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ACUTE GASTROENTERITIS (AGE) DUE TO *VIBRIO CHOLERAE* EL-TOR OGAWA IN MUMBAI – A 5 YEAR STUDY

VAISHALI R. WABALE*, AMEETA A. JOSHI, RENU S. BHARADWAJ***, ABHAY S. CHOWDHARY**** AND B. L. SARKAR*****

** Assistant Professor, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.*
*** Associate Professor, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.*
**** Professor & Head, Department of Microbiology, B. J. Medical College & Sassoon Hospital, Pune, Maharashtra, India.*
***** Professor & Head, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.*
****** Senior Deputy Director (Scientist F) Vibrio Phage Reference Laboratory Department of Microbiology National Institute of Cholera & Enteric Diseases (ICMR) WHO Collaborating Centre for Diarrheal Diseases Research & training . P-33, CIT Road, scheme XM, KOLKATA -700 010, INDIA*

ABSTRACT

Vibrio cholerae erupted as leading, unpredictable, potent pathogen to the global fear of reemergence with an ability to cause epidemics and antibiotic resistance. Newer molecular and more discriminatory procedures helping epidemiologists to understand evolution, spread and emergence of newer variants. To study prevalence of *V. cholerae* and to identify current circulating biotype, serotype, phage type and changing pattern of antibiotic resistogram for epidemiological purpose. Hanging drop preparations (HDP) technique performed on all samples. Identified strains were serotyped using high titer antisera against O1, Ogawa, Inaba and O139. Phage typing by 'Basu and Mukherjee' & 'New Scheme' performed. Antibiogram had done using modified Kirby-Bauer's disc diffusion method by standard CLSI guidelines. HDP showed more specificity (75.77%, HDP + culture positive) than sensitivity (24.22%, culture positive, HDP negative). Gentamycin showed 100% sensitivity. Co-trimoxazol showed 100% resistance from 2008 onwards. T-4 was common phage type been replaced by T-2 by 'Basu and Mukherjee' scheme while, T-27 was the only predominant one by 'New scheme'. In 2009, newer phage variants observed. High incidence rate of diarrhea due to *V. cholerae* is of utmost importance. HDP still has a limited role in diagnosis. Important to study biotype, serotype, phage type and resistogram prevalent in that area for epidemiological control purpose. . Appearance of different circulating phage types in Mumbai, probably originating from different parts of country.

KEY WORDS: **Acute gastroenteritis, Cholera, Biotype, Serotype, Phage type.**

***Corresponding author**

VAISHALI R. WABALE
Assistant Professor, Department of Microbiology,
Grant Government Medical College,
Mumbai, Maharashtra, India.

INTRODUCTION

Acute gastroenteritis (AGE) is an inflammation of gastrointestinal tract. It involves the stomach as well as small intestine which results in acute diarrhea. Bacteria which can cause AGE are: Salmonella, Shigella, Staphylococcus, Vibrio, *Campylobacter jejuni*, Clostridium, *Escherichia coli*, Yersinia etc. However, in India, *Vibrio cholerae* leads the list[1]. In humans *V. cholerae* is an etiologic agent of Asiatic cholera. It is a severe diarrheal disease and has been responsible for causing seven pandemics so far[2, 3, 4]. It is an acute diarrheal illness and commonly characterized by the symptoms of sudden vomiting as well as rice watery stools; which leads to dehydration, anuria and hypovolemic shock. It has been said that there can be a fluid loss of 15-20 liters per day[5]. However, in developing countries like India, diarrheal illness ranks first among infectious diseases in terms of incidence. Due to the high mortality rate, it is often under reported and hence always has a fear complex associated with it [4]. Cholera, being an acute intestinal infection occurs due to the ingestion of food or water contaminated with the causative agent *V. cholerae* [6]. This organism is a curved gram negative bacillus and belongs to the family Vibrionaeceae[7, 8]. It has a short incubation period and produces an enterotoxin which causes copious painless, watery diarrhea. This can quickly leads to severe dehydration and death if immediate treatment is not administered. In many of the patients vomiting also occurs [6]. *V. cholerae* have both pathogenic and non-pathogenic strains, differing in their virulence, gene contents and polysaccharide surface antigens [7]."Only *V. cholerae* O1 and O139 have so far been reported to cause a disease defined clinically and epidemiologically as Cholera"[9]. It is divided into classical and El Tor biotypes and into three sero-subtypes namely Ogawa, Inaba and Hikojima. In India, El Tor biotype has characteristics common with O139 but differs from O1 in its polysaccharide surface antigens [10, 11, 12]. Most of the time people infected with *V. cholerae* do not become ill although they continue to shed bacterium in feces (7-14 days). However, when illness does occur, about 80-90% of episodes is of mild or moderate severity and usually becomes difficult to distinguish clinically from other types of acute diarrhea. Overall, less than 20% of ill persons develop typical cholera with signs and symptoms of moderate to severe dehydration [6]. This organism is erratic in nature and nobody can predict when a non-toxigenic strain transforms itself into potent, virulent strain, possibly causing havoc or pandemic throughout the world. Hence, a constant vigilance of this pathogen is of paramount importance [4]. The present study was undertaken to see the prevalence of *V. cholerae* in Mumbai, Maharashtra, India. To identify the current circulating biotype, serotype, phage type in the area for epidemiological purpose and to study the changing pattern of antibiotic resistogram in the strains isolated in our hospital.

MATERIALS AND METHODS

The Grant Government Medical College (GGMC) and Sir J. J. Hospitals is situated in the South Mumbai and caters primarily to all the population of Mumbai city. The study was conducted in the Department of Microbiology, GGMC, over a period of 5 years from January 2005 to December 2009. A total of 355(22.44%) strains of *V. cholerae* were isolated from 1596 stool samples from patients were clinically suspected of AGE attending Sir J. J. Hospitals. The stool samples were collected from all patients having signs and symptoms of acute gastroenteritis. All the specimens were collected in sterile container before starting antibiotics and transported to the laboratory for processing. Whenever necessary rectal swabs were collected with the help of sterile swab sticks and were immediately inoculated in alkaline peptone water (APN) and then transported to the Microbiology laboratory within 2 hours. Hanging drop preparations (HDP) were examined as soon as samples reached the laboratory. The gross examination of all samples was done and a provisional HDP report was communicated immediately to the patients. Simultaneously, all samples were inoculated into 10 ml sterile APN and incubated for 6 hours at 37^{o}C [3]. These samples were further inoculated on Blood agar, MacConkey agar, Nutrient agar as well as on Thiosulphate-Citrate-Bile Salts-Sucrose (TCBS) agar ((HIMEDIA, Mumbai). These media were incubated aerobically for 24 hours at 37^{o}C. Next day the growth was observed and colonies were further subjected to a battery of biochemical tests. Identification of biotypes, and serotypes were done using standard microbiological procedures [2,3,13]. Serotyping of the strains was performed in our laboratory by using *V. cholerae* high titer antisera against O1, Ogawa, and Inaba received from Central Research Institute (CRI), Kasauli and O139 obtained from National Institute of Cholera and Enteric Diseases (NICED), Kolkata. All identified serotypes were sent to NICED for further phage type identification. At NICED, phage typing of the strains was done by both, conventional 'Basu and Mukherjee' phage typing scheme as well as 'New Scheme 'of phage typing. Antibiotic susceptibility testing was done using modified Kirby-Bauer's disc diffusion method. Isolates were characterized as sensitive and resistant based on diameter size of the Zones of Inhibition (ZOI), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[14]. The commercial antibiotic discs (Hi Media Laboratories, Mumbai) used in the present study were Chloramphenicol (30µg), Tetracycline (30µg), Ampicillin (10µg), Ciprofloxacin (10µg), Co-trimoxazole (25µg), Nalidixic acid (30µg), Gentamicin (10µg), and Polymyxin B (50µg). After confirmation, all the isolates were inoculated into alkaline nutrient agar.

RESULTS

During the 5 year study period, a total of 1596 stool samples from AGE cases were processed in our laboratory and from that 355 (22.24%) *V. cholerae* were isolated. The isolation rate per year ranged from 11.67% to 32.85% [Table 1]. There was a significant rise observed in the incidence of AGE. However, maximum isolation rate was found in the year 2009 (32.85%). There was no significant difference observed in the incidence of AGE in adults compare to children. However, a clear male preponderance was observed in both age groups. Table 2 shows the results of HDP

positive Vis a Vis culture positive taking culture as a gold standard. Out of 355 grown *V. cholerae* isolates, 269 i.e. 75.77% were identified by HDP as well as grew on culture. While, 86 i.e. 24.22% were picked up on culture although HDP was negative. Hence, though HDP is an emergency diagnosis, culture is the gold standard method. All the isolates showed 100% sensitivity to Chloramphenicol, 92.6% to Gentamycin, 87.8% to Tetracycline and 85.3% to Netilmycin and Amikacin respectively. However, 100 % Resistance was reported only to Co-trimoxazole from year 2008 onwards. According to conventional phage typing method of 'Basu and Mukherjee', T-4 phage type was common in the year 2005 and 2006; however, it was been replaced by T-2 from year 2007 onwards. The more discriminatory 'New Scheme' revealed that T-27 the only phage type predominantly identified over the years, followed by T-26 and T-24 respectively [Table 3]. In 2009 many new phage types have made an appearance i.e.T-21, T-23, T-25, T-16 and T-13. These have never been seen earlier in Mumbai. Thus, the 'New Scheme' is more discriminatory as compared with 'Basu and Mukherjee' phage typing scheme and gives an indication of the newer variant phage types introduced into Mumbai.

DISCUSSION

A sharp increase in the global incidence of several emerging and reemerging infectious diseases has been reported in the last decade of the 20th century [15]. In the recent years, drug resistances to antibiotics among gram negative organisms have been increased rapidly. The epidemiological importance of preventing these drug resistant strains from spreading in the community has become a global problem [16]. *V. cholerae* erupted as one of the leading organism contributing to this global fear of reemergence. This organism reappeared in Latin America in January 1991 after the absence of 100 years in that continent. In the very next year, a novel strain 'O139 Bengal' arrived throughout the Indian Peninsula and subsequently disappeared after few years. Thus, such unexplained outbreaks of this pathogen continue to occur from time to time making it the most unpredictable one. The newer molecular biology techniques and more discriminatory procedures are now helping the epidemiologist's to understand the evolution and spread of *V. cholerae* and the emergence of newer variants. However, all this can be possible only when the diagnostic laboratories maintains proper records of the detected isolates, preserves the isolates and take effort to interpreted it in the light of its public health importance [15]. In developing countries like India, where required sanitary measures are not in place, this pathogen can be easily transmitted by the faeco-oral route. Being a potent pathogen with an ability to cause epidemics and emergence of antibiotic resistance; in the current scenario, it poses a serious threat. All *V. cholerae* isolates in this study belonged to sero group Ogawa and biotype El Tor. Severe dehydration and rice water stool were the significant parameters associated with positive stool culture in our study. Overall positivity rate was 22.24% which was similar to the studies reported by Barve S et al (27%) [6] and Karki R et al (27%) [14] from Kathmandu, Nepal respectively. Shah HD et al [7] also reported 16.23% isolation rate from Lalpur town in Jamnagar in year 2010. A low rate as compared to our study was reported by Mandal J et al (5.79%) [16] and Narang P et al (5.14%) [15]. Contaminated water remains the prime vehicle for outbreaks of cholera in developing countries like India. In Mumbai, the affected areas are crowded slums, with poor sanitation practices and lack of sewerages. Corrosion of old pipes resulting in contamination of drinking water results in large outbreaks. Fecal contamination of water sources of households is a common cause of outbreak [17]. As per the United Nations criteria, improved drinking water sources includes public water pipelines, household water connections, protected dug wells, bore wells, springs and rain water collection. In spite of possible commitment and well directed efforts till today in some parts of India, people do not have access to safe drinking water [18]. Sporadic cases of acute diarrhea occur throughout the year in western parts of India. Cholera is a great threat in those people who lived in shanties and in crowded places where no sanitary facilities available. In our study occurrence of AGE cases were observed throughout the year with a peak in monsoon, a finding similar to that reported by Nitsure S et al, [5] Karki R et al [14, 19] and Stine OC et al [20]. In the present study 42.98% culture positivity was seen in children of age less than 14 years with clinical suspicion of AGE than in the adults (40.93%). Similar findings were reported by Karki R et al, [19] Barve Set al [6] and Mandal J et al [16]. However, Shah HD et al [7] reported more isolation in adults as compared to pediatric age group. An earlier study from this institute also reported the maximum isolation in adults [4]. A clear male preponderance was seen in the present study which was similar to the findings of our previous study[4]. However, Albert MJ et al [21] reported the incidence of AGE more in male children of age 5 years as compared to female children of same age. Barve et al [6] reported positivity rate similar in both the genders when compared to Karki R et al [19] who reported no significant differences between the genders. Rice water stool and dehydration were the common significant parameters associated with the positivity in our study over a period of 5 years. HDP was having more specificity rather than sensitivity as most of HDP negative samples were turned to be culture positive. Thus, the HDP method can be helpful in the rapid diagnosis of cholera but it has limitations, as the results may depend on the individual expertise and can be affected by the transport time of the sample. In the present study, HDP positive and Culture positive were 75.77%; however, HDP negative and Culture positive were 24.22%, which were similar to our previous study (76.66% and 23.33% respectively) [4]. In the present study, from year 2005 to 2007 the strains were susceptible to Co-trimoxazole, however, the resistance was observed from year 2008 onwards. Similar findings were reported by Narang P et al [15] with 100% sensitivity to Co-trimoxazole till 1996, however, the resistance was observed from year 1997 up to 2005 in the range of 29-65%. Other studies conducted by Karki et al [14] and Mandal J et al [16] reported similar findings. Co-trimoxazole resistance is a serious problem in a country like Nepal where, this drug is commonly used to treat patients with AGE [19]. The reason behind such increased drug resistance could be

the widespread irrational use of antibiotics for AGE even when not indicated. Easy availability of the drug over the counter only worsens the situation [16]. Such indiscriminate use of Co-trimoxazole and other antimicrobials is a matter of concern as therapeutic options are very limited. This problem becomes more severe while treating children, women in the antenatal period and lactating mothers. Hence, there is a need for constant surveillance of *V. cholerae* isolates. A structured planning is needed to prevent epidemics arising from such drug resistant strains [16]. In the present study, phage typing was performed at NICED [22]. According to 'Basu and Mukherjee' phage typing scheme T-4 phage type was common in year 2005 and 2006. However, it was being replaced by T-2 phage type from year 2007 onwards. Similar findings were reported by Narang P et al, [15] Nitsure S et al [5] and Turbadkar et al [23]. According to the 'New Scheme', we had T-27 as the predominant phage type throughout the study period. These findings exactly correlates with our previous study where predominant phage was T-27 followed by T-26, T-12 and T-24 [4]. Similar findings were reported by Narang P et al [15] in their 16 year study, where T-27 was the predominant phage followed by T-23, T-21, T24, T28 and T-20. In another study conducted by Nitsure S et al [5], the predominant phage was T-27 followed by T-20, T-26 and T-13. The study done by Turbadkar SD et al [23] reported similar predominant phage type T-27, followed by T-23 from the same institute. From Mysore, Srirangaraj S et al [24] also reported T-27 as a predominant one followed by T-21, T-25 and T-23. Thus 'New Scheme' is more discriminatory and could identify four circulating phage types when compared with a single phage type identified by conventional old 'Basu and Mukherjee' scheme. The spectrum of phage types found over a period of 5 years in Mumbai suggests that, they originated from different cities and some of them may have been imported from other cities. AGE is an important global threat to health and remains a key indicator for socio-economic development of a country. However, the disease is not a problem in the countries where adequate hygiene standards are present. In fact, it is a challenge to the developing and under-developing countries; where safe drinking water supply and adequate sanitation measures are neither in place nor guaranteed by the government. The threat of cholera is imminent in most developing countries of the world [6]. Genome of *V. cholerae* is constantly changing and drug resistant species are emerging. This statement is supported by the study conducted by R Saha [25] who stated that 'The development of multiple resistances in *V. cholerae* is no doubt alarming'. They have the ability to cause outbreak even an epidemic in spite of the presence of good herd immunity in the population. A vigilant, constant monitoring from clinical laboratories especially in academic institutes is the need of the hour. This would help to monitor the strain changes and phenotypic or genotypic variations in the genome. The isolates should be typed or sent to the reference laboratory for phage typing. Being a notifiable disease, accessibility of information to the concerned health authorities and exchange of data with academicians is essential [15].

Table 1
Shows year-wise distribution of stool samples

Year	No of Samples	No of Isolates (%)
2005	150	25(16.66)
2006	257	30(11.67)
2007	342	85(24.85)
2008	497	100(20.12)
2009	350	115(32.85)
Total	1596	355(22.24)

Table 2
Shows the results of HDP positive Vis a Vis Culture positive

Year	Isolates	HDP positive/ Culture positive (%)	HDP negative / Culture positive (%)
2005	25	20	5
2006	30	23	7
2007	85	83	2
2008	100	80	20
2009	115	63	52
Total	355	269 (75.77%)	86 (24.22%)

Table 3
Year -wise distribution of Predominant Circulating Phage types

Year	Isolate	Predominant Phage type	
		Basu &Mukherjee Scheme	New Scheme
2005	25	T-4	11 T-27, 8 T-26, 5T-12 & 1T-24.
2006	30	T-4	T-27, few T-7
2007	85	T-2	T-27
2008	100	T-2	T-27, few T-7
2009	115	T-2, UT	43T-27, 20-UT, 3T-4, 3T-21, 2T-24, 2T-26, 2T-25, 2T-23,1T-2, 1T-16, 1T-13

UT: - Untypable

CONCLUSION

To conclude, the high incidence rate of diarrhea due to *V. cholerae* is of utmost importance. Hanging drop preparation method still has a limited role to play in the diagnosis of *V.cholerae* infection. It is important to study the biotype, serotype and phage type and resistogram prevalent in that area for epidemiological control purpose. Different circulating phage types seem to be making an appearance in Mumbai, probably originating from different parts of the country.

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