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Epidemiological Distribution of Different *Vibrio cholerae* Strains Causing Cholera Disease in Endemic Countries: A Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Cholera is an acute severe watery diarrhoeal disease caused by a toxigenic strains of *Vibrio cholerae.* Significant clinical presentations characteristic of this disease include; *a*symptomatic intestinal colonization, accompanied by rapid fluid and electrolyte loss, cholera sicca (unusual accumulation of fluid in the intestinal lumen leading to circulatory collapse and death), significant hypovolemia and electrolyte abnormalities, abdominal pain and discomfort, borborygmi, and vomiting. However, diagnostic pointers like hypokalemia, hypo or hypernatremia, isonatremic dehydration, hypocalcemia, and acidosis may be seen. Yet, in complicated cases, Kidney failure with acute tubular necrosis due to anuria, low glycogen, inadequate gluconeogenesis, severe hypoglycemia, coma (in rare occasions), chronic enteropathy, malnutrition and pneumonia in children may occur. Since man is the natural host of this pathogenic bacterium (*Vibio cholera)*, which he sheds into the environment through faeces or vomitus, its eradication has become difficult especially from their natural warm aquatic environmental reservoirs. The association of this disease with drinking water, and food, has been traced to the pollution of water sources (wells, rivers, streams and ponds) as well as food sources like vegetables (from gardens which are watered with sewage contaminated water from the environment). Cholera has become a night mare especially in continents like Asia and Africa, which lack adequate healthcare facilities and

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infrastructures. These areas represent the most endemic regions of the nearly 50 countries recorded to be endemic to cholera in the world. The World Health Organization reports that only an estimated 5–10% of the actual case number are officially reported annually worldwide. This review attempts to describe the epidemiological distribution of the various strains of *Vibrio cholerae* causing epidemics all over the globe.

Keywords: Vibrio; cholera; epidemiological; distribution; endemic; countries.

1. INTRODUCTION

Following the World Health Organization (WHO) Standard definition of a case of cholera, it can only be described as a case of cholera when: a five years and above individual living in a locality that is not endemic to cholera becomes infected and presents with the symptoms (in this case, acute watery diarrhoea with or without vomiting, leading to severe dehydration and or death). It is termed a confirmed case only when after Laboratory culture of the diarrheal patient's sample, the bacteria (Vibrio *cholerae* O1 or O139) are isolated [1].

The notable sero-groups of *Vibrio cholerae* known to be most often associated with epidemic cholera outbreaks include the toxigenic strains of *Vibrio cholerae* O1 and O139. The non-O1 and non-O139 *Vibrio cholera* have also been reported to have been associated with severe diarrhoeal disease, but not with disease outbreaks as those seen with cholera. These diarrhoeal diseases are only increasingly reported towards the end of summer and during the early rainy season, when the waters are warmest and the temperatures are favourable. However, the causative agents (Non-O1 and non-O139 *Vibrio cholerae)* are classified as the third among the group of most frequently reported bacteria [2].

Since man is the natural host of this pathogenic bacterium *Vibio cholerae*, it is shed into the environment through faeces or vomitus and the natural reservoirs for the bacterium in warm aquatic environments make their eradication very difficult. Therefore, the association of this disease with drinking water sources in Nigeria (the northern region most especially) and other parts of the cholera endemic regions of the world cannot be overemphasized. This is because most out breaks have been traced to the pollution of water sources like wells, rivers, streams and ponds as well as food sources like vegetables from gardens which are watered with sewage contaminated water from the environment.

A high infectious dose of about 108 cells is required because it takes just approximately 2-5hours after infection before the onset of disease in a healthy person [3].

V. cholerae infection, causes several disease manifestations, including; asymptomatic intestinal colonization, acute watery diarrhea accompanied by rapid fluid and electrolyte loss, cholera sicca (unusual accumulation of fluid in the intestinal lumen leading to circulatory collapse and death), significant hypovolemia and electrolyte abnormalities, abdominal pain, discomfort, borborygmi, and vomiting. Characteristic diagnostic features may include; hypokalemia, hyponatremia or hypernatremia, isonatremic dehydration, hypocalcemia, and acidosis. Complications such as Kidney failure with acute tubular necrosis due to anuria, low glycogen, inadequate gluconeogenesis, severe hypoglycemia or even coma, chronic enteropathy, malnutrition and pneumonia in children are common [4].

The individuals at high risk of being infected with this toxin-producing strain of *Vibrio cholerae* are healthcare workers, cholera response workers, and migrants, especially those travelling to endemic regions and those who cannot with limited resources, take care of their food, drinks and personal hygiene. Most people, especially those with achlorhydria, those belonging to blood group O, those under prolonged ill heath, and those who cannot have easily available health care services are more susceptible to cholera disease [5].

It has been observed that Asia and Africa, represent the most endemic regions of the close to 50 recorded countries, endemic to cholera in the world [4].

The existence of cholera can be traced to India for many centuries now; since the disease was declared a public health challenge in many areas of this country [6]. However, West Africa has remained the seat of cholera since its first appearance in the continent in 1970, during the

seventh pandemic wave. African countries, one after the other, have faced the squad of epidemics repeatedly, year after year. The World Health Organization reports of [7], stated that from Benin-Mauritania, 11 African countries which are served by the Atlantic Ocean, sent in reports of approximately 30,475 cases of cholera. It also reported that Ghana alone, recorded 52.4% (51,333 suspected cases) out of the 97,887cases of cholera reported from the above African counties from 2009-2015 [5].

In Nigeria, Cholera has become a serious course for concern and a major public health challenge for close to forty –nine years. Since the first outbreak in 1970, many states of the country have been experiencing outbreaks with many cases and fatalities reported yearly. The number of cholera cases might be under estimated because only areas with reported cases are recorded, while unreported cases remain silent and unknown. This is same with the global estimates of the disease made public annually, since epidemiological surveillance and other public health facilities do not reach most interior parts of the less developed and most affected countries [8]. The World Health Organization reports that only an estimated 5–10% of the actual case number are reported officially annually worldwide [9]. This review attempts to describe the characteristics and epidemiological distribution of the various strains of *Vibrio cholerae* causing epidemics all over the globe.

2. HISTORY OF CHOLERA

The Ganges River in Sanskrit India, as far back as the 5th century BC was reported to have been serving as a reservoir, from where people who presented with a disease like cholera, were being infected [10,11]. From the Indian peninsula, Hippocrates in 460-377 BC, recorded the first descriptions of an illness that might have been cholera [12]. John Snow, while working with infected stool in 1813-1858, observed an infectious material that could contaminate drinking water sources and cause a communicable disease [10,12,13] and an Anglican minister Henry Whitehead traced this infectious material to an infected well in London [11]. Furthermore, Filippo Pacini in Italy, in 1854, was the first to note microscopically, that this bacterium has the characteristic comma-shape described today [11,13-17]. The presence of this pathogenic agent in stool was later on proven by Robert Koch, during his research in Egypt and Calcutta (Kolkata) India from 1883/1884. He isolated pure cultures of this infectious material from stool [18] and later identified it in 1885 as *V. cholerae* [11].

However, the acute dehydrating effects of this night mare of a disease, led to the development of intravenous fluids therapy for cholera. In 1830, R. Hermann gave intravenous infusion of fluids to some cholera victims as treatment. This German chemist in Moscow's trial, spurred up his medical colleague (Jaehnichen) to infuse a patient with six ounces of fluid. His trial restored the pulse of the cholera patient, though the patient died later. This success story, led to the conclusion made by William Brooke O'Shaughnessy, a young Irish physician, in Great Britain; in his study of Cholera pathology, Brooke concluded that to treat Cholera, the saline matter and specific blood gravity should be restored, and this statement was then later on propagated by Thomas Latta of Scotland in 1832. He treated 15 cholera victims, by a trial of O'Shaughnessy's idea and this rescued five of them. Then in the late nineteenth century, Rogers, confirmed all these successful trials by producing a hypertonic saline which demonstrated the effectiveness of intravenous fluid, by reducing the fatality rate due to cholera from 60-70% to 30%, and saving many [19].

3. THE *Vibrio cholerae* GENOME

Heidelberg et al. [20], asserts that, through comparative genomics of the virulence genes and the growth locations, the complete genome of *Vibrio cholera,* has been shown to have two asymmetrically pronounced circular chromosomes; the large chromosome or chromosome 1 and the small chromosome or chromosome 2). The large chromosome or chromosome 1, encodes for the following genes; the DNA replication and repair, transcription and translation, catabolic and anabolic pathway genes, genes encoding for surface antigens, toxin co-regulated pili/adhesion as well as endo and exotoxins [20]. An enzyme, (3-hydroxy-3-methylglutaryl CoA reductase), a gene, suspected to have been a fall out product of an interaction with Achaea, and a gene capture system (an integron island, which is thought to be a large plasmid, integrated in to the original *Vibrio species*), all form part of the small chromosome (chromosome 2) [21]. These two chromosomes, which may be products of lateral gene transfer, have various ORFs that carry out almost the same functions. For example, the gene glyA encodes for serine hydroxymethyl

transferase and appears only on a single location on both chromosome1 and 2. On chromosome 1, the gene glyA leads to the α-Proteobacteria, while on chromosome 2, glyA gene, (sandwiched by transposases encoding genes and suggested to have originated from mutation by transposition reactions) is with the g-Proteobacteria [20].

The *Vibrio* strains from the 1960s and the 1970s, have been grouped into gene Cluster I, while the second cluster (Cluster II), contains strains from the 1980s and 1990. This grouping was according to how they were revealed by the Amplified fragment length polymorphism (AFLP), [22].

4. VIBRIO SPECIES AND DISEASES

There have been several reports on the emerging hazards associated with the consumption of foods contaminated with *V. fluvialis.* This is indicated by the isolation of these emerging pathogens from sporadic cases of acute diarrheas and outbreaks [23]. This pathogen has been indicated in extra intestinal infections, such as hemorrhagic cellulites and cerebritis, peritonitis, acute otitis, biliary tract infection, bacteraemia, and even ocular infections [23].

Moreover, other Vibrio species (eg. *V. alginolyticus)* have also been incriminated as agents of food poisoning [24,25], wound infections [26], profuse watery diarrhea that leads to rapid dehydration and death if not treated [27-29]. It has also been noted that this species can easily be isolated from the stools, (mostly from gastroenteritis cases), dead diseased shellfish, waters (especially coastal, estuarine, temperate and tropical waters), seafoods, human wounds etc [30].

However, strains of other pathogenic species such as *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae* [31] have also been reported in Europe and sub-Saharan Africa [32,33] as emerging causes of wound infections, food poisoning, mild to moderate, self-limiting and critical gastrointestinal infections or as agents implicated in disease outbreaks [34]. Infection with *Vibrio vulnificus* from seafood is usually severe with a high mortality rate [35]. However, in recent years, *V. mimicus* and *V. alginolyticus* (with pathogenicity almost like that of *V. paraheamolyticus*) have been increasingly recognized as other sea food borne pathogens [35-37].

5. VIBRIOSIS

As defined by Singleton and Sainbury, [30], vibriosis refers to any disease, usually other than cholera, of man or animals (e.g. fish) caused by a *Vibrio* species and occurs by the faecal–oral route, usually via contaminated water or food. The manifestation of this disease is influenced by the number of organisms ingested and this is harnessed by low stomach acidity as a predisposing factor. An incubation period of 1–5 (usually 2–3) days is common observed. The stools containing faecal material, rapidly becomes pale-grey and watery ('rice-water stools'); with about 1–30 litres of fluid passed out per day. This precarious volumes of fluid loss, results in severe dehydration and electrolyte depletion, which in untreated cases, may lead to high mortality rates of about 50–75%. Death may occur within hours, but usually within 1–2 days even though mild cases can also occur, particularly in endemic areas.

This diarrhoeal disease may be caused by non-cholera toxin producing O1 strains; as a result of the production of Ace and/or Zot toxins (Accesory cholera enterotoxin and Zonula occludens toxin), encoded by bacteriophage CTX8.

6. THE DISEASE CHOLERA

Cholera is an acute infectious, secretory, and painless diarrheal disease [38], caused by the Gram-negative bacterium *Vibrio cholerae* [11,38]. This infection primarily originates, when water or food, contaminated by the faeces of a *Vibrio cholera* infected individual (even that with no apparent symptoms), is consumed by an apparently healthy person [7,39].

After about 7-14 days of infection with *V. cholerae*, the bacteria can be found in faeces, the major source of infection to potential victims. Symptoms which may include major indicators such as watery stool and vomiting [40], may not be developed in about 75% of *V. cholera* infected people. About 20% out of the 25% of infected people who develop symptoms, present with severe dehydration from acute diarrhea ("rice water stools"), as a result of a great percentage of water and salts lost per hour [22].Watery, gray, flaky mucus stool, intense thirst, muscle cramps, weakness, anuria, sunken eyes and consequently kidney failure, shock, coma, and death may occur if untreated. A carrier state is usually observed in some people after recovery from the disease [22].

Among French speakers" cholera was nicknamed 'mort-de-chien' or 'dog's death,' due to the acute awful effects and the dehumanizing symptoms of the disease [41].

The disease appears mostly, as explosive outbreaks and pandemics [22]. The cholera burden per year has been estimated to be 1-4million cases with a total of 21,000-143,000 deaths globally, each year [42-44].

The most important virulence factor associated with this *V. cholerae* O1 and O139 life-threatening diarrhea is the cholera toxin (ctx) [45]. This is a pentameric toxin that triggers signals for elevation of intracellular cAMP and consequently, stimulates luminal secretion of electrolytes and water from enterocytes. This is the basis of loss of water and electrolytes from the gut, weight loss, dehydration and death [46,47].

7. CHOLERA TOXIN (CHOLERAGEN) AND ITS MECHANISM OF ACTION

The Choleragen is a protein enterotoxin, responsible for the symptoms seen in cholera. Structurally, CT is composed of B subunits that form a pentameric ring around a central A subunit to one side of the plane of the ring (AB5 arrangement of subunits). A variant of *V. cholerae* produces B subunits ('choleragenoid') without an A subunit [49].

The GM1 gangliosides in the brush border membranes of cells lining the small intestines are the glycolipid receptors for the B subunits. Internalization of the toxin occurs via a non-clathrin coated vesicle. While inside the cell, proteolytic cleavage, fragments the A subunit in to two temporarily bonded disulphide parts; the active catalytic fragment is responsible for CT activity and acts as an ADP-ribosyl transferase. It is activated only when the disulphide bond is reduced and ADP-ribosylates the Gs protein. This protein stimulates the conversion of the Adenylate Cyclase (AC) to a permanently active form. This raises the cAMP level within the target cell and activates the cystic fibrosis transmembrane conductance regulator (CFTR), resulting in the secretion of chloride ions into the lumen of the intestines. The effect of Zot (Zonula occludens toxin) on the structure of TJs causes more membrane permeability. Furthermore, the intestinal cells are stimulated by the Accesory cholera enterotoxin (ACE), to secrete Ca^{2+}-dependent $Cl^{-}/HCO3^{-}$, while the Heat stable enterotoxin (Sta) causes increased cGMP secretion and inhibition of the regulatory mechanism of Na^{+}/Cl. These thus lead to an efflux of water and electrolytes (sodium and chloride ions) into the gut lumen, causing the profuse watery diarrhoeal characteristic seen in cholera disease [46].

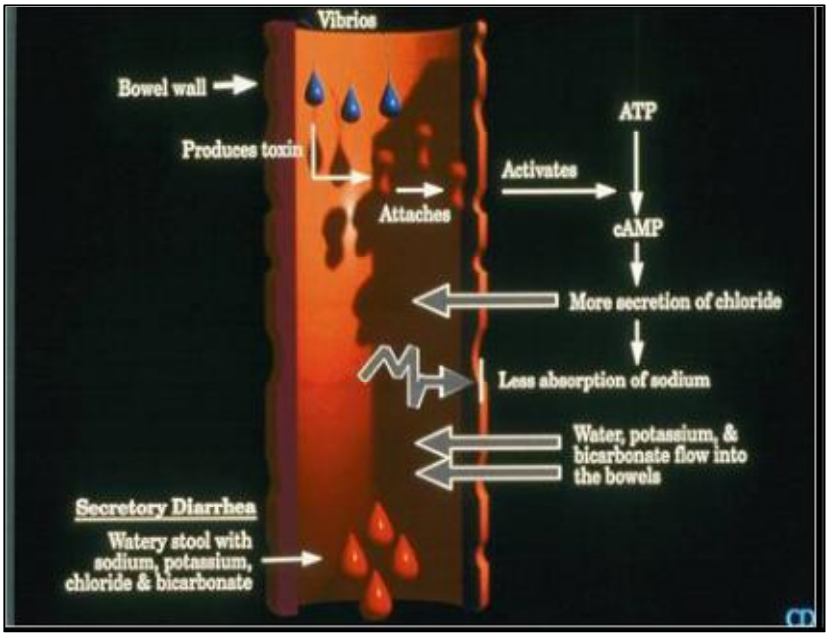

Fig. 1. Pathogenesis of *V. cholerae* in the bowels; Source: [48]

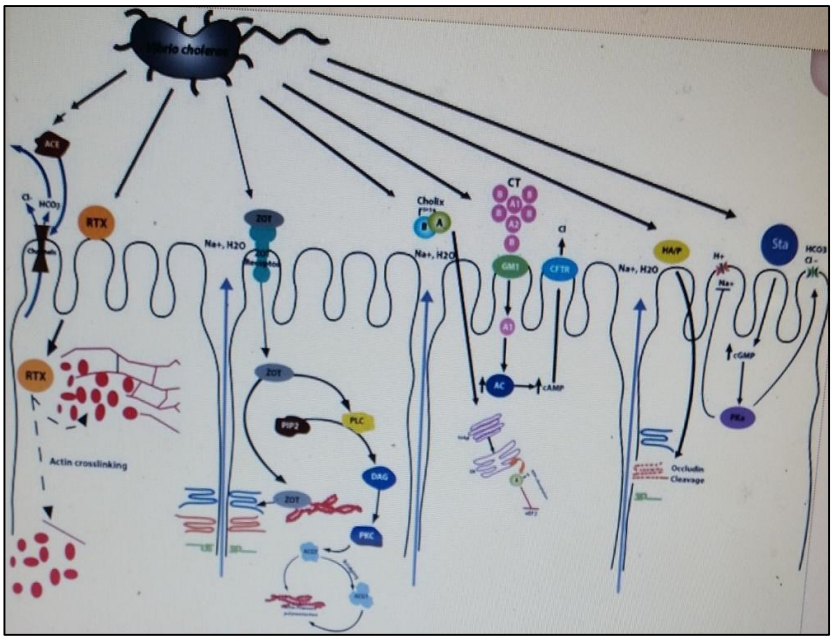

Fig. 2. Mechanism of action of *V. cholerae* toxins; Source: Dalian et al. [46]

8. OTHER *Vibrio* VIRULENCE FACTORS

V. parahaemolyticus produces a thermo stable direct haemolysin (*tdh*) and thermo stable direct haemolysin-related haemolysin (*trh1 and trh*2) genes, which are virulence associated genes [36]. The pathogencity of *V. mimicus* which is genetically and biochemically closely similar to *V. cholera,* involves several toxins [35]. Virulence factors associated with pathogenesis reported in *V. fluvialis* include a Chinese hamster ovary (CHO) cell elongation factor, CHO cell-killing factor, enterotoxin-like substance, lipase, protease, cytotoxin, and hemolysin [23].

The strains of *V. cholerae* which do not produce cholera toxin but may cause less severe form of disease, produce the Ace and Zot toxins [20].

Virulence in pathogenic O1 and O139 *V. cholerae* is associated with two genetic elements; Cholera Toxin (CTX), which is of the AB5 family of ADP-ribosyltransferase, typical of the profuse rice-watery diarrheal disease [49]. This toxin is encoded by genes *ctxA* and *ctxB* of the Bacteriophage CTXf8, for which toxigenic strains of *V. cholerae* are thus lysogenic), and the type IV toxin co-regulated pili (encoded by a chromosomal pathogenicity island which also encodes factors responsible for intestinal colonization) [50]. These appendages help the bacterium to adhere to the intestine [29,51]. TCP is the receptor for CTX prophage during *V. cholerae* infection and integration of the prophage into the bacterial chromosome.

Other Virulence factors include VSP-II (VC0511, VC0513), VPI-I (tcpA), CTXW (ctxB), heat stable enterotoxin (NAG-ST), type three secretion system (T3SS), type VI secretion system (T6SS), [29,52], enterotoxigenic hemolysin (hlyA) [36]) and RTX toxin (rtxA) [29]. Resistance factors include: IntI1, IntI2 integrases, variable regions from class 1 and 2 integrons, the 39 conserved sequence from class 1 integron, SXT element-integrase gene and associated SXT resistance genes (floR, sul2, dfrA1 and strAB), genes related to quinolone resistance (gyrA, gyrB, parC and parE), biotype-specific repeat sequence transcriptional regulator (rstR), ctxB alleles and rfb gene specific for O1 serogroup 1 [29]. All these, create an environmental reservoir of critical virulence genes, which may contribute to the evolution of pathogenic *V. cholerae* [52]. This has been suggested as the reason why severe disease has been associated with the non-O1/O139 strains that carry the genes for the CTX prophage, TCPand express CT. The pre-CTX prophage and TCP genes demonstrate a variety of different *rstR* and *tcpA* alleles suggestive of ongoing genetic recombination among non-O1/O139 strains in environmental reservoirs.

Whereas El Tor strain N16961 carries only a single copy of the cholera toxin prophage (the

integrated genome of CTXf, a temperate phage with a single copy of the cholera toxin (CT) genes, ctxAB, located on chromosome 1), other *V. cholerae* strains carry several copies of this element. However, strains of the classical biotype have a second copy of the prophage that is localized on chromosome 2 [20]. The cholera toxin prophage region (CTXU) in toxigenic *V. cholerae* O1 El Tor and O139 strains is often flanked by RS1 containing rstC gene which is not present in RS2. The El Tor biotype also has the rtxC gene (which is a component of a gene cluster of10 kb size), comprising four ORFs, rtxABCD, that encodes the Repeat in Toxin (RTX) [51].

A region encoding an RTX toxin (rtxA), (which is suggested to have been horizontally acquired along with the sensor histidine kinase/response regulator) together with its activator (rtxC) and transporters (rtxBD) is located on the other side of CTXf prophage. Two genes encoding a sensor histidine kinase and response regulator, downstream a third transporter gene, a paralogue of rtxB, is transcribed in the same direction as rtxBD [20].

Genes involved in `quorum sensing', or cell-density-dependent regulation, also exist on both chromosomes of *V. cholera* [53-56]. In bioluminescent *Vibrio species* (notably *Aliivibrio fischeri* gen. nov., comb. Nov. formally *V. fischeri* and *V. harveyi*), quorum sensing is used to control light production. Although this strain of *V. cholerae* lacks the genes for bioluminescence, it does have the genes required for the autoinducer-2 (AI-2) quorum sensing mechanism (luxOPQSU) but this pathway is split between the chromosomes with luxOSU on chromosome 1 and luxPQ on chromosome 2 [53-56].

Vibrio cholerae has also been reported to produce shiga-like toxins as well as the TCP, a type IV pilus, whose genes involved in the assembly of TCP (ABCDEFGHIJNQRST) reside on chromosome 1 [19].

Also, Part of a proposed `pathogenicity island' (VPI) is a critical intestinal colonization factor of *V. cholera.* These genes are acquired DNA, which encodes for TCP and other genes (such as acfABCD, toxT, aldA and tagAB), which are associated with the ToxR regulatory cascade [19] CTXf cellular receptor is the toxin-coregulated pilus (TCP) whose gene cluster, as well as the regulatory genes for the control of these gene clusters (toxR), are located on chromosome 1 [20].

VSP-I and VSP-II are linked with the pandemic potential of seventh pandemic El Tor isolates [51]. Thus, these rstR, tcpA, ctxB genes, the *Vibrio* seventh pandemic island-I (VSP-I), VSP-II and the number of genes in the repeat toxin region (RTX) are used asgenetic markers to differentiate the Classical from the El Tor biotypes [29].

Genes encoding haemolysins (hlyA) (for enterotoxic activity), lipases, proteases, including the haemagluttinin protease (hap), (a secreted metalloprotease that seems to attack proteins involved in maintaining the integrity of epithelial cell tight junctions), virulence factors reside on chromosomes unlike those of CTX, RTX and all known intestinal colonization factors.

The haemolysin (HlyA) genes (a 19-base-pair oligodeoxynucleotide probe) are developed for the purpose of distinguishing the Classical from the El Tor biotypes. In the El Tor strains, HlyA is an intact,82 kDa and biologically active protein but that of the classical biotype is a non-haemolytic, truncated protein product of 27 k Da which is formed from an 11-base-pair deletion [36].

Two regions, identified exclusively among El Tor biotype and O139 serogroup isolates, are the Vibrio seventh pandemic island-I (VSP-I), (including VC0175–VC0185) and VSP-II, enclosing VC0490–VC0497 (later shown to include a region of 26.9 kb- VC0490–VC0516)., The classical biotype strains and U.S. Gulf Coast strains possess the CT1 prototype of the Cholera toxin B subunit while the El Tor biotype and O139 strains produce the CT2.

The deduced amino acid sequence in the biotypes has undergone some three non-random base change mutations that have resulted to the three types of ctxB genes. The classical biotype worldwide and the US Gulf Coast belong to the first group with Genotype 1, El Tor biotype strains from Australia; belong to genotype 2 and the seventh pandemic and the Latin American epidemic strains of El Tor biotype, all grouped as genotype 3. A strain of *V. cholerae* O139 re-emerged in Kolkata in 1996 carrying new genotype of ctxB that had CTX Calcutta phage. This was placed in the fourth genotype. However, in 1998 the fifth genotype was detected and prevailed mostly in CTX phages with El Tor rstR [57]. They also reported that O139 strains have been detected to be of five types classified using the CT genotypes.

In Asia, Naha et al. [58], reported the presence El Tor variant strains producing classical toxin from different outbreaks. These variants were found to produce an amount of cholera toxin equivalent to that produced by classical strains [58,59], a characteristic not portrayed by the prototype El Tor strains.

Furthermore, the strain of *V. cholerae* isolated in the Haitian epidemic, carrying the Haitian ctxB, is alleged to have been isolated in Kolkata in 2006 and in 93.3% of strains isolated in Kolkata during 2011 epidemic [60]. The mature TcpA subunit of this strain carried a point mutation at the 64th amino acid [51] and this variant had also been isolated in Kolkata 2003 outbreak which later replaced all the El Tor tcpA [61]. This is an indication that the Haitian epidemic strain carrying the Haitian ctxB and tcpA alleles could have originated from Kalkota, from where it has now spread to other regions. However, it still has some of its own native peculiarities, not found in any other strain, already isolated elsewhere [51]. The double-mismatch amplification mutation assay (DMAMA)-PCR of the Nepalese *V. cholerae* strain has revealed that this strain is a clone, that is closely related to the Bangladesh and Haitian *V. cholerae s*trains. They possess the B-7 allele of *ctx* subunit B, with a point mutation at amino acid position 64 (N → S) of *tcpA,* while the *ctxAB* promoter revealed four copies of the tandem heptamer repeat sequence 5′-TTTTGAT-3′. The strains possessed all the ORFs of the Vibrio seventh pandemic island (VSP)-I, but lacked the ORFs 498–511 of VSP-II and also carried the SXT genetic element. The *gyrA* and *parC* of the NA^R strains (n = 4) carried a point mutations at amino acid positions 83 (S → I), and 85 (S → L), respectively. Grim *et al*. [62], observed that the new *V. cholerae* , El Tor variant has a more competitive ecological edge and greater infectivity over that of other pathogenic clones. This they noted is as a result of the acquisition of a combination of the new characteristics [63], or due to the fact that since CT is directly responsible for the major clinical signs of the disease, genetic changes in the CT genes could alter the clinical manifestation of cholera [64]. A combination of the improved environmental fitness of the, El Tor strains and the production of a more severe form of the disease by the classical strain (through recombination and lateral gene transfer), resulted in changes that conferred the new *V. cholerae* strain, the qualities of infectivity, expanded ecological persistence, and dispersion. This thus, gives the atypical El Tor strains a better ability to succeed in its environment and transmit progeny through human populations [62,65,66].

Wah et al. [66], reported that the predominant clones causing cholera in Asia and Africa are atypical El Tor *V. cholerae*, CIRS101, and CIRS101-like variants. They also observed that the Myanmar isolates from 2012 and the CIRS101 strain contained a single nucleotide polymorphism in the *tcpA* gene at 266 (A→G) of the prototype seventh pandemic El Tor (N16961) strain.

9. DEVELOPMENT OF *Vibrio cholerae* HYBRID STRAINS

This facultative pathogen (*Vibrio cholerae*), naturally inhabits temperate aquatic environments, where the β-1,4-linked GlcNAc, (an insoluble polysaccharide found in the chitinous exoskeleton of zooplankton), serves as the preferred source of nutrient. Chitin induces the homologous recombination of DNA from the extracellular environment and DNA in chromosomes to cause natural transformation in *V. cholera,a* process described as natural competence. This horizontal gene transfer (HGT) process in *V. cholera* and other pathogenic microbes, leads to the acquisition of virulence and multiple drug resistance mechanisms. Thus it is thought to have been the genesis of the O139 variant or the outbreak strain, and the replacement of the O-antigen biosynthetic locus [67].

The Integration of Conjugative Elements (ICEs of the SXT/R391 family which range from ~80 to 110 Kb in size) into the 5′end of the highly conserved prfC (peptide-chain-release factor C) gene can invariably result in HGT in these group of bacteria. This region contains all of the genes required for gene transfer into naive hosts by conjugation and those present in *V. cholerae* strains are characterized by a genetic core made up of not less than ten component groups of genes, with different genetic compositions at particular locations [67]. Recently, these later researchers reported that, close to 77% of the sequenced clinical isolates of *V. cholera* possess the seeming most common, of the ICEs (VchInd5). The identification of these strains in several areas of the globe, signifies therefore that, VchInd5, could have been part of the ancestor original Bengal strain that was now later distributed to other strains globally. A pointer to conclusive statement is the 2010 Haitian epidemic outbreak strain that through

Phylogenetic and Bayesian analyses was revealed to have emanated from the lineage, having the VchInd5 ICE [68].

10. THE VIABLE BUT NON-CULTURABLE STATE

The viable but non-culturable (VBNC) state of bacteria, is a state in which live bacteria, cannot grow on the frequent and commonly used bacteriological media [54] but can live on host cells. Many human pathogens (e.g.*V. cholerae*, and other Gram-negative bacteria), have been shown to revert to the VBNC state when subjected to natural stresses conditions like starvation, temperature changes as well as osmotic pressure [69-71].

Pathways, leading to the regulation of the response signals of starvation, quorum sensing, production of enterotoxigenic haemolysin (HlyA) etc, are expressed by the chromosomes 1 and 2 [69,70]. The alternative sigma factor j38 (rpoS) which is important for survival of *V. cholerae* in the environment, although it has no known association with pathogenicity, is necessary for the commencement of the VBNC state in these bacteria [50]. On chromosome 1, near the oriC, is a copy ofrpoS, which regulates expression of catalase, cyclopropane-fatty-acyl-phospholipid synthase and HA/protease, as well as many other proteins which are present on the two chromosomes [50].

There are reports on the conversion and resuscitation of VBNC *V. cholerae* and *Vibrio vulnificus* in environmental water samples [53], back to the culturable states by the quorum-sensing molecules (CAI-1 and AI-2) [53-56] previously, demonstrated this phenomenon in the *V. cholerae* O1, O139 and some other enteric bacteria using coculture with the human colonic epithelial cell line (HT-29) (or cells from other eukaryotic origin) and temperature up shift. They extracted a natural protein factor (FCVC) [72], from HT-29 cells and observed that it could convert VBNC *V. cholerae* to culturable *V. cholerae.* They later characterized and identified FCVC to be a catalase [73].

VBNC *V. cholerae* O1 El Tor variant strains harboring a gene for the cholera toxin had been isolated from environmental water samples in Kolkata, India. This is an indication that VBNC *V. cholera* when taken in to the human intestine can by the action of catalase, be converted to cause the disease cholera in humans [72]. This therefore implies that VBNC *V. cholerae* O1 and O139 species in environmental water poses a great hazard because they can act as a source of infection in these areas when converted and resuscitated [53,72].

11. THE EPIDEMIOLOGICAL DISTRIBUTION AND SPREAD OF *Vibrio cholerae* SEROTYPES

Approximately twelve out of the >60 reported *Vibrio* species (amongst which include *Vibrio cholerae),* are pathogenic to humans [35]. These have been isolated from water, sea foods etc [34,35,74].

V. cholerae is classified into more than 200 serogroups using the polysaccharide composition of their somatic antigens, although, the O1, and O139 Serogroups [28,75], are those associated with epidemic cholera disease [11].

The O1-serogroup is further grouped in to biotypes El Tor and Classical [64,75,51,66] and into serotypes Ogawa, Inaba and Hikojima [24). These O1 strains can interconvert and switch between the Ogawa and Inaba serotypes [74], and this has been the major cause of outbreaks all over the world [76]. Classical and El Tor biotypes phenotypically, can be differentiated using their ability to hemolyze sheep erythrocytes, their Voges–Proskauer (VP) test reactions, susceptibility to polymyxin B, their ability to agglutinate chicken red cells (erythrocytes) (CCA), and their susceptibilities to phages.

The biotypes El Tor, which was first isolated in 1905 in El Tor Egypt, emerged on the Indonesian island of Sulawesi in 1961. Subsequently, it spread to Asia and Africa [77]. Having Sparked up the seventh cholera pandemic that began in 1961 [22,77,78], V. *cholerae* O1 biotype El Tor, since then has become endemic (isolated from epidemics and out breaks of cholera) in Africa [22].

Through genetic exchange, hybrid strains of classical and El Tor biotypes (strains with intermediate characteristics), have developed and this is a pointer, showing that there is some degree of ancestral relatedness between the two biotypes of *V. cholera* [75].

Furthermore, a more virulent EL Tor strain from India is reported in Haiti and produces the

classical Cholera Toxin [11], though it has low endemicity. It is reportedly slowly displacing the original El Tor and replacing the Classical strain [11,45,61]. It has virtually been attested to be behind three most severe cholera epidemics in the last four years of the Seventh Pandemic [79]. This strain that was isolated in Asia since the 1990s [79], gained its roots only in Mozambique, Zambia and South Africa [80,81]. The CT-B subunit of this variant is different from the southern Africa variants, and this may be used to trace the geneology of the central or western African hybrid strains of *V. cholerae* O1 El Tor [81]. In Garoua, Pasteur Center, Cameroon, an atypical El Tor strain of *V. cholerae* O1 biotype El Tor, serotype Ogawa, was reportedly isolated from human stool samples and shown to possess a DNA sequence of *ctxB,* similar to that of the Orissa variant, identified in India in 2007 [77]. More so, Mahmud et al. [82], reported the involvement of four clonal types of altered variants of *V. cholerae* O1 biotype El Tor, serotypes Ogawa in the 2012 outbreak of cholera in Sierra Leone.

Vibrio cholerae O139 Bengal is a substitution mutation product of lateral gene transfer, which caused the genomic island of O139 to be substituted for the O1 antigen [10] and this except for the polysaccharide surface antigen, gave this strain, characteristics similar to *V. cholerae* O1, biotype El Tor [22]. Infections with O139 Bengal, began in India and Bangladesh [83], then spread to south-East Asia [11], Africa [22], and in coexistence with the El Tor Vibrios, the 0139 Vibrios, continued to spread causing Outbreaks or cases in many countries of the world [84].

The non-O1/non-O139 strains have not been associated with epidemic cholera because, they do not produce major virulence factors, although, genes coding for virulence factors have been identified in certain strains of both clinical and environmental origins [76]. However, sporadic cases or outbreaks of diseases which simulate cholera have been reported [22,45,76].

The non-O1 and non-O139 (non-choleragenic) strains are serovars of *V. cholerae* [22,45]. Reports of conversion of non-O1 to O1 serotype and its relationship with the O139 Bengal, in cholera epidemics, has increased research interest in non-O1/non-O139 *V. cholerae* strains. For example, a non-O1/non-O139 lineage of *V. cholerae,* has been traced recently to an outbreak in Nigeria [29].

Some non-O1/O139 *V. cholerae* isolates have also been reported to cause outbreaks or sporadic cases of non-cholera gastroenteritis [45].

Other strains which have been designated as the Matlab (MT) variants (strains which have not been biotype into any of the groups yet) have also been reported in Bangladesh [75].

Further reports coming from National Center for Biotechnology Information, NIH, Bethesda, USA, showed that the sequencing center (16-MAR-2007) of the Institute for Genomic Research, isolated from the 6th pandemic strain, a *Vibrio cholerae* O395 strain, with characteristics similar to other *Vibrio species*; although, the epidemiological distribution of this O395 strain and spread of new infection is still not known [21].

12. CONCLUSION

Cholera has become endemic in the African continent (Nigeria inclusive) for over a long period of time and the *Vibrio cholerae* O1 and O139 have been the major causative agents associated with most outbreak reports. Although there have been reports about the isolation of a new strain of *V. cholerae* (O395, 6th pandemic strain), with characteristics similar to other *Vibrio species,* the epidemiological distribution of this O395 strain and its spread of new infection is still not understood. However there are several reports of *A*typical El Tor strains of *V. cholerae* O1 biotype El Tor, serotype Ogawa, isolated from human stool, as well as the development of hybrid strains with characteristics that appear to be midway between the classical and El Tor biotypes. This atypical EL Tor genetic content has been identified in a virulent strain in Haiti from India. It seems to potentially manifest more toxigenic characteristics (producing the classical CT), low endemicity and more virulence. Slowly, it is replacing the Classical strain and has virtually been attested to be the one behind the three most severe cholera epidemics in the last four years (The Seventh Pandemic). Gradually it is displacing the original El Tor strains. More cholera awareness and surveillance schemes, prompt response as well as prevention, control and treatment strategies need to be developed to help curb the spread of these new strains to other vulnerable parts of the world.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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