

## Hepatitis B in the Northwestern region of Sao Paulo State: genotypes and resistance mutations

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### ABSTRACT

In Brazil, few studies on the molecular aspects of hepatitis B virus (HBV) infection have been conducted in the interior regions of Sao Paulo State. This study aimed to identify HBV genotypes and evaluate strains with resistance mutations for nucleoside analogues in the Administrative Region (AR) of the municipality of Sao Jose do Rio Preto. We performed nested PCRs of 127 samples from the Health Care Services of the AR to amplify, sequence and analyze fragments of the HBV DNA, in order to identify genotypes and resistance mutations. The HBV S/Pol regions of 126 samples were successfully amplified and sequenced. Five different genotypes were found, and the main ones were A, D and F; a greater number of samples contained the subgenotypes A1 (n = 51; 40.5%), D3 (n = 36; 28.6%), A2 (n = 14; 11.1%) and F2a (n = 9; 7.1%). Resistance mutations (rtM204V/I/S) associated or not with compensatory mutations (rtL180M, rtV173L) were identified in 13.9% (5/36) of patients undergoing viral treatment and 1.1% (1/90) of naïve patients. The diversity of genotypes/subgenotypes found is probably due to the intense migration occurring in the region. These data can complement epidemiological and clinical surveillance, and can be used for a more effective management of chronic HBV patients.

**KEYWORDS:** Hepatitis B virus. HBV genotypes. Resistant mutations. Brazil.

### INTRODUCTION

Chronic hepatitis B virus (HBV) infections affect millions of people worldwide and are associated with a range of clinical manifestations, including cirrhosis, liver failure and hepatocellular carcinoma (HCC). Despite the availability of an effective vaccine, HBV causes nearly 900,000 deaths every year. According to the World Health Organization (WHO), at least 2 billion people live with serological evidence of past or present infection by HBV<sup>1</sup>.

Ten HBV genotypes (A to J) have been characterized. The classification of these genotypes is based on a sequence divergence greater than 8% for the entire genome. In Brazil, genotype A is the most common, followed by D and F. Genotype A is most prevalent in the Northern, Northeastern and Southeastern regions; genotype D is most prevalent in the Southern and Midwestern regions. There is an equal distribution of genotypes A and D. Genotype F has been found in almost all regions of Brazil, with the exception of the South<sup>2</sup>. Approximately 14,000 new cases are detected and reported annually, which highlights the impact of the disease in the Brazilian territory<sup>3</sup>.

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The Administrative Region (AR) of Sao Jose do Rio Preto is located in the Northwestern region of Sao Paulo State and comprises 102 municipalities divided into seven health regions: Santa Fe do Sul, Jales, Fernandopolis, Votuporanga, Sao Jose do Rio Preto, Jose Bonifacio and Catanduva. Approximately 1.5 million people live in this AR<sup>4</sup>, which has one of the largest and most important hospital complexes in Sao Paulo State, the Hospital de Base, which offers several health services, including highly complex procedures, constituting a reference in the care for patients from all Brazilian regions and even from Latin America.

Astbury *et al.*<sup>5</sup> conducted the only study of HBV genotyping and resistance mutations analysis in Sao Jose do Rio Preto. They identified the subgenotypes A1, A2, B1, D1, D2, D3, F1 and F2, in addition to strains resistant to the nucleoside analogues (NA) lamivudine (LAM), entecavir (ETV), and telbivudine (LDT). Few studies have been carried out in other regions of the interior of the state. Chachá *et al.*<sup>6</sup> detected the subgenotypes A1, A2, D1-D4, F2a and F4 circulating in Ribeirao Preto. Tonetto *et al.*<sup>7</sup> observed genotypes A, C, D and F in the municipality of Campinas. These studies contributed to the knowledge on the molecular aspects of HBV infection in the interior of Sao Paulo State and complemented the epidemiological and clinical surveillance in the region.

The aim of this study was to identify HBV genotypes and subgenotypes circulating in the region of Sao Jose do Rio Preto and to evaluate the frequency of strains with resistance mutations for nucleoside analogues that are used in the treatment of hepatitis B.

## MATERIALS AND METHODS

### Study population

This was a retrospective study carried out in the AR of Sao Jose do Rio Preto, Sao Paulo State, Brazil (latitude -49.797363 and longitude -20.623609), involving 127 patients from the Health Care Services, who were sent for HBV viral load test, from September 2015 to January 2018. The patients' information was obtained from the HBV viral load request form, prepared by the Department of STI/AIDS and Viral Hepatitis, Ministry of Health. This form was filled by the physician with the patient's name, age, self-declared skin color, gender, diagnostic definition, clinical stage of HBV disease, comorbidities (cirrhosis and coinfections) and treatment.

### Extraction, amplification, and sequencing of HBV DNA

A commercial kit, QIAamp® DNA Mini kit, Qiagen

(QIAGEN, Hilden, Germany) was used, according to the manufacturer's guidelines, to extract HBV-DNA from serum or plasma samples. Amplification and sequencing of the S and polymerase (S/Pol) regions of the HBV genome were performed using in-house nested PCR with the primers FHBS1 and FHBS2, described by Sitnik *et al.*<sup>8</sup>, and the primers RADE1 and RADE2, described by Gomes-Gouvêa *et al.*<sup>9</sup> which amplify a 734 bp fragment. The amplified DNA was sequenced using an automatic 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), with the primers FHBS2 and RADE2.

### Identification of HBV genotypes and analysis of resistance mutations

After sequencing, the quality of the sequences was analyzed using the Sequence Scanner program (Thermo Fisher Scientific, Waltham, MA, USA). The consensus sequence of each sample was obtained using the Sequencher® version 4.1.4 DNA sequence analysis software (Gene Codes Corporation, Ann Arbor, MI, USA), and analyzed using the online software VirusTools.

VirusTools was developed by researchers from the Hospital Israelita Albert Einstein, in the city of Sao Paulo, for HBV genotyping and detection of HBV strains with resistance mutations for NA, based on HBV sequences that have been cataloged using phylogenetic methods. For this, the Bayesian estimate of the evolutionary scale of HBV was used, based on the reference genome sequences deposited at the National Center for Biotechnology Information – NCBI, and the phylogenetic tree topology was estimated using the maximum likelihood criterion. This software facilitated the analysis of viral sequencing for the genotyping and investigation of antiviral resistance mutations (unpublished data).

These resistance mutations can occur during the natural course of chronic hepatitis B in untreated individuals, or during treatment with NA that promotes selective pressure, favoring the emergence of strains with resistance mutations, so the patients were divided into two groups: patients not undergoing treatment, and patients undergoing treatment. In both groups, the presence of resistance mutations was searched for the NA lamivudine (LAM), adefovir (ADF), entecavir (ETV), emtricitabine (FTC), tenofovir (TDF) and telbivudine (LDT); partial resistance was described in cases in which the presence of mutations indicated a reduced sensitivity to NA; total resistance was present when the mutation was associated with resistance to the investigated NA.

## Statistical analysis

Data were organized in an Excel spreadsheet, exported to the SPSS 21 software (IBM Corp., Armonk, New York, USA), and descriptive statistics was applied. To test the significance level of associations, the Pearson's chi-squared and the Fisher's Exact test were used to evaluate categorical variables. A value of  $p < 0.05$  was considered statistically significant.

## Ethical standards

The procedures described herein were only initiated after obtaining the approval from the Ethics Committees of the participating institutions. This study was approved by the Ethical Committee in Research at the Instituto Adolfo Lutz (CEPIAL) (N° 466/2012). All the patients provided written consents.

## RESULTS

Table 1 shows the demographic characteristics of 127 patients enrolled in the study. The average age was  $45.8 \pm 12.1$  years, with a minimum and maximum of 20 and 75 years, respectively. The age groups with the largest number of patients were 35-39 ( $n = 21$ ), 40-44 ( $n = 20$ ), and 45-49 ( $n = 18$ ), and together, they represented 46.4% of the participants, followed by age groups 55-59 ( $n = 16$ ) and 30-34 ( $n = 14$ ). The number of patients according to the health region of the AR is shown in Figure 1.

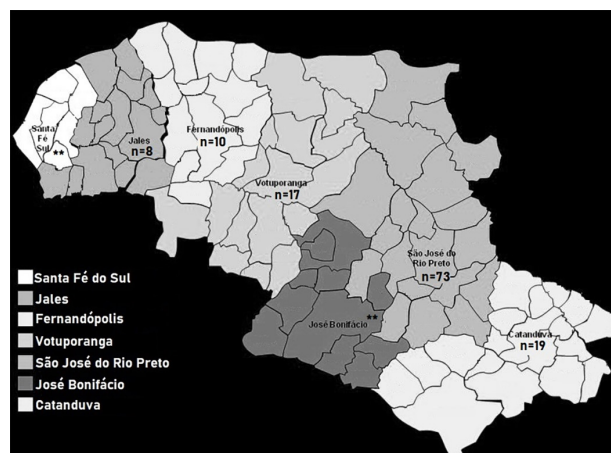
Regarding the stage of the disease, all the samples analyzed came from patients with chronic hepatitis B, with the presence of cirrhosis in 4.7% (6/127) and coinfection with HCV and/or HIV in 12.6% (16/127) of the patients. HBV/HIV co-infection was present in 7.9% (10/127), HBV/HCV in 3.1% (4/127), and HBV/HCV/HIV in 1.6% (2/127) of the patients.

It was possible to amplify the HBV S/Pol region of the 127 samples included in the study; however, one sample was not of sufficient quality to perform the genotyping through sequencing, and was excluded from the analysis. Among the 126 amplified samples, the genotype/subgenotype distribution was as follows: A1 ( $n = 51$ ; 40.5%), A2 ( $n = 14$ ; 11.1%), B1 ( $n = 1$ ; 0.8%), C1 ( $n = 1$ ; 0.8%), D1 ( $n = 2$ ; 1.6%), D2 ( $n = 7$ ; 5.6%), D3 ( $n = 36$ ; 28.6%), D4 ( $n = 2$ ; 1.6%), E ( $n = 1$ ; 0.8%), F2a ( $n = 9$ ; 7.1%) and F4 ( $n = 2$ ; 1.6%).

The 126 genotyped samples were analyzed for the presence of resistance mutations for the nucleoside analogues LAM, ADF, ETV, FTC, TDF and LDT, in the groups: patients undergoing treatment and patients not undergoing treatment.

**Table 1** - Distribution of gender, age, and skin color of HBV carriers.

	HBV samples	
	n	%
<b>Gender</b>		
Male	80	63
Female	47	37
<b>Age Group</b>		
20-24	3	2.4
25-29	6	4.7
30-34	14	11.0
35-39	21	16.5
40-44	20	15.7
45-49	18	14.2
50-54	12	9.5
55-59	16	12.6
60-64	6	4.7
65-69	4	3.2
70-75	7	5.5
<b>Skin color</b>		
White	85	66.9
Mixed ethnicity	26	20.5
Black	12	9.4
Yellow	4	3.2



**Figure 1** - Number of samples according to the health region of the Administrative Region (AR) of Sao Jose do Rio Preto; \*\* The samples collected in the regions of Santa Fe do Sul and Jose Bonifacio were not counted individually, but they were inserted in the neighboring regions, due to changes in the AR division.

In the group of patients undergoing treatment, strains of HBV with resistance mutations (rtM204V/I/S) associated or not with compensatory mutations (rtL180M, rtV173L) were identified in 13.9% (5/36) of the analyzed samples. Strains with partial resistance mutations for ETV

(rtM204V/I/S) or potentially associated with resistance to ADF (rtV214A, rtL217R, rtQ215S, rtN238T, and rtP237H) were identified in 13.9% (5/36) and 19.4% (7/36) of the samples, respectively.

The resistance profile of each sample, with its respective mutations and clinical information is described in [Table 2](#); five samples with resistance to LAM and FTC, and partial resistance to ETV were identified. One sample also showed resistance to LTD. The HBV viral load levels varied, with an average log of 5.33 ( $\pm$  1.51); the HBV/D3 subgenotype was the most prevalent (40%); and the rate of HIV and/or HCV coinfection was 50%.

In the group of patients who were not undergoing treatment, 1.1% (1/90) of the samples presented resistance mutations (rtM204V) to LAM, ADF and FTC, as well as partial resistance to ETV, in addition to a mutation (rtA181T) that confers partial resistance *in vitro*, but does not imply *in vivo* resistance to TDF. One sample (1.1%) presented a compensatory mutation (rtV/F/L/M207I), which typically appears after other primary variants of resistance to LAM; and 26.7% (24/90) of the samples showed potential resistance mutations for ADF (rtV214A, rtL217R, rtP237H, rtN238T, rtQ215S, and rtI233V). The

viral load levels showed an average log of 4.18 ( $\pm$  1.15); the HBV/D3 subgenotype was the most prevalent (46.2%); there were no patients with coinfections in this group.

Statistical analyses to evaluate the relationship between the genotypes (A, B, C, D, E and F) and gender, age groups, skin color, the patient group (undergoing treatment and not undergoing treatment), and presence of total/partial resistance mutations did not reveal any significant relationship. However, we found a higher percentage of patients with resistance mutations in the group of patients undergoing treatment than in the group of patients who were not undergoing treatment ( $p=0.020$ ), as shown in [Table 3](#).

## DISCUSSION

The AR of Sao Jose do Rio Preto is located in the Northwestern region of Sao Paulo State, bordering Mato Grosso do Sul and Minas Gerais, and is among the three regions with the highest migration rates in the state<sup>10</sup>, contributing to the intense circulation and fluctuation of the population. In this region, there are many patients under follow-up for hepatitis B; 191 new cases were reported in the studied period<sup>11</sup>.

**Table 2** - Resistance profiles of patients undergoing treatment for hepatitis B.

Patient ID	HBV Subgenotype	Treatment	Viral Load Log	Mutations	Resistance Profile						Coinfection
					LAM	ADF	ETV	FTC	TDF	LDT	
99	D2	LAM/TDF/EFV	6.36	rtV214A	S	<b>PTR</b>	S	S	S	S	HIV/HCV
101	F2a	TDF	5.64	rtM204V + rtL180M	<b>R</b>	S	<b>PR</b>	<b>R</b>	S	S	HIV
102	A2	LAM/ADF	3.82	rtL217R	S	<b>PTR</b>	S	S	S	S	-
104	D3	LAM	3.34	rtM204I + rtL180M	<b>R</b>	S	<b>PR</b>	<b>R</b>	S	<b>R</b>	-
106	D3	TDF	7.14	rtV214A + rtP237H	S	<b>PTR</b>	S	S	S	S	-
108	A1	LAM/TDF/EFV	7.08	rtM204V + rtV173L + rtL180M	<b>R</b>	S	<b>PR</b>	<b>R</b>	S	S	HIV
111	D3	ETV	3.77	rtM204S + rtQ215S	<b>R</b>	<b>PTR</b>	<b>PR</b>	<b>R</b>	S	S	-
112	F2a	TDF	5.30	rtN238T	S	<b>PTR</b>	S	S	S	S	HIV
121	D2	TDF/LAM	3.99	rtM204S + rtQ215S	<b>R</b>	<b>PTR</b>	<b>PR</b>	<b>R</b>	S	S	HIV
126	D3	TDF (irregular)	6.89	rtV214A + rtP237H	S	<b>PTR</b>	S	S	S	S	-

LAM = Lamivudine; ADF = Adefovir; ETV = Entecavir; FTC = Emtricitabine; TDF = Tenofovir; LDT = Telbivudine; EFV = Efavirenz; S = Sensitive; R = Resistant; PR = Partially Resistant; PTR = Potentially Resistant.

**Table 3** - Relationship between the groups of patients and the presence of resistance mutations.

Groups of patients		Resistance mutations (total/partial)		Total	p value
		No	Yes		
Groups of patients	Not undergoing treatment	88 (73.9%)	2 (28.6%)	90 (71.4%)	0.020
	Undergoing treatment	31 (26.1%)	5 (71.4%)	36 (28.6%)	
Total		119	7	126	

Among the studied HBV patients, males were predominant, they had an average age of approximately 46 years. Similar characteristics were verified in other Brazilian investigations carried out in Ribeirao Preto<sup>6</sup> and Campinas<sup>12</sup>, both in Sao Paulo State and Maranhao State<sup>13</sup>. In our study, the age group with the highest number of cases differed from the national scenario, which, in the last 10 years, has been characterized by a decrease in the detection of hepatitis B in individuals up to 44 years old, and an increase among those over the age of 60 years<sup>3</sup>.

The distribution of cases according to the skin color showed a higher concentration among white people, in contrast to the findings of Nabuco *et al.*<sup>14</sup> and Cruz-Santos *et al.*<sup>13</sup> in Rio de Janeiro and Maranhao States, respectively. These data also differ from the racial distribution in the country, which for the first time in 2019 showed a higher concentration among self-declared people of mixed ethnicity (black and white) and black people, followed by white, yellow and indigenous people<sup>3</sup>.

Five different genotypes were found in our samples, and the main ones were genotypes A (A1 and A2), D (D1, D2, D3, and D4) and F (F2a and F4). These findings corroborate those of other studies carried out in different Brazilian regions, in which the same genotypes were found, showing that there is a high genetic diversity among circulating HBV genotypes<sup>7,15-23</sup>.

Regarding the circulation of subgenotypes in the country, D4 and A1 were reported in Maranhao State<sup>13</sup>; A1, A2, D1-D4, F2a and F4 in Ribeirao Preto<sup>6</sup>; A1, D3, and F2 in the city of Goiania<sup>24</sup>; F2a in the city of Sao Paulo<sup>25</sup>; and A1, D2-D4, and F2a in Rondonia State<sup>26</sup>, with A1 and D3 being the most frequently found, as in the present study.

Astbury *et al.*<sup>5</sup> conducted the only HBV study in the region of Sao Jose do Rio Preto, and identified the subgenotypes A1, A2, B1, D1, D2, D3, F1 and F2. In the present study, we detected the same subgenotypes but we collected a larger number of samples in a much larger area of the region. This has enabled the identification of a high variability in the profile of viral strains and to verify the occurrence of immigration in the region.

This diversity of genotypes distribution is a result of population migrations, and several studies suggested that the current distribution of HBV genotypes/subgenotypes in Brazil is due to the migrations from Europe and Africa that occurred during the colonial period<sup>6</sup>, since A1 and D4 were probably introduced in South America by African slaves, who were the carriers of these subgenotypes<sup>19,27</sup>, whereas the introduction of A2, D2, and D3 was related to the European immigration<sup>18,22,27</sup>. On the other hand, the presence of HBV/F2a has been related to the presence of indigenous people, who live in the Northern region of the country<sup>21</sup>.

Wolf *et al.*<sup>28</sup> demonstrated that the subgenotypes A1 and A2 were introduced in Brazil between the 16<sup>th</sup> and 20<sup>th</sup> centuries. The spread of HBV-A1 across the country probably occurred between 1600 and 1740, introduced directly from Africa through the slave trade. HBV-A2 was introduced later, probably between the 1950s and 1970s in the 20<sup>th</sup> century, coming from Europe. The first introduction and the high spread across the country led to a much higher frequency of A1 than A2. However, HBV-A2 also showed a consistent increase and became a prevalent subgenotype. Possibly due to this early introduction of HBV-A by human migration processes and their continuous spreading along the time, HBV-A is the most frequent genotype in Brazil.

The HBV-D, the second most frequent genotype detected in the country, was introduced in South Brazil, firstly as the subgenotype HBV-D3, probably between 1904 and 1942, and then as HBV-D2, probably between 1946 and 1953, and HBV-D1 possibly between 1954 and 1969. The introduction of HBV-D3 at the beginning of the 20<sup>th</sup> century was characterized by intense Italian immigration, evidencing the probable contribution of this immigration to the high prevalence of this subgenotype in the Southern region. HBV-D1 and D2 strains currently circulating in South Brazil are probably descendants of HBV introduced by immigrants from Europe and Middle East countries<sup>29</sup>.

The third most frequent genotype detected, HBV-F has already been in South America for more than three centuries, and their current lineages have a common ancestor in the Amerindian population thousands of years ago<sup>30,31</sup>. In Brazil, this genotype is frequently observed in the Amazonian region, commonly detected in indigenous people. It was possibly introduced in Brazil centuries ago, possibly in the pre-Columbian era in a similar way that other South American countries, by the first pre-historical settlers before the arrival of the European conquerors. It was more largely disseminated from the early 1800s because of the increase of the Latin American population<sup>21,30</sup>.

In this study, genotypes B, C and E were also found. Genotype B, subgenotype B1 was identified in a 57-year-old male patient who descended from Asians and was born in Brazil. He did not develop cirrhosis or HCC, despite this genotype being associated with the development of fulminant hepatitis, and did not undergo treatment. He had a viral load of 2,529 IU/mL, without coinfections.

Genotype C, subgenotype C1 was identified in a 34-year-old female patient born in China. The patient was being treated with TDF, had a viral load of 2,358 IU/mL, and showed neither coinfections nor the development of cirrhosis or HCC. Genotype E was identified in a 38-year-old black male born in Angola. The patient had a viral load

of 12,930 IU/mL, without coinfections, and he had not yet undergone treatment.

Considering that in Asia the genotypes B and C are more prevalent, the main route of transmission is vertical<sup>32</sup>, and that the referred patients who presented these genotypes descended from or came from this region, it can be hypothesized that most HBV infections occur during pregnancy, childbirth or breastfeeding. In addition, perinatal infection causes chronic infection in 90% of newborns, whereas early childhood infection leads to 20-40% chronicity and infections in adulthood result in 0-10% of chronicity<sup>33,34</sup>. On the other hand, horizontal transmission is the main route in West Africa, where genotype E is more prevalent, and was probably the type of exposure to HBV of that patient. This happens because seroconversion occurs at a younger age in patients with this genotype, which may be one of the reasons why vertical transmission does not occur in West Africa<sup>35</sup>.

Thus, the HBV genotype can provide clues on the possible type of exposure to this virus in certain geographic areas, and serve as an epidemiological tool to follow the routes of transmission<sup>32</sup>. In the latter case, these genotypes reflect the origin of immigrants or the country where they may have acquired the virus, reinforcing the concept that the HBV genotype can act as a valuable tool in the surveillance of viral dissemination, since the circulation of genotypes B, C and E is generally restricted to specific geographic regions (HBV/B and HBV/C in East Asia and HBV/E in West Africa) and is rarely reported in Brazil<sup>15</sup>.

In the last 20 years, significant advances have been made in the treatment of chronic hepatitis B in Brazil; the prolonged use of antivirals can lead to the development of resistance, although it has become increasingly evident that mutations related to resistance to antivirals are not always induced by therapy and can occur in treatment-naïve individuals<sup>16,36</sup>.

In our group of treated patients, the mutation patterns in the HBV strains conferred resistance to LAM, FTC and LDT, partial resistance to ETV, and a potential resistance to ADF. Furthermore, the presence of resistance mutations was greater in this group ( $p = 0.020$ ), which demonstrates the role of treatment in the emergence of resistant HBV strains. It is known that certain drugs have a low genetic barrier to resistance, such as LAM, ADF and LDT, and that the probability of appearance of resistant mutants increases with the treatment duration<sup>37</sup>.

The early detection of these patterns is extremely important for the success of the therapy; however, few studies were carried on these groups of patients, and in clinical practice, a resistance test for nucleoside analogs is only performed after therapeutic failure. To prevent and minimize the emergence of drug resistance, treatment is

currently performed with nucleoside analogs that cause rapid viral suppression acting as a genetic barrier to resistance, such as ETV and TDF<sup>38</sup>.

In the group of treatment-naïve patients, only 1.1% of the samples presented resistance mutations for LAM, ADF and FTC, and partial resistance to ETV. In a study with samples from several regions of Brazil, Gomes-Gouvêa *et al.*<sup>17</sup> found that the resistance rate of mutations associated with nucleoside analogs was 1.6% in naïve patients. In another study conducted in the Northern and Northeastern regions, the prevalence of these resistance mutations was 2.6%<sup>16</sup>. The resistance mutation rates in naïve patients are approximately 1-20% in several studies, most of them carried out in Asian populations<sup>16</sup>.

On the contrary, the frequency of strains that showed potential resistance mutations for ADF (26.7%) was higher in the present study than in Gomes-Gouvêa *et al.*<sup>17</sup> (7.7%) that studied several regions of the country. However, some studies have shown that these mutations are not uncommon, and may only represent natural polymorphisms that are common in some genotypes, which are unimportant in the development of resistance to this drug<sup>17</sup>.

Likewise, it is important to monitor the presence of resistance in treatment-naïve patients, owing to the pre-existence of mutations that need to be investigated and screened. The therapeutic effectiveness of antiviral treatments may be affected by infection with drug-resistant viral strains. This information, before the beginning of treatment, can contribute to clinical decisions, reduce therapeutic failures and the risk of progression to cirrhosis and HCC<sup>16</sup>. However, more studies are necessary to justify the implementation of resistance tests before prescribing the treatment.

## CONCLUSION

In summary, different HBV genotypes were found, similar to the results of studies in other Brazilian regions. The high diversity of genotypes/subgenotypes reflected the intense migration that occurred in the studied region, owing to its importance as a reference center in health. Among treated patients who had strains of HBV with resistance mutations for nucleoside analogues, half had a profile of total resistance to LAM and FTC and partial resistance to ETV. Among the treatment-naïve patients, only one had this profile.

Some genotypes previously unidentified in the Sao Jose do Rio Preto region were found in this study (genotypes C and E). Moreover, the data draw attention to the importance of the surveillance of viral hepatitis throughout Sao Paulo State, especially in cities with a large influx of immigrants and fluctuating populations.

Finally, this study demonstrates the seriousness of hepatitis B as a health problem in the region of Sao Jose do Rio Preto, and proves the need for an always-active surveillance, for the effective management of chronic HBV patients in order to reduce the mortality and morbidity associated with this viral infection.

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