Phage Types of *Vibrio cholerae* O1 Biotype ElTor Strains Isolated from India during 2012–2017

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Abstract

Background: Cholera is a primordial disease caused by *Vibrio cholerae* which existed from centuries in different parts of the world and still shows its periodic, endemic and epidemic presence. Thousands of cholera cases are reported from different parts of India and the disease remains endemic throughout the year. At present, we do not have enough knowledge about the phenotypic nature of the circulating *V. cholerae* strains in this part of the world. **Objectives:** This study was carried out over a period of 6 years with the aim defer with the changes in the prevalence and distribution of biotypes, serotypes and phage types of *V. cholerae* clinical isolates from various endemic regions of the country to determine phenotypic characteristics of the circulating strains and also to predict the attributes of cholera strains responsible for causing significant outbreaks in future. **Materials and Methods:** A total of 1882 *V.cholerae* O1 isolates from different cholera endemic areas of India were included in this study. *V.cholerae* strains which were identified as O1 biotype ElTor further analyzed for serotype and phage types using the standard methodologies. Polyvalent O1 and monospecific Inaba and Ogawa antisera were used for serotyping. A panel of five phages of Basu and Mukherjee phage typing scheme and five phages from the new phage typing scheme were used for phage typing analysis following standard methodology. **Results:** Maximum numbers of strains were isolated from cholera-endemic states like Gujarat and Maharashtra. All the isolates were confirmed as *V. cholerae* O1 biotype ElTor and majority of them were serotype Ogawa (93.2%). New phage typing scheme resulted in almost 100% typeable *V. cholerae* O1 strains included in this study and phage type 27 was the predominant type. Although 80% of the strains used in this study were sensitive to all the vibrio phages, S5 phage was found most efficient in lysing cholera strains indicating its broader host range. **Conclusion:** The current study identified phage type 27 as the most dominant type and serotype Ogawa was found continuous in circulation throughout the year which has caused recent cholera outbreaks in India during the past years. Phage sensitivity data propose an alternative cost-effective approach to prevent cholera outbreak by therapeutic uses of typing phages irrespective of origin or clonality of the strains.

Keywords: ElTor, O1, Ogawa, phage typing, S5 phage, *Vibrio cholera*

INTRODUCTION

Cholera remains a significant global health burden and is endemic in many parts of the third world countries due to inadequate sanitation and safe drinking water resources mainly during the rainy season.[1] *Vibrio cholerae*, an extremely motile, halophilic, curved shaped, Gram-negative bacterium is known for causing profound acute secretory diarrhea, cholera. Although *V. cholerae* is a natural member of aquatic environments, only a small fraction of environmental strains are capable of causing the disease. Among the several known serogroups, only two serogroups, *V. cholerae* O1 and O139 can produce cholera toxin and *V. cholerae* O1 often accountable for causing the vast majority of the disease including recent cholera outbreaks throughout the world.[2]

Serogroup O1 consists of biotypes ElTor and classical with distinct phenotypes. Both the biotypes carry two serotypes, Inaba and Ogawa with different disease characteristics.[3] Cholera has a long history; in the past 200 years, seven cholera pandemics have killed millions across the globe including the Indian subcontinent (specifically in Gangetic Bengal).[4] After

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several devastating pandemics in the different corners of the globe, the seventh pandemic which originated in Indonesia is still ongoing.[5,6] Most recent cholera outbreaks caused by *V. cholerae* O1 occurred in Haiti, causing at least 8,183 deaths and above 665,000 cholera incidences till date (https://www.cdc.gov/cholera/haiti/index.html#two).[7] Global estimates suggest that every year 1.3 million to 4.0 million cases of cholera are responsible for 21,000–143,000 deaths.[8] In India, 27,615 cholera cases were reported between 2010 and 2015, reflecting the sustained presence of *V. cholerae* O1 in the country and cholera is considered to be one of the underreported pathogens throughout the world.[9]

Several serotypes of *V. cholerae* strains harbor variety of bacteriophages, and these are beneficial from the clinical viewpoint with the command to control the bacterial population leading to controlling the disease.[10-12] 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.[13] Lysogenic filamentous phage, CTXΦ, is capable of transferring cholera toxin genes to nontoxigenic strains leading to the conversion of nontoxigenic strains to toxigenic strain.[14-16] The presence of cholera bacteriophages was found inversely associated with the occurrence of viable *V. cholerae* in the aquatic environment which was found in correlation with the number of locally reported cholera cases.[12] Lysogenic phage present in some environmental *V. cholerae* strains of both epidemic and nonepidemic serogroups has the power to kill the epidemic strains and thus contributes to the control of the disease.[16]

Different bacteriophages have been used to distinguish pathogenic strains of bacteria from clinical isolates based on their phage susceptibility. Due to their natural specificity, ease of maintenance and straightforward production, phage owns several desirable characteristics which makes them especially suited as bacterial strains discriminator. Since the late 1960s when molecular and modern methods were not widely available, lytic phages of *V. cholerae* have been used for typing of *V. cholerae* O1 which remained primary choice for classification of *V. cholerae* strains. Basu and Mukherjee initially proposed a phage typing scheme for *V. cholerae* O1 biotype ElTor in 1968, which was considered as a primary choice and a gold standard approach for differentiating *V. cholerae* biotype ElTor strains in the past.[17] Six phage types were identified by Basu and Mukherjee to characterize *V. cholerae* O1 biotype ElTor strains, among them phage type 2 and 4 are predominant whereas types 1, 3, 5, and 6 are not in circulation due to some unknown reason, investigation of which may require a separate study. A fundamental feature of the phage typing of *V. cholerae* was its usefulness in differentiating strains into several types as well as for differentiating classical and ElTor biotypes. Phage typing schemes for *V. cholerae* O1 and O139 were developed form NICED over the last few decades.[17-20]

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.[21,22] An earlier study has found that *V. cholerae* isolates from Kolkata, India are strongly correlated with resistance to some antibiotics such as streptomycin, tetracycline, and trimethoprim–sulfamethoxazole.[23] Other studies conducted in Nepal on *V. cholerae* strains isolated from cholera patients have found that 6.45% of the strains were multidrug resistant.[24,25] These studies suggest that antibiotic-resistant strains of *V. cholerae* in the Indian subcontinent are on the rise.[26-28] Horizontal gene transfer is an widely accepted methods of antibiotics resistance spreads.[29]

To use bacteriophages for therapeutic application in cholera, phage candidate or phage cocktail must have a broad host range and minimum effect on commensal organisms. A recent epidemiological study had identified the cholera hot spot region in India, but data on the phenotypic nature of presently circulating vibrio strains are missing in this part of the world. In this study, we have not only characterized the *V. cholerae* strains but did a comparative analysis on the host range of these phages for future therapeutic applications. Phenotypic characteristics including their susceptibility to vibrio phages described in this study are beneficial to keep the scientific community better equipped with the knowledge about the nature of circulating strains and the capability of phages to kill circulating *V.cholerae* strains to subvert future epidemic.

MATERIALS AND METHODS

Bacterial strains
V. cholerae O1 biotype ElTor strain MAK 757 (ATCC 51352) was used as the standard propagating strain or as the host for the propagation of *V. cholerae* O1 phages. We received clinical and environmental samples used in this study from different parts of the country. We included a total of 1882 *V. cholerae* O1 strains isolated from different cholera endemic areas in India between 2012 and 2017 in this study. We also included *V. cholerae* O139 strain NPR-4, classical biotype strains *V. cholerae* 154 and 569B which were used as negative control. Bacterial strains used in the recent study were grown in selected media (TCBS) using the standard methodology.[30]

Bacteriophages
The panel of five phages of Basu and Mukherjee phage typing scheme (Gr 1 to Gr V) and five phages from the new phage typing scheme (i.e., N4, S5, S20, M4, and D10] were used for phage typing study. These phages are in regular use in our laboratory which were reported earlier as different from each other and biotype specific.[18,19] All the phages were purified from the stock by successive single-plaque isolation against the respective standard propagating strain.

Propagation of bacteriophages
Standard broth lysis procedure was used to prepare phage lysates. Nutrient broth inoculated with 1% (vol/vol) cell

suspension from the overnight grown culture of MAK757 and grown at 37°C for 4–6 h in a shaker incubator. Mid-logarithmic-phase cultures of *V. cholerae* MAK757 were infected with respective phages at a multiplicity of infection of 0.1, and the infected cultures were incubated with shaking at 37°C until complete lysis occurred. After complete lysis of the culture solution, few drops of chloroform was added in the lysate, mixed well and centrifuged at 10,000 rpm for 20 min at 4°C.Phage lysate was collected and stored at 4^0C followed by centrifugation. For concentrating, phage lysate was centrifuged at 30,000 rpm for 2 h at 4°C using a Beckman 50.2 Ti rotor to pellet down the phage particles, resuspended in 0.05 M Tris [pH 7.5] with 0.02 M MgCl2 and kept at 4°C.[19] Broth lysis was used for preparation of larger stock. However, to get small volume high titer stock of phages, alternate to the above lysis method, plate lysis procedure was used for phage propagation following the standard methodology.[19] Bacteriophages used in the present study were purified using cesium chloride density (1.3–1.7 g/ml) gradient as per the standard protocol.[18,19]

Serotyping

Serological identification was carried out with each strain by using polyvalent O1 and consequently monospecific Inaba and Ogawa antisera (Difco, Detroit, Mich., USA) according to the WHO guideline (World Health Organization [1993]: Guidelines for Cholera Control. World Health Organization, Geneva).

Determination of routine test dilution and Phage typing

Phage typing was carried out based on the standard technique regularly performed in our laboratory.[18,19] A panel of the typing phages were used to type all the 1882 *V. cholerae* O1 biotype ElTor strains used in this study. Briefly, a single colony of *V. cholerae* strain grown in nutrient agar media was inoculated in nutrient broth and incubated under static conditions for 4–5 h at 37°C. An aliquot of the fresh culture (0.1 ml) was mixed with molten 0.8% soft agar (3.5 ml, maintained at 42°C) and poured onto a nutrient agar plate to prepare a uniform lawn of bacteria. Routine test dilutions (RTDs) of the phages were determined by the method as described by Chattopadhyay *et al.*[18] In brief, bacteriophage stock was serially diluted and the dilutions were spotted onto the lawn of the standard propagating host strain. Lowest dilution which was capable to lyse or react positively (appearance of at least five plaques) was considered as RTD and used for phage typing.[18,19]

Phage typing was performed as per the standard protocol of our laboratory.[19] Mid logarithmic phase young culture of *V. cholerae* (0.1 ml.) was mixed with molten soft agar and poured onto a nutrient agar plate. The plates were then kept to dry at room temperature (20–30 min) followed by the application of small drops of all the typing phages at the respective RTD dilutions. The drops on the plates were allowed to dry at room temperature for around 10 min and incubated

at 37°C for 16–18 h. *V. cholerae* MAK757 was used in each set of an experiment as a positive control due to the ability of all the typing phages to lyse MAK757. Presence of a clear lytic zone or appearance of at least five or more plaques for individual phage drop was considered as positive reaction.

Phage sensitivity

We have calculated individual phage sensitivity by dividing the total number of strains sensitive for specific phage with the total number strain studied.[18,20]

RESULTS

Bacteriophage and host range

A total of ten bacteriophages which are in routine use for phage typing in Vibrio phage reference laboratory were used in this study to determine phenotypic characterization of *V. cholerae* strains circulating in India. *V. cholerae* O1 biotype ElTor strain MAK757 (ATCC 51352) was found sensitive to all the ten bacteriophages but *V. cholerae* O1 classical biotype strain 569B and *V. cholerae* 154 were found resistant to these ten ElTor phages. Strains of common enteropathogens such as *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* were not sensitive to these phages.

Biotyping and serotyping

All the 1882 strains used in this study were characterized and confirmed as *V. cholerae*. Our result showed that all the strains included in this study belonged to *V. cholerae* O1 biotype ElTor [Table 1]. This result is indicative of complete replacement of classical biotypes by ElTor and revealed that *V. cholerae* O1 biotype ElTor is the only circulating pathogenic strain in India.

Based on cholera strains O1 antigen seroreactivity *V. cholerae* O1 strains can be divided into three groups, Inaba, Ogawa, and Hikojama. 1882 strains of *V. cholerae* O1 biotype ElTor were serotyped in our laboratory. Results indicated 1754 (93.2%) strains belonged to serotype Ogawa. Only 128 (6.8%) strains were found as serotype Inaba. We did not found any Hikojima serotype in our analysis [Table 1].

Phage typing

A total of 1882 *V. cholerae* O1 biotype ElTor strains isolated from different cholera endemic areas in India were used for phage typing with the set of typing phages available in the Vibrio Phage Reference Laboratory. Out of the ten phages, five phages were from Basu and Mukherjee scheme and five were from the new phage typing scheme.

Basu and Mukherjee phage typing

Five phages of the Basu and Mukherjee phage typing scheme was used to discriminate the ElTor strains and our results indicated that the strains were typed into two different phage types, phage type 2 (T-2) and phage type 4 (T-4). It was found that 1529 (81.25%) strains were grouped as T-2 and 334 (17.75%) were grouped as T-4. Almost 1% strains were found untypeable (UT) [Table 1].

State	No of Strains	Biotype		Serotype		Basu & Makherjee			New Phage type						
		Classical	Eltor	Ogawa	Inaba	T-2	T-4	UT	27	26	23	24	13	21	25
Gujrat	604	0	604	549	55	521	80	3	338	47	27	25	20	13	25
Andhra Pradesh	180	0	180	172	8	144	36	0	114	18	10	10	11	5	2
New Delhi	82	0	82	78	4	75	4	3	47	9	5	4	5	6	0
Karnataka	18	0	18	18	0	15	3	0	9	2	3	3	0	0	0
Maharastra	503	0	503	468	35	400	93	10	311	55	21	24	19	19	15
Madhyapradesh	69	0	69	59	10	39	29	1	41	5	2	3	4	0	1
Tamil Nadu	67	0	67	65	2	60	5	2	29	5	6	7	3	5	
West Bengal	136	0	136	128	8	102	34	0	110	13	2	3	1	0	2
Punjab	223	0	223	217	6	173	50	0	138	9	17	4	9	10	10
Total %			100	93.2	6.8	81.25	17.75	1	60.41	8.66	4.94	4.41	3.82	3.08	2.92
Total	1882	0	1882	1754	128	1529	334	19	1137	163	93	83	72	58	55

Table 1: Distribution of phenotypes of *Vibrio cholerae* O1 strain isolated from different states in India

New phage typing

Along with the Basu and Mukherjee typing phages, new phage typing[18] scheme was simultaneously used to classify the 1882 *V. cholerae* O1 isolates. In contrast to Basu and Mukherjee's scheme, new phage typing scheme, developed in our institute has a better discriminatory power and is capable of differentiating *V.cholerae* O1 biotype ElTor strains into 27 different types. Phage typing analysis based on the lytic reaction with the phages from the new phage typing scheme [Table 1] indicated 100% type ability of *V. cholerae* O1 biotype El Tor strains. Analysis revealed that *V. cholerae* O1 biotype ElTor strains were distributed into several different phage tapes of which (Types 27, 26, 23.24,13,21 and 25) were the major types that represented almost 81% of the total typed strains in this study [Table 2]. Majority of the 1882 strains belonged to phage type 27 (60.41 %). Phage type 26 (8.66%) was the next major phage type followed by type 23 (4.94%), type 24 (4.41%), type 13 (3.82%), type 21 (3.08%) and type 25 (2.92%) [Table 1]. It was found that the *V. cholerae* O1 biotype ElTor strains belonging to phage type 27 were widely and almost equally distributed in all the nine states from where strains were selected for the present study.

Identification of candidate phage for an alternative therapy

Sensitivity of the circulating *V. cholerae* strains against the five phages of the new phage typing scheme was calculated and it was found that all the phages have the ability to lyse at least 80% of the circulating strains in India. As shown in Table 3, phage N4 can lyse 91.17% strains, phage S20 can lyse 83.95%, phage M4 can lyse 86.29% of strains and phage D10 can lyse 80.49%. However, phage S5 has shown highest lethality which can kill or lyse 94% of the circulating *V. cholerae* O1 biotype El Tor strains.

DISCUSSION

We are using phage typing as one of the primary methods in monitoring the *V. cholerae* strains responsible for cholera infection in India at Vibrio Phage Typing Reference Laboratory. Besides potential application of phage typing scheme to distinguish cholera strains, our study will definitely contribute to an improved understanding of possible application of the phages as biocontrol agents during cholera outbreak.

Most of the cholera cases during the period from 2010 to 2015 in India were reported from West Bengal, Karnataka, Assam, Madhya Pradesh, Maharastra, Gujrat, and Punjab.[9] We incorporated *V. cholerae* strains in our present study which were received from these cholera endemic states of India as shown in Table 1. West Bengal reported 5,914 cholera cases in this time frame and other states were also considered to be cholera endemic due to the frequent cholera outbreaks in recent past.[7]

We have characterized and confirmed all the strains used in this study as *V. cholerae* O1 biotype ElTor. The frequency of serogroup O139 has declined significantly over the past few years consistent with our findings as well. Analysis of serotype distribution in our present study indicates the dominance of Ogawa serotype among the circulating *V. cholerae* O1 strains in India irrespective of demography and period which matches well with the earlier findings in India.[20,31] The capability of the strains belonging to Inaba serotype to convert into Ogawa serotype may explain the dominance of Ogawa serotype in our present study.[32,33] Clinical symptoms of patients infected with Ogawa or Inaba serotype of *V. cholerae* showed different disease pattern as explained elsewhere.[34] Infection with Ogawa strains are associated with shorter duration of diarrhea with more frequent abdominal pain, vomiting and need for more intravenous fluids when compared with infection with Inaba strain of *V. cholerae*.[34] Recent Cholera outbreaks in Haiti indicates that toxigenic *V. cholerae* O1 serotype Ogawa can rapidly change into Inaba serotype with the potential to cause disease in individuals who have acquired immunity against Ogawa serotype which supports the hypothesis that periodic shift may occur due to a consequence of host immunity against different serotypes of *V. cholerae* O1.[32] Findings of our present study may challenge the previous hypothesis of cyclic shift of *V. cholerae* O1 serotype due to induction of population-level immunity against dominant strain during an outbreak season. In India, so far, there is no report available regarding the shift in serotypes during different outbreak seasons irrespective

Table 2: Major Phage type classification based upon sensitivity to phages N4, S5, S20, M4 and D10. (+) indicates sensitive to specific phage, (−) indicates resistance to specific phage

Phage Type	N4	S5	S20	M4	D10	no of strain
13	-	+	+	+	+	72
21	+	+	-	-	+	58
23	+	+	-	+	+	93
24	+	+	+	-	-	83
25	+	+	+	-	+	55
26	+	+	+	+	-	163
27	+	+	+	+	+	1137

Table 3: Comparative analysis of vibriophage susceptibility. All the 1882 strains used in the present study were analyzed for sensitivity against the individual phages. Maximum number of strains were found sensitive to phage S5 followed by N4, M4, S20 and D10

Phage	No of specific phage sensitive strain out of total 1882 *Vibrio* strains	Sensitivity to individual phages (%)
N4	1716	91.17
S5	1770	94.04
S20	1580	83.95
M4	1624	86.29
D10	1515	80.49

of the geography or ethnicity of the infected host indicating equilibrium may have achieved between Ogawa and Inaba strains which require further investigation on responsible host factors associated with dominating serotypes to fine-tune the earlier proposed hypothesis.

Phage typing is a classical method to discriminate bacterial strains into different phage types based on the sensitivity pattern of a panel of typing phages with the bacterial host of the same species. It is a phenotypic characterization method of bacterial strains; much used in the microbiological community and has been around for several decades. The method itself requires that the different phages are available and therefore can generally only be performed at reference laboratories. Vibrio phage Reference Laboratory is involved in phage typing study over the decades using different phage typing schemes. Although Basu and Mukherjee scheme can classify *V. cholerae* O1 strains into six different types, over the years only two dominating types was found in circulation, reasons for abolition of other four types may demand a different study. In the current study, all of the 1882 strains clustered into two phage types only (either type 2 or type 4) when phage typing was performed using Basu and Mukerjee's scheme [Table 1]. Moreover 1% of the isolates remained untypeable. An earlier study also reported existence of significant number of untypable strains of *V. cholerae* O1 biotype ElTor when phage typing was performed using Basu and Mukherjee's scheme.[20] Another drawback of this scheme is that the finer distinction is missing and our result indicates that this scheme

lacks true identification of origin or clonality of the disease dissemination. Therefore, a different scheme with better discrimination power and better typeability was essential. New phage typing scheme which was developed based upon 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 ElTor strains to get a better idea on the origin and clonality route of the outbreak.[18] The present study has shown that type 27 is the dominant phage type in every state with maximum percentage [80%] from West Bengal [Figure 1], indicating the dissemination of cholera outbreak may have occurred from common precursor strain. These observations reveal a common origin and probably the pathogenic strains of cholera belong to the same clone. O1 strains from Maharashtra, Andhra Pradesh and Gujarat exhibited the maximum number of phage types where chorea is endemic over last 5 years. Co-occurrence type 27 and incidence of cholera indicates that *V. cholerea* O1 strains belonging to phage type 27 might be primarily responsible for outbreaks in recent past in India.

Apart from the phages characterized in our laboratory to distinguish *V. cholerae* O1, naturally occurring similar kind of lytic phages may play an essential role in controlling the spread of cholera infection. In the recent past, a model was proposed to understand how equilibrium exists between vibriophages and its host contributing to an outbreak. In an outbreak, increase load of *V. cholerae* leads to an increase in vibriophage population, which subsequently results in declining of vibrio population and helps in controlling outbreak.[35] A recent study suggests that a cholera patient having vibriophages in their stool less likely to transmit cholera infection through household contact when compared with patients without any vibrophage in their stool.[36] Later it was found that phage positive vibrio samples are non-motile under dark field microscope supporting the role bacteriophages in controlling and spread of an outbreak.

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.[37-41] In India, the history of phage therapy dates back to 1930s when vibriophages had been successfully used to treat cholera infection in the pre-antibiotic era.[42-44]

Phage therapy remained a standard part of the healthcare systems in Eastern Europe and the USSR since the second half of the 20th century.[45] However, it was largely neglected in the other part of the world due to easy availability of antibiotics for treatment. 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 ElTor makes the population vulnerable to a new pandemic.[46,47] To overcome the problem of MDR phenotypes of *V. cholerae*, phage therapy may be a better option. Earlier studies have shown that cocktail of the five bacteriophages reduced the *V. cholerae* load in a
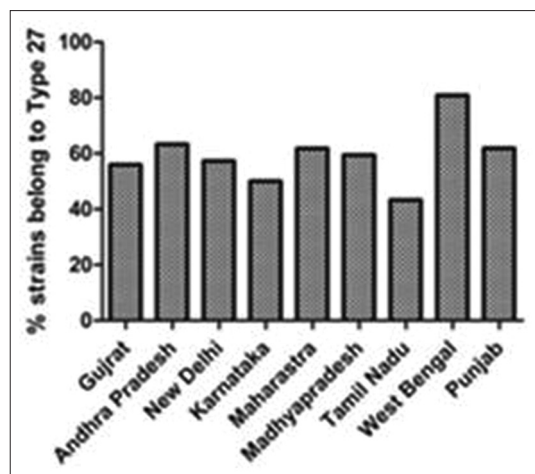
Figure 1: Distribution of strains belong to phage Type 27 [T-27] in different states of India isolated from 2012 to 2017

rabbit model of infection when administered 6 or 12 h after the pathogen challenge.[48] In other similar studies virulent vibriophages were used to control diseases in the animal model.[10,21] We found that typing phages are highly reactive to circulating ElTor strains [Table 2]. Our analysis revealed that the ability of the phages to cause lysis of *V.cholerae* strains ranges between 80%–94%, signifying their probable usages as candidate for phage cocktail for therapeutic uses. Moreover, as 94% of the circulating strains are susceptible to S5 phage, vibriophage S5 alone may also be used as an ideal candidate for phage therapy. Therefore a cocktail of these vibriophages or vibriophage S5 in combination with lytic phages of other diarrhea causing organisms may be considered as an universal cocktail for the prophylaxis of diarrheal diseases.[41,49-51] Hopefully future studies will be helpful to ascertain the decisive use of phages to treat diarrheal diseases. Cholera typing phages used in this study were found nonreactive (inability to cause bacterial lysis) to other commensals which makes them more suitable than antibiotics to manage cholera outbreak.

Conclusion

We have demonstrated that phage typing is still an internationally recognized method of choice for characterizing circulating strains. The present study has identified and characterized dominant cholera strains circulating in India throughout the year. This knowledge will be helpful to design a novel strategy to manage cholera outbreak in different parts of the country. Moreover, this study indirectly supports the use of phages as a cost-effective alternative to control the disease and antibiotic resistance burden of *V. cholerae* strains. We also advocate the use of these typing phages by directly applying them to water bodies and drinking water supply systems to reduce the burden of cholera incidence in the cholera hot spot region in India or anywhere in the world.

Conflicts of interest

There are no conflicts of interest.

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