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Increased levels of pre-treatment drug resistance of human immunodeficiency virus type 1 subtypes in people living with HIV in Ghana: A cross-sectional study

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Abstract

Background: Antiretroviral therapy (ART) has significantly reduced the burden of human immunodeficiency virus (HIV-1) infection. However, the emergence of drug resistance (DR) during therapy and the transmission of resistant strains contribute to treatment failure and may compromise ART efficiency. Genotypic-DR testing guides the selection of drugs for initiation of therapy or a switch to new regimens. However, such precision medicine practice among persons living with HIV (PLWH) is not available in Ghana, thus creating a critical gap in knowledge of the contribution of pre-treatment drug resistance to treatment failure.

Objective: The study aimed to determine the presence of drug-resistant HIV-1 strains in ART-naive people living with HIV (PLWH) in Ghana.

Methods: Sixty-nine (69) ART-naive PLWH were enrolled from three hospitals in Accra. Demographics and clinical data were documented, and venous blood samples were collected. HIV-1 protease and reverse transcriptase genes were amplified by conventional polymerase chain reaction (PCR) and directly sequenced. Sequences were assembled and edited and submitted to the Stanford HIV Drug Resistance Database (HIVdb) for subtyping and DR analyses.

Results: The mean viral load and CD4+ counts were 1.38 x 10^5 copies/ml and 409 cells/µl, respectively. We found 10.8% resistance (K103N, V179I, A98G, E138A) against non-nucleoside reverse transcriptase inhibitors, three accessory protease inhibitors mutations (V32L, V11L, L10LF) in nine participants, but no NRTI mutations. Sixty-four percent (64%) of participants carried HIV-1 subtype CRF02_AG, 26% carried subtype B, and the remaining were subtypes CRF06_cpx, A, C and G.

Conclusion: Our data confirmed CRF02_AG as the predominant HIV-1 subtype in Ghana, with increasing occurrence of subtype B. These findings indicate the need for continuous monitoring of subtype dynamics and drug resistance to guide the national ART program and enhance the clinical management of PLWH.

Keywords: ART, HIV-1, pre-treatment drug resistance, NNRTI, PI, CRF

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INTRODUCTION

Human immunodeficiency virus (HIV) type-1 (HIV-1) infection occurs globally, with an estimated 37.9 million people living with the virus [1]. About 25.8 million people living with HIV-1 (PLWH) reside in sub-Saharan Africa [2-5]. Globally, HIV-1 infections have declined, and the UNAIDS has projected that by 2030, effective antiretroviral therapy (ART) should be available for all infected people in all HIV-1-endemic regions [3,6]. Antiretroviral therapy (ART) has effectively transformed HIV-1 into a manageable chronic illness by

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reducing HIV-1 replication. Many antiretroviral drugs belonging to different drug classes have been licensed, but the most widely used drugs in sub-Saharan Africa target protease, reverse-transcriptase (RT) and recently, integrase proteins of HIV [7,8]. Most patients on combination ART experience virologic suppression, delayed progression to Acquired Immune Deficiency Syndrome (AIDS), and increased life expectancy [9].

However, the emergence of drug resistance poses a major challenge to the effective management of HIV-1 with ART [10-12]. Studies conducted in HIV-1-endemic but well-resourced regions globally have identified and established drug resistance as a leading cause of treatment failures [10,11,13]. Recent studies have observed an increasing trend in the prevalence of transmitted HIV-1 drug resistance, ranging between 3% and 7% in some African countries and at about 10% globally [13-16].

In Ghana, previous studies have documented HIV drug resistance mutations in both ART-naive and ART-experienced PLWH [17-20]. However, due to the increasing trends of transmitted drug resistance reports in other parts of the world [13-16], it is imperative to regularly profile pre-treatment HIV-1 drug resistance mutations, particularly among PLWH and those naive to ART, to inform the choice of antiretrovirals in the first-line regimen. HIV-1 genotypic testing is done in developed countries prior to the initiation of ART to guide physicians in prescribing the appropriate drugs for each patient [13,21] and reduce treatment failure. However, in most African countries, including Ghana, pre-treatment resistance testing is not performed for PLWH prior to ART initiation. This situation could lead to the emergence and spread of drug-resistant mutations and compromise the effectiveness of ART [21,22]. Previous studies have documented a high prevalence of resistance mutations in HIV patients on ART in Ghana. However, the contribution of pre-treatment drug resistance to this is unknown [2,19,23,24].

This study, therefore, determined circulating HIV-1 subtypes and protease-inhibitor and/or reverse transcriptase-inhibitor resistance mutations in ART-naive PLWH in Ghana.

MATERIALS AND METHODS

A cross-sectional design with a purposive sampling strategy was used to enrol sixty-nine (69) ART-naive HIV-1 seropositive participants. The study participants were enrolled from the Korle-Bu Teaching Hospital (KBTH), the Greater Accra Regional Hospital (GARH), and the La General Hospital (LGH), Accra, Ghana. These hospitals have well-structured HIV clinics that provide ART and care for PLWH. Sample and data collection were done from May 2017 to December 2019. We obtained consent and enrolled people living with HIV who had not yet initiated antiretroviral therapy (ART) from three hospitals during the study period, prior to the start of their treatment. All participants already on ART or who had prior initiated and discontinued ART were not enrolled in the study. Only participants who signed the informed consent form were recruited for the study. Socio-demographic and clinical data were accessed from clinical records and interviews with the aid of a semi-structured questionnaire. Data collected included age, gender, date of HIV-1 diagnosis and risk behaviours.

About 5 ml of venous blood was collected in EDTA tube by phlebotomy, from which an aliquot of 50 µl was used for CD4+ T cell count estimation by fluorescence-activated cell sorter (FACS) Count equipment from Becton Dickinson (BD) Biosciences (San Jose, CA, USA). The remaining sample was spun in a microcentrifuge at 1,500 rpm for 10 minutes to obtain plasma. Aliquots of the plasma were used to measure viral load using the Cobas Amplicor HIV-1 MonitorTM Test from Roche (Indianapolis, IN, USA). To determine circulating HIV-1 subtypes, HIV-1 RNA was purified from plasma using nucleic acid purification reagent (Quick-RNA Viral Kit-R1034) from Zymo Research (Irvine, California, USA). The viral RNA was converted into complementary DNA (cDNA) using ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs Inc.) and stored at -20 ℃ until use. HIV-1 protease and reverse transcriptase genes were amplified from the viral cDNA using a nested polymerase chain reaction (PCR) protocol adopted from the HIV Genotyping Laboratory at the Noguchi Memorial Institute for Medical Research, University of Ghana.

HIV-1 protease was amplified using primer sets DRPR05: PR outer sense (AGACAGGTTAATTTTTTAGGGA), DRPRO2L; PR outer anti-sense (TATGGATTTTCAGGCCCAATTTTTGA), DRPRO1M; PR inner sense (AGAGCCAACAGCCCCACCAG) and DRPRO6; PR inner anti-sense (ACTTTTGGGCCATCCATTCC). HIV-1 reverse transcriptase was amplified in a nested reaction using primer sets DRRT1L: RT outer sense (ATGATAGGGGGAATTGGAGGTTT), DRRT4L; RT outer anti-sense (TACTTCTGTTAGTGCTTTGGTTCC), DRRT7L; RT inner sense (GACCTACACCTGTCAACATAATTGG) and DRRT6L; RT inner anti-sense (TAATCCCTGCATAAATCTGACTTGC) as previously described (18).

Phusion High-Fidelity PCR Master Mix with HF Buffer from New England Biolabs was used for the PCR reactions. Each reaction mixture consisted of 12.5 µl of the master mix, 1.25 µl of each primer (sense and anti-sense), 5 µl of nuclease-free water, and 5 µl of cDNA or first-round PCR product. The thermal cycling was done in a SimpliAmp Thermal Cycler (Applied Biosystems, ThermoFisher Scientific) with the following conditions: initial denaturation at 98 ℃ for 30 seconds, followed by 30 cycles of 98 ℃ for 10 seconds, 58 ℃ for 30 seconds, 72 ℃ for 90 seconds, and then a final extension at 72 ℃ for 10 minutes

for both first round and nested PCR runs. The nested PCR products were resolved on a 1.5% agarose gel electrophoresis and visualised under ultraviolet (UV) light in a UV imager (UVP BioDoc-It2 Imager, Analytina). The expected sizes of the nested PCR products were 463 bp for the protease and 887 bp for the reverse transcriptase genes and this was confirmed by agarose gel electrophoresis.

The PCR products with the right sizes were purified and directly sequenced using a Sanger sequencing platform. Primer sequences and methods used in generating the data were adopted from previous studies [25,26]. The raw sequences were assembled in Seqman Pro software version 13 (DNASTAR, USA) to generate a consensus sequence and edited for correction of base calling. The edited consensus sequences were exported into BioEdit version 7 software and aligned with the reference HIV-1 HXB2 sequence to verify that they were in the right frames and that any occurring frameshifts were corrected.

Data analysis

The consensus sequences were submitted to the Stanford University HIV Drug Resistance Database (Stanford HIVdb version 9.0) to identify HIV-1 drug-resistant mutants and determine a score of susceptibility of the respective class of antiretroviral drugs. Mutations were compared to the World Health Organisation (WHO) drug resistance surveillance list for monitoring local, national, and regional drug resistance [27,28]. The Context-based Modelling for Expeditious Typing (COMET HIV-1) platform from the Luxembourg Institute of Health was used to determine the HIV-1 subtypes of the sequences. Statistical computations for each data set were applied, where necessary, in GraphPad Prism version 8, and descriptive statistics were used to analyse the demographic and clinical history data of the participants.

RESULTS

Sixty-nine patients were enrolled, comprising 19 males and 47 females, representing 27.5% and 68.1%, respectively. Three participants (4.4%) did not indicate their gender. The demographic and clinical characteristics of the participants have been summarised in Tables 1 and 2, respectively.

HIV-1 Drug Resistance Mutations and HIV-1 Subtype Distribution

Out of the 69 samples, 62% (n = 43) and 54% (n = 37) were successfully sequenced for protease and reverse transcriptase coding regions, respectively, and submitted to the Stanford HIVdb for drug resistance mutation analysis. We found HIV-1 drug resistance mutations to NNRTIs and PIs (Table 3), some of which are on the WHO's drug resistance surveillance list [27,29]. However, no NRTI mutations were observed in this study (Table 3). Out of the 37 RT sequences obtained, four (10.8%) had NNRTI drug resistance mutations, K103N, E138A, V179I and A98G. The K103N is a major resistance and induces high-level resistance to Nevirapine and Efavirenz, the two NNRTIs used in Ghana at the time of the study. The A98G mutation induces low-level resistance. A polymorphic mutation E138A was observed in two different individuals with CRF02_AG subtypes. A minor NNRTI mutation V179I, selected in patients receiving etravirine and rilpivirine but with little effect on susceptibility, was also found in one individual. We also detected three protease inhibitor accessory mutations (V32L, V11L, L10LF). All drug resistance mutations found in this study have been summarised in Table 3. Out of the 9 patients with documented resistance mutations, 6 participants had HIV-1 subtype CRF02_AG while one each had subtypes G, A, C and B. Sixty nine percent of the circulating subtypes were CRF02_AG strains, followed by subtypes B and G being 26% and 4%, respectively. The minority populations were subtypes A, C and CRF06_cpx, which accounted for 2% each (Figure 1).

Table 1. Demographic characteristics of the study participants

Variables	n = 69	Percent (%)
Age in years (Range 8-66) Mean = 38 years		
<21	4	5.80
21 – 40	24	34.78
>40	33	47.83
Undisclosed	8	11.59
Duration since HIV-1 diagnosis		
<2years	32	46.38
2 to 5 years	4	5.80
>5 years	33	47.83
Risk behavior		
Men who have sex with men	5	7.25
Others	4	5.80
Heterosexual contact	30	43.48
Unidentified	22	31.88
Unwilling to disclose	8	11.59

Table 2. Clinical characteristics of the study participants

CD4+ lymphocyte counts (cells/mm^3)	n = 69	Percent (%)
<200	22	31.88
200 to 500	17	24.64
>500	22	31.88
Undetermined	8	11.59
Viral load (copies/µl)		
Target not detected	4	5.80
<50	3	4.35
51 to 999	10	14.49
1,000 to 99,000	36	52.17
>100,000	16	23.19

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Table 3. HIV-1 drug resistance mutations observed in the study

NNRTI Mutations (frequency of occurrence)		PI Mutations (frequency of occurrence)	
K103N (1)	NVP/EFV	V32I (1)	(ATV/LPV/DRV) *r
V179I (1)	ETR/RPV	V11L (1)	DRV*r
A98G (1)	EFV/ETR/NVP/RPV	L10LF (2)	(ATV/LPV/DRV) *r
E138A (2)	ETR/RPV		

(N)– Frequency of the mutation, NVP – nevirapine, EFV- efavirenz, RPV – rilpivirine, ETR – etravirine, ATV – atazanavir, LPV – lopinavir, DRV – darunavir, *r – ritonavir-boosted

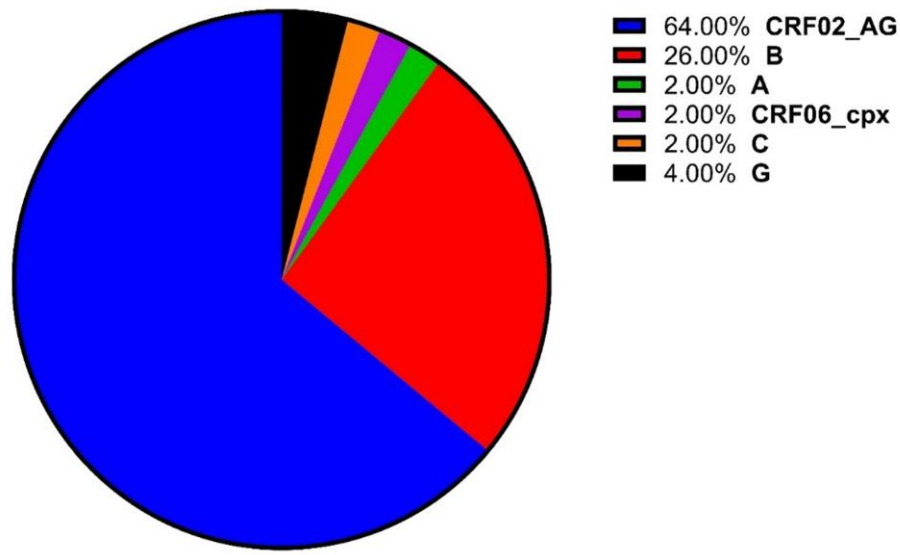

Figure 1. HIV-1 subtype distribution in the study population
A pie chart showing the percentages of the circulating HIV-1 subtypes CRF02_AG, B, A, CRF06_cpx, C and subtype G, respectively. CRF02_AG is dominant, followed by subtype B.

DISCUSSION

Globally, HIV is still a pandemic, with infections higher in females than in males in sub-Saharan Africa [4,5,30,31], an observation which has also been reported in nearly all HIV-1 endemic populations in the West African sub-region, including Ghana. In this study, more females were recorded than males, which is consistent with Ghana's HIV-1 patient demographics, where the female-to-male ratio is about 2:1 [32]. While several reasons may explain this phenomenon, the most frequently attributed reason is that more than 90% of HIV-1 transmissions in the region are via heterosexual contact, and the female genitalia predisposes women to easier acquisition of sexually transmitted diseases from men [31,33].

Participants in this study were adults aged 18 to 66 years with a mean age of 38 years, suggesting a possible higher risk of transmission of HIV-1, including but not limited to transmission due to sexual contact. At least 30 participants indicated HIV infection was through sexual contact, and was mostly heterosexual. This finding is consistent with previous reports that HIV-1 is mainly transmitted through sexual contact and accounts for more than 90% of all new infections [33].

HIV-1 primarily infects CD4+ T lymphocytes, which also serve as a marker of immune activation. Consequently, untreated HIV-1 infection depletes CD4+ T lymphocyte count with a corresponding increase in viremia. These clinical events compromise the host immune response and increase the risks of acquiring other opportunistic infections [31,34]. All the participants enrolled in this study had not initiated ART at the time of sampling, and their enrollment criteria were confirmed with their clinical records. The participants were yet to initiate ART because they did not indicate their consent to initiation at the time of first diagnosis, suggesting that there remains a subpopulation of HIV-infected persons who are yet to initiate therapy despite the gains of the 'treat all infected persons' policy. The

immunological indicator (CD4+ T lymphocyte count), with a mean of 409 cells/mm3, was consistent with their clinical records, as all participants showed no symptoms of disease, suggesting a delayed progression from infection to disease. Considering that the participants were ART-naive and carried infection for at least two years, this observation was important, though in sharp contrast with expectations of a significant reduction in the CD4+ T cell population during untreated HIV-1 infection.

In HIV-1 infection, disease progression has been strongly linked with subtypes. HIV-1 subtype B has been shown to be more virulent and accounts for the most severe cases of morbidity reported [35-37]. Previous reports have shown that HIV-1 subtypes affect drug mutation patterns, and these differences are attributed to differences in codon calls [38,39]. A significant number (74%) of the participants in this study had non-B clades of HIV-1, and this could explain the delayed disease. While the delayed progression to disease of participants is good for the HIV situation in Ghana, the gradual increase in Subtype B (26%) compared to earlier reports is worthy of note and, hence, suggests enhanced surveillance.

Nearly all HIV-1 endemic countries have reported diverse CRFs from Group M due to the rapid replication and high mutation rate of the virus. Infection of host cells by one or two viral subtypes results in recombinant forms, which have stemmed mainly from Group M HIV-1. The development of these CRFs can have a selection advantage in the host, as noted in the CRF02_AG in West Africa, CRF01_AE in Central Africa, and CRF07_BC and CRF08_BC in China (40). Studies have shown that there may be recombinants between different HIV-1 groups but not between HIV-1 and HIV-2 [41]. The increasing frequency of HIV-1 subtype B in Ghana could be because of the observation of the year of return, during which many people from the global North visited Ghana, and the number of tourists to Ghana increased. The trend is of concern because subtype B, which drives the epidemic in Europe and North America, is more transmissible and virulent than some other subtypes [35–37]. In communities in which subtype B is the more prevalent circulating strain, it is known that the transmission and reports of new cases and drug resistance are relatively higher compared to areas where non-B subtypes are the majority strains in circulation [35,42-45]

Considering that the subtype B is associated with incidental increases in drug resistance and disease progression, it will be important to monitor the HIV-1 subtype dynamics in Ghana actively through the collection of geospatial data from multiple sites in order to understand the host and pathogen genomics of subtype B as well as other subtypes circulating the country. This will help to identify regions and communities that are lagging in each stage of the HIV care continuum and, hence, upscale efforts to consolidate the gains of antiretroviral therapy and sustain viral suppression. Our study found CRF02_AG as the predominant circulating subtype of HIV-1, followed by subtype B with minority populations of subtypes A, C, G and CRF06_cpx. Even though the majority of the global HIV population lives in sub-Saharan Africa, subtype B is not the dominant clade in this region (31,46,47). HIV-1 subtypes reported in African countries showed that newly infected individuals carry clades other than B (18,48,49). A study conducted in 2016 by Colleen et al. [41] in Cameroon found different subtypes and CRFs, with 64.9% CRF02_AG. Another study in Nigeria revealed a more diversified and prevalent HIV-1 epidemic with CRF06 cpx and CRF02_AG recombinant strains in circulation [50]. The findings from our study corroborate these previous findings. HIV-1 subtype B has accounted for the majority of newly diagnosed patients in Europe, North America, and Australia, scoring as high as 70-80% of newly diagnosed cases in Europe and in the USA [36,38,48], and has been the basis for most drug resistance analysis algorithms [22,38,51]. Even though drug resistance profiles between B and non-B subtypes may be different, the established reports of drug resistance in B subtypes are applicable to non-B subtypes due to resistance-related positions that are common to many of the HIV-1 strains [22,49]. Studies have shown that the non-B and CRF clades have mainly been associated with immigration and heterosexual transmission [48].

ART initiation in many resource-limited countries, including Ghana, is usually without prior genotypic testing [49]. In advanced and some developing countries, such precision medicine is common at the initiation of ART and could explain the sustained benefits of ART [52-54]. Generally, drug-resistant mutations are driven by the susceptibility or response to the drugs and occur at very different rates in different populations. The mutations are largely caused by factors including circulating subtypes, drug adherence, and random replication-driven mutations [39,55].

A limited number of studies have investigated pre-treatment drug resistance in Ghana [56,57]. An HIV-DR threshold survey that was conducted in 2009 reported low levels of transmitted drug resistance according to WHO guidelines for characterising transmitted drug resistance [57]. The study reported M184V and Y181C as major mutations in one person and A98G, K103R, K101Q and E138Q as minor mutations. It was recommended that the study be repeated every two years [56,57]. Contrary to the 2009 study, resistance to NNRTI mutations was observed in 4 of 37 people, representing 10.8%. A major NNRTI resistance mutation K103N was found in addition to A98G and E138A, which confer low resistance to NNRTIs [22,49].

This study found no major PI-associated mutations, but accessory PI resistance mutations (V32L, V11L and L10LF) were detected. This suggests the virus's susceptibility to protease inhibitors. This observation is expected because, at the time of the study, protease

inhibitors were not extensively used but were reserved as a second-line regimen. It is important to note that the PI accessory mutations found in our study only reduce susceptibility to protease inhibitors when present with other mutations [58]. Thus, investigating their occurrence and frequencies is strongly recommended in future studies. The K20I mutation, which confers resistance to PI in HIV-1 subtype B, was only seen in non-subtype B samples, in which it is the consensus amino acid and does not confer any drug resistance.

In HIV-endemic countries, including Botswana and Eswatini, the success of the ART program and achievement of the UNAIDS 95-95-95 targets reflect a strong comprehensive clinical management, including genotypic testing services, linkage to high-quality care, retention in care and a scale-up of periodic surveillance [54,59,60]. However, this is not the case in Ghana, and this may be the reason why Ghana is far behind in achieving its UNAIDS goals. Considering the emergence of drug resistance mutations in our population of PLWH, it will be imperative to incorporate genotypic drug resistance tests into the national response to HIV in order to monitor treatment with drug resistance testing and also conduct regular national surveys on HIV drug resistance. Introducing these objectives into the current HIV clinical management to monitor the 95-95-95 progress will consolidate the intended gains of ART and minimise the risks of treatment failure.

The limitations of our present study include the failure to enrol sufficient participants proportional to the sample population. This was due to the rollout of the treat-all policy, which made it difficult to easily identify PLWH, and we have yet to initiate therapy. To the best of our knowledge, the evolution of DR mutants is best studied in HIV infection acquired for at least a year and has yet to initiate ART. Secondly, the samples studied were collected between 2017 and 2019, all from HIV ART clinics within Accra. A larger sample size from a broader population, including all the HIV ART clinics, is therefore recommended in future studies to establish the pre-treatment HIV drug resistance situation in Ghana. Additionally, there is a need for periodic surveillance studies to establish a database comprising a stable trend of HIVDR information in Ghana, considering the relevance of longitudinal studies in providing quality health care [61,62].

Conclusion

Low but increased levels of pre-treatment HIV-1 DR compared to the previous national threshold survey were observed in this study. We also found that HIV-1 subtype CRF02_AG is still dominant in Ghana, followed by subtypes B, A, C, G and CRF06_cpx. Surprisingly, we recorded a higher proportion of subtype B compared to previous studies. Although the level of PDR was low, the increasing trend strongly suggests that active monitoring of emergence of DR strains to guide the national ART program. It is therefore important to continually conduct active surveillance for subtype dynamics and drug resistance and integrate genotypic HIV drug resistance testing into routine monitoring of ART and public health campaigns. This will guide the national ART program, enhance the clinical management of PLWH and thus enable Ghana to attain the third ‘95 (viral suppression) of the UNAIDS goals.

DECLARATIONS

Ethical consideration

The protocol for this study was approved by the Institutional Review Board of the University of Ghana’s Noguchi Memorial Institute for Medical Research (NMIMR), Study 030/16-17, and the Ghana Health Service (GHS) Ethics Review Board. All participants consented to participate in the study, with parents consenting for their underage children.

Consent to publish

All authors agreed on the content of the final paper.

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Competing Interest

The authors declare no conflict of interest

Author contribution

DNKQ, PKQ, OQ, GBK and EYB designed the study. DNKQ, AAM, ATB, SA and EYB performed the laboratory experiments. DNKQ, AAM, PKQ and EYB analysed the data. DNKQ wrote the first draft. GBK and EYB edited the draft manuscript. All authors revised the manuscript, contributed to the final draft and consented to its submission for publication.

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Availability of data

The datasets generated and analysed in this work are included in this published article. The sequence dataset(s) supporting the conclusions of this article have been deposited at the GenBank with Accession

numbers ON411805 to ON411840 in https://www.ncbi.nlm.nih.gov/Genbank

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