CHANGING EPIDEMIOLOGICAL TREND OF CHOLERA IN WEST BENGAL: THE GIANT IS BACK

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ABSTRACT: Cholera is a devastating diarrheal disease caused by V. cholera. Two biotypes of V. cholerae O1, classical and El-Tor, are distinguished. Each biotype is further subdivided into two serotypes, termed Inaba and Ogawa. As large deltaic areas of the Ganges and Brahmaputra rivers are considered to be the homeland of cholera, objective of our study was to detect the circulating strain of Vibrio causing Cholera outbreaks in different pockets of West Bengal. Water samples collected from the water sources of the affected areas and stool samples and or rectal swabs of suspected cases were tested according to standard bacteriological protocol.

In July 2008, Cholera outbreak was caused by Vibrio cholerae O1 El-Tor Inaba serotype in Darjeeling district affecting 176 persons. Mean age of the affected people was 15years. Thereafter in the June, 2010, there was an outbreak of Vibrio cholerae El Tor Ogawa affecting 87 people in Maldah. Mean age of the cases was 25years. Similar type of Vibrio cholerae O1 El-Tor Ogawa strain was isolated in the outbreak of June to September 2012 in Maldah with lower mean age of cases i.e.7years. A total of 93 patients suffered from Cholera during this outbreak.

Cholera outbreak in West Bengal was caused by classical Vibrio cholerae O1 Ogawa serotype affecting 803 people in North 24 Paragana in October, 2013. Mean age of the patients was 31year. As classical Vibrio causes more severe disease than El-Tor, its reemergence with multidrug resistant properties is no doubt an upcoming threat.

INTRODUCTION: Cholera is a devastating diarrheal disease caused by V. cholerae that has been responsible for seven global pandemics and much suffering over the past two centuries. It is an acute diarrheal disease that can, in a matter of hours, result in profound, rapidly progressive dehydration and death. Accordingly, cholera gravis (the severe form of cholera) is a much-feared disease, particularly in its epidemic presentation. Epidemic cholera remains a significant public health concern in the developing world today.

The species V. cholerae is classified into more than 200 serogroups based on the carbohydrate determinants of their lipopolysaccharide (LPS) O antigens. Although some non-O1 V. cholerae serogroups (strains that do not agglutinate in antisera to the O1 group antigen) have occasionally caused sporadic outbreaks of diarrhea, serogroup O1 was, until the emergence of serogroup O139 in 1992, the exclusive cause of epidemic cholera. Two biotypes of V. cholerae O1, classical and El Tor, are distinguished. Each biotype is further subdivided into two serotypes, termed Inaba and Ogawa.

Cholera is native to the Ganges delta in the Indian subcontinent. Since 1817, seven global pandemics have occurred. The seventh pandemic—the first due to the El Tor biotype—began in Indonesia in 1961 and spread throughout Asia as V. cholerae El Tor displaced the endemic classical

biotype. In the early 1970s, El Tor cholera erupted in Africa, causing major epidemics before becoming a persistent endemic problem. Currently, >90% of cholera cases reported annually to the World Health Organization (WHO) are from Africa, but the true burden in Africa as well as in Asia is unknown since diagnosis is often syndromic and since many countries with endemic cholera do not report cholera to the WHO. It is possible that >3 million cases of cholera occur yearly (of which only 200,000 are reported to the WHO), resulting in >100,000 deaths annually (of which <5000 are reported to the WHO).

Now the world is facing the eighth pandemic. In October 1992, a large-scale outbreak of clinical cholera caused by a new serogroup, O139, occurred in southeastern India. The organism appears to be a derivative of El Tor O1 but has a distinct LPS and an immunologically related O-antigen polysaccharide capsule. (O1 organisms are not encapsulated.) After an initial spread across 11 Asian countries, V. cholerae O139 has once again been largely replaced by O1, although O139 still causes a minority of cases in some Asian countries. The clinical manifestations of disease caused by V. cholerae O139 are indistinguishable from those of O1 cholera. Immunity to one, however, is not protective against the other (1).

AIMS AND OBJECTIVE(S): As large deltaic areas of the Ganges and Brahmaputra rivers are considered to be the homeland of cholera, objective of our study was to detect the circulating strain of Vibrio causing Cholera outbreaks in different pockets of West Bengal with an ultimate goal to find out the epidemiological pattern of Cholera in the Ganges belt of West Bengal in last five years.

MATERIALS AND METHODS: Properly collected Stool samples from suspected cases in a clean universal container or collected rectal swabs were transported in Cary Blair's media which also acted as enrichment media. Further enrichment was done in Alkaline Peptone Water followed by inoculation in Mac Conkey's agar media and TCBS (Thiosulfate-citrate-bile salts-sucrose agar) media for overnight incubation at 37^0c. Next day, the discrete colonies were further studied by Gram staining, tests for motility and a battery of biochemical tests which phenotypically implied the organism to be V. cholerae.

VP test reactivity, polymyxin-B susceptibility (150µg) and hemolytic properties were exploited to differentiate between classical and El Tor. Serotyping was done by slide agglutination test using corresponding antisera [supplied by DIFCO BACTO Detroit Michigan 48232-7058 USA Lot No. (17050131(10)166528LA,(17)041231(10)166079LA and (17)050502(10)2119465]. Antisera for O1 and O139 were supplied by National Institute of Cholera and Enteric Diseases (NICED). Antibiogram was done by modified Kirby-Bauer disk diffusion technique using commercially available appropriate antibiotic disks.

Water samples collected from the tube wells or water source of the affected areas were also tested according to standard bacteriological protocol.

Cases suffering from other causes of diarrhea were excluded.

RESULTS: In the year 2008, there was an outbreak caused by Vibrio cholera O1 El Tor Inaba serotype in Kharibari (Darjeeling district). The outbreak affected 176 persons. Mean age of the affected people was 15 years (range was 1.5 year to 35 years). The index case was reported in the month of July.

Thereafter in the year 2010, there was an outbreak of Vibrio cholerae O1 El Tor Ogawa affecting 87 people in Maldah. Mean age of the cases were 25 (range was 5 to 55 years). The index case was reported on 25th June, 2010.

Similar type of Vibrio cholerae O1 El Tor Ogawa strain was isolated in the outbreak of June, 2012 in Maldah with lower mean age of cases i.e., 7 years (range from 4 to 16 years). This outbreak continued up to the month of September 2012. A total of 93 patients suffered from Cholera during this outbreak.

Surprisingly enough, recent Cholera outbreak in post Phailin (super cyclone) West Bengal was caused by Vibrio cholerae classical biover Ogawa serotype affecting 803 people in Dumdum region (North 24 Paragana, suburban regions adjacent to Kolkata). The outbreak has occurred in the month of October, 2013. Mean age of the patients was 31year (range from 9 years to 65 years).

According to our present study, Ogawa serotype is being circulating and causing outbreaks in West Bengal since 2010 (Table-1).

Year of outbreak	Affected district	Number of cases	Time of onset (month)	Biotype	Serotype
2008	Darjeeling	176	July	El Tor	Inaba
2010	Maldah	87	June	El Tor	Ogawa
2012	Maldah	93	June	El Tor	Ogawa
2013	North 24 paragana	803	October	Classical	Ogawa

Table-1: CHOLERA OUTBREAK IN DIFFERENT DISTRICTS OF WESTBENGAL SINCE 2008

Apart from the re-emergence of V. Cholerae O1 Classical Biotype, increasing trend of multidrug resistance has also been established in circulating Ogawa serotype (Table-2).

	2008 El Tor, Inaba	2010 El Tor, Ogawa	2012 El Tor, Ogawa	2013 Classical Ogawa
Tetracycline	S	S	S	R
Cotrimoxazole	S	S	R	R
Chloramphenicol	S	S	R	R
Furazolidone	S	S	R	R
Norfloxacin /Ciprofloxacin	S	S	S	S
Erythromycin/ Azithromycin	S	S	S	S
Gentamicin	S	S	S	R
Polymyxin -B	R	R	R	S

Table-2: Drug sensitivity pattern of Vibrio cholerae (Year wise isolation)

Legend:
S- Sensitive
R- Resistant

Multidrug resistant Vibrio has emerged in West Bengal since 2012 and in the 2013 outbreak we have encountered Tetracycline resistant Classical Vibrio biotype which is also resistant to Cotrimoxazole, Chloramphenicol, Furazolidone and Gentamicin.

DISCUSSION: There is a constant tug of war between the serotype Inaba and Ogawa in West Bengal since 1999. According to Anuradha et al,Eighty-three V. cholerae O1 isolates were sent to the National Institute of Cholera and Enteric Diseases (NICED) in Kolkata for serotyping and phage typing. All the V. cholerae O1 isolates were of El Tor biotype. Among the two nonO1 vibrios isolated in 2005, neither agglutinated with O-139 antisera. V. cholerae El Tor serotype Inaba was found only in 2006. The isolates of 2004 and 2005 were of the Ogawa serotype. In a previous study in the same institute, all isolates detected over a period of 5 years (1996-2000) were of the Ogawa serotype.(2) From 2001 to 2005, all isolates were V. cholerae El Tor Ogawa (unpublished). Anuradha et al had isolated serotype Inaba in 2006 for the first time.

Jabeen et al performed a study from January to September 2005 with a total of 3290 stool samples received for culture at Aga Khan University laboratory, Pakistan. Of these 245 (7.4 %) yielded growth of V. cholerae. The total number of V. cholerae serotype Inaba (VCI) strains isolated were 243(3). In our study, Inaba was isolated in the year 2008, since 2010, the circulating serotype is Ogawa; whereas in another study done by Pallavi Garg et al (in the year 2000), emergence of Vibrio cholerae O1Bio type El Tor Serotype Inaba from the Prevailing O1 Ogawa Serotype Strains in Kolkata (West Bengal) was reported (4).

In the year 2012, Multidrug-resistant Vibrio cholerae O1El Tor was isolated by Sangita Roy et al. in Belgaum, south India (5). In the contrary to these two studies, in the year 2013, in our study, we have found out Vibrio cholerae O1 Classical Biotype to be the circulating pathogen in West Bengal.

With a discordance to the classical belief that El Tor biotype (6) is more drug resistant, in our study, Classical biotype was resistant to almost all drugs except Fluoroquinolones, Azithromycin and Polymyxin-B. Multidrug resistance pattern of Classical biotype is not well documented by other workers.

CONCLUSION: As Vibrio cholerae classical causes more severe disease than El Tor, its reemergence is no doubt an upcoming threat; moreover, its multidrug resistant pattern leaves behind very few therapeutic options especially for the pregnant women.

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