

## Surveillance of Molecular Markers of *Plasmodium falciparum* Resistance to Sulphadoxine-Pyrimethamine 5 Years after the Change of Malaria Treatment Policy in Ghana

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**Abstract.** In 2005, sulphadoxine-pyrimethamine (SP) became the drug of choice for intermittent preventive treatment of *Plasmodium falciparum* malaria in pregnancy (IPTp) in Ghana. Reports suggest the use of SP by others to treat uncomplicated malaria. Because of the increased use of SP, the prevalence of mutations in the genes, dihydrofolate reductase (*dhfr*), and dihydropteroate synthetase (*dhps*), linked to SP resistance in *P. falciparum* were determined. Blood samples from 945 children with uncomplicated malaria collected at nine sites from 2003 to 2010 were analyzed using polymerase chain reaction and restriction fragment length polymorphism. Prevalence of the *dhfr* triple and *dhfr* plus *dhps* quadruple mutations showed significant increase in trend from 2003 to 2010 ( $\chi^2 = 18.78$ ,  $P < 0.001$ ,  $\chi^2 = 15.11$ ,  $P < 0.001$ , respectively). For *dhps* double mutant G437 + E540 the prevalence was low (1.12%) caused by the very low prevalence of E540. Our findings show the wide use of SP in Ghana and therefore its use for IPTp needs to be closely monitored.

### INTRODUCTION

In the absence of an effective vaccine, chemotherapy remains one of the mainstays for the control and management of *Plasmodium falciparum* malaria. However, chemotherapy has suffered a setback caused by the emergence and spread of strains of *P. falciparum* resistant to available antimalarial drugs. Continuous monitoring of the effectiveness of antimalarial drugs in disease-endemic areas is therefore crucial for early detection of reduced parasite susceptibilities to the drugs.<sup>1</sup>

Malaria in pregnancy is associated with significantly increased morbidity and mortality for both mother and neonate, in part as a result of reduced immunity to the disease in women during pregnancy, especially during first pregnancies.<sup>2</sup> Malaria causes miscarriages, anemia in pregnancy, stillbirth, and low birth weight (and may predispose to neonatal death). It is the leading cause of childhood deaths in endemic areas.<sup>3</sup> In Ghana, malaria in pregnant women accounts for 28.1% of out-patient department attendance, 13.7% of admissions, and 9% of maternal deaths.<sup>4</sup> The World Health Organization (WHO) approach to reduce malaria in pregnancy includes the use of intermittent preventive treatment in pregnancy (IPTp), insecticide-treated nets, and case management (early detection and rapid treatment). In 2005, sulphadoxine-pyrimethamine (SP) replaced chloroquine in the National Malaria Control Program guidelines for IPTp because of the high parasite resistance rates reported for the latter drug.<sup>5</sup> For IPTp, pregnant women in Ghana are required to take a curative treatment dose of SP every month after 16 weeks of conception for a maximum of three doses. Over 90% of pregnant women attend antenatal clinic at least once during their pregnancies, and staff have been trained to monitor the administration of IPTp to enhance compliance in taking of the drug.<sup>6</sup> With the increased use of SP in the country, there is the possibility of parasite resistance to the drug as observed in some malaria-endemic countries such as Mozambique and Senegal.<sup>7,8</sup>

The SP mainly targets the inhibition of folate synthesis in the parasite, in particular the activity of dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*). The magnitude of SP susceptibility in the parasite depends on the frequency of mutations identified in the genes that encode for these enzymes.<sup>9,10</sup> *Plasmodium falciparum* molecular markers of SP resistance are located on chromosomes 4 and 8 respectively for *dhfr* and *dhps* genes. Point mutations resulting in amino acid change occurring in codons N51I, C59R, and S108N, also known as the *dhfr* triple mutant have been associated with pyrimethamine resistance,<sup>9,10</sup> whereas S436A, A437G, and K540E of *dhps* have been linked to sulphadoxine resistance.<sup>11</sup> The *dhfr* gene core mutation is the S108N that predicts a 10-fold increased risk of resistance to pyrimethamine,<sup>12</sup> whereas the core mutations of the *dhps* genes are K540E and A437G mutations for sulphadoxine resistance.<sup>13,14</sup> The quintuple mutant, which involves mutants of codons 51, 59, 108, 437, and 540 from the two genes, synergistically confers *in vivo* SP treatment failure.<sup>15</sup> Suggestions have been made about different levels of *in vivo* resistance being determined by specific sets of *dhfr* and *dhps* mutations.<sup>16,17</sup> Mockenhaupt and others<sup>14</sup> reported the association of *dhfr* mutations alone with SP treatment failures in northern Ghana, while suggesting a minor role of mutations in the *dhps* gene. Mutations in the *dhfr* gene also confer resistance to drugs such as proguanil, which is not widely used in Africa, but is used in combination with atovaquone (malarone) for prophylaxis for non-immune travelers. The mutation S108N with either N51I or C59R or both in the parasite reduces the efficacy of the active ingredient of proguanil, which is cycloguanil.<sup>18</sup>

Recent studies on the origins and spread of the *dhfr* and *dhps* mutation have been conducted using isolates from different malaria-endemic areas to ascertain evolution and distribution of SP resistance across African countries. The report indicated a differential molecular basis of resistance between East and West African countries and that the spread of resistant parasites was caused by recent migration patterns.<sup>19</sup> For Ghana, Alam and others<sup>20</sup> have reported that the triple mutant alleles of *dhfr* (I51, R59, N108) were of Southeast Asian lineage, whereas the double mutant *dhfr* (R59 and N108) and *dhps* (A436 and G437) were of African

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lineage. They also indicated a possible sulphadoxine resistance emerging from Africa.

In 2005, Ghana reviewed its malaria treatment policy as a result of field-based evidence of significant parasite resistance to available antimalarial drugs provided by Koram's group at the Noguchi Memorial Institute for Medical Research (NMIMR).<sup>5,21,22</sup> Chloroquine was replaced with artemisinin combination therapy (ACT) as the first-line drug for treatment. As part of the change in policy, SP was selected for IPTp in Ghana.<sup>4,23</sup> However, since the change, there has been speculation that SP is still being used to treat any malaria-like symptoms in non-pregnant patients in addition to its use in IPTp. The widespread or increased availability of the drug through the IPTp program and the continued use of SP for treating uncomplicated malaria will increase drug pressure and lead to increased prevalence of molecular markers of SP resistance. A recent study in Senegal where SP is being used as an intermittent preventive treatment in infants (IPTi) has shown an increase in the prevalence of SP mutations after 2 years of IPTi.<sup>7</sup> The investigation involved the frequency of *dhfr* and *dhps* mutations in two study areas, one with IPTi and one without. The authors reported an increase in the *dhfr* triple mutant frequency in the area with IPTi after 2 years but not at the other site. They also observed that the frequency of the quadruple mutation was comparatively the same for the two areas and no quintuple mutation was observed at either site after the 2 years of monitoring. The authors therefore concluded that IPTi using SP did not impact the frequency of the combined *dhfr* and *dhps* mutations.<sup>7</sup> Another study in Mali observed no increase in the prevalence of the *dhfr* and *dhps* mutations after a year of IPTi.<sup>24</sup> In countries where SP is now being used in combination with artemisinin derivatives, a rapid increase in the prevalence of quintuple mutations have been reported. This trend has been observed in Mozambique, 5 years after the change of treatment policy.<sup>8</sup> Therefore, the situation in Ghana calls for an urgent assessment of SP to duly advise the National Malaria Control Program (NMCP). The WHO recommends IPTi only in areas with a prevalence of *dhps* E540 < 50%.<sup>25,26</sup> An estimation of the prevalence of the molecular markers of SP, chloroquine, amodiaquine, mefloquine, and artemisinin resistance and validation of the association of mutations with resistance and clinical efficacy in different settings is needed data for local policy guidance and for contributing to global monitoring for anti-malarial drug resistance.

Therefore, our major objective was to characterize mutations in *dhfr* and *dhps* genes that are reportedly linked to SP resistance and determine the prevalence of mutations over a period spanning 2 years before the change in policy and 5 years after the policy was instituted using samples from nine sentinel sites in Ghana. We hope to establish baseline rates of SP resistance alleles in malaria cases and evaluate the trend in prevalence of these mutations through the period when new guidelines promoted the use of SP for IPTp.

## MATERIALS AND METHODS

**Study sites and population.** Samples used in this study were collected from children 6–59 months of age presenting at health centers in Ghana with uncomplicated malaria from 2003 to 2010. These samples were collected from nine sentinel sites designated as part of a surveillance program for monitor-

ing malaria drug resistance in Ghana (Figure 1). The sites were established by the NMIMR in collaboration with the NMCP to represent the eco-epidemiological settings in Ghana. The sites Begoro, Bekwai, Hohoe, and Tarkwa are in the tropical forest zone with perennial malaria transmission; Navrongo, Wa, and Yendi are in the Guinea savanna zone with seasonal transmission; Cape-Coast and Prampram are in the coastal savanna with perennial transmission of malaria. Cape-Coast, Hohoe, Tarkwa, and Sunyani are urban areas, whereas Begoro, Bekwai, Navrongo, Wa, and Yendi are rural areas. Samples were obtained after parents or guardians gave informed consent for the participation of their children in the study. Filter paper blood blots were prepared for each patient, air dried, and placed in a zip-locked bag containing a few crystals of desiccant. Bags were stored at room temperature in a clean and dry environment until tested. The study was approved by the NMIMR IRB and the U.S. Naval Medical Research Unit No. 3 (NAMRU-3) IRB.

**Molecular analysis.** The DNA was extracted from 945 filter paper blood blots using the Tris-EDTA buffer extraction method.<sup>27</sup> About 3 mm blood blot filter paper was cut and soaked in 65  $\mu$ L of TE buffer in a microtube. The microtube was then incubated at 50°C for 15 minutes and then at 97°C for 15 minutes for DNA elution. After centrifugation of the tube, the supernatant, which is rich in parasite DNA, was transferred into a new microtube and stored at –20°C until use. Five microliters of the supernatant was used for the molecular characterization of mutations. Detection of *dhfr* and *dhps* genetic mutations was carried out by nested polymerase chain reaction (PCR) followed by restriction fragment



FIGURE 1. A map of Ghana showing the 10 sentinel sites for monitoring antimalarial drug resistance. U = urban; R = rural.

length polymorphism (RFLP) following a published protocol.<sup>28</sup> The *Dhfr* mutations at codons 51, 59, 108, and 164 and *dhps* mutations at codons 437 and 540 were characterized. In cases of samples with no visible amplification, PCR/RFLP was repeated for clarification. The trends in the prevalence of *dhfr* triple IRN (I51, R59, and N108), *dhfr* and *dhps* quadruple IRNG (*dhfr* I51, R59, N108, and *dhps* G437), and *dhfr* plus *dhps* quintuple IRNGE (*dhfr* I51, R59, N108, and *dhps* G437, E540) mutants from 2003 to 2004 and 2005 to 2010 were determined.

**Data analysis.** The prevalence of mutations in *dhfr* I51, R59, N108, and L164 and *dhps* G437 and E540 were determined for each sentinel site and overall for Ghana. The proportion of samples harboring mutations was determined for each site for the years 2003–4, 2005–6, 2007–8, and 2010. In samples where both mutant and wild-type alleles were identified, they were scored as mutants. The  $\chi^2$  test for trends (EpiCalc 2000) was used to determine the significance in increasing trends of the prevalence of resistance alleles over the time points.

## RESULTS

**Trends in prevalence of resistance alleles of *dhfr* and *dhps* before and after the change in drug policy.** There were 212 samples (2003–2004) used for baseline analysis and were

from four sites, Hohoe, Navrongo, Sunyani, and Tarkwa. A total of 733 samples (232 for 2005–6, 410 for 2007–8, and 89 from 2010) representing samples after the change in drug policy in Ghana (2005–2010) were also analyzed for the presence of the mutations for comparison. No samples were available for 2009 because of financial constraints and sample numbers in 2010 were lower as a result of administrative difficulties in restarting surveillance. The details of the prevalence of the resistance alleles from the sites and for the time points are shown in Table 1 for *dhfr* and *dhps*, respectively. The prevalence of the alleles showed an annual increase in the majority of the sentinel sites for *dhfr* and *dhps* genes (Table 1). There was continuous increase in the *dhfr* triple mutants for all the sites except Cape-Coast and Hohoe.

Because of heterogeneity in the transmission dynamics in the study sites, the data was analyzed according to urbanicity and ecological zones. For the urban setting, data from Cape-Coast, Hohoe, Tarkwa, and Sunyani were used, whereas Begoro, Bekwai, Navrongo, Wa, and Yendi were considered for the rural setting. The prevalence of resistance alleles was comparatively higher in the urban setting than the rural setting (Figure 2). Both settings had an increasing trend in the resistance alleles over time. For urban, triple mutant, IRN, and quadruple mutant, IRNG, increasing trends were significant,  $\chi^2 = 7.72$ ,  $P = 0.005$  and  $\chi^2 = 10.63$ ,  $P < 0.001$ , respectively.

TABLE 1  
Prevalence of *Plasmodium falciparum* infections with *dhfr* and *dhps* resistance alleles in the nine sentinel sites in Ghana\*

| Site       | Year    | n  | I51 | R59  | N108 | IN | RN | IRN  | G437 | E540 | GE |
|------------|---------|----|-----|------|------|----|----|------|------|------|----|
| Begoro     | 2003–04 | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2005–06 | 40 | 45  | 90   | 83   | 5  | 43 | 33   | 88   | 3    | 3  |
|            | 2007–08 | 50 | 56  | 86   | 88   | 4  | 30 | 52   | 92   | 0    | 0  |
|            | 2010    | 15 | 87  | 100  | 93   | 0  | 13 | 80   | 100  | 0    | 0  |
| Bekwai     | 2003–04 | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2005–06 | 20 | 55  | 70   | 80   | 15 | 20 | 30   | 75   | 0    | 0  |
|            | 2007–08 | 50 | 68  | 86   | 94   | 8  | 24 | 58   | 88   | 0    | 0  |
|            | 2010    | 14 | 64  | 86   | 86   | 0  | 21 | 64   | 86   | 0    | 0  |
| Cape-Coast | 2003–03 | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2005–06 | 40 | 55  | 75   | 82.5 | 8  | 23 | 42.5 | 72.5 | 0    | 0  |
|            | 2007–08 | 50 | 66  | 88   | 88   | 6  | 20 | 60   | 100  | 0    | 0  |
|            | 2010    | 20 | 60  | 85   | 80   | 0  | 20 | 60   | 80   | 5    | 5  |
| Hohoe      | 2003–04 | 78 | 65  | 77   | 87   | 5  | 19 | 55   | 89   | 0    | 0  |
|            | 2005–06 | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2007–08 | 50 | 60  | 68   | 80   | 14 | 16 | 42   | 100  | 0    | 0  |
|            | 2010    | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
| Navrongo   | 2003–04 | 34 | 62  | 52   | 70   | 12 | 12 | 41   | 73.5 | 0    | 0  |
|            | 2005–06 | 40 | 30  | 45   | 55   | 5  | 23 | 20   | 50   | 0    | 0  |
|            | 2007–08 | 50 | 62  | 66   | 74   | 2  | 12 | 54   | 66   | 0    | 0  |
|            | 2010    | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
| Sunyani    | 2003–04 | 50 | 30  | 42   | 46   | 4  | 20 | 22   | 40   | 0    | 0  |
|            | 2005–06 | 35 | 51  | 48.6 | 51   | 11 | 14 | 20   | 74.3 | 0    | 0  |
|            | 2007–08 | 50 | 68  | 64   | 70   | 8  | 6  | 54   | 80   | 0    | 0  |
|            | 2010    | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
| Tarkwa     | 2003–04 | 50 | 34  | 24   | 66   | 18 | 6  | 14   | 22   | 0    | 0  |
|            | 2005–06 | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2007–08 | 18 | 61  | 56   | 50   | 11 | 11 | 22   | 61   | 0    | 0  |
|            | 2010    | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
| Wa         | 2003–04 | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2005–06 | 39 | 59  | 56   | 62   | 18 | 10 | 31   | 67   | 0    | 0  |
|            | 2007–08 | 42 | 41  | 64   | 74   | 10 | 19 | 29   | 62   | 0    | 0  |
|            | 2010    | 20 | 55  | 70   | 65   | 5  | 15 | 45   | 50   | 0    | 0  |
| Yendi      | 2003–4  | –  | –   | –    | –    | –  | –  | –    | –    | –    | –  |
|            | 2005–06 | 20 | 55  | 40   | 75   | 25 | 15 | 20   | 80   | 0    | 0  |
|            | 2007–08 | 50 | 62  | 58   | 54   | 8  | 14 | 26   | 56   | 2    | 2  |
|            | 2010    | 20 | 45  | 70   | 85   | 10 | 35 | 30   | 80   | 0    | 0  |

\*(-) = data not available; n = number of samples. Figures are in percentages.

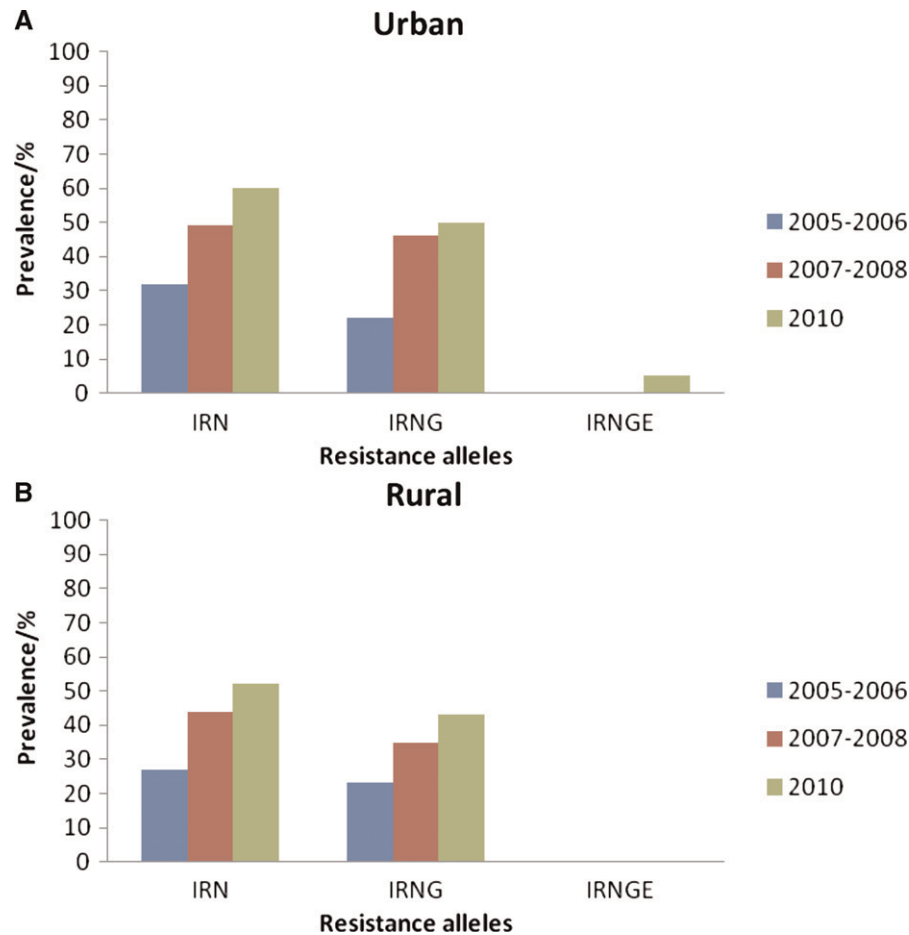


FIGURE 2. Trends in prevalence of the triple (IRN), quadruple (IRNG), and quintuple (IRNGE) resistance alleles after the change in treatment policy. A = urban setting; B = rural setting.

For rural, significant increasing trends in the prevalence were  $\chi^2 = 14.38$ ,  $P < 0.001$  and  $\chi^2 = 10.50$ ,  $P < 0.001$  for IRN and IRNG, respectively. There was no significant difference between the prevalence of the triple mutation and quadruple mutations between the urban and rural areas. The general trend of the prevalence over the time points is shown in Figure 2A and B. One quintuple mutant was found in the urban setting and no sample had that mutant in the rural setting.

In terms of ecological zones, the forest zone (represented by Begoro, Bekwai, Hohoe, and Tarkwa) showed the highest prevalence of resistance alleles followed by the coastal savannah zone (Cape-Coast) and then the Guinea savannah zone (Navrongo, Wa, and Yendi) (Figure 3A–C). The increasing trend in the prevalence of the mutant alleles after the change in drug policy was significant for the forest (IRN,  $\chi^2 = 21.89$ ,  $P < 0.001$ ; IRNG,  $\chi^2 = 19.85$ ,  $P < 0.001$ ) and savannah zones (IRN,  $\chi^2 = 27.04$ ,  $P < 0.001$ ) but not for the coastal savannah zone (IRN,  $\chi^2 = 1.92$ ,  $P = 0.16$ ; IRNG,  $\chi^2 = 3.53$ ,  $P = 0.06$ ). Prevalence of IRNG was significantly higher in the forest zone than the savannah zone in 2007–08 and 2010 (44% [95% confidence interval [CI]: 37.5, 50.9] versus 25% [18.3, 33.1]) and (69% [49.1, 84.1] versus 25% [13.2, 41.5]), respectively.

Generally, there was an increase in the percentage of mutations in the *dhfr* and *dhps* genes after the change in drug policy. The increasing trends of *dhfr* and *dhps* mutations in the total Ghanaian *P. falciparum* isolates from 2003 to 2010

are shown in Figure 4. The  $\chi^2$  analysis for trends showed significant increase in the triple (IRN,  $\chi^2 = 18.78$ ,  $P < 0.001$ ) and quadruple (IRNG,  $\chi^2 = 15.11$ ,  $P < 0.001$ ) resistance alleles from 2003 to 2010 in Ghana (Figure 4). No L164 allele was detected in the samples and the prevalence of E540 alleles was very low (range 0–1.12%). Of the 945 samples analyzed, only two showed the presence of the quintuple mutation (IRNGE).

## DISCUSSION

Study results provide evidence for the high prevalence of strains of *P. falciparum* in Ghana with the resistance alleles of the *dhfr* or *dhps* genes. This observation is a result of the increased use of SP in terms of its use for IPTp and the unauthorized use for the treatment of uncomplicated malaria. Although triple and quadruple mutants were detected in the samples analyzed in this study, it must be emphasized that the predictive value of these markers varies geographically and involves a complex interplay between host immunity, parasites, and drug.<sup>29–31</sup>

Overall, the results of this study showed a significant increase in the prevalence of mutations in the *P. falciparum* *dhfr* and *dhps* genes from 2003 to 2010. The combined benefits of low-cost, single-dose administration, and better tolerability as compared with ACTs currently in use in Ghana might

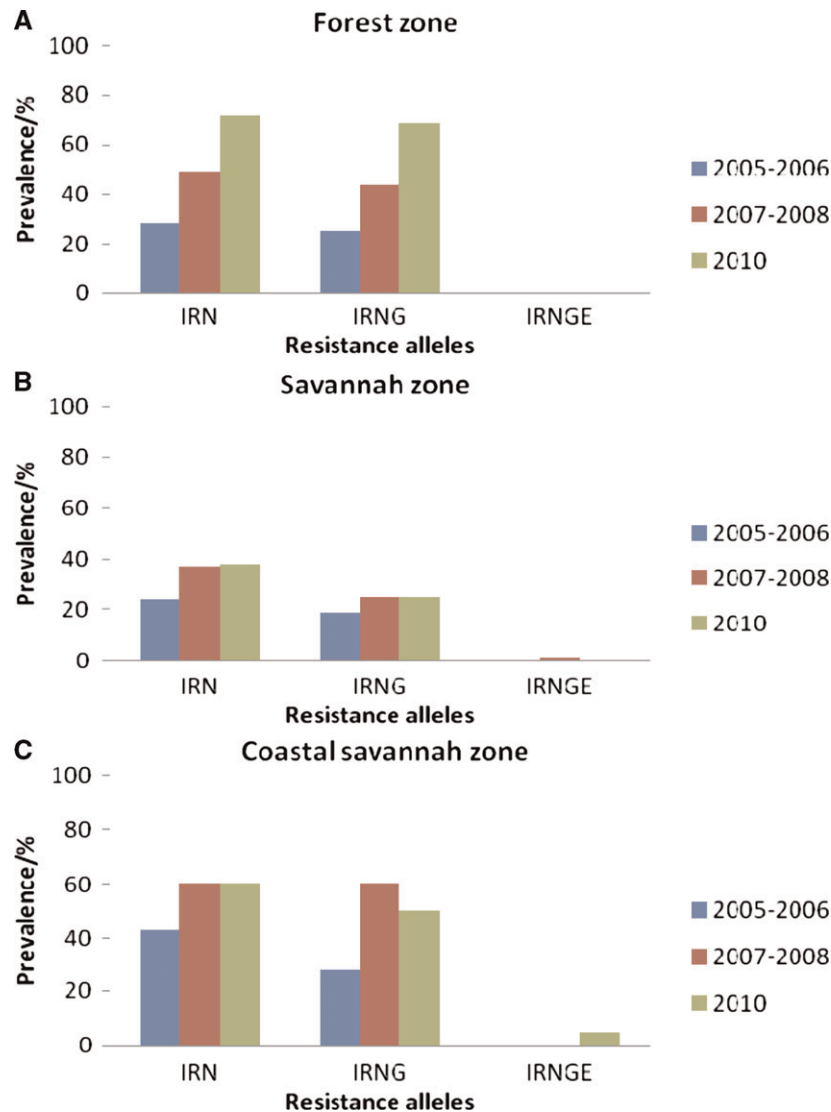


FIGURE 3. Trends in prevalence of the triple (IRN), quadruple (IRNG), and quintuple (IRNGE) resistance alleles after the change in treatment policy. A = forest zone; B = guinea savannah zone; C = coastal savannah zone.

have influenced the choice of SP for the treatment of “malaria-like” diseases in the country in addition to the IPTp program. It is unclear whether the use of SP for IPTp alone or the use of SP in the general population for the treatment of uncomplicated malaria has contributed to the drug pressure. The SP mutations have increased dramatically since the inception of its use in malaria treatment in most malaria-endemic areas. A study in Cameroon reported that codon 108 (S108N) has increased inexorably from 48% in 1994–1995, 71% in 1997–1998, and 93% in 2000–2001.<sup>32</sup> The triple mutations, S108N, N51I, and C59R were evident in 100%, 93%, and 57% of isolates, respectively, in recent surveys in Angola<sup>33</sup> and in Tanzania, 100%, 100%, 100%, and 100% of isolates for 59R, 108N, 436S/437G, and 540E, respectively, in 2007 from a low transmission area.<sup>34</sup> Data from Uganda also showed within 99%, near 99%, and 57–94% of isolates respectively for the 108, 51, and 59 mutations.<sup>35</sup> Mockenhaupt and others<sup>36</sup> reported 72%, 56%, and 65% of isolates respectively for 108, 51, and 59 in Ghanaian isolates from samples collected in 2005 from Tamale in the northern region when the implementation

of the new policy began. From one of our study sites close to the area mentioned previously, similar prevalence data were observed for I51 and N108, 55% and 75%, respectively, for the year 2005. Before the change in treatment policy, a study conducted in Ghana specifically in the Ashanti region (in the forest zone) by Marks and others,<sup>37</sup> showed 86.5%, 65.5%, and 77.8% for samples collected in 2001 and 88.1%, 66.7%, and 79.7% for 2003 samples respectively for N108, I51, and R59 mutations.

In areas where the frequency of SP use in the population for the treatment of uncomplicated malaria was high before the change in treatment policy to the use of ACTs as was the situation in Peru, studies conducted there revealed a significant decline in the prevalence of the highly resistant triple mutant *dhfr* and *dhps* in accordance with the decreased SP use.<sup>38,39</sup> This finding suggests that SP-resistant parasites have lower fitness in the absence of continuous drug pressure. There was an observed drop in IRN prevalence in Navrongo from 41% in 2003–04 and 20% in 2005–06 for unclear reasons. It should be noted that Navrongo is the only site with a

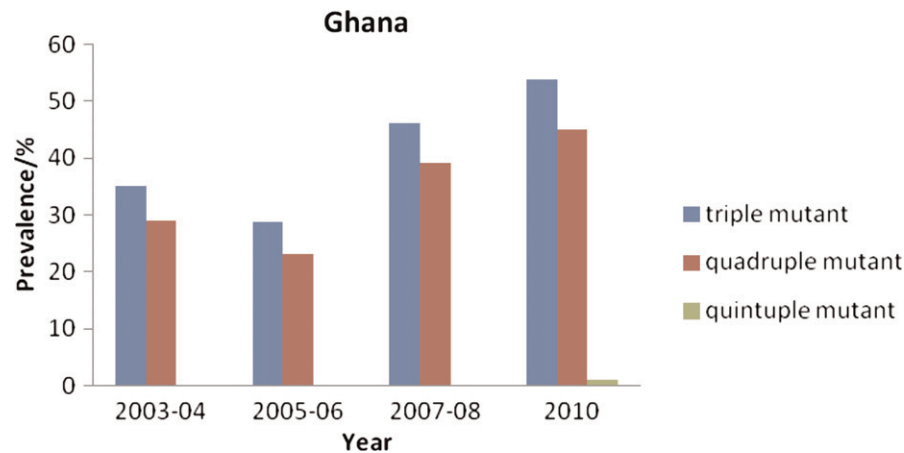


FIGURE 4. Trends in prevalence of the triple (IRN), quadruple (IRNG), and quintuple (IRNGE) resistance alleles prior to and after the change in treatment policy.

demographic surveillance system and a Ghana Health Service (GHS) supported research center, and consequently use of ACTs in the treatment of uncomplicated malaria may have been more rapidly and thoroughly instituted as compared with the other sites, which would have been more likely to use more familiar treatments such as chloroquine and SP. As such the strict monitoring and the accompanying compliance of the use of ACTs in the treatment of uncomplicated malaria in the year of policy implementation may have resulted in the reduction of triple mutant parasites in 2005–06. From our study, we have shown a continuous increase in the SP-resistant genotypes over a 5-year period of drug policy change in association with increased drug use in the population consistent with the selective advantage for the SP-resistant parasites caused by drug pressure in Ghana. Efforts to reverse this trend are crucial to prevent the establishment of multidrug-resistant parasites in the country. This includes drug resistance surveillance, which acts as an early warning system to predict reduced susceptibilities of parasites to drugs and also to inform on appropriate treatment policies.

Assuming the increase in prevalence of the mutation was solely caused by drug pressure, the observed increased drug-resistant genotypes in the urban setting over the rural setting can possibly be anticipated (Figure 1A and B), although not statistically significant. This could be caused by the fact that SP as an over-the-counter drug in Ghana is more readily available and/or affordable for those in the urban areas of the country. A study conducted by Abuaku and others<sup>40</sup> on drug use among caregivers in some parts of Ghana, revealed that SP use is common among the study participants because it is a single dose therapy and therefore has increased compliance among users. The analysis on the ecological zones revealed differences in the prevalence of resistance alleles at the three zones. Although information on the intensity of transmission in these areas is not available, using the pattern of transmission, it was speculated that the forest area with perennial transmission may be of low intensity and the seasonal transmission in the savannah area may be of relatively high intensity. So far, the studies on transmission intensity and drug resistance have produced conflicting results. Malaria drug resistance often appears first in areas of low transmission intensity, suggesting that the conditions in such areas may enhance the selection of drug resistance<sup>41</sup> and may be related

to the modestly higher prevalence of mutants observed in the low transmission intensity forest area of our study.

Apart from drug pressure from the use of SP, other antifolates being used in the treatment of respiratory tract infections in children such as co-trimoxazole (trimethoprim and sulfamethoxazole) may also contribute to the selection of resistant parasites because co-infections of bacteria and malaria are common.<sup>42</sup> Co-trimoxazole is also used for prophylaxis in human immunodeficiency virus (HIV)-exposed infants and HIV-infected individuals in Africa, which may also enhance the effect of drug pressure in countries with a high prevalence of HIV.<sup>43–45</sup>

With the current trend in prevalence of mutations in circulating parasites, the use of SP alone for IPTp needs to be closely monitored to avoid putting women at risk of getting malaria during pregnancy. Should SP be maintained as the drug of choice for IPTp/IPTi/IPTc in most endemic countries, we then suggest a review of the mode of dispensing that should be preserved for IPT use only. We recommend that it should be stocked only by antenatal service providers. However, pharmacists and chemical sellers should be given the necessary education on the use of the drug. The reason for using only SP for IPTp is the lack of ACT safety data in pregnancy. Thus, as more of the data on safety of ACT in pregnancy is obtained, SP could be co-administered with an artemisinin derivative for IPT in pregnant women and also in infants and children. Combination therapy may not only reduce malaria transmission but also slow down the spread of resistance in malaria-endemic areas.

The annual increase in prevalence of mutations in the SP resistance-associated *dhfr* and *dhps* genes observed in this study raises concerns about the indiscriminate use of this drug by others in addition to its use for IPTp in Ghana. The continuous use of SP could increase drug-based selective pressure leading to the spread of drug-resistant parasites. Our findings highlight the need to continually monitor all the antimalarial drugs currently in use in Ghana to allow for early detection of reduced parasite susceptibility to antimalarial drugs. As the resistant genotypes increase in frequency, it may eventually lower the efficacy of SP use for IPTp in Ghana. Studies are being planned to investigate the efficacy of SP for IPTp in pregnant women and also determine how to potentially limit the use of SP for indications other than for IPTp.

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