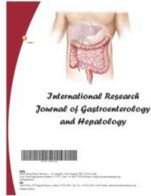

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Detection and Genotypic Characterization of Hepatitis B Virus among Prospective Blood Donors at a Tertiary Healthcare Facility in Central Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author HCE designed the study, collected samples, performed laboratory and statistical analyses and wrote the first draft of the manuscript. Authors HIM and GRP designed and supervised the study, manage literature searches, wrote the protocols and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to detect and type hepatitis B virus circulating among prospective blood donors at a tertiary healthcare Facility in Central Nigeria
Study Design: The study was a cross sectional study.
Place and Duration of Study: Keffi, Nasarawa State, between January and October, 2018.
Methodology: Blood sample (3 ml) was collected from each of the 400 consenting blood donors at Federal Medical Centre, Keffi, Nasarawa State and their socio-demographic information obtained using structured questionnaires. The sera were screened for HBV infection serologic markers (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb) using HBV-5 rapid panel test kit (CTK Biotech. Inc. San Diego, USA). All samples positive for HBsAg, HBeAg and those negative for HBsAg but positive for HBcAb were genotyped by PCR using type-specific primers. Data collected were analysed using Smith's Statistical Package (version 2.8, California, USA) and P value of ≤ 0.05 was considered statistically significant.

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Results: Of the 400 blood donors screened, 31(7.8%) were positive for HBsAg, 113(28.3%) for HBsAb, 11(2.8%) for HBeAg, 18(4.5%) for HBeAb and 78(19.5%) for HBcAb. A total of 34 samples (HBsAg-positive and HBsAg-negative but HBcAb-positive) were genotyped and of these, 32 (94.1%) had HBV-DNA bands while the remaining 2(5.9%) samples were not-typable. Furthermore, Of the 32 HBV-DNA-positive samples successfully genotyped, 17(53%) had single HBV genotype infection while the remaining 15 (46.8%) had mixed HBV genotype infection. In relation to frequency of occurrence, single HBV/E genotype was predominant (37.5%), followed HBV/F (9.4%) and HBV/A (6.3%). Meanwhile, double mixed-infection of HBV genotypes B/E had the highest rate (18.8%), followed by B/D (12.5%) and A/B (3.1%). Finally, triple infections with both A/B/D and A/B/E genotypes occurred at the same rate of 6.3%

Conclusion: This study reported the circulation of HBV genotypes A, B, D, E and F in the study population with predominance of genotype E and a novel appearance of genotype F in Nigeria. These findings are of public health significance particularly in antiviral therapy.

Keywords: Hepatitis B virus; infection; seromarkers; HBV genotypes; blood donors; Central Nigeria.

1. INTRODUCTION

Hepatitis B virus (HBV) infection remains a major global health problem in the world, with approximately 257 million people chronically infected and accounting for over 620,000 deaths per year [1]. HBV, the causative organism of the infection is a double-stranded DNA virus of a complex structure that causes infection of the liver [2]. It belongs to the *Hepadnaviridae* family and is the most common cause of chronic liver disease; hepatocellular carcinoma and necrotizing vasculitis [3]. Most infected individuals are asymptomatic [1]. However, others may have symptoms including yellowing of the skin and eyes (jaundice), nausea, dark urine, extreme fatigue, abdominal pain and vomiting [1,3].

The most common outcome after HBV infection is the expression of diverse serological markers of varying epidemiological and clinical significance namely; hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (HBeAb), hepatitis B core antigen (HBcAg) and hepatitis B core antibody (HBcAb) [4]. Symptomatic and asymptomatic forms of both acute and chronic infections may be discovered incidentally only through laboratory assay of these viral markers [5]. These markers may occur singly or in various combinations depending on the natural history of the infection [4,6].

Genotypically, HBV genomes have been classified according to their genetic variability (>8% for whole genome) into 10 major genotypes designated A to J [7] with different geographical distributions, clinical features and responses to treatment [8,9]. This genetic diversity occurs due to the replication error of the virus during its replication [7]. HBV genotype A is predominant in Eastern and Southern Africa, Europe and South-east Asia [10]. Genotypes B and C are found in Asia [11]; genotype D is common in the Mediterranean area, the Middle East and India [12,13]; genotype F is restricted to Central and South America [10]; Genotype G has been found in France and Germany [11] while genotype E is the predominant strain found in Sub-Saharan Africa followed by A and D genotypes respectively [14-17]. Studies conducted in Nigeria have reported the circulation of genotypes A, B and E in different locations and population groups with E being the most predominant genotype [14,18,19].

It was documented that infections caused by HBV genotypes A and D tend to progress to chronic stage than one as a result of genotypes B and C, while genotypes A and B have higher rates of spontaneous HBeAg seroconversion compared to C and D [20]. Genotype E is associated with poor response to interferon-based therapy [8].

There still paucity of published data on HBV genotypes in Nigeria. Thus, this study was conducted to detect and type hepatitis B virus circulating among prospective blood donors at a tertiary healthcare Facility in Central Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at Federal Medical Centre, Keffi, Nasarawa State, Nigeria. It is one of the 22 Federal Medical Centres established by

the Federal Government of Nigeria to provide basic and advanced healthcare needs of people living in the State and other neighboring States. Keffi city, where the centre is located is approximately 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the capital of Nasarawa State. It is located geographically between latitude $8^0$3'N of the equator and longitude $7^0$50'E and situated on an altitude of 850m above sea level. Additionally, it has an area of 138km^2 and a population of about 92,664 at the 2006 census [21].

2.2 Study Population

The study population comprises of male and female commercial donors and adult relatives of patients requiring blood transfusion aged 18-65 years who came to donate blood at the Blood Group and Serology unit of the Federal Medical Centre, Keffi.

2.3 Ethical Approval and Consent

Formal ethical approval to conduct this study was obtained from the Research Ethics Committee of Federal Medical Centre, Keffi (FMC/KF/HREC/212/17). In addition, All individuals included in this study willingly completed and signed an informed consent form. Individual anonymity was treated with confidentiality and for the purpose of this study.

2.4 Sample Size Determination

The sample size for this study was determined using the formula by Naing et al. [22] for sample size calculation at 0.05 level of precision;

$$n = \frac{Z^2pq}{d^2}$$

Where:

n = required sample size
Z = standard normal deviation at the required confidence interval (1.96) which corresponds to 95% confidence interval.
P = prevalence of HBV infection from previous study (8.3%) (0.1) [15].
Q = 1 – p = 0.9
d = degree of precision expected (0.05)

$$n = \frac{(1.96)^2(0.1)(0.9)}{(0.05)^2} = \frac{3.8416 \times 0.09}{0.0025} = \frac{0.3457}{0.0025} = 138.3$$

$$n = 138$$

To minimize error, this was however rounded up to 400 samples.

2.5 Sample Collection, Processing and Storage

The Blood Group and Serology unit of the Medical laboratory was used as the sample collection point. Three mls of blood sample was obtained from each consenting participant by venepuncture and placed in an appropriately labeled plain tube. This was allowed to clot at room temperature and spun for 5 min at 3000 rpm to separate out the serum [23]. The resultant sera were harvested into well-labeled cryovials and stored at −20 °C until use.

2.6 Laboratory Analyses

2.6.1 Detection of HBV infection serologic markers

All sera were screened for HBV infection serologic markers (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb) using HBV-5 rapid panel test kit (CTK Biotech. Inc. San Diego, USA). The test was conducted and results interpreted according to manufacturer's instructions.

2.6.2 Molecular genotyping of hepatitis B virus

A genotyping system based on Polymerase Chain Reaction (PCR) using type-specific primers was used for the determination of HBV genotypes (A to F) according to previously described methods [16]. Samples positive for HBsAg, HBeAg and those negative for HBsAg but positive for HBcAb (because HBcAb is an indicator of HBV occult infection) were selected for molecular typing of HBV. It was conducted at DNA Labs. No. Q5 Danja Road, Angwan Sarki, Kaduna State.

2.6.3 Hepatitis B virus DNA extraction

The HBV viral DNA was extracted using AccuPrep genomic DNA extraction kit (BIONEER Daejeon, North Korea) according to the manufacturer's instructions.

2.6.4 Polymerase Chain Reaction (PCR)

PCR was carried out in two rounds with MJ Research PTC-100 programmable thermal cycler (MJ Research Inc., Water- town, USA), using oligonucleotide primers that were adopted from the work of Doumbia et al. [16].

For the first round PCR, the following primers were used;

P1 5'- TCACCATATTCTTGGGAACAAGA-3' (universal sense)

S1-2 5'- CGAACCACTGAACAAATGGC-3' (universal antisense)

For the second round PCR, the following primers were used;

Mix A;

B2 5'- GGCTCAAGTTCAGGAACAGT-3' (types A to C specific, sense)

BA1R 5'- CTCGCGGAGATTGACGAGATGT-3' (type A specific, antisense)

BB1R 5'- CAGGTTGGTGAGTGACTGGAGA-3' (type B specific, antisense)

BC1R 5'- GGTCCTAGGAATCCTGATGTTG-3' (type C specific, antisense)
Mix B;

B2R 5'- GGAGGCGGATCTGCTGGCAA-3' (types D to F specific, antisense)

BD1 5'- GCCAACAAGGTAGGAGCT-3' (type D specific, sense)

BE1 5'- CACCAGAAATCCAGATTGGGACCA-3' (type E specific, sense)

BF1 5'- GCTACGGTCCAGGGTTACCA-3' (type F specific, sense)

2.6.5 Polymerase chain reaction (PCR) procedure

For each PCR mix tube (containing Taq DNA Polymerase, dNTPs, MgCl 2 and 1 x PCR buffer), 17 µl of master mix was added. This was followed by the addition of 3 µl of the extracted DNA to make 20 µl total volume. Positive and negative control tubes were prepared by adding the same 17 µl of the master mix into each tube. To the positive control tube, 3 µl of a known DNA (genotype E) sample was added while 3 µl of deionised water was added to the negative control tube making 20 µl total volume. The content of the tube was centrifuged for 30 s using a micro-centrifuge and the tubes were loaded into the PCR machine. The first round PCR was carried out in a PTC-100 programmable thermal controller that was programmed to first incubate the samples for 5 min at 95°C, followed by 40 cycles consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. During the second round, two reactions were performed for each sample (including positive and negative controls), with the common universal sense primer (B2) and mix A for types A through C and the common universal antisense primer (B2R) and mix B for types D through F. Fresh PCR mix tubes were labelled in twos for each sample (A and B) for mix A and B where 2 µl each of the primer mix A and B was added to the corresponding tubes followed by the addition of 1 µl each of the aliquot of the first PCR product to all the samples (As and Bs). Finally, 17 µl of deionised water was added to each tube to make 20 µl total volume. The content of each tube was centrifuged to mix properly, loaded into the PCR machine and allowed to run using the following parameters; one amplification for 40 cycles consisting of preheating at 95°C for 5 min, 30 cycles of amplification at 94°C for 2 min, 58°C for 1 min and 75°C for 5 min.

2.6.6 Agarose gel electrophoresis

The PCR products were analyzed by running a 2% agarose gel stained with ethidium bromide. The sizes of PCR products were estimated in relation to the migration pattern of a 100bp to 1000bp increments plus DNA molecular marker (BIONEER Daejeon, North Korea). The results were interpreted considering the specific size of each genotype as follows; in mix A; type A 68bp, type B 281bp and type C 122bp; in mix B; type D 119bp, type E 169bp and type F 97bp.

2.7 Data Analysis

The data obtained were analyzed using Smith's Statistical Package (version 2.8, California, USA). Chi-square test was conducted at 95% confidence interval and P values ≤ 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

HBV Genotyping is important in determining HBV disease progression and treatment [24]. In this study, a total of 400 eligible blood donors majority of who were males (391/400) and aged 25-34 years (203/400) were screened for HBV infection serologic markers. Of these, 31(7.8%) were positive for HBsAg, 113(28.3%) for HBsAb, 11(2.8%) for HBeAg, 18(4.5%) for HBeAb and 78(19.5%) for HBcAb (Table 1). However, a total of 34 samples (HBsAg-positive and HBsAg-negative but HBcAb-positive) were genotyped by

PCR using type specific primers. Of the 34 samples genotyped, 32 (94.1%) had the HBV-DNA bands while the remaining 2(5.9%) samples were not-typable (Figs. 1A-2B). The 2(5.9%) HBV positive samples that were not-typable in this study might probably be due to mutation in the viral genome, errors in DNA extraction protocols or PCR procedures.

This study was able to detect and genotype infecting HBV in 94.1% (32/34) of the collected samples that were HBV-DNA positive. This was consistent with previous findings in Nigeria where Ahmad et al. [14] and Pennap et al. [15] were able to detect and characterize 83.6% and 100% HBV respectively using nested PCR. Researchers from other countries of the world also reported similar observations. For instance, in Egypt by Khaled et al. [25] using innogenetics line probe assay (INNO-LiPA), in the United Arab Emirates by Al-Shaer et al. [26] using the nucleic acid testing (NAT), in Cote d'Ivoire by Doumbia et al. [16] using nested PCR; in India by Pandey et al. [27] using the NAT and in Iraq by Al-Suraifi et al. [28] using nested PCR where they were able detect and genotype infecting HBV in 71.4%, 95.0%, 68.7%, 69.7% and 60.5% of the collected samples respectively. The observed varying rates of isolation may be impacted by different methodologies of detection and characterization of the virus employed in the different studies.

Furthermore, Of the 32 HBV-DNA-positive samples genotyped, 17(53%) had single HBV genotype infection while the remaining 15 (46.8%) had mixed HBV genotype infection (Fig. 3). This agrees with the report of Pennap et al. [15] in Central Nigeria were single genotype infection was predominant. However, it disagrees with the findings of Ahmad et al. [14] in Zaria who reported mixed genotype infection of 82.6% as against single genotype infection of 17.4% respectively. The mixed genotype infection recorded in this study could be as a result of possible infection with more than one genotype of the virus. It could also be as a result of migrations and long-term travels for instance, inter and intra continental travels for trade, studies or peacekeeping missions by military personnel [29,30].

In this current studies, single HBV/E genotype was predominant (37.5%), followed HBV/F (9.4%) and HBV/A (6.3%). Meanwhile, double mixed-infection of HBV genotypes B/E had the highest rate of occurrence (18.8%) followed by B/D (12.5%) and A/B (3.1%). Finally, triple infections with both A/B/D and A/B/E genotypes occurred at the same rate of 6.3% (Fig. 4).

Interestingly, HBV genotypes E has been reported to be the predominant genotype in Nigeria [31-33] and Sub-Saharan Africa in general [16,17]. This study is in agreement with previous studies that showed Genotype E to be the prevalent genotype in Nigeria with Genotype B being the second most prevalent [15]. Mixed infection of genotype B/E in this study is in consonance with the earlier reports of Pennap et al. [15] and Ahmad et al. [14] in Nasarawa and Kaduna State respectively. Mono-infection genotype F was identified in this study, which has not been common in Nigeria. However, it is either that this genotype has always been in the population without identification due to limited study on HBV genotypes in Nigeria or that the virus keeps evolving in the Nigerian population.

Table 1. Prevalence and distribution of HBV infection serologic markers in relation to age and gender among prospective blood donors at Federal Medical Centre Keffi, Nigeria

Parameter	No. Examined	No. Positive (%)				
		HBsAg	HBsAb	HBeAg	HBeAb	HBcAb
Age (Years)						
15-24	50	7(14.0)	13(26.0)	3(6.0)	2(4.0)	13(26.0)
25-34	203	13(6.4)	52(25.6)	3(1.5)	8(3.9)	35(17.2)
35-44	131	10(7.6)	41(31.3)	4(3.0)	8(6.1)	24(18.3)
≥45	16	1(6.3)	7(43.8)	1(6.3)	0(0.0)	6(37.5)
Total	400	31(7.8)	113(28.3)	11(2.8)	18(4.5)	78(19.5)
p-value		0.4621	0.1001	0.9999	0.1777	0.0005*
Gender						
Male	391	31(7.9)	111(28.4)	11(2.8)	17(4.3)	77(19.7)
Total	400	31(7.8)	113(28.3)	11(2.8)	18(4.5)	78(19.5)
p-value		0.1435	0.0735	0.2646	0.9920	0.0222*

**Statistically significant*

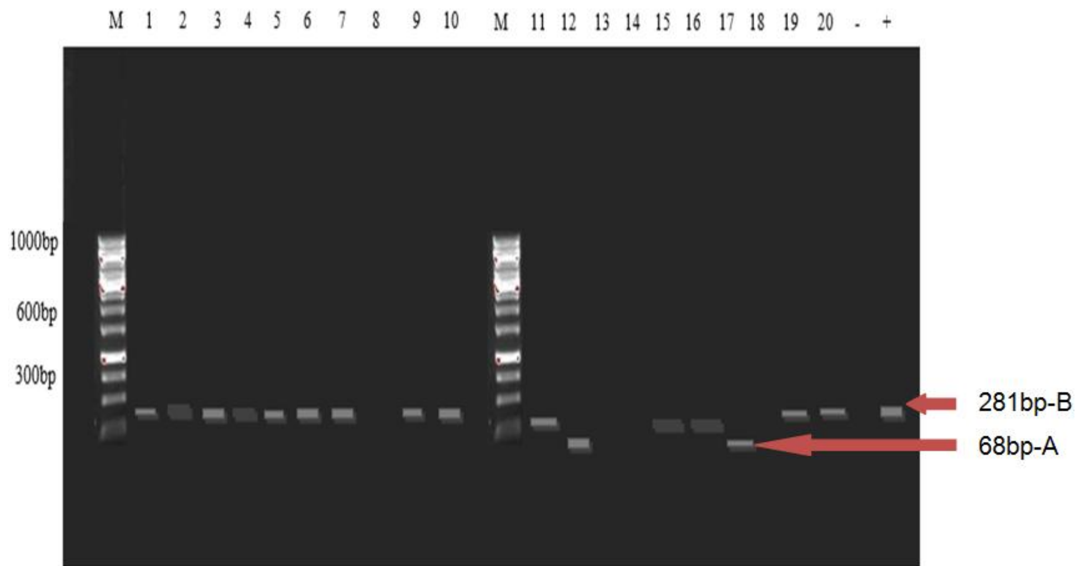

Fig. 1A. Agarose gel electrophoretogram of Mix A preparation for samples 1–20. Samples 5, 6, 8, 16 and 18 were positive for genotype A (68bp) while samples 3, 4, 5, 6, 10, 11, 16, 18 and 19 were positive for genotype B (281bp). *M represents the molecular ladder, ' −'is the negative control while ' + ' is the positive control

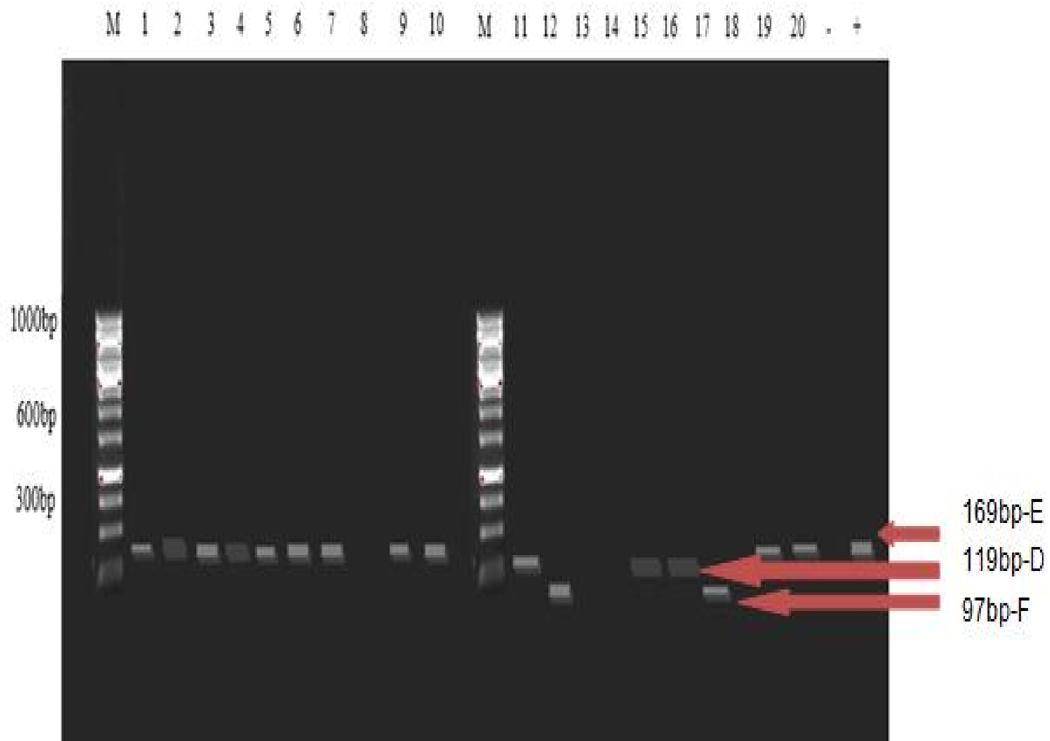

Fig. 1B. Agarose gel electrophoretogram of Mix B preparation for samples 1–20. Samples 11, 15, 16 were positive for genotype D (119bp), samples 1, 2, 3, 4, 5, 6, 7, 9, 10, 19 and 20 were positive for genotype E (169bp) while samples 12 and 17 were positive genotype F (97bp). *M represents the molecular ladder, ' −'is the negative control while ' + ' is the positive control

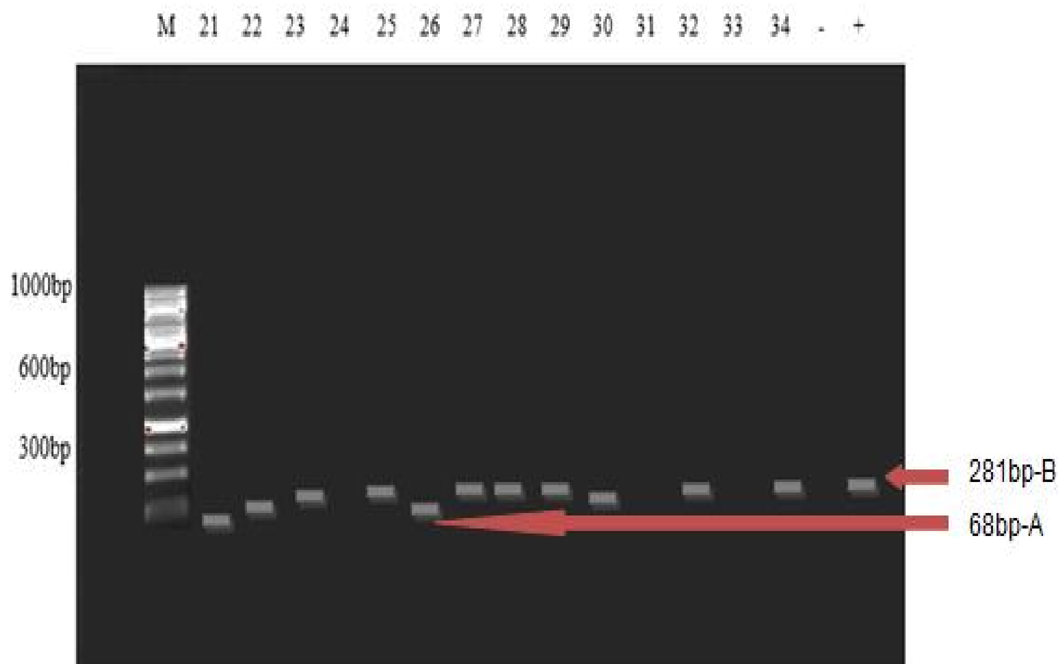

Fig. 2A. Agarose gel electrophoretogram of Mix A preparation for samples 21–34. Samples 24 and 26 were positive for genotype A (68bp), samples 22, 24, 25, 26 and 30 were positive for genotype B (281bp) while samples 31 and 33 were not-typable. *M represents the molecular ladder, ‘ −’is the negative control while ‘ + ’ is the positive control

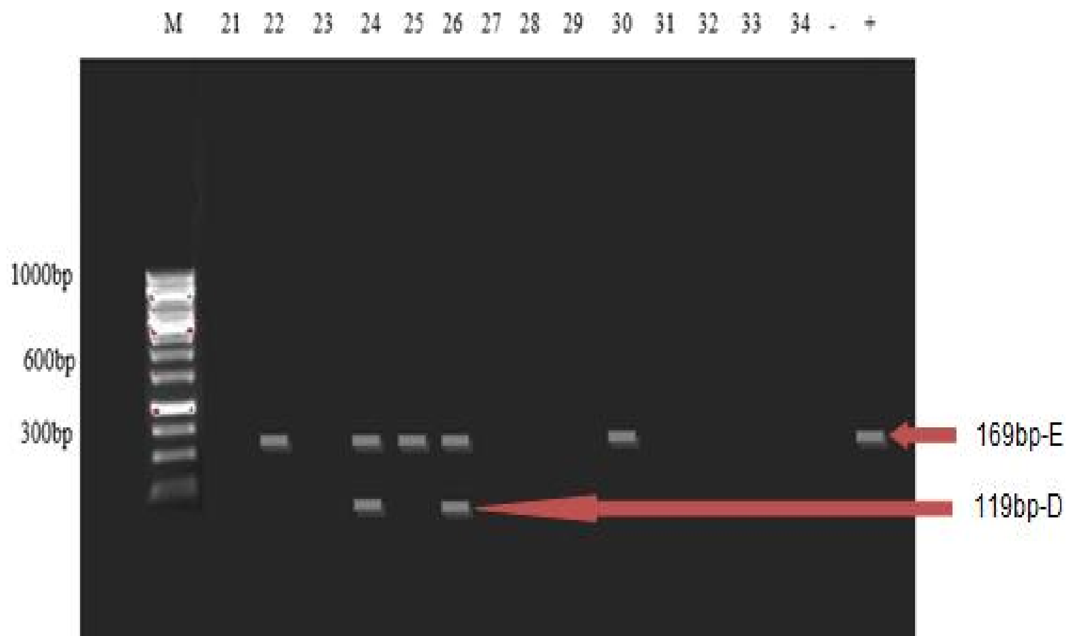

Fig. 2B. Agarose gel electrophoretogram of Mix B preparation for samples 21–34. Samples 22, 26, and 30 were positive for genotype D (119bp), Samples 23, 25, 27, 28, 29, 32 and 34 were positive for genotype E (169bp), sample 21 was positive for genotype F (97bp) while samples 31 and 33 were not-typable. *M represents the molecular ladder, ‘ −’is the negative control while ‘ + ’ is the positive control

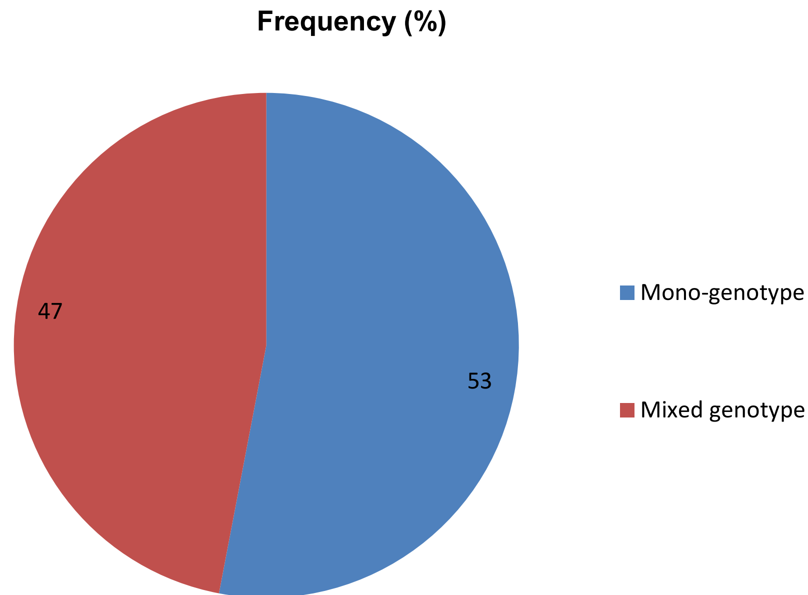

Fig. 3. Proportions of HBV mono and mixed genotype infection among prospective blood donors at Federal Medical Centre Keffi, Nigeria

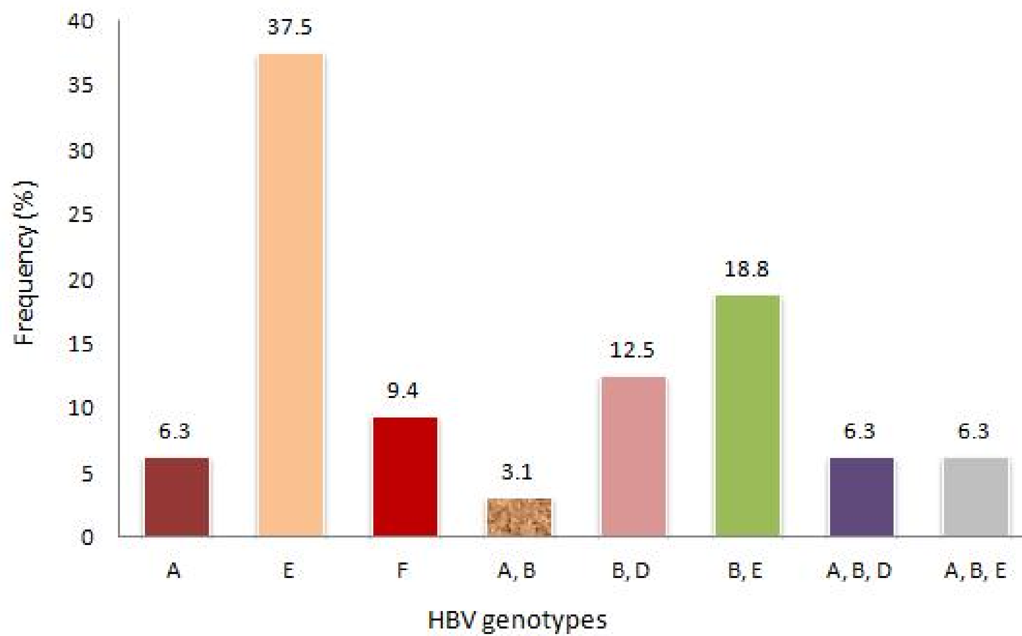

Fig. 4. HBV genotype distribution among prospective blood donors at Federal Medical Centre Keffi, Nigeria

4. CONCLUSION

This study reveals the presence HBV infection among prospective blood donors in the study area. This is frightening since the infected donors may serve as source of infection to blood recipients. The study also reported the circulation of HBV genotypes A, B, D, E and F in the study

population with predominance of genotype E and a novel appearance of genotype F in Nigeria. Hence, it is recommended that HBV genotyping become a routine exercise in clinical medicine since these genotypes have different response to treatment.

CONSENT

All authors declare that written informed consent was obtained from each participant (or other approved parties) for publication of this research work.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have been conducted in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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