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Polymorphism of the Merozoite Surface Protein-1 Block 2 Region in Plasmodium Falciparum Isolates from Symptomatic Individual Living in Rural Area of Senegal

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Abstract

Background: Polymorphism and antigenic variation of malaria parasites determine malaria episode and its outcome. The aim of this study was to determine *Plasmodium falciparum* genetic diversity over time in population with uncomplicated malaria under ACT exposure in Senegal.

Method: *P. falciparum* isolates collected from 300 individuals with uncomplicated malaria infection living in a rural area of Senegal from 2004 to 2008 were analyzed by a nested PCR amplification of *msp1* and *msp2* genes to compare *P. falciparum* allelic families' diversity.

Results: Allelic variation in both *msp1* and *msp2* genes were identified in the samples analyzed. For *msp1* gene, 10 different alleles were found (3 msp1_K1, 4 msp1_Mad20 and 3 msp1_Ro33). Among them msp1_k1 allelic family was predominant (>70%) over year. Regarding *msp2* gene, 7.0 different alleles were found (3 msp2_3D7, 4 msp2_FC27). However msp2_FC27 strain was predominant, especially in 2006 and 2007. Monoclonal infections were more frequent for *msp1* gene, in 2004 (48.78%) and 2005 (45.05%) and for *msp2* gene than polyclonal ones.

Conclusion: This study demonstrated some differences in the *P. falciparum* diversity between symptomatic subjects over years living in rural area in Senegal and this should be taken into account when designing *msp1* or *msp2* malaria vaccine.

Keywords: *P. falciparum*; Polymorphism; MSP1; MSP2; Genetic diversity

Background

Malaria remains a leading cause of ill health, causing an estimated 243 million cases of clinical malaria and 863 thousand deaths. More than 85% of malaria cases and 90% of malaria deaths occur in sub Saharan Africa [1]. In Africa, the vast majority of cases and deaths occur in young children [1]. Efforts to control this disease in Africa have been hindered by the spread of resistance to chloroquine and more recently, to sulfadoxine-pyrimethamine [2,3]. Currently key interventions are recommended by World Health Organization (WHO) for the control of malaria such as: use of insecticidal treated nets (ITNs) or indoor residual spraying (IRS) for vector control, and prompt access to diagnosis and treatment with Artemisin Combination Therapy (ACT) for uncomplicated *P. falciparum* malaria. Following the WHO recommendation, the National Malaria Control Programme (NMCP) of Senegal adopted ACT since 2006 as first line treatment of confirmed malaria. Furthermore, since 2004 several clinical trials using ACT derivate were performed in Senegal particularly in Kaolack region. As any drug ACT could have an impact in the *msp1* and *msp2* genes polymorphism over year. The relationship between parasites genotypes and clinical outcome has been reported in several studies [4-6]. Genetic diversity of *P. falciparum* field isolates, the occurrence of variant forms of the parasite in different geographic areas, and occultation of multiple genotypes during a single mosquito, constitute one of the main obstacles to the design of a effective malaria vaccine [7,8]. The objectives of this study was to determine the genetic diversity of *P. falciparum* parasites under ACT exposure and to compare the polymorphism of two highly polymorphic genes encoding the merozoite surface protein-1 (*msp-1*) and the merozoite surface protein-2 (*msp-2*) [9,10] in infected individuals living in rural areas in Senegal.

Methods

Study site

This study was conducted in rural area of Keur Soce, located from 200 km Southern-East of Dakar. The study was carried out in the health post of Lamarame belonging to the medical district of Ndoffane. Ndoffane is located at 17 km to the south of Kaolack. The terrain is generally flat with some depressions promoting the presence of water collection that are potential breeding sites. The climate is Sudano-Sahelien type with temperatures oscillating between 24°C and 40°C; extremes of 15°C in January and 44°C in July have been recorded. Rainfall is on average 600 mm per year. The district of Ndoffane host approximately 200 villages with a total population of nearly 75,000 inhabitants of which about 7% are aged up to 24 months. Malaria is the most important public health problem, accounting for 45% of general morbidity. Latest entomological surveys showed an entomological

inoculation rate between nine and 12 bites per night during the period of high transmission.

Sample collection

This study was part of several studies assessing anti-malarial drug efficacy that were performed from 2004 to 2008 in Keur Soce area. Patients were enrolled in accordance with the World Health Organization (WHO) protocol for antimalarial assessment [11], with sligh modifications. The inclusion criteria are: 1) patient of any age; 2) fever (axillary temperature >= 37.5°C) or a history of fever within the past 48 hours; 3) *P. falciparum* mono infection with parasite density between 1,000 and 100,000 asexual forms per microliter, identified microscopically on blood smears; 4) no history of anti-malarial drug administration in the last two weeks; 5) no history of serious adverse events to the study drugs; 6) no evidence of a concomitant febrile illness; 7) no sign/symptoms of severe malaria as defined by WHO [12]. Informed consent was obtained from parents or legal guardians of children and individual prior to their enrolment. A total of 300 positives samples were included in our study. At the time of the study, the studies protocols were reviewed and approved by the Ethical committee of Senegal.

Finger prick blood samples were collected from consenting patients, thick and thin blood smears were prepared for microscopic identification of *P. falciparum*. Blood from a finger prick was blotted onto Whatman filter paper, labeled with patients' study numbers and stored dry at room temperature. Dry filter paper was individually placed into plastic bag protected with silica gel desiccant.

DNA extraction and analysis

P. falciparum DNA was extracted from blood collected on 3 mm Whatman filter paper by methanol method describe by Djimde et al. [13]. Briefly, tree our four fragments of filter paper sample were incubated with 500 µl of methanol in 1.5 ml eppendorf tubes.

Gene	Primers	Sequencies	Notes	PCR Conditions
MSP1	F	5'AAGCCTTAGAAGATGCAGTATTGAC3'	NEST1	94°C-3 min
	R	5'ATTCATTAATTTCTTCATATCCATTATC3'		
	K1_F	5'AAGAAATTACTACAAAAGGTG3'	Family specific NEST2	94°C-25 s, 50°C-35 s, 68°C-2 min 30 s × 30
	K1_R	5'TGCATCAGCTGGAGGGCTTGCACCAC3'		72°C-3 min
	RO33_F	5'AGGATTTGCAGCACCTGGAGATCT3'		4°C ∞
	RO33_R	5'GAGCAAATACTCAAGTTGTTGCA3'		For PCR1 and PCR2
	Mad20_F	5'TGAATTATCTGAAGGATTTGTACGTC3'		
	Mad20_R	5'GAACAAGTCGAACAGCTGTTA3'		
MSP2			NEST1	94°C-3 min
	S2	5'GAGGGATGTTGCTGCTCCACAG3'		94°C-25 s, 42°C-60 s, 65°C-2 min 30 s × 30
	S3	5'GAAGGTAATTAAAACATTGTC3'		72°C-3 min; 4°C ∞
	S1	5'GAGTATAAGGAGAAGTATG3'	NEST2	94°C-3 min
	S4	5'CTAGAACCATGCATATGTCC3'		94°C-25 s, 50°C-60 s, 70°C-2 min 30 s × 30
				72°C-3 min ; 4°C ∞

Table 1: Sequence of oligonucleotide primers used to genotype *P. falciparum* and PCR conditions.

The mixture was incubated 15 minutes at room temperature before removing the methanol. Eppendorf tubes with fragment of filter paper were left unmoved until they completely dry. Then 50-70 µl of distilled water were added in each tube with dry filter paper and heated at 95-100°C for 15 mn. During the incubation steps, tubes were vortexed and returned to the heat block every five minutes. Samples were centrifuged and extracted DNA was transferred into new, labeled tube and stored at +4°C for immediate use. PCR was performed following a 2-step amplification scheme, in which two microliter product of the first reaction (outer PCR) was used as the template for the second reaction (nested PCR) for both *msp1* and *msp-2*. Outer and nested PCR reactions were performed in 25 µl final volume. For each reaction, 200 µM each of dNTP, 1 µM of each primer, 0.5 UI of Taq DNA polymerase and PCR Buffer 10X containing 100 mM Tris-HCl, pH 8.3 at 25°C, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin were used. The Outer and the Nested amplification were performed on an Applied Biosystem 2720 and PTC-100 Peltier Thermal Cycler. For *msp-1*, the primers used in the first amplification reaction were conserved among all isolates. Allelic family-specific primers were used for the nested amplification reaction for block 2 of *msp-1* corresponding to msp1_Mad 20, msp1_K1 and msp1_Ro33 allelic families. For *msp2* gene, PCR amplifications of *P. falciparum msp2* genes were done as

described by Foley et al. [14]: the primary reaction was performed with 5 µl of DNA with S2 and S3 *msp2* primers. Two microliters of the outer reaction was used for the nested PCR with S1 and S4 primers [14]. HinfI restriction enzyme was used to distinguish *msp2* allelic families: msp2_FC27 and msp2_3D7. A total volume of 25 µl reaction mixture with the restriction enzyme (HinfI) was incubated at 37°C overnight. Primers sequence and PCR conditions are listed in Table 1. For *msp-2* gene, the number of infecting genotypes in an isolate (multiplicity of infection) was detected by restriction enzyme using Hinfl enzyme (New England Biolabs) fellow by agarose gel electrophoresis (2%). Among the 198 samples analyzed, restriction digestion was succeful in 72% (144/198) of PCR products. After digestion, any sample with conserved 110 and 130 pb fragments and fragments varying between 150 -350 pb was considered as belonging to the msp-2_FC27 family and any samples with size 70pb-105 pb and fragments varying between 250 -500 pb was deemed to belong to the msp2_3D7/IC family.

DNA visualization

The PCR products were subjected to electrophoresis on 2% agarose gels (Gibco-BRL) in Tris acetate-EDTA buffer for various lengths of time depending of the predicted size of the PCR products and visualized with ethidium bromide by transillumination with ultraviolet light. We used 100 base pair ladders (Pharmacia Biotech, Piscataway, NJ) as standards for estimation of the sizes of DNA fragments.

Allelic distribution and multiplicity of infection

The *msp-1* and *msp-2* alleles were categorized by their molecular weights and considered the same if their molecular weights were approximately within 10 bp [15]. The multiplicity of infection (MOI), or complexity of infection, was estimated by the average number of PCR fragments per infected individual [15]. The detection of a single PCR fragment for each allele was classified as a single genotype infection. Detection of more than one PCR fragment for either *msp1*, *msp2* was considered as multiple *P. falciparum* genotype infection (MOI).

Statistical analysis

Data were entered in a Microsoft Excel sheet, and the haplotype of each positive sample was determined. Statistical analyses of data were performed with Epi info 6.04a (http://www.cdc.gov/epiinfo/Epi6/EI6dnjp.htm). A χ2-test was used to compare differences in proportions of parasites genotypes. Significance level of statistical tests was set at 0.05 with two sides.

Results

PCR efficacy

Among the 300 samples analyzed, 247 (82.33%) and 198 (66%) were positives respectively for *msp-1* and *msp-2* genes. Failure amplification could be done by extraction efficiency. Negative PCR samples were excluded from further analysis.

Allelic polymorphism of msp-1 and msp-2 genes

A highly polymorphic nature of Senegalese *P. falciparum* isolates with respect to *msp-1* and *msp-2* genes were noted. For *msp-1*, the main allelic type (K1, Mad_20 and RO_33) were identified. Regarding *msp-2*, 3D7/IC and FC27 alleles were identified.

For *msp-1* gene, the total number of different sized alleles (MOI) detected in samples analyzed was 10. Among them, 3 for K1 (160-220 bp), 4 for Mad_20 (200-360 bp) and 3 for RO_33 (160-360 bp) allelic families were noted.

Over year, results shown that msp-1_K1 monoclonal infections was more frequent with 56%, 74.5%, 83.3%, 78.9% and 76.7% respectively in 2004, 2005, 2006, 2007 and 2008 than infections with msp1_Mad20 and msp1_Ro33 strains (Table 2). Our results also showed infections with msp1_Ro33 type strains was higher than infections with msp1_Mad20 from 2004 to 2007 with no significant difference [(2005: mad20 21.5%; RO33 68.6%) (2006: Mad20 45.2%; Ro33 80.9%)] in 2005 and 2006 ($p=10^{-4}$). However in 2008, msp1_Mad20 (60.7%) was more frequent than msp1_RO33 (33.9%) (p=0.004) (Table 2).

	2004	2005	2006	2007	2008
MSP1_K1	56% (23/41)	74.5% (38/51)	83.3% (35/42)	78.9% (45/57)	76.7% (43/56)
MSP1_Mad 20	31.7% (13/41)	21.5% (11/51)	45.2% (19/42)	45.6% (26/57)	60.7% (34/56)
MSP1_RO3 3	48.7% (20/41)	68.6% (35/51)	80.9% (34/42)	61.4% (35/57)	33.9% (19/56)

Table 2: Prevalence of *P. falciparum* K1, RO33 and Mad 20 allelic family over year.

For *msp-2* gene, the overall MOI was 7.0; with 3 alleles for 3D7 (250-500 bp) and 4 alleles for FC27 (150-350 bp).

Considering allelic families, results showed that msp-2_FC27 strains were more frequent among our patients over year, especially in 2006 and 2007 except in 2008, where infections with msp-2_3D7 strains were predominant (Table 3).

	2006	2007	2008
MSP2_3D7	28.20% (17/58)	32.25% (20/62)	56.66% (26/60)
MSP2_FC27	71.80% (41/58)	67.75% (42/62)	43.34% (34/60)
P value	10^{-4}	10^{-4}	0.1

Table 3: Prevalence of *P. falciparum* 3D7 and IC/FC27 allelic family.

Multiplicity of infection (MOI)

In respect of gene *msp-1*, result showed that monoclonal infections were more frequent in 2004 (48.78%) and 2005 (45.05%) than polyclonal (double and triple *msp1* allelic family) with significant differences between monoclonal and triple infections (2004: p=0.002; 2005: p=0.0006) (Table 4). However significant decrease of monoclonal infections was noted: more than 45% in 2004-2005 and 19% in 2006 were noted. For the overall MOI of *msp1* gene, our results show an increase of double infections overtime: from 2004 (23.52%), 2005 (35.29%) to 2006 (52.4%) (Either K1/Mad_20, K1/RO_33 or Mad20/RO33). An increase of triple infection (K1/Mad_20/RO_33) was also noted overtime from 17.07%, 15.68% to 28.6% respectively in 2004, 2005 and 2006 with no significant difference (Table 4).

From 2006 to 2008, a slight reduction of double clonal infections was noted from 52.4% to 45% respectively in 2007 and 2008. Similarly for infections with triple clonal strains, have decrease over year (Table 4).

For *msp-2* gene, considering family allelic, 71.80%, 67.75% and 43.34% of patients carried FC27 allelic family while 28.20%, 32.25% and 56.66% carried 3D7 allelic family in 2006, 2007 and 2008 respectively (Table 3). Furthermore, monoclonal infection was more frequent than multiple infections over year Table 5.

	2004	2005	2006	2007	2008
Single_inf	48.78%	47.05%	19.04%	33.33%	37.50%
	(20/41)	(24/51)	(8/42)	(19/57)	(21/56)
Double_inf	23.52%	35.29%	52.40%	45.60%	44.64%
	(12/41)	(18/51)	(22/42)	(26/57)	(25/56)
Triple_inf	17.07%	15.68%	28.60%	21.10%	17.85%
	(7/41)	(8/51)	(12/42)	(12/57)	(10/56)

Table 4: Overall Multiplicity of infection of the MSP1 genotypes of the *P. falciparum* K1, RO33 and Mad2O allelic family.

	2006	2007	2008
MSP2_Single	51.71% (30/58)	54.83% (34/62)	68.3% (41/60)
MSP2_double	48.27% (28/58)	45.16% (28/62)	31.66% (19/60)
P value	0.71	0.28	10^{-4}

Table 5: Overall MOI of the *MSP2* genotypes of the *P. falciparum* 3D7 and IC/FC27 allelic family.

Discussion

This study was undertaken to assess the polymorphism of the merozoite surface protein-1 block 2 allelic types in *P. falciparum* isolates from rural area in central Senegal. *P. falciparum* is thought to be responsible of nearly 95% of diagnosed malaria cases in the country. A better understanding of population structure of *P. falciparum* genotypes may be an important element for implementing malaria control strategies in the country. Data showed a relatively high polymorphic nature of K1, Mad_20 and RO_33 *msp-1* allelic types according to the number of band sizes (10 different PCR products: 3 K1, 4 Mad20 and 3Ro33) and 3D7 and FC27 *msp-2* allelic types among senegalese *P. falciparum* isolates. The number of different *msp-1* alleles observed among *P. falciparum* isolates was lower than those found by Konate et al. [16] in the holoendemic area of Dielmo (Senegal) where 33 *msp-1* alleles were identified. This study also revealed the predominance of K1 type alleles at study site level or throughout the parasite population compared to Mad20 and Ro33 over year. Similar results were found in several studies conducted in Gabon [17-21], in Central Africa or in Honduras [22] in Central America. Ours results showed also that the msp1_Mad20 family was less frequent except in 2008 where this pattern was more frequent than msp1_RO33. Our findings are supported by similar study conducted in Burkina Faso where authors found that msp1_Mad20 allelic family was less frequent in infected children from rural area than those from urban area [23]. The difference in rural symptomatic malaria in 2008 may suggest a specific immune response against this allele (msp1_RO33), or a probable efficacy of ACTs introduced in Senegal in 2006 against this allele. ACT has been in use since 2006 but up scaling was done in 2008. RDTs were also introduced in 2007, and in 2008 following guideline of the Ministry of Health (MoH) through the National malaria Control Program (NMCP) ACTs are the first line treatment for uncomplicated *P. falciparum* malaria in Senegal. In Keur Soce area ACTs drug are highly effectives with an adequate clinical and parasitological cure rate over 95% [24]. As any drug, ACT could result in genetic modification of the *P. falciparum* parasites. A random event due to a genetic drift can also explain the opposite we observed in 2008 [25]. *msp-2* allelic families diversity is also noted in Keur Soce area with a predominance of msp-2_IC/FC27 in 2006-2007 while in 2008, the frequency of msp2_3D7 was more frequent than msp2_3D7. During these two years, msp2_3D7 and msp2_IC/FC27 alleles were nearly equally frequent. Similar results were found in Tanzania [26] and more or less similar results were reported in Namawala, an area lying along the northern edge of flood plains of the Kililomero River [27].

Regarding the multiplicity of infections (MOI), our results showed that MSP1 single infection was more frequent from 2004 to 2005. From 2006 to 2008 MSP1 double infection become more frequent than single infection. Triple infection increased over time. Regarding *MSP2* pattern, it appeared clearly that the single infection was more frequent than the double and triple infection was not found in our study. Differences in transmission level over time and anti-malarial immunity known to be strain specific [28], could partly explained the differences in the distribution of the different alleles over year. In a given locality, the parasite genetic pool may be relatively stable, perhaps related to the stable parasite life cycle; thus, the distribution of alleles may be determined randomly so that certain alleles will predominate by chance. Nevertheless, there are certain factors external to the natural life cycle that are known to affect allelic distribution, notably, anti-malarial drug pressure and result in a higher prevalence of drug resistance markers, the proximity of populations to water reservoirs, as it has been observed in Keur Soce, the use of insecticide-treated nets, indoor insecticide spraying or certain human genetic factors (hemoglobin types, G6PD), that could explain the difference in the distribution of *P. falciparum* genotypes over years.

Conclusion

This study demonstrated that there were some differences in the *P. falciparum* diversity between symptomatic subjects over year living in rural area in Senegal and this should be taken into account when designing *msp1* or *msp2* malaria vaccine. The study also emphasizes the importance of evaluating the extent of parasite genetic variation in different part of Senegal and the factors affecting this variation. In this context, longitudinal studies examining the dynamics of the *P.*

falciparum genetic diversity, including genes conferring drug resistance, could have public health implications for malaria control.

Competing Interest

The authors declare that they have no competing interest.

Authors' Contribution

Ndiaye JL, Ndiaye M, Faye B and Gaye O were responsible for the concept and the design of the study. Ndiaye M, Ndiaye JL, Lo AC, Abiola A collected the study data. Ndiaye M, Lo AC, Abiola A were responsible for PCR analysis. Ndiaye JL, Ndiaye M, Faye B and Gaye O were involved in the preparation of the manuscript, which was read and approved by all the authors.

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References

1. World Health Organization (2009) World Malaria Report, Geneva.
2. Trigg JK, Mbwana H, Chambo O, Hills E, Watkins W, et al. (1997) Resistance to pyrimethamine/sulfadoxine in Plasmodium falciparum in 12 villages in north east Tanzania and a test of chlorproguanil/dapsone. Acta Trop 63: 185-189.
3. EANMAT (2003) The efficacy of antimalarial monotherapies, sulphadoxine-pyrimethamine and amodiaquine in East Africa: implications for sub-regional policy. Trop Med Int Health 8: 860-867.
4. Ariey F, Hommel D, Le Scanf C, Duchemin JB, Peneau C, et al. (2001) Association of severe malaria with a specific Plasmodium falciparum genotype in French Guiana. J Infect Dis 184: 237-2414.
5. Chen Q, Schlichtherle M, Wahlgren M (2000) Molecular aspects of severe malaria. Clin Microbiol Rev 13: 439-450.
6. Gupta S, Hill AV, Kwiatkowski D, Greenwood AM, Greenwood BM, et al. (1994) Parasite virulence and disease patterns in Plasmodium falciparum malaria. Proc Natl Acad Sci 91: 3715-3719.
7. Raj DK, Das BR, Dash AP, Supakar PC (2004). Genetic diversity in the msp1 gene of Plasmodium falciparum in different malaria endemic localities. Am J Trop Med Hyg 71: 285-289.
8. Moore SA, Surgey EG, Cadwgan AM (2002) Malaria vaccines: where are we and where are we going? Lancet Infect Dis 2: 737-743.
9. Farnert A, Snounou G, Rooth I, Bjorkman A (1997) Daily dynamics of Plasmodium falciparum subpopulations in asymptomatic children in a holoendemic area. Am J Trop Med Hyg 56: 538-547.
10. Viriyakosol S, Siripoon N, Petcharapirat C, Petcharapirat P, Jarra W, et al. (1995) Genotyping of Plasmodium falciparum isolates by the polymerase chain reaction and potential uses in epidemiological studies. Bull World Health Organ 73: 85-95.
11. WHO (2001) Monitoring antimalarial drug resistance. Geneva.
12. WHO (2000) Severe falciparum malaria. Trans R Soc Trop Med Hyg 94: 90.
13. Djimdé A, Doumbo OK, Cortese JF, Kayentao K , Doumbo S, et al. (2001) A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med 344: 4.
14. Foley M, Ranford-Cartwright LC, Babiker HA (1992) Rapid and simple method for isolating malaria DNA from fingerprick samples of blood. Mol Biochem Parasitol 53: 241–244.
15. Soulama I, Nébié I, Ouédraogo A, Gansane A, Diarra A, et al. (2009) Plasmodium falciparum genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in Burkina Faso. Malar J 8: 135.
16. Konate L, Zwetyenga J, Rogier C, Bischoff E, Fontenille D, et al. (1999) Variation of Plasmodium falciparum msp1 block 2 and msp2 allele prevalence and of infection complexity in two neighbouring Senegalese villages with different transmission conditions. Trans R Soc Trop Med Hyg 93: 21–28.
17. Snewin VA, Herrera M, Sanchez G, Scherf A (1991) Polymorphism of the alleles of the merozoite surface antigens MSAl and MSA2 in Plasmodium falciparum wild isolates from Colombia. Molecular and Biochemical Parasitology 49: 265-275.
18. Snounou G, Viriyakosol S, Zhu X, Jarra W, Pinheiro L, et al. (1993) High sensitivity of detection ofhuman malaria parasites by the use of nested polyrnerase chain reaction. Molecular and Biochemical Parasitology 61: 315-320.
19. Viriyakosol S, Siripoon N, Petcharapirat C, Petcharapirat P, Jarra W, et al. (1995) Genotyping of Plasmodium falciparum isolates by the polymerase chain reaction and potential uses in epidemiological studies. Bulletin of the World Health Organization 73: 85-95.
20. Aubouy A, Bakary M, Keundjian A, Mbomat B, Makita JR, et al. (2003) Combination of drug level measurement and parasite genotyping data for improved assessment of amodiaquine and sulfadoxine-pyrimethamine efficacies in treating Plasmodium falciparum malaria in Gabonese children. Antimicrob Agents Chemother 47: 231-237.
21. Missinou MA, Kun JF, Lell B, Kremsner PG (2001) Change in Plasmodium falciparum genotype during successive malaria episodes in Gabonese children. Parasitol Res 87: 1020-1023.
22. Haddad D, Snounou G, Mattei D, Enamorado IG, Figueroa J, et al. (1999) Limited genetic diversity of Plasmodium falciparum in field isolates from Honduras. Am J Trop Med Hyg 60: 30-34.
23. Issiaka S, Issa N, Alpnonse O, Adama G, Amidou D, et al. (2009) Plasmodium falciparum genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in Burkina Faso. Malaria J 8: 135.
24. Ndiaye JL, Randrianarivelojosia M, Sagara I, Brasseur P, Ndiaye I, et al. (2009) Randomized, multicentre assessment of the efficacy and safety of ASAQ--a fixed-dose artesunate-amodiaquine combination therapy in the treatment of uncomplicated Plasmodium falciparum malaria. Malar 8: 125.
25. Bogreau H, Renaud F, Bouchiba H, Durand P, Assi S, et al. (2006) Genetic diversity and structure of African Plasmodium falciparum populations in urban and rural areas. Am J Trop Med Hyg 74: 953-959.
26. Eresto V Mbugi, Benezeth MM, Sakurani TB, Allen LM, Thomas BN, et al. (2006) Multiplicity of infections and level of recrudescence in Plasmodium falciparum malaria in Mlinba, Tanzania. African J of Biotech 5: 1655-1662.
27. Smith T, Beck HP, Mwankusye S, Felger I, Fraser-Hurt N, et al. (1999) The epidemiology multiplicity of Plasmodium falciparum infections 3 Age dependence of the multiplicity of Plasmodium falciparum infections and other malariological indices in an area of high endemicity. Transactions Roy Soc Trop Med Hyg 93: 11–14.
28. Magesa SM, Mdira KY, Babiker HA, Alifrangis M, Farnert A, et al. (2002) Diversity of Plasmodium falciparum clones infecting children living in a holoendemic area in north-eastern Tanzania. Acta Trop 84: 83-92.