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Investigation of a hepatitis A outbreak from Shimla Himachal Pradesh

L.P. Chobe & V.A. Arankalle

National Institute of Virology (ICMR), Pune, India

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***Background & objective*: Hepatitis A is an enterically transmitted viral disease, highly prevalent in India and mainly presents as a paediatric sporadic disease. This study investigated an outbreak of viral hepatitis at Shimla, Himachal Pradesh, India, during January-March 2007.**

***Methods*: Eighty seven blood samples, 3 water samples and 2 sewage samples were collected. Serum samples were tested for IgM and IgG anti HAV and IgM and IgG anti HEV antibodies. Serum, sewage and water samples were tested for HAV-RNA by nested RT- PCR. Nearly complete full genome (excluding extreme 5' end) was amplified from one serum sample.**

***Results*: The hepatitis cases were mainly seen among children and young adults and 63.2 per cent (55/88) were positive for anti-HAV IgM. These cases were reported from the areas getting water supply from Ashwani Khud water supply system. This water purification system received water from a natural stream in which treated sewage water was let into 4 km upstream the collection point since one year. HAV-RNA present in serum, sewage and water samples showed 100 per cent sequence homology. Phylogenetic analysis based on 5' non coding (5' NC) and nearly complete genome showed the evidence of HAV genotype IIIA in all the samples.**

***Interpretation and conclusion*: The aetiological agent of the present outbreak was hepatitis A virus which is emerging in an outbreak form in India, emphasizing a definite need for formulating vaccination / control strategies.**

Key words Genotype - hepatitis A virus - molecular epidemiology

Hepatitis A is an enterically transmitted viral disease. The infection occurs primarily due to ingestion of contaminated food and water and person-to-person contacts[1]. Common source outbreaks occur due to faecally contaminated food and water[2]. The average incubation period is 25-30 days. Hepatitis A virus (HAV) is the only member of the genus Hepatovirus within the family *Picornaviridae*[3]. It is a non-enveloped virus, 27-30 nm in diameter. The genome is 7.5 Kb, positive strand RNA with single open reading frame (ORF)[4]. HAV isolates are classified into 6 genotypes (I-III human, IV-VI simian), but a single serotype exists[5,6].

In most developing countries in Asia and Africa, hepatitis A is highly endemic and large population acquires immunity through asymtomatic infection

early in life[7]. However, several recent reports documented changing epidemiology of hepatitis A in these countries from hyperendemicity to intermediate endemicity[8-11]. In India recent surveillance studies carried out from different areas documented decline in anti-HAV prevalence associated with improved hygienic standards and socio-economic conditions[10,12-14], and possibility of hepatitis A outbreak was suspected[10]. In the past few years outbreaks of hepatitis A have been reported among children and adults from India[15-18] (NIV unpublished data).

This study was carried out to investigate an outbreak of hepatitis A at Shimla, Himachal Pradesh during January - March 2007.

Material & Methods

Study area: The outbreak occurred at Shimla, Himachal Pradesh between the period January-March 2007. The cases were mainly noted from Kusumpti, Vikas Nagar, Panthaghati, Mehi and New Shimla locality having the population around 50,000. The first case was reported on January 21, 2007 and approximately 450 cases were reported till February 23, 2007. National Institute of Virology (NIV), Pune was informed about the outbreak on February 15, 2007 and investigations were undertaken immediately thereafter.

Collection of samples

Blood samples - A total of 87 blood samples were collected either from indoor or OPD patients of Reepen Hospital (Deen Dayal Hospital) or by house-to-house survey. Detailed history was recorded for each patient in a pre set proforma.

Environmental samples - Affluent and effluent sewage samples (5 litre each) (SIME1, SIME2) from Malayana sewage treatment plant, 5 litres water from Ashwani Khud water supply system before treatment (SIME3), 15 litres treated water from Ashwani Khud water supply system (SIME4) and 15 litres water from Kusumpti water tank (SIME5) were collected. The sewage and water samples were transported to NIV at room temperature, while the serum samples were transported on wet ice.

Serology: All the serum samples were screened for the presence of IgM and IgG antibodies against hepatitis A (General Biologicals, Taiwan) and hepatitis E virus[19].

Nested reverse transcription PCR (RT-PCR): Affluent and effluent sewage samples (140 µl each) were directly processed for RT-PCR without any concentration. Water samples were concentrated using two step membrane based ultrafiltration technology according to the protocol described previously[16] and used for RT-PCR.

Affluent and effluent sewage samples (140 µl each), concentrated water sample and representative 7 serum samples (SIM1, SIM25, SIM27, SIM28, SIM33, SIM34, SIM35) were screened for the detection of HAV RNA by RT-PCR. Viral RNA was isolated by using QIAamp viral RNA mini kit (Qiagen, USA) according to the manufacturer's instruction. Primers representing the conserved 5' non-coding region (5'NC) were used[16]. In addition, entire genome (excluding extreme 5'end) was amplified from one serum sample (SIM27) using combinations of different sets of primers based on the consciences nucleotide sequence of genotype IIIA strains covering entire genome (Table I).

Table I. Position and nucleotide sequence of oligonucleotide primers used for PCR and sequencing of nearly complete genome of hepatitis A virus isolate from Shimla

Nucleotide position/ polarity	Nucleotide sequence (5' 3')
F1	GCCTAGGCTATAGGCTAAAT
F2	TCTCATCCAGTGGATGCATT
F3	TCTGTTGAGGTATCACACTTAT
F4	TCAATTCCCACTTTGGCTGCT
F5	TCTCTCAGCTATTAATCTAG
F6	CTACTGATGTTGATGGAATGGC
F7	TCATTATTCTACACAGAAGAT
F8	TCTGTCAGGGATACAGGAGATT
F9	TATTATGATCTGAATTATGGT
F10	TACAACTGATGAGGATTGGTCT
F11	TTCTGGTGAGCCATCTGGGT
F12	CAGGATGTGGTTCTAATGAAAGT
F13	TTGATCCTATGGCAGTGATGCTGT
F14	CACACTGGTGTTGCAATTGG
F15	TGATAGACAGTGGGACCAACT
F16	GTTATGGAGATGATGTTCTTAT
R1	TGTCCAGCATTCAATGGCGAGGT
R2	TAGATCCATAGCTCTGATCTCCT
R3	TTCAGGATTTGTGTTAGTCATCT
R4	TCCAGAGTCATCTCCAACCT
R5	TAACAGCACCAAGAGCTGTCT
R6	GCAGTGATTCCTTTCCAGGAG
R7	GGTACTAGTTATGGAATCTAG
R8	TCAGCCTCCTCAATTGCCCT
R9	CTCCATTGGACATCCAGACACC
R10	TTGTGATTGGTAACTGATTGG
R11	CAAAGCTCTAGGCACATCACTC
R12	TCTTTATAATCATCTGGCTCAT
R13	CCAACATCTCCAAATCTAATC
R14	TCCAATAGAATCCGAATTGT
R15	TGAAAAGATAAAATAAACAAACCT

Oligonucleotides are based on the consciences sequences of genotype IIIA hepatitis A virus strains

PCR products were column purified (QIA gel purification kit, Qiagen, USA) and both the strands were sequenced using Big Dye Terminator cycle sequencing Ready Reaction Kit (Applied Biosystems, USA) and an automatic sequencer (ABI PRISM 310 Genetic Analyser, Applied Biosystems, USA).

Phylogenetic analysis: Sequence alignments were generated by CIUSTAL X (version 1.83)[20]. The phylogenetic status was assessed employing the software molecular evolutionary genetic analysis (MEGA) version 4[21]. For analysis in MEGA, Jukes Cantor (JC) distance was utilized employing the neighbour joining (NJ) algorithm. The reliability of different phylogenetic groupings was evaluated using the bootstrap test (1000 bootstrap replications) available in MEGA.

Accession numbers and designations of the complete or partial genome sequences employed for the analysis in the present study were as follows;

Complete genome sequences:

Genotype IA- K02990 (LA), AF35722 (LU38), AB021565 (AH2), AB020564 (AH1), AB020567 (FH1), AB020566 (AH3), AB020568 (FH2), AB020569 (FH3), AF485328 (LY6), AF512536 (DL3), X75215 (GBM/WT), X83302 (FG), AY974170 (M2).

Genotype IB- M14707 (HM-175), M20273 (MBB), AF268396 (HAF-203), AF314208 (L-A-1).

Genotype IIA- AY644676 (CF53/Berne).

Genotype IIB- AY644670 (SLF88).

Genotype IIIA- AJ299464 (NOR-21), AY644337 (HMH), AB279732 (HA-JNG04-90F), AB279733 (HA-JNG08-92F), AB279734 (HAJ95-8F).

Genotype IIIB- AB258387 (HA-JNG06-90F), AB279735 (HAJ85-1F).

Genotype V- D00924 (AGM-27).

5' noncoding region sequences (300nt): DQ172956 Murud (2004) 3, DQ172953 Tathawade (2003), DQ172951 Srigaon (2004) 3, DQ172947 Lonand (2004) 1, DQ172946 Savali (2004) 4, DQ172941 Sarawade (2003) 1, DQ172940 (Dorli), DQ172939 Daund (2004) S8, DQ172961 Budhgaon (2004) 5, DQ004693 KOT-1,

Results & Discussion

This hepatitis outbreak occurred between January - March 2007 at Shimla, capital of Himachal Pradesh situated at the 2100 m height in North West Himalayan ranges. The cases were mainly among children and young adults (2.5-25 yr). No deaths were reported and generally mild clinical symptoms were observed.

Drinking water is supplied through five water supply systems namely Seog, Cherot and Jagroti Nallh, Chair Nallh, Nauti Khud and Ashwani Khud to Shimla. Jaundice cases were noted mainly from the localities receiving water from Ashwani Khud water supply system. This system was installed in 1992 wherein the water gets collected from Ashwani khud, a natural stream, chlorinated, pumped to Kawalag storage plant and then to Kusumpti tank where it is rechlorinated and distributed. Since one year treated sewage water from Malayana sewage treatment plant was let in to the Ashwani Khud water stream, four kilometers upstream of the collection point. Two months before the onset of the outbreak treatment by silver ionization was introduced instead of chlorination at Kusumpti storage tank. Chlorination was reintroduced since February 28, 2007.

Of the 87 serum samples collected 55 (63.2%) were positive for anti HAV IgM antibodies. Of the remaining 17 anti HAV IgM negative samples, 13 were positive for anti HAV IgG in the age group <25 yr while all 15 samples were positive for anti HAV IgG in the age group >26 yr (Table II).

In order to assess the involvement of hepatitis E virus, most common epidemic causing virus in India, all serum samples were screened for IgM and IgG anti HEV antibodies. One patient (15 M) was co-infected with HEV (anti HEV IgM positive) while 40.2 per cent (35 of 87) were positive for anti HEV IgG.

Both the affluent and effluent sewage samples from Malayana sewage treatment plant, treated and untreated water samples from Ashwani khud water supply system and eight serum samples were positive for HAV RNA. Water sample from Kusumpti water storage tank did not show the presence of HAV RNA.

Table II. Prevalence of HAV IgM in different age groups

Age groups (yr)	Anti HAV-IgM Pos / No. of samples tested
0-5	1/2
6-10	16/19
11-15	18/22
16-20	12/19
21-25	8/10
26+	0/15
Total	55/87 (63.2%)

The phylogenetic status of the HAV isolates sequenced during the present study on the basis of 5' non coding (5' NC) and ORF region is presented in Figs 1 nd 2. Both regions placed the Shimla isolates in genotype IIIA.

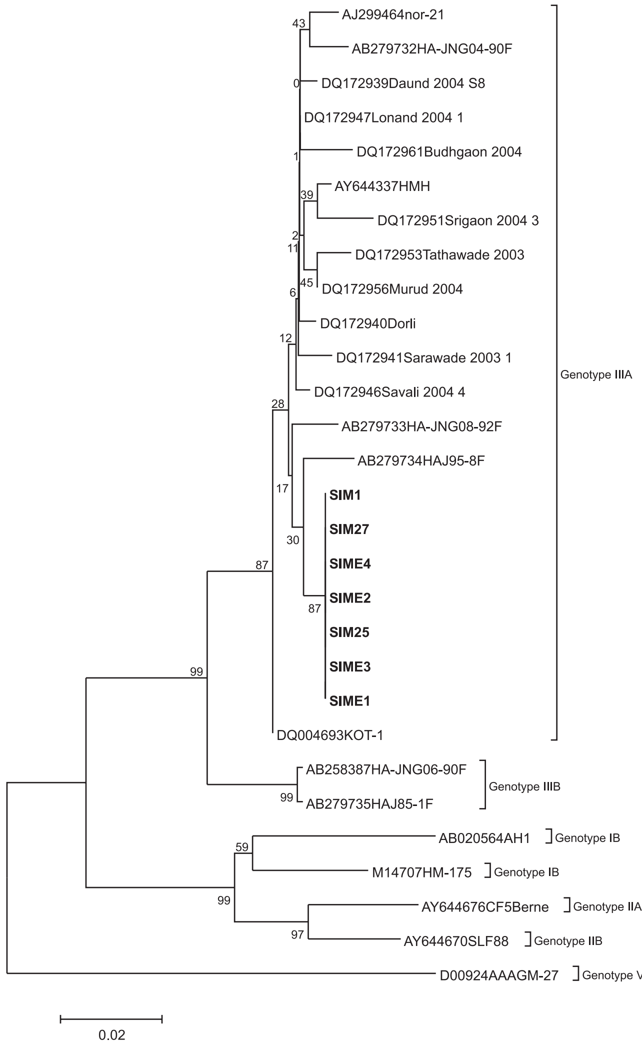

Fig. 1. Phylogenetic analysis of 5' NCR sequences. Sequences for each major genotype were selected from the GenBank database for Genotype IIIA: AY644337 (HMH), AJ299464 (nor-21), AB 279732 (HA-JNG0-90F), AB279733 (HA-JNG08-92F), AB279734 (HA-195-8F) genotype IIIA sequences generated from western India during our previous studies[22], DQ172939 (Daund 2004. S8), DQ 172947 (Lonand 2004 1) , DQ 172961 (Budhgaon 2004), DQ 172951 (Srigaon 20043), DQ 172953 (Tathawade 2003), DQ 172956 (Murud 2004), DQ 172940 (Dorli 2007), DQ 17294 1 (Sarawade 2003), DQ 172946 (Savali 2004 4), genotype IIIA sequence from Kerla 16 (DQ 004693 KOT-1), genotype IIB: AB258387 (HA – JNG06-90F), AB279735 (HAJ 85-1F), genotype IB: AB020564 (AH1), M14707 (HM-175), genotype IIA: AY 644676 (CF5 Berne); genotype IIB: AY644670 (SLF88); genotype V: D00924 (AGM27). Sequences generated in the present study are in red colour.

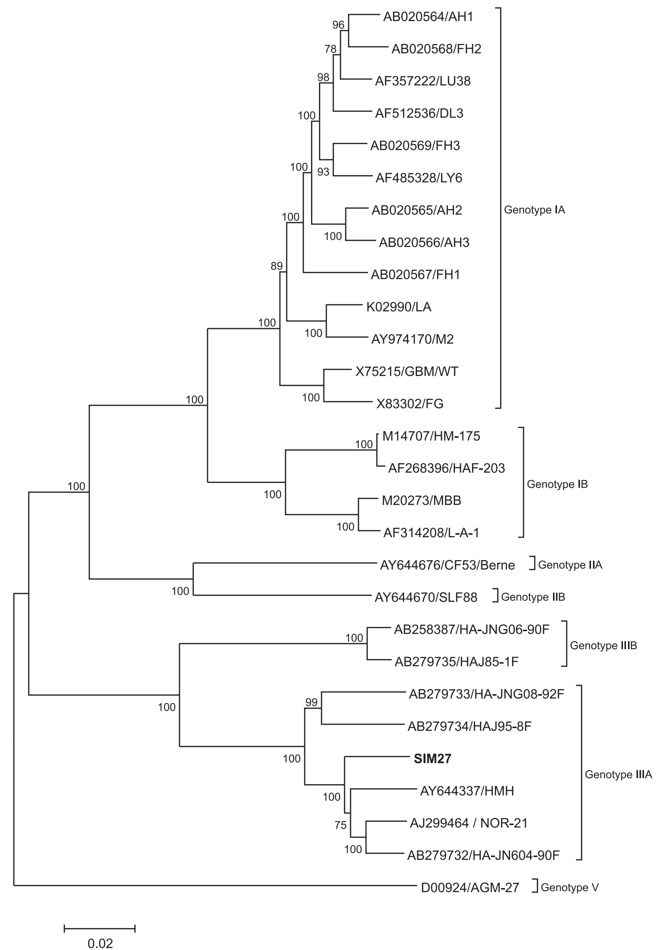

Fig. 2. Phylogenetic analysis based on open reading frame (ORF) region. ORF sequence of SIM 27 (6684NT) was compared with representative number of sequence selected from GenBank Genotype IA: K02990 (LA), AF35722 (LU38), AB021565 (AH2), AB020564 (AH1), AB020567 (FH1), AB020566 (AH3), AB020568 (FH2), AB020569 (FH3), AF485328 (LY6), AF512536 (DL3), X75215 (GBM/WT), X83302 (FG), AY974170 (M2). Genotype IB: M14707 (HM-175), M20273 (MBB), AF268396 (HAF-203), AF314208 (L-A-1). Genotype IIA: AY644676 (CF53/Berne). Genotype IIB: AY644670 (SLF88). Genotype IIIA: AJ299464 (NOR-21), AY644337 (HMH), AB279732 (HA-JNG04-90F), AB279733 (HA-JNG08-92F), AB279734 (HAJ95-8F). Genotype IIIB: AB258387 (HA-JNG06-90F), AB279735 (HAJ85-1F). Genotype V: D00924 (AGM-27) take list from document 1) SIM 27 is represented in red colour.

In 5'non-coding region all the Shimla isolates had 100 PNI (per cent nucleotide identity) with each other, 98.4 PNI with other Indian HAV isolates from various outbreaks from Maharashtra, 98.6 PNI with genotype IIIA and 96.2 PNI with genotype IIIB isolates which were previously generated in our lab and taken for comparison[22]. The accession numbers for the sequences generated during this study were FJ227129-FJ227135.

The genomic length of SIM27 isolate was 7347 nucleotides. It possessed a single long ORF of 6684 nucleotides encoding the polyprotein of 2228 aa. The 3'UTR was of 70 nucleotides. ORF region of SIM27 had 95.5 PNI with IIIA, 88.7 PNI with genotype IIIB and 82.1 PNI with the genotype I and II isolates. At amino acid level ORF showed 99.3 per cent identity with IIIA, 98.1 per cent with IIIB and 94.2, 93.5 per cent with the genotype I and II isolates respectively.

With the changing epidemiology of hepatitis A virus, outbreaks of hepatitis A have been recorded in India[17,18]. The Kerala epidemic affecting over 110 adults being noteworthy[16]. The population under study belonged to the locality of Shimla receiving drinking water from a single water supply system. Mixing of effluent water from the sewage treatment plant into the water four kilometers upstream the collection point of this water supply system and lapses in the chlorination treatment seem to be the major causes of this outbreak. Newly introduced less efficient silver ionization treatment, two months prior to the onset of the outbreak as compared to chlorination may be an important contributory factor. Possibility of role of less than average rainfall cannot be ruled out. Hundred per cent sequence homology between the environmental samples *i.e.*, sewage and water samples and clinical samples further confirms that water contaminated with sewage was responsible for the outbreak. Such concordance between the environmental and clinical specimens has been reported earlier[16,23,24].

Eight sera, affluent and effluent sewage samples and treated and untreated water samples were positive for HAV-RNA. However, the viral RNA could not be detected in a water sample from the storage tank. This could be either due to the very low viral load or due to super chlorination of water that is reintroduced after the onset of outbreak.

On molecular characterization, all isolates were of genotype IIIA having 98.4 PNI with other Indian isolates. Genotype IIIA has been reported earlier from north India[25], Kerala[16] and various outbreaks from Maharashtra (our unpublished observations). Even though equal prevalence of two genotypes *i.e.*, IA and IIIA was reported from acute hepatitis cases in north India[26], a single genotype, IIIA seems to be predominantly circulating from northern to southern States of India.

In conclusion, to avoid such outbreaks of HAV in future, there is a need of disposal of sewage water after the collection point of the water treatment plant and adequate chlorination of drinking water. In addition, there is an urgent need of preparing a model guideline for public works department regarding proper planning and execution of water supply system.

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References

1. Krugman S, Ward R, Giles JP. The natural history of infectious hepatitis. *Am J Med* 1962; *32* : 717-28.
2. Bean NH, Goulding JS, Lao C, Anulo FJ. Surveillance for food borne disease outbreaks-United States.1988-1992. *MMWR CDC Sueveill Summ* 1996; *45* : 1-66.
3. Minor PD. Picornaviridae, classification and nomenclature of viruses. The Fifth Report of the International Committee on Taxonomy of Viruses. *Arch Virol* 1991; *2* (Suppl): 320-6.
4. Hoolinger FB, Emerson SU. Hepatitis A virus. In: Knipe DM, Hoeley PM, Graffin DE, Martin MA, Lamb RA, Roizman B, Straus SE, editors. *Fields virology*, 4th ed. Philadelphia PA: Lippincott Williams and Wilkins; 2001. p. 799-840.
5. Lu L, Ching KZ, de Paula VS, Nakono T, Seigl G, Weitz M, *et al*. Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF3/Berne isolate). *J Gen Virol* 2004; *85* : 2943-52.
6. Robertson BH, Jansen RW, Khanna B, Totsuka A, Nainan OV, Siegl G, *et al*. Genetc relatedness of hepatitis A virus strain recovered from different geographical regions. *J Gen Virol* 1992; *73* : 1365-77.
7. Gust ID. Epidemiological patterns of hepatitis A in different parts of the world. *Vaccine* 1992; *10* : S56-62.
8. Innis BL, Snitbhan R, Hoke CH, Munindhorn W, Laorakpongse T. The declining transmission of hepatitis A in Thailand. *J Infect Dis* 1991; *163* : 989-95.
9. Hong-Yuan H, Hsu HY, Chang MH, Chen DS, Lee CY, Sung JL, *et al*. Changing seroepidemiology of hepatitis A virus infection in Taiwan. *J Med Cirol* 1985; *17* : 297-301.
10. Arankalle VA, Chadha MS, Chitambar SD, Walimbe AM, Chobe LP, Gandhe SS. Changing epidemiology of hepatitis A and Hepatitis E in urban and rural India (1982-1998). *J Viral Hepat* 2001; *8* : 293-303.
11. Chitamber SD, Chadha MS, Joshi MS, Arankalle VA. Prevalence of hepatitis A antibodies in Western Indian population: changing pattern. *Southeast Asian J Trop Med Public Health* 1999; *30* : 273-6.
12. Das K. The changing epidemiological pattern of hepatitis A in an urban population of India: emergence of a trend similar to the European countries. *Eur J Epidemiol* 2000; *16* : 507-10.
13. Mall ML, Rai RR, Philip M, Naik G, Parekh P, Bhawnani SC, *et al*. Seroepidemiology of hepatitis A infection in India: changing pattern. *Indian J Gastroenterol* 2001; *20* : 132-5.

14. Jindal M, Rana SS, Gupta RK, Das K, Kar P. Serological study of hepatitis A virus infection amongst the students of medical college in Delhi and evaluation of the need of vaccination. *Indian J Med Res* 2002; *115* : 1-4.

15. Joshi YK, Tandon BN, Gandhi BM. Hepatitis A epidemic in Kerala (India) in 1980. *Indian J Med Res* 1985; *81* : 96-101.

16. Arankalle VA, Sarada Devi KL, Lole KS, Shenoy KT, Verma V, Haneephabi M. Molecular characterization of hepatitis A virus from a large outbreak from Kerala. *Indian J Med Res* 2006; *123* : 760-9.

17. Patnaik SK. An outbreak of viral hepatitis A in Ibrahimpatanam town of Andhra Pradesh. *J Commun Dis* 1995; *27* : 118-9.

18. Chitambar SD, Chadha MS, Yeolekar LR, Arankalle VA. Hepatitis A in Day Care Centre. *Indian J Pediatr* 1996; *63* : 781-3.

19. Tsarev SA, Tsareva TS, Emerson SU, Kapikian AZ, Ticehurst J, London W, *et al*. ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: Identification of HEV infection in primates. *J Infect Dis* 1993; *168* : 369-78.

20. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; *25* : 4876-82.

21. Tamura K, Dudley J, Nei M, Kumar S. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007; *24* : 1596-9.

22. Chadha MS, Lole KS, Bora MH, Arankalle VA. Outbreaks of hepatitis A among children in western India. *Trans R Soc Trop Med Hyg* 2009; *103* : 911-6.

23. De Serres G, Cromeans TL, Levesque B, Brassard N, Barthe C, Dionne M, *et al*. Molecular confirmation of hepatitis A virus from well water; epidemiology and public health implications. *J Infect Dis* 1999; *179* : 37-43.

24. Pina S, Buti M, Jardi R, Clemente-Casares P, Jofre J, Girones R. Genetic analysis of hepatitis A virus strains recovered from the environment and from patients with acute hepatitis. *J Gen Virol* 2001; *82* : 2953-63.

25. Khanna B, Spelbring JE, Innis BL, Robertson BH. Characterization of a genetic variant of human hepatitis A virus. *J Med Virol* 1992; *36* : 118-24.

26. Hussain Z, Das BC, Husain SA, Asim M, Chattopadhyay S, Malik A, *et al*. Hepatitis A viral genotypes and clinical relevance: Clinical and molecular characterization of hepatitis A virus isolates from northern India. *Hepatol Res* 2005; *32* : 16-24.

Reprint requests: Dr Vidya A. Arankalle, National Institute of Virology (ICMR), Microbial Containment Complex
Sus Road, Pashan, Pune 411 021, India
e-mail: varankalle@yahoo.com