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SHORT COMMUNICATION

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Identification of group B rotavirus as an etiological agent in the gastroenteritis outbreak in Maharashtra, India

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Acute gastroenteritis outbreak occurred at Pargaon, Maharashtra, India in 1789 cases with an attack rate of 32.5% between November to December 2015. The stool specimens ($n = 32$) were investigated for different enteric viral agents using conventional methods. Transmission electron microscopy and RNA polyacrylamide gel electrophoresis respectively identified morphologically distinct rotavirus particles in 28% and RNA migration pattern of Group B Rotavirus (GBR) in 72% of the specimens. Reverse transcription polymerase chain reaction and nucleotide sequencing confirmed presence of GBR in 97% of the samples analyzed. The predominance of GBR infections and absence or insignificant presence of other agents confirmed GBR as an etiological agent of the gastroenteritis outbreak occurred in Maharashtra, India.

KEYWORDS
diarrhea, electron microscopy, outbreak, RNA-PAGE, rotavirus group B, RT-PCR

1 | INTRODUCTION

Viral gastroenteritis continues to be an important cause of morbidity and mortality globally despite the improvements in sanitation and hygiene. The known causative agents are rotaviruses (Group A [GAR], Group B [GBR], and Group C [GCR]), calciviruses (norovirus and sapovirus), enteric adenoviruses, human astroviruses, aichiviruses, toroviruses, coronaviruses, picobirnaviruses, enteroviruses, and Sali/Klassi viruses. Among these, rotavirus, norovirus, astrovirus, and adenovirus infections are most predominant.

GAR is the leading cause of gastroenteritis in children and has been linked to diarrheal outbreaks among hospitalized infants, young children attending day care centers, and old age individuals.[1] GBR and GCR infections have been reported more frequently in adult cases of gastroenteritis outbreak.[2,3] Globally, outbreaks due to norovirus, adenovirus, and astrovirus have also been reported.[4–6]

An outbreak of acute gastroenteritis started on 29th November, 2015 with epicenter around the Bhimashankar sugar factory, Pargaon, Maharashtra, western India with around 116 hospital inpatient care (IPD) cases. The total population of Paragon is recorded to be 5500. Overall 1789 (IPD = 321; Hospital outpatient care [OPD] $N = 1468$) cases were reported from 29th November to 5th December, 2015. Among 321 IPD and 1468 OPD patients, 90% were in the age group of 15-59 years. The source of drinking water was a well situated near Bhima river. Water samples collected from well and water storage tank, showed presence of >16 coliforms and declared as unpotable by the state/sub divisional Health Laboratory on 1st December, 2015. Epidemiological investigations were immediately conducted to determine the etiology of the gastroenteritis outbreak. A total of 15 stool specimens were tested by state/sub divisional Health Laboratory, Pune for Enterotoxigenic *Escherichia coli*, *Shigella*, *Vibrio cholerae*, *Salmonella*, and *Klebsiella* showed absence of bacterial agent. We report here the findings of viral investigations carried out for rotaviruses (GAR, GBR, GCR), norovirus, adenovirus, and enterovirus.

2 | MATERIALS AND METHODS

2.1 | Study design

A total of 32 stool samples were collected from patients ($n = 32$) within 24 h of hospitalization. In the present study, a case of acute

gastroenteritis was defined as the passage of ≥3 watery stools in a day with or without associated symptoms such as vomiting, fever, and abdominal pain. All patients were examined for fever, number of episodes, and duration of vomiting and diarrhea, extent of dehydration, and treatment for the assessment of disease severity score (DSS).[7] According to the scores obtained, the disease condition of each of the patients was categorized as mild (scores 0-5), moderate (scores 6-10), severe (scores 11-15), and very severe (scores 16-20). The mean vesikary score between the two groups was compared using *t*-test.

2.2 | ELISA for detection of GAR

Thirty percent stool suspensions were prepared in 0.01M Phosphate Buffered Saline (PBS), pH 7.4. All specimens were tested for the presence of GAR by using antigen capture ELISA (Premier Rotaclone, Meridian Bioscience, Inc.) as per manufacturer's instructions. The fecal suspensions were stored at −70°C for further analysis.

2.3 | Electron microscopy

All specimens were examined for virus like particles by Electron microscopy (EM) using clarified 10% stool supernatant by negative staining as described earlier.[8]

2.4 | Viral RNA electrophoresis

Viral RNA was extracted from stool specimens using TRIzol LS reagent (Invitrogen, Waltham, MA) according to the manufacturer's instructions. The RNA electrophoresis in polyacrylamide gel (PAGE) was carried out for the detection of rotavirus dsRNA genome segments. The electropherotyping of viral RNA was carried out in 10% PAGE at 100 V using Tris-Glycine buffer. The gel was stained with silver nitrate as described earlier.[9]

2.5 | RNA extraction, PCR/RT-PCR, and nucleotide sequencing

Viral nucleic acid extraction and detection of GAR, GCR, Norovirus, Adenovirus, and Enterovirus was carried out by PCR/RT-PCR as described earlier.[3,10–14] The use of NSP2 gene based semi-nested RT-PCR assay reported for detection of GBR was modified to single RT-PCR assay using the modified primers (Forward-5′ GCCATCA-GACAGAGAATGTGT 3′ [CAL-1 strain- AY238383.1, nucleotide position 112-132] and Reverse-5′ TTGTCTGCCGAAGCTAAAACA 3′ [CAL-1 strain- AY238383.1, nucleotide position 432-412]) of the earlier study.[15] GBR positive stool specimens were genotyped using primers published earlier for VP7 gene.[16] RNA was denatured at 97°C for 5 min and was rapidly chilled on ice for 5 min. Briefly, the RT-PCR reaction was carried out with an initial reverse transcription step at 50°C for 30 min followed by PCR activation at 95°C for 5 min followed by 35 cycles of amplification (60 s at 94°C, 30 s at 55°C, and 60 s at 68°C) with final extension at 68°C for 7 min in a thermal cycler. The SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase kit (Invitrogen) was used for both cDNA synthesis and PCR amplification in a single tube for detection of RNA viruses. All the PCR products were electrophoresed in 2% agarose gel containing ethidium bromide (0.5%) and visualized under UV transiluminator. PCR amplicons were excised from the gel for purification (QIAquick, Qiagen, Hilden Germany) and cycle sequencing was carried out using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and ABI 3130XL genetic analyzer (Applied Biosystems). Nucleotide sequence identity was determined through BLAST (www.ncbi.nlm.nih.gov/blast). The phylogenetic tree was generated with Maxiumum Likelihood method using MEGA 6 software.[17] The nucleotide sequences of the strains examined in the study have been deposited in GenBank under the accession numbers KY196517-KY196528 and MF066701-MF066707.

3 | RESULTS

The outbreak of acute gastroenteritis occurred at Pargaon, Maharashtra, India lasted for 6 days and affected a total of 1789 individuals with an attack rate of 32.5%. The stool specimens collected from 32 patients were subjected to laboratory investigations. Rotavirus like particles visualized in 28.1% of the specimens and no other enteropathogens were detected by using EM. The representative EM image of the rotavirus particle with wheel shaped structure is shown in Figure 1.

RNA-PAGE analysis indicated RNA migration pattern of GBR (4-2-1-1-1-1-1) in 24 (71.8%) stool specimens. Ninety seven percent (31/32) of the specimens showed positivity for GBR using partial NSP2 gene based RT-PCR. Representative GBR RT-PCR

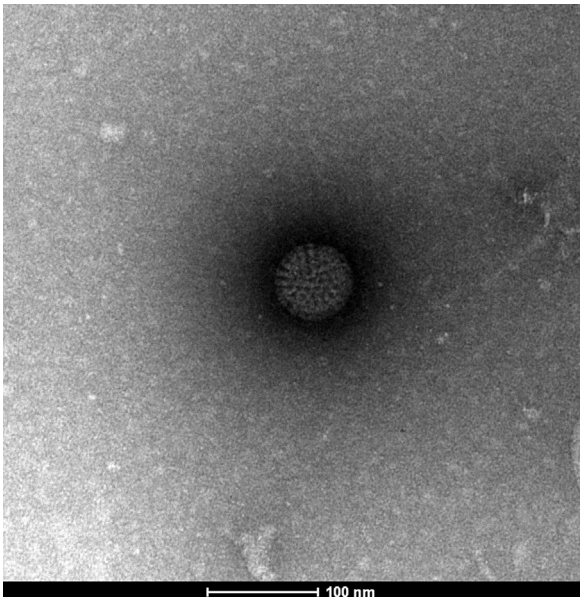

FIGURE 1 Electron micrograph showing rotavirus particle in stool suspension of a patient from gastroenteritis outbreak

positive samples were subjected for VP7 genotyping and nucleotide sequencing ($n = 7$). Phylogenetic analysis revealed that the study strains were closer to the strains from Indian-Bangladeshi lineage (96.4-100 and 96.7-98.7% nucleotide identity) than with the Chinese lineage (92.5-95.1% and 91.2-91.9% nucleotide identity) of N1 and G2 genotype of NSP2 and VP7 genes respectively (Figure 2). A unique nucleotide and amino acid change in comparison to all global GBR strains was observed in all study strains at nucleotide position NSP2-287 and amino acid position NSP2-78 (Isoleucine→Valine). Among all strains of the present study, amino acid Arginine at VP7-76 position observed in Indian-Bangladeshi lineage strains has been replaced by Lysine. The occurrence of the same amino acid (Lysine) at this position has been noted in the Chinese lineage strains.

The age distribution analysis indicated occurrence of GBR infections in patients aged between 12 and 50 years (median 30 years) with exception of a single male patient of 4 years. The male: female ratio in GBR positive patients was observed to be 0.55:1 (male 11, female 20). Clinical severity score of the patients with GBR infection ($n = 27$) indicated severe disease in 70.3% and moderate disease in 29.6% patients. Severity score was not available for four GBR positive patients.

The severity of the infection using DSS system among GBR RNA positive patients was analyzed for 26 patients (age: 12-50 years, median 30 years). No significant difference was observed in mean DSS with age (≤median, $n = 15$, mean DSS 11.1 ± 2.6; >median, $n = 11$, mean DSS 12.1 ± 1.6) as well as gender (Male, $n = 7$, mean DSS 10.6 ± 2.2; Female, $n = 19$, mean DSS 11.8 ± 2.2) of the patients (t-test, $P > 0.05$ for each comparison). The mean DSS of the GBR RT-PCR and RNA-PAGE positive patients ($n = 21$, mean DSS 11.6 ± 2.1) was not significantly different from those of GBR RT-PCR positive and RNA-PAGE negative ($n = 5$, mean DSS 11.0 ± 2.9) patients ($P > 0.05$).

GAR was detected by ELISA and RT-PCR respectively in one and two stool specimens. Enterovirus was detected in only one specimen by RT-PCR. All GAR and enterovirus positive specimens were also positive for GBR-RT PCR. All 32 stool specimens were found to be negative for Norovirus and GCR by RT-PCR and Adenovirus by PCR.

4 | DISCUSSION

Twenty-eight percent of the stool specimens with rotavirus like particles, majority with GBR RNA positivity (RNA-PAGE: 72%; RT-PCR: 97%) and very low occurrence of other enteric pathogens indicated GBR as the major etiological agent of the gastroenteritis outbreak occurred at Pargaon, Maharashtra, India. The predominance of GBR infections observed in adults (median age 30 years) was in concordance with earlier reports.[18,19] The low prevalence in children compared to adults has been suggested to be either due to low exposure of children to GBR or faecal shedding below the detection limit of the assay employed in the study.[20]

In China, a series of widespread gastroenteritis epidemics affecting millions of people were reported to be caused by GBR.[18] Over the period, epidemics settled to sporadic focal outbreaks and after 1987, no such reports were available from China. In India, circulation of GBR in sporadic cases of gastroenteritis was revealed from the retrospective analysis of stool samples collected in 1993.[21]

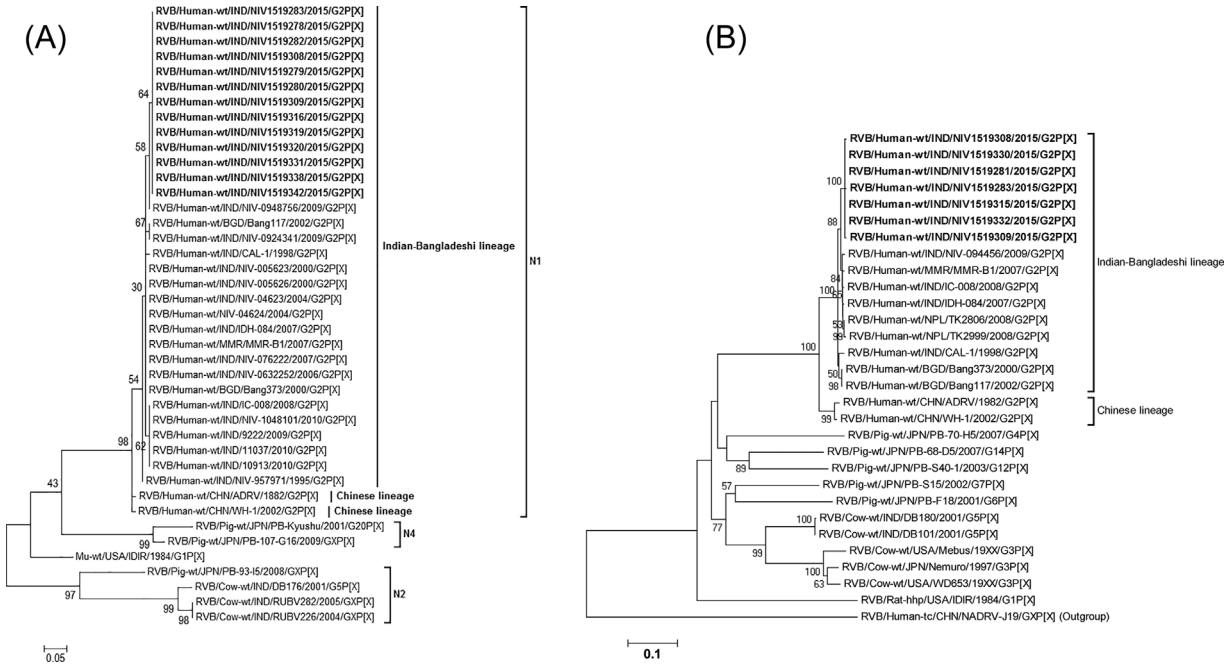

FIGURE 2 A, Phylogenetic dendrogram of partial NSP2 gene (112-432 bp) of group B rotavirus (GBR) strains. The strains of the present study are indicated by the bold letters. The scale represents genetic distance. B, Phylogenetic dendrogram of VP7 gene (63-755 bp) of group B rotavirus (GBR) strains. The strains of the present study are indicated by the bold letters. The scale represents genetic distance

Further, the occurrence of GBR in the gastroenteritis outbreaks in India was reported from union territory of Daman, Gujarat, and Maharashtra states of India.[2,19] After 2009 till the year 2015 of the present study, no report was available on the association of GBR as the causative agent of gastroenteritis outbreak from India. It is possible that in India fecal excretion as well as circulation of GBR is at a low level and that there is under-reporting of GBR infection due to lack of easy and rapid diagnostic methods. In addition, GBR mainly infects adults and etiology of majority of the diarrheal episodes among adults normally remains unknown.

RNA PAGE analysis has been reported to be 100 000 times less sensitive as compared to RT-PCR assay[22] and data point out requirement of high viral load for visualization of RNA-PAGE pattern as compared that required for RT-PCR detection. Therefore, among GBR RT-PCR positive specimens of the study, RNA PAGE positive are expected to have high viral load as compared to RNA-PAGE negative specimens. Comparison of mean DSS of RNA PAGE positive and RNA PAGE negative patients showed no significant difference ($P > 0.05$). The correlation between viral load and severity of the disease has been shown earlier among gastroenteritis patients infected with GAR using quantitative real time PCR assay.[23] In view of this, GBR RNA PAGE positive and negative faecal specimens need to be tested by quantitative Real time PCR assay.

The study strains were closer to Indian Bangladeshi strains of GBR and highly conserved in nature as has been demonstrated previously.[24] However, further studies are necessary to ascertain the role of subtle genetic substitutions observed in the study strains coupled with nucleotide analysis of remaining genes to understand the genetic evolution of the virus over the period, its role in pathogenicity and epidemiology of the disease.[25]

The state/sub divisional Health Laboratory declared that the piped well water with a high coli form count was the common source of infection and that the leakage in the pipeline and irregularity in chlorination of water led to gastroenteritis outbreak. Even though, the analysis of piped and well water samples for viral agents was not conducted in the present study, the predominance of GBR infections among outbreak cases and the common source of water with high coli form count indicate spread of GBR through fecal contaminated water. The use of well water was completely stopped and the alternative arrangement was made to supply the drinking water. To prevent the GBR spread, regular chlorination of well water was performed. Training was also provided to the family member of every house for hygienic practices and decontamination of water.

The occurrence of GBR as an etiological agent of the gastroenteritis outbreak in Pargaon demonstrated in the study highlights its importance in public health policy as the virus causes severe and large gastroenteritis outbreaks in adults. Further, the surveillance studies should be conducted to better understand the role of GBR in outbreak as well as sporadic cases of gastroenteritis from different parts of India. It would be necessary to extend such studies in pediatric population while group A rotavirus vaccines are being implemented in India under National Rotavirus Immunization Programme.

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CONFLICTS OF INTERESTS

The authors declared that there is no conflict of interest.

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