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1 **Prevalence and molecular characterization of hepatitis B virus** 2 **infection in HIV-infected children in Senegal**

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35 **Abstract**

36 **Background and aims**

37 Sub-Saharan Africa (SSA) is the region with the most patients co-infected with the human
38 immunodeficiency virus (HIV) and the hepatitis B virus (HBV) worldwide. However, few
39 studies have focused on SSA children who are at a higher risk of developing a chronic
40 infection than adults. Furthermore, children on first-line antiretroviral therapy (ART)
41 including low genetic barrier drugs may develop both HBV and HIV resistance mutations.
42 The aim of this work was to document HIV-HBV co-infection and to characterize the HBV
43 isolates in children in Senegal.

44 **Methods**

45 This is a retrospective study of 613 children infected with HIV on ART or not. Dried blood
46 spot (DBS) specimens were used to detect hepatitis B surface antigen (HBsAg) with a rapid
47 diagnostic test (RDT). Confirmation of HBsAg status and hepatitis B e antigen (HBeAg)
48 detection was performed on an automated platform using the chemiluminescence assay
49 technology. HBV viral DNA was quantified by real-time polymerase chain reaction (PCR)
50 and the preS1/preS2/HBsAg region was genotyped by nested PCR followed by sequencing
51 using the Sanger technique.

52 **Results**

53 The prevalence of HIV-HBV co-infection was 4.1% (25/613). The median age of co-infected
54 children was 13 years (2 years-16 years) and 40% (10/25) were girls. Almost all 19/20 (95%)
55 were infected with HIV-1 and 79% (19/24) were treated with 3TC-based triple combination
56 ART. The median duration of time on ART was 15 months (3 months-80 months). More than
57 half of the children 53% (9/17) were experiencing HIV virologic failure and 75% (6/8) had at
58 least one HIV-related RAM. Of the six children with resistance, none of the three
59 administered treatments were effective on HIV. Of the 25 co-infected children, 82% (18/22)
60 were HBeAg-positive, while the median HBV viral load (VL) was 6.20 log₁₀ IU/mL (24/25
61 patients), and 62,5% (10/16) of the children had a persistent HBV viremia. Combination of
62 ART was the only factor associated with HBV viremia persistence. Amplification was
63 successful in 15 out of 16 patients (rate of 94%), and the ensuing phylogenetic analysis
64 revealed that eight strains (53%) belonged to genotype A and seven (47%) to genotype E.
65 HBV-related 3TC RAMs were uncovered in 20% of these patients (3/15). HBsAg escape
66 mutations were found in 20% of the children (3/15).

67 **Conclusions**

68 Our results showed a high level of drug resistance mutations to both HIV and HBV, a
69 significant level of HBsAg escape mutations, HBV DNA persistence and HIV virologic
70 failure in co-infected children in Senegal. The HBV genotypes found were A and E.

71 **Keywords**

72 HIV, HBV, children, Senegal, genotypes, resistance mutations.

73 **Abbreviations**

74 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AIDS, acquired
75 immunodeficiency syndrome; AZT, azidothymidine; CHB, chronic hepatitis B; CNLS,
76 Conseil National de Lutte contre le Sida; DBS, dried blood spot; EFV, efavirenz; EPI,
77 Expanded Program on Immunization; FTC, emtricitabine; HBeAg, hepatitis B e antigen;
78 HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma;
79 HIV, human immunodeficiency virus; Ig, immunoglobulin; K2P, Kimura 2 parameters;
80 LPV/r, lopinavir/ritonavir; MTCT, mother-to-child transmission; NJ, Neighbor Joining; nt,
81 nucleotide; NVP, nevirapine; PBS, phosphate buffered saline; PCR, polymerase chain
82 reaction; PMTCT, prevention of mother-to-child transmission; RAM, resistance-associated
83 mutation; RDT, rapid diagnostic-test; RT, reverse transcription; SSA, Sub-Saharan Africa;
84 TDF, tenofovir disoproxil fumarate; UNAIDS, Joint United Nations Program on HIV/AIDS;
85 VL, viral load; WHO, World Health Organization.

86 **Introduction**

87 Both human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are major health
88 issues worldwide. According to the World Health Organization (WHO), Sub-Saharan Africa
89 (SSA) alone encompasses 27 of the 36 million HIV-infected adults and children worldwide,
90 and approximately 6.1% (70 million) of the African population is infected with HBV [1].
91 Furthermore, although 7.4% of HIV-infected patients are co-infected with HBV worldwide
92 [1], this figure rises to 71-74% in SSA (the highest worldwide) [2,3]. Co-infection is well-
93 known to aggravate the prognosis of chronic hepatitis B (CHB), and poses a particular
94 challenge in children as the disease progresses rapidly towards chronicity leading to an
95 increased risk, over time, of death by cirrhosis or hepatocellular carcinoma (HCC) [1,4-7]. In
96 addition, children are highly vulnerable to HIV infection and display antiretroviral therapy
97 (ART) failure, especially in resource-limited settings [8]. However, few studies have focused
98 on co-infected SSA children.
99 According to the data obtained by the Joint United Nations Program on HIV/AIDS
100 (UNAIDS) in 2019, 1.7 million children are infected with HIV worldwide [9]. In addition,

101 according to the WHO, 5-8% of SSA children harbor HBV [2]. Mother-to-child transmission
102 (MTCT) of the viruses can be antenatal, perinatal or horizontal [5] and is still an important
103 public health issue. In Senegal, according to the Conseil National de Lutte contre le Sida
104 (CNLS), approximately 5,000 children are infected with HIV [10]. Most of these children are
105 on first-line ART including lamivudine (3TC) and/or other low genetic barrier molecules. The
106 reverse transcription (RT) step of HBV is prone to error owing to the lack of proofreading
107 ability of the polymerase [5]. Ten subgroups of HBV have been described (genotypes A to J)
108 with a distinct geographical distribution, three genotypes of which are primarily found in
109 Africa (A, D and E) [5,11,12]. Numerous studies have reported a predominance of genotype E
110 in West Africa [13] and in Senegal [14,15]. Finally, HBV genotype has been shown to play a
111 major role in disease progression and response to treatment [16,17]. The error-prone step also
112 explains the appearance of “quasi-species” in patients and possibly drug resistance variants
113 under nucleoside analog treatment. These mutations reduce the success of existing treatment
114 strategies [11]. Thus, in cases of HIV-HBV pediatric co-infection with treatments including
115 low genetic barrier drugs, these children may develop resistance mutations.

116 A recent study in children born to HIV-infected mothers in Dakar, capital of Senegal, reported
117 a significant prevalence of HBsAg carriage (3%) [18]. These results prompted us to conduct
118 the present study to document the rate of HIV and HBV co-infection and to characterize the
119 HBV strains in Senegalese children in other regions outside Dakar.

120 **Patients and methods**

121 **Study population**

122 This is a retrospective study, ancillary to the EnPRISE study that aimed at evaluating the
123 efficacy of ART in Senegalese children [19]. Samples were collected between March and
124 June 2015. The National Ethics Committee (n° 377/MSAS/DPRS/CNERS) validated this
125 study.

126 The samples were collected in health centers and regional hospitals in all regions of Senegal,
127 except in Dakar in which a similar study had previously been conducted. Samples were sent
128 to the Laboratoire de Bactériologie-Virologie (LBV) of the University Teaching Hospital A.
129 Le Dantec where the EnPRISE study analyzes were performed. They were then transferred to
130 the Centre Médical Inter-Armées laboratory and then to the molecular biology platform of the
131 Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formations (Iressef)
132 for the purpose of the study.

133 The study population consisted of dried blood spot (DBS) specimens of 613 HIV-infected
134 children (out of the 666 analyzed in EnPRISE), followed at 72 pediatric management sites,

135 treated or not with ART and included in the EnPRISE study. Most of these children were
136 administered triple combination therapy mainly AZT/3TC/NVP.

137 **HBV serology**

138 For each sample, the three remaining blood spots were eluted for at least two hours in 600
139 microliters (μL) of phosphate buffered saline (PBS) and the eluates were used for assays. All
140 analyzes were performed according to the manufacturers' instructions.

141 Immuno-chromatographic detection of HBsAg was performed using the Determine TM
142 HBsAg RDT (Alere TM Inc., Waltham Massachusetts, USA). The Architect® i1000SR
143 platform (Abbott Diagnostics, North Ryde, NSW, 2113 Australia) was used to confirm
144 HBsAg status and to detect HBeAg in co-infected individuals. This technique uses
145 chemiluminescent microparticle assay technology. HBV VL was determined in co-infected
146 patients by real-time PCR using the Amplix® automated platform (Biosynex, 67038
147 Strasbourg, France). The detection threshold of the device is 26 copies/mL.

148 **HBV DNA extraction, amplification and sequencing**

149 HBV viral DNA was extracted from 200 μL eluted DBS from the co-infected patients with a
150 VL greater than three \log_{10} IU/mL using the NucliSENS easyMAG commercial kit
151 (bioMérieux, Inc., 100 Rodolphe Street, Durham NC 27712 USA). This is an automated
152 magnetic extraction technique based on the BOOM technology, which uses silica particles.
153 The final elution volume was 50 μL and these extracts served as a template for the PCR.

154 The HBV S gene was amplified using five μL of the extracted DNA with the HBPr1 and
155 HBPr135 primers (outer PCR) followed by a nested PCR using HBPr2 and HBPr94 to
156 generate a fragment of about 1,197 nucleotides (nt). DNA in the 45 μL reaction mix was
157 amplified over 40 cycles with denaturing step at 94°C for 30s, annealing at 50°C for 30s and
158 elongation at 72°C for 30s [12]. The second round PCR products were enzymatically purified
159 using ExoSAP-ITTM PCR Product Cleanup (Affymetrix Inc., 3450 Central Expressway, Santa
160 Clara, CA 95051 USA) and sequenced.

161 The amplicons were sequenced by the Sanger method on an automated DNA sequencer ABI
162 3130 (Applied Biosystems, 50 Lincoln Centre Drive Foster City, CA 94404 USA) using five
163 primers (HBPr2, HBPr3, HBPr14, HBPr94 and HBPr134) covering the preS1/preS2/HBsAg
164 region [12].

165 **Data analysis**

166 The resulting sequences were assembled and edited with the SeqMan TM software
167 (DNASTAR, Inc., USA); the consensus sequences obtained were then submitted to the
168 HBVseq (<https://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html>) and HBVdb

169 (<https://hbvdb.ibcp.fr/HBVdb/>) databases for genotype determination and to search for
170 possible mutations. The Seaview version 4.5.4 platform enabled sequence alignment using the
171 muscle software, estimation of genetic distance using the Kimura 2 parameters (K2P) method
172 and construction of the phylogenetic tree using the Neighbor Joining (NJ) method (1000
173 bootstrap replicas). Reference sequences from the different genotypes and sub-genotypes
174 (only for genotype A) were included in the analysis, as well as closely related sequences
175 obtained by BLAST analysis. The sequences were also submitted to the Genafor
176 (<https://hbv.geno2pheno.org/>) database to search for HBsAg escape mutations. Associations
177 between the variables were tested using a Fisher's exact test. A p-value greater than or equal
178 to 0.05 was considered statistically significant.

179 **Results**

180 A total of 613 DBS from HIV-infected children were analyzed. Twenty-five were positive for
181 HBsAg, representing a global prevalence of 4.1%.

182 **Characteristics of the co-infected children**

183 The characteristics of the 25 co-infected children are shown in **Table I**.

184 The median age of co-infected children was 13 years (2 years-16 years) and 75% (15/20) were
185 aged ten years or more at the time of the study, thus born before 2005 when HBV vaccine was
186 not implemented in the Expanded Program on Immunization (EPI) in Senegal. Ten children
187 (40%) were girls. Almost all of the patients 96% (24/25) were infected with HIV-1 and were
188 treated with ART combination, mainly 3TC-based (79%) or TDF-based (21%) therapies,
189 except for one untreated child. The median duration of time on ART was 15 months (3
190 months-80 months) and 71% (17/24) were treated for at least six months.

191 The physicians estimated that 83% (20/24) of the children had a regular follow-up and 71%
192 (15/21) a good adherence. There was a significant correlation between the regularity of
193 follow-up and adherence to ART ($p < 0.01$). Among the 22 children with available
194 information, only one child had received prevention of mother-to-child transmission
195 (PMTCT) care.

196 **HIV virology and resistance data (obtained from the EnPRISE study results)**

197 HIV RNA results showed that only 47% (8/17) of children treated for at least six months had
198 an undetectable VL. There was no correlation between VL and duration on ART ($p = 1$). The
199 median HIV VL was 3.2 \log_{10} IU/mL (2.2 – 4.8). More than half of the children 53% (9/17)
200 were experiencing virologic failure ($VL > 3 \log_{10}$ IU/mL).

201 Among the eight children for whom HIV genotyping was done, 75% (6/8) had at least one
202 RAM. The HIV-related 3TC/FTC, AZT, NVP, TDF and EFV were found in 100% (6/6), 83%
203 (5/6), 83% (5/6), 50% (3/6) (though only one had taken it) and 17% (1/6) of the co-infected
204 children with RAMs. Of the six children with resistance, none of the three administered
205 treatments were effective. The 3TC/FTC RAM found was M184V. All of the patients with
206 HIV-related RAMs had received 3TC-based therapy, except one who was receiving
207 TDF/FTC/EFV. There was no correlation between HIV RAM and combination of ART ($p =$
208 0.46). All six children had a VL greater than three \log_{10} IU/mL. No correlation was found
209 between HIV RAM and HIV VL ($p = 1$). They were all on ART for at least six months. No
210 correlation between HIV RAM and duration on ART ($p = 1$).

211 In terms of genetic diversity, CRF02_AG was the predominant form 88% (7/8).

212 **HBeAg prevalence and HBV viral load**

213 The HBeAg marker was successfully tested in 22 patients, leading to a prevalence of 82%
214 (18/22) (Table I).

215 HBV DNA results showed that only 29% (5/17) of children treated for at least six months had
216 an undetectable VL, three of which were under TDF-based therapy. The median HBV VL in
217 24 co-infected patients was 6.20 \log_{10} IU/mL, with a VL greater than six \log_{10} IU/mL in 50%
218 (12/24) of children and an undetectable VL in 25% (6/24) of children (Table I). The VL was
219 greater than three \log_{10} IU/mL in 16 of the children. However, VL was undetectable in six
220 children despite them being positive for HBsAg and HBeAg in three cases.

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Table I. Characteristics of the 25 co-infected children

Characteristic	Number	Percentage (%)
Sex		
F	10/25	40
M	15/25	60
Sex ratio	1.5	
Age groups (years)		
[2 – 10[5/20	25
[10 – 16]	15/20 ¹	75
Median age (min-max)	13 (2-16)	
Average age ± SD	10.8 ± 4.4	
Regularity of follow up		
Regular	20/24	83
Not regular	4/24	17
Adherence to ART		
Good	15/21	71
Average	2/21	10
Bad	4/21	19
Access to PMTCT		
Yes	1/22	5
No	21/22	95
HBeAg-positive	18/22 ¹	82
HBV VL (log₁₀ IU/mL)		
<1.31	6/24	25
[1.31 – 3]	2/24	8
[4 – 6]	4/24	17
>6	12/24 ²	50
Combination of ART		
AZT+3TC+NVP	16/24	67
AZT+3TC+EFV	1/24	4
AZT+3TC+LPV/r	1/24	4
ABC+3TC+NVP	1/24	4
TDF+3TC+EFV	2/24	8
TDF+3TC+LPV/r	1/24	4
TDF+FTC+EFV	2/24	8
PCR positive	15/16	94
HBV genotype		
A	8/15	53
E	7/15	47
HIV profile		
HIV-1	19/20	95
HIV-2	1/20	5
HIV VL (log₁₀ IU/mL)		
<3	12/25	48
>3	13/25	52
HIV genotypes performed	8/13	62
RAMs		
HBV-related	3/15	20
HIV-related	6/8	75
HBsAg escape mutation	3/15	20

238 ¹Three co-infected children could not be tested for HBeAg for technical reasons.

239 ²One co-infected child could not be tested for VL for technical reasons.

240

241 **HBV genotype distribution**

242 Among the 25 co-infected children, 16 were eligible for genotyping as their VL was greater
243 than three log₁₀ IU/mL, 15 of which were successfully amplified and sequenced,

244 corresponding to an amplification rate of 94%. The characteristics of those 16 children are
245 shown in **Table II**.

246 The molecular analysis of HBV sequences found in the study showed that eight (53%) were
247 genotype A and seven (47%) genotype E. Two sequences, one genotype A and one genotype
248 E, were excluded from the phylogenetic analysis because they were too short, 860 nt and 732
249 nt respectively. The 13 remaining sequences were trimmed to have the same size for
250 alignment purposes. The final sequences were 1108 nt long for genotype A and 1105 nt for
251 genotype E. Partial preS1/preS2/S gene phylogenetic analysis of the 13 Senegalese HBV
252 sequences with the references from other countries is shown in **Figure 1**. All Senegalese HBV
253 A sequences were closely related to the Cuban HBV A4 isolate. HBV sequence data
254 generated in this study have been deposited into the GenBank database and were attributed
255 accession numbers MT010581-MT010595.

256 **Resistance-associated mutations (RAMs)**

257 The submission of the sequences to the databases showed that three samples had at least one
258 HBV-related 3TC RAM, with an overall resistance rate of 20% (3/15). The RAMs found were
259 L180M, M204V/I and S202N. Two mutations occurred in genotype A and one in genotype E.
260 There was no correlation between HBV RAM and HBV genotype ($p = 1$). No HBV-related
261 TDF RAM was found. All of the patients with RAMs had received 3TC-based therapy and
262 had an HBV VL of at least five \log_{10} IU/mL. No correlation was found between HBV RAM
263 and HBV VL ($p = 1$).

264 HBsAg escape mutations were detected in three patients, with a prevalence of 20% (3/15) in
265 the co-infected children. The mutations found were 120P/120T, 129H and 133L. They were
266 all vaccine and therapy escape mutations. Two occurred in genotype A and one in genotype E.
267 The patients with HBsAg escape mutants were all treated with ART combination
268 AZT/3TC/NVP and had an HBV VL greater than six \log_{10} IU/mL.

269 The higher HBV VL (7.8 \log_{10} IU/mL) was found in a male patient aged 14, under triple
270 combination ART including AZT+3TC+NVP for 44 months, with a low-intermediate HIV
271 VL (3.2 \log_{10} IU/mL); HBeAg-positive and genotype A, presenting resistance mutation to
272 both HIV and HBV.

273 **Factors associated with HBV persistence**

274 Among the 16 children with an HBV VL greater than three \log_{10} IU/mL, ten (62,5%) were
275 treated for at least six months. The only factor associated with persistent HBV viremia was
276 the combination of ART ($p = 0.047$).

277 **Table II.** Detailed characteristics of the 16 co-infected children tested for molecular analysis

Patient	Sex	Age	DBS sampling date	Regularity of follow up	Adherence to ART	Access to PMTCT	Combination of ART	3TC based ART	TDF based ART	Duration of ART (months)	HIV profile	HIV RNA (IU/mL)	HBV DNA (IU/mL)	HBeAg	HBV PCR	HBV genotype	HIV related RAM	HBV related RAM	HBsAg escape mutation
1	M	14	19/03/2015	Yes	Good	ND	AZT+3TC+NVP	Yes	No	44	HIV-1	1.50 x 10 ³	7.00 x 10 ⁷	Pos	Pos	A	Yes	Yes	No
2	M	13	19/03/2015	No	Bad	No	AZT+3TC+NVP	Yes	No	29	HIV-1	2.70 x 10 ⁴	2.00 x 10 ⁷	Pos	Pos	A	No	No	No
3	M	12	24/03/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	35	HIV-1	< 800	1.66 x 10 ⁵	NT	Pos	A	NT	Yes	Yes
4	M	4.5	19/03/2015	ND	Good	No	AZT+3TC+NVP	Yes	No	18	HIV-1	< 800	1.10 x 10 ⁶	Pos	Pos	A	NT	No	Yes
5	M	ND	25/03/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	19	HIV-1	< 800	5.80 x 10 ⁶	Neg	Pos	E	NT	No	No
6	F	16	25/03/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	80	HIV-1	2.40 x 10 ⁴	3.90 x 10 ⁷	Pos	Pos	A	Yes	No	No
7	F	14	21/04/2015	Yes	ND	No	TDF+3TC+EFV	Yes	Yes	14	HIV-1	<800	1.89 x 10 ⁴	Pos	Pos	A	NT	No	No
8	F	11	21/04/2015	Yes	ND	No	AZT+3TC+NVP	Yes	No	13	HIV-1	1.20 x 10 ⁴	1.50 x 10 ⁶	Pos	Pos	A	Yes	No	No
9	F	10	21/04/2015	Yes	Average	No	AZT+3TC+NVP	Yes	No	5	ND	< 800	2.50 x 10 ⁶	Pos	Pos	E	NT	No	No
10	M	5	21/04/2015	No	Bad	No	AZT+3TC+NVP	Yes	No	3	HIV-1	< 800	1.30 x 10 ⁶	Pos	Pos	E	NT	Yes	No
11	M	2.4	21/04/2015	No	Bad	No	AZT+3TC+LPV/r	Yes	No	3	HIV-1	3.30 x 10 ³	6.00 x 10 ⁷	Pos	Pos	E	NT	No	No
12	M	ND	08/06/2015	Yes	Good	Yes	TDF+3TC+LPV/r	Yes	Yes	5	HIV-2	5.80 x 10 ³	5.60 x 10 ⁶	Pos	Pos	A	NT	No	No
13	F	16	10/06/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	17	HIV-1	< 800	1.90 x 10 ⁵	Pos	Pos	E	NT	No	No
14	M	10	10/06/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	4	ND	< 800	3.50 x 10 ⁶	Pos	Pos	E	NT	No	Yes
15	M	13	10/06/2015	Yes	Average	No	AZT+3TC+NVP	Yes	No	40	HIV-1	4.30 x 10 ⁴	1.29 x 10 ⁴	Pos	Neg	NT	Yes	NT	NT
16	M	13	10/06/2015	No	NA	No	NA	NA	NA	NA	HIV-1	6.90 x 10 ⁴	1.60 x 10 ⁵	Pos	Pos	E	NT	No	No

278 F, female; M, male; NA, not applicable; neg, negative; ND, not documented; NT, not tested; pos, positive.

280 **Discussion**

281 The aim of this study was to determine the rate of HIV-HBV co-infection and to characterize
282 the associated HBV isolates in children in Senegal.

283 In our study, the overall prevalence of HBsAg was 4.1% (25/613). These results are greater
284 than those of preliminary studies in Senegalese children recruited in three of the main
285 children's hospitals [20] and in HIV infected children in Tanzania that documented a HBsAg
286 prevalence of 1.1% (3/295) and 1.2% (2/167) [21], respectively. This discrepancy may be due
287 to the fact that the majority of children in the latter Senegalese study were vaccinated against
288 HBV. However, the vaccine is only administered to children older than six weeks, leaving a
289 gap for potential MTCT, which would explain the positive cases observed despite the vaccine.
290 A dose administered at birth, as recommended by the WHO [1], could lead to an even greater
291 reduction in this prevalence, if not an elimination. Regarding the Tanzanian study, the
292 observed difference may be due to the lower prevalence of HBV of 6% in the general
293 population [22] compared to the prevalence of 11% in Senegal [20].

294 Although HBV vaccine was implemented at around the same time in these countries, a higher
295 HBsAg prevalence than in our study was found in HIV-infected children in Ivory Coast [23],
296 Nigeria [24], [25] and Zambia [26], 12.1% (34/280), 7.8% (31/395), 7.7% (12/155) and
297 10.5% (43/411), respectively.

298 In terms of HIV results, more than half of the children 53% (9/17) were experiencing
299 virologic failure. These results are consistent with those obtained in HIV-infected children in
300 Senegal 56% (70/125) [8]. Moreover, 75% (6/8) had at least one RAM. In the latter
301 Senegalese study, a higher rate of 92% (48/52) was found.

302 In addition, the immunization status of children in our cohort was unknown, while in most
303 studies, children had received at least one dose or all three doses of the HBV vaccine [20,27].
304 However, in our study, the majority of HBsAg-positive children 75% (15/20) were aged ten
305 years or more, thus born before 2005 when HBV vaccine was not implemented in the EPI in
306 Senegal. We can therefore assume that they were not immune. In this context, it would be
307 urgent to organize HBV screening campaigns in people born before the implementation of the
308 vaccine for preventive or therapeutic management if necessary.

309 Regarding mothers, their HBsAg status or any other HBV marker was not documented in our
310 study; we have thus not been able to establish whether this is MTCT of the infection.
311 However, our results suggest HIV-HBV co-infection of mothers. A previous study showed
312 that all children infected with HBV were born to mothers carrying HBeAg [27], which was

313 corroborated by Senegalese [18] and South African [28] studies in which 89% (25/28) and
314 11% of mothers of these children were HBsAg-positive. In addition, in Senegal, a previous
315 study estimated a prevalence of HBsAg carriage in pregnant women around 11% [29].
316 Moreover, only 5% (1/22) of the children had access to PMTCT in our study. These data
317 suggest a vertical transmission of the infection.

318 In our study, the prevalence of HBeAg was 82% (18/22). Similar results were obtained in
319 HIV-infected children in the Ivory Coast, 82% (28/34) [23].

320 Moreover, 53% (8/15) of strains tested were genotype A and 47% (7/15) genotype E. These
321 results are similar to those found in adult patients in Senegal [30], displaying 55% (17/31) of
322 genotype A and 42% (13/31) of E. However, several studies have reported different genotype
323 E ratios of 70.1% (47/67), 70% (19/27), 75% (47/63), 72% (23/32) and 92.1% (58/63), in
324 Australia (in children from SSA) [31], in Senegal in adult patients [32], [15], [14] and in
325 Ghana [6], respectively. Some studies demonstrated that patients with genotype A tended to
326 be younger [14]. This could explain the higher rate of genotype A in our young population
327 than in the adult population of the same region. Moreover, a recent study in The Gambia
328 showed that genotype A is a significant risk factor for fibrosis [17] often associated with the
329 development of HCC. In addition, all Senegalese HBV A sequences were closely related to
330 the Cuban HBV A4 isolate. The A4 isolate from the Cuban study was related to an isolate
331 from The Gambia, suggesting a probable African origin [33].

332 HBsAg vaccine and therapy (immunoglobulin (Ig)) escape mutations including 120P/120T,
333 129H and 133L were detected in three patients, with a prevalence of 20% (3/15) in the co-
334 infected children. A study in Ghana reported a lower vaccine escape mutation rate of 11%
335 (7/63) and an immunoprophylaxis escape mutation rate of 100% [6].

336 In our study, the HBV drug resistance mutation rate was 20% (3/15), while a higher rate of
337 33.3% (21/63) was found in Ghana [6] and 75% (9/12) in Thailand [34]. These results could
338 be due to the fact that in this latter case, adolescents were on 3TC monotherapy. Furthermore,
339 67% (2/3) of children with at least one 3TC resistance mutation were genotype A and 33%
340 (1/3) genotype E, whereas different results were reported in adult patients in Ghana with a
341 ratio of 95.2% (20/21) of genotype E [6].

342 The HBV-related 3TC RAMs found in the present study were L180M, M204V/I and S202N,
343 corroborating former studies that found the same mutations [6]. Moreover, as in our own
344 study, none of the patients receiving TDF displayed HBV-related RAMs. Our present study
345 thus suggests that the use of 3TC as the only HBV-active molecule in HIV-HBV patients is an

346 ineffective treatment plan. In addition, our results showed that the only factor associated with
347 persistent HBV viremia was the combination of ART.

348 The most recent treatment guidelines recommend the use of TDF in treatment regimens for
349 both HIV and HBV carriers and HIV-HBV co-infected patients [1] and this may be most
350 useful in the SSA context and easily implementable.

351 **Conclusion**

352 Our study shows that pediatric HIV-HBV co-infection remains a significant health issue.
353 Better management strategies for HIV-infected children are necessary, including regular
354 monitoring for the early diagnosis of treatment failure, systematic detection of co-infections
355 and setting-up of appropriate therapeutic combinations (high genetic barrier ART when
356 available) to prevent the selection of resistance mutations in this sensitive population. These
357 measures are urgently needed to achieve both the WHO and UNAIDS targets for the global
358 elimination of viral hepatitis and acquired immunodeficiency syndrome (AIDS) epidemic by
359 2030 [1,35].

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363 **Authors' contribution**

364 HDN, CTK and GL: conceptualization and supervision of the study; KKF, AD, AMC, GLB
365 and BT: providing samples and data from patients; GL, AJN and RMT: performing
366 experiments; RMT, IC, FHP and DC: phylogenetic analysis; RMT: drafting of the
367 manuscript. RMT, IC, FHP, CTK, HDN, SM and CSB: critical reading of the manuscript; All
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