et al. 1992a,b,c, 2004). It is also applied in the genealogy of environmental microbes since it is less cost effective and fast in determination with no advance knowledge, negative influence of aged culture and computer assisted methods (Kang and Dunne 2003; Tuang et al. 1999). Although the technique may be limited in consistency of reproducible results, banding patterns has remained constant (Yang and Yen 2012). The study of Marques et al. (2008) reported the application of the technique in delineating genotypic species of Pseudomonas syringae while other investigators applied in racial differentiation, biotypes of Ralstonia solanacearum and typing of Aeromonas members (Taca et al. 2005; Galal et al. 2003; Paola et al. 1984). In virobiology, epidemiological relationship of clinical strains and environmental strains are studied amongst choleragenic V. cholerae O1 members (Goel et al. 2010; Kumar et al. 2009; Tulatorn et al. 2018) and other non-cholera Vibrio members (Sellek et al. 2012; Rameshkumar et al. 2011; Gordan 2008; Maluping et al. 2008; Rameshkumar and Nair 2007; Gomez-Gil et al. 2004). It may also be applicable in the diversity study of SAAT-1/139 members in a polyphasic molecular approach for novel strains.

Occurrence of pathogen in the environment (waste and domestic water)

Although various investigators of Vibrio members have observed and reported numerous cases of V. cholerae occurrence in different estuarine environments, plankton, crab, fish, shellfish, ready-to-eat foods and vegetables, most of their report has shown a high prevalence of Non-O1/Non-O139 V. cholerae in these specimen than the choleragenic O1 Vibrio members (Fri et al. 2017; Deshayes et al. 2015; Trubiano et al. 2014; Yadava et al. 2013; Rashid et al. 2013; Hasan et al. 2012b, c; Kwon 2010). NSAG/NAG-O1/O139 V. cholerae members have occurred and persisted in various geographical regions as either free living or in association with other organisms such as mollusces, copepod, zooplankton and sponges (Blackwell and Oliver 2008). Their survival and persistence in the environment are influenced by atmospheric pressure, oxidation reduction potential, temperature, total chlorine, free/residual chlorine and salinity (Owoseni et al. 2017; Beaz-Hidalgo et al. 2010, Ighinosoa et al. 2009). Some of these environmental dynamics also ensure its formation of viable but not culturable (VBNC) state, rugose type, smooth types and biofilm formation (Visick 2009; Yildiz and Visick 2009; Asakura et al. 2007; Oliver et al. 2005; De et al. 2004; Islam et al. 2004; Wong and Wang, 2004). It was also observed that the cholera toxin (CT) and tcp gene were present in some Non-O1/Non-O139 members isolated from the environment which indicated their epidemic causing tendency and ability to cause cholera/diarrhea/diarrhea-related diseases as well as other extra-intestinal infections (Feghali and Adib 2011; Restrepo et al. 2006; Park et al. 2004; Morris and Acheson 2003; Sharma et al. 1998). This was also previously reported in Calcutta, India by Sharma and his group and recently by Madhusudana and Surendran (2013). The SANAG-1/139 V. cholerae have been implicated in sporadic epidemic and marine associated infection in crustacean, fishes, shell fish, sediments etc. (Raghu and Bhat 2011; Frans et al. 2011; Ji et al. 2011; Raissy et al. 2011; Soto-Rodriguez et al. 2010; Alagappan et al. 2010; Beaz-Hidalgo et al. 2010; Pal and Das, 2010).

Another area of the SANAG-1/139 V. cholerae studies of salient documentation is their diversity. The diversity of the SANAG-1/139 V. cholerae has also been a concern especially environmental strains which have been reported to be implicated in current human related gastritis, septicaemia, meningitis, wound or soft tissue infection as well as diarrhea (Madhusudana and Surendran 2013).

SANAG-1/139 association with planktons

Huq and other investigators have pointed that SANAG-1/139 Vibrio association with other environmental organisms (plankton) is noteworthy in the occurrence and their persistence in the environment (Huq et al. 2005; Grossart et al. 2005; Lipp et al. 2003). Planktons (Phytoplankton and zooplankton) are categorized as marine floating organisms whose products bacterial recognizes, source for livelihood and tends to associate, with a bid to survive within the marine environment (Long et al. 2005; Thompson et al. 2004). Planktons may serve as a vehicle for transmission or spread of pathogens as pointed by various investigators (Rehnstam-Holm et al. 2010; Huq et al. 2005).

Biofilm formation

Vibrio cholerae survival in the environment is associated with ability of the flagella to aid movement and adapt in changing environmental situation (Beshiru and Igbinoso, 2018; Odjadjare et al. 2012; Broberg and Calder 2011). These mobile microbial structures are mainly of two categories which are the polar flagellum and the lateral flagellum. Whilst polar flagella ensures swimming, the lateral flagella alines with other genes for biofilm formation and cellular transformation (Wang et al. 2015). Biofilm is a cell-surface associated matrix, it is refered to as a bacterial community
were in a bid to survive harsh conditions utilize trapped/absorbed nutrients to form protective coat using structural genes and regulatory appendages (Yildiz and Visick 2009; Wai et al. 1999). These genetic appendages are exopolysaccharide biosynthetic gene, lateral flagella and pili gene, c-di-GMP signaling gene, quorum sensing gene and other component regulatory genes (Yildiz and Visick 2009; Snoussi et al. 2008). This is similar to the characteristics exhibited by Vibrio members as they try to survive enhanced environmental stress, antibacterial and depleted nutrient survival. The activity also favors the inter-relationship of Vibrio members with other bacteria (Yildiz and Visick 2009; Visick 2009) as the biosynthesis of exopolysaccharide occurs and flagellar/fimbrial alteration develops to form a three dimensional structure dubbed biofilm (Fong et al. 2010). V. cholerae has shown rigid biofilm formation, a characteristic that is traced to its environmental stress survival. Such survival has also be reported to have been facilitated by biofilm formation especially in other Vibrio members (V. parahaemolyticus, V. fischeri and V. alginolyticus) in the environment (Beshiru and Igbinosa 2018; Chavez-Dozal and Nishiguchi 2011). Currently, Vibrio investigators have purified an antibiofilm activity bioagent (exopolysaccharide A101) from culture supernatant of some marine Vibrio members. Some of its activities act by inhibiting both biofilm formation and disruption of biofilm establishment (Jiang et al. 2011). Other antibiofilm biomolecule produced is exopolysaccharide QY101, which has similar activity as exopolysaccharide A101. It also eliminate V. cholerae cells from surface material serving as a protection of material and deprivation of Vibrio survival (Jiang et al. 2011; Matz et al. 2005).

**Multiple antibiotic resistance amongst SAAT/SANAS-1/139**

Regular exposure of Vibrio strains to harsh environmental condition as well as antibiotics has been associated with the incidence of multiple drug resistant strains of SANAG-1/139 V. cholerae. The use of residual chlorine in wastewater treatment facilities and other chemical elements has also been associated with the incidence of drug resistance since bacterial now possess tolerance to chlorine treatment in waste water (Owoseni et al. 2017; Owoseni and Okoh 2017a,b). Such resistance acquisition is evident amongst NSAG/NAG-O1/O139 V. cholerae members since they are residential floral of the environment and wastewater (Ojadjare et al. 2012). The study of Chandrasekhar and his group showed that somatic antigen non-agglutinating strains of 1/139 V. cholerae isolated from the environment in Hubli, India are resistant to Fluroquinolone antibiotic members (Chandrasekhar et al. 2008). A similar antibiotic resistant profile was also found amongst O1 V. cholerae members to ciprofloxacin on isolates from Bangladesh (Kim et al. 2010). Isolates of NSAG/NAG-O1/O139 or non-O1/non-O139 V. cholerae collected from the Kerala Environment also shows multiple antibiotic resistance at varying degree to various antibiotics eg; nalidixic acid, chloramphenicol, cefotaxime, Streptomycin, trimethoprim, tetracycline, cotrimoxazole, neomycin, ofloxacin, furazolidone, norfloxacin, ciprofloxacin, gentamicin and spectinomycin (Bernard 2006; Jagadeeshan et al. 2009). Other multiple antibiotic resistance reported amongst the V. cholerae members were found in the El Tor biotype (Goel et al. 2010) which is still increasing.

**Future prospects**

The clinical, environmental and epidemiological relevance of somatic antigen NAGVc-1/139 or SANAG-1/139 V. cholerae members are today receiving low attention yet reports of its implication in disease cases are on the increase. Various investigators have reported the possible change in the LPS gene structure of V. cholerae members which portend a future association with epidemic outbreaks. Suffice to say that various previous observations have shown that SANAG-1/139 V. cholerae strains possess in their LPS gene structure a tendency for gene duplication which in the future may enhance epidemic and increase pathogenesis. It is also suggestive that future strains associated antigenic lineage may occur as the changing tendency continues in successive outbreak endemic areas. These possible future occurrences pose threat to the management and control of the pathogen if blind research focus and interest is given to the study of the NSAG/NAG-O1/O139 V. cholerae members. The rapid detection of the NSAG/NAG-O1/O139 V. cholerae strains is another area with paucity of reports in vibriology. The reports that the LPS subunits of the somatic antigen consists of sugar and aminoglycosides (such as glucose, glycero-D-manno-heptose fructose, fucosamine, quinovosamine, galactosamine, glucosamine and 2,4-Diacetyl-amido-2,4,6-trideoxyglucose {QuiNAc4NAc}) is another area that has not been harnessed. It has been shown that the serogroup classification of the various NSAG/NAG-O1/O139 Vibrios was informed by the somatic antigen subunit sugars and aminoglycosides. We are optimistic that in the future, if these sugars and aminoglycosides are well studied, a prototype of serogroup antisera for the rapid differentiation of the various NSAG/NAG-O1/O139 V. cholerae members would have been designed and applied. This should be a roadmap to the differentiation and identification of the various serogroup members as well as delineating their pathogenic potential and/or implication in outbreak cases. Other studies on the molecular weight nature of the capsules would also be differentiated using various genetic techniques such as Sodium deodecylSulfate-polyacrylamide gel electrophoresis.
Conclusion

The clinical relevance of NSAG/NAG-O1/O139 *V. cholerae* members has remained unclear since most of its members are neglected in the field and/or research driven interest. Standard microbiological, molecular and serological methods for detecting these potential outbreak causing pathogens in the environment are also limited. A possible future direction may be a redirected attention as well as research driven interest on these members and their respective pathogenic potential using effective molecular biology, microbiological protocols and Molecular typing methods. These methods should also employ a well-structured novel technology as an environmental monitoring strategy toward enhancing the detection of the pathogen since they are residential floral of the estuaries and coastal region. A novel technique which may be use for pathogen enumeration, serotyping using the various surface sugar and aminoglycosides to differentiate the members and application of “DNA chip” that will structurally bind to both mobile DNA as well as environmental bacterial of the group be employed. In addition to this, countries and specific health organizations in endemic and pandemic regions of cholera should encourage reporting cases of O1/O139-NAGVc members in any observed region. An awareness campaign and peer educator program must be initiated with a view to eliminating related *Vibrio* pathogens. The application of these relevant advance methods may also provide result to the detection of Viable but Non-culturable strains (VBNC) of the *Vibrio* members which are posing threat to the environment. It will also describe a model for the resuscitation, enumeration and detection of the NSAG/NAG-O1/O139 Vibrio members in aquatic environment. The poor understanding and dynamics for the serological detection of non-O1/non-O139 *Vibrio cholerae* and the standard method for detecting NSAG/NAG-O1/O139 members has presented this emerging pathogenic group as public health risk associated. A risk situation such that, if not properly handle as early as possible would result a world outbreak situation of cholera. Although the point of discuss that NSAG/NAG-O1/O139 members may play an incidental role in re-establishing epidemic *V. cholerae* O1 strain has not been justified, it is envisaged that future position of the potential pathogen is not far from such. We believe that a more intense research driven studies be conducted on the emerging pathogen. This strategic intervention study would specifically focus on the environmental resident etiologic agent known as NSAG/NAG-O1/O139 *V. cholerae* members. A special interest should be directed at those members with the choleraigen (ie the *ctx*-gene, *tcp*-gene, *cep*-gene, *ace*-gene etc.) and the epidemic associated virulence genes. These are some of our ongoing research, interest and study in our laboratory. The clonal association of these NSAG/NAG-O1/O139 members in the *V. cholerae* study is another study area which we are exploring with a view to understanding the dynamism and relationship of the *V. cholerae* strains. Understanding these will enhance current surveillance of the emerging pathogen to provide the adequate management of infection cases.

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Data availability  The datasets as well as results presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest  The authors declared no conflict of interest.
Ethical approval This study reviewed the emerging nature of some neglected strains of Vibrio members which are genealogically and phylogenetic related to the somatic antigen agglutinatable type Vibrio cholerae of 1/139 (SAAT-Vc-1/139) members or O1/O139 Vibrio cholerae somatic antigen agglutinatable strains of 1/139 Vibrio cholera. These strains are the somatic antigen non-agglutinatable strains of 1/139 Vibrio cholerae (SANAS-Vc -1/139) or O1/O139 non-agglutinating Vibrio cholerae (O1/O139-NAG-Vc).

Consent to participate There was no clinically associated specimen hence ethical concerns, consent and approval were not necessary.

Consent for publication The authors of this manuscript have read, correct and agreed on the final copy reports contained in this study.

References

Aguinaga A, Portillo ME, Yuste JR, del Pozo JL, García-Tutor E, Pérez-Gracia JL, Leiva J (2009) Non-O1 Vibrio cholerae inguinal skin and soft tissue infection with bullous skin lesions in a patient with a penis squamous cell carcinoma. Ann Clin Microbiol Antimicrob 8(1):1–4

Aguirre-López M, Sánchez IP, Arias AA, Giraldo ML, Velásquez-Velásquez EM, Franco JL, Trujillo-Vargas CM (2017) Neonatal screening for congenital lymphopenia and other rare diseases in the world. Rev Esp Pediatr [internet] 73(2):61–74

Alagappan KM, Deivasigamani B, Somasundaram ST, Kumaran S (2010) Occurrence of Vibrio parahaemolyticus and its specific phages from shrimp ponds in east coast of India. Curr Microbiol 61(4):235–240

Albuquerque A, Cardoso H, Pinheiro D, Macedo G (2013) Vibrio cholerae non-O1 and non-O139 bacteremia in a non-traveler Portuguese cirrhotic patient: first case report. Gastroenterol Hepatol 36:309–310

Ali M, Nelson AR, Lopez AL, Sack DA (2015) Updated global burden of cholera in endemic countries. PLoS Negl Trop Dis 9(6):e0003832. https://doi.org/10.1371/journal.pntd.0003832

Alphonsa VJ (2013) PhD thesis titled characterization and pathogenicity of Vibrio cholerae and Vibrio vulnificus from marine environments

APHA, AWWA, WEF (2012) Standard methods for examination of water and wastewater, 22nd edn. American Public Health Association, Washington

Arnett MV, Fraser SL, McFadden PE (2008) Non-O1 Vibrio cholerae epideral brain infection in a 12-year-old boy after a depressed skull fracture. Pediatr Infect Dis J 27(3):284–285

Asakura H, Ishiwa A, Arakawa E, Makino SI, Okada Y, Yamamoto S, Igimi S (2007) Gene expression profile of Vibrio cholerae in the cold stress induced viable but nonculturable state. Environ Microbiol 9(4):869–879

Ateba CN, Mbewe M (2013) Determination of the genetic similarities of fingerprints from Escherichia coli O157: H7 isolated from different sources in the North West Province, South Africa using ISR BOXAIR and REP-PCR analysis. Microbiol Res 168(7):438–446

Aydianian A, Tang L, Morris JG, Johnson JA, Stine OC (2011) Genetic diversity of O-antigen biosynthetic regions in Vibrio cholerae. Appl Environ Microbiol 77:2247

Aydianian A, Tang L, Chen Y, Morris JG Jr, Olsen P, Johnson JA, Stine OC (2015) Genetic relatedness of selected clinical and environmental non-O1/O139 Vibrio cholerae. Int J Infect Dis 37:152–158

Bachellier S, Clement JM, Hofnung M (1999) Short palindromic repetitive DNA elements in enterobacteria: a survey. Res Microbiol 150(9):627–639

Baig MZ, Abdullah UH, Shaqruit Y, Humayun KN, Zafar A (2018) Non O1, non O139 Vibrio cholerae bacteraemia in an infant; case report and literature review. JPMA J Pak Med Assoc 68(4):650–652

Balcazar JL, Loureiro S, Da Silva YJ, Pintado J, Planas M (2010) Identification and characterization of bacteria with antibacterial activities isolated from seahorses (Hippocampus guttulatus). J Antimicrob Chemother 63(5):271–274

Basu A, Garg P, Datta S, Chakrabarty B, Bhattacharya T, Khan A, Nair GB (2000) Vibrio cholerae O139 in Calcutta, 1992–1998: incidence, antibiograms, and genotypes. Emerg Infect Dis 6(2):139

Bauer A, Östensvik Ö, Florvåg M, Ørmen Ø, Ørvik LM (2006) Occurrence of Vibrio parahaemolyticus, V. cholerae, and V. vulnificus in Norwegian Blue Mussels (Mytilus edulis). Appl Environ microbiol 72(4):3058–3061

Baumann P, Furniss AL, Lee JV (1984) Vibrio. In: Kreig NR, Holt JG (eds) Bergey’s manual of systematic bacteriology. Williams and Wilkins Co., Baltimore, pp 518–538

Beaz-Hidalgo R, Balboa S, Romalde JL, Figueras MJ (2010) Diversity and pathogenicity of Vibrio species in cultured bivalve molluscs. Environ Microbiol Rep 2(1):34–43

Bennett SD, Otieno R, Ayers TL, Odhiambwo A, Faith SH, Quick R (2015) Acceptability and use of portable drinking water and hand washing stations in health care facilities and their impact on patient hygiene practices, Western Kenya. PloS One 10(5):e0126916

Bernard RSW (2006) Studies on ribotyping, integron genes and pathogenicity of marine Vibrios. PhD Thesis, Cochin University of Science and Technology, India.

Beshiru A, Igbinoso EO (2018) Characterization of extracellular virulence properties and biofilm-formation capacity of Vibrio species recovered from ready-to-eat (RTE) shrimps. Microb Pathog 119:93–102

Bhattacharya T, Chatterjee S, Maiti D, Bhadra RK, Takeda Y, Nair GB, Nandy RK (2006) Molecular analysis of the rstR and orfU genes of the CTX prophages integrated in the small chromosomes of environmental Vibrio cholerae non-O1, non-O139 strains. Environ Microbiol 8(3):526–634

Bhuyan SK, Vairelle MG, Arya N, Yadav P, Veer V, Singh L, Yadava PK, Kumar P (2016) Molecular epidemiology of Vibrio cholerae associated with flood in Brahmaputra River valley, Assam, India. Infect Genet Evol 40:352–356

Bi K, Miyoshi SI, Tomochika KI, Shinoda S (2001) Detection of virulence associated genes in clinical strains of vibrio mimicus. Microbiol Immunol 45:613

Bik EM, Bunschoten AE, Gouw RD, Moodi FR, Imbro P, DePaola A (2007) Use of a real time PCR assay for detection of the ctxA gene of Vibrio cholerae O139 strains: evidence for horizontal transfer of genes involved in polysaccharide synthetase. EMBO J 14:209–216

Blackstone GM, Nordstrom JL, Bowen MD, Meyer RF, Imbro P, DePaola A (2007) Use of a real time PCR assay for detection of the ctxA gene of Vibrio cholerae in an environmental survey of Mobile Bay. J Microbiol Methods 68(2):254–259

Blackwell KD, Oliver JD (2008) The ecology of Vibrio vulnificus, Vibrio cholerae and Vibrio parahaemolyticus in North Carolina estuaries. J Microbiol 46(2):146–153

Blake PA, Weaver RE, Hollis DG (1980) Diseases of humans (other than cholera) caused by Vibrios. Annu Rev Microbiol 34(1):341–367

Blokesch M, Schoolnik GK (2007) Serogroup conversion of Vibrio cholerae in aquatic reservoirs. PLoS Pathog 3(6):e81
Broberg CA, Calder TJ, Orth K (2011) Vibrio parahaemolyticus cell biology and pathogenicity determinants. Microbes Infect 13(12–13):992–1001
Buck JD, McCarthy SA (1994) Occurrence of non-O1 Vibrio cholerae in Texas Gulf Coast dolphins (Tursiops truncatus). Lett Appl Microbiol 18(1):45–46
Buck JD, Wells RS, Rhinehart HL, Hansen LJ (2006) Aerobic microorganisms associated with free-ranging bottlenose dolphins in coastal Gulf of Mexico and Atlantic ocean waters. J Wildl Dis 42(3):536–544
Cariri FA, Costa AP, Melo CC, Theophilo GN, Hofer E, de Melo NOP, Leal NC (2010) Characterization of potentially virulent non-O1/non-O139 Vibrio cholerae strains isolated from human patients. Clin Microbiol Infect 16:62–67
Carvajal GH, Sanchez J, Ayala ME, Hase A (1998) Differences among marine and hospital strains of Vibrio cholerae during Peruvian epidemic. J Gen Appl Microbiol 44(1):27–33
CDC (1996) Case definitions: CDC division of public health surveillance and informatics. Available at: www.cdc.gov/nndss/
CDC (2011a) National enteric disease surveillance: COVIS annual summary, 2011a. http://www.cdc.gov/nccezid/dfwed/edebrerports.html. Accessed 9 Nov 2013
CDC (2011b) Vital Signs: incidence and trends of infection with pathogens transmitted commonly through food—foodborne diseases active surveillance network 10 U.S. sites 1996–2010. Morb Mortal Wkly Rep 60(22):749–755
CDC (2012) Cholera and other vibrio illness surveillance system. http://www.cdc.gov/nationalsurveillance/cholera_vibrio_surveillance.html. Accessed Sept 2013
CDC (2013) Incidence and trends of infection with pathogens transmitted commonly through food—foodborne diseases active surveillance network 10 U.S. sites 1996–2012. Morb Mortal Wkly Rep 62(15):283–287
CDC (2014) National enteric disease surveillance: COVIS annual summary; Centers for disease control and prevention. Department of Health and Human Services, Atlanta, GA, USA
CDC (2017) Centers for disease control and prevention (CDC), national enteric disease surveillance: COVIS annual summary, 2017. https://www.cdc.gov/nationalsurveillance/pdfs/covis-annual-summary-2017–732c.pdf. Accessed on 31 Oct 2017
Ceccarelli D, Chen A, Hasan NA, Rashid SM, Huq A, Colwell RR (2015) Vibrio cholerae non-O1/non-O139 carrying multiple virulence factors and V. cholerae O1 in the Chesapeake Bay, Maryland. Appl Environ Microbiol. 81(6):1909–1918
Centers for Disease Control and Prevention (2010a) Update on cholera—Haiti, Dominican Republic, and Florida. MMWR Morb Mortal Wkly Rep 59:1637–1641
Centers for Disease Control and Prevention (2010b) Update: outbreak of cholera—Haiti, 2010. MMWR Morb Mortal Wkly Rep 59:1586–1590
Centers for Disease Control and Prevention (2012a) National enteric disease surveillance: COVIS annual summary, 2012. US Department of Health and Human Services, Atlanta, GA
Centers for Disease Control and Prevention (CDC) (2012b) Cholera and other vibrio illness surveillance overview. Atlanta, Georgia: US Department of Health and Human Services, CDC, http://www.cdc.gov/nccezid/dfwed/PDFs/nat-covis-surr-overview-508e.pdf. Accessed 1 Nov 2013
Centers for Disease Control and Prevention (CDC) (2015) CDC laboratory methods for the diagnosis of Vibrio cholerae. Laboratory methods for the diagnosis of Vibrio cholerae. Ch 6. Atlanta: CDC. Available from: http://www.cdc.gov/cholera/pdf/Laboratory-Methods-for-the-Diagnosis-of-Vibrio-cholerae-chapter-6.pdf. Accessed 13 Apr 2016

Centers for disease control and prevention (2017). Cholera in Africa. Available online: https://www.cdc.gov/cholera/africa/index.html. Accessed on 27 Aug 2017
Chandrasekhar MR, Krishna BV, Patil AB (2008) Changing characteristics of Vibrio cholerae: emergence of multidrug resistance and non-O1, non-O139 serogroups. Southeast Asian J Trop Med Public Health 39(6):1092–1097
Chatterjee SN, Chaudhuri K (2003) Lipopolysaccharides of Vibrio cholerae: I. Physical and chemical characterization. Biochim Biophys Acta (BBA)-Mol Basis Dis 1639(2):65–79
Chatterjee SN, Chaudhuri K (2004) Lipopolysaccharides of Vibrio cholerae: II. Genetics of biosynthesis. Biochim Biophys Acta (BBA)-Mol Basis Dis 1690(2):93–109
Chatterjee T, Mukherjee D, Dey S, Pal A, Hoque KM, Chakrabarti P (2011) Accessory cholera enterotoxin, Ace, from Vibrio cholerae: structure, unfolding, and virstatin binding. Biochemistry 50(14):2962–2972
Chavez-Dozal A, Nishiguchi MK (2011) Variation in biofilm formation among symbiotic and free-living strains of Vibrio fischeri. J Basic Microbiol 51(5):452–458
Chen Y, Johnson JA, Pusch GD, Morris JG, Stine OC (2007) The genome of non-O1 Vibrio cholerae NRT365 demonstrates the presence of pathogenic mechanisms that are distinct from those of O1 Vibrio cholerae. Infect Immun 75(5):2645–2647
Chen W, Zhang J, Lu G, Yuan Z, Wu Q, Li J, Zhang J (2014) Development of an immunochromatographic lateral flow device for rapid diagnosis of Vibrio cholerae O1 serotype Ogawa. Clin Biochem 47(6):448–454
Chen YT, Tang HJ, Chao CM, Lai CC (2015) Clinical manifestations of non-O1 Vibrio cholerae infections. PLoS One 10(1):e0116904
Choi SM, Lee DG, Kim YH, Kim YJ, Lee S, Kim CC (2003) Bacteremic cellulitis caused by non-O1, non-O139 Vibrio cholerae in a patient following hematopoietic cell transplantation. Bone Marrow Transplant 31(12):1181–1182
Chokesajjawatee N, Zo YG, Colwell RR (2008) Determination of clonality and relatedness of Vibrio cholerae isolates by genomic fingerprinting, using long-range repetitive element sequence-based PCR. Appl Environ Microbiol 74(17):5392–5401
Chowdhury N, Asakura M, Neogi SB, Hinenoya A, Haldar S, Ramamurthy T, Yamasaki S (2010) Development of simple and rapid PCR-fingerprinting methods for Vibrio cholerae on the basis of genetic diversity of the superintegron. J Appl Microbiol 109(1):304–312
Collin B, Rehnstam-Holm AS (2011) Occurrence and potential pathogenesis of Vibrio cholerae, Vibrio parahaemolyticus and Vibrio vulnificus on the South Coast of Sweden. FEMS Microbiol Ecol 78(2):306–313
Comstock LE, Johnson JA, Michalski JM, Morris JG Jr, Kaper JB (1996) Cloning and sequence of a region encoding a surface polysaccharide of Vibrio cholerae O139 and characterization of the insertion site in the chromosome of Vibrio cholerae O1. Mol Microbiol 19(4):815–826
Cowan ST, Steel KJ (1974) Enterobacteriaceae. In: Buchanan RE, Gibbons NE (eds) Bergey’s manual of determinative bacteriology, 8th edn. The Williams and Wilkins Co., Baltimore, pp 290–293
Crowe SJ, Newton AE, Gould LH, Parsons MB, Stroika S, Bopp CA, Mahon BE (2016) Vibriosis, not cholera: toxigenic Vibrio cholerae in Lima, Peru. J Clin Microbiol 53(4):815–826
Dalsgaard A, Serichantalergs O, Forslund A, Lin W, Mekalanos J, Mintz E, Shimada T, Wells JG (2001) Clinical and environmental isolates of Vibrio cholerae serogroup O141 carry the CTX...
phage and the genes encoding the toxin-coregulated pili. J Clin Microbiol 39(11):4086–4092
Dalusi L, Lyimo TJ, Lugomela C, Hosea KM, Sjöling S (2015) Toxigenic Vibrio cholerae identified in estuaries of Tanzania using PCR techniques. FEMS Microbiol Lett 362(5):009
Dalusi L, Saareheimo J, Lyimo TJ, Lugomela C (2015) Genetic relationship between clinical and environmental Vibrio cholerae isolates in Tanzania: a comparison using repetitive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) fingerprinting approach. Afr J Microbiol Res 9(7):455
De K, Ramamurthy T, Faruque SM, Yamauchi S, Takeda Y, Nair GB, Nandy RK (2004) Molecular characterisation of rough strains of Vibrio cholerae isolated from diarrhoeal cases in India and their comparison to smooth strains. FEBS Microbiol Lett 232(1):23–30
de Magny GC, Mozumder PK, Grim CJ, Hasan NA, Naser MN, Alam M, Colwell RR (2011) Role of zooplankton diversity in Vibrio cholerae population dynamics and in the incidence of cholera in the Bangladesh Sundarbans. Appl Environ Microbiol 77(17):6125–6132
Deshayes S, Daurel C, Cattoir V, Parienti JJ, Quilici ML, de la Blanchardière A (2015) Non-O1: non-O139 Vibrio cholerae bacteraemia in splenectomised thalassaemic patient from Malaysia. Trop Biomed 36(3):320–325
Deshayes S, Daurel C, Cattoir V, Parienti JJ, Quilici ML, de la Blanchardière A (2015) Non-O1: non-O139 Vibrio cholerae bacteraemia: case report and literature review. Springerplus 4:575
Devi MS, Paria P, Kumar V, Parida PK, Maurye P, Behera BK, Das BK (2022) Molecular identification and pathogenicity study of virulent Vibrio cholerae non-O1/O139 serotype associated with mortality of farmed Labeo rohita (Hamilton, 1822), in India. Aquaculture 547:735729
Dhar R, Badawi M, Qabazard Z, Albert MJ (2004) Vibrio cholerae (non-O1, non-O139) sepsis in a child with Fanconi anemia. Diag Microbiol Infect Dis 50:287–289. https://doi.org/10.1016/j.diagmicrobio.2004.08.004
Diaz-Quínez A, Hérnandez-Monroy I, Montes-Colima N, Moreno-Pérez A, Galicia-Nicolás A, Martínez-Rojano J, Kuri-Morales P (2014) Outbreak of Vibrio cholerae serogroup O1, serotype Ogawa, biotype El Tor strain—La Huasteca region, Mexico, 2013. MMWR Morb Mortal Wkly Rep 63(25):552
Dutta D, Chowdhury G, Pazhani GP, Guin S, Dutta S, Ghosh S, Ramamurthy T (2013) Vibrio cholerae non-O1, non-O139 serogroups and cholera-like diarrhea, Kolkata, India. Emerg Infect Dis 19(3):464
Dziezman M, Serruto D, Tam VC, Sturtevant D, Diraphat P, Faruque SM, Mekalanos JJ (2005) Genomic characterization of non-O1, non-O139 Vibrio cholerae reveals genes for a type III secretion system. Proc Natl Acad Sci 102(9):3465–3470
Eddabba R, Moussaoui W, Prévost G, Delalande F, Van Dorselaer A, Meunier O, Mimouni O (2011) Occurrence of Vibrio cholerae non-O1 in three wastewater treatment plants in Agadir (Morocco). World J Microbiol Biotechnol 27(5):1099–1108
Engel MF, Musken MA, Mooi-Kokenberg E, Kuiper EJ, van Westerloo DJ (2016) Vibrio cholerae non-O1 bacteraemia: description of three cases in the Netherlands and a literature review. Euro Surveill. 21(15):30197. https://doi.org/10.2807/1560-7917.ES.2016.21.15.30197
Farina C, Marin F, Sciaffino E, Luzzi I, Dionisi AM, Leonì F, Bordoni S (2010) A fatal Vibrio cholerae O37 enteritis. J Med Microbiol 59(12):1538–1540
Farrer JJ, Janda MJ, Birkhead K (2003) Vibrio. In: Murray PR, Baron EJ, Jorgensen JH, Pfister MA, Yakrus RH (eds) Manual of clinical microbiology, 8th edn. ASM Press, Washington, DC, USA, pp 706–718
Farmer P, Almazor CP, Bahnsten ET, Barry D, Bazile J, Bloom BR, Weigel JL (2011) Meeting cholera’s challenge to Haiti and the world: a joint statement on cholera prevention and care. PLoS Negl Trop Dis 5(5):e1145
Faruque SM, Mekalanos JJ (2003) Pathogenicity islands and phages in Vibrio cholerae evolution. Trends Microbiol 11(11):505–510
Faruque SM, Alim AA, Roy SK, Khan F, Nair GB, Sack RB, Albert MJ (1994) Molecular analysis of rRNA and cholera toxin genes carried by the new epidemic strain of toxigenic Vibrio cholerae O139 synonym Bengal. J Clin Microbiol 32(4):1050–1053
Faruque SM, Albert MJ, Mekalanos JJ (1998a) Epidemiology, genetics, and ecology of toxigenic Vibrio cholerae. Microbiol Mol Biol Rev 62(4):1301–1314
Faruque SM, Saha MN, Alim AA, Albert MJ, Islam KN, Mekalanos JJ (1998b) Analysis of clinical and environmental strains of nontoxigenic Vibrio cholerae for susceptibility to CTXΦ: molecular basis for origination of new strains with epidemic potential. Infect Immum 66(12):5819–5825
Faruque SM, Saha MN, Sack DA, Sack RB, Takeda Y, Nair GB (2000) The O139 serogroup of Vibrio cholerae comprises diverse clones of epidemic and nonepidemic strains derived from multiple V. cholerae O1 or non-O1 progenitors. J Infect Dis 182(4):1161–1168
Faruque SM, Chowdhury N, Kamruzzaman M, Dziejman M, Rahman MH, Sack DA, Mekalanos JJ (2004) Genetic diversity and virulence potential of environmental Vibrio cholerae population in a cholera-endemic area. Proc Natl Acad Sci 101(7):2123–2128
Feghali R, Adib SM (2011) Two cases of Vibrio cholerae non-O1/non-O139 septicaemia with favourable outcome in Lebanon. East Mediterr Health J 17(8):722–724
Filetici E, Bonadonna L, Ciccozzi M, Anastasio MP, Fantasia M, Shima t M (1997) Phenotypic and genotypic biotyping of environmental strains of Vibrio cholerae non-O1 isolated in Italy. Appl Environ Microbiol 63(10):4102–4106
Fong JC, Syed KA, Klose KE, Yildiz FH (2010) Role of Vibrio poly saccharide (vps) genes in VPS production, biofilm formation and Vibrio cholerae pathogenesis. Microbiology 156(9):2757–2769
Fooladi I, Ali A, Alishahi A, Fallah Mehrabadi J, Mahmoodzadeh Hosseini H (2013) Facile and rapid detection of Vibrio cholerae by multiplex PCR based on ompU, ctxA, and toxR genes. Jundishapur J Microbiol 6(10):1–5
Frans I, Michiels CW, Bossier P, Willems KA, Lievens B, Rediers H, Bossier P, Meuleman E, Vaneechoutte M (1997) Comparative study on the identification of races and biovars of Ralstonia solanacearum. J Environ Res Public Health 14(10):1111
Galal AA, Kehil Y, El-Daoudi YH, Shihata ZA, Ouf MF (2003) A comparative study on the identification of races and biovars of some Egyptian isolates of Ralstonia solanacearum. Egypt J Phytopathol 31(1–2):103–117
Garrison GM, Brenner DJ, Krieg NR, Staley JT (2005) Bergey’s manual of systematic bacteriology. Volume 2: the proteobacteria. Bergey’s Manual Trust, Berlin, pp 735–769
Goel AK, Tamrakar AK, Nema V, Kamboj DV, Singh L (2005) Detection of viable toxigenic Vibrio cholerae from environmental water sources by direct cell duplex PCR assay. World J Microbiol Biotechnol 21(6–7):973–976
Interscience conference on antimicrobial agents and chemotherapy. Anaheim, California, pp 11–14
Johnson LK, Brown MB, Carruthers EA, Ferguson JA, Dombeck PE, Sadowsky MJ (2004) Sample size, library composition, and genotypic diversity among natural populations of Escherichia coli from different animals influence accuracy of determining sources of faecal pollution. Appl Environ Microbiol 70(8):4478–4485
Jones J, Benner R Jr, DePaola A, Hara-Kudo Y (2013) Vibrio Densities in the intestinal contents of fish from coastal Alabama. Agric Food Anal Bacteriol 3:186–194
Kang HP, Dunne WM (2003) Stability of repetitive-sequence PCR patterns with respect to culture age and subculture frequency. J Clin Microbiol 41(6):2694–2696
Karaolis DK, Lan R, Kaper JB, Reeves PR (2001) Comparison of Vibrio cholerae pathogenicity islands in sixth and seventh pandemic strains. Infect Immun 69(3):1947–1952
Katz LS, Petkau A, Beaulaurier J, Tyler S, Antonova ES, Turnsek MA, Tarr CL (2013) Evolutionary dynamics of Vibrio cholerae O1 following a single-source introduction to Haiti. Mbio 4(4):e00398-e143
Kaysner CA, Abeita C Jr, Wekell MM, DePaola A Jr, Stott RF, Leitch JM (1987) Incidence of Vibrio cholerae from estuaries of the United States West Coast. Appl Environ Microbiol 53(6):1344–1348
KDHE (2015) Foodborne illness and outbreak manual. www.kdheks.gov/public/epi/downloads/kansas_foodborne_illness_manual.pdf.
Kecker P, Senderovich Y, Ken-Dror S, Laviad-Shitrit S, Arakawa E, Halpern M (2017) Ottis media caused by V. cholerae O100: a case report and review of the literature. Front Microbiol 8:1619
Keddy KH, Sooka A, Parsons MB, Njankop-Lafourcade BM, Fistch K, Smith AM (2013) Diagnosis of Vibrio cholerae O1 infection in Africa. J Infect Dis 208(1):23–31
Kerketta JA, Paul AC, Balaji V, Jesudason MV, Moses K, Wong KB, Chuang YC, Huang GC, Hsu SY (1998) Infections due to Vibrio vulnificus in the intestinal contents of finfish from coastal Alabama. Agric Food Anal Bacteriol 3:186–194
Kim HS, Lee MA, Chun SJ, Park SJ, Lee KH (2007) Role of NtrC in pathogenicity islands with respect to culture age and subculture frequency. J Clin Microbiol 41(6):2694–2696
Kondo S, Kawamura Y, Sano Y, Iguchi T, Hisatsune K (1997) A chemical study of the sugar composition of the polysaccharide portion of lipopolysaccharides isolated from Vibrio cholerae non-O1 from O2 to O155. Syst Appl Microbiol 20(1):1–11
Kong RYC, Lee SKY, Law TWF, Law SHW, Wu RSS (2002) Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. Water Res 36(11):2802–2812
Kumar P, Jain M, Goel AK, Bhadouria S, Sharma SK, Kamboj DV, Nair GB (2009) A large cholera outbreak due to a new cholera toxin variant of the Vibrio cholerae O1 El Tor biotype in Orissa, Eastern India. J Med Microbiol 58(2):234–238
Kumar P, Yadav F, Deshmukh DG, Bulle PA, Singh D, Singh N, Yadava PK (2017) Vibrio cholerae O1 with ctxB variant genotypic acquired qnrVC mediated ciprofloxacin resistance in Yamatmal, India. Clin Microbiol Infect 23(12):1005–1006
Kwon JD (2010) Occurrence of non-O1/non-O139 Vibrio Cholerae and Aeromonas spp. in Arizona recreational waters. Thesis, University of Arizona, Tucson, Arizona, USA. http://hdl.handle.net/10150/146600
Lai CC, Liu WL, Chiu YH, Gau SJ, Hsieh PR (2012) Spontaneous bacterial empyema due to non-O1, non-O139 Vibrio cholerae in a cirrhotic patient with hepatocellular carcinoma. Diagn Microbiol Infect Dis 73(1):84–85
Lee J, Akhtar M, Kwon SY, Lim SS, Hwang HJ, Jee JS, Yoon HJ, Kim HS, Seo YH, Yoon K (2013) Septic shock due to non-O1 Vibrio cholerae in a type 2 diabetes mellitus patient in mainland China. Int J Infect Dis 25:116–118
Lin CJ, Chiu CT, Lin DY, Sheen IS, Lien JM (1996) Non-O1 Vibrio cholerae bacteremia in patients with cirrhosis: a 5-yr experience from a single medical center. Am J Gastroenterol 91:336–340
Lipp EK, Rivera IN, Choopun N, Louis VR, Lipp ES, Mekalanos JJ, Colwell RR (2003) Direct detection of Vibrio cholerae and ctxA using multiplex PCR. Water Res 36(11):2802–2812
Loharikar A, Newton AE, Stroika S, Freeman M, Greene KD, Parsons MB, Mahon BE (2015) Cholera in the United States, 2001–2011: a reflection of patterns of global epidemiology and travel. Epidemiol Infect 143(4):695–703
Long RA, Rowley DC, Zamora E, Liu J, Bartlett DH, Azam F (2005) Antagonistic interactions among marine bacteria impede the proliferation of Vibrio cholerae. Appl Environ Microbiol 71(12):8531–8536
Lu B, Zhou H, Li D, Li F, Zhu F, Cui Y, Wang D (2014) The first case of bacteraemia due to non-O1/non-O139 Vibrio cholerae in China. J Med Microbiol 71(8):975–977
Livas S, Wang H, Chen M, Wang L, Zhang Z, Zhou B, Zhang B, Sun H, Gu X, Hu X, Kang Y et al. (2014) A case of bacteraemia due to non-O1/non-O139 Vibrio cholerae isolated from hospitalised patients in China. BMC Microbiol 14:1–12
and clinical samples collected in Italy. Int J Food Microbiol 132(1):47–53
Ottaviani D, Leoni F, Rocchegiani E, Canonico C, Masini L, Pianetti A, Carratufo A (2011) Unusual case of necrotizing fasciitis caused by *Vibrio cholerae* O137. J Clin Microbiol 49(2):757–759
Owoseni M, Okoh A (2017a) Assessment of chlorine tolerance profile of *Citrobacter* species recovered from wastewater treatment plants in Eastern Cape, South Africa. Environ Monit Assess 189(4):201
Owoseni M, Okoh A (2017b) Evidence of emerging challenge of chlorine tolerance of *Enteroxoccus* species recovered from wastewater treatment plants. Int Biodeterior Biodegrad 120:216–223
Owoseni MC, Olaraniran AO, Okoh AI (2017) Chlorine tolerance and inactivation of *Escherichia coli* recovered from wastewater treatment plants in the Eastern cape, South Africa. Appl Sci 7(8):810
Pal D, Das N (2010) Isolation, identification and molecular characterization of *Vibrio parahaemolyticus* from fish samples in Kolkata. Eur Rev Med Pharmacol Sci 14(6):545–549
Park KS, Ono T, Rakuda M, Jang MH, Okada K, Iida T, Honda T (2004) Functional characterization of two type III secretion systems of *Vibrio parahaemolyticus*. Infect Immun 72(11):6659–6665
Patel NM, Wong M, Little E, Ramos AX, Kolli G, Fox KM, Manch R (2009) *Vibrio cholerae* non-O1 infection in cirrhotics: case report and literature review. Transpl Infect Dis 11(1):54–56
Peng S, Hao W, Li Y, Wang L, Sun T, Zhao J, Dong Z (2021) Bacterial communities associated with four blooming scyphozoan jellyfish: potential species-specific consequences for marine organisms and humans health. Front Microbiol 12:1104
Petsaris O, Nousbaum JB, Quilici ML, Le Coadou G, Payan C, Abalain P, Meunier A, Lesueur A (2008) Distribution of genes for virulence and ecological communities associated with four blooming scyphozoan jellyfish: potential species-specific consequences for marine organisms and humans health. Front Microbiol 12:1104
Peters O, Nousbaum JB, Quilici ML, Le Coadou G, Payan C, Abalain P, Meunier A, Lesueur A (2008) Distribution of genes for virulence and ecological communities associated with four blooming scyphozoan jellyfish: potential species-specific consequences for marine organisms and humans health. Front Microbiol 12:1104
Parodi A, Rohwer F, Edwards R, Azam F, Bartlett DH (2005) A glimpse into the expanded genome content of *Vibrio cholerae* through identification of genes present in environmental strains. J Bacteriol 187(9):2992–3001
Raghul SS, Bhat SG (2011) Seasonal variation in the hydrolytic exoenzyme profile of *Vibrio* sp. associated with the marine benthic environment of South India. Indian J Geo-Mar Sci 40(6):826–833
Rahman MH, Biswas K, Karunasagar I (2010) Non-O1, non-O139 *Vibrio cholerae* bacteraemia in a cirrhotic patient. J Med Microbiol 59(Pt. 10):1260–1262
Piergentili P, Castellani-Pastoris M, Fellin RD, Farisano G, Bonello C, Rigoll E, Zampieri A (1984) Transmission of O non O group 1 *Vibrio cholerae* by Raw Oyster consumption. Int J Epidemiol 13(3):340–343. https://doi.org/10.1093/ije/dj3.3.340
Purdy A, Rohwer F, Edwards R, Azam F, Bartlett DH (2005) A glimpse into the expanded genome content of *Vibrio cholerae* through identification of genes present in environmental strains. J Bacteriol 187(9):2992–3001
Raghul SS, Bhat SG (2011) Seasonal variation in the hydrolytic exoenzyme profile of *Vibrio* sp. associated with the marine benthic environment of South India. Indian J Geo-Mar Sci 40(6):826–833
Rahman MH, Biswas K, Karunasagar I (2010) Non-O1, non-O139 *Vibrio cholerae* bacteraemia in a cirrhotic patient. J Med Microbiol 59(Pt. 10):1260–1262
Piergentili P, Castellani-Pastoris M, Fellin RD, Farisano G, Bonello C, Rigoll E, Zampieri A (1984) Transmission of O non O group 1 *Vibrio cholerae* by Raw Oyster consumption. Int J Epidemiol 13(3):340–343. https://doi.org/10.1093/ije/dj3.3.340
Purdy A, Rohwer F, Edwards R, Azam F, Bartlett DH (2005) A glimpse into the expanded genome content of *Vibrio cholerae* through identification of genes present in environmental strains. J Bacteriol 187(9):2992–3001
Raghul SS, Bhat SG (2011) Seasonal variation in the hydrolytic exoenzyme profile of *Vibrio* sp. associated with the marine benthic environment of South India. Indian J Geo-Mar Sci 40(6):826–833
Rahman MH, Biswas K, Karunasagar I (2010) Non-O1, non-O139 *Vibrio cholerae* bacteraemia in a cirrhotic patient. J Med Microbiol 59(Pt. 10):1260–1262
Piergentili P, Castellani-Pastoris M, Fellin RD, Farisano G, Bonello C, Rigoll E, Zampieri A (1984) Transmission of O non O group 1 *Vibrio cholerae* by Raw Oyster consumption. Int J Epidemiol 13(3):340–343. https://doi.org/10.1093/ije/dj3.3.340
Purdy A, Rohwer F, Edwards R, Azam F, Bartlett DH (2005) A glimpse into the expanded genome content of *Vibrio cholerae* through identification of genes present in environmental strains. J Bacteriol 187(9):2992–3001
Raghul SS, Bhat SG (2011) Seasonal variation in the hydrolytic exoenzyme profile of *Vibrio* sp. associated with the marine benthic environment of South India. Indian J Geo-Mar Sci 40(6):826–833
Rahman MH, Biswas K, Karunasagar I (2010) Non-O1, non-O139 *Vibrio cholerae* bacteraemia in a cirrhotic patient. J Med Microbiol 59(Pt. 10):1260–1262
Rahman MH, Biswas K, Karunasagar I (2010) Non-O1, non-O139 *Vibrio cholerae* bacteraemia in a cirrhotic patient. J Med Microbiol 59(Pt. 10):1260–1262
Rahman MH, Biswas K, Karunasagar I (2010) Non-O1, non-O139 *Vibrio cholerae* bacteraemia in a cirrhotic patient. J Med Microbiol 59(Pt. 10):1260–1262
Sellek RE, Niemiec, M. Olsen JS, Bassy O, Lorenzo P, Marti L, Roszkowiak A, Kocik J, Cabria JC (2012) Phenotypic and genetic analyses of 111 clinical and environmental O1, O139, and non-O1/O139 Vibrio cholerae strains from different geographical areas. Epidemiol Infect 140(8):1389–1399

Senderovich Y, Izhaki I, Halpern M (2010) Fish as reservoirs and vectors of Vibrio cholerae. PloS One 5(1):e8607

Shan X, Fu J, Li X, Peng X, Chen L (2022) Comparative proteomics and secretomics revealed virulence, and coexistence-related factors in non O1/O139 Vibrio cholerae recovered from 16 species of consumable aquatic animals. J Proteom 251:104408

Sharma C, Thungapathra M, Ghosh A, Mukhopadhyay AK, Basu A, Mitra R, Nair GB (1998) Molecular analysis of non-O1, non-O139 Vibrio cholerae associated with an unusual spouse in the incidence of cholera-like disease in Cuttaca, India. J Clin Microbiol 36(3):756–763

Snoussi M, Noumi E, Usai D, Zanetti S, Bakhrouf A (2008) Distribution of some virulence related-properties of Vibrio alginolyticus strains isolated from Mediterranean seawater (Bay of Khenis, Tunisia): investigation of eight Vibrio cholerae virulence genes. World J Microbiol Biotechnol 24(10):2133–2141

Son MS, Megli CJ, Kovacikova G, Qadri F, Taylor RK (2011) Characterization of Vibrio cholerae O1 El Tor biotype variant clinical isolates from Bangladesh and Haiti, including a molecular genetic analysis of virulence genes. J Clin Microbiol. https://doi.org/10.1128/CM.01286-11

Soto-Rodriguez SA, Gomez-Gil B, Lozano R (2010) Bright-red syndrome in Pacific white shrimp Litopenaeus vannamei is caused by Vibrio harveyi. Dis Aquat Org 92(1):11–19

Stine OC, Sozhamannan S, Gou Q, Zheng S, Morris JG Jr, Johnson JA (2000) Phylogeny of Vibrio cholerae based on recA sequence. Infect Immun 68(12):7180–7185

Stroeher UH, Jedani KE, Dredge BK, Morona R, Brown MH, Karaoguzos LE, Manning PA (1995) Genetic rearrangements in the rfb regions of Vibrio cholerae O1 and O139. Proc Natl Acad Sci U S A 92(22):10374–10378

Sujatha K, Senthilkumaar P, Sangeetha S, Gopalakrishnan MD (2011) Isolation of human pathogenic bacteria in two edible fishes, Pria-canthus hamrur and Megalaspis cordyla at Royapuram waters of Chennai, India. Indian J Sci Technol 4(5):539–541

Taoção M, Alves A, Saavedra MJ, Correia A (2005) BOX-PCR is an isolator of Vibrio cholerae serogroup O75 infections acquired in the southeastern United States. Clin Infect Dis 47(8):1035–1040

Tong TH, Janssens L, Noseda B, Samapundo S, Nguyen BL, Heyndrickx M, Devlieghere F (2014) Evaluation of the microbiological safety and quality of Vietnamese Pangasius hypophthalmus during processing by a microbiol assessment scheme in combination with a self-assessment questionnaire. Fish Sci 80(5):1117–1128

Traroe O, Maritkainen O, Siitonen A, Barro N, Haukka K (2014) Occurrence of Vibrio cholerae in fish and water from a reservoir and a neighboring channel in Ouagadougou, Burkina Faso. J Infect Dev Ctries 8(10):1334–1338

Trubiano JA, Lee JYH, Valcanis M, Gregory J, Sutton BA, Holmes NE (2014) Non-O1, non-O139 Vibrio cholerae bacteriaemia in an Australian population. Intern Med J 44(5):508–511

Tuang FN, Rademaker JI, Aloclitia EC, Louws FJ, Bruin FJ (1999) Identification of bacterial repPCR genomic fingerprints using a backpropagation neural network. FEMS Microbiol Lett 177(2):249–256

Tulatorn S, Preeprem S, Vuddhakul V. Mittrarpap-Arthorn P (2018) Comparison of virulence gene profiles and genomic fingerprints of Vibrio cholerae O1 and non-O1/non-O139 isolates from diarrheal patients in southern Thailand. Trop Med Health 46(1):1–8

Uracaka H, Kitatsukamoto K, Ohwada K (1997) 16S rDNA genotyping using PCR/RFLP (restriction fragment length polymorphism) analysis among the family Vibrionaceae. FEMS Microbiol Lett 152(3):125–132

Versalovic J, Koehler T, Lupski R (1991) Distribution of repetitive DNA sequences in eubacteria and application to finnerprinting of bacterial enomes. Nucleic Acids Res 19(24):6823–6831

Vissick KL (2009) An intricate network of regulators controls biofilm formation and colonization by Vibrio fischeri. Mol Microbiol 74(4):782–789

Wai SN, Mizuoe Y, Yoshida SI (1999) How Vibrio cholerae survive during starvation. FEMS Microbiol Lett 180(2):123–131

Wang XM, Wang DC, Tan HL, Zhong HJ, Chen JD, Li BS, Kan B (2007) Development and application of real-time polymerase chain reaction to detect Vibrio cholerae O1 and O139 in river water. Zhonghua liu xing bing xe za zhi=Zhonghua liuxingbingxe zazhi 28(8):768–771

Wang D, Xu X, Deng X, Chen CJ, Li B, Tan H, Kan B (2010) Detection of Vibrio cholerae O1 and O139 in environmental water samples by an immunofluorescent-aggregation assay. Appl Environ Microbiol 76(16):5520–5525

Wang S, Wang J, Mou H, Luo B, Jiang X (2015) Inhibition of adhesion of intestinal pathogens (Escherichia coli, Vibrio cholerae, Campylobacter jejuni, and Salmonella Typhimurium) by common oligosaccharides. Foodborne Pathog Dis 12(4):360–365

Wang Q, Zhang Y, Yang Q, Fu S, Qu B, Defoirdt T (2021) One health pathogen surveillance demonstrated the dissemination of gut pathogens within the two coastal regions associated with intensive farming. Gut Pathogens 13(1):1–16

Waturangi DE, Joanito I, Yogi Y, Thomas S (2012) Use of REP- and primed polymerase chain reaction (AP-PCR) In: Persing DH (ed) Diagnostic molecular microbiology. ASM press, Washington, pp 595–602

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18(22):6531–6535
Wong HC, Wang P (2004) Induction of viable but nonculturable state in *Vibrio parahaemolyticus* and its susceptibility to environmental stresses. J Appl Microbiol 96(2):359–366

World Health Organization (2014) Cholera, number of cases. Global health observatory data repository. Retrieved 25 June 2015. http://apps.who.int/ghodata/

World Health Organization (2017) Diarrhoeal disease. WHO fact sheet. Updated May 2017. http://www.who.int/mediacentre/factsheets/fs330/en/. Accessed 20 Aug 2018. Deployments from the oral cholera vaccine stockpile, 2013–2017. Wkly Epidemiol Rec 2017; 92:437–42.

Xing M, Hou Z, Yuan J, Liu Y, Qu Y, Liu B (2013) Taxonomic and functional metagenomic profiling of gastrointestinal tract microbiome of the farmed adult turbot (*Scophthalmus maximus*). FEMS Microbiol Ecol 86(3):432–443

Xu M, Cao C, Wang D, Kan B, Jia H, Xu Y, Li X (2013) District prediction of cholera risk in China based on environmental factors. Chin Sci Bull 58(23):2798–2804

Yadava JP, Jain M, Goel AK (2013) Detection and confirmation of toxigenic *Vibrio cholerae* O1 in environmental and clinical samples by a direct cell multiplex PCR. Water SA 39(5):611–614

Yamai S, Okitsu T, Shimada T, Katsube Y (1997) Distribution of serogroups of *Vibrio cholerae* non-O1 non-O139 with specific reference to their ability to produce cholera toxin, and addition of novel serogroups. Kansenshogaku zasshi Jpn Assoc Infect Dis 71(10):1037–1045

Yamazaki S, Garg S, Nair GB, Takeda Y (1999) Distribution of *Vibrio cholerae* O1 antigen biosynthesis genes among O139 and other non-O1 serogroups of *Vibrio cholerae*. FEMS Microbiol Lett 179:115–121

Yamazaki E, Sakamoto R, Matsumoto T, Morimatsu F, Kurazono T, Hiroi T, Kurazono H (2013) Development of an immunochromatographic test strip for detection of cholera toxin. BioMed Res Int. https://doi.org/10.1155/2013/679038

Yang A, Yen C (2012) PCR optimization of BOX-A1R PCR for microbial source tracking of *Escherichia coli* in waterways. J Exp Microbiol Immunol 16:85–89

Yildiz FH, Visick KL (2009) *Vibrio* biofilms: so much the same yet so different. Trends Microbiol 17:109–118. https://doi.org/10.1016/j.tim.2008.12.004

Yu CY, Ang GY, Chan KG, Singh KKB, Chan YY (2015) Enzymatic electrochemical detection of epidemic-causing *Vibrio cholerae* with a disposable oligonucleotide-modified screen-printed biosensor coupled to a dry-reagent-based nucleic acid amplification assay. Biosens Bioelectron 70:282–288

Zmeter C, Hussam T, Ala IS, Souha SK (2018) Non-O1, non-O139 *V. cholerae* septicemia at a tertiary care center in Beirut, Lebanon; A case report and review. J Infect Public Health. https://doi.org/10.1016/j.jiph.2018.01.001

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