P. falciparum msp1 and *msp2* genetic diversity in *P. falciparum* single and mixed infection with *P. malariae* among the asymptomatic population in Southern Benin

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Abstract

Plasmodium falciparum and *Plasmodium malariae* infections are prevalent in malaria-endemic countries. However, very little is known about their interactions especially the effect of *P. malariae* on *P. falciparum* genetic diversity. This study aimed to assess *P. falciparum* genetic diversity in *P. falciparum* and mixed infection *P. falciparum/ P. malariae* isolates among the asymptomatic populations in Southern Benin. Two hundred and fifty blood samples (125 of *P. falciparum* and 125 *P. falciparum/ P. malariae* isolates) were analysed by a nested PCR amplification of *msp1* and *msp2* genes. The R033 allelic family was the most represented for the *msp1* gene in mono and mixed infection isolates (99.2% vs 86.4%), while the K1 family had the lowest frequency (38.3% vs 20.4%). However, with the *msp2* gene, the two allelic families displayed similar frequencies in *P. falciparum* isolates while the 3D7 allelic family was more represented *in P. falciparum/P. malariae* isolates (88.7%). Polyclonal infections were also lower (62.9%) in *P. falciparum/P. malariae* isolates ($p< 0.05$). Overall, 96 individual alleles were identified (47 for *msp1* and 49 for *msp2*) in *P. falciparum* isolates while a total of 50 individual alleles were identified (23 for *msp1* and 27 for *msp2*) in *P. falciparum/ P. malariae* isolates. The Multiplicity of Infection (MOI) was lower in *P. falciparum/ P. malariae* isolates ($p< 0.05$). This study revealed a lower genetic diversity of *P. falciparum* in *P. falciparum/ P. malariae* isolates using *msp1* and *msp2* genes among the asymptomatic population in Southern Benin.

Keywords: *P. falciparum*, *P. malariae*, Genetic diversity, merozoite surface protein, Asymptomatic.

1. Introduction

Malaria is one of the major public health issues globally, especially in tropical and sub-tropical parts of the world where this disease is endemic and its transmission is perennial [1]. A global estimate of 229 million new malaria cases and around 409,000 malaria deaths were reported in 2019, with 92% of the disease burden recorded in the African regions [2].

To date, five parasites from the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*) are involved in malaria transmission [3]. It has been observed that a large proportion of *Plasmodium* spp. infections are asymptomatic in endemic countries [4,5]. However, in most African countries, asymptomatic malaria is not currently included in national malaria intervention strategies and thus represents a silent reservoir that sustains malaria transmission [6,7]. A high prevalence (over 70%) of asymptomatic malaria cases was reported in Ghana [8], Nigeria [9], Senegal [4], and over 50% in Southern Benin [10]. Given the high prevalence of these infections, the World Health Organization (WHO) suggests that intervention strategies should be extended to asymptomatic malaria cases for effective malaria control [11].

To control and eventually eliminate malaria, an effective vaccine is considered very important to be used in addition to the existing control measures such as the Indoor Residual Sprayings (IRS), Long-Lasting Insecticide Treated Nets (LLINs) as well as the Mass Drug Administration (MDA) of Artemisinin-based Combination Therapy (ACT) [12]. Nevertheless, the initiative to have an effective vaccine is hampered by the parasite diversity and complexity [13–16] that reduce the efficacy of the antimalaria drugs in high malaria transmission areas and complicate the development of efficient malaria vaccines.

The current parasite control tools exploit the blood-stage antigens such as Erythrocyte Binding proteins (EBA), Apical Membrane Antigen (AMA), merozoite surface proteins 1 and 2 (msp1 and

msp2) for vaccine development [17,18]. Indeed they play a key role in malaria transmission including invasion of erythrocytes [19], and are targeted by the host immune responses [20,21].

The *msp1* and *msp2* genes are highly polymorphic in malaria-endemic countries. They are used in various studies to assess the different parasite clones in circulation (genetic diversity), the number of clones infecting individuals (multiplicity of infection), and determine the impact of malaria interventions [22,23].

The polymorphism of *msp1* and *msp2* genes has also been correlated with annual malaria incidence in populations from Indonesia [24] and Papua New Guinea [25]. Several studies have further reported correlations with age, parasitemia, and genetic diversity [26,27], while some have found no association [13,27,28]. Furthermore, it has been shown that there is competition within the host, with some clones of the parasite, sometimes being suppressed or diminished when other genotypes are present [29]. Indeed, an intra-host competition was demonstrated in studies using the malaria mouse model inoculating with 3 different clones of the *P. chabaudi* strain [30,31].

Plasmodium species infections can often be presented as mono-infection (one species) or mixed infections within one individual [32,33]. These mixed infections, especially, *P. falciparum/P. malariae* is increasing in endemic countries [34,35]. It is already the case in southern Benin where 8.8% of the population was infected by mixed infections *P. falciparum/P. malariae* [10]. However, the interactions between mixed infections have not been well characterised [36]. It could be possible that there is an inter-species competition between these two species.

The genetic diversity of *P. falciparum* is poorly investigated in Benin [13,28,37]. To the best of our knowledge, only one study was reported about 20 years ago, where the diversity of *P. falciparum* in asymptomatic subjects was investigated in Southern Benin [13]. Here, we aim to provide updated data on the genetic diversity and complexity of *P. falciparum* infection using *msp1* and *msp2* genes and then compare the outcome to the genetic diversity and complexity of *P.*

falciparum infections in mixed *P. falciparum / P. malariae* isolates in the Ouidah-Kpomasse-Tori Bossito (OKT) health district in Southern Benin. These data will be helpful when selecting and designing appropriate intervention tools.

2. Materials and Methods

2.1. Study population and sampling procedure

The study was conducted in the OKT health district from June 2019 to August 2019 and from September 2019 to October 2019. A detailed description of the study area has been published in previous studies [38,39]. A total of 2289 participants aged from 0 to 105 years were recruited in the rural localities in the OKT health district. Briefly, all the volunteers who signed the informed consent and willing to participate in the study were included. A structured questionnaire was administered to record participants' socio-demographic data (body temperature, history of fever, age, sex, bed net use, and location). Approximately 60 µL of capillary blood were collected from each participant, 20 µL were used to prepare a thick and thin smear on a glass slide for detection and quantification of the asexual stages and/or gametocytes of *Plasmodium* spp using microscopy, 40 µL were used to prepare two biological replicates of blood spots on filter paper for further molecular analysis.

2.2.Microscopic and molecular identification of *Plasmodium* spp.

The collected samples were screened for *Plasmodium* spp. infection using both microscopy (thick and thin blood smear) and Nested PCR to identify *P. falciparum* and *P. malariae.* The details of the description of the methods (microscopy and Nested PCR) and the results have already been published [10]. Briefly, the thick and thin blood smears prepared were stained with 10% Giemsa and read according to WHO recommendations [40]. Furthermore, qualitative detection of *P. falciparum* and *P. malariae* was based on a nested PCR as described previously [41]. The samples

coinfected by *P. falciparum* and *P. malariae* were selected for *Pfmsp1* and *Pfmsp2* genotyping as well as *P. falciparum* mono-infections. These mono- and co-infected samples were collected from the same localities.

2.3.Allelic genotyping of *Pfmsp1* and *Pfmsp2* genes from mono and mixed infection *P. falciparum/ P. malariae* samples

PCR-positive samples were used for the genotyping. For all samples selected, nested PCR targeting the *Pfmsp1* and *Pfmsp2* genes was performed using a previously described method [42] with slight modifications. Nested PCR was performed within two reactions and the location of *msp1* and *msp2* primers used have been shown in **Figure1**: The first round of DNA amplification was performed using M1-OF/M1-OR primers for *Pfmsp1* and M2-OF/M2-OR primers for *Pfmsp2* (**Supplementary data. Table 1**) to amplify *msp1* (Block 2) and *msp2* (Block 3). The PCR mixture had a total volume of 15 µL, containing 5 µL of DNA template, 3.55 µL of water, 5 µl of buffer 5x, 4 µl of 25 mM $MgCl_2$, 1 µL of 5 mM dNTPs, and 0.25 µL of Taq DNA Polymerase (BioLabs®Inc.). In the second round of amplification reactions (nested 2), the polymorphic allelic families of the *msp1* (K1, MAD20, and RO33) and *msp2* (FC27 and 3D7) genes were amplified using the following primers pairs: K1/K2 for the K1 family, MAD20-1/ MAD20-2 for the MAD20 family and RO33-1/RO33-2 for the RO33 family. For the allelic families of the *Pfmsp2* gene, the primer pairs, FC27-1/FC27-2 and 3D7-1/3D7-2 were used for the FC27 and 3D7 families respectively (**Supplementary data. Table 1)**. The template for the secondary reaction was 2 µL of the primary reaction product. The primary and nested PCR reaction cycling conditions included an initial denaturation at 95 °C for 5 min followed by 35 cycles of a second denaturation at 94 °C for 30 sec, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were run for 60 mins on a 2% agarose gel stained with a 0.5 µg/mL ethidium bromide solution and visualised under an ultraviolet transilluminator.

2.4. Data analysis

Asymptomatic carriers were defined as participants with asexual forms of Plasmodium spp. with no history of fever within the past 48 hours (temperature ≥ 37.5°C) and who did not show any other clinical signs of malaria before inclusion in the study. Age was stratified into three groups: < 5; 5–14 and ≥ 15 years. The multiplicity of infection (MOI) or the number of genotypes per infection was calculated by dividing the total number of fragments detected for *msp1* or *msp2* markers by the number of samples positive for each marker. The frequency of polyclonal infection was calculated using the number of samples with more than one amplified fragment out of the total number of positive samples for each marker. The expected heterozygosity (HE) was calculated by use of the formula HE = [n/(n-1)] [(1-∑Pi2)], where n = sample size, Pi = allele frequency as described by [43]. The proportions were compared for significance using the Chi-square and Fisher exact tests. The MOI was compared using student t-tests (between the *P. falciparum* and *P. falciparum/ P. malariae* isolates; the *Plasmodium* spp. microscopic and submicroscopic infections) or variance analysis ANOVA (between the three age groups) in R software version 3.5.3. Graphical representations were performed using GraphPad Prism 8.0.2 software (San Diego, California USA). Spearman's rank correlation coefficient was calculated to assess the possible associations between MOI of *msp1* and *msp2* and parasite density. $p < 0.05$ was considered indicative of statistical significance.

3. Results

3.1. Demographic characteristics of the study population

Two hundred fifty (250) samples have been analysed including 125 samples infected by *P. falciparum/P. malariae* and 125 mono-infected by *P. falciparum* samples. Of the 250 subjects included in our study, 109 and 141 were male and female respectively. The participant were 1.33 to 85 years old with an average of 19± 16.3 years. A total of 77 (61.6%) and 48 (38.4%) of females

and males were included in *P. falciparum* isolates. Similarly, 64 (51.2%) and 61 (48.8%) of females and males were included in *P. falciparum/P. malariae* isolates *malariae* isolates (**Table 1).**

3.2.Frequency and diversity of allelic families of *Pfmsp1* and *Pfmsp2* in mono-infection *P. falciparum* isolates group

The proportion of positive *msp1* samples was 96% (120/125). In the *P. falciparum* isolates group, 38.3%, 66.7% and 99.2% had K1, MAD20 and RO33 allelic types, respectively. For individual infections, no K1 allele was detected. However, 0.8% carried MAD20 type, 26.7% carried RO33 type (**Table 2**), and 82.5% displayed multiple infections (**Table 3**).

The proportion of positive *msp2* samples was 97.6% (122/125). In the *P. falciparum* isolates group 3D7 and FC27 allelic families had similar frequencies of 88.5% and 89.3% respectively (**Table 2**). However, for individual infections, 10.7% contained only 3D7 type, 11.5% carried only FC27 type (**Table 2**) and 90.2% had multiple infections (**Table 3**).

A total of 96 individual alleles including 47 for *msp1* and 49 for *msp2* were identified. For the *msp1* gene, 12 K1 type alleles (150–1100 bp), 16 MAD20-type alleles (100-1100 bp) and 19 RO33 type alleles (100–1100 bp) were identified (**Figure 2**). For the *msp2* gene, 24 different 3D7 type alleles (100–1200 bp) and 25 FC27 type alleles (100–1200 bp) were detected (**Figure 3)**.

3.3. Frequency and diversity of allelic families of *Pfmsp1* and *Pfmsp2* in mixed infections *P. falciparum /P. malariae* isolates group

The proportion of positive *msp1* samples was 82.4% (103/125) in mixed infection *P. falciparum/ P. malariae* isolates. In this group, 20.4% of parasites were K1 type; 67% were MAD20 type and 86.4% were RO33 type. For individual infections, there was no K1 type while11.7% had MAD20 type, 26.2% had RO33 type (**Table 2**) and 73.8% had multiple infections (**Table 3**). The multiple infection decreased from 82.5% in single *P. falciparum* isolates to 73.8% in mixed *P. falciparum*

/*P. malariae* samples but was not significantly different (p=0.1), whereas the individual infection frequency with MAD20 allelic types increased significantly (p= 0.0007) (**Table 2**).

Furthermore, the proportion of positive *msp2* samples was 77.6% (97/125). In this group, 88.7% and 56.7% had 3D7 and FC27 allelic types, respectively (**Table 2**). For individual infections, 43.3% of the infections contained only 3D7 type, and 11.3% carried only FC27 type. The multiple infections rate was (62.9%) and decreased significantly compared to that in *P. falciparum* mono-infection isolates (p <0.0001) (**Table 3**).

A total of 50 individual alleles including 23 for *msp1* and 27 for *msp2* were identified. For the *msp1* gene, 8 K1 type alleles (120 -700 bp) and 9 MAD20 type alleles (100 - 800 bp) were identified. For the RO33 allelic family, 6 type alleles (100 – 700 bp) were identified (**Figure 3**). For the *msp2* gene, 14 different 3D7 type alleles (180 - 550 bp) and 13 FC27 type alleles (100 - 700 bp) were detected (**Figure 3**).

3.4. Multiplicity of infection (MOI) in mono-infection *P. falciparum* and *in* mixed infections *P. falciparum/ P. malariae* isolates

Several samples showed multiple distinct amplified fragments, indicating the presence of more than one genotype per infection. The MOI was 3.7 and 5.8 respectively for *msp1* and msp2 in *P. falciparum* isolates (**Table 3**). According to the age, the MOI of *msp1* and *msp2* was not significantly different (p > 0.05) (**Table 4**).

The MOI was 2.7 and 2.3 for *msp1* and *msp2*, respectively in *P. falciparum/ P. malariae* isolates (**Table 3**). MOIs for both *msp1* and *msp2* were significantly higher in *P. falciparum* isolates than in *P. falciparum/ P. malariae* isolates (p <0.05). Consequently, the heterozygosity of the *msp1* and *msp2* gene was higher in *P. falciparum* isolates 0.93 and 0.96 than in isolates from *P. falciparum/ P. malariae* 0.86 and 0.71.

3.5.Genetic diversity of microscopic and submicroscopic *Plasmodium* spp. infections

A total of 42.4% (106/250) displayed a submicroscopic *Plasmodium* infection. Among these 106 submicroscopic *Plasmodium* infections, 53.8% (57/106) were submicroscopic *P. falciparum* mono-infection, and 46.2% (49/106) were submicroscopic *P. falciparum/P. malariae* coinfections. In the mono-infection *P. falciparum* group, specific allelic types of the *msp1* gene frequencies were significantly higher in microscopic isolates than that of the submicroscopic ones. They were respectively 58.2% vs 13.2% for K1 (p <0.0001) and 76.1% vs 54.7% for MAD20 (p= 0.01) (**Table 5**). The MOI for *msp1* and *msp2* were higher in microscopic isolates than that of the submicroscopic ones (p<0.05) (**Table 7**).

In the mixed infections group, the frequencies of R033 and K1 allelic types were higher in microscopic isolates than that of the submicroscopic ones (R033: 94.2% vs 70.6%; p= 0.001) and (K1: 26.1% vs 8.8%; p= 0.04) (**Table 6**). The MOI for *msp1* was higher in microscopic isolates than in the submicroscopic ones (3 vs 2; p <0.005), while for the *msp2* gene, there was no significant difference (2.6 vs 1.8; p= 0.12) (**Table 7**).

Moreover, *msp1* (Spearman's r= 0.203; p=0.04) showed a significant positive correlation between MOI and parasite density in *P. falciparum* isolates. However, no statistical difference was observed between MOI and parasite density using the *msp2* marker in *P. falciparum* isolates; and in *P. falciparum/ P. malariae* isolates using both markers (*msp1* and *msp2*) (p> 0.05).

4. Discussion

Several studies from different African regions have devoted efforts to characterize the genetic diversity and complexity of *P. falciparum* infection at the community level. However, there is limited information on the *msp1* and *msp2* genetic diversity of *P. falciparum* in asymptomatic populations in the OKT health district in Southern Benin, a high malaria transmission area. Moreover, there is no data on the genetic diversity of *P. falciparum* in mixed infection isolates. To fill this gap, this study aimed to assess the genetic diversity and complexity of *P. falciparum*

infection in mono-infected *P. falciparum* isolates. The same parameters were then evaluated in asymptomatic individuals co-infected by *P. falciparum* and *P. malariae*. This study is important to understand the impacts of malaria control measures on parasite populations. In addition, knowing the genetic diversity and the MOI of *P. falciparum* infection in *P. falciparum/P. malariae* mixed infection isolates collected from field samples, will allow us to further understand the interaction between *Plasmodium* spp. to improve malaria control strategies.

This study showed that the *msp1* (K1, MAD20, RO33) and *msp2* (3D7 and FC27) allelic families were present in *P. falciparum* isolates. It also revealed that the RO33 allelic family of *msp1* was the most prevalent while a similar frequency was observed for both *msp2* allelic families (3D7 and FC27). Studies carried out to assess the association between the distribution of *msp1* allelic families and malaria status (asymptomatic and symptomatic) revealed different observations [44–46]. However, the RO33 allelic family was frequently reported in asymptomatic malaria cases [47]. These findings are supported by a community-based study in Papua New Guinea that reported an association between a reduced risk of symptomatic malaria and a high frequency of the RO33 allelic family [48]. The predominance of this allelic family was highlighted in studies conducted in Benin [28], in Malaysia [44] , Island [49], Brazil [50], and Gabon [51]. In spite of the predominance of RO33 observed in the present study, other studies identified the *msp1* allelic family as the least predominant gene [46,52]. Further investigations with large sample sizes and various sites including symptomatic and asymptomatic patients of *P. falciparum* infections in Southern Benin are required to characterize better the allelic family polymorphism of *P. falciparum* in this region. Knowledge of the different genetic profiles of malaria parasites, even within and between countries is necessary to improve vaccine design, which will be very useful for malaria control.

The present study reported high numbers of alleles (47 alleles for *msp1* and 49 alleles for *msp2*) circulating in the study population. This represents the highest allele variability either for *msp1* or

msp2 genes compared to the genetic diversity observed in Southern Benin [13,28]. This finding may be related to the intensity of malaria transmission in the OKT health district, where malaria prevalence was recently shown to be increasing among inhabitants [10]. However, it has been shown that most alleles exhibit significant temporal fluctuations through the years [53]. Hence, the polymorphism of the parasite is poorly evaluated by examining a single blood sample since genotypes can appear and disappear in a short time [53,54]. Furthermore, it has been shown that there is intra- and inter-individual variation in the number of parasite genotypes present in different malaria episodes [52], suggesting the need for multiple studies in the same region to assess the real parasites genetic profile. A high number of alleles was reported in the previous survey in Gabon [55] but contrasted what was observed in Benin [28]. Knowing the alleles circulating in a region is important for malaria control because the allelic diversity could influence the degree and spread of parasite resistance to antimalarial drugs. In addition, the different circulating alleles in malaria-endemic areas must be targeted to develop second-generation vaccines.

Our results, showed that 82.5% and 90.2% of the participants carried multiple infections of *msp1* and *msp2* genes, respectively. In addition, a high MOI (3.7 for *msp1* and 5.8 for *msp2*) was found in the study area. These results suggest that *P. falciparum* isolates exhibit a high complexity of infection in the study population. It has been reported that in meso-endemic areas, the polyclonal infection can occur up to 100% [56–58]. The high complexity of infection could be explained by the fact that in high malaria-endemic areas, individuals are more exposed to mosquito bites and consequently to a high inoculation of genetically different parasite populations. This high exposition influence the intensity of malaria parasite transmission which affects the occurrence of mixed clones as initially demonstrated by Snewin and Scherf [59]. Several studies have reported a significant association between the complexity of infection and polyclonal infections in asymptomatic subjects [60,61]. The observation of a high rate of MOI and polyclonal infection in

the present study leads us to recommend to NMCP the implementation of mass drug administration in this region since this preventive measure has been previously reported to contribute to the reduction of malaria transmission [49,62].

The polyclonal infection, the number of alleles in *msp1* and *msp2* genes, and the MOI of *msp1* and *msp2* were all significantly lower in the co-infected *P. falciparum/P. malariae* group compared to that of *P. falciparum* group. The polyclonal *msp1* and *msp2* infections were reduced to about 73.8% and 62.9%, respectively in the *P. falciparum/P. malariae* group and the MOI of *msp1* and *msp2* also decreased to about 2.7 and 2.3, respectively. There was also a reduction in the *msp1* alleles (23 alleles) and the *msp2* alleles (27 alleles) in the *P. falciparum/P. malariae* group, which suggests a decrease in the genetic diversity and the complexity of the infection in the case of co-infection. These observations allow speculation on inter-species (*P. falciparum* and *P. malariae*) competition. This is very plausible because it has been shown in animal models, notably with the *P. chabaudi* strain, that there is an intra-competition between *P. chabaudi* clones in the host. Indeed, when mice were infected with three distinct clones of this parasite, there was competition and reduction of some clones [30,31]. Thus, we hypothesized that the clones of the two different species (*P. falciparum* and *P. malariae*) could compete with each other. In *Plasmodium* spp. infections , within-host competition is most likely caused by competition for resources, such as red blood cells, and strain-transcending immunity [30,63,64].

In addition, some alleles of the *msp1* and *msp2* allelic families were only observed in *P. falciparum/P. malariae* group. Further studies are needed to provide more data on within-host competition of *P. falciparum* and *P. malariae* the probability of finding particular alleles in mixed infection groups. This allelic variation should be better explored by sequencing parasites from both single and mixed isolates. Moreover, a study on the genetic diversity of *P. malariae* is needed in the study area. This could be useful for designing an effective malaria vaccine.

A lower genetic diversity was also observed in submicroscopic *Plasmodium* spp. isolates compared to microscopic ones. This may establish a correlation between the complexity of infection (MOI) and parasitaemia as previously reported [65,66].

In this study, the definition of the alleles was only based on their size differences. Therefore, there might be more valuable SNPs that could induce a significant difference in their sequences. Consequently, PCR genotyping used in this study lacks sufficient sensitivity to show SNP. Alternative methods such as capillary electrophoresis genotyping or whole genome sequencing are better and offer a higher resolution to distinguish parasite populations.

Conclusion

Our study revealed a low genetic diversity and complexity of *P. falciparum* infection in mixed *P. falciparum*/*P. malariae* infection compared to *P. falciparum* mono-infection isolates. The RO33 allelic family of *msp1* was the most prevalent among the two groups. Moreover, some alleles of *msp1* and *msp2* allelic families were only observed in *P. falciparum/P. malariae* isolates.

A better characterisation of *P. falciparum* and *P. malariae* genetic diversity in the study area with a more sensitive and specific method (sequencing) is needed to guide targeted interventions for effective malaria control in the country.

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Ethics approval and consent to participate

Ethical clearance (N°115/2018/CER-ISBA/FSS/UAC of 29th October 2018) was given for the study by the Beninese Ethical Committee of the Faculty of Sciences and Health. The study got the approval of the authorities of the Ouidah-Kpomasse-Tori Bossito health district. Written informed consent was obtained from the volunteer inhabitants enrolled. The informed consent form and survey were signed/filled out by a parent or tutor for participants aged less than 18 years-old. Malaria positive cases among recruited participants were treated with ACT, according to NMCP guidelines.

Conflict of interests

The authors have declared that they have no competing interests

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Author's contributions

Romuald Agonhossou: Conceptualization, investigation, methodology, writing - original draft, molecular and statistical analysis, review & editing**. Romaric Akoton:** Conceptualization, investigation, methodology, writing - original draft, review & editing**. Hamirath Lagnika:** Investigation**,** molecular analysis, review & editing**. Oswald Y. Djihinto:** Review & editing. **Pierre M. Sovegnon**: Review & editing. **Helga D. Saizonou:** Review & editing. **Francine Ntoumi:** Review & editing**. Charles S. Wondji:** Review & editing**. Steffen Borrmann:** review & editing**. Ayola A. Adegnika:** Review & editing**. Luc S Djogbénou:** Conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, visualisation, review & editing

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