Целью исследования являлось охарактеризовать генетические варианты ВГВ, циркулирующие в Гвинейской Республике, на основе нуклеотидных последовательностей полного генома вируса, а также проанализировать мутации вируса в регионах Core и HBsAg при моноинфекции и ВГВ/ВИЧ-коинфекции.

Материалы и методы. Материалом для исследования послужили 2616 проб сыворотки крови жителей Гвинейской Республики. Пациенты были обследованы на наличие маркеров ВГВ: HBsAg, HBs IgG и HBCore IgG. Нуклеотидные последовательности полного генома ВГВ были получены для 298 образцов, включая пациентов с коинфекцией ВИЧ/ВГВ. Амплификацию и последующее секвенирование вируса проводили с использованием вложенной ПЦР с парными перекрывающимися праймерами, совместно фланкирующими полный геном ВГВ (ген S, P, C, X).

Результаты. Серологические маркеры ВГВ выявлены в 80,77% проб, HBsAg — у 16,01% обследованных. ДНК ВГВ выявили в 22,36% случаев. Распространенность HBsAg-отрицательного ВГВ у пациентов с РНК ВИЧ составляет 45,16%, что значительно выше, чем в группе без ВИЧ-инфекции (6,07%). В обследованной группе преобладал ВГВ генотипа Е — 75,5%, по сравнению с D1 — 9,39%, D2 — 4,02%, D3 — 6,37%, D2 — 4,7%. Вариабельность аминокислот среди образцов ВГВ была выше в регионе PreCore/Core, чем в регионе PreS1/PreS2/S. Мутации SHB были обнаружены в 83,89% случаев, мутации Core у 94,29%, замены аминокислот PreCore у 16,77% пациентов соответственно.

Результаты, полученные в этой работе, демонстрируют высокую распространенность ВГВ в регионе и указывают на необходимость дальнейших крупномасштабных исследований мутаций ВГВ с целью улучшения стратегий контроля и профилактики заболеваний в Гвинейской Республике.

Ключевые слова: ВГВ, генотипы, аминокислотные замены, мутации вакцинного бегства, мутации лекарственной устойчивости, молекулярная эпидемиология, Гвинейская Республика

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IgG, and HBCore IgG. HBV complete genome nucleotide sequences were obtained for 298 samples including HIV/HBV co-infected patients. Amplification and subsequent sequencing of HBV were performed using nested PCR with pair’s overlapping primers jointly flanking the complete HBV genome (S, P, C, X genes).

Results. HBV serological markers were detected in 80.77% samples, while HBsAg was detected in 16.01% of the examined group. HBV DNA we detected in 22.36%. The prevalence of HBsAg-negative HBV in patients with HIV RNA is 45.16%, which is significantly higher than 6.07% found in the group without HIV infection. Phylogenetic analysis of HBV in the examined samples showed that HBV genotype E (75.5%) predominates in the group compared to HBV genotype D1 (9.39%), D2 (4.02%), D3 (6.37%), and A2 (4.7%). In the tested group, the variability of amino acids among the HBV samples was higher in the PreCore/Core region than in the PreS1/PreS2/S region. SHB mutations were detected in 83.89%, Core mutations in 94.29%, PreCore amino acid substitutions in 16.77% of the patients, respectively.

The results obtained in this work demonstrate a high prevalence of HBV in the region and indicate the need for further large-scale studies of HBV mutations in order to improve strategies for disease control and prevention in the Republic of Guinea.

Key words: HBV, genotypes, amino acid substitutions, vaccine escape mutations, drug resistance mutations, molecular epidemiology, Republic of Guinea

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Introduction. Hepatitis B virus (HBV) is one of the most common hepatotropic viruses which can cause both acute and chronic liver diseases. More than 240 million people have been diagnosed with chronic hepatitis B infection (CHB) [1]. The main laboratory diagnostic marker is the HBV surface antigen (HBsAg), the occurrence of which in population varies by geographic region.

HBV infection is prevalent in the African continent; the number of people in Africa with CHB is over 75 million, 25% of whom will presumably die from liver disorders. The occurrence of the HBV surface antigen in African countries exceeds 8% and may reach 25% [1]. There is a wide variation in HBsAg prevalence estimates in these countries. The highest prevalence of the virus has been found in sub-Saharan Africa, in such West African countries as Liberia (17.55%), Mali (15.5%), Burkina Faso (14.5%), Ghana (13.8%), Senegal (13.8%), Nigeria (13.6%), and Mauritania (10.9%) [2, 3]. In East and South Africa, the HBsAg prevalence is relatively low and corresponds to the average level [1], whereas in such North African countries as Tunisia, Algeria, Egypt, and Morocco, the level is below 2% [4]. This can partially be explained by differences in risk factors and transmission routes in different countries. In general, the prevalence estimates at the country level indicate a high infection burden in sub-Saharan Africa [1].

Along with the differences in HBsAg prevalence between the countries, the occurrence of HBV surface antigen varies among different groups in the same region or country. However, it should be noted that data on the serological prevalence of HBV in the population are limited, since serological screening is often carried out only in certain population groups — in the high-risk groups (HIV-infected people, prisoners, IDUs, etc.) and in the groups where the prevalence of infection significantly affects the health of the population (blood donors, pregnant women). For instance, in Sierra Leone, the HBsAg+ prevalence among children was 18.18% in 1998, whereas the HBCore IgG antibodies were detected in 71% of children; only seven years later, when assessing the HBV prevalence in pregnant women from the middle and high social and economic classes, the HBsAg+ occurrence was found to comprise 6.2%, which
It is worth noting that in Africa HBV is transmitted mainly at an early age. Horizontal transmission takes place at the age of 2 to 10 years; children with high-level viremia are highly likely to transmit the virus through cuts and scratches to their susceptible siblings and playmates. Although horizontal transmission is the main route of virus transmission, perinatal transmission is believed to cause about 10% of chronic infection, and the low level of HBeAg occurrence in HBsAg-positive pregnant women in most African countries correlates with the low frequency of perinatal transmission. Moreover, 20–30% of patients infected in their early childhood become chronic carriers, while only 10% of them remain HBeAg-positive in adolescence [8].

Regarding the high occurrence of HBV in African countries, it should also be noted that these countries have the high prevalence of hepatocellular carcinoma which is known to account for almost 85% of all primary liver tumours and is the fifth most frequently diagnosed cancer disease in the world. In sub-Saharan Africa, HCC makes a significant contribution to the liver disease mortality due to the occurrence of high risk factors in the continent. West African countries are slightly behind, with HCC mortality in West Africa reaching 200,000 people per year. Moreover, due to late visits to doctors, most patients with HCC die within a few weeks after the diagnosis. In fact, the mortality from HCC in this region is almost the same as the morbidity. Gambia is the most affected country, followed by the Republic of Guinea, Liberia, and Sierra Leone [9]. One of the main reasons for the high prevalence of HCC in African countries is the HBV infection at an early age, late virus detection, and inadequate treatment, which is associated inter alia with insufficient diagnostic means available in the region.

Occult hepatitis B infection (OBI) is a phase of CHB in which HBV DNA is detected in the liver tissue with undetectable HBsAg levels in peripheral blood serum, whether or not HBV DNA is detected in peripheral blood [10]. Despite the absence of HBsAg in peripheral blood, most patients with OBI are seropositive for one or more serological markers, depending on the phase of disease progression, anti-HBs IgG, HBeAg, anti-HBe IgG, anti-HBCore IgG; however, over 20% of the patients are seronegative for all HBV markers [10]. The prevalence of OBI correlates with the prevalence of HBsAg-positive CHB.

A large proportion of HCC cases in African countries is associated with occult HB; OBI is detected in more than 75% of HBsAg-negative HCC patients [11]. Such a high prevalence of HBV is attributed inter alia to the OBI occurrence in the region. It should be noted that the methods for HBV detection and diagnostics of HBV-associated liver disease in low- and middle-income countries differ significantly from those used in countries with access to expensive technologies that require qualified personnel. Most research on this topic in African countries is limited to detection of HBV surface antigen, while molecular genetic methods that make it possible to more accurately assess the HBV prevalence, genotype the virus, quantify HBV DNA, detect the infection at low virus load in the case of occult CHB are available only in central laboratories of large cities.

HBV is characterized by a high degree of genetic heterogeneity; it is currently subdivided into ten geno-
types designated as A-I genotypes which differ from each other in the composition of nucleotide sequences by more than 8%. Additionally, genotypes A, B, C, D, F, and H are subdivided into 45 subgenotypes which show complete divergence of nucleotide sequences in the range from 4 to 7.5% [12]. Studies of the prevalence of HBV variants in different regions of the world have been actively carried out since the discovery of the genotype up to the present. All genotypes and serotypes differ in their geographic distribution which changes very slowly over time, reflecting the virus propagation pathways associated with human migration, as well as the possible different geographical origin of different genotypes, which allows to use them as epidemiological markers [1]. In most regions, with rare exceptions, there are 1–2 prevailing genotypes and several minor ones, including those imported from other regions [13]. Despite significant public health problems associated with HBV in sub-Saharan Africa, many countries in this region do not perform systematic HBV monitoring and there is no information on genetic characteristics of virus variants prevailing in a particular region. Nonetheless, even though molecular genetic methods are insufficiently used in the region, some genetic variants of the virus are known that are characteristic of African countries. A, D and E genotypes are the most common HBV genotypes found in Africa. For instance, although genotype A is widespread throughout the world, on the African continent it is predominant in South, Central, and East African countries. In general, genotype E is prevalent in West and Central Africa. However, the prevalence of these genotypes can vary significantly even within the same country.

Vaccination and anti-viral therapy are key approaches to reducing morbidity and mortality from HBV infection. However, after nearly half a century of the development and implementation of therapeutic drugs and effective HBV vaccine, and significant global vaccination coverage, HBV infection is still a serious health problem worldwide. A high degree of HBV genetic variability allows the virus to respond to endogenous and exogenous selective pressures by further modulating its genome structure. During prolonged infection and under different selective pressures, some variants, particularly in the S gene, might evolve and thereby assist the virus to escape therapeutic, prophylactic, and diagnostic measures.

**Mutations in the PreS/S gene.** The envelope gene of HBV has three open reading frames (ORFs), PreS1, PreS2, and S, which encode three proteins, the small, middle, and large HBsAg translated from distinct mRNAs. The large S gene of HBV encodes the preS1 protein (108 aa), the preS2 protein (55 aa), and the small S protein — HBsAg (226 aa). Mutations in the S region (the region of the hepatitis B small surface antigen, SHB) mainly occur in the N-terminal region (aa 1–99) and in the major hydrophilic region (MHR) (aa 100–169), rather than in the C-terminal area (aa 170–226), both before and after antiviral therapy. MHR, for which a relatively large number of amino acid substitutions were shown, includes the “α” determinant (aa 124–147), the tertiary structure of which determines antigenic specificity. Thus, the efficacy of vaccination and therapy may be reduced due to the emergence of clinically significant mutations: immune-escape mutations (IEMs), drug resistance mutations (DRMs), and mutations affecting the progression of the disease. Due to the overlap of the S and the reverse transcriptase (RT) genes, DRMs in the RT gene can lead to the emergence of IEMs in the MHR and vice versa.

Mutations occur due to the high error rate of the RT enzyme without the possibility of proofreading and provide a selective advantage in avoiding the effect of drug therapy and spreading in the population. Moreover, some mutations lead to resistance to only one agent, while others are associated with resistance to several agents. Depending on the drug to which mutations cause resistance, resistance to 3TC (primary and compensatory), ETV (combination of amino acid substitutions), and TFV is known. In African countries, drug choices are usually limited to 3TC or tenofovir. Among patients with chronic HBV monoinfection, the incidence of HBV 3TC resistance reaches 20% per year. In patients with HIV + HBV coinfection, this indicator can reach 90% after 5 years of treatment, since the development of resistance is accelerated by HIV coinfection [14]. The presence of amino-acid substitutions in the MHR of HBsAg can alter the immunogenicity of HBsAg and can contribute to immune escape. Immune-escape variants of HBV may be associated with the failure of diagnostic tests. Because an escape mutation alters the protruding loop of the “α” determinant, pre-existing neutralizing antibodies cannot adequately recognize the altered epitope [15]. These mutants can cause HBV reactivation even in anti-HBs-positive patients and can spread despite correct active/passive vaccination strategies. Thus, immune-escape variants of HBV present a clinical challenge, because these mutations can lead to the diagnostic failure of
commercial assays for HBsAg, as well as to the pro-
phylactic failure of immunoglobulins or vaccines and
to HBV reactivation. Some Pre-S mutations are associ-
ated with the progression of liver disease. For
example, Pre-S2 deletions are frequently observed in
cases of liver cirrhosis and HCC. Pre-S2 start codon
mutations are associated with cases of liver cirrhosis
and HCC as well; they can also impair secretion of
the virus. In addition, mutations in the vicinity of the
Pre-S2/S splice donor site were common in occult
HBV infections.

Mutations in the PreCore/Core gene. The HBV
core protein encoded by the HBV core gene from the
PreCore/Core ORF has 183 aa and is essential for
viral replication. It is a 21 kDa structural protein
involved in both structural and functional processes.
It plays a key role in the HBV life cycle, and alter-
ations in the protein sequence may serve as potential
markers of disease progression [16]. Some of the
basal core mutations promoters were identified as
possible prognostic markers for the development of
cirrhosis and HCC.

The Republic of Guinea is a region with a high
prevalence of many viral infectious diseases, including
those caused by hepatotropic viruses, which empha-
sizes the importance of the epidemiologic assess-
ment of this territory for determining the HBV prev-
ance [17]. However, studies in this region are limited in both
the quantity and quality of the diagnostic methods
used. In Guinea, an assessment of the HBsAg preva-
ience in various groups showed that the incidence
among HIV-infected people, diabetics and prisoners
was 8%, 8–9%, and 27.7%, respectively [18]. An ear-
lier phylogenetic analysis of samples obtained in 2006
from asymptomatic HBsAg carriers from Conakry con-
firmed the prevalence of genotype E which comprised
95.1% and a wide representation of deletions in the
core region among circulating strains [19]. While sev-
eral dozens of studies have been published for some
countries, allowing to summarize the available data
and obtain reliable HBV information for these territ-
ories, very few publications can be found for other coun-
tries, and they are frequently not only limited in terms
of the methodology, but also contradict each other. We
found no publications on the prevalence of OBI or
mutations of HBV strains circulating in the Republic of
Guinea. In this region, practically no studies on geno-
typic characterization, analysis of HBV vaccine escape
mutants and drug resistance have been carried out.

Aim. The aim of this study was to characterize the
genetic variants of HBV currently circulating in the
Republic of Guinea, based on the nucleotide
sequences of the complete virus genome, and to ana-
lyze clinically significant mutations in the Core and
HBsAg regions during HBV monoinfection and
HBV/HIV coinfection.

Materials and Methods. The study material was
represented by 2616 blood serum samples collected
from residents of the Republic of Guinea — blood
donors, conditionally healthy people. The donors
denied the anamnesis of HBV infection.

The subjects were examined for the presence of
HBV markers with a qualitative detection of HBsAg,
HBs IgG, and HBCore IgG using test-systems man-
ufactured by Vector-Best (Russia) and Diagnostic
Systems (Russia) in accordance with the manufac-
turer’s instructions.

For the primary detection of HBV, nucleic acids were
extracted from blood serum using the AmplPrime Ribo-
Prep kit from the Central Research Institute of
Epidemiology (CRIE, Russia). The test for the presence
of the virus was performed by real-time polymerase
chain reaction (RT-PCR) with hybridization fluores-
cence detection using the AmpliSens® HBV-FL kit
(CRIE, Russia) with a sensitivity of 50 IU/ml. Next, we
used a PCR-based method developed by the
St. Petersburg Pasteur Institute which allows to detect
low concentrations of HBV DNA in various clinical sam-
ple and uses amplified products for sequence analysis;
the sensitivity was 5 IU/ml [20]. Amplification and sub-
sequent sequencing of HBV were performed using nest-
ed PCR. At the first stage, asymmetric PCR with
extended oligonucleotides was carried out; at the second
stage, to increase the sensitivity, PCR was carried out
using the amplification products of the first reaction and
one of the nested pair’s overlapping primers jointly
flanking the complete HBV genome (S, P, C, X genes).
The products of the sequencing reaction were analyzed
using an ABI Prism 3500 genetic analyzer (Applied
Biosystems, USA). The primary analysis of the obtained
fragments was performed using the BLAST algorithm
(http://www.ncbi.nlm.nih.gov/BLAST) on the
nucleotide sequences provided in the GenBank
sequence database. The resulting sequences were
aligned in the MEGA 7.0 program using the ClustalW
algorithm. The phylogenetic tree was constructed using
the neighbor-joining method; the significance of the tree
was assessed using bootstrap analysis with 1000 repli-
cates. The amino acid sequence of the envelope protein
was determined by translating the corresponding
nucleotide sequence according to the open reading
frame.
The nucleotide sequences of the complete genomes of some HBV isolates examined in this work were deposited to the GenBank database under the accession numbers MN507840-MN507849, MW455161-MW455166.

Statistical data processing was carried out using the MS Excel (Microsoft, USA) and Prizm 5.0 software (GraphPad Software, USA). To assess the statistical significance of the numerical data obtained during pairwise comparison depending on the sample characteristics, the Fisher’s exact test or the Chi-square test with Yates’ correction were used. The probability value \( p<0.05 \) was taken as the statistical significance threshold.

**Results and Discussion.** We examined 2616 clinical blood serum samples; HBV serological markers were detected in 2113 samples, or 80.77%, while HBsAg was detected in 16.01% of the examined group. When examining 2,616 clinical blood serum samples for the presence of HBV DNA using the AmpliSens® HBV-FL kit, we detected the virus in 426 samples, or 16.28%. However, although HBsAg was detected in combination with other markers in most samples, there were also seven HBsAg-negative samples. In five cases, the viral load was below 100 IU/ml, in two cases it was slightly above 50 IU/ml, however, the viral load of about 50 IU/ml was detected in only one out of seven HBV DNA-positive HBsAg-negative samples. When examining serum samples for the presence of HBV DNA using the PCR-based method developed by the St. Petersburg Pasteur Institute, we detected the virus in 585 samples, or 22.36%.

We previously showed that serological markers of HBV are detected in 29.03% of HIV-positive patients, including 16.12% of HBsAg. HBV DNA was detected in all HBsAg-positive and two anti-HBCore IgG-positive patients, as well as in twelve patients who were negative for all HBV serological markers analyzed in the work. Thus, HBV DNA was found in 61.29% of HIV-positive patients [21].

The probability of detecting HBV serological markers in HIV-infected patients is significantly lower than that in patients from the group without HIV infection: \( \chi^2=48.379, \ p<0.0001, \text{ df}=1, \text{ RR}=2.741, \text{ CI: } 2.053–3.658 \). The prevalence of HBsAg-negative HBV in patients with HIV RNA is 45.16%, which is significantly higher than 6.07% found in the group without HIV infection: \( \chi^2=115.07, \ p<0.0001, \text{ df}=1 \). The risk of developing HBsAg-negative CHB in HIV-infected patients is more than ten times higher than in patients without HIV: \( \text{RR}=10.969, \ p<0.0001, \text{ CI: } 7.823–15.379 \).

The development of OBI is determined by suppression of the endonuclear transcription of sub-genomic HBV RNA from the covalently closed circular HBV DNA matrix; the RNA is implicated in the synthesis of the viral genome and viral proteins. The suppression may be caused by a variety of factors which are not yet fully understood, including genetic characteristics of the virus itself and/or of its host, or by external interference. HIV coinfection is one of these factors. A reactivation of OBI can be caused by immunodepression/immunosuppression induced by HIV infection. These results are consistent with published reports that the OBI prevalence in HIV-infected patients ranged from 0 to 10% when using PCR-based testing with standard commercial kits and up to 35–89% when using more sensitive versions of the method. OBI has been shown to be associated with high levels of HIV RNA. Since HIV is capable of suppressing HBV replication, this leads to the prevalence of occult CHB among HIV-infected patients, whereas HIV-induced immunosuppression can lead to low antibody response to HBsAg, as well as HBV reactivation [4]. It should be noted that the registered high incidence rates of OBI among HIV-infected patients reflect the assumption of higher rates of HIV/HBV prevalence in West and South African countries that can be as high as 30% [5].

Nucleotide sequences of the complete HBV genome were obtained from 298 samples, including patients with HIV/HBV coinfection. Phylogenetic analysis of HBV in the examined samples showed that HBV genotype E (75.5%) predominates in the examined group compared to HBV genotype D (19.8%) and HBV genotype A (4.7%). It should be noted that genotype A is represented by subgenotype A2, whereas genotype D — by subgenotypes D1, D2, and D3. Thus, HBV genotype E comprising 75.5% predominates in the group compared to HBV genotype D1 (9.39%), D2 (4.02%), D3 (6.37%), and A2 (4.7%). The phylogenetic relationships between the examined HBV isolates from patients living in the Republic of Guinea and the reference sequences from the GenBank database are shown in Figure.
It should be noted that genotype E and genotype D were detected in both HBsAg-negative and HBsAg-positive samples. We previously showed that among patients with HIV/HBV coinfection, HBV genotype E and HBV genotype D have equal representation in the group (47.36%), while HBV genotype A was detected in only one case (5.26%) [3]. A comparative analysis of the prevalence of HBV subgenotypes among patients with or without HIV infection showed a significant difference: $\chi^2 = 12.597$, $p = 0.0134$, df = 4. Thus, the prevalence of HBV genotype D among patients with HIV/HBV coinfection is higher than among patients with HBV monoinfection: $\chi^2 = 7.948$, $p = 0.0048$, df = 1, RR = 2.643, 95% CI = 1.546–4.520.
In regions that are geographically close to the Republic of Guinea, the prevalence of HBV genotypes is generally consistent with our results. For example, in Côte d’Ivoire, the distribution of HBV genotypes was 6.3% for genotype A, 6.3% for genotype D, and 87.4% for genotype E. In Burkina Faso, genotype E comprises 72%, while subgenotype A3 — 25%. In Mali and Senegal, HBV genotype E is also predominant — 91.1% and 75%, respectively. In Côte d’Ivoire and Uganda, HBV/A2 was present as a minority HBV/A variant. HBV/A2 strains similar to some isolates characteristic of Europe suggest the spread of HBV from South Africa to other regions [17]. Genotype D is widespread throughout the world, whereas its subgenotypes have distinctive geographic distribution. Subgenotype D1 is found in North African countries, while subgenotype D3 in South African countries. Genotype D which is presumably associated with more severe liver diseases was identified mainly in North Africa and some East African countries [17]. HBV genotype E is highly epidemic in most South and West sub-Saharan countries, where it accounts for more than 90% of total HBV, while regions in which genotype E is endemic have a higher incidence of HCC [9, 17]. Despite its wide geographic distribution, genotype E is characterized by a low genetic diversity, which apparently indicates its short evolutionary history. HBV genotype E is epidemiologically associated with chronic and occult HBV infections, as well as with HCC, drug resistance, and vaccine escape.

In the tested group, the variability of amino acids among the HBV samples was higher in the PreCore/Core region than in the PreS1/PreS2/S region. SHB mutations were detected in 83.89%, Core mutations in 94.29%, PreCore amino acid substitutions in 16.77% of the patients, respectively. Information on the most common clinically significant mutations identified in the examined group is presented in the Table.

Mutations associated with amino acid substitutions in the reverse transcriptase domain are classified as primary or secondary compensatory resistance mutations. None of the patients had known primary drug resistance HBV mutations; however, among samples with HBV genotype E, we identified mutations at specific positions of reverse transcriptase associated with drug resistance, including primary mutations that are potentially associated with reduced sensitivity to antivirals and the secondary compensatory resistance mutations that generally restore the replication capability of the viral polymerase (Table).

Due to the overlap between the genes encoding reverse transcriptase and HBsAg, drug resistance mutations can introduce mutations in the MHR of HBsAg. To date, more than 30 immune-escape mutations in HBsAg have been identified; they allow the virus to evade neutralizing antibodies and facilitate persistent HBV infection and viral fitness. Immune-associated escape mutations can also interfere with HBsAg recognition by antibodies induced by the vaccine, thus posing a potential threat to the global vaccination program [22]. We determined the prevalence of 10 immune-associated escape mutations affecting HBsAg-recognition by antibodies. Most of these substitutions are known to act as vaccine-escape mutations (Table). Mutations in the aa 124–137 region are believed to interact with protective or neutralizing anti-HBs antibodies resulting from natural infection as well as from vaccination with HBsAg. HBV genotype E is known to exhibit clear genotypic divergence from all other genotypes within the «a» determinant which can have escape mutations. The most common polymorphisms in genotype E are known to be T116N, P120L/S, Q129H/R, M133I, D144E, and G145I. In our work, we see the confirmation of this data.

Of particular interest is the detection of HBV DNA in six samples (HBsAg- HBCore IgG+ HBs IgG+). Five samples belonged to genotype E and one to genotype D1. Anti-HBCore IgG+ usually appears in the acute phase of HBV infection and persists for a long time after the virus clearance, while the presence of anti-HBs IgG+ together with anti-HBCore IgG+ usually indicates, resolved infection. In the absence of HBsAg, serum anti-HBs IgG+ indicate protective immunity against HBV acquired through vaccination (HBCore IgG-) or natural infection (HBCore IgG+) [23]. In patients with CHB, the coexistence of HBsAg and HBs IgG+ is associated with an increase in the variability of the “a” determinant, suggesting the selection of HBV immune-escape mutants during chronic carriage. The most frequent changes are localized at positions 123, 126, 129, 130, 133, 144, 145 and 181, as described for immune-escape variants, and some of them, including aa 119–123, have been reported to play a role in the mechanism of occult HBV infection [6, 14, 15, 17]. However, in each of the six cases we described, escape mutations associated with diagnostic failure and vaccine escape were also found. These patients may develop liver cir-
rhosis, HCC, and liver failure. Vaccinated people are at risk of contracting strains with such mutations. Of particular importance are 31 samples with a combination of T127P + S204R mutations in the SHB region: this combination, together with the F170L mutation, has previously been observed in an HBsAg-negative patient with HBV reactivation 3 months after the termination of long-term prophylactic treatment with lamivudine [Ошибка: источник перекрёстной ссылки не найден]. The authors suggested that these mutations may interfere with the HBsAg recognition in the diagnostic assays.

The incidence of the G1896A mutation was significantly higher among HBV genotype E samples (47.11%) compared to samples of all other genotypes (5.48%): \( \chi^2 = 39.254, p<0.0001, \text{df}=1, \text{RR}=8.598, 95\% \text{CI}=3.282–22.522 \). The correlation of HBV genotype E with an increased risk of developing HCC can also be explained by the substantial proportion of patients harbouring a viral strain with the G1896A mutation [25].

### Table

| HBV genotype region | Mutation | Prevalence in all group | Genotype; prevalence in genotype | Description |
|---------------------|----------|-------------------------|-----------------------------------|-------------|
| RT                  | L80F     | 3.69%                   | E (4.88%)                         | The mutations are not described, however, as change at given positions were can be considered as possible mutations of resistance to lamivudine, telbivudine, and entecavir |
| RT                  | S202R    | 5.03%                   | E (6.66%)                         | Associated with HBsAg-negative CHB |
| RT                  | S204R    | 10.4%                   | E (13.77%)                        | Associated with HBsAg-negative CHB |
| MHR                 | Y100C    | 4.69%                   | A (7.14%), E (5.77%)              | Escape-mutant (immune escape, vaccine escape, diagnostic escape). Since MHR is the most important antigenic determinant in envelope proteins and is composed of two loops bounded by disulfide bridges between cys124 and cys137, and cys139 and cys147, mutations in this region breaks disulfide bridges. In-silico structure prediction studies suggested that the three dimensional conformation of the extravirion loop of isolates with aa substitutions between residues 133 and 144 are different from that of the wild-type genotype E, and the loop is embedded in the polar head-groups of the extravirion leaflet of the virion membrane. Some of them leading to surface antigen production different from the usual one |
| MHR                 | M103I    | 3.02%                   | E (4%)                            | |
| MHR                 | T127P    | 19.12%                  | D (96.61%)                        | |
| MHR                 | Q129H/R  | 19.79%                  | E (20.44%), D (22.03%)           | |
| MHR                 | M133I    | 22.14%                  | E (25.33%), D (11.86%), A (14.28%) | |
| MHR                 | Y134H    | 5.7%                    | E (7.55%)                         | |
| MHR                 | C137Y    | 10.4%                   | E (13.7%)                         | |
| MHR                 | K141E    | 8.38%                   | E (11.11%)                        | |
| MHR                 | D144E    | 5.36%                   | E (7.11%)                         | |
| MHR                 | G145R    | 4.69%                   | E (6.22%)                         | |
| MHR                 | Y147C    | 3.69%                   | E (4.88%)                         | |
| PreCore             | H5D      | 29.19%                  | E (38.66%)                        | In HBsAg-negative HBV genotype E. Associated with severe liver disease. This mutation could partially explain the high prevalence of HCC in Africa |
| PreCore             | K21N     | 6.37%                   | D3 (100%)                         | Suspected of being associated with severe disease in HBsAg-negative patients |
| PreCore             | W28* (G1896A) | 36.91% | E (47.11%), D + A (5.48%) | To affect HBeAg production negatively. Creates a stop codon and prevents the synthesis of HBeAg. Responsible for more than 90% of defective HBeAg secretion and to affect the HBeAg serostatus |
| PreCore             | G29D     | 18.79%                  | E (24.88%)                        | The mutation is associated with hepatocellular carcinoma development and affects the HBeAg serostatus |
| Core                | L116I    | 94.63%                  | E (97.33%), D (88.13%), A (78.57%) | Changes between aa 113 and 143 influence the antigenicity and stability of the particle. May create immune escape mutants leading to chronic viral persistence and severe liver disease. Located within B-cell epitopes, and is associated with disease progression, cirrhosis, and hepatocellular carcinoma development |
| Core                | T146N    | 75.5%                   | E (100%)                          | Possibly characteristic of HBV genotype E |
It is interesting that the same HBV mutations were detected both in the studied group among HIV-infected patients and in patients without HIV infection. Moreover, the incidence of certain mutations in the subgroups did not differ significantly. The absence of significant differences in the prevalence of HBV mutations between mono- and coinfected patients that were either HBsAg-negative or HBsAg-positive may indicate an independent natural development that is not associated with the coinfection or the occult CHB infection. Immune-escape variants do not usually occur alone in HBV strains of infected patients, but in combination with polymerase and/or core mutations. It is therefore important to understand the functional implications of immune-escape mutations for the replication of HBV strains, especially for strains with common treatment-associated mutations, such as drug resistance. Quasispecies with higher complexity should theoretically tend to breach genetic barriers, thus leading to drug resistance. The discovery of the coexistence of complex HBV mutants, for example, immune escape and drug resistance, represents a serious challenge requiring antiviral therapy adjustments.

Screening for HBV DNA is not routinely done in the Republic of Guinea due, at least in part, to the high cost and lack of HBV treatment programs outside of coinfection with HIV. Patients infected with HBV either are left untreated (HBV monoinfection) or undergo ART (HIV + HBV coinfection) without proper monitoring, which greatly increases the risk of developing DRMs.

**Conclusion.** Although the information on HBV genetic diversity and clinically significant mutations is crucial for patient management, it is scarce for the Republic of Guinea. The results obtained in this work demonstrate a high prevalence of HBV in the region and indicate the need for further large-scale studies of HBV mutations in order to improve strategies for disease control and prevention in the Republic of Guinea. At the same time, methods should be used that allow not only detecting manifest and occult CHB forms, but also sequencing identified isolates, since although vaccination protects against a wide range of strains, it cannot neutralize the so-called vaccine-escape strains, which can lead to seronegative OBI with subsequent reactivation.

In this study, we for the first time showed the presence of HBV isolates with immune-escape mutations in the Republic of Guinea. These findings emphasize the need for careful monitoring of specific mutational patterns, as these data raise the question of possible transmission of HBV genotype E to vaccinated individuals.

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