

## Serotyping and Phage Typing of *Vibrio cholerae* Isolated at Tertiary Care Hospital, Ahmedabad, Gujarat

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### ABSTRACT

**Introduction:** Cholera continues to be a growing concern in most developing countries. Cholera is an acute diarrheal disease endemic in India. Yet there are few reliable population-based estimates of laboratory-confirmed cholera in endemic areas around the world. The aim of this hospital-based study was to isolate and serogrouping of *Vibrio cholerae* in patients with diarrhea at tertiary care hospital, Ahmedabad during January 2021 to July 2022. **Material & Methods:** A retrospective study involving cases of acute watery diarrhea was done during January 2021 to July 2022. All stool samples from suspected cases were tested for *Vibrio cholerae* by standard microbiological procedures. Out of total 1294 stool samples *Vibrio cholerae* were isolated in 179 samples and sent to the NICED (National Institute of Cholera and Enteric Diseases) for serotyping and phage typing. **Results:** In present study rate of isolates of *V. cholerae* was 13.83 % (179 out of 1294 cases). *V. cholerae* O1 serotype Ogawa (78.77%) belonging to phage type 27 (54.74%) was the most common in the cases of acute diarrhea in present study. **Conclusion:** The present study identified serotype Ogawa and phage type 27 as the most dominant type and was found continuous in circulation throughout the study period. Phage typing is still an internationally recognized method of choice for characterizing circulating strains. This knowledge will be helpful to design a novel strategy to manage future cholera outbreaks.

**Keywords:** Cholera, *Vibrio cholerae*, Serotyping, Phage typing

### INTRODUCTION

Cholera is an acute diarrheal illness caused by toxigenic strains of *Vibrio cholera* serogroups O1 and O139. Presently, *V. cholerae* O1 belonging to the El Tor biotype is the most common serogroup in India, while the frequency of serogroup O139 has declined considerably over the past few years<sup>1</sup>. Depending on somatic antigens, *Vibrio cholerae* have more than 200 serogroups, of which O1 and O139 are outbreak/epidemic strains<sup>2</sup>. *V. cholerae* O1 has two well-established biotypes, namely, classical and El Tor, which are differentiated primarily based on phenotypic characters<sup>3</sup>. *Vibrio cholerae* O1 has been responsible for several cholera outbreaks in developing countries<sup>4</sup>. Outbreaks of cholera are generally due to lack of sanitation or contamination of drinking water<sup>5</sup>. Cholera is also changing epidemiologically. Multiple antibiotic resistant strains of *V. cholerae* have emerged, along with the El Tor variants that produce the cholera toxin of the classical biotype that has spread into Asia and parts of Africa<sup>1</sup>. Cholera produces acute severely dehydrating diarrhea, often requiring intravenous fluids. Thus despite the widespread

availability of oral rehydration solution, cholera continues to be a serious public health problem with outbreaks easily overwhelming treatment facilities<sup>6</sup>.

Among the several known serogroups, only two serogroups, *V. cholerae* O1 and O139 serogroups produce cholera toxin (CT), a critical virulence factor and express toxin coregulated pilus (TCP), which are responsible for secretory diarrhea and intestinal colonization, respectively<sup>4</sup>. Serogroup O1 consists of biotypes El Tor and classical with distinct phenotypes. Both the biotypes carry two serotypes, Inaba and Ogawa with different disease characteristics<sup>7</sup>. Serogroups other than O1 and O139 are designated as *V. cholerae* non-O1/O139 and such serogroups have >200 somatic (O) antigens and mostly lack CT- and TCP-coding genes<sup>4</sup>.

Several serotypes of *V. cholerae* strains harbor variety of bacteriophages, and these are beneficial from the clinical point with the command to control the bacterial population leading to controlling the disease. Vibrio phage plays a critical role in the evolution of pathogenic *V. cholerae* by mediating horizontal transfer of clusters of virulence genes, genomic rearrangements, as well as by bactericidal selection. Lysogenic filamentous phage, CTX is capable of transferring cholera toxin genes to nontoxigenic strains leading to the conversion of nontoxigenic strains to toxigenic strain. Lysogenic phage present in some environmental *V. cholerae* strains of both epidemic and non-epidemic serogroups has the power to kill the epidemic strains and thus contributes to the control of the disease<sup>7</sup>.

Apart from the importance of cholera bacteriophages to discriminate cholera strains, phages have been used as an alternative to antibiotic therapy to circumvent the burden of antibiotic resistance<sup>8</sup>.

### **AIMS AND OBJECTIVES**

1. To Isolate *Vibrio cholerae* from stool samples in cases of acute watery diarrhea.
2. To know serotyping and phage typing pattern of isolated *Vibrio cholerae*.
3. To determine any change in the trend of phenotypic pattern of *Vibrio cholerae* isolates.

### **MATERIAL AND METHODS**

This retrospective study was carried out at Narendra Modi Medical College, L.G. Hospital, Ahmedabad, Gujarat, India, from January 2021 to July 2022. The study protocol was approved by Institutional Review Board (IRB). The stool samples received from all acute watery diarrhea (AWD) cases with or without vomiting and/ or dehydration of any age group from different wards /locations were included in the study. Those presenting with acute bloody diarrhea (acute diarrhea with visible blood in stool with or without fever and/or pain in abdomen), and patients with acute watery diarrhea who were on antibiotics were excluded from the study.

During the study period, total 1294 stool samples were collected in sterile, leak proof container. Enrichment was done in alkaline peptone water (APW, pH8.0) for 6-8 hours. Before subculture darting motility of *V. cholerae* was checked direct from sample as well as after enrichment.

The subculture was done on Nutrient agar, Mac Conkey agar and Thiosulphate-citrate-bile salt-sucrose agar (TCBS). The isolates were identified as *V. cholerae* according to the standard microbiological methods using hanging drop preparation for motility, white translucent dew drop colonies on Nutrient agar, yellow colonies on TCBS agar, Gram stain, oxidase test, string test, triple sugar iron agar test, cholera red reaction. The culture media and reagents were obtained from Hi Media Laboratories, Mumbai.

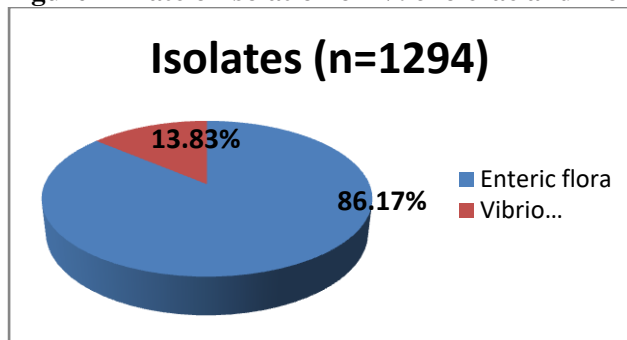
All the isolated strains were sent to National Institute of Cholera and Enteric Diseases (ICMR-NICED), Kolkata for serotyping and phage typing. Data were collected and recorded to study the rate of isolation of *V. cholerae* during the study period. Proportions of cholera cases by age and gender were analyzed to determine the trend.

### **RESULTS**

In present study rate of isolation of *V. cholerae* was 13.83 % (179 out of 1294 cases).

In cases of acute watery diarrhea (n=1294), 86.17 % cases showed normal enteric flora in which E. coli was predominant.

**Figure -1 Rate of isolation of V. cholerae and normal enteric flora**



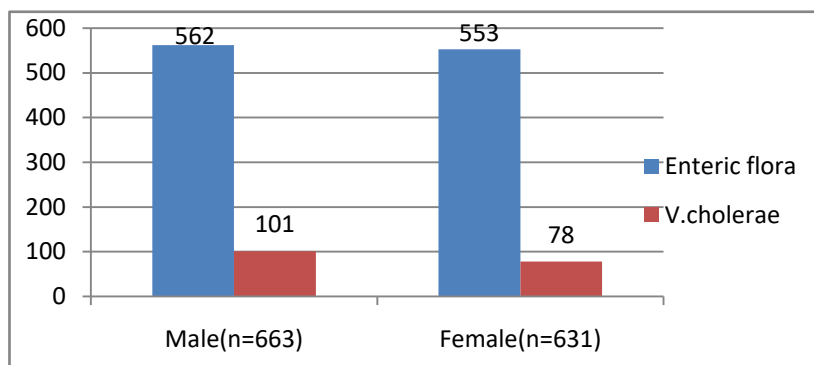
In present study children between 0-10 years age group were most commonly affected (17.35 %), followed by 11-20 years (12.95%), 21-30 years (12.09%) and >30 years (11.92%).

**Table -1 Age wise distribution of affected people**

Age group(Years)	Percentage(%)
0-10	17.35 %
11-20	12.95%
21-30	12.09%
>30	11.92%

Out of the 1294 cases, 663 (51.23%) were males and 631 (48.76%) were females. V. cholerae was isolated in 101 males (15.23 %) and in 78 females (12.36 %).

**Figure - 2 Sex wise distribution of data**



The predominant serotype was V. cholerae O1 serotype Ogawa 141 (78.77%) followed by Inaba 15 (8.37%) and 23 (12.84%) non O1 / O139 Vibrio cholerae.

**Table -2 Percentage of isolation of different serotypes of V. cholerae**

V. cholerae (n=179)	No.	Percentage (%)
V. cholerae O1 serotype Ogawa	141	78.77%
V. cholerae O1 serotype Inaba	15	8.37%
V. cholerae non O1 / O139	23	12.84%

Out of 179 strains of *V. cholerae* 98 belonged to phage type 27(54.74%), followed by phage type 19 (10.61%), phage type 23 (6.14%), phage type 26 and 25(4.46% each), phage type 16 and 13(2.23% each), phage type 21(1.11%) cases.

**Table -3 Percentage of isolation of different Phage types of *V. cholerae***

Phage type(n=179)	No.	Percentage (%)
Phage type 27	98	54.74%
Phage type 19	19	10.61%
Phage type 23	11	6.14%
Phage type 26	8	4.46%
Phage type 25	8	4.46%
Phage type 16	4	2.23%
Phage type 13	4	2.23%
Phage type 21	2	1.11%

It was found that the *V. cholerae* O1 serotype Ogawa strains belonging to phage type 27 were widely distributed in the present study.

## DISCUSSION

The present study showed high isolation rate of *V. cholerae*(13.83%) among all acute watery diarrhea cases, which is similar to other studies which reported higher rates 14.03 per cent<sup>5</sup>, 20.8 per cent<sup>9</sup> and 16.2 per cent<sup>10</sup>. However Sur et al<sup>6</sup> reported a prevalence rate (4%) of culture confirmed cholera cases among all the acute watery diarrhea cases in a community based study in slums of Kolkata, India. Kaur and Lal<sup>11</sup> also reported similar isolation rate of *V. cholerae* 2%, 2.6%, 6.7%, 7.08%, 0.9% and 2.6% during 1992-1997, respectively, in a hospital-based study in north India.

The predominant isolate in present study was *V. cholerae* O1 serotype Ogawa (78.77%), which was comparable to many studies done in India<sup>5,9,12,13,14</sup>. Analysis of serotype distribution in present study indicates the dominance of Oga wa serotype among the circulating *V. cholerae* O1 strains in India irrespective of demographical distribution and time period which matches well with the earlier findings in India<sup>3</sup>.

Young children bear the greatest burden of cholera. Cholera has traditionally been considered to occur infrequently in young children and consequently, the WHO recommends that cholera should be suspected among those over two years of age who have acute watery diarrhea and severe dehydration if cholera is endemic in the local area<sup>15</sup>. Aside from present study, two other studies have shown that cholera is a significant problem in young children<sup>16,17</sup>.

Male preponderance was found in cholera cases in present study. Increased prevalence among females has been reported by Pal et al<sup>9</sup>, however, many studies have reported higher attack rate among males than females or no variation was reported in the attack rate between genders<sup>2,10,14</sup>.

*Vibrio* phage Reference Laboratory is involved in phage typing study over the decades using different phage typing schemes. As Basu and Mukherjee scheme can classify *V. cholerae* O1 strains into six different phage types, 1% of the isolates remained un-typeable. Another drawback of this scheme is low discrimination power<sup>18</sup>.

So New phage typing scheme was developed, based upon which phages isolated from NICED, Kolkata can distinguish *V. cholerae* O1 strains into 27 different types and is useful to achieve more type distinction and improved discrimination within the biotype El Tor strains to get a better idea on the origin and clonality route of the outbreak<sup>7,19</sup>.

In present study, the *V. cholerae* O1 serotype Ogawa strains belonging to phage type 27 were the dominant phage type with maximum 54.74%, which is similar to other studies done in past. Co-occurrence of type 27 and incidence of cholera indicates that *V. cholerae* O1

strains belonging to phagetype 27 might be primarily responsible for outbreaks in recent past in India<sup>7</sup>.

In an outbreak, increase load of *V. cholerae* leads to an increase in vibrio phage population, which subsequently results in declining of *Vibrio* population and helps in controlling outbreak<sup>19</sup>.

A recent study suggests that a cholera patient having vibrio phages in their stool less likely to transmit cholera infection through household contact when compared with patients without any *Vibrio* phage in their stool. Later it was found that phage positive *Vibrio* samples are non-motile under dark field microscope supporting the role of bacteriophages in controlling the spread of an outbreak<sup>20</sup>.

Widespread use of antibiotics resulted in the development of MDR bacteria which is a major problem in healthcare units today. Increase in multidrug resistance phenotype of *V. cholerae* O1 biotype El Tor makes the population vulnerable to a new pandemic.<sup>21,22</sup> Five bacteriophages reduced the *V. cholerae* load in a rabbit model of infection when administered 6 or 12 h after the pathogen challenge<sup>23</sup>.

Bacteriophages are a cost effective alternative to antibiotics and sometimes may be used for therapeutic purposes to treat multi-drug resistance (MDR) bacteria with limited success. To overcome the problem of multidrug resistance (MDR) phenotypes of *V. cholerae*, phagetherapy may be a better option<sup>7</sup>.

## CONCLUSION

In present study, 179 *V. cholerae* have been identified and phage type 27 was the dominant *V. cholerae* strain. This knowledge will be helpful to manage future cholera outbreaks. This study also indirectly supports the use of phages as a cost-effective alternative to control the disease and antibiotic resistance burden of *V. cholerae* strains.

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