VIBRIO CHOLERAE AND CHOLERA TOXIN: FROM CALCUTTA TO KOLKATA

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Robert Koch in Calcutta

On December 11, 1883, Robert Koch and his team arrived in Calcutta\(^1\). Their mission was to identify the causative agent of cholera. Immediately after their arrival, they started to work at the Medical College Hospital, Calcutta. In Koch’s report submitted to the Home Minister of the Imperial German Government on January 7, 1884\(^2\), he described the exclusive presence of “cholera bacillus” in the cholera materials and the correlation between its emergence and the process of disease. Further in his report on February 2, 1884\(^3\), he, for the first time, stated that the bacillus was not straight, but “a little curved, like a comma” in shape. Around the end of April, 1884, Koch and his team left Calcutta and sailed back to their home country.

On July 26 and 29, 1884, the “Die Conferenz zur Erörterung der Cholerafrage (Conference on the Cholera Problem)” was held at the Imperial Department of Health, Berlin\(^4\). In this conference, Koch predicted the presence of the cholera toxin. He discussed, “What an assumption that the Kommabacillus produces a specific toxin, symptoms and development of cholera can be explained as follows:……The symptoms of true cholera as a whole, which is thought to be a result of dehydration and concentration of blood, is essentially a result of intoxication, in my opinion.” And also, “I might only emphasize that in the intestinal content under the influence of Kommabacillus a toxic substance is produced.”

Discovery of Cholera Toxin by S. N. De

In 1953, S. N. De who was working at Department of Pathology, Nilratan Sircar Medical College, Calcutta developed an unique animal model to work on the pathogenicity of Vibrio cholerae. In the summary of the paper reporting this model\(^5\), he describes “Injection of living Vibrio cholerae into the lumen of a loop of rabbit small intestine isolated by ligature is followed after twenty-four hours by accumulation within this loop of a large amount of fluid having gross, microscopic and cultural similarity with the cholera stool.”

By applying this unique rabbit ileal loop test to the culture filtrate of V. cholerae in which no bacillus exist, he succeeded to demonstrate that the culture filtrate also gave the accumulation of a large amount of fluid. This is the first demonstration of cholera toxin produced by V. cholerae. The paper was published in Nature in 1959\(^6\), 75 years after Koch proposed a hypothesis that cholera is caused by a toxin produce by V. cholerae.

This rabbit ileal loop test is now known as De’s test and is widely used as the most popular model with which to demonstrate the biological activity of cholera toxin and other related toxin, such as heat-labile enterotoxin of enterotoxigenic Escherichia coli.

Almost the same time as De discovered the cholera toxin, Dutta and Habbu\(^7\) also reported the evidence that cell-free preparation of V. cholerae caused the similar symptoms to cholera in infant rabbit model. This model was used by Finkelstein and LaSpalluto\(^8\) to isolate and purify cholera toxin.

Structure and Function of Cholera Toxin

In 1969, Finkelstein and LaSpalluto\(^8\) purified cholera toxin to its homogeneity. They used a hypertoxicogenic strain 569B of classical biotype. Since then the 569B became a prototype strain for the purification of cholera toxin. The availability of the purified cholera toxin opened up a new era on cholera toxin research and several important findings were reported during several years after the success of the purification. The most important one is the...
finding that cholera toxin is composed of two subunits, A and B.

In October 1973 at the 9th US-Japan Joint Cholera Conference held at Grand Canyon, Arizona, US, different research groups reported the subunit structure and the relevant functions of cholera toxin. Ohtomo et al.\(^9\) reported the two distinguished antigenic components in cholera toxin complex. Holmgren et al.\(^10\) reported the existence of two components of cholera toxin and the interaction of one of them with GM\(_1\) ganglioside. Antigenic difference of two subunits and receptor binding property of cholera toxin subunit were also presented by van Heyningen et al.\(^11\). Similarly, Cuatrecasas et al.\(^12\) reported on the membrane receptor and function of cholera toxin.

It has now become evident that cholera toxin is the A-B subunit toxin. The A subunit is composed of the A\(_1\) peptide of 195 amino acid residues and the A\(_2\) peptide of 45 amino acid residues. A\(_1\) and A\(_2\) peptides are linked by a disulfide bond. The B subunit is a molecule of 103 amino acid residues. The A subunit has ADP ribosyltransferase activity and stimulates the membrane bound adenylate cyclase through its A\(_1\) peptide, resulting in an accumulation of intracellular cAMP. The B subunit is to bind to the cell receptor, GM\(_1\) ganglioside.

**Detection of Cholera Toxin**

Symptoms of cholera primarily resulted from the action of cholera toxin. Therefore, after a pioneering work of S. N. De\(^6\), several biological, biochemical, immunological and genetic methods for detection of cholera toxin have been developed. As for immunological assays, GM\(_1\) ganglioside ELISA and reversed passive latex agglutination (RPLA) are mostly used in cholera research laboratory. Oku et al\(^13\) reported a very sensitive bead ELISA. By using the bead-ELISA, Nair et al.\(^14\) identified the cholera outbreak that occurred in a cruise ship. When the ship returned her mother port after sometime, no diarrheal symptoms were observed in all patients and only diarrheal stools were kept in refrigerator. Although *V. cholerae* was not isolated from the stool samples, cholera toxin was detected by the bead ELISA.

Quite recently, a dipstick named Crystal VC\(^TM\) was made commercially available by an Indian company for the diagnosis of cholera. Originally developed by an investigator in the Pasteur Institute, Paris, dipstick test is a lateral flow immunochromatographic test for the qualitative determination of LPS antigen of *V. cholerae* O1 and O139 serogroups. Specificity against O1 and O139 antigens is derived from the monoclonal antibodies specific to each antigens. Mukherjee et al.\(^15\) applied the dipstick test to stool specimens from hospitalized diarrheal patients and reported that the sensitivity and specificity of the test were 92% and 73%, respectively. As the dipstick test requires minimal technical skill and the results can be available within about 10 minutes, this test is useful in the remotely affected areas where alternative diagnostic tests including culture method are not available.

**Discovery of *V. cholerae* O139**

In November 1992, a large outbreak of cholera occurred in Chennai (then Madras). The strains of *V. cholerae* that caused the outbreak were examined at National Institute of Cholera and Enteric Diseases (NICED) and found that they were not agglutinated with the O1 antiserum. This was quite unique as it was a consensus that O1 serotype of *V. cholerae* among existing 138 serotypes is the only serotype to cause epidemic cholera. Strains of *V. cholerae* belonging to serotypes other than O1 are collectively called non-O1 and believed to cause only sporadic diarrhea. Extensive studies to characterize these non-O1 *V. cholerae* strains were carried out by G. B. Nair at NICED, T. Shimada at National Institute of Health, Tokyo, and myself at Department of Microbiology, School of Medicine, Kyoto University.

The unexpected results were obtained: (i) none of the strains agglutinated with polyvalent O1 antiserum or with monoclonal antibodies against factor A, B, and C of the O1 serogroup, (ii) none of the strains agglutinated with antisera against any of the existing 137 serogroups of *V. cholerae* non-O1 recognized at that time, (iii) all the strains produced cholera toxin when examined by bead-ELISA. Consequently, these strains were assigned to a new serogroup O139 and given a synonym “Bengal” to symbolize the first isolation of these strains from coastal areas located on the Bay of Bengal\(^16,17\).

**V. cholerae El Tor Variant**

There are two biotypes in *Vibrio cholerae* O1, that is, classical and El Tor. The former was responsible for the fifth and sixth cholera pandemics while the latter is responsible for the seventh pandemic that is ongoing. The biotypes are differentiated by phenotypic properties, such as hemolysis, agglutination of chicken erythrocytes, Voges-Proskauer test, inhibition by 50 units of polymyxin B, lysis by classical phage IV and lysis by El Tor phage S. Each biotype also differs from each other in its unique gene sequences encoding cholera toxin B subunit (CTB); classical biotype carries classical *ctxB* and El Tor biotype does El tor *ctxB*. 
In 2002, Nair et al.\(^{18}\) reported the isolation of strains of \(V.\) cholerae O1 that have phenotypic properties of both classical and El Tor biotype. The gene of this strain was classical ctxB. Further, they isolated El Tor biotype \(V.\) cholerae O1 strains carrying classical ctxB \(^{19}\). For these new types of strains of \(V.\) cholerae O1, we have recently proposed the designation of hybrid and El Tor variants, respectively\(^ {20}\).

Raychoudhuri et al.\(^ {21}\) examined 123 strains of \(V.\) cholerae O1 isolated in Kolkata during 1989-2005. It was found that all strains, which were of a variant El Tor biotype, that is, El Tor strain carrying classical ctxB, emerged in 1990. Since 1995, prototype El Tor strains were completely replaced by El Tor variant strains. Isolation of El Tor variants strains are now reported from many Asian and African countries.

It is known that clinical manifestation of cholera caused by classical strains is more severe than that caused by prototype El Tor strains. WHO recently claimed that El Tor variant causes more severe episodes of cholera with higher case fatality rates\(^ {22}\). In relation to this, it is interesting to note that recent report by Ghosh et al.\(^ {23}\) shows that El Tor variant strains produce much more cholera toxin than El Tor variant strains and the amount of cholera toxin produced is more or less the same to that produced by classical strains of \(V.\) cholerae O1.

**Cholera in Kolkata**

Active surveillance of acute diarrheal diseases is ongoing from November 2007 at the Infectious Diseases and Beliaghata General Hospital (ID Hospital), Kolkata. Nair et al.\(^ {24}\) reported the results obtained during November 2007 and June 2009. On two random days per week, every fifth diarrheal patient admitted to ID Hospital was enrolled for the study. Fecal specimens of a total 2,001 (5.6%) acute diarrheal patient admitted to ID Hospital was enrolled for the study. Fecal specimens of a total 35,780 patients hospitalized were examined for the following enteropathogens:

**Bacteria** : \(V.\) cholerae O1, O139, and nonO1-nonO139, \(V.\) parahaemolyticus, \(V.\) fluvialis, Aeromonas spp., Campylobacter jejuni, C. coli, Shigella dysenteriae, S. flexneri, S. boydii, S. sonnei, Salmonella spp., enteropathogenic E. coli, enterotoxigenic E. coli, enteraggregative E. coli.

**Virus** : Rotavirus, Adenovirus, Norovirus G1 and G2, Sapovirus, Astrovirus.

**Parasites** : Blastocystis hominis, Entamaeba histolytica, Giardis lamblia, Cryptosporidium spp.

For identification of these pathogens, in addition to conventional culture methods, several immunological and molecular methods were employed. \(V.\) cholerae was isolated from 496 cases, which was 24.8% of the patients examined. \(V.\) cholerae was the most frequently isolated enteropathogens among 26 enteropathogens examined. These data shows that one of four admitted patients is cholera patient.

One hundred and sixteen years and seventy-five years, respectively, have passed since the discovery of \(V.\) cholerae by Robert Koch and of cholera toxin by S. N. De, but cholera is still a big public health problem in Kolkata.

**References**