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Vibrio cholerae: A historical perspective and current trend

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ABSTRACT

Vibrio cholerae (*V. cholerae*) is a Gram-negative, curved, rod-shaped bacteria with two of its strains *V. cholerae* O1 and *V. cholerae* O139) known to cause cholera, a deadly diarrheal disease that has repeatedly plagued the world in pandemics since 1817 and still remains a public health problem globally till today. The pathogens' persistence in aquatic milieux during inter-epidemic periods is facilitated by the production of a biofilm, thus evolving from being an infection of oral-fecal transmission to a more composite ecological framework of a communicable disease. The outbreaks of cholera spread rapidly in various intensities within and among countries and even continents and the World Health Organization estimates that 3–5 million cases outbreak and over 200 000 die yearly from cholera. Also, the impact of a cholera epidemic is not limited to its high morbidity and mortality rates alone, but also the grievous impact on the economy of the countries experiencing the outbreaks. In this review, we carried out an overview of *V. cholerae* including its isolation and detection, genetics as well as a comparison of the toxigenic and non-toxigenic determinants in the human host and the host defences. Furthermore, the history of global pandemics, cost implications, conflict and ecological methodologies of cholera prevention and control. The management of disease and antibiotic resistance in *V. cholerae* are also highlighted.

1. Introduction

Vibrio cholerae (*V. cholerae*) belongs to the family of Vibrionaceae, a facultative anaerobe with a flagellum used for mobility[1]. It is Gram-negative, bean-rod shaped, and oxidase positive, but it does not form spores[2]. Fresh isolates are prototrophic, and in suitable media they breed very fast within a maximum growth rate of 30 min. They exhibit maximum growth in an aerobic condition, though they are facultative organisms. *V. cholerae* fare well in an alkaline medium but are destroyed in any condition below pH 6 and can be found mainly in aquatic habitats (freshwater, saltwater or brackish water) or in the intestine, vomit and stool of a human host[3].

The bacterium is distinguished serologically on the O antigen of its lipopolysaccharide into cholera vibrio (pathogenic) and non-cholera vibrio (non-pathogenic) variants. Cholera toxin-producing strains, O1 and O139 serogroups, cause cholera disease (acute enteric human diarrhoea), while the non-toxigenic O1/O139 group causes non-epidemic periodic diarrhoea, wound infection, gastroenteritis, septicemia and skin infections[4]. *V. cholerae* O1 is further classified into Ogawa, Inaba and Hikojima biotypes that may either be classical or El Tor serotypes.

Although, all *Vibrio* strains exist in an aquatic environment, the non-toxigenic strains are more dominant in this environment[5]. In their aquatic habitations, these bacteria are found mostly attached to the exoskeletons of phytoplanktons and zooplanktons to improve their adaptation to the aquatic habitat. Because they have to be modified to suit both environments (aquatic habitats or in the intestine of a human host), many *V. cholerae* structures, such as their pili, have to be strongly heritably structured, with the ability to colonize surfaces. The organism has different ways of colonizing surfaces. This depends on the presence and characters of both preserved and mutable genetic factors[6]. In the marine

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environs, different surfaces are accessible for colonization (biofilm development). These include suspended mineral particles that are made up of majorly negatively charged silicates, cellulosic plants and the exoskeletons of crustaceans, which include zooplankton organisms and they consist principally of chitin[7]. Ecological reports reveal that an add-on to the chitin outer walls is a fundamental habitual routine of *V. cholerae* in the aquatic habitat and biofilm development establishes an efficacious endurance device[7,8]. Biofilms formation is critical for the ecological existence, spread and infectivity of the *Vibrio* species[9].

Previous project reported a universal model for *V. cholerae* biofilm growth, consisting of distinctive forms of transcript inside plankton with a layer of a molecule thick[10]. Planktonic phase involves transitory exchanges and with a surface which may be umpired by mannose-sensitive haemagglutinin. This shallow relationship brings about flagella genetic copy suppression leading to an enduring add-on of the organism to the external layer of the plankton. Once fashioned, this add-on leads to the establishment of a well-developed biofilm comprising packs of bacteria colonizing the surface[8,10].

Extended incubation in the course of a biofilm assay can lead to a rugose morphotype of *V. cholerae*[11]. The occurrence of the rugose variant makes the organisms, as biofilms, predation resilient[12]. Actually, it is a vital protection mechanism that contributes immensely in preserving the *Vibrio* species attached to floating materials, chitin and other surfaces[13]. Hence, the presence of biofilm in water bodies promotes the endurance and perseverance of the organism as well as serving as a nutrient source[10]. It is also responsible for the survival of the pathogenic *V. cholerae* against the acidic medium of the stomach of the infested human host. Persat *et al*. described *V. cholerae* in biofilm-clumps as well as in a planktonic form in stool samples of cholera patients[13]. The regular contamination of clusters like biofilm in water, always exceeds those that exist in planktonic form, and also that evolution instigates an increase in the infectious character of *V. cholerae* in biofilm; hence biofilms are more significant in the infection process[13]. The development of biofilm rests solely on the reaction of exopolysaccharides, proteins and nucleic acids. among which exo-polysaccharides are the key portion of the biofilm matrix and their absence diminishes the development of biofilm[14,15].

V. cholerae can exist as free-living bacteria in aquatic environments, especially warmer water, hence, it is more prevalent in Africa and Southeast Asia[16]. As a result of this environmental factor, cholera can remain dormant in water, attaching to plankton and the chitin in the shells of mollusks and other crustaceans, resulting in seasonal outbreaks[16].

2. Segregation and identification of *V. cholerae*

2.1. Collection of samples

Samples collection for isolation of *V. cholera* depends on the scope of the study and could be from water, faeces, food or aquatic animals. Samples are taken at different sites according to the justification of the study, in order to have good standard results. In non-cholera epidemic areas or developed countries, determination of the concentration of *V. cholerae* in sea animals such as oysters, the water bodies where the oyster is harvested and the underlying sediment should be of paramount importance[17].

Water collection bottles should be washed and autoclaved for 20 to 25 min at 121 °C preceding their use. For water sampling, polypropylene bottles must be used while glass bottles are standard for plankton sampling[18]. Collection of adequate volume and sample size is crucial to ensure the completion of all appropriate analyses. Also the simple physiochemical parameters of water should be determined on site at the same time and samples are collected and these parameters may include temperature, salinity, pH, dissolved oxygen and conductivity of the water, depending on study targets[18]. The nature of the water current when sampling, the water depth, and rainfall can also be measured if necessary[18].

2.2. Isolation and identification of *V. cholerae*

In recent time, modern science have improved technologically in the area of isolation, identification and characterization of *V. cholerae*. Both the virulent and non-virulent strains can be found throughout the year in aquatic environments, not necessarily in cholera epidemic environment[19]. Unless appropriate methods are used, the organisms may not be detected especially if they are in their viable but non-culturable (VBNC) state *i.e.* the VBNC *V. cholerae* will not form colonies on culture plates when a traditional culturing method is used but can cause disease[20,21].

To detect these organisms irrespective of their cultivability, two methods can be used. These are fluorescence *in situ* hybridization, which is used to identify the arrangements of DNA genes specific to all serogroups of *V. cholerae* and fluorescent antibody plus direct viable count (FA-DVC). These are direct detection methods which can only detect serogroups associated with cholera epidemics and cholera toxin-producing vibrio[18]. Fluorescence *in situ* hybridization is a quantification method that can give an accurate estimation of vibrio cells in an environmental water sample. This is accomplished by visualizing a fluorescently-labelled oligonucleotide probe using epi-fluorescence or con-focal laser scanning microscopy.

The FA-DVC method used for the fast discovery of virulent *V. cholerae* is also direct, without any culturing. When carried out with the direct viable count according to the method of Kogure *et al*., it can be used to differentiate between the cells that can be cultured and VBNC cells of *V. cholerae*[22,23]. It can also distinguish the pathogenic strains from the non-pathogenic serotypes. Direct FA-DVC is accurately used to detect the presence of *V. cholerae* within 8 h out of which 6 h is used for incubation.

Xu *et al*. introduced the indirect fluorescent antibody method for the enumeration of *V. cholerae* serogroup O1 in conservational water samples[24]. It is used mostly when commercial direct fluorescent antibody kits are not available. It is also very useful in counter checking the presence of non-culturable organisms in negative cultured samples[25]. PCR is another method for evaluating directly the existence of *V. cholerae* in ecological samples or after the sample has been enriched with alkaline peptone water. These methods work