pISSN: 0976 3325 eISSN: 2229 6816

ORIGINAL ARTICLE

ISOLATION OF VIBRIO CHOLERA O1 DURING AN OUTBREAK OF ACUTE GASTROENTERITIS IN DAHOD DISTRICT, GUJARAT

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ABSTRACT

Background: Epidemics of cholera caused by Vibrio cholerae O1 or O139 have been reported from different parts of India. It is necessary to investigate Acute Gastroenteritis cases for the presence of V. Cholerae by stool sample analysis in the reference laboratory.
Objectives: To analyze stool samples for the presence of V. cholerae and subtypes in confirmed cases of cholera during an outbreak of Acute Gastroenteritis.
Materials and Methods: During an outbreak of Acute Gastroenteritis, total 171 stool samples and 5 water samples were collected by local health authorities and sent to the Microbiology Department, Medical College, Baroda for the analysis.
Results: Out of total 171 stool samples, 46 stool samples were found positive (27%) for V. cholerae O1 biotype El Tor, serotype Ogawa as a sole pathogen. Highest positivity (50%) was found in age group of 0-15 years. Positivity rate was same among both the genders. Water samples showed presence of coliform bacilli with high MPN (most probable number) count. Faecal coliform was also found in both the water samples.
Conclusion: V. cholerae O1 biotype El Tor, serotype Ogawa was confirmed as a sole pathogen in stool samples confirm the cholera outbreak and water contamination was supported by the presence of Faecal coliform organisms in water samples.

Keywords: Acute Gastroenteritis, stool samples, Water samples, Vibrio Cholerae, Biotype, El tor, Ogawa, Faecal coliform

INTRODUCTION

Cholera is an acute intestinal infection caused by ingestion of food or water contaminated with the bacterium Vibrio cholera. It has a short incubation period and produces an enterotoxin that causes copious, painless, watery diarrhoea that can quickly lead to severe dehydration and death if treatment is not promptly given. Vomiting also occurs in most patients.[1]

Most persons infected with V. cholera do not become ill, although the bacterium is present in their faeces for 7-14 days. When illness does occur, about 80-90% of episodes are of mild or moderate severity and is difficult to distinguish clinically from other types of acute diarrhoea. Less than 20% of ill persons develop typical cholera with signs of moderate or severe dehydration.[1]

Cholera remains a global threat and is one of the key indicators of social development. While the disease no longer poses a threat to countries with minimum standards of hygiene, it remains a challenge to countries where access to safe

drinking water and adequate sanitation cannot be guaranteed. Almost every developing country faces cholera outbreaks or the threat of a cholera epidemic.[1]

Cholera has been endemic in India since time immemorial. Bengal has remained home to cholera since long along with adjoining region of Bangladesh. The number of cases in India has declined significantly in last century but the frequency has continued at low level with large variations. The reported deaths due to cholera have reduced significantly from 150 in 1991 to 09 in 2010 reflecting increased availability of treatment facilities.[2] El Tor Biotype has largely replaced the Classical one. Outbreaks of cholera continue to be reported from various parts of India.[3]

It is necessary to investigate cases of Acute Gastroenteritis for lab confirmation by stool sample in reference laboratory. The organism that causes cholera can be serotyped using polyvalent cholera O1 antiserum and labelled as Vibrio cholera serogroup O1. If agglutination with anticholera O1 antiserum is negative, then an attempt should be made with Vibrio cholera O 139 antiserum. If agglutination does not occur with VC O1 or O 139 then the isolate is labelled as Non agglutinable (NAG) vibrios or Non cholera vibrios (NCV) or Non O1 Vibrios. This is a misnomer as the isolate can still be typed with some antiserum provided antisera for all cholera serogroups are made available. Both VC O1 and O139 serogroups can be further divided into 3 serological sub- types namely Inaba, Ogawa and Hikojima.[3]

During 1992, VC O139 Bengal was first discovered from Bangladesh. Due to non-availability of specific O139 antiserum, initial O139 serotypes were diagnosed as NAG. Later specific O139 cholera antiserum became available. This can now be used routinely. Therefore, isolation of NAG strains in large numbers should arouse suspicion for a new emerging serogroup.[3]

Biotyping: There are two biotypes: classical and El-tor. The classical biotype that used to cause severe cholera outbreaks in the past has been replaced these days by El –Tor biotypes (Characterized by resistance to Mukherjee cholera phage V, hemolysis on blood agar plates and chicken cell agglutination) that are less severe but leads to more extensive morbidity.[3]

Present study was conducted with the objectives to analyze stool samples collected during an outbreak of Acute Gastroenteritis for the presence of V. cholerae and subtypes in confirmed cases of cholera and analysis water samples.

MATERIAL AND METHODS

An outbreak of Acute Gastroenteritis was reported from Zalod Taluka of Dahod District, Gujarat during the last week of May 2010. Some cases being reported from adjoining areas of Rajasthan and the adjoining Fatehpura and Limkheda Talukas as well. All the patients were admitted to Community Health Center (CHC), Zalod with for Acute Gastroenteritis and Dehydration. A Rapid Response Team from the Medical College of the region was immediately sprung into action and necessary investigations and control measures taken immediately for the control of outbreak.

Local Health Authorities had collected 2-5 ml of liquid faeces stool samples in a sterile, wide-mouth container from the indoor cases of Gastro enteritis as per the guidelines of IDSP and NICD in the V.R. transport media before starting of Antibiotics, in a cold chain with all the details of the samples including Name, Age, sex, residential address, date of onset and date of collection within 4 to 6 hours were sent to the Microbiology Department, Medical College, Baroda. Water samples were also collected from the different water sources in the surrounding affected villages for bacteriological analysis. Department of Microbiology, Medical College, Vadodara is the reference laboratory for the surrounding districts of Vadodara.

Physical examination was done for Colour, Appearance and Consistency. The samples were examined under hanging drop preparation for motility. The isolation procedure included inoculating about 1 ml of stool specimen into 10ml of sterile alkaline peptone water and incubating it for 5-6 hrs at 37^0 C. Also a loopful of stool sample was inoculated on TCBS, MacConkey agar, Nutrient agar and incubated at 37^0 C aerobically. Subculture loopful of suspension from alkaline peptone water after incubation on TCBS, MacConkey agar, Nutrient agar. Incubate it at 37^0 C aerobically.

After incubation media were examined for Vibrio like colonies and a set of conventional biochemical tests were performed to those

colonies. After confirmation by biochemical tests serotyping was done to further ascertaining the serotype. Antibiotic sensitivity testing was done by Kirby Bauer technique on the isolates.

On confirmation of V. Cholerae, lab report were immediately informed to local health authorities and state IDSP for taking preventive and control measures. Out of total V. Cholera positive samples, randomly selected samples were sent for further bio typing to NICD, Kolkata and their reports were also analyzed.

A total of 5 water samples were also received from different affected areas viz. Main pump-Sampoi village, last point of Tank of Bhaman village, Dig Well of Hirola village, Well of Kaljini Saraswani and Hand pump of Jetpur School of Zalod Taluka. Pre Sterilized glass bottles of 250 ml capacity with Ground glass stoppers containing 0.25 ml of fresh 1.8% solution of sodium thiosulfate crystal were used for collection of water. Sampling was done from tap or pump outlet, reservoir (stream/river/lakes), well. Multiple Tube Test Method was done for examination of water. MacConkey DS Broth is used for culture and McCrady's Table was used to determine no. of coliforms per 100 ml water sample (MPN).

Results of the stool samples and water samples analysis were entered in MS Excel sheet and analyzed accordingly.

RESULTS

During May & June 2010, total 171 stool samples were received at the Microbiology laboratory for analysis. 46 stool samples were found positive (27%) for V.Cholerae O1.subtype. V. cholerae O1 biotype ElTor, serotype Ogawa was isolated as a sole pathogen from 5 randomly selected stool samples sent to NICD, Kolkata. Highest positivity 23 samples(50%) was found in pediatric age group (up to 15yrs) and then most affected age group is 36 to 45 yrs of age with 40% positivity rate for V. Cholera. Older age group is least affected. Gender wise positivity rate was same in both male and female. Subtype. V. cholerae O1 biotype El Tor, serotype Ogawa was isolated as a sole pathogen from 5 randomly selected stool samples sent to NICD, Kolkata.

Water samples

2 out of the 5 water samples (1 from Main Pump, Sampoi village, 1 from Dig Well, Hirola village) showed presence of coliform bacilli with high MPN (most probable number) count. Faecal coliform was also found in both the water samples which suggest the contamination of water.

Table 1: Age & Gender wise analysis of Stool Samples for Cholera

Age (years)	Stool Samples	V. Cholera Positive		
		Positive	Males	Females
0-5	18	7 (39%)	2	5
6-15	55	16 (29%)	10	6
16-25	25	5 (20%)	2	3
26-35	30	7 (23%)	4	3
36-45	20	8 (40%)	4	4
46-55	10	1 (10%)	0	1
56-65	10	2 (20%)	1	1
>65	3	0	0	0
Total	**171**	**46(27.0%)**	**23**	**23**

DISCUSSION

Sporadic cases of acute diarrhea occur frequently in most parts of India throughout the year. However, at times explosive outbreaks of diarrhea occur due to cholera. Cholera is one of the major infectious diseases with epidemic potentials especially amongst Communities living in congested slums without proper sanitary facilities, which are typical of endemic area. The present outbreak mainly affected residents of tribal zone, Zalod Taluka and surrounding villages. Few villages from adjoining border area of Rajasthan State were also affected. The population is sharing the common source of water supply and practicing poor domestic as well as personal hygiene. V. cholerae 01 (Ogawa) biotype ElTor was isolated during this outbreak and there was no isolation of V. cholerae 0139.

In this study, presence of phages in the water samples is an additional indicator for cholera contamination in this community. Moreover, low level of personal and domestic hygiene led to extensive environmental contamination, resulting in contamination of these water sources. Earlier, in countries where cholera is endemic, V. cholerae O1 bacteriophages (i.e., vibriophages) have been detected in sewage water and served as strain markers.[4]

Until 1992, epidemics of cholera were caused by Vibrio cholerae classical or El Tor biotypes of serogroup O1. The classical biotype is believed

to have caused first six pandemics, which occurred in the Indian subcontinent and subsequently in other areas of the world between 1817 and 1923.[5] Vibrio cholerae O1 biotype El Tor was first reported in 1905,[6]and was found to be the causative agent for most of cholera outbreaks. It was isolated as a sole pathogen in the hospitalized acute diarrhea patients; 40.0% in Tripura, 52.9% in West Bengal and 63.0% in Assam outbreaks[7, 8, 9] and from water samples examined.[10]

Vibrio cholerae O1 El Tor biotype, Ogawa serotype has been causing most of the cholera outbreaks in India till recently. It was also involved in Delhi outbreak in the year 2005[10]Bacteriological analysis of 431 rectal swabs, collected from acute diarrhea cases at a surveillance site and in different diarrhea outbreak areas of Orissa from May to October 2005 had V. cholerae. Out of 265 culture-positive samples, Vibrio cholerae O1 was isolated in 56 samples (20.8%)[11] Cholera outbreaks have been reported due to contaminated water source for human consumption.[12,13,14]

As per the WHO, The number of officially notified cases from Asia decreased nearly 3-fold compared with 2004. A total of 2472 cases and 28 deaths were reported from 6 Asian countries. The Indian subcontinent reported 78% of all cases notifi ed from Asia, with India notifying a total of 1939 cases and 3 deaths. China, Malaysia, the Philippines and Thailand reported respectively 161, 237, 66 and 35 cases. Japan reported 34 cases, of which 28 were imported cases. However, many more unreported cases occur in Central and South-East Asia. No surveillancedata were received from Central Asia for 2006, but there is heightened concern about the occurrence of epidemicprone diarrhoeal diseases in the region.[15]

CONCLUSION

The present findings definitely support the outbreak of cholera. It appeared from the explosive nature of this outbreak that the most probable cause was contamination of drinking water source and transmission was through faeco-oral route. It is important to provide safe drinking water, adequate sanitary facilities in tribal areas and people should be motivated to use sanitary facilities and maintain proper personal hygiene.

ACKNOWLEDGEMENT

We are thankful to Chief District Health officer, Additional District Health officer and District Surveillance Officer of Dahod District and Block Health Officer, Zalod for providing us all details pertaining to this outbreak.

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