



## RESEARCH NOTE

# Analysis of the complete genome of hepatitis B virus subgenotype C2 isolate NHB17965 from a patient with uncomplicated chronicity [version 1; referees: 1 approved, 1 not approved]

Modhusudon Shaha <sup>1</sup>, Palash Kumar Sarker<sup>1</sup>, Md. Saddam Hossain<sup>1</sup>, Keshob Chandra Das<sup>2</sup>, Munira Jahan <sup>3</sup>, Shuvra Kanti Dey<sup>4</sup>, Shahina Tabassum<sup>3</sup>, Abu Hashem <sup>1</sup>, Md. Salimullah<sup>2</sup>

<sup>1</sup>Microbial Biotechnology Division, National Institute of Biotechnology, Dhaka, 1349, Bangladesh

<sup>2</sup>Molecular Biotechnology Division, National Institute of Biotechnology, Dhaka, 1349, Bangladesh

<sup>3</sup>Department of Virology, Bangabandhu Sheikh Mujib Medical University, Dhaka, 1000, Bangladesh

<sup>4</sup>Department of Microbiology, Jahangirnagar University, Dhaka, 1342, Bangladesh

**v1** First published: 09 Jul 2018, 7:1023 (doi: [10.12688/f1000research.15090.1](https://doi.org/10.12688/f1000research.15090.1))  
 Second version: 13 Aug 2018, 7:1023 (doi: [10.12688/f1000research.15090.2](https://doi.org/10.12688/f1000research.15090.2))  
 Latest published: 10 Sep 2018, 7:1023 (doi: [10.12688/f1000research.15090.3](https://doi.org/10.12688/f1000research.15090.3))

## Abstract

The number of chronic cases of hepatitis B virus (HBV) is increasing rapidly in the world. Herein, we report a complete genome of HBV subgenotype C2 (HBV/C2) with current common amino acid substitutions from a patient with chronic HBV without liver complications. Complete genome analysis revealed that the isolated strain was a non-recombinant wild type and had several regular substitutions in the reverse transcriptase domain and small surface proteins of HBV. The isolated complete sequence could be considered as a chronic reference strain of HBV/C2 in Bangladesh. This study may help clinicians and scientists gain in-depth knowledge on common substitutions of HBV/C2 genome and to identify potential therapies against chronic HBV infections.

## Keywords

HBV/C2, Chronic, Non-recombinant, Bangladesh

## Open Peer Review

Referee Status:

	Invited Referees		
	1	2	3
<b>REVISED</b>			
<b>version 3</b> published 10 Sep 2018			
<b>REVISED</b>			
<b>version 2</b> published 13 Aug 2018		report	report
<b>version 1</b> published 09 Jul 2018			
	report		report

- 1 **Mohammad Ariful Islam** , Jagannath University, Bangladesh
- 2 **Paul Klapper** , University of Manchester, UK
- 3 **Ashesh Kumar Chowdhury** , BIRDEM General Hospital, Bangladesh

## Discuss this article

Comments (0)

**Corresponding author:** Md. Salimullah ([salim2969@gmail.com](mailto:salim2969@gmail.com))

**Author roles:** **Shaha M:** Methodology, Writing – Original Draft Preparation; **Sarker PK:** Investigation, Validation, Writing – Review & Editing; **Hossain MS:** Data Curation, Methodology; **Das KC:** Conceptualization, Writing – Review & Editing; **Jahan M:** Data Curation, Methodology, Writing – Review & Editing; **Dey SK:** Investigation, Validation, Writing – Review & Editing; **Tabassum S:** Data Curation, Writing – Review & Editing; **Hashem A:** Supervision, Writing – Review & Editing; **Salimullah M:** Conceptualization, Supervision, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** The study was supported by the National Institute of Biotechnology, Ministry of Science and Technology, Bangladesh.

**Copyright:** © 2018 Shaha M *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Shaha M, Sarker PK, Hossain MS *et al.* **Analysis of the complete genome of hepatitis B virus subgenotype C2 isolate NHB17965 from a patient with uncomplicated chronicity [version 1; referees: 1 approved, 1 not approved]** *F1000Research* 2018, 7:1023 (doi: [10.12688/f1000research.15090.1](https://doi.org/10.12688/f1000research.15090.1))

**First published:** 09 Jul 2018, 7:1023 (doi: [10.12688/f1000research.15090.1](https://doi.org/10.12688/f1000research.15090.1))

## Introduction

The number of cases of chronic liver disease caused by hepatitis B virus (HBV) are increasing markedly<sup>1</sup>. Globally, more than 2 billion people have been infected by HBV<sup>2</sup> and, according to the World Health Organization (WHO) **approximately 257 million were living with HBV in 2017**. In Bangladesh, the rate of HBV chronicity is 2–6%<sup>3</sup>, which makes it relatively higher risk than other infectious diseases.

HBV genome comprises a partially double-stranded covalently closed circular DNA that encodes four highly overlapping major open reading frames<sup>4</sup>. Due to the absence of proof-reading activity, the mutation rate of HBV is high<sup>5</sup>; hence, recombinant strains are evolving with a common pattern. Most of the HBV cases are chronic, which has a high possibility of causing liver cirrhosis<sup>6</sup> and hepatocellular carcinoma<sup>7</sup>. In Bangladesh, there are no reported reference complete sequence of HBV chronic strain of subgenotype C2. Hence, we isolated the complete genome of a HBV/C2 strain collected from a patient without liver complications, though carrying the virus for a long time.

## Methods

### Isolation and sequencing

An HBV-positive plasma sample was collected from a 45-year-old male patient in a tertiary hospital in Dhaka, Bangladesh after obtaining the patient's written informed consent. The infected patient had chronic liver disease, as determined by ultrasonography. The patient was diagnosed with chronic HBV infection recently, with a high viral load in the plasma. However, the patient was not showing signs of jaundice, though was affected by fever, nausea, vomiting and fatigue. The study was approved by the Research Ethics Committee of National Institute of Biotechnology, Bangladesh (NIBREC2015-01). The patient was not taking any antiviral therapy and was diagnosed 1 month prior to obtainment of the plasma sample. HBV DNA was extracted from the sample using the QIAamp MinElute Virus Spin kit (Qiagen, Germany). The complete HBV genome was amplified by six sets of primer pairs used previously in another study<sup>8</sup> using a conventional PCR method. The primer sequences and their annealing temperatures were as follows: set 1, forward-AAGCTCTGCTAGATCCCAGAGT, reverse- AGTTGGCGA-GAAAGTGAAAGCCTG, 56°C; set 2, forward- CCTATTGATT-GGAAAGTATGTCA, reverse- AACAGACCAATTTATGCCTA, 48°C; set 3, forward- GAGACCACCGTGAACGCCCA, reverse-CCTGAGTGCTGTATGGTGAGG, 56°C; set 4, forward- TTCAC-CTCTGCCTAATCATC, reverse- ATAGGGGCAATTTGGTGGTCT, 52°C; set 5, forward- TCAGGCAACTATTGTGGTTTCA, reverse- GGGTTGAAGTCCCAATCTGGATT, 51°C; set 6, forward-GGGTCACCATATTCTTGGA, reverse- CGAGTCTA-GACTCTGTGGTA, 51°C. For a mixture of 25 µl reaction volume, 12.5 µl of 2X MasterMix (Thermo Fisher Scientific, USA), 1 µl each of forward and reverse primers (IDT, USA), 9.5 µl of

nuclease-free water (Thermo Fisher Scientific, USA) and 2 µl of template DNA were used. The condition of the PCR reaction was 1 cycle at 95°C for 10 min, 35 cycles at 95°C for 1 min, with the aforementioned annealing temperatures for 1 min and 72°C for 1 min, and a final cycle for 10 min at 72°C. Sanger sequencing was performed using the BigDye Terminator version 3.1 cycling sequencing kit (Applied Biosystems, USA) by ABI 3130 Genetic Analyser (SeqGen, CA, USA) and by thermal cycler (Sigma-Aldrich, Germany) using the described annealing temperatures as per manufacturer's instructions after the purification of PCR products using PureLink PCR Purification Kit (Thermo Fisher Scientific, USA), performed in accordance with the manufacturer's protocol. Next, the sequenced contigs were assembled using the Seqman tool of DNASTAR Lasergene version 7.2<sup>9</sup>.

## Analysis

The subgenotyping and mutation analysis of the sequenced genome were performed using the **HBV Geno2Pheno** tool version 2 using the default parameters, comparing against the HBV genotype D consensus sequence. Recombination analysis of the sequence was performed using the **NCBI genotyping tool**. The complete genome was deposited in the GenBank under the accession number **MH220971**.

## Results and discussion

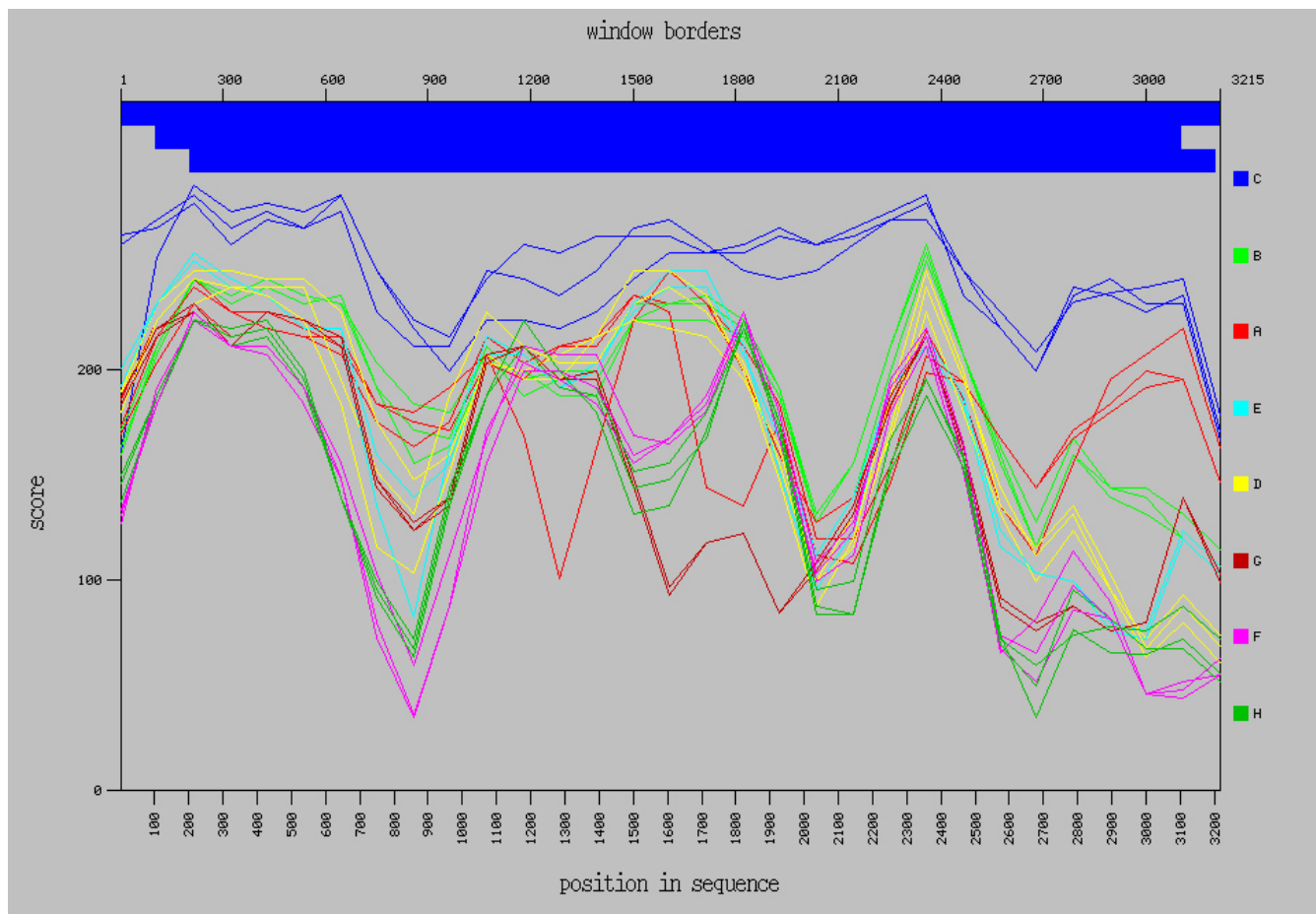
Analysis of the complete genome denotes that the isolate studied here, termed NHB17965, comprises HBV genotype C and subgenotype C2 (HBV/C2) with a GC content of 48.77%. Recombination analysis using the NCBI Genotyping tool showed that NHB17965 is a non-recombinant wild-type HBV isolate (**Figure 1**).

Isolate NHB17965 was observed to have amino acid substitutions H9Y, N13H, I91L, P109S, T128N, I269L and V278I in the polymerase domain and S53L, P120T, I126T and S210N in the small hepatitis B surface protein as analysed by **HBV Geno2Pheno** tool, compared against the HBV genotype D consensus sequence. These substitutions may be the results of regular genomic changes to HBV because of a lack of proof-reading activity of the viral reverse transcriptase, and may not signify any danger. Hence, the isolate NHB17965 could be considered as a reference strain of chronic HBV/C2 infection in Bangladesh.

The findings of this study will help clinicians and scientists to gain substantial knowledge about the current common genomic substitutions of HBV/C2 and to develop treatments against chronic HBV infections.

## Data availability

Genome of the HBV strain isolated in this study, **MH220971**.



**Figure 1. Recombination analysis of the isolate NHB17965.** The Simplot diagram was generated using the NCBI Genotyping tool.

### Competing interests

No competing interests were disclosed.

### Grant information

The study was supported by the National Institute of Biotechnology, Ministry of Science and Technology, Bangladesh.

### References

- MacLachlan JH, Cowie BC: **Hepatitis B virus epidemiology.** *Cold Spring Harb Perspect Med.* 2015; 5(5): a021410.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shaha M, Hoque SA, Rahman SR: **Molecular epidemiology of hepatitis B virus isolated from Bangladesh.** *SpringerPlus.* 2016; 5(1): 1513.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Munshi SU, Tran TTT, Vo TNT, *et al.*: **Molecular characterization of hepatitis B virus in Bangladesh reveals a highly recombinant population.** *PLoS One.* 2017; 12(12): e0188944.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shaha M, Das KC, Hossain MS, *et al.*: **Complete Genome Sequence of a Circulating Hepatitis B Virus Genotype C Strain Isolated from a Chronically Infected Patient Identified at an Outdoor Hospital in Bangladesh.** *Genome Announc.* 2018; 6(9): pii: e01601-17.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Caligiuri P, Cerruti R, Icardi G, *et al.*: **Overview of hepatitis B virus mutations and their implications in the management of infection.** *World J Gastroenterol.* 2016; 22(1): 145–154.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lin J, Wu JF, Zhang Q, *et al.*: **Virus-related liver cirrhosis: molecular basis and therapeutic options.** *World J Gastroenterol.* 2014; 20(21): 6457–6469.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Di Bisceglie AM: **Hepatitis B and hepatocellular carcinoma.** *Hepatology.* 2009; 49(5 Suppl): S56–S60.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sugauchi F, Mizokami M, Orito E, *et al.*: **A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness.** *J Gen Virol.* 2001; 82(Pt 4): 883–892.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Burland TG: **DNASTAR's Lasergene sequence analysis software.** *Methods Mol Biol.* 2000; 132: 71–91.  
[PubMed Abstract](#) | [Publisher Full Text](#)

# Open Peer Review

Current Referee Status:



Version 1

Referee Report 07 August 2018

doi:10.5256/f1000research.16434.r36692



**Paul Klapper** 

University of Manchester, Manchester, UK

This paper reports a sequence analysis of a strain of hepatitis B virus. However, there are some aspects of the paper that merit attention. In the Abstract and again in the Introduction the authors state: *The number of chronic cases of hepatitis B virus (HBV) is increasing rapidly in the world*". I found this an interesting statement and wondered what was the evidence base for this. In the Introduction the authors cite MacLachlan and Cowie (2015). It appears to me that the authors are mis-quoting this reference. The reference actually says "*The burden of chronic HBV infection is increasingly being recognized*", this is substantially different from suggesting that the number of cases is increasing. There is a general lack of comprehensive epidemiological information on chronic hepatitis B infection as many developing countries (the epicentre of chronic HBV infections) lack surveillance to provide data. We do not know how WHO programmes to prevent vertical transmission of HBV are impacting on chronic hepatitis B and I believe we lack information to support the assertion of a global increase in numbers.

Also in the abstract - the third line "*with current common amino acid substitutions*" is not a meaningful statement. The sentence needs rewriting to make clear what the authors actually mean.

Introduction 1st paragraph

last 2 lines: "*which makes it relatively higher risk than other infectious diseases*"; it is unclear what is meant here, a rate of 2-6% is clearly low compared with risk of, for example, influenza or rotavirus infection.

Introduction, 2nd Paragraph "*the mutation rate of hepatitis B is high*" and "*hence, recombinant strains are evolving with a common pattern*". Mutation is a random event how can a random event lead to a commonly evolving pattern. The authors need to recast the sentence to explain what they really mean.

Introduction, end of 2nd paragraph. the genome was isolated from "*a patient without liver complication*" yet in methods we are told the patient has chronic liver disease as adjudged by ultrasonography.

Methods: line 4. Was formal staging not used to describe liver disease e.g. relating shear wave elastography to fibrosis score? Were liver function tests performed?

Methods, line 5 "*The patient was diagnosed with chronic hepatitis B recently*". This seems strange, the standard definition of chronic hepatitis B infection is the detection of hepatitis B surface antigen in serum for more than 6 months. This patient - with blood taken only one month post diagnosis would not seem to meet the definition. No hepatitis B markers results are given for the patient and so understanding of the

phase of chronic illness (see EASL guidance; Journal of Hepatology 2017 vol. 67: 370–398) is not possible

Analysis - reference needed for the HBV Geno2Pheno software used.

What evidence is there that the HBV/C2 isolate sequenced is a common phenotype in chronic hepatitis B virus infection in Bangladesh? Without such evidence it is difficult to see how the conclusions "*The findings of this study will help....*" are justified. This could simply be an single instance of this virus produced through random mutation. It is therefore also difficult to understand how this could be considered a 'reference strain' for chronic hepatitis B as it may represent a single instance and further as the patient does not appear to meet a case definition of having chronic hepatitis B infection, can it be considered as a reference strain for chronic hepatitis B infection.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Author Response 08 Aug 2018

**Modhusudon Shaha**, National Institute of Biotechnology, Bangladesh

We would like to thank the reviewer for his constructive comments on the manuscript. Herein, the responses to the comments are given.

*'The number of chronic cases of hepatitis B virus (HBV) is increasing rapidly in the world'*- The sentence is corrected in the revised manuscript.

*"with current common amino acid substitutions"*- this portion of the sentence is removed from the revised manuscript.

*"which makes it relatively higher risk than other infectious diseases"*- the sentence is re-written with substantial clarity.

*"the mutation rate of hepatitis B is high" and "hence, recombinant strains are evolving with a*

*common pattern*"- the sentence is re-written in the revised manuscript.

*"a patient without liver complication"*- the sentence is corrected in the revised manuscript.

Particular liver function tests were not performed. However, the liver was observed normal using ultrasonography.

*"The patient was diagnosed with chronic hepatitis B recently"*- The sentence is edited in the revised manuscript. The detail of diagnosis of the chronicity is described in the revised manuscript. Reference is given that study used the HBV Geno2Pheno tool.

*"The findings of this study will help...."*- the sentence is re-written in the revised manuscript.

**Competing Interests:** No competing interests were disclosed.

Referee Report 23 July 2018

doi:10.5256/f1000research.16434.r35849



**Mohammad Ariful Islam** 

Jagannath University, Dhaka, Bangladesh

The overall level of the paper is good: even if it is quite simple, it is well written and some important considerations are highlighted.

The manuscript talks about the complete genome analysis of hepatitis B virus and represent the complete genome sequence of HBV subgenotype C2 in Bangladesh.

The study is a short research note. Although the findings of the study is limited and like a genome announcement, it signifies to be documented. The study is scientifically acceptable.

I have some minor queries

- what does the isolate NHB17965 indicates?
- any reference sequences used in this study? If used, what are these?"

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.



**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 01 Aug 2018

**Modhusudon Shaha**, National Institute of Biotechnology, Bangladesh

We would like to thank the reviewer for his constructive comments on the manuscript.

NHB17965 indicates the sample identification number given by the laboratory.

There are no reference sequences used in this study for the analysis. The isolated sequence was analyzed using NCBI Genotyping tool and Geno2Pheno tools as given in the manuscript.

**Competing Interests:** No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact [research@f1000.com](mailto:research@f1000.com)

**F1000Research**