



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 cholera toxin. Reported cholera outbreak cases around the world have been associated with these cholerae *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-NAG-*Vc* 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-NAG-*Vc*) yet there is a sustained paucity of research interest on the non-agglutinable *V. 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 Cholerae · 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 *Vibrionaceae* members. According to the Bergeys manual of Systematic Bacteriology (Garrity et al. 2005; George et al. 2005), the family of *Vibrionaceae* 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 (*Vibrionaceae*) 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 chemoheterotrophs, 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 (cholerae) 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-agglutinable 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, septicemia, 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 cholerae *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 cholerae 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/rasff_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 truncatus*, 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-cholerae, non-pathogenic, they are asymptomatic colonizers of human subjects, they are associated with mild and/or sporadic illness (such as 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-COVIDS-WHO revealed that members of O75 and O141 which are NAG-O1/O139 have been observed in outbreak cases (WHO 2017; CDC-COVIDS 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 where 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 (choleraogenic *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 vibriosis cases globally (Ali et al. 2015), which may be traceable 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) as at then but it shows remarkable 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 choleraogenic potential. Some of such characters are acquisition of enterotoxin (CT) and other epidemic associated genes (tcp-gene) (Shan et al. 2022; Faruque et al. 2003; Basu et al. 2000). From the foregoing, 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 Aydanian 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 its major host. Suffice 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 Aydanian 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)

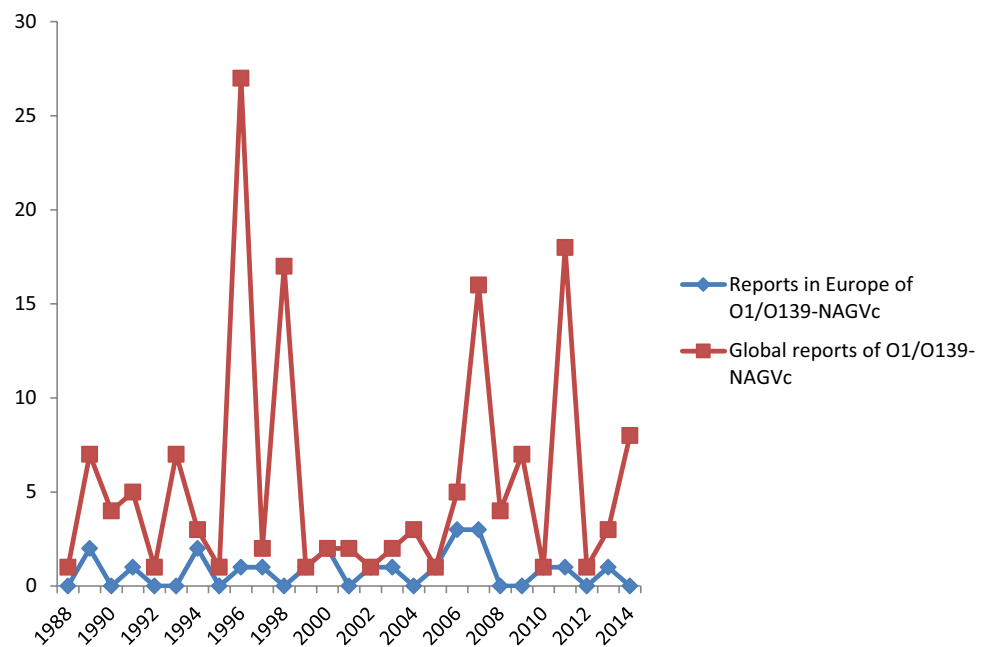
Identified O1/O139-NAGVc	Source	Diseases	Country/place of isolation	Clonal complexity	Year isolated	Ref
O77	C	Intestinal diarrhea	India	S	1976	Octavia et al. 2013
O79	E	Intestinal diarrhea	Bangladesh	S	2002	Dziejman et al. 2005
O80	C	Acute gastroenteritis	India	S	1977	Octavia et al. 2013
O83	E	Intestinal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

Fig. 1 Pattern of O1/O139-NAGVc reported cases between 1988 and 2014 in the Globe and Europe (Adopted from Engel et al. 2016)



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-agglutinating 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 Chatterjee 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 (Chatterjee 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 documentation 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 unreserved attention is given to any suspected source of a problem, hence the need for an unbiased research attention on the SANAS-*Vc* is reasonable.

Geneology 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 cholera toxin 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. Suffice 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 interpret 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-cholera toxin *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; Strocher 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 United States 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-cholera toxin situation, cholera-like and cholera toxin vibriosis (CDC-COVIS 2011, 2012). Other investigators prefer cholera to be a case if a diarrhea disease can be reproduced 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-choleraegenic (non-toxigenic) and the choleraegenic (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 choleraegenic 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 (<i>Oreochromis aureus</i>)	Senderovich et al. 2010
Water from marine inshore	Peru	Lorna fish (<i>Sciaena deliciosa</i>)	Carvajal et al. 1998
Lake	Sea of Galilea Israel	Longhead barbel (<i>Barbus longiceps</i>)	Senderovich et al. 2010
Fish pond	Kfar Rupin, Israel	Galilee St. Peter's fish (<i>Sarotherodon galilaeus</i>)	Senderovich et al. 2010
Fish pond	Atlit, Israel	Grass carp, white-amur (<i>Ctenopharyngodon idella</i>)	Senderovich et al. 2010
Mediterranean Sea (Marine water)	Akko, Israel	Blotcheye soldierfish (<i>Myripristis murdjan</i>)	Senderovich et al. 2010
Royapuram coast (Marine water)	Chennai, Tamil Nadu, India	Bulls eye (<i>Priacanthus hamrur</i>)	Sujatha et al. 2011
Adult zebrafish cultured in tanks	Auckland, New Zealand	Zebrafish (<i>Danio rerio</i>)	Lan and Love 2012
Marine aquaculture	Qingdao, China	Turbot fish (<i>Scophthalmus maximus</i>)	Xing et al. 2013
Tanghin freshwater reservoir	Ouagadougou, Burkina Faso (Africa)	Tilapia (<i>Oreochromis niloticus</i>)	Traoré et al. 2014
Fowl river (estuarine)	Gulf of Mexico	Sheepshead (<i>Archosargus probatocephalus</i>)	Jones et al. 2013
Food industry	Vietnam	Frozen tra fish (<i>Pangasius hypophthalmus</i>) fillet	Thi et al. 2014

Table 4 Clinical and hospital reported cases of O1/O139-NAGVc

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	Biliary 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	Austria	21 yrs	Hirk et al. 2016
2012	Gastroenteritis, septicemia/stool, blood	China/Beijing	70 yrs	Lu et al. 2014
	Gastroenteritis, septicemia/stool, blood	Lebanon	54 yrs	Feghali and Adib 2011
	Liver cirrhosis biopsized tissue, acute gastroenteritis/blood, stool			Patel et al. 2009
2006 and 2009	Liver cirrhosis biopsized tissue, acute gastroenteritis/blood, stool food material	Italy/Apulian region	49 yrs	Ottaviani et al. 2009, 2011
	Septicemia/blood	Lebanon	Preterm baby	Feghali and Adib, 2011
2000–2013	Gastroenteritis, septicemia/stool, blood	Australian population	40–86 yrs	Trubiano et al. 2014
2008	Gastroenteritis, septicemia/stool, blood	Italy and Egypt	54 yrs	Farina et al. 2010
1982	Gastroenteritis, septicemia/stool, blood	USA/Dolo, Venice	Not reported	Piergentili et al. 1984
2011	Liver cirrhosis biopsized tissue, septicemia/blood, tissue	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, Ashok, 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

Table 4 (continued)

Year of occurrence	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

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 (KDH) and Environment in conjunction with the Bureau of Epidemiology and Public Health Informatics (BEPHI) documented in 2016 that Non-O1/Non-O139 *V. cholerae* has been associated with over 2–3% of reported diarrhea cases/illnesses in all developing tropical countries where 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).

About fifty percent of all the cases of cholera reported are traceable to the Haitian epidemic (58,000) of 2010 and the Dominican Republic epidemic (2000) (CDC-COVIS 2017). Cases of cholera reported in the United States between 2001 and 2010 were all associated with international travel to Asia while the 2010 and 2011 epidemic is associated with travel to Haiti and the Dominican Republic (Loharikar et al. 2015). The case fatality rate (CFR) of about 26 reporting countries is 2.43% with Guinea and Congo recording the highest CFR. Non-O1/Non-O139 and SAAT-1/139 *V. cholerae* are known to be implicated in various gastrointestinal infections which are associated with the consumption of contaminated sea food. According to the current Centre for Disease Control Surveillance data on cholera, it was reported that Cholera and Other *Vibrio* Illness Surveillance (CDC-COVIS 2017) show about 39.9% association with NSAG/NAG-O1/O139. Their report connote that Non-O1/Non-O139 *V. cholerae* are implicated in over 697 infection cases within a 15 year period as about 39.9% of these cases were hospitalized and 5% mortality recorded (Aguirre-López et al. 2017; Janda et al. 2015; CDC-COVIS 2014; Diez-Quinonez et al. 2014; Newton et al. 2011, 2012). Other recent study from the Asian continent reveals that Non-O1/Non-O139-Vc is

linked to 70% of case that resemble cholera. Some refers to such pathogens as the choleraogenic Non-O1/Non-O139 *V. cholerae* since the various symptoms of SAAT *V. cholerae* infected patients were observed in NAG infected patients (Dutta et al. 2013; Ismail et al. 2012). This rare gastroenteritis are today becoming prevalent amongst hospitalized monomicrobial patients of greater than five years of age and a history of non-choleraeogenic *Vibrio* infections (Janda et al. 2015).

Pathogenesis of non-O1/non-O139 *Vibrio cholerae*

The NSAG/NAG-O1/O139 or Non-O1/Non-O139 *V. cholerae* members have shown genetic heterogeneity as reported 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 tcp-gene (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

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 swimming 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

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-O139Vc ensures the acquisition of the CT (Aydanian et al. 2011; Chatterjee 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. cholerae* as reported by various investigators has further substantiate

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, 2012; 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 (*CTX ϕ*), they are classified/biyped 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 cholera toxin 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 epidemic associated genes of *V. cholerae* O1/O139 in the

environment (Zmeter et al. 2018; Baig et al. 2018; Kechker 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 meningitis of patients resulting remarkable weight loss, increased pulse (about 150 beats/mins), a temperature of 38–40 °C, abnormal white blood cell count ($6.65 \times 10^9 L^{-1}$), 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 $\times 10^9$ 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 Kechker and his team (Kechker 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; Kechker 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 Igbinsosa 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 water 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 Vibriosis/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 Vibriosis/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 Independent 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 order 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 that report of suspected cases of all *Vibrio* associated diarrhea be sent for confirmation and a document for updated surveillance initiated. The cases should include O1/O139 *V. cholerae* cases, all Non-O1/Non-O139 *V. cholerae* cases (O2, O4, O75, O79 etc.) and *Grimontia hollisae* (*Vibrio hollisae*) cases (Moph.gov.ib 2017). Between 1981 and 1993, more than one hundred and thirty Non-O1 cholera-related case have been reported in Florida. (Daniels and Shafaie 2000; Dalsgaard et al. 2001). Non O1/Non-O139 *V.cholerae* members were initially after identification reported as causative agent of both small outbreaks relative cases and sporadic cases of cholera-like disease (Okada et al. 2014; Bhattachaiya et al. 2006; Klontz 1990; Morris et al. 1990). Other isolates of SANAS *V. cholerae* which belongs to higher numerical serogroups apart from O1 and O139 does not harbour 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. 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. Suffice 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; septicaemia) 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 harbours choleraogenic/toxigenic strains of O1/O139 *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/choleraogenic and non-toxigenic/non-choleraogenic 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 (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 shows 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 sub-parent 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; Teklehaimanot 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 choleraogenic 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/choleraogenic 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 Length 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*, *ctxAB* etc.) in a specimen extracted DNA and treatment with the endonuclease to fragment into varying/polymorphic 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* (Urakawa 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 been applied in typing various members of the *Vibrionaceae* eg *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* etc. (Balcazar et al. 2010; Maluping et al. 2005, 2008; Rodriguez et al. 2006).

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. (Bachelier 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 genomic species of *Pseudomonas syringae* while other investigators applied in racial differentiation, biovars of *Ralstonia solanacearum* and typing of *Aeromonas* members (Tacao et al. 2005; Galal et al. 2003; Paola et al. 1984). In vibriology, epidemiological relationship of clinical strains and environmental strains are studied amongst choleraogenic *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 food stuffs and vegetables, most of their report has shown a high prevalence of Non-O1/Non-O139 *V. cholerae* in these specimen than the choleraogenic 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 molluscs, 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, Ighinosa 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. (Raghul 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 Igbinsosa, 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 referred 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 flagella/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 been 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 Igbiosa 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, glycerol-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 dodecylSulfate-polyacrylamide gel electrophoresis

(SDS-PAGE), thin layer chromatography and subsequent development of chromatogram on a Sephadex LH20. This application would differentiate the size of digested capsules in molecular sizes, which may enhance identification of the various high molecular weight capsules carrying *V. cholerae* members. The nature of the polysaccharide biosynthetic genes would also be characterized which may also aid identification. Another area of future research interest is the control of biofilm formation which has enhanced the pathogen environmental survival. Various actinobacterial members are being studied to develop an antibiofilm agent to control and eradicate recalcitrant NSAG/NAG-O1/O139 *V. cholerae* members. Recently, various *Vibrio* members have shown production of antibiofilm activity bioagent which are exopolysaccharide-A101, QY101 from culture supernatant of some marine *Vibrio cholerae* members. The survival and recalcitrancy observed amongst the *Vibrio* members in the environment and Coastal water bodies would also have been reduced with the advent of antibiofilm biomolecule. There is currently an ongoing study to produced antibiofilm agent from the actinobacterial diverse nature. The future presents possible eradication and control strategy for the pathogen as interest is engendered toward the study of the NSAG/NAG-O1/O139 *V. cholerae* members.

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 ethiologic agent known as NSAG/NAG-O1/O139 *V. cholerae* members. A special interest should be directed at those members with the cholera toxin (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 agglutinable type *Vibrio cholerae* of 1/139 (SAAT-Vc-1/139) members or O1/O139 *Vibrio cholerae* somatic antigen agglutinable strains of 1/139 *Vibrio cholerae*. These strains are the somatic antigen non-agglutinable 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.

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