A systematic review of Hepatitis B virus (HBV) drug and vaccine escape mutations in Africa: a call for urgent action

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ABSTRACT:

International sustainable development goals for the elimination of viral hepatitis as a public health problem by 2030 highlight the pressing need to optimize strategies for prevention, diagnosis and treatment. Selected or transmitted resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) in hepatitis B virus (HBV) may reduce the success of existing treatment and prevention strategies. These issues are particularly pertinent for many settings in Africa where there is high HBV prevalence and co-endemic HIV infection, but lack of robust epidemiological data and limited education, diagnostics and clinical care. The prevalence, distribution and impact of RAMs and VEMs in these populations are neglected in the current literature. We therefore set out to assimilate data for sub-Saharan Africa through a systematic literature review and analysis of published sequence data, and present these in an on-line database (https://livedataoxford.shinyapps.io/1510659619-3Xkoe2NKkKJ7Drg/). The majority of the data were from HIV/HBV coinfected cohorts. The commonest RAM was rtM204I/V, either alone or in combination with compensatory mutations, and identified in both reportedly treatment-naïve and treatment-experienced adults. We also identified the suite of mutations rtM204V/I + rtL180M + rtV173L, that has been associated with vaccine escape, in over 1/3 of cohorts. Although tenofovir has a high genetic barrier to resistance, it is of concern that emerging data suggest polymorphisms that may be associated with resistance, although the precise clinical impact of these is unknown. Overall, there is an urgent need for improved diagnostic screening, enhanced laboratory assessment of HBV before and during therapy, and sustained roll out of tenofovir in preference to lamivudine alone. Further data are needed in order to inform population and

individual approaches to HBV diagnosis, monitoring and therapy in these highly vulnerable settings.

Author's summary

The Global Hepatitis Health Sector Strategy is aiming for the elimination of viral hepatitis as a public health threat by 2030. However, mutations associated with drug resistance and vaccine escape may reduce the success of existing treatment and prevention strategies. In the current literature, the prevalence, distribution and impact of hepatitis B virus (HBV) mutations in many settings in Africa are neglected, despite the high prevalence of HBV and co-endemic HIV infection. This systematic review describes the frequency, prevalence and co-occurrence of mutations associated with HBV drug resistance and vaccine escape mutations in Africa. The findings suggest a high prevalence of these mutations in some populations in sub-Saharan Africa. Scarce resources have contributed to the lack of HBV diagnostic screening, inconsistent supply of drugs, and poor access to clinical monitoring, all of which contribute to drug and vaccine resistance. Sustainable long-term investment is required to expand consistent drug and vaccine supply, to provide screening to diagnose infection and to detect drug resistance, and to provide appropriate targeted clinical monitoring for treated patients.

INTRODUCTION

In 2015, the World Health Organisation (WHO) estimated that 3.5% of the world's population (257 million people) were living with Hepatitis B virus (HBV) infection, resulting in 887,000 deaths each year, mostly from complications including cirrhosis and hepatocellular carcinoma (HCC) [1]. United Nations Sustainable Development Goals set out the challenge of elimination of viral hepatitis as a public health threat by the year 2030 [2]. One of the existing strategies in the elimination toolbox is use of antiviral drugs in the form of nucleos(t)ide analogues (NAs). Suppression of viraemia not only reduces inflammatory and fibrotic liver disease in the individual receiving treatment but also reduces the risk of transmission. However, the emergence of HBV resistance-associated mutations (RAMs) is a potentially significant concern for the success of this strategy.

Africa is the continent with the second largest number of individuals with chronic HBV (CHB) infection, with an estimated 6.1% of the adult population infected [1]. However, there is little commitment and resource invested into the burden of this disease, and many barriers are contributing to the epidemic [3,4]. Globally, less than 10% of the population with CHB are diagnosed, with an even smaller proportion on treatment [1,4]. This proportion is likely to be even lower in Africa. The situation in Africa is further complicated by the substantial public health challenge of coendemic human immunodeficiency virus (HIV) and HBV; coinfection worsens the prognosis in dually infected individuals [5]. There is also a lack of robust epidemiological data on HBV from Africa [3,4].

Widespread use of antiretroviral therapy (ART) for HIV, incorporating NAs that also have activity against HBV, may have an impact on HBV through improved rates of viraemic suppression, but also potentially by driving the selection of RAMs. The WHO recommends screening for Hepatitis B virus surface antigen (HBsAg) in all HIV-1 infected individuals prior to ART initiation, and for all pregnant women during antenatal visits, to improve the clinical outcomes of people living with CHB and to enhance interventions that reduce the incidence of new cases [6]. However, screening of HBsAg is not routinely performed in many settings in Africa, with lack of implementation at least partially driven by cost and lack of programmes for HBV treatment outside the setting of HIV coinfection. HBV infected patients either remain untreated (most typical in the setting of monoinfection), or are exposed to antiviral drugs without proper monitoring and often intermittently, putting them at risk of developing RAMs (more likely in the setting of HIV coinfection) [4,7–10].

HBV is a DNA virus that replicates via an RNA intermediate, with reverse transcriptase (RT) catalysing the transcription of RNA into DNA [7]. NAs that inhibit RT are therefore used to prevent HBV replication, including lamivudine (3TC), entecavir (ETV) and tenofovir (conventionally in the form of tenofovir disoproxil fumarate (TDF), but more recently available as the prodrug, tenofovir alafenamide fumarate (TAF)), with mostly historical use of other agents including telbivudine (LdT) and adefovir (ADV) [6,11]. Choice of TDF/TAF or ETV is determined by availability, cost, safety profile and barrier to resistance [4]. In Africa, the choice of agent is usually limited to 3TC and TDF. Emergence of mutations happens because the RT enzyme is error-prone and lacks the proofreading function required to repair errors during transcription [7]; when these mutations confer a selective advantage by

allowing the virus to escape the effect of drug therapy, they will become amplified in the viral population. Some RAMs confer resistance to one agent only, while others are associated with resistance to several agents (Fig 1).

Fig 1: HBV drug resistance associated mutations (RAMs), vaccine escape mutations (VEMs) and mutations associated with Hepatitis B immunoglobulin (HBIg) resistance. HBV genes are shown in the coloured ovals. TDF = tenofovir, ETV = entecavir, 3TC = lamivudine. This figure incorporates data from eight studies; three were identified by the systematic review presented in this manuscript [12–14] and five from the wider literature [7,15–18].

3TC was originally seen as a major breakthrough in treating HBV [19]. However, it is now known to have a low genetic barrier to resistance and its long-term effectiveness is limited as a result of resistance mutations in the 'YMDD' motif (tyrosine, methionine, aspartate, aspartate; amino acids 203-206) in domain C of the viral polymerase (Pol). These occur with associated upstream compensatory mutations in Pol domains A, B and in the B-C interdomain [7,15,16]. Among chronic HBV monoinfected patients, incidence of HBV resistance to 3TC is as high as 20% per year. In HIV/HBV coinfected patients, this can reach 90% over 5 years of treatment, as development of resistance is accelerated in HIV coinfection [5,20]. 3TC has also been associated with the induction of cross-resistance to emtricitabine (FTC), LdT, and at least partially ETV, thus reducing the options for subsequent treatment [10].

TDF is widely used in treatment of both HIV and HBV and is generally well tolerated. TDF has a high genetic barrier to resistance and maintains effective suppression of HBV in both monoinfected and HIV/HBV coinfected individuals [5,7,10,21,22]. Although it has a recognised association with nephrotoxicity in HIV treatment, current literature suggests it may be better tolerated in HBV infection [11]. Conversely, African populations have a higher background of renal disease [23] and could be potentially more vulnerable to nephrotoxitiy from TDF [24]. TAF delivers equally potent viraemic suppression at lower plasma levels, and is therefore associated with reduced nephrotoxicity [25], but is not available in Africa at present. HBV resistance to TDF is not well characterised, but there are emerging data from *in vitro* studies associating Pol mutations rtA194T and rtN236T with decreased susceptibility [11,21]. Virological breakthrough on TDF therapy has been reported in two patients harbouring rtS78T/sC69 mutations [17], and in another patient with multi-site polymerase mutations; rtL80M, rtL180M, rtM204V/I, rtA200V, rtF221Y, rtS223A, rtT184A/L, rtR153Q, and rtV191I [26]. The significance of these mutations needs to be further explored in clinical studies.

First line ART treatment regimens for HIV in sSA now almost universally include TDF, and current guidelines also recommend TDF-based regimens in individuals with HBV/HIV coinfection [27]. Accordingly, in both HIV monoinfection and HBV/HIV coinfection, use of TDF has increased across much of Africa. Nevertheless, it remains the case that 3TC is used as the only HBV-active agent in some settings [7,8], as well as in second line regimens, exemplified by South Africa where second line ART substitutes Zidovudine (AZT) for TDF leaving only 3TC coverage for HBV [28]. Among HBV/HIV coinfected children in South Africa treated with regimens

169 including 3TC and/or TDF, HBV viraemia has been demonstrated, highlighting
170 potential underlying HBV drug resistance [29].
171
172 ETV is another active agent, and is safe and well tolerated. However it is not active
173 against HIV and therefore has to be added to ART regimens rather than being part of
174 the primary backbone, is not recommended in pregnancy, and is not routinely
175 available in most African settings [30]. Resistance arises more commonly in the
176 context of prior 3TC exposure [11,31], which may limit its future potential in Africa,
177 particularly in HIV endemic populations.
178
179 As a component of the Expanded Programme on Immunization (EPI), HBV
180 preventive vaccines have been rolled out in Africa since 1995 [4]. HBV vaccine is
181 highly effective in prevention of mother to child transmission (PMTCT); when
182 administered to infants within 24 hours of birth followed by a dose given at 6 and
183 another at 14 weeks to complete the primary series, it reduces the rate of mother to
184 child transmission by 85% - 95% [32,33]. However, by 2016 only 11 countries in
185 Africa had adopted birth dose HBV vaccination as part of the routine infant
186 immunisation schedule [34]. Changes in the S protein can result in vaccine escape
187 mutants (VEMs) [16,18], and also diagnostic escape mutations which result in false
188 negative HBsAg testing [16]. Mutations in HBV Pol can also lead to amino acid
189 changes in the Surface (S) protein due to overlapping reading frames (ORFs) in the
190 genome [16]. Whilst the S protein mutation sG145R has been identified as the major
191 VEM, recently other mutations in S protein have been associated with immune
192 escape [16] Fig 1. There are very few data for VEMs in Africa, but in other settings of

high endemicity, VEMs can be common, as evidenced by a reported prevalence of 28% in vaccinated HBV-infected children in Taiwan [35].

To date, no systematic review has assessed the geography and prevalence of HBV RAMs and VEMs in Africa. An understanding of the extent to which these mutations circulate in Africa is essential to improving HBV therapy in patients with and without HIV coinfection. We therefore set out to describe the frequency, co-occurrence and distribution of RAMs and VEMs in Africa, and to suggest whether changes are needed in recommendations for laboratory diagnostics and/or approaches to drug therapy or vaccine deployment. This will underpin further research to identify and track relevant mutations in these populations.

METHODS

Search strategy

Between October 2017 and January 2018, we searched the published literature, in MEDLINE (PubMed; https://www.ncbi.nlm.nih.gov/pubmed), SCOPUS (https://www.elsevier.com/solutions/scopus) and EMBASE (https://www.elsevier.com/en-gb/solutions/embase-biomedical-research). Our search strategy is detailed in S1 Table (documenting use of PRISMA criteria and selection of studies) and S2 Table (listing our search criteria). The earliest paper we identified on HBV drug resistance in Africa was published in 2007. We reviewed the titles and abstracts matching the search terms and only included those relating to drug or vaccine resistance in HBV infection, including only those that presented original data and had undergone peer review. All retrieved articles were in English, therefore no exclusion in relation to language was required.

For each publication we recorded reference, publication year, study design, sample size, study population, proportion of participants who tested HBsAg+ or HBV DNA+, country, year(s) of specimen collection, genotype identified, antiviral treatment, sequencing method, gene sequenced, number of sequenced samples, participant recruitment site and sequence accession number. Data were curated using MS Excel software (Microsoft, Redmond, WA).

RAMs reported in published sequences not represented in primary studies

We expanded our search for evidence of RAMs by identifying publicly available HBV sequences from Africa, that had not been included in the results of our primary literature search. We used both the Hepatitis B Virus database (https://hbvdb.ibcp.fr/ [36] and Hepatitis Virus Diversity Research Alignments database (http://hvdr.bioinf.wits.ac.za/alignments/) [37].

Analysis

In order to determine the prevalence of RAMs and VEMs, we first reported these using the denominator (total number of HBV positive patients) and numerator (total number of HBV positive patients with the specified mutation) as reported in published studies. We also pooled data by country in order to provide regional estimates. Downloaded sequences were managed using Sequence editor, database and analysis platform, SSE version 1.3, for analysis [38].

Data visualisation

We developed an R package, gene.alignment.tables, for the visualisation of the sequence data in this study; this is available on Github [39] and can be used for visualising generic gene sequence datasets. The package was developed by University of Oxford's Interactive Data Network and a specific instance of the visualisation is hosted as a Shiny app which can be viewed here: https://livedataoxford.shinyapps.io/1510659619-3Xkoe2NKkKJ7Drg/ [40].

RESULTS

The initial search yielded 56 articles in MEDLINE, 150 in SCOPUS and 150 in EMBASE. Of these, 32, 136 and 119 were excluded from search results of MEDLINE, SCOPUS and EMBASE respectively, as they did not did not meet the inclusion criteria. After de-duplication, 37 articles were included. 27 articles identified from MEDLINE, SCOPUS and EMBASE were identical; five unique articles were included from EMBASE, four from SCOPUS and one from MEDLINE. A total of 37 articles were downloaded in full (S1 Table (part II) ; S3 Table).

Study characteristics

Epidemiological data for HBV represented by the 37 studies we identified are summarised in Table 1. Studies included were from Southern Africa (Botswana, Mozambique, South Africa, Zambia and Zimbabwe), East Africa (Ethiopia, Kenya, Malawi, Sudan and Uganda), West Africa (Cote d'Ivoire, Gambia, Ghana, Guinea-Bissau and Nigeria) and Central Africa (Cameroon, Gabon). There was considerable heterogeneity in recruitment protocols and exposure to anti-viral treatment. Twenty-six studies recruited from hospitals, three studies recruited from the community

266 [8,41,42] and eight studies did not specify where recruitment was undertaken
267 [10,43–49]. All studies were observational.
268
269

Table 1: Prevalence of HBsAg and HBeAg from 37 studies of HBV drug resistance in Africa

	Study (Reference)	Country	Characteristics of study population	HIV co-infection status of cohort[a]	HBV prevalence in this cohort (reported as HBsAg prevalence unless otherwise specified)	HBeAg prevalence among HBV-positive individuals
East Africa	Deressa et al 2017 [50]	Ethiopia	Patients attending outpatient ART clinic at tertiary referral university hospital	+	17/308 (6%)	Not reported
	Hundie et al 2016 [41]	Ethiopia	Stored plasma samples from HBV infected blood donors obtained from blood bank centres	±	391/391 (100%)	Not reported
	Day et al 2013 [8]	Kenya	Longitudinal cohort study of female sex workers in an urban setting	+	11/159 (7%)	6/11 (55%)
	Kim et al 2011 [9]	Kenya	Individuals from an urban centre enrolled in randomised controlled trial of adherence to ART	+	27/389 (7%)	24/27 (89%)
	Mabeya et al 2017 [13]	Kenya	Individuals seeking treatment at the comprehensive HIV Clinic at tertiary referral university hospital	+	29/400 (7%)	Not reported
	Auodjane et al 2014 [20]	Malawi	Individuals starting ART treatment at a tertiary referral university hospital	+	133/1117 (12%)	67/133 (50%)
	Galluzzo et al 2012 [45]	Malawi	Pregnant women enrolled in a PMTCT study on safety and pharmacokinetics of antiretroviral drugs	+	21/21 (100%)	7/21 (33%)
	Mahgoub et al 2011 [42]	Sudan	Plasma samples from blood donors from capital city in Sudan	±	16/404 (4%)	Not reported
	Yousif et al 2014 [47]	Sudan	Individuals seeking treatment at a AIDS care unit and HIV treatment centre	+	96/358 (27%)[b]	32/ 50 (64%)

	Calisti et al 2015 [51]	Uganda	All HIV patients attending a regional referral hospital	+	109/2820 (4%)	Not reported
West Africa	Boyd et al 2015 [52]	Cote d'Ivoire	Individuals enrolled in randomised multi centre trials of benefits and risks of early ART initiation	+	259/ 2465 (11%)	39/168 (23%)
	Archampong et al 2017 [12]	Ghana	Serum samples from HBV-HIV co-infected patients collected at tertiary referral university hospital	+	235/235 (100%)	Not reported
	Chadwick et al 2012 [53][d]	Ghana	Stored sera from all adult patients attending the HIV clinic at a tertiary referral university hospital	+	143/371 (39%)	Not reported
	Geretti et al 2010 [54][d]	Ghana	Consecutive serum samples collected from unselected HIV-infected patients attending a tertiary referral university hospital	+	140/838 (17%)	37/140 (26%)
	Ndow et al 2017 [55]	Gambia	Individuals attending HIV clinic	+	23/187 (12%)	Not reported
	Stewart et al 2011 (44)	Gambia	Individuals receiving HAART; recruitment site not specified	+	70/ 570 (12%)	6/21 (29%)
	Langhoff Honge et al 2014 [56]	Guinea Bissau	Patients attending outpatient ART clinic at tertiary referral university hospital	+	94/576 (16%)	16/94 (17%), HDV prevalence : 18/72 (25%)
	Faleye et al 2015 [57]	Nigeria	Pregnant women attending antenatal clinics from two tertiary university hospitals	±	15/272 (6%)	Not reported
Central Africa	Gachara et al 2017 [58]	Cameroon	Patients attending outpatient ART health centre	+	20/337 (6%)[c]	Not reported
	Kouanfack et al 2012 [59]	Cameroon	Patients attending outpatient ART clinic at tertiary hospitals	+	54/552 (10%)	Not reported
	Magoro et al 2016 [60]	Cameroon	Patients attending outpatient ART health centre	+	116/445 (26%)	16/102 (16%)
	Bivigou-Mboumba et al 2016 [61]	Gabon	Patients attending outpatient ART clinic	+	71/762 (9%)	Not reported
	Bivigou-Mboumba et al 2018 [62]	Gabon	Patients attending HIV care centers	+	43/487 (9%)	Not reported

Southern Africa	Anderson et al 2015 [48]	Botswana	Stored plasma samples of HIV/HBV co-infected individuals collected from studies conducted in a Research Institution	+	81/81 (100%)	Not reported
	Matthews et al 2015 [63]	Botswana	Women attending antenatal and paediatric clinics	±	17/443 (4%)	16/60 (27%); HDV: negative
	Chambal et al 2017 [64]	Mozambique	Patients attending outpatient ART health centre	+	47/518 (9%)	Not reported
	Wandeler et al 2016 [14]	Mozambique	Individuals starting ART treatment at urban clinic in Mozambique and rural clinic in Zambia	+	78/1032 (8%)	24/168 (14%)
	Andersson et al 2013 [65]	South Africa	Stored serum of women infected with HIV enrolled in an Antenatal Sentinel HIV and Syphilis Prevalence Survey	±	97/3089 (3%)	17/94 (18%); HDV: negative
	Amponsah-Dacosta et al 2015 [43]	South Africa	Stored serum of individuals exposed to HBV participating in a health facility-based hepatitis B serosurvey conducted at a provincial level.	±	33/201 (16%)	Not reported
	Amponsah-Dacosta et al 2016 [49]	South Africa	Individuals due to HAART initiation enrolled in longitudinal study	+	5/5 (100%)	5/5 (100%)
	Hamers et al 2013 [10]	South Africa	Individuals enrolled in a multicentre prospective study of ART resistance monitoring	+	37/175 (21%)	Not reported
	Gededzha et al 2016 [66]	South Africa	Stored sera from HBV infected individuals attending a tertiary referral university hospital	±	8/9 (89%)	Not reported
	Makondo et al 2012 [46]	South Africa	Stored sera from HIV infected individuals prior to ART initiation, recruitment site not specified	+	71/298 (24%)[b]	Not reported

	Matthews et al 2015 [63]	South Africa	Women attending antenatal and paediatric clinics in South Africa and Botswana	±	49/507 (10%)	Not reported; HDV: negative
	Powell et al 2015 [67]	South Africa	Stored serum samples of individuals infected with HIV receiving care at a tertiary university hospital	+	37/394 (9%)	Not reported
	Selabe et al 2007 [68]	South Africa	Individuals infected with HBV admitted at tertiary University hospital	±	35/35 (100%)	Not reported
	Selabe et al 2009 [69]	South Africa	Individuals infected with HBV admitted at tertiary University hospital	-	17/17 (100)	9/17 (53%)
	Wandeler et al 2016 [14]	Zambia	Individuals starting ART treatment at urban clinic in Mozambique and rural clinic in Zambia	+	90/797 (11%)	24/168 (14%)
	Hamers et al 2013 [10]	Zambia	Individuals enrolled in a multicentre prospective study of ART resistance monitoring	+	55/523 (11%)	Not reported
	Baudi et al 2017 [70]	Zimbabwe	Stored plasma samples of individuals attending HIV support clinic	+	19/176 (11%)	Not reported

HBsAg and HBeAg prevalence were determined from 37 studies (Treatment naïve: n= 8 studies, 566 individuals with HBsAg; Treatment experienced: n= 19 studies, 1243 individuals with HBsAg; Mixed regimen where some were treatment experienced, naïve or treatment status not specified: n= 10 studies, 1046 individuals with HBsAg). Studies were identified by a systematic literature search of HBV resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) from African cohorts published between 2007 and 2017 (inclusive).

[a] HIV status is designated '+' whole cohort HIV-positive; '±' some of cohort HIV-positive; '-'none of cohort HIV-positive

[b] HBV prevalence in these cohorts was reported using HBV DNA detection rather than HBsAg

[c] Occult HBV prevalence reported in these cohorts

[d] These two studies recruited from the same overall cohort in Ghana.

Study populations were categorised as follows:

- HBV/HIV coinfected patients: (n=28 studies), [8-10,12-14,20,43-48,50–56,58-,–62,64,67,70];
- HBV infected with and without HIV coinfection: (n=8 studies), [41–43,57,63,65,66,68];
- Chronic HBV monoinfection: (n=1 study), [69].

Antiviral treatment exposure varied as follows:

- Treatment-naïve: (n=8 studies), [14,46–48,63,64,68,70];
- 3TC-based regimen only: (n=10 studies), [8,9,20,44,45,49,53,59,61,69];
- Regimens including 3TC or TDF: (n=6 studies), [10,13,51,52,55,65];
- Mixed regimen where some received 3TC, others TDF, while others left untreated; (n=7 studies), [12,50,54,56,60,62,66];
- Treatment regimen not specified: (n=6 studies), [41–43,57,58,67].

HBV amino acid polymorphisms were studied from within the following proteins;

- Pol only (n=13 studies), [8,9,12-14,20,44,53,55,56,59,63,68]; only one of these used a deep sequencing method [20];
- S only (n=3 studies), [43,57,65];
- Pol and S (n=12 studies),[10,41,45,48,51,52,54,58,60,62,64,67];
- Pol, S and PC/BCP (n=4 studies), [47,50,61,69];
- S and PC/BCP region (n=3 studies), [42,46,70];
- Whole genome (n=2 studies), [49,66].

All studies, except for two [53,68], specified the HBV genotype (S3 Table & S2 Fig).

318

319 **Prevalence of HBsAg, HBeAg and HDV coinfection**

320 The prevalence rates of HBsAg in these study cohorts ranged from 3%-26%;
321 however, the populations included were highly selected and therefore not
322 necessarily representative of the general population, particularly as a result of a
323 strong bias towards HIV-infection (Table 1). Only three studies included in this
324 review reported on HDV prevalence: two studies did not detect any HDV antibodies
325 [63,65], whereas the other study reported a HDV prevalence of 25% in Guinea-
326 Bissau [56].

327

328 **RAMs identified in African cohorts**

329 The co-occurrence and distribution of HBV RAMs and VEMs are summarised
330 according to the region where they were identified (Fig 2). This illustrates the patchy
331 and limited data that are available, with South Africa, Ghana and Cameroon best
332 represented, but with large areas (especially in northern and central Africa) not
333 represented at all in the literature.

334

335 **Figure 2: Annotated map to summarise HBV drug Resistance Associated**
336 **Mutations (RAMs) and Vaccine Escape Mutations (VEMs).** Mutations identified
337 from 33 studies of African cohorts published between 2007 and 2017 (inclusive).
338 Four studies identified by our systematic literature review were not represented here
339 as they did not report any RAMs.

340

341 Although 35 studies specified the HBV genotype, it was only possible to group RAMs
342 according to genotype in fourteen studies [8,9,13,14,44,46,47,50,51,56,60-62,69]

(S1 Fig; S2 Fig). The remaining 21 studies generally reported the genotypes identified, but did not specifically state the genotype of HBV within which RAMs were identified.

We have developed an interactive tool to display the genomic positions of RAMs identified through our literature review alongside relevant metadata. This can be accessed on-line here: https://livedataoxford.shinyapps.io/1510659619-3Xkoe2NKkKJ7Drg/ [40].

Overall, the most prevalent RAM was rtM204V/I in both treatment experienced and treatment naïve individuals, and occurring either alone or in combination with other polymorphisms rtL80I/V, rtV173L, rtL180M, rtA181S, rtT184S, rtA200V and/or rtS202S (Fig 3); mutations among individuals with and without exposure to HBV therapy are listed in S4 Table and S5 Table, respectively). This mutation was present in 29 studies at a highly variable prevalence of between 0.4% [12] and 76% [69]. Across all cohorts, the mutation was present in 208/2569 (8%) of all individuals represented. The mutation, by itself, was most prevalent in South Africa; on pooling data for three studies from this setting, it was present in both treatment experienced and treatment naïve patients (n=13/17, 76% [69] and n=16/72, 22% [67,68] respectively). In addition to South Africa, rtM204I/V was also frequent in Malawi among treatment experienced patients (n=24/154, 16% [20,45]) (Fig 3), and in genotype non-A infection: in this setting, the mutation was detected in genotype C infection (n=2/17, 12% [69]) (S2 Fig).

Figure 3: Prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins among HBV infected patients in Africa.

These data are derived from 27 studies of HBV drug resistance in Africa published between 2007 and 2017 (inclusive). The countries represented are listed in alphabetical order. A detailed summary of RAMs identified from each study is presented (Fig 2, S4 Table, S5 Table). Prevalence of RAMs for a specific country was determined by grouping all studies from that country that reported a specific mutation. We used all individuals who tested HBsAg positive to generate a denominator in order to provide a conservative estimate of RAM prevalence, and the numerator was the total number of individuals with that specific mutation from these studies.

A: treatment naïve;

B: treatment experienced.

The rtM204I/V mutation by itself confers resistance to 3TC; in combination with A194T it may also be associated with reduced efficacy to TDF, and in combination with L180M and V173L with vaccine escape, through corresponding substitutions in the surface antigen sites targeted by neutralising antibodies. Although TDF has a high genetic barrier to resistance, and is associated with reliable suppression of HBV viraemia [7,10,21,22], mutations rtN236T and rtA194T, which have been linked with resistance to both TDF and ADV [7], have been identified in Southern Africa in both treatment naïve [14] and treatment experienced [10] patients.

WHO guidelines recommend a first-line regimen including TDF in HIV/HBV coinfected patients [6], and the South African Department of Health HIV/AIDS treatment guideline included TDF as first-line regimen from 2010 [71], however we

found a minority of studies (9/37, 24%) reporting TDF-containing regimens for HIV/HBV coinfected individuals. As anticipated, most of the studies that did use TDF were carried out after 2010, whereas those that used 3TC were generally earlier (S3 Table).

From this dataset, it is difficult to ascertain whether RAMs are genuinely more prevalent in genotype A infection, or this simply reflects enrichment of genotype A in sub-Saharan African populations (S2 Fig). Interpreting RAMs according to sub-genotypes was difficult since most studies did not specify sub-genotype and others did not indicate which RAMs were identified in which genotype. Of concern is the detection of RAMs even in reportedly treatment naïve individuals (Fig 3 & S4 Table), suggesting that RAMs are being transmitted. A study in South Africa that recruited 3TC-naïve HBV infected adults with or without HIV, reported rtM204I in 13/35 (37%) individuals [68].

HBV RAMs in published sequences from Africa

We searched the Hepatitis B Virus database and GenBank to identify HBV sequences derived from Africa, from studies not already included in our review. We identified an additional 69 isolates: 23 had undergone full length genome sequencing whereas 46 isolates represented either the polymerase (n=3) or S region (n= 43) of the HBV genome Table 2. To avoid duplication of results, we excluded fourteen studies already identified by our literature review that had submitted their sequences to GenBank (S3 Table). RAMs in the additional 69 isolates were as follows:

- rtM204V in genotype A (2/69, 2.9% of sequences), this occurred in combination with rtL180M;

- rtM204V + rtL180M in genotype E (1/69, 1.5%);
- rt180M + rtA181V in genotype E (1/69 (1.5%);
- rtQ215S identified in genotype D (4/69, 5.8%).

All these mutations are associated with 3TC resistance; rtA181V has also been associated with reduced susceptibility to TDF [7,15].

In the S gene, the most prevalent mutations were:

- sD144A/E/G occurring in genotype A (6/69, 8.7%), D (10/69, 14.5%) and E (7/69, 10.1%) associated with VEM;
- sI110L occurring in genotype A (3/69, 4.3%), D (4/69, 5.8%) and E (11/69, 15.9%) associated with immunoglobulin resistance.

Table 2: HBV drug resistant mutations (RAMs) identified from HBV genome sequences from Africa downloaded from the Hepatitis B Virus database (https://hbvdb.ibcp.fr/**)** [36] **and GenBank database (http://hvdr.bioinf.wits.ac.za/alignments/)** [37]

HBV Protein	HBV Genotype	Position and nature of the mutation	Number (%) of HBV sequences with mutation	Accession number	Region sequenced	Country of origin
Polymerase (Pol)	A	rtL180M	2/69 (2.9%)	KM519454	Full length	South Africa
				FM199980	Full length	Rwanda
		rtM204V	2/69 (2.9%)	KM519454	Full length	South Africa
				FM199980	Full length	Rwanda
		rtI233V	1/69 (1.4%)	HM535205	Full length	Zimbabwe
	D	rtV214A	1/69 (1.4%)	FJ904395	Polymerase	Tunisia
		rtQ215S	4/69 (5.8%)	FJ904414	Full length	Tunisia
				FJ904431	Full length	Tunisia
				FJ904436	Full length	Tunisia
				FJ904438	Full length	Tunisia
	E	rtV173L	1/69 (1.4%)	KF849723	Full length	Angola
		rtL180M	2/69 (2.9%)	KF849720	Full length	Angola
				KF849723	Full length	Angola
		rtA194T	1/69 (1.4%)	GQ161771	Full length	Guinea
		rtM204V	1/69 (1.4%)	KF849723	Full length	Angola

		rtA181V	1/69 (1.4%)	KF849720	Full length	Angola
		rtN238D	2/69 (2.9%)	HM363566	Polymerase	Nigeria
				HM363587	Polymerase	Nigeria
Surface (S)	A	sI110L	3/69 (4.3%)	KY493896	S	Cameroon
				KP168431	Full length	Kenya
				AY233286	Full length	South Africa
		sP120S	1/69 (1.4%)	KX648547	S	Zimbabwe
		sG129R	1/69 (1.4%)	FN547352	S	Cameroon
		sT126A	1/69 (1.4%)	JN182330	S	South Africa
		sD144A/E/G	6/69 (8.7%)	FN547249	S	Cameroon
				KX493873	S	Cameroon
				FM199980	Full length	Rwanda
				FM200180	S	Rwanda
				FM200189	S	Rwanda
				KF467020	S	South Africa
		sG145R	1/69 (1.4%)	FM200185	S	Rwanda
		sC149R	1/69 (1.4%)	KF476024	S	South Africa
	D	sI110L	4/69 (5.8%)	AB561830	S	Egypt
				KX357627	Full length	Ethiopia
				FJ904429	S	Tunisia
				KJ416196	S	Tunisia
		sP120S	1/69 (1.4%)	KX357636	Full length	Ethiopia
		sM133T	1/69 (1.4%)	KM108592	S	Sudan
		sD144E	10/69 (14.5%)	FJ904427	Full length	Tunisia
				FN547165	S	Cameroon
				FN547179	S	Cameroon
				FN547239	S	Cameroon
				FN547255	S	Cameroon
				FN547258	S	Cameroon
				FN547262	S	Cameroon
				FN547281	S	Cameroon
				FN547318	S	Cameroon
				FN547319	S	Cameroon
	E	sI110L	11/69 (15.9%)	KY494047	S	Cameroon
				AM494711	S	Central Africa Republic
				AM494720	S	Central Africa Republic
				AM494725	S	Central Africa Republic
				AM494727	S	Central Africa Republic
				JQ972822	S	Central Africa Republic

				AB205190	Full length	Ghana
				GQ161756	S	Guinea
				GQ161768	Full length	Guinea
				GQ161795		Guinea
				DQ060822	Full length	South Western Africa and Madagascar
		sT126N	1/69 (1.4%)	HM363608	S	Nigeria
		sP120S	1/69 (1.4%)	HM363599	Full length	Nigeria
		sM133T	3/69 (4.3%)	HM363603	S	Nigeria
				KF170751	S	Sudan
				KF170752	S	Sudan
		sD144E	7/69 (10.1%)	FN547300	S	Cameroon
				KY494047	S	Cameroon
				AM494719	S	Central African Republic
				AM494726	S	Central African Republic
				FN594756	Full length	Niger
				HM363565	Full length	Nigeria
				HM363590	S	Nigeria
		G145A/R	3/69 (4.3%)	KY493921	S	Cameroon
				AB205327	S	Ghana
				AM494741	S	Central African Republic

VEMs

VEMs were identified in Central, East, West and Southern Africa (Fig 2). However, it was not possible to ascertain whether individuals harbouring these mutations had been vaccinated against HBV infection. The most common VEM was the triple mutation rtV173L + rtL180M + rtM204I/V, found in the *Pol* gene. This suite of mutations was identified in 14 studies [12,13,20,44,50-55,58-60,62], at a pooled prevalence of 4% (57/1462). Another significant VEM, sG145K/R [16], was identified in six studies [12,42,47,57,60,62] and sM133L/T, associated with VEM,

immunoglobulin and diagnostic escape mutation [12,48], was identified in seven studies [12,41,47,48,57,62,70] (Fig. 2).

DISCUSSION

Summary

To our knowledge, this is the first systematic review that assesses RAMs and VEMs for HBV in Africa. The high rates of HBV infection among HIV infected individuals in some locations including Cameroon [60] and South Africa [10] could be an indication that HBV infection has been previously under-reported, possibly due to lack of routine screening, poor awareness, stigma, high costs and limited clinical and laboratory infrastructure [4,8–10,45,53]. The literature suggests a widespread exposure of the HIV-infected population to 3TC-based treatment. This may be changing over time in line with current ART treatment recommendations (regimens for Africa summarised in S6 table), but the introduction of TDF-based regimens for HIV treatment has been inconsistent, and TDF monotherapy is not consistently available for HBV infection in the absence of HIV.

In keeping with other settings, the most common RAM identified here was rtM204I/V, either alone or in combination with compensatory mutations rtL180M ± rtV173L. Of concern, rtM204V/I was seen in 76% of treatment experienced patients [69] and 22% of treatment naïve patients [67,68] in South Africa. A review of worldwide incidence of RAMs among treatment naïve patients also described rtM204V/I as the most frequent, but with a much lower prevalence of 5% [72]. The contribution of unreported or undocumented 3TC exposure in the reportedly treatment naïve populations remains to be determined. A European study demonstrated that the

most frequent primary mutation was rtM204V/I, found in 49% of treatment experienced patients [73], while in China rtM204I, rtN236T and rtL180M+rtM204V+rtV173L/rtS202G were also the most prevalent RAMs [74].

The triple mutation rtM204V + rtL180M + rtV173L has been identified in East, West and Central Africa [20,44,51–54,59]. This combination of polymorphisms is associated with both vaccine escape and resistance to 3TC and other □-nucleoside analogues [20,44,51,54,59,60]. Interestingly, this triple mutation has not been reported in the Southern African region to date, which is likely to reflect the composition of the study populations.

Clinical and public health significance of RAMs

Apart from the nature of drug being used for HBV treatment, other predictors of HBV drug resistance include HBV viral load, HBV intra host heterogeneity, HBeAg status, host body mass index and serum alanine aminotransferase (ALT) activity [20,75,76]. Individuals with rtM204V/I plus compensatory mutations typically exhibit high HBV DNA levels [20] and are therefore highly infectious to others. The spread of RAMs may lead to a rise in drug resistance in treatment naïve chronic HBV infection, representing a substantial challenge for Africa and highlighting an imperative to ensure routine use of TDF in preference to, or in combination with, 3TC-based therapy.

Although these data provide a preliminary picture of the prevalence of RAMs in some settings, there are no recommendations to stipulate any specific prevalence threshold above which HBV drug resistance mutations represent a significant barrier

to successful treatment at a population level, and/or RAM prevalence thresholds that should trigger a switch to alternative first-line therapy. For HIV, surveillance for transmission of RAMs is based on screening recently infected, treatment naive individuals, and classifies drug resistance using thresholds of <5%, 5-15%, and >15% to stratify the risk to public health [77]. Similar thresholds and recommendations for HBV could help to underpin the assimilation of epidemiological data and to unify treatment approaches.

TDF resistance

The identification of mutations associated with reduced TDF susceptibility are of concern, as they suggest the potential for increasing prevalence of polymorphisms that confer partial or complete viral escape from a drug that to date has not been widely associated with resistance. There is now potential for increasing selection of TDF resistance as this drug becomes more widely used. However, as a new first line single tablet option incorporating 3TC, TDF and Dolutegravir (DTG) (triple therapy abbreviated to 'LTD') emerges as a recommended option for HIV treatment in Africa, surveillance is needed to determine the clinical outcomes for HBV [78].

If clinically significant, TDF resistance mutations may still represent a particular problem for many African settings, as resource constraints make it unrealistic to provide baseline screening for RAMs, or to monitor patients on treatment with serial viral load measurements. Despite these potential concerns, it has been shown that TDF is effective even in the presence of RAMs and that there is comparable efficacy among 3TC-experienced and NA-naïve patients [79].

VEM

VEM were identified in 16 different countries in East, West, Central and Southern Africa. Information on vaccine exposure was not available, but there are two strands of evidence to support significant population exposure to HBV vaccination. First, vaccination has been progressively rolled out in most countries in sSA since the mid-1990's; second, most HBsAg mutations reported by these studies are located within the common immunodominant B cell epitope (aa 124-147) in which selection of polymorphisms is associated with HBV vaccination [80,81].

VEM have been more robustly reported from Asia, in settings where the HBV infant vaccination programme is well established; for example, in Taiwan, VEM prevalence among vaccinated children increased from 7.8% to 23.1% within 15 years of the launch of the universal vaccine program, although the decline in VEM prevalence thereafter may be partly related to a smaller HBV carrier pool [80]. HBV infection despite immunoprophylaxis can occur either as a consequence of MTCT of pre-existing VEM, or as a result of *de novo* selection of escape mutations from vaccine-induced immune responses, particularly in the setting of delayed vaccination [80,81].The HBV genotype sequence used for vaccines may potentially have an influence on immunogenicity against non-vaccine genotypes, but there are limited data to support this [82]. Only 11 African countries recommend the first HBV vaccine dose at birth, in contrast to the majority of African countries in which HBV vaccination is delayed until 6 weeks of age [33]. It is likely that this delay not only provides a window of infection but also increases the possibility of transmitted VEM and/or emergence of new escape mutations.

High maternal HBV viral load and immunosuppression are other risk factors associated with VEM among infants [80]; both of these are pertinent for emergence of VEMs in Africa given that HBV viral load testing is not routinely available, and HIV is highly prevalent in some populations. Effective PMTCT strategies in Africa, including screening and treating antenatal women, increasing access to viral load monitoring, and introducing HBV birth dose vaccine will help to decrease the prevalence of VEM [4,33,83].

HDV/HBV coinfection

One study from our literature review reported a high HDV prevalence of 25%; however, in this cohort, RAMs occurred in individuals with HBV monoinfection [56]. Given that HDV is characteristically associated with decreased HBV replication [84], it is possible that emergence of HBV RAMs is altered in this setting. However, as the true prevalence and impact of HDV in sSA is not known [85], further studies are needed to determine the impact of HDV coinfection on HBV RAMs.

Limitations of current data

Screening for HBV infection is not routinely performed in many African settings and therefore the true prevalence and characteristics of HBV infection are not known [4,7–10]. We identified very few published studies; only a minority of patients had HBV sequencing undertaken, and there were no data from certain regions of Africa. This highlights the substantial problem of HBV neglect in Africa , and a specific blind-spot relating to sequence data [4]. Identifying the true prevalence of resistance mutations, and characterising the populations in which these are selected and enriched, is currently not possible due to sparse data and lack of clear descriptions

of the denominator population. Most such studies do not perform a truly systematic assessment, but focus on high risk groups – particularly including those with HIV/HBV coinfection: of the 37 studies included here, only one exclusively reported on participants who were HBV mono-infected [69]. Although we have made every effort to assimilate the relevant data to build up a regional picture for Africa, the heterogeneity between studies makes it difficult to draw robust conclusions from pooled data. These findings are a reflection of the little attention paid towards the burden of this disease in Africa and the neglect in robust epidemiological data.

Only nine studies undertook a longitudinal approach to detection of drug resistance [8–10,20,44,45,49,52,53]. The results of the other 28 studies that undertook a cross-sectional approach could be skewed by the timing of recruitment of study participants, with a risk of under-representation of drug resistance if screening is undertaken only at baseline, and potentially an over-representation if screening is undertaken in patients with HIV coinfection, who are more at risk of advanced disease and prolonged drug exposure. As most of these studies recruited individuals from hospital settings, this raises the latter possibility.

Mutations across the whole genome might be relevant in determining resistance [86]. However, most of the included studies analysed only defined genes from within the HBV genome; only two sequenced the whole genome, and these determined consensus sequence. This potentially results in an under-representation of RAMs and VEMs that may be present as low numbers of quasispecies, but could become significant if selected out by exposure to drug or vaccine.

593 In studies that reported RAMs among treatment naïve individuals, the literature
594 suggests that sequence analysis was performed prior to ART initiation. However, we
595 cannot exclude the possibility that that some of these participants had prior ART
596 exposure. Due to the nature of the cohorts that have been studied, most of the
597 RAMs identified were from HIV/HBV coinfected individuals. It is possible that HIV
598 increases the risk of HBV RAMs both in terms of drug exposure, and also as a
599 function of increased HBV viral loads. A study from Malawi demonstrated the rapid
600 emergence of 3TC resistance in HIV coinfection, with virtually all treatment naïve
601 HBeAg positive individuals starting antiviral treatment showing emergence of rtM204I
602 by six months. Likewise, a study carried out in Italy revealed that patients with HIV
603 coinfection were more likely to harbour the rtM204V mutation and to show multiple
604 mutations compared to HBV monoinfected patients [87]. It would be worth further
605 exploration of this observation in Africa, as there are currently very limited data.

606

607 **Challenges and opportunities for Africa**

608 A major challenge for Africa is to improve coverage rates of infant vaccination,
609 deploy catch-up vaccination programmes for older children and adults, adopt
610 widespread screening and develop treatment programmes for HBV. While HBV
611 vaccine is effective, gaps in vaccine coverage in Africa can be demonstrated by the
612 high perinatal transmission rate of HBV in sSA (estimated at 38% among women
613 with a high HBV viral load) and the observation that up to 1% of newborns in sSA are
614 still infected with HBV [88]. Sustained efforts are required to build robust PMTCT
615 programmes that deliver screening and treatment for antenatal women, and timely
616 administration of HBV birth vaccine for their babies [33,83].

617

Although the WHO recommends monitoring for the development of drug resistance once on therapy [6], implementation remains challenging as viral load monitoring and sequencing are both rarely available [7]; despite the advancement and availability of HIV testing and monitoring, in many settings it remains uncommon to monitor HIV viral load after ART initiation [89,90]. Affordable, accessible and sustainable platforms for quantifying both HIV and HBV viral loads remain an important priority for many settings in Africa, given the lack of on-treatment monitoring in many settings. Given the simplicity and relative ease of collection, preparation and transport of dried-blood-spot (DBS) samples [91], adopting DBS testing could improve access to HBV diagnosis, viral load monitoring and linkage to care, especially in areas with limited access to laboratory facilities.

Development of a cheap, rapid test for the detection of the most frequently observed RAMs and VEMs should be considered as a potentially cost-effective strategy for Africa. Proof of principle for a rapid test for diagnosis and detection of resistance has been demonstrated by the GeneXpert MTB/RIF assay for *Mycobacterium tuberculosis* (MTB) [92]. A similar approach has been applied for HBV through use of a multiplex ligation-dependent probe real time PCR (MLP-RT-PCR) [93]. Although this assay is able to detect RAMs quickly and cheaply, there are still limitations as the test requires high viral load samples, is based on detection of known RAMs from within discrete regions of the genome, and may not identify RAMs that are present as minor quasispecies.

New metagenomic sequencing platforms, such as Illumina and Nanopore, provide the opportunity for whole deep genome sequencing, which can reveal the full

643 landscape of HBV mutations in individual patients, quantify the prevalence of drug
644 resistance mutations among HBV quasi-species, and determine the relationship
645 between these polymorphisms and treatment outcomes [87]. Nanopore technology
646 also has the potential to develop into an efficient point of care test that could detect
647 viral infection and coinfection, as well as determining the presence of VEMs and
648 RAMs [94], but is currently limited by cost and concerns about high error rates.
649
650 There have been few studies looking at the correlation between genotype, clinical
651 outcomes of disease, response to antiviral therapy and RAMs/VEMs, but none from
652 Africa. Studies outside Africa have shown that genotype A is more prone to
653 immune/vaccine escape mutants, pre-S mutants associated with immune
654 suppression, drug associated mutations and HCC in HIV/HBV coinfected participants
655 [46,87,95]. Studies investigating the role of genotypes in predicting response to
656 antiviral therapy and their association with various types of mutations are urgently
657 needed in Africa, particularly in light of the high frequency of genotype A infection
658 and high population exposure to antiviral agents that have been rolled out over the
659 past two decades as a component of first-line ART.
660
661 Existing infrastructure for diagnosis, clinical monitoring and drug therapy for HIV
662 represents an opportunity for linkage with HBV care. Particularly in settings of limited
663 resource, joining up services for screening and management of blood-borne virus
664 infection could be a cost-effective pathway to service improvements.
665
666 **Conclusions**

667 This review highlights the very limited data for HBV RAMs and VEMs that are
668 available from Africa. Scarce resources resulting in lack of diagnostic screening,
669 inconsistent supply of HBV drugs and vaccines, and poor access to clinical
670 monitoring contribute to drug and vaccine resistance, potentially amplifying the risk
671 of ongoing transmission and adding to the long-term burden of HBV morbidity and
672 mortality in Africa. We call for urgent action to gather and analyse better data,
673 particularly representing the HBV monoinfected population, and for improved access
674 to TDF.
675
676 HBV RAMs and VEMs have been identified in several African countries among
677 HIV/HBV coinfected and HBV monoinfected patients, before and during treatment
678 with NAs but the data are currently insufficient to allow us to form a clear picture of
679 the prevalence, distribution or clinical significance of these mutations. Overall, the
680 data we describe suggest a significantly higher prevalence of drug resistance in
681 some African populations than has been described elsewhere, and that is not
682 confined only to drug-exposed populations, highlighting an urgent need for better
683 population screening, assessment of HBV infection before and during therapy, and
684 increasing roll out of TDF in preference to 3TC. At present, TDF accessibility is
685 largely confined to HIV/HBV coinfected individuals; we now need to advocate to
686 make monotherapy available for HBV monoinfected individuals. However, there are
687 uncertainties as to whether its long-term use might result in nephrotoxicity, and
688 potentially in an increase in selection of TDF RAMs.
689
690 We should ideally aim for the goals of a combined HBV test that includes diagnosis
691 of infection, genotype and presence of RAMs/VEMs; new sequencing platforms such

as Nanopore make this technically possible, although cost remains a significant barrier at present. Sustainable long-term investment is required to expand consistent drug and vaccine supply, to provide screening infection and for drug resistance, and to provide appropriate targeted clinical monitoring for treated patients.

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SUPPORTING INFORMATION:

S1 Fig: HBV drug Resistance Associated Mutations (RAMs) grouped according to genotype. Data summarised from fourteen studies published between 2009-2017 (inclusive). 21 studies were not represented here as they did not specifically indicate which genotype individuals with RAMs belonged to. **Available at** https://doi.org/10.6084/m9.figshare.5774091 [96].

S2 Fig: Distribution of HBV genotypes and prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins in geno-A and geno-non-A samples.

A: Distribution of HBV genotypes derived from 35 studies reporting resistance associated mutations (RAMs) in Africa published between 2009 to 2017 (inclusive);

B: Prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins in geno-A and geno-non-A samples. These data are derived from 14 studies of HBV drug resistance in Africa published between 2007 and 2017 (inclusive). 21 studies were not represented here as they did not specifically indicate which genotype individuals with RAMs belonged to. We had more geno-A samples represented than other samples, we therefore combined samples from other genotypes that had RAMs (B, C, D, E, D/E) to form geno-non-A samples. We then compared prevalence of Pol/RT mutation between geno-A samples to geno-non-A samples. Prevalence of RT/Pol mutations for a specific genotype(geno-A/geno-non-A) was determined by grouping all studies with geno-A/geno-non-A infection that reported a specific mutation; the denominator was the total number of individuals infected with geno-A/geno-non-A from these studies and the numerator was the total number of individuals infected with geno-A/geno-non-A with that specific mutation. **Available at** https://doi.org/10.6084/m9.figshare.5774091 [96].

S1 Table: PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) criteria for a systematic review of hepatitis B virus (HBV) drug and vaccine escape mutations in Africa. Available at https://doi.org/10.6084/m9.figshare.5774091 [96].

I. Checklist to demonstrate how PRISMA criteria (2009) have been met in this review;

II. Flow diagram illustrating identification and inclusion of studies for a systematic review of drug and vaccine resistance mutations in Africa.

S2 Table: Details of search strategy used to identify studies on HBV resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) conducted in Africa. A: PubMed database; B: SCOPUS and EMBASE database. **Available at** https://doi.org/10.6084/m9.figshare.5774091 [96].

S3 Table: Full details of 37 studies identified by a systematic literature search of HBV resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) from African cohorts published between 2007 and 2017 (inclusive). Available at https://doi.org/10.6084/m9.figshare.5774091 [96].

S4 Table: HBV Pol/RT mutations among treatment-naïve HBV infected patients in Africa from 12 studies published between 2007 and 2017 (inclusive). Available at https://doi.org/10.6084/m9.figshare.5774091 [96].

S5 Table: HBV Pol/RT mutations among treatment-experienced HBV infected patients in Africa, from 25 studies published between 2009 and 2017 (inclusive). Available at https://doi.org/10.6084/m9.figshare.5774091 [96].

S6 Table: First line ART regimen for adults in Africa, and overlap with HBV therapy. Information derived from published ART guidelines in all cases where these are available in the public domain. This information was collated in May 2018. Available at https://doi.org/10.6084/m9.figshare.5774091 [96].

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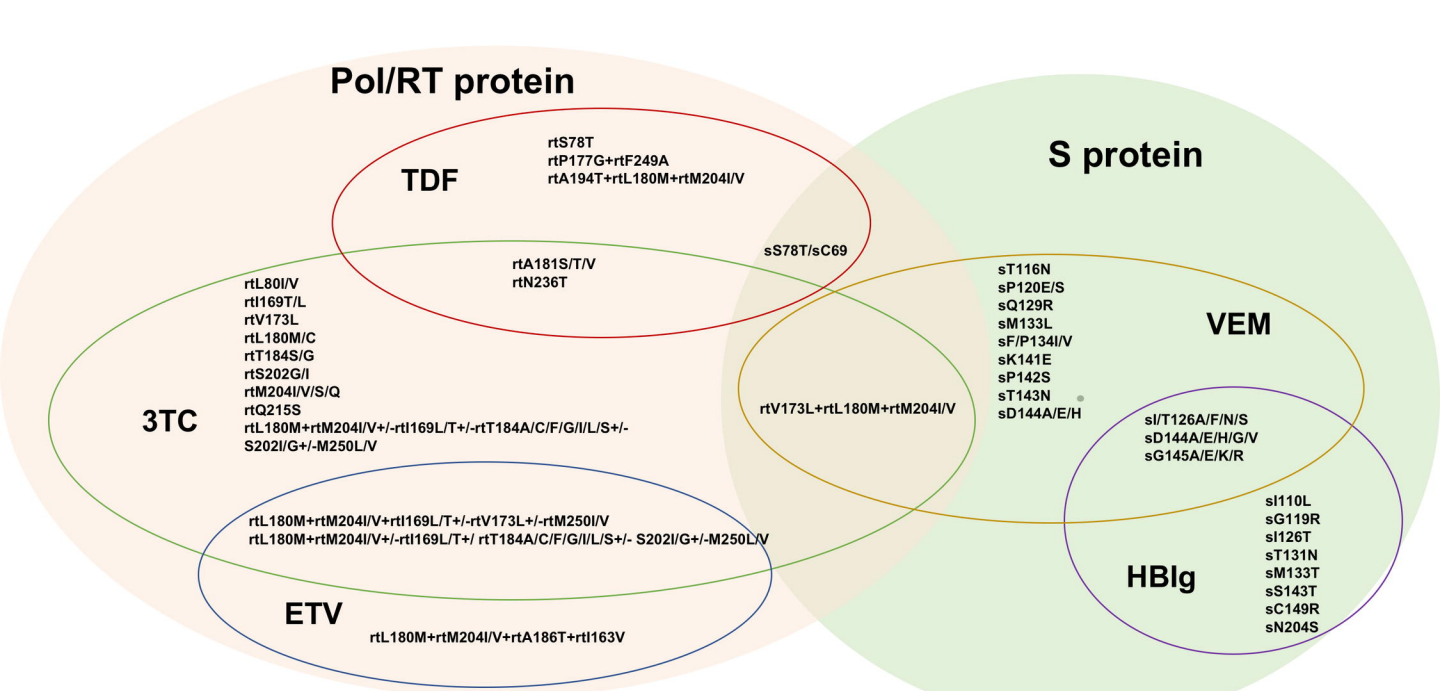

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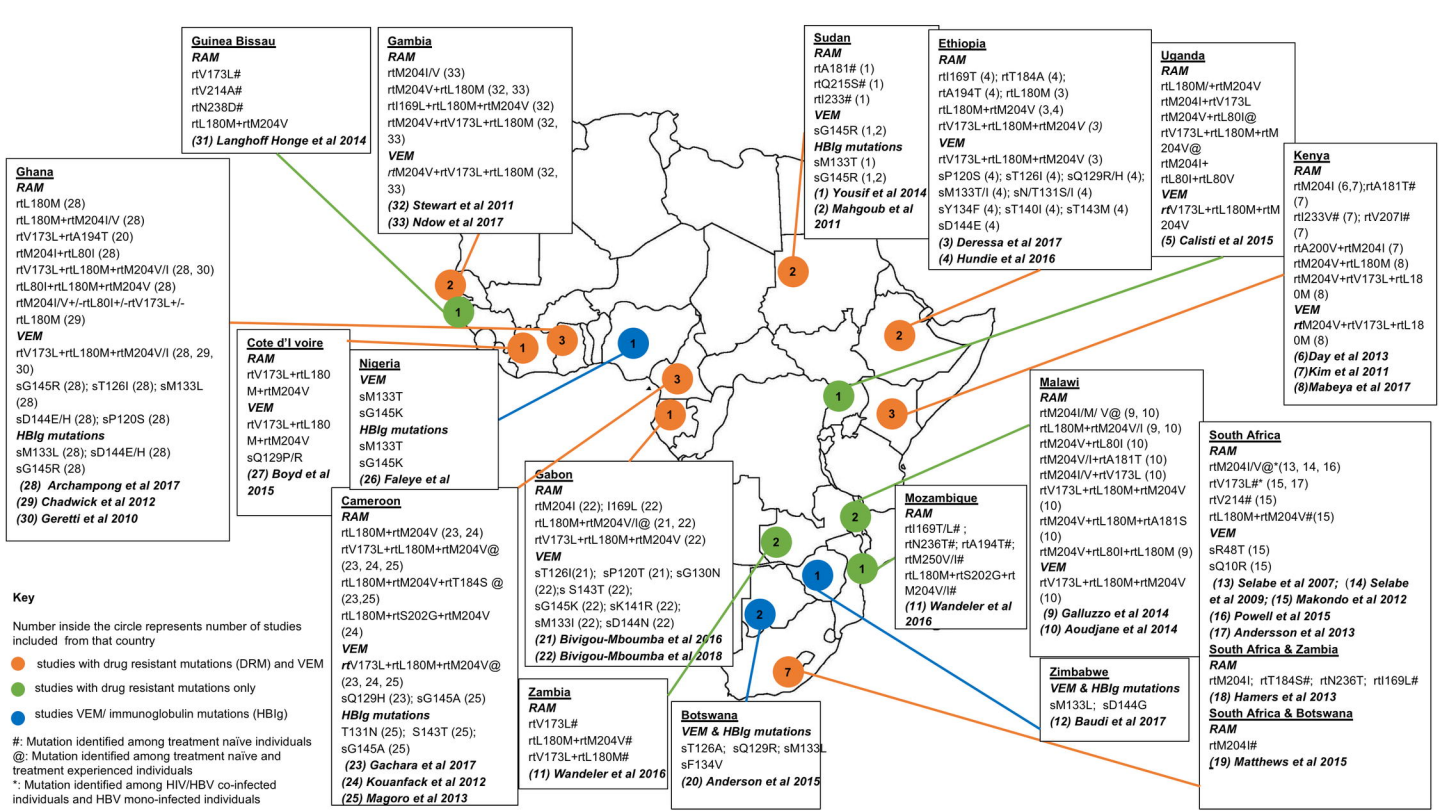
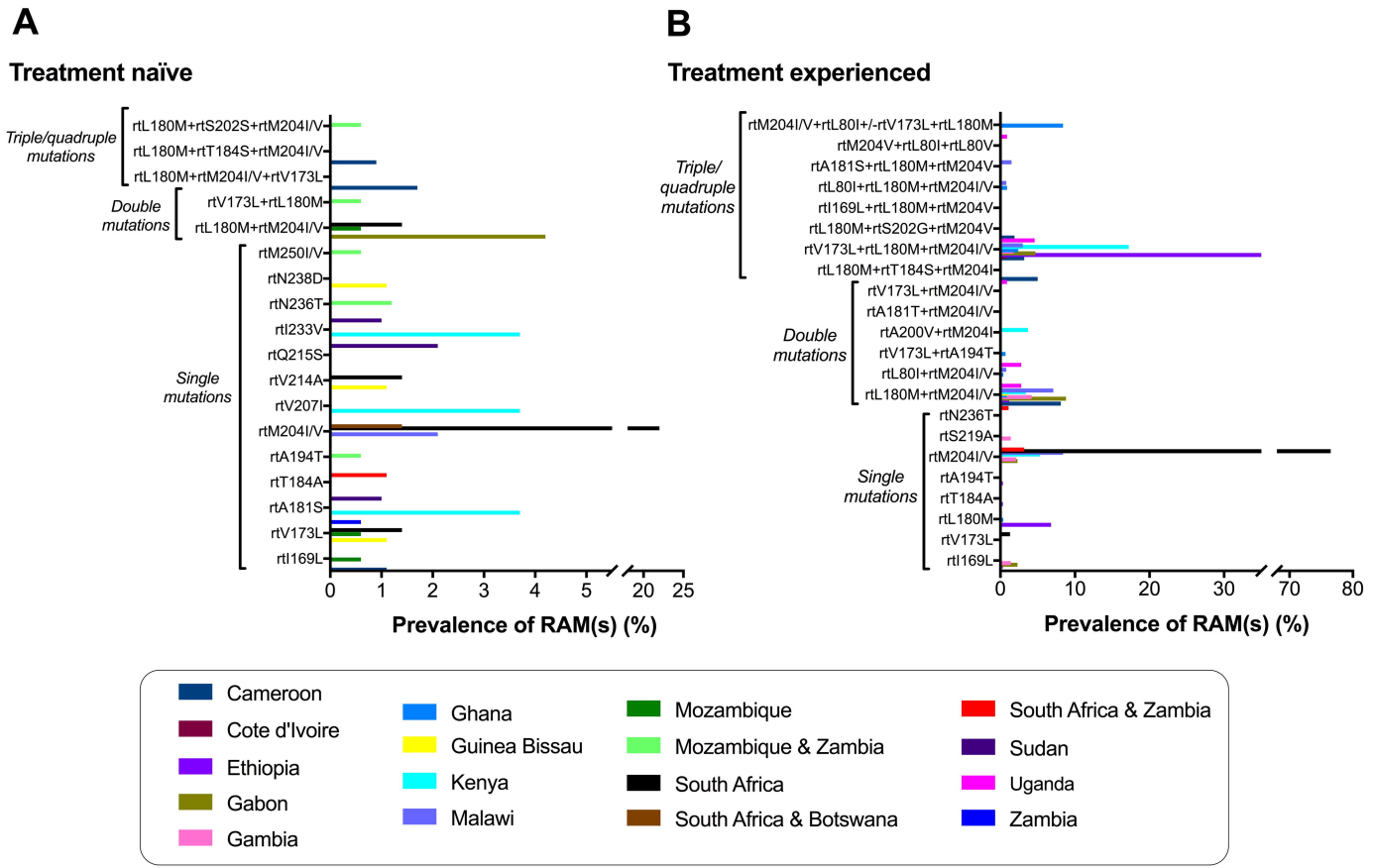

Guinea Bissau
RAM
rtV173L#
rtV214A#
rtN238D#
rtL180M+rtM204V
(31) Langhoff Honge et al 2014
Gambia
RAM
rtM204I/V (33)
rtM204V+rtL180M (32, 33)
rtI169L+rtL180M+rtM204V (32)
rtM204V+rtV173L+rtL180M (32, 33)
VEM
rtM204V+rtV173L+rtL180M (32, 33)
(32) Stewart et al 2011
(33) Ndow et al 2017
Sudan
RAM
rtA181#; (1)
rtQ215S#; (1)
rtI233#; (1)
VEM
sG145R (1,2)
HBIg mutations
sM133T (1)
sG145R (1,2)
(1) Yousif et al 2014
(2) Mahgoub et al 2011
Ethiopia
RAM
rtI169T (4); rtT184A (4);
rtA194T (4); rtL180M (3)
rtL180M+rtM204V (3,4)
rtV173L+rtL180M+rtM204V (3)
VEM
rtV173L+rtL180M+rtM204V (3)
sP120S (4); sT126I (4); sQ129R/H (4);
sM133T/I (4); sN/T131S/I (4)
sY134F (4); sT140I (4); sT143M (4)
sD144E (4)
(3) Deressa et al 2017
(4) Hundie et al 2016
Uganda
RAM
rtL180M/+rtM204V
rtM204I+rtV173L
rtM204V+rtL80I@
rtV173L+rtL180M+rtM204V@
rtM204I+
rtL80I+rtL80V
VEM
rtV173L+rtL180M+rtM204V
(5) Calisti et al 2015
Kenya
RAM
rtM204I (6,7);rtA181T# (7)
rtI233V# (7); rtV207I# (7)
rtA200V+rtM204I (7)
rtM204V+rtL180M (8)
rtM204V+rtV173L+rtL180M (8)
VEM
rtM204V+rtV173L+rtL180M (8)
(6)Day et al 2013
(7)Kim et al 2011
(8)Mabeya et al 2017
Ghana
RAM
rtL180M (28)
rtL180M+rtM204I/V (28)
rtV173L+rtA194T (20)
rtM204I+rtL80I (28)
rtV173L+rtL180M+rtM204V/I (28, 30)
rtL80I+rtL180M+rtM204V (28)
rtM204I/V+/-rtL80I+/-rtV173L+/-rtL180M (29)
VEM
rtV173L+rtL180M+rtM204V/I (28, 29, 30)
sG145R (28); sT126I (28); sM133L (28)
sD144E/H (28); sP120S (28)
HBIg mutations
sM133L (28); sD144E/H (28)
sG145R (28)
(28) Archampong et al 2017
(29) Chadwick et al 2012
(30) Geretti et al 2010
Cote d'I voire
RAM
rtV173L+rtL180M+rtM204V
VEM
rtV173L+rtL180M+rtM204V
sQ129P/R
(27) Boyd et al 2015
Nigeria
VEM
sM133T
sG145K
HBIg mutations
sM133T
sG145K
(26) Faleye et al
Gabon
RAM
rtM204I (22); I169L (22)
rtL180M+rtM204V/I@ (21, 22)
rtV173L+rtL180M+rtM204V (22)
VEM
sT126I(21); sP120T (21); sG130N (22);s S143T (22);
sG145K (22); sK141R (22);
sM133I (22); sD144N (22)
(21) Bivigou-Mboumba et al 2016
(22) Bivigou-Mboumba et al 2018
Cameroon
RAM
rtL180M+rtM204V (23, 24)
rtV173L+rtL180M+rtM204V@ (23, 24, 25)
rtL180M+rtM204V+rtT184S @ (23,25)
rtL180M+rtS202G+rtM204V (24)
VEM
rtV173L+rtL180M+rtM204V@ (23, 24, 25)
sQ129H (23); sG145A (25)
HBIg mutations
T131N (25); S143T (25);
sG145A (25)
(23) Gachara et al 2017
(24) Kouanfack et al 2012
(25) Magoro et al 2013
Zambia
RAM
rtV173L#
rtL180M+rtM204V#
rtV173L+rtL180M#
(11) Wandeler et al 2016
Botswana
VEM & HBIg mutations
sT126A; sQ129R; sM133L
sF134V
(20) Anderson et al 2015
Mozambique
RAM
rtI169T/L# ;
rtN236T#; rtA194T#;
rtM250V/I#
rtL180M+rtS202G+rtM204V/I#
(11) Wandeler et al 2016
Malawi
RAM
rtM204I/M/ V@ (9, 10)
rtL180M+rtM204V/I (9, 10)
rtM204V+rtL80I (10)
rtM204V/I+rtA181T (10)
rtM204I/V+rtV173L (10)
rtV173L+rtL180M+rtM204V (10)
rtM204V+rtL180M+rtA181S (10)
rtM204V+rtL80I+rtL180M (9)
VEM
rtV173L+rtL180M+rtM204V (10)
(9) Galluzzo et al 2014
(10) Aoudjane et al 2014
Zimbabwe
VEM & HBIg mutations
sM133L; sD144G
(12) Baudi et al 2017
South Africa
RAM
rtM204I/V@*(13, 14, 16)
rtV173L#* (15, 17)
rtV214# (15)
rtL180M+rtM204V#(15)
VEM
sR48T (15)
sQ10R (15)
(13) Selabe et al 2007; (14) Selabe et al 2009; (15) Makondo et al 2012
(16) Powell et al 2015
(17) Andersson et al 2013
South Africa & Zambia
RAM
rtM204I; rtT184S#; rtN236T; rtI169L#
(18) Hamers et al 2013
South Africa & Botswana
RAM
rtM204I#
(19) Matthews et al 2015
Key
Number inside the circle represents number of studies included from that country
studies with drug resistant mutations (DRM) and VEM
studies with drug resistant mutations only
studies VEM/ immunoglobulin mutations (HBIg)
#: Mutation identified among treatment naïve individuals
@: Mutation identified among treatment naïve and treatment experienced individuals
*: Mutation identified among HIV/HBV co-infected individuals and HBV mono-infected individuals


A
Treatment naïve
Triple/quadruple mutations
rtL180M+rtS202S+rtM204I/V
rtL180M+rtT184S+rtM204I/V
rtL180M+rtM204I/V+rtV173L
Double mutations
rtV173L+rtL180M
rtL180M+rtM204I/V
Single mutations
rtM250I/V
rtN238D
rtN236T
rtI233V
rtQ215S
rtV214A
rtV207I
rtM204I/V
rtA194T
rtT184A
rtA181S
rtV173L
rtI169L
0 1 2 3 4 5 20 25
Prevalence of RAM(s) (%)
B
Treatment experienced
Triple/ quadruple mutations
rtM204I/V+rtL80I+/-rtV173L+rtL180M
rtM204V+rtL80I+rtL80V
rtA181S+rtL180M+rtM204V
rtL80I+rtL180M+rtM204I/V
rtI169L+rtL180M+rtM204V
rtL180M+rtS202G+rtM204V
rtV173L+rtL180M+rtM204I/V
rtL180M+rtT184S+rtM204I
Double mutations
rtV173L+rtM204I/V
rtA181T+rtM204I/V
rtA200V+rtM204I
rtV173L+rtA194T
rtL80I+rtM204I/V
rtL180M+rtM204I/V
Single mutations
rtN236T
rtS219A
rtM204I/V
rtA194T
rtT184A
rtL180M
rtV173L
rtI169L
0 10 20 30 70 80
Prevalence of RAM(s) (%)
Cameroon
Cote d'Ivoire
Ethiopia
Gabon
Gambia
Ghana
Guinea Bissau
Kenya
Malawi
Mozambique
Mozambique & Zambia
South Africa
South Africa & Botswana
South Africa & Zambia
Sudan
Uganda
Zambia