Prevalence, Risk Behaviors, and Virological Characteristics of Hepatitis B Virus Infection in a Group of Men Who Have Sex with Men in Brazil: Results from a Respondent-Driven Sampling Survey

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Abstract

Background

Men who have sex with men (MSM) are at increased risk of exposure to hepatitis B virus (HBV) compared with the general population. This study aims to assess the epidemiological and virological characteristics of HBV infection in a sample of MSM in Brazil, where data are scarce.

Methods

A cross-sectional study was conducted among MSM in the City of Goiânia, Central Brazil, from March to November 2014, using Respondent-Driven Sampling (RDS). After signing the consent form, participants were interviewed and a blood sample collected. All samples were tested for HBV serological markers and HBV DNA. HBV nucleotide sequence analysis was also performed.

Results

A total of 522 MSM were recruited in the study. The prevalence of HBV infection (current or past [presence of anti-HBc marker]) was 15.4% (95% CI: 8.7–25.8) and the rate of HBsAg carriers was 0.6% (95% CI: 0.2–1.6). About 40% (95% CI: 32.3–48.8) of the participants had serological evidence of previous HBV vaccination (reactive for isolated anti-HBs). In addition, 44.3% (95% CI: 36.1–52.9) were seronegative for all HBV markers. Age over 25 years old, receptive anal intercourse, previous sex with women, and history of sexually transmitted infections (STIs) were factors associated with HBV infection. HBV DNA was
detected only in HBsAg-positive individuals. HBV isolates were classified into genotype A (subgenotypes A1 and A2), and some mutations were identified throughout the genome. Therefore, occult HBV infection was not observed in the study population.

Conclusions
Public health strategies should be improved for the MSM population in order to prevent HBV and other STIs, as well as to provide appropriate management of patients with active infections.

Introduction
Hepatitis B virus (HBV) infection has a wide global distribution. The World Health Organization (WHO) estimates that there are over 240 million chronically infected people worldwide. The chronic form of this infection is associated with a variety of clinical manifestations, ranging from an asymptomatic carrier state to severe liver disease, including cirrhosis and hepatocellular carcinoma (HCC) [1].

The traditional serological marker of hepatitis B is the HBV surface antigen (HBsAg), which is detectable in serum during acute and chronic infection. However, with the development of highly sensitive molecular biology techniques, HBV DNA has been detected in serum and/or liver without detectable HBsAg. This profile is called occult HBV infection (OBI) and has been associated with hepatitis B reactivation, increased severity of liver disease, and HCC development. In addition, an increased risk of HBV transmission through blood transfusion, organ transplantation, and hemodialysis due to OBI has been reported [2,3].

HBV isolates are classified into ten genotypes, designated A–J, based on sequence divergence greater than 7.5% in the complete genome. Most genotypes further segregate into subgenotypes that differ from each other by 4–7.5%. HBV genotypes/subgenotypes have different geographical distributions, and influence clinical outcomes and response to treatment [4,5]. In Brazil, genotype A is the most prevalent, followed by genotypes D and F, with a higher proportion of subgenotype A1 [6,7].

The genetic variability of HBV can lead to the occurrence of different diagnosis and clinical profiles. Mutations in the Pre-S/S gene region have been linked to low HBsAg levels and HBV vaccine/immunoglobulin escape, whereas those found in the Pre-C/C and BCP (basal core promoter) gene regions can lead to decreased hepatitis B e antigen (HBeAg) expression and to progression to severe liver disease [8,9].

HBV infection occurs in a considerable number of men who have sex with men (MSM). According to the US CDC [10], about 20% of new cases of HBV infection in adults in the United States of America occur among gay and bisexual men. In addition, a high rate of chronic hepatitis B was reported in MSM [11]. Some studies have shown that MSM are at high risk of sexually transmitted infections (STIs), including hepatitis B [12–18]. This greater vulnerability involves the context of violence, conditions of sexual practices such as unprotected anal intercourse and multiple sexual partners in addition to low access to health services and social integration, which can lead to unsafe sexual practices [13,17].

Worldwide, HBV infection prevalence in MSM varies according to geographical region as well as with the characteristics of the selected subgroup studied. Thus, the prevalence of this infection in MSM has ranged from 0.9% in Lebanon [19] to 44% in the Netherlands [11]. In addition, higher HBV rates have been described in specific subgroups of MSM, such as gays
and bisexuals homeless young adults in USA (52.4%) [20], HIV-positive MSM in Taiwan (52.9%) [16] and male transvestite commercial sex workers in Uruguay (50.5%) [21].

In Brazil, previous [22,23] and current [15] studies concerning HBV prevalence among MSM are still rare, and no data regarding the molecular epidemiology of HBV in MSM has been published thus far. Therefore, this study was conducted in order to investigate the HBV prevalence, associated factors, occult infection rate and molecular characterization of viral isolates in MSM in Central Brazil.

**Material and Methods**

**Study population and sampling method**

A cross-sectional study among MSM was conducted in Goiânia (1,256,514 inhabitants), the capital of the State of Goiás in Central Brazil. From March to November 2014, a total of 522 MSM were recruited using Respondent-Driven Sampling (RDS), a chain-referral sampling method in which initial participants, called seeds, recruit a limited number of additional participants. These participants recruit more participants and this process goes on until the desired sample size is reached. This recruitment strategy is an efficient data collection method and it is useful to study hidden populations such as MSM [24,25]. Initially, formative research was conducted to determine study logistics and to select the seeds. Based on their extensive social network, three seeds were first selected by collaborating with governmental and non-governmental organizations supporting LGBT (Lesbian, Gay, Bisexual and Transgender) individuals and during the gay pride parade. Additionally, two more seeds were included during the study to enroll additional participants. Each seed received three numbered referral coupons to invite other eligible MSM to the study (first wave). Eligibility criteria for participation were being born male sex, aged 18 years or older, leaving in the Goiânia metropolitan area, reporting have had sex with another man in the 12 months preceding the study, do not identify as transsexual, and presenting a valid recruitment coupon.

Considering that MSM is a hard-to-reach population, the sample size calculation took into consideration the expected design effect of 2.0 [26]. The minimum sample size of 418 participants was calculated using an estimated prevalence for exposure to HBV of 11.4% [15], with confidence interval of 95% and a precision of 4.4%. Due to the return of some coupons previously issued, the sample size reached 522.

After being informed of the objectives and methodology of the study, individuals signed the consent form and answered an interviewer-administered structured questionnaire to collect information about their personal network size, relationship with the recruiter, sociodemographic characteristics and possible factors associated with HBV infection. Then, a blood sample was collected from each participant for laboratory testing. All participants received three referral coupons to recruit new MSM of their social relationships (friends and/or sexual partners), in addition to educational materials and condoms. As an incentive, each participant received four public transportation tickets and two additional transportation tickets for each participant they recruited into the study.

The procedures of this study were approved by the Ethics Committee of the Federal University of Goiás (UFG), Goiânia, Goiás, Brazil. All participants signed informed written consent. This consent form was approved by the ethics committee.

**Serological tests**

Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for detection of HBsAg (Hepanostika HBsAg Ultra, Biomérieux, Boxtel, The Netherlands), antibodies against hepatitis B core antigen (anti-HBc) (Hepanostika anti-HBc Uni-form, Biomérieux), and
antibodies against HBsAg (anti-HBs) (Bioelisa anti-HBs, Biokit, Barcelona, Spain). HBsAg reactive samples were subsequently tested for the presence of anti-HBc IgM (Bioelisa anti-HBc IgM, Biokit), HBeAg and antibodies against HBeAg (anti-HBe) (Eti-Ab/Ebk Plus, Diasorin, Italy).

**Molecular tests**

DNA was extracted from all samples using phenol-chloroform method [27]. The Pre-S/S region was amplified by semi-nested polymerase chain reaction (PCR) [28]. HBV DNA-positive samples were submitted to amplification of the complete genome [29]. When the full-length genome amplification was not successfully achieved, the S, BCP, and Pre-C/C gene regions were further amplified, as previously described [28]. Nucleotide sequences of the amplified regions were determined by direct sequencing using a BigDye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), on an ABI 3130 automated sequencer (Applied Biosystems). Sequences were aligned and edited using SeqMan II v.5.01 (DNASTAR), Clustal W and BioEdit. HBV genotypes and subgenotypes were determined by phylogenetic analysis using MEGA program v.6.0 (Molecular Evolutionary Genetics Analysis) and published reference sequences available in GenBank database (http://www.ncbi.nlm.nih.gov/). To identify mutations in the HBV genome, the deduction of amino acids (aa) was performed from nucleotide sequences by using MEGA program v.6.0. The nucleotide sequences obtained in this study were deposited in GenBank under the accession numbers KU900750 to KU900754.

**Data analysis**

Prevalence and 95% confidence intervals (CI) were estimated using RDS Analysis Tool (RDSAT) v.5.6 (http://rds-analysis-tool.software.informer.com/versions/). To reduce possible biases associated with chain referral sampling, RDSAT provides weights for each participant based on his social network size and recruitment patterns [24]. Data and weights generated through RDSAT were exported to SPSS v.20 (SPSS Inc., IBM, Chicago, US) for weighted analysis of variables associated with HBV infection [current or past (defined by the presence of anti-HBc serological marker)] using Pearson’s chi-square test and Fisher’s exact test. Variables associated with this infection at $p < 0.10$ in the univariate analysis were included in the multivariable analysis using a logistic regression model. Finally, a $p$-value less than 0.05 was considered statistically significant. The design of social networks to visualize the distribution of the HBV exposure in the recruitment network was performed using NetDraw software (http://www.analytictech.com/downloadnd.htm).

**Results**

**Sampling**

Of a total of 1,227 coupons issued, 530 (43.2%) were redeemed. Among the 530 MSM who presented a valid recruitment coupon, eight were not selected (two refused to give a blood sample and six were under 18 years old). Therefore, 522 MSM were included in the analysis (5 seeds and 517 recruits). The median number of waves by seed was 9 (range 3–15), and the median number of recruits by seed was 103 (range 15–169).

**Characteristics of participants**

The sociodemographic characteristics of the study population are shown in Table 1. Participants were mostly young ($\leq$ 25 years old, 60%). The majority self-identified as gay (74.9%), followed by bisexual (19.4%) and transvestite (5.7%). Skin color was assessed by self-report (59% claimed brown or mixed (pardo), 18.9% white, 16.6% black and 5.6% others). Most of MSM
were single (76.9%), previously attended high school (10–12 years of study, 63.9%) and were in the lowest tier of Brazilian social class (61.3%).

### Prevalence of HBV serological markers

Table 2 shows the prevalence of HBV serological markers. Among the 522 MSM studied, 77 (15.4%; 95% CI: 8.7 to 25.8) had been exposed to HBV. Of these, 5 (0.6%) were anti-HBc/HBsAg carriers, 60 (9.3%) were reactive for anti-HBc/anti-HBs and 12 (5.5%) were positive for anti-HBc only. Moreover, 206 (40.3%) were reactive for isolated anti-HBs. In addition, 239 (44.3%) were seronegative for all hepatitis B serological markers. The representation of the recruitment network indicating cases of exposure to HBV is presented in Fig 1.

### Factors associated with HBV infection

Univariate analysis of demographic, behavioral characteristics, and HIV serological status among unvaccinated MSM (Table 3) revealed that age over 25 years, more than 10 sex partners

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**Table 1. Sociodemographic characteristics of 522 MSM in Goiânia, Central Brazil.**

| Characteristics | N     | Unweighted % | Weighted % | 95% CI  |
|-----------------|-------|--------------|------------|---------|
| Age (median: 23.6; range: 18–62) |       |              |            |         |
| ≤ 25 years      | 338   | 64.75        | 60.0       | 50.8–68.5 |
| > 25 years      | 184   | 35.25        | 40.0       | 31.5–49.2 |
| Self-identification |       |              |            |         |
| Gay             | 355   | 68.0         | 74.9       | 67.2–81.4 |
| Bisexual        | 76    | 14.6         | 19.4       | 13.4–27.2 |
| Transvestite    | 91    | 17.4         | 5.7        | 3.8–8.3   |
| Skin color/race |       |              |            |         |
| Brown (pardo)   | 296   | 56.7         | 59.0       | 50.6–67.4 |
| White           | 103   | 19.7         | 18.9       | 13.5–25.7 |
| Black           | 91    | 17.4         | 16.6       | 9.9–26.4  |
| Yellow (Asian)  | 21    | 4.0          | 4.1        | 1.7–9.2   |
| Indigenous      | 11    | 2.1          | 1.5        | 0.7–3.4   |
| Marital status  |       |              |            |         |
| Single          | 420   | 80.5         | 76.9       | 66.9–84.5 |
| Married/in stable relationship | 94  | 18.0         | 20.1       | 12.9–9.9  |
| Separated/divorced | 8   | 1.5          | 3.1        | 0.9–9.4   |
| Education (years) (n = 520)a |       |              |            |         |
| ≤ 9             | 77    | 14.8         | 13.7       | 9.0–20.3  |
| 10–12           | 307   | 59.0         | 63.9       | 55.9–71.3 |
| ≥ 13            | 136   | 26.2         | 22.4       | 17.0–28.9 |
| Social class (Brazilian criteria)b |       |              |            |         |
| A/B (≥ R$ 8,641) | 7  | 1.4          | 0.4        | 0.1–1.7   |
| C (R$ 2,005–8,640) | 81 | 15.5         | 9.9        | 6.6–14.5  |
| D (R$ 1,255–2,004) | 140 | 26.8         | 28.4       | 20.3–38.3 |
| E (≤ R$ 1,254)  | 294   | 56.3         | 61.3       | 52.1–69.7 |

MSM, men who have sex with men; CI, confidence interval; R$, Brazilian real.

aMissing data are not shown.

bBrazilian criteria classifies individuals into five economic classes [30]: “A” constitutes the highest tier of social class, while “E” the lowest one (US$ 1.00 is approximately equal to R$ 2.32).

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in lifetime, history of drugs or alcohol use during sex, receptive anal intercourse, previous sex with women and history of STIs were statistically associated with HBV infection (p<0.05). These variables in addition to ever having received payment for sex (p = 0.055) and HIV seropositivity (p = 0.079) were included in a multivariate analysis using a logistic regression model. Age over 25 years old (p<0.001), receptive anal intercourse (p = 0.001), previous sex with women (p = 0.038), and history of STIs (p = 0.001) were independent factors associated with HBV infection in the study population (Table 4).

### Table 2. Prevalence of HBV serological markers among 522 MSM in Goiânia, Central Brazil.

| HBV markers                  | Total | Unweighted % | Weighted % (95% CI) |
|------------------------------|-------|--------------|---------------------|
| Anti-HBc/ HBsAg              | 5     | 1.0          | 0.6 (0.2–1.6)       |
| Anti-HBc/ anti-HBs           | 60    | 11.5         | 9.3 (5.9–14.5)      |
| Anti-HBc only                | 12    | 2.3          | 5.5 (1.2–21.8)      |
| Any exposure marker          | 77    | 14.8         | 15.4 (8.7–25.8)     |
| Anti-HBs (immunized)         | 206   | 39.5         | 40.3 (32.3–48.8)    |
| Absence of any marker (susceptible) | 239   | 45.8         | 44.3 (36.1–52.9)    |

HBV, hepatitis B virus; MSM, men who have sex with men; CI, confidence interval; HBsAg, hepatitis B surface antigen; anti-HBc, antibodies against hepatitis B core antigen; anti-HBs, antibodies against HBsAg.

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### Fig 1. Recruitment networks of 522 men who have sex with men in Goiânia, Central Brazil.

The seeds are indicated by large squares and recruits by small squares. Individuals who had been exposed to HBV are in black and others in gray.

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Table 3. Demographic, behavioral characteristics and HIV serological status associated with HBV infection among unvaccinated MSM in Goiânia, Central Brazil.

| Variable                          | HBV Pos./total | Unweighted % | Weighted % (95% CI) | OR (95% CI) | P value |
|-----------------------------------|----------------|--------------|---------------------|-------------|---------|
| **Age (years)**                   |                |              |                     |             |         |
| ≤ 25                              | 22/183         | 12.0         | 5.2 (2.4–10.8)      | 1           |         |
| > 25                              | 55/133         | 41.3         | 46.3 (28.1–65.5)    | 15.73 (5.10–48.39) | 0.000   |
| **Self-identification**           |                |              |                     |             |         |
| Gay                               | 49/207         | 23.7         | 28.0 (14.8–46.6)    | 1.96 (0.48–8.02) |         |
| Bisexual                          | 17/61          | 27.9         | 27.7 (12.7–50.4)    | 1.94 (0.43–8.78) | 0.493   |
| Transvestite                      | 11/48          | 22.9         | 16.5 (5.9–38.4)     | 1           |         |
| **Number of sexual partners in lifetime** |        |              |                     |             |         |
| ≤ 10                              | 16/93          | 17.2         | 11.9 (5.9–22.6)     | 1           |         |
| > 10                              | 61/223         | 27.3         | 35.1 (19.3–55.0)    | 3.99 (1.30–12.23) | 0.012   |
| **Ever used drugs or alcohol during sex** |        |              |                     |             |         |
| No                                | 20/102         | 19.6         | 13.6 (7.0–24.8)     | 1           |         |
| Yes                               | 57/214         | 26.6         | 33.4 (18.0–53.4)    | 3.19 (1.10–9.65) | 0.034   |
| **Ever received payment for sex** |                |              |                     |             |         |
| No                                | 45/173         | 26.0         | 17.6 (11.5–26.1)    | 1           |         |
| Yes                               | 32/143         | 22.4         | 41.5 (18.3–69.3)    | 3.32 (0.94–11.69) | 0.055   |
| **Receptive anal intercourse**    |                |              |                     |             |         |
| No                                | 11/57          | 19.3         | 7.0 (2.6–17.5)      | 1           |         |
| Yes                               | 66/259         | 25.5         | 32.3 (18.9–49.5)    | 6.35 (1.80–22.53) | 0.002   |
| **Condom use at anal intercourse**|                |              |                     |             |         |
| Always                            | 30/157         | 19.1         | 22.5 (9.0–46.1)     | 1           |         |
| Not Always                        | 47/159         | 29.5         | 31.3 (18.9–47.2)    | 1.57 (0.44–5.62) | 0.484   |
| **Sex with women**                |                |              |                     |             |         |
| No                                | 32/169         | 18.9         | 15.2 (9.4–23.7)     | 1           |         |
| Yes                               | 45/147         | 30.6         | 34.3 (17.3–56.6)    | 2.91 (1.00–8.48) | 0.045   |
| **Group sex**                     |                |              |                     |             |         |
| No                                | 28/142         | 19.7         | 26.3 (11.2–50.1)    | 1           |         |
| Yes                               | 49/172         | 28.5         | 26.5 (15.4–41.5)    | 1.01 (0.29–3.49) | 0.987   |
| **Sex against will**              |                |              |                     |             |         |
| No                                | 57/216         | 26.4         | 24.2 (12.2–42.4)    | 1           |         |
| Yes                               | 19/98          | 19.4         | 32.1 (15.4–55.0)    | 1.48 (0.42–5.24) | 0.543   |
| **STIs**                          |                |              |                     |             |         |
| No                                | 44/232         | 18.9         | 12.0 (7.4–18.8)     | 1           |         |
| Yes                               | 33/82          | 40.2         | 57.7 (31.9–79.9)    | 10.02 (3.04–33.08) | <0.001  |
| **HIV status**                    |                |              |                     |             |         |
| Negative                          | 54/246         | 21.9         | 20.6 (9.2–39.9)     | 1           |         |
| Positive                          | 23/70          | 32.8         | 45.2 (24.4–67.8)    | 3.17 (0.84–11.93) | 0.079   |
| **Ever used any illicit drug**    |                |              |                     |             |         |
| No                                | 35/155         | 22.6         | 23.6 (14.2–36.6)    | 1           |         |
| Yes                               | 42/160         | 26.2         | 26.5 (13.4–45.5)    | 1.17 (0.41–3.32) | 0.772   |
| **Blood transfusion lifetime**    |                |              |                     |             |         |
| No                                | 70/294         | 23.8         | 25.5 (14.4–41.0)    | 1           |         |
| Yes                               | 6/17           | 35.3         | 31.3 (9.4–66.6)     | 1.33 (0.26–6.87) | 0.117   |

HBV, hepatitis B virus; MSM, men who have sex with men; CI, confidence interval; OR, odds ratio; STIs, sexually transmitted infections; HIV, human immunodeficiency virus.

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Characteristics of HBV DNA positive samples

Of the 522 samples, HBV DNA was detected only in the five anti-HBc/HBsAg positive ones. Thus, occult HBV infection was not observed in the study population. Relative to HBV DNA positive samples (Table 5), two and three were HBeAg and anti-HBe reactive, respectively.

Table 4. Multivariate analysis of factors associated with HBV infection among MSM in Goiânia, Central Brazil.

| Variable                        | Adjusted OR (95% CI) | P value |
|---------------------------------|----------------------|---------|
| Age (years)                     |                      |         |
| ≤ 25                            | 1                    |         |
| > 25                            | 11.32 (4.08–31.42)   | <0.001  |
| Number of sexual partners in lifetime |                    |         |
| ≤ 10                            | 1                    |         |
| > 10                            | 1.68 (0.52–5.45)     | 0.386   |
| Ever used drugs or alcohol during sex |                  |         |
| No                              | 1                    |         |
| Yes                             | 1.65 (0.61–4.47)     | 0.321   |
| Ever received payment for sex   |                      |         |
| No                              | 1                    |         |
| Yes                             | 0.81 (0.27–2.44)     | 0.702   |
| Receptive anal intercourse      |                      |         |
| No                              | 1                    |         |
| Yes                             | 11.60 (2.60–51.72)   | 0.001   |
| Sex with women                  |                      |         |
| No                              | 1                    |         |
| Yes                             | 2.83 (1.06–7.56)     | 0.038   |
| STIs                            |                      |         |
| No                              | 1                    |         |
| Yes                             | 5.79 (2.16–15.56)    | 0.001   |
| HIV status                      |                      |         |
| Negative                        | 1                    |         |
| Positive                        | 2.01 (0.75–5.42)     | 0.164   |

HBV, hepatitis B virus; MSM, men who have sex with men; CI, confidence interval; STIs, sexually transmitted infections; HIV, human immunodeficiency virus; aAdjusted odds ratio for the following variables: age, number of sexual partners in lifetime, use of drugs or alcohol during sex, received payment for sex, receptive anal intercourse, sex with women, STIs and HIV.

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Characteristics of HBV DNA positive samples

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Table 5. Hepatitis B virus biomarkers in MSM with HBV DNA positive in Goiânia, Central Brazil.

| HSH | Age (years) | Serological markers | Genome/ regions amplified | Mutations | Subgenotype |
|-----|-------------|---------------------|---------------------------|-----------|-------------|
|     |             | HBeAg | Anti-HBe | S region | BCP and Pre-C/C region |         |         |
| Y02 | 37          | -     | -       | Pre-S/S  | T131N     | -          | A2       |
| Y413| 39          | -     | +       | S        | T131N     | -          | A1       |
| Y431| 52          | -     | +       | Full genome | T131N    | A1762T, G1764A | A2       |
| Y494| 37          | +     | -       | Full genome | T131N    | G1862T, G1888A | A1       |
| Y513| 26          | -     | +       | Pre-S/S, BCP and Pre-C/C | T131N    | G1862T   | A1       |

HBV, hepatitis B virus; MSM, men who have sex with men; HBeAg, hepatitis B e antigen; anti-HBe, antibodies against HBeAg; BPC, basal core promoter.

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Anti-HBc IgM marker was not detected in any of these samples. Two samples (Y431 and Y494) were successfully amplified for full-length HBV genome, one (Y513) for Pre-S/S, BCP and Pre-C/C, one (Y02) for Pre-S/S, and one (Y413) for S gene region. Sequence analysis of these samples revealed that all HBV isolates had the T131N amino acid substitution in the S gene. In addition, analysis of BCP and Pre-C/C gene regions showed the double mutation A1762T/G1764A in sample Y431, G1862T/G1888A in Y494, and G1862T in Y513. No mutations were detected in X and overlapping HBV polymerase gene regions.

Phylogenetic analysis of the S gene showed that the five isolates belonged to HBV genotype A, subgenotypes A1 (n = 3) and A2 (n = 2) (Fig 2). These results were further confirmed by analysis of other amplified genomic regions.

Discussion

To our knowledge, this is the first study to investigate the epidemiological and virological aspects of HBV infection in MSM in Brazil using RDS as a recruitment and data analysis method. The sociodemographic characteristics of the studied population were similar to previous reports for Brazilian MSM, such as age under 25 years old [15], self-identification as brown [31], single status [31], high school attended, and lowest tier of Brazilian social class [15,32]. Although these characteristics were similar to those reported previously, they can also be influenced by RDS. This recruitment method of participants depends on a connected social network, and like other sampling methods for hidden populations, there are tradeoffs [25,33].

The prevalence of HBV infection (15.4%; 95% CI: 8.7–25.8) estimated in this study was similar to those found in MSM in Argentina (22.9%; 95% CI: 19.9–26.9) [34], Australia (10.6%; 95% CI: 10.0–11.4) [35], and the USA (19.4%; 95% CI: 15.0–24.0) [36]. These are countries with a low endemicity for this infection, similar to that reported in Brazil [37]. This prevalence was also similar to that obtained in another recent study among MSM in Brazil (Campinas City, São Paulo State: 11.4%; 95% CI: 7.8–14.9) [15]. Nevertheless, the prevalence determined in this study was higher than that observed in the general population in Central Brazil (5.3%; 95% CI: 4.6–6.1) [38], suggesting that MSM still have a greater vulnerability to HBV acquisition.

Moreover, the prevalence of anti-HBs alone showed that about 40% of the population had serological evidence of immunization against HBV. In addition, a high percentage (44.3%) of MSM was seronegative for all hepatitis B serological markers, indicating susceptibility to HBV infection. These data suggest lower vaccination coverage than desired, since the HBV vaccine is recommended and available free to all MSM in Brazil. Therefore, it is important to emphasize the need to reassess and improve strategies for prevention and control of hepatitis B in this group, mainly to increase the number of MSM vaccinated against HBV.

As described by other authors [14,36,39], multivariate analysis shows that age over 25 years old was independently associated with HBV infection in this study. The association between HBV infection and older age has been widely reported and it is probably due to the increased risk of exposure over time and also to the greater vaccination coverage especially among children and young people in the last decades [36,40].

Receptive anal intercourse and history of STIs were factors associated with HBV infection in this population, as well as in other MSM [14,39]. Indeed, mucosal lesions caused by anal sex have been associated with transmission of several STIs [41]. Additionally, sex with women was associated with HBV infection in this study. Although most participants have self-identified as gay, almost half (47%) reported previous sex with women. Studies indicate that men who have sex with both genders are a potential bridge for STIs between MSM and women [34]. A previous study conducted among Brazilian MSM has suggested that bisexual men who have
Fig 2. Phylogenetic tree analysis of the S region of hepatitis B virus (HBV). The phylogenetic tree performed by using the CLUSTAL W program and analyzed by Kimura two-parameter methods. Genetic distances were calculated by the maximum composite likelihood. Phylogenetic tree was constructed by the neighbor-joining method using MEGA v.6.0 software (bootstrap resampling test with 1,000 replicates), including 5 isolates from Brazilian MSM (black square), 33 GenBank sequences of genotypes A–J (GenBank...
practiced unprotected vaginal and anal sex with their female partners as well as unprotected anal intercourse with their male partners probably have an increased risk for HIV exposure and, consequently, for other STIs [23].

HBV DNA was found in all HBsAg-positive MSM, showing that they have active hepatitis B and a higher potential for HBV transmission. Additionally, this risk is directly related to the level of HBV DNA in serum, and it is usually higher in those HBeAg-positive patients [41,42].

Despite the importance of researching occult HBV infection due to the risk of progression to serious liver disease and potential transmission of HBV [2], there is only one study concerning to OBI in MSM in the world, showing an OBI prevalence of 0.2% in HIV-positive individuals in Germany [41]. Reflecting this low prevalence, OBI was not observed in MSM analyzed in this study.

Phylogenetic analysis showed that HBV genotype A (A1 and A2) was identified in the study population, corroborating previous studies which have indicated that this genotype is the most prevalent in Brazil [7,38]. In addition, all HBV isolates analyzed here presented the T131N substitution in the S gene. It has been suggested that this mutation is a natural polymorphism in HBV genotypes A and G, being associated with persistence of the HBV even after loss of HBsAg and anti-HBs seroconversion as well as with vaccine escape [43].

The typical double mutation in BCP (A1762T and G1764A) found in one HBeAg-negative individual (Y431) is responsible for decreased HBeAg expression and has been linked to HBV oncogenesis [9]. In this study, the Pre-C G1862T mutation was found in one HBeAg-negative (Y513) and in one HBeAg-positive (Y494) participant (along with G1888A), both belonging to subgenotype A1. As reported elsewhere [9,44], the presence of G1862T in some HBeAg-positive patients suggests that this mutation can be genotype specific, since it was predominantly found in HBV/A1 isolates. G1888A is also characteristic of subgenotype A1 [45]. These findings indicate that HBV genotypes/subgenotypes may display different clinical implications on the variability of BCP, Pre-C/C and S gene regions and may impact hepatitis B prognosis.

These results must be considered in the context of the study’s limitations. First, as this was a cross-sectional study, it could not establish the causality between associated factors and HBV infection; therefore a longitudinal study is needed to explore these factors among Brazilian MSM. Second, as interviews were conducted face-to-face, it is possible that behavioral responses may be sensitive for some participants resulting in information biases, although interviewers were trained to minimize these biases. Third, RDS is a relatively recent method and has its own limitations regarding calculation of the sample size and appropriate tools for data analysis, as well as definition of estimators [46]. In this study, although we have considered a design effect of 2 to the sample size calculation, as often recommended in the literature [26,33], and some findings on multivariate associations were significant, CIs were wide due to the modest sample size. Lastly, the incentive reward offered by the RDS may attract more low-income MSM to participate in the study. In addition, this was also limited to MSM aged 18 years or older, potentially limiting the representativeness of the studied sample. Despite these limitations, RDS is an effective approach to access hidden populations such as MSM. Additionally, this study provided a comprehensive investigation of the epidemiological and virological characteristics of HBV in a group of MSM in Brazil. Therefore, further national study is necessary to confirm our findings.

In conclusion, this study shows that the HBV prevalence among MSM was higher than that previously reported in the general population in Central Brazil, and was associated with sexual
risk behaviors. The large proportion of the study population showed susceptibility to HBV infection, highlighting the need to increase HBV immunization coverage in MSM, as well as the sexual health education programs in Brazil. It is also worth mentioning the need for expert assistance and monitoring of HBV DNA-positive individuals to prevent progression to more severe diseases.

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Author Contributions

Conceived and designed the experiments: RMBM MPO CLRL SAT MADM MAM LRFSK.
Performed the experiments: AMCS MPO NS.
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Contributed reagents/materials/analysis tools: RMBM NMA.
Wrote the paper: MPO RMBM.
Involved in study implementation and participated of data collection: MPO RMBM CLRL SAT MADM MAM AMCS. Assisted the molecular analyses: MADM NMA. Provided critical revisions of the manuscript: LRFSK NMA.

References

1. World Health Organization (WHO). Hepatitis B. Fact sheet n°204. WHO, 2015. Available: http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed 11 Jan 2016.
2. Kwak MS, Kim YJ. Occult hepatitis B virus infection. World J Hepatol 2014; 6(12): 860–869. doi: 10.4254/wjh.v6.i12.860 PMID: 25544873
3. Pondé RA. Molecular mechanisms underlying HBsAg negativity in occult HBV infection. Eur J Clin Microbiol Infect Dis. 2015; 34(9): 1709–1731. doi: 10.1007/s10096-015-2422-x PMID: 26105620
4. Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology. 2014; 57(3–4): 141–150. doi: 10.1159/000360947 PMID: 25034481
5. Pourkarim MR, Amini-Bavil-Olyaee S, Kurbanov F, Van Ranst M, Tacke F. Molecular identification of hepatitis B virus genotypes/subgenotypes: revised classification hurdles and updated resolutions. World J Gastroenterol. 2014; 20(23): 7152–7168. doi: 10.3748/wjg.v20.i23.7152 PMID: 24966586
6. Lago BV, Mello FC, Kramvis A, Niel C, Gomes SA. Hepatitis B virus subgenotype A1: evolutionary relationships between Brazilian, African and Asian isolates. PLoS One, 2014; 9(8): 1–9.
7. Mello FC, Souto FJ, Nabuco LC, Villela-Nogueira CA, Coelho HS, Franz HC et al. Hepatitis B virus genotypes circulating in Brazil: molecular characterization of genotype F isolates. BMC Microbiol. 2007; 7: 103. PMID: 18036224
8. Gao S, Duan ZP, Coffin CS. Clinical relevance of hepatitis B virus variants. World J Hepatol. 2015; 7(8): 1086–1096. doi: 10.4254/wjh.v7.i8.1086 PMID: 26052397
9. Lazarevic I. Clinical implications of hepatitis B virus mutations: recent advances. World J Gastroenterol. 2014; 20(24): 7653–7664. doi: 10.3748/wjg.v20.i24.7653 PMID: 24976703
10. Centers for Disease Control and Prevention (CDC). Viral Hepatitis. Information for Gay and Bisexual Men. CDC, 2013. Available: www.cdc.gov/hepatitis. Accessed 11 Jan 2016.
11. van Houdt R, Bruisten SM, Speksnijder AG, Prins M. Unexpectedly high proportion of drug users and men having sex with men who develop chronic hepatitis B infection. J Hepatol. 2012; 57(3): 529–533. doi: 10.1016/j.jhep.2012.04.030 PMID: 22612997
12. Chow EP, Tucker JD, Wong FY, Nehl EJ, Wang Y, Zhuang X, et al. Disparities and risks of sexually transmissible infections among men who have sex with men in China: a meta-analysis and data synthesis. PLoS One. 2014; 9(2): 1–13.

13. de Vries HJ. Sexually transmitted infections in men who have sex with men. Clin Dermatol. 2014; 32(2): 181–188. doi: 10.1016/j.clindermatol.2013.08.001 PMID: 24559552

14. Lama JR, Agurto HS, Guanira JV, Ganoza C, Casapia M, Ojeda N, et al. Hepatitis B infection and association with other sexually transmitted infections among men who have sex with men in Peru. Am J Trop Med Hyg. 2010; 83(1): 194–200. doi: 10.4269/ajtmh.2010.10-0003 PMID: 20595501

15. Soares CC, Georg I, Lampe E, Lewis L, Morgado MG, Nicol AF, et al. HIV-1, HBV, HCV, HTLV, HPV-16/18, and Treponema pallidum infections in a sample of Brazilian men who have sex with men. PLoS One. 2014; 9(8): e102676. doi: 10.1371/journal.pone.0102676 PMID: 25083768

16. Tseng YT, Sun HY, Chang SY, Wu CH, Liu WC, Wu PY, et al. Seroprevalence of hepatitis virus infection in men who have sex with men aged 18–40 years in Taiwan. J Formos Med Assoc. 2012; 111(8): 431–438. doi: 10.1016/j.jfma.2011.06.022 PMID: 22939661

17. Urbanus AT, van Houdt R, van de Laar TJ, Coutinho RA. Viral hepatitis among men who have sex with men, epidemiology and public health consequences. Euro Surveill. 2009; 14(47): 19421. PMID: 19941800

18. World Health Organization (WHO). Guidelines: Prevention and treatment of HIV and other sexually transmitted infections among men who have sex with men and transgender people. Recommendations for a public health approach. 2011.

19. Kassak K, Mahfoud Z, Kreidieh K, Shamra S, Affif R, Ramia S. Hepatitis B virus and hepatitis C virus infections among female sex workers and men who have sex with men in Lebanon: prevalence, risk behaviour and immune status. Sex Health. 2011; 8(2): 229–233. doi: 10.1071/S10080 PMID: 21592438

20. Nyamathi A, Salem B, Reback CJ, Shoplistaw S, Branson CM, Idemundia FE, et al. Correlates of hepatitis B virus and HIV knowledge among gay and bisexual homeless young adults in Hollywood. Am J Mens Health. 2013; 7(1): 18–26. doi: 10.1177/1557988312456068 PMID: 22879650

21. Salganik MJ. Variance estimation, design effects, and sample size calculations for respondent-driven sampling. J Urban Health. 2006; 83(Suppl 6): i98–104.

22. Motta-Castro AR, Martins RM, Araujo NM, Niel C, Facholi GB, Lago BV, et al. Molecular epidemiology of hepatitis B virus in an isolated Afro-Brazilian community. J Med Virol. 1994; 44: 180–186. PMID: 7852959

23. Suttmoller F, de Souza CT, Monteiro JC, et al. The Rio de Janeiro HIV vaccine site-II. Recruitment strategies and socio-demographic data of a HIV negative homosexual and bisexual male cohort in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 1997; 92(1): 39–46. PMID: 9302413

24. Suttmoller F, Penna TL, de Souza CT, Lambert J. Human immunodeficiency virus incidence and risk behavior in the 'Projeto Rio': results of the first 5 years of the Rio de Janeiro open cohort of homosexual and bisexual men, 1994–98. Int J Infect Dis. 2002; 6(4): 259–265. PMID: 12718818

25. Soares CC, Georg I, Lampe E, Lewis L, Morgado MG, Nicol AF, et al. HIV-1, HBV, HCV, HTLV, HPV-16/18, and Treponema pallidum infections in a sample of Brazilian men who have sex with men. PLoS One. 2014; 9(8): e102676. doi: 10.1371/journal.pone.0102676 PMID: 25083768

26. Kassak K, Mahfoud Z, Kreidieh K, Shamra S, Affif R, Ramia S. Hepatitis B virus and hepatitis C virus infections among female sex workers and men who have sex with men in Lebanon: prevalence, risk behaviour and immune status. Sex Health. 2011; 8(2): 229–233. doi: 10.1071/S10080 PMID: 21592438

27. Motta-Castro AR, Martins RM, Araujo NM, Niel C, Facholi GB, Lago BV, et al. Molecular epidemiology of hepatitis B virus in an isolated Afro-Brazilian community. J Med Virol. 1994; 44: 180–186. PMID: 7852959

28. Motta-Castro AR, Martins RM, Araujo NM, Niel C, Facholi GB, Lago BV, et al. Molecular epidemiology of hepatitis B virus in an isolated Afro-Brazilian community. Arch Virol. 2008; 153: 2197–2205. doi: 10.1007/s00705-008-0237-0 PMID: 19941800

29. Kerr LR, Mota RS, Kendall C, Pinho A de A, Mello MB, Guimaraes MD, et al. Sexual violence against men who have sex with men in Brazil: A Respondent-Driven Sampling Survey. AIDS Behav. 2015; 19(9): 1630–1641. doi: 10.1007/s10461-015-1016-z PMID: 25666270

30. Brasil. Secretaria de Assuntos Estratégicos da Presidência da República do Brasil. Assuntos estratégicos: Social e renda—A Classe Média Brasileira. Brasília, DF, 2014.

31. Salganik MJ. Variance estimation, design effects, and sample size calculations for respondent-driven sampling. J Urban Health. 2006; 83(Suppl 6): i98–112.

32. Niel C, Moraes MTB, Gaspar AMC, Yoshida CFT, Gomes AS. Genetic diversity of hepatitis B virus strains isolated in Rio de Janeiro, Brazil. J Med Virol. 1994; 44: 180–186. PMID: 7852959

33. Motta-Castro AR, Martins RM, Araujo NM, Niel C, Facholi GB, Lago BV, et al. Molecular epidemiology of hepatitis B virus in an isolated Afro-Brazilian community. Arch Virol. 2008; 153: 2197–2205. doi: 10.1007/s00705-008-0237-0 PMID: 19941800

34. Günther S, Li BC, Miska S, Krüger DH, Meisel H, Will H. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. J Virol. 1995; 69(9): 5437–5444. PMID: 7636989

35. Brasil. Secretaria de Assuntos Estratégicos da Presidência da República do Brasil. Assuntos estratégicos: Social e renda—A Classe Média Brasileira. Brasília, DF, 2014.

36. Salganik MJ. Variance estimation, design effects, and sample size calculations for respondent-driven sampling. J Urban Health. 2006; 83(Suppl 6): i98–112.

37. Niel C, Moraes MTB, Gaspar AMC, Yoshida CFT, Gomes AS. Genetic diversity of hepatitis B virus strains isolated in Rio de Janeiro, Brazil. J Med Virol. 1994; 44: 180–186. PMID: 7852959

38. Motta-Castro AR, Martins RM, Araujo NM, Niel C, Facholi GB, Lago BV, et al. Molecular epidemiology of hepatitis B virus in an isolated Afro-Brazilian community. Arch Virol. 2008; 153: 2197–2205. doi: 10.1007/s00705-008-0237-0 PMID: 19941800

39. Günther S, Li BC, Miska S, Krüger DH, Meisel H, Will H. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. J Virol. 1995; 69(9): 5437–5444. PMID: 7636989

40. Brasil. Secretaria de Assuntos Estratégicos da Presidência da República do Brasil. Assuntos estratégicos: Social e renda—A Classe Média Brasileira. Brasília, DF, 2014.
33. Johnston LG, Hakim AJ, Dittrich S, Burnett J, Kim E, White RG. A Systematic Review of Published Respondent-Driven Sampling Surveys Collecting Behavioral and Biologic Data. AIDS Behav. 2016; doi: 10.1007/s10461-016-1346-5

34. Pando MA, Balán IC, Marone R, Dolezal C, Leu CS, Squiquera L, et al. HIV and other sexually transmitted infections among men who have sex with men recruited by RDS in Buenos Aires, Argentina: high HIV and HPV infection. PLoS One. 2012; 7(6): e39834. doi: 10.1371/journal.pone.0039834 PMID: 22768137

35. Gamagedara N, Weerakoon AP, Zou H, Fehler G, Chen MY, Read TR, et al. Cross-sectional study of hepatitis B immunity in MSM between 2002 and 2012. Sex Transm Infect. 2014; 90(1): 41–45. doi: 10.1136/sextrans-2013-051131 PMID: 23920399

36. Pitasi MA, Bingham TA, Sey EK, Smith AJ, Teshale EH. Hepatitis B virus (HBV) infection, immunity and susceptibility among men who have sex with men (MSM), Los Angeles County, USA. AIDS Behav. 2014; 18: 248–255. doi: 10.1007/s10461-013-0670-2 PMID: 24276792

37. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet, 2015; 386(10003): 1546–1555. doi: 10.1016/S0140-6736(15)61412-X PMID: 26231459

38. Pereira LM, Martelli CM, Merchán-Hamann E, Montarroyos UR, Braga MC, de Lima ML, et al. Population-based multicentric survey of hepatitis B infection and risk factor differences among three regions in Brazil. Am J Trop Med Hyg. 2009; 81(2): 240–247. PMID: 19635877

39. Linkins RW, Chonwattana W, Holtz TH, Wasinrapee P, Chaikummao S, Varangrat A, et al. Hepatitis A and hepatitis B infection prevalence and associated risk factors in men who have sex with men, Bangkok, 2006–2008. J Med Virol. 2013; 85(9): 1499–505. doi: 10.1002/jmv.23637 PMID: 23797893

40. Souto FJ. Distribution of hepatitis B infection in Brazil: the epidemiological situation at the beginning of the 21st century. Rev Soc Bras Med Trop. 2016; 49(1): 11–23. doi: 10.1590/0037-8682-0176-2015 PMID: 26689276

41. Jansen K, Thamm M, Bock CT, Scheufele R, Kücherer C, Muenstermann D, et al. High prevalence and high incidence of coinfection with hepatitis B, hepatitis C, and syphilis and low rate of effective vaccination against hepatitis B in HIV-positive men who have sex with men with known date of HIV seroconversion in Germany. PLoS One. 2015; 10(11): e0142515. doi: 10.1371/journal.pone.0142515 PMID: 26555244

42. Shiffman ML. Management of cute hepatitis B. Clin Liver Dis. 2010; 4: 75–91.

43. Svicher V, Cento V, Salpini R, Mercurio F, Fraune M, Beggel B, et al. Role of hepatitis B virus genetic barrier in drug-resistance and immune-escape development. Dig Liver Dis. 2011; 43(12): 975–83. doi: 10.1016/j.dld.2011.07.002 PMID: 21831732

44. Chandra PK, Banerjee A, Datta S, Chakravarty R. G1862T mutation among hepatitis B virus-infected individuals: association with viral genotypes and disease outcome in Kolkata, Eastern India. Intervirology. 2007; 50(3): 173–180. PMID: 17259736

45. Kimbi GC, Kew MC, Kramvis A. The effect of the G1888A mutation of subgenotype A1 of hepatitis B virus on the translation of the core protein. Virus Res. 2012; 163(1): 334–340. doi: 10.1016/j.virusres.2011.10.024 PMID: 22100339

46. White RG, Lansky A, Goel S, Wilson D, Hladik W, Hakim A, et al. Respondent driven sampling—where we are and where should we be going? Sex Transm Infect. 2012; 88(6): 397–399. PMID: 23012492