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Original article

CIRCULATING AND CHANGING PHAGE TYPES OF *V CHOLERAE* ISOLATES AT LG GENERAL HOSPITAL AHMEDABAD, GUJARAT

Dr B.K.Prajapati Dr A.H.Rajput Dr M.U.Vinzuda,Dr T.K.Trivedi, Dr S.D.Rathod, Dr M.T.Kadam , Dr B.L.Sarkar*.

Department of Microbiology, AMCMET Medical College, L.G. General Hospital, Ahmedabad, Gujarat. National Institute of Cholera & enteric Diseases, Kolkata, India*

Corresponding author :A.H.Rajput Assistant professor *

ABSTRACT:

Introduction:

Cholera is endemic in Ahmedabad and its surrounding area. The retrospective study was under taken from Jan 2011-Aug 2015 to understand Circulating and Changing Phage Types of V. cholerae.

Method: A total 171 strains of V. cholerae were isolated from total no. of 688 stool samples collected from suspected case of cholera at L.G.General Hospital, Ahmedabad from Jan 2011- Aug 2015. V.cholerae was identified by standard microbiology procedure. Isolates were sent to NICED, Kolkata for further confirmation and phage typing.

Result: Out of 171 strains 150strains were revived at NICED. 148 isolates were identified O1 Biotype El tor serotype Ogawa. Phage T27 was remained the predominant type in all the year according to new phage typing scheme. As per Basu and Mukherjee phage typing scheme T2 phage was predominant in Jan 2011- 2013 and T4 phage was predominant in 2014-2015. Two strains of V. cholerae were untypable.

Conclusion: During 2011-13 all strains were belong to Basu & Mukherjee Type 2 Phage type which was totally replaced by Basu & Mukherjee Type 4 in 2013- 2015. Phage T27 was remained the predominant phage type in all the years. Monitoring of the prevalent phage types in area is important as introduction of new phage may herald the onset of an outbreak.

Key Word: V. cholerae, Phage type, Cholera, NICED Kolkata

INDTRODUCTION:

Cholera is an important public health problem in India. Cholera outbreaks occur seasonally and are associated with monsoon season, warm temperature, heavy rain fall and increased plankton population. Vibrio cholerae has a unique ability to exist in an autochthonous state in riverine and brackish water estuaries and coastal waters and can exist in dwarfish forms in response to nutrient deprivation as a viable but non culturable form. It can exist in the gut and attached to the surface of both fresh water and marine copepods. In addition, the genetic assortments and reassortments that are going on in these isolates, equip them appropriately to survive better in the changing environmental conditions. These also contribute to the increase in drug resistance amongst the V. cholerae strains. (1,2)

The geographical distribution of cholera is changing and so is often considered as a re-emerging disease, in part because infections are appearing in novel communities or in communities where the disease had been absent for many years.(3) This may be due to the changes in the environment or climate, following the EI Nino phenomenon which has made conditions favourable for cholera worldwide.(4) The problem of global warming(5) and inland incursion of sea water covering more and more of the coastal stretches of land could lead us to the brink of a resurgent pandemic. Worldwide there has been increase in the number of cholera cases and outbreaks in the new communities and with changing profiles.(3,6)

In the Asian region, the Indian subcontinent continues to harbour a major chunk (78%) of cholera cases(3). Outbreaks of cholera including major epidemics have occurred from time to time at various places in India.(6) We studied circulating and changing phage typesof V. cholerae isolated during Jan 2011 to Aug 2015 at L.G.Hospital ,Ahmedabad, Gujarat. Bacteriophage typing is a convenient and highly discriminating method of identifying epidemic strain of V.cholerae. (7) V. cholerae has been recognised as one of the most common cause of bacterial diarrhoea throughout the world. In an outbreak of an infectious disease such as cholera , it is very important to determine whether the strain have a common origin or different origin. Although a number of problem exist, phage typing of V.cholerae is useful for differentiating classical and El tor strains and for epidemiological purpose. (8, 9)

MATERIAL AND METHOD:

A total l71 strains of V. cholerae were obtained between Jan 2011-Aug 2015 from suspected cholera patients at L.G. General Hospital , Ahmedabad. The stool samples of such patients were collected in a sterile container and enrichment was done in alkaline peptone water (APW, pH -8.0) for 6-8 hour. Before the sample was plated, hanging drop preparation was made to confirm the typical darting motility of the V. cholerae. If present, both directly from the sample and after enrichments in alkaline peptone water . The Samples were plated on MacConkey agar, Blood agar and Thiosulphate citrate bile salt sucrose agar (TCBS).(10) The suspected colonies were subjected to Gram stain, oxidase, motility and string tests. The Gram negative rods that were oxidase positive, actively motile and string test positive were

subjected to further biochemical tests.[10] These were indole, triple sugar iron agar, cholerared reaction, citrate utilization, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase and sugar fermentation tests using sucrose, mannitol, arabinose and mannose.(10) Biotyping was performed by the Voges– Proskauer test, chick red cell agglutination test, sheep RBC haemolysis test and Polymyxin-B (50 unit disc) sensitivity test.[10] Serotyping was carried out by slide agglutination using Ogawa and Inaba antisera.[2] All the isolates belonged to the V. cholerae ElTor biotype and Ogawa serotype. All isolates were sent to National Institute of Cholera and Enteric Disease (NICED), Kolkata, for Confirmation and Phage typing.

RESULT AND DISCUSSION:

The phage typing scheme was proposed by Basu and Mukherjee in1968, and since it has been in continuous use for typing of V.cholerae biotype El tor strain. In 1969, all six phage types were reported in India and abroad. However, Phage types 2and 4 were predominant.

In our studynumber of cases were from slum dwellings or suburban areas in and around Ahmedabad.. V. cholerae is a known faeco-oral pathogen and indeed, infection rates were significantly higher in areas with poor sanitation. Mostly, the slums are occupied by migrant population where the hygienic conditions are quite often compromised. Interestingly, the importance of weather and climate as having effect on water quality is being increasingly recognized.(4) In this study, all the cases presented during the months of June to October. The occurrence of cases correlated well with the onset of monsoon in this region. Rains increase the level of surface water and have been shown to be linked to leakage of water pumps and mixing of stagnant water through broken pipelines.(11,12) Such untreated water sources are used by people living in slum dwellings and suburban areas for bathing, cooking and drinking which enhances the chances of infection. All the isolates were V. cholerae O1, biotype El Tor, serotypr Ogawa. Phage typing was done for the isolates collected during Jan 2011 to Aug 2015.

During the period of Jan 2011-Aug 2015, 150 strains were identified as V. cholerae at NICED, Kolkata. Out of 150, two strains of V. cholerae were untypable during the year 2013. 12 strains were not revived at NICED and 9 isolates were contaminated. All isolates were V. cholerae O1, Bio types El Tor. From 2011- 2013 all strains were belong to Basu & Mukherjee Type 2 Phage type which were totally replaced by Basu & Mukherjee Type 4 Phage in 2013- 2015. According to new phage typing scheme T27 was predominated phage type during Jan 2011-Aug 2015(Table 1). The results of phage typing were consistent with the overall countrywide epidemiological data21 which report type 27 to be the predominant one. The present findings corroborated well with previous studies(13). This suggests that a particular clone of V. cholerae O1 strain is probably circulating all over India. Two Untyable strain were found during 2013.

Though fl uid and electrolyte replacement either by oral rehydration or intravenous fl uid therapy is the treatment of choice for acute diarrhoea, antibacterial agents are indicated as useful adjuncts for the treatment of cholera as these shorten the duration of hospital stay, stop excretion of vibrios in the stool and also minimize the requirement for fluid .(14)

The study showed that there was no readily discernible sex pattern but male outnumbered the females (Table 3). Most cases were in age group of 0-5 years followed by 6-10 years. However Cholera affects all ages and both sexes, infection rate is increasingly reported in paediatrics(Table 2).

This finding is in contrast with several studies from Mumbai,[7] Bikaner [16] and Ludhiana [17], were all strains were belonged to type T4. At present ,the prevalent biotypes in India are T2 and T4.[6]But ,in the present study prevalent biotypes as per Basu & Mukherjee typing scheme are T2 and T4 and New phage typing scheme are T 27, T 26.T 21 and T7.

Continuous monitoring of the changing trend of phage type is a must, as introduction of new phage may herald the onset of an outbreak and resistance to antibiotic. Strong regional commitment to surveillance and preparedness for outbreaks should be maintained and timely information should be given to the health authorities as well as to the public.

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Table:1 Distribution of Phage types Years wise

Phage group

Years	Type																			
	Basu & Mukherjee (N-148)		New Scheme(N-148)																	Total
	2	4	4	7	13	15	16	17	19	20	21	22	23	24	25	26	27	NG	UT	Cont.	
2011	9	-	-	1	-	-	-	-		-	-	-	-	-	-	1	7	2	-	2	
2012	15	-	1	-	-	-	1	-		-	1	-	-	-	-	2	10	3	-	2	
2013	46	24	-	1	2	1	-	1		1	3	-	3	2	1	1	54	1	2	4	
2014	-	24	-	3	-	-	-	-	4	1	0	-	0	0	-	3	13	2	-	1	
2015	-	30	-	5	-	-	-	-	4	4	-	1	-	-	-	5	11	4	-		
Total	70	78																12	2	9	171

Table 1: Total 171 ,Tyapable:148,Untypable:2,Nogrowth:12,Contaminated:9

Table: 2 Distribution of Cholera case amongst different age groups (n-171)

Years	0-5	6-10	11-15	16-20	21-25	16-30	31-35	36-40	41-45	46-50	51-55	Total

2011	**5**	**7**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**13**
2012	**13**	**6**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**20**
2013	**44**	**20**	**8**	**-**	**2**	**-**	**-**	**-**	**1**	**-**	**2**	**77**
2014	**13**	**9**	**4**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**27**
2015	**21**	**06**	**3**	**2**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**34**
Total	**96**	**48**	**17**	**2**	**2**	**2**	**0**	**1**	**1**	**0**	**2**	**171**

Table 3: Sex wise distribution of cholera case (n- 171)

Years	Male	Female	Total
2011	**8**	**5**	**13**
2012	**11**	**9**	**20**
2013	**36**	**41**	**77**
2014	**16**	**11**	**27**
2015	**17**	**17**	**34**
Total	**88**	**83**	**171**

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