# Drug resistance maps to guide intermittent preventive treatment of malaria in African infants

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SUMMARY

Intermittent preventive treatment of infants (IPTi) with sulphadoxine pyrimethamine (SP) is recommended as an additional malaria control intervention in high transmission areas of sub-Saharan Africa, provided its protective efficacy is not compromised by SP resistance. A significant obstacle in implementing SP-IPTi, is in establishing the degree of resistance in an area. Since SP monotherapy is discontinued, no contemporary measures of *in vivo* efficacy can be made, so the World Health Organisation has recommended a cut-off based upon molecular markers, stating that SP-IPTi should not be implemented when the prevalence of the *dhps* 540E mutation among infections exceeds 50%. We created a geo-referenced database of SP resistance markers in Africa from published literature. By selecting surveys of malaria infected blood samples conducted since 2004 we have mapped the contemporary prevalence of *dhps* 540E. Additional maps are freely available in interactive form at http://www.drugresistancemaps.org/ipti/. Eight countries in East Africa are classified as unsuitable for SP-IPTi when data are considered at a national level. Fourteen countries in Central and West Africa were classified as suitable while seven countries had no available contemporary data to guide policy. There are clear deficiencies in molecular surveillance data coverage. We discuss requirements for ongoing surveillance of SP resistance markers in support of the use of SP-IPTi.

Key words: Antimalarial resistance, maps, *dhps* 540, intermittent preventive treatment infants.

## INTRODUCTION

IPTi is a promising intervention for reducing malaria and anaemia in young children in malaria endemic countries which can be rolled out within the World Health Organisation's (WHO) Expanded Programme of Immunisation (EPI). A treatment dose of sulphadoxine pyrimethamine (SP) is given at the time of routine vaccination of infants and studies have shown this reduces the incidence of clinical malaria in under-ones by about 30% (Aponte *et al*. 2009). The intensification of SP resistance in many parts of Africa has led to concerns that the protective efficacy will be compromised. Since SP is the only drug tested for safety, efficacy and EPI interactions, no alternative drugs are currently available for use in IPTi.

Randomised controlled trials of IPTi using sulphadoxine pyrimethamine reported protective efficacies against clinical episodes of malaria ranging from 20% to 59% and against anaemia from 10% to 50% up to 12 months of age (Schellenberg *et al*. 2001, 2005; Chandramohan *et al*. 2005; Macete *et al*. 2006; Mockenhaupt *et al*. 2007; Grobusch *et al*. 2007*a*, *b*; Kobbe *et al*. 2007). The first study of IPTi with SP conducted in Ifakara Tanzania during 1999–2000 showed the highest protection against malaria (Schellenberg *et al*. 2001) but resistance has risen steeply in the following years (Malisa *et al*. 2010) raising concerns about efficacy. A later trial in an area of exceptionally high SP resistance in Northern Tanzania reported no protective efficacy with SP-IPTi (Gosling *et al*. 2009). To examine the relationship between the protective efficacy of SP-IPTi and resistance, an analysis of seven SP-IPTi trials was carried out (Griffin *et al*. 2010). Two measures of SP resistance were used; contemporaneous data from six *in vivo* efficacy studies using SP and seven molecular studies reporting frequency of *dhfr* triple and *dhps* double mutations within 50 km of the trial sites. The results suggested that there was a reduction in the protective efficacy of SP-IPTi with increasing molecular markers of SP resistance.

SP resistance occurs via substitutions in the target enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) coded by point mutations in the *dhfr* and *dhps genes* (Cowman *et al*. 1988; Peterson *et al*. 1988; Brooks *et al*. 1994; Triglia and Cowman, 1994). Mutant *dhfr* alleles are varied and code for a range of tolerance to pyrimethamine from intermediate to high, depending upon the number of mutations present. The triple mutant *dhfr* allele (N51I + C59R + S108N) is a significant contributor to SP treatment failure. It originated in

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Asia (Roper *et al*. 2004) but now has a pan-African distribution dating back to the 1980s (Certain *et al*. 2008; Maiga *et al*. 2007).

Sulphadoxine resistant *dhps* emerged during the 1990s, against this background of high level pyrimethamine resistance. In East Africa the appearance of a *dhps* double mutant A437G + K540E heralded the arrival of significant SP treatment failures in the region (Naidoo and Roper 2010). The combination of the *dhps* double mutant A437G + K540E and the *dhfr* triple mutant were shown to be predictive of early SP treatment failure among malaria patients in Kenya, Uganda and Malawi (Omar *et al*. 2001; Staedke *et al*. 2004; Kublin *et al*. 2002). In West and Central Africa the *dhps* K540E mutation is rare but the *dhps* A437G single mutant, combined with triple mutant *dhfr* was found to be associated with treatment failure in Gabon (Kun *et al*. 1999), Ghana (Mockenhaupt *et al*. 2005), the Gambia (Dunyo *et al*. 2006) and Republic of Congo, Brazzaville (Ndounga *et al*. 2007). An exception is a study by Marks *et al*. (2005) in Ghana who reported no association of A437G with treatment outcome *in vivo*. Studies done *in vitro* indicate that the 437G single mutant confers a lesser degree of drug tolerance than the A437G and K540E combined (Brooks *et al*. 1994; Triglia *et al*. 1997). Hence it is considered very likely that the consequences for IPTi efficacy are less severe.

The WHO Technical Consultation on Intermittent Preventive Treatment of Infants (WHO, 2009) took account of the differing sensitivity of single and double mutant forms of *dhps*, noting that 540E mutations are indicative of high level SP resistance. A prevalence of 50% of the *dhps* 540E mutation is the threshold recommended by WHO to determine whether or not SP-IPTi should be implemented.

In addition to the *dhps* 540E, there are two other mutations which are newly emerging in Africa which, when found in conjunction with the *dhfr* triple and *dhps* double mutants, indicate extremely high levels of parasite insensitivity to SP which abrogate SP efficacy for both IPTi and IPTp. These are the *dhfr* 164L mutation and the *dhps* 581G. Harrington *et al*. (2009) in a study in Muheza, Tanga region, Tanzania showed that the efficiency of IPTp was largely compromised when pregnant women were infected with parasites expressing the *dhps*- A437G + K540E + A581G haplotype. Another recent study at Hale Health Centre, situated 32 km north of Muheza, reported 55% prevalence of the same haplotype. The clinical and parasitological cure rate at day 28 was less than 20% in children aged 6–59 months treated for clinical malaria with SP (Gesase *et al*. 2009). The establishment of A437G + K540E + A581 G may explain the total lack of protective effect of SP for IPTi in the same area reported by Gosling *et al*. (2009).

Currently there is a need for standardized information about the *dhps* 540E, 581G and *dhfr* 164L mutations in African sites where implementation of SP-IPTi is being used or considered for use. The purpose of this review is to summarise current knowledge about the prevalence of the *dhps* 540E and 581G and the *dhfr* 164L markers in a form which is freely accessible and easily understood. We have created maps which are available in interactive form on the worldwide web. They have utility not only in guiding IPTi – SP policy but also in highlighting the gaps in surveillance coverage which need to be addressed.

## MATERIALS AND METHODS

### *Data collection*

We conducted online literature searches periodically during October 2005 to February 2011 using the Pubmed, African Journal Online and Bioline databases using the search terms 'malaria', '*dhps*' and '*dhfr*' (Naidoo and Roper, 2010). We included published studies which had been conducted in any malarious African country excluding studies of malaria imported from Africa to non-African countries, animal studies, vaccine trials, prophylaxis studies, methodology studies and *in vitro* studies of resistance. There were no restrictions applied on the basis of malaria case detection, age, pregnancy status, transmission intensity or molecular method used to detect resistant mutations.

We reviewed the full text of suitable studies and extracted the prevalence of *dhfr* and *dhps* mutations. Data were first written on a proforma containing predesigned data fields, including geo-referenced study site and study year, and subsequently double entered into a data entry system to ensure quality control. We recorded the proportion of infections containing individual *dhfr* or *dhps* point mutations and where possible, the haplotypes of associated point mutations in each gene. The details of studies that were identified and included in our analyses are provided on our website (www.drugresistancemaps.org).

### *Data analysis*

We analysed the prevalence of *dhps* K540E, A581G and *dhfr* I164L. Graphs showing 540E prevalence through time were generated in Intercooled Stata ver 9.2 with calculated 95% exact binomial confidence intervals for each study sample. Studies where the year of sampling was not reported were not included in the plots of prevalence through time. We generated graphs to illustrate the prevalence of *dhps* 540E over a 20 year period, from 1988 to 2009 in six geographical categories. Pearce *et al*. (2009) identified five geographical regions of mainland Africa consisting of neighbouring countries which share the same *dhps* resistance allele lineages. In this study, we added a

Table 1. Regional sub-division of African countries based on shared *dhps* resistance lineages (Pearce *et al*. 2009) and including islands as a separate category

| Countries | Regional category |
|---|---|
| Democratic Republic of Congo (East), Kenya, Malawi, Mozambique, Rwanda, South Africa, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe | Southeast |
| Djibouti, Ethiopia, Sudan | Northeast |
| Benin, Burkina Faso, Ivory Coast, Equatorial Guinea, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal | West |
| Angola, Democratic Republic of Congo (West), Gabon, Namibia, Republic of Congo | Southwest |
| Cameroon, Central African Republic | Central |
| Comoros, Madagascar, Sao Tome/Principe | Islands |

sixth cluster consisting of island populations and these are listed in Table 1.

Vector maps were created in Mapinfo ver 9.0 to illustrate the spatial distribution of *dhfr* 164L and *dhps* 581G prevalence reports. All studies, including those whose study year was not stated were included in these maps. To illustrate the recent prevalence of 540E we selected only those studies where the samples were collected in 2004 or later and created a composite map showing sites where 540E was examined and highlighting those where prevalence exceeded the recommended threshold of 50%.

## RESULTS

### *Prevalence of the* dhps *540E mutation*

We identified 119 publications describing 260 unique surveys of 540E prevalence which fulfilled the inclusion criteria and these are listed in full in the supplementary bibliography S1 (see http://journals.cambridge.org/PAR). The cumulative total number of isolates tested for 540E was 30 224, of which 10 339 (36%) tested positive with 540E. The regional distribution of surveys is summarized in Table 2. The most intensively studied area was the Southeast region where 129 surveys were carried out in 83 unique study sites in the 11 Southeast region countries listed in Table 1. The prevalence of 540E for successive surveys in each region is plotted with 95% exact binomial confidence intervals in Fig. 1. The 540E was common in surveys conducted throughout Southeast and Northeast Africa and its prevalence, which tended to increase over time, was seen to approach 100% in some areas. In Northeast Africa 27 surveys were conducted in 13 unique study sites in three countries and a prevalence of 100% 540E was reported at one site in 2004.

In the West, Central, Southwest and Island regions, the prevalence of 540E never exceeded 50%. In the West we recorded 45 surveys in 35 unique study sites within 14 countries. Among these the majority of surveys reported the 540E mutation was rare or absent, An exception was a reported prevalence of 23% in Ibadan, Nigeria. In the five countries of the Southwest region, there were 21 surveys in 16 study sites and the prevalence of 540E was universally less than 50%. Within the Central region 21 surveys from 11 different study sites all reported 540E prevalences of under 6%. Similarly the 17 surveys at 12 sites among island populations reported low prevalences of 540E and the maximum value recorded was 20% in 2004 in Sao Tome and Principe.

### *Prevalence of the* dhfr *164L mutation*

All publications in which surveys of *dhfr* 164L were carried out are listed in the supplementary bibliography S2 (see http://journals.cambridge.org/PAR). Of 19 597 isolates tested for 164L in 183 surveys and 114 unique sites, 164L was detected in just 130 isolates (0·7%). The regional distribution of the surveys and mutant positive isolates are shown in Table 2. In Fig. 2 a map illustrating the prevalence of 164L shows all the surveys which examined codon 164 in African parasites. Detection rates for the 164L mutation ranged from 0·6% to 13·7% of isolates tested. The 164L mutation was found in the Central African Republic, Comoros, Kenya, Madagascar, Malawi, Rwanda and Uganda but was absent in 21 other countries where surveys were conducted.

### *Prevalence of the* dhps *581G mutation*

All publications in which surveys of *dhps* 581G were carried out are listed in the supplementary bibliography S3 (see http://journals.cambridge.org/PAR). Of 15 331 isolates tested for 581G in Africa, the mutation occurred in 782 (5%) isolates in 13 countries namely Cameroon, Democratic Republic of Congo, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mali, Niger, Rwanda, Sudan, Tanzania and Uganda. The 581G was reported in all the regions except for the Southwest (Table 2). The survey sites and prevalence measurements are recorded in the map in Fig. 3 which shows 14 other countries where surveys were negative. The majority of the surveys recorded 581G < 50% although in one study carried out prior to 1997 in Sotuba, Mali the 581G mutation was reported to be 100%.

### *Recent surveys of the* dhps *540E resistance marker and their application for SP-IPTi*

To focus on the current situation, a subset of recent data was selected using only the surveys of *dhps* 540

Table 2. Prevalence of *dhps* K540E, *dhps* A581G and *dhfr* I164L

| | Southeast | Northeast | Central | West | Southwest | Islands | All Regions |
|---|---|---|---|---|---|---|---|
| All surveys of K540E | | | | | | | |
| number of surveys | 129 | 27 | 21 | 45 | 21 | 17 | 260 |
| samples tested for K540E | 18751 | 1537 | 2109 | 4573 | 1976 | 1278 | 30224 |
| samples positive for 540E | 9531 | 609 | 9 | 65 | 95 | 30 | 10339 |
| unique sites surveyed | 83 | 13 | 11 | 35 | 16 | 12 | 170 |
| countries surveyed | 11 | 3 | 2 | 14 | 5 | 3 | |
| Surveys of K540E since 2004 | | | | | | | |
| number of surveys | 42 | 3 | 7 | 11 | 12 | 15 | 90 |
| samples tested for K540E | 6313 | 280 | 932 | 2392 | 1104 | 1146 | 12167 |
| samples positive for 540E | 3840 | 276 | 7 | 31 | 64 | 25 | 4243 |
| surveys with >50% 540E | 34 | 3 | 0 | 0 | 0 | 0 | 37 |
| surveys with <50% 540E | 8 | 0 | 7 | 11 | 12 | 15 | 53 |
| unique sites surveyed | 32 | 3 | 4 | 10 | 10 | 11 | 70 |
| countries surveyed | 9 | 1 | 2 | 9 | 5 | 3 | |
| Surveys of A581G | | | | | | | |
| number of surveys | 71 | 12 | 9 | 18 | 5 | 5 | 120 |
| samples tested for A581G | 11041 | 835 | 1220 | 889 | 275 | 1071 | 15331 |
| samples positive for A581G | 690 | 34 | 14 | 43 | 0 | 1 | 782 |
| unique sites surveyed | 45 | 9 | 4 | 14 | 4 | 3 | 79 |
| countries surveyed | 9 | 3 | 2 | 8 | 3 | 3 | |
| Surveys of I164L | | | | | | | |
| number of surveys | 103 | 13 | 21 | 35 | 5 | 6 | 183 |
| samples tested for I164L | 13393 | 1099 | 1513 | 1829 | 395 | 1368 | 19597 |
| samples positive for I164L | 78 | 0 | 1 | 0 | 0 | 51 | 130 |
| unique sites surveyed | 60 | 9 | 15 | 21 | 5 | 4 | 114 |
| countries surveyed | 10 | 3 | 2 | 8 | 3 | 3 | |

carried out between 2004 and 2009. In all, 90 recent surveys were identified in which 4243 (35%) of 12167 samples were positive for the 540E mutation. The regional distribution of these surveys and the proportion which reported prevalences higher than the 50% cut-off are summarised in Table 2, while their geographical locations are shown in the map in Fig. 4. The Southeast and Northeast regions contained all the survey sites with prevalence of the 540E mutation >50%. Prevalence exceeded 50% in eight countries namely Ethiopia, Kenya, Uganda, Rwanda, Tanzania, Malawi, Zambia and Mozambique. Within this subgroup 40 recent surveys were conducted of which 34 surveys recorded a prevalence of 50% or more.Exceptions being four studies in Maputo, Mozambique (2004 6% and 11%, 2005 16%. 2006 49%) and 2 studies in Zambia (Macha 2006 11%, Mpongwe 2004 46%). Within individual countries surveys recorded broadly consistent prevalences of the 540E mutation.

In Ethiopia, three surveys were conducted in Jimma, Dilla and Humera all in 2004 and these reported consistently high 540E prevalences of 97%, 97% and 100% respectively (Gebru-Woldearegai *et al*. 2005; Schunk *et al*. 2006; Pearce *et al*. 2009). The sites cover a wide area – Jimma and Dilla are approximately 220 km apart and Humera is 900 km from Dilla. Although there have been no surveys published subsequently,and no surveys detected *dhfr* 164L the extreme prevalence of the 540E in 2004 and early reports of low level 581G (< 2%) indicate that SP-IPTi is likely to be compromised by drug resistance in Ethiopia.

In Kenya, nine surveys were carried out in nine unique sites between 2004 and 2006. The *dhps* K540E prevalence was always greater than 50% and ranged from 74% in Iguhu, Kakamega District to 99% in Kombewa, (these sites are about 12 km apart) (Bonizzoni *et al*. 2009; Zhong *et al*. 2008). There are reports of *dhfr* 164L at prevalences around 3% in western Kenya suggesting that a very highly SP resistant form might be emerging here (McCollum *et al*. 2006).

In Uganda, 540E prevalence has exceeded 50% since 1999 (Staedke *et al*. 2004). Surveys conducted during 2002–2004 in six national surveillance sites recorded prevalences of between 80% (in Mubende) and 98% (in Tororo) (Francis *et al*. 2006), and most recently *dhps* 540E prevalences of 98% and 100% were recorded in Rukungiri and Kabale in 2005 (Lynch *et al*. 2008). Overlaid upon *dhps* 540E there are emerging foci of *dhps* 581G (up to 45% prevalence) and *dhfr* 164L (up to 14% prevalence) in southwest Uganda recorded in 2005 (Francis *et al*. 2006; Lynch *et al*. 2008) indicating that SP-IPTi will be severely compromised by resistance in this area.

In Rwanda, surveys at Rukara and Mashesha in 2005 and reported prevalences of 97% and 84% 540E respectively (Karema *et al*. 2010). The same surveys revealed emergence of the *dhfr* 164L (12%) and *dhps* 581G (61%) at Rukara which is approximately 120 km from the highly resistant populations

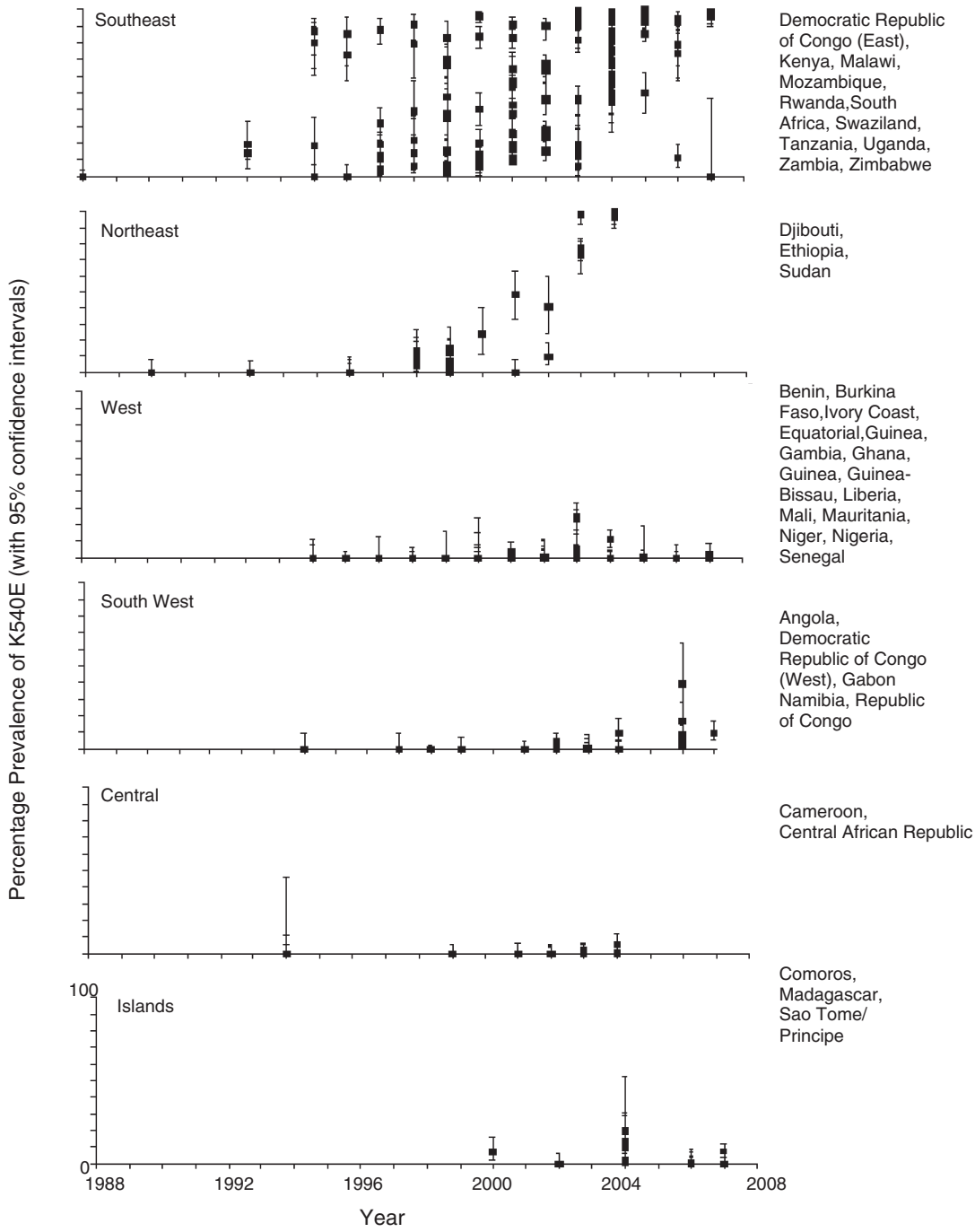


Fig. 1. Surveys of 540E prevalence (with 95% confidence intervals) conducted during 1988–2008 and displayed according to their geographic region.

in southwest Uganda. At the more distant survey site at Mashesha (approximately 170 km from southwest Uganda) *dhfr* 164L was still absent but *dhps* 581 was 30%. The intensification of resistance in both sites predicts that SP efficacy will be exceptionally poor.

The collation of 540E data from seven recent surveys in five different sites in Tanzania indicated a range in prevalence from 50% in 2005 in Chamwino to 97% in 2007 in Korogwe District (Enevold *et al*. 2007; Alifrangis *et al*. 2009). In Korogwe, Alifrangis *et al*. (2009) also reported *dhps* 581G at 12% but no *dhfr* 164L in surveys conducted between 2003 and 2007.

In Malawi, five surveys were conducted in 2005 and 2007 in four unique sites spanning the length of country. The prevalence of 540E reached extremes of 96%–100% (Bridges *et al*. 2009; Nkhoma *et al*. 2007). Additionally in 2003, the prevalence of 581G was 3% and 164L was 4% in Blantyre (Alker *et al*. 2005) although both these mutations remained absent in the other Malawian sites surveyed (Plowe *et al*. 1997; Bwijo *et al*. 2003; Bell *et al*. 2008; Bridges *et al*. 2009; Ochong *et al*. 2008).

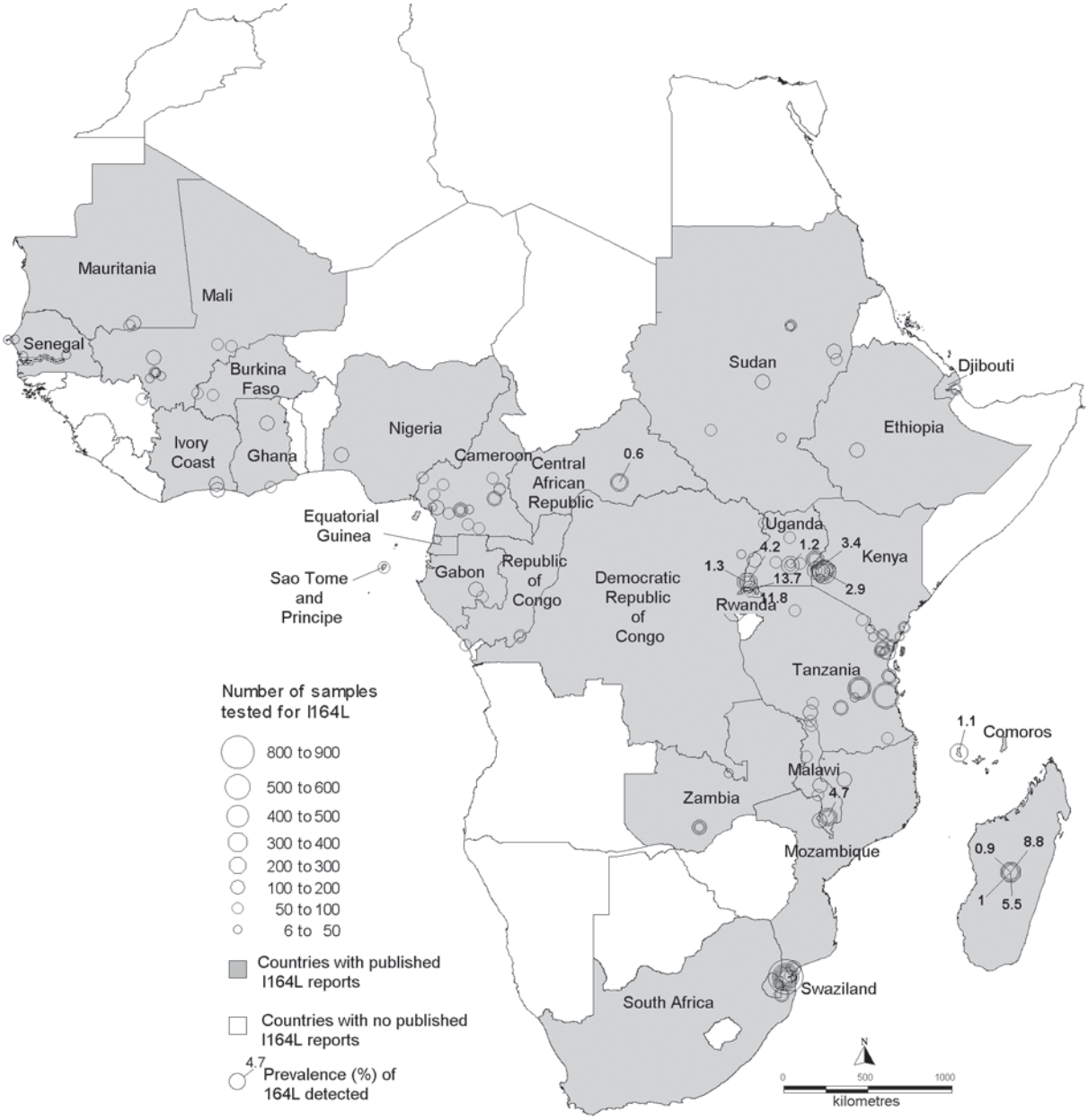


Fig 2. Map of *dhfr* 164L survey sites. Countries where surveys were conducted are shaded and the survey sites are indicated by open circles. For positive surveys the prevalence of 164L is indicated.

The picture in Zambia is mixed. Surveys in six Zambian sites during 2004 recorded 540E prevalences of between 46% – 75% (Pearce *et al*. 2009) with just one survey at Mpongwe falling below the 50% prevalence cut-off. However a survey in Macha in 2006 reported prevalence of 10% 540E (Mkulama *et al*. 2008). No 164L was detected in Macha (Mkulama *et al*.2008) and there is no recent data on 581G. These surveys indicate limited efficacy of SP in Zambia although additional monitoring of SP resistance genes including 581G are required.

In Mozambique, annual surveys between 2004 and 2008 at sentinel sites across Maputo district recorded the rise in prevalence of 540E across that region (Raman *et al*. 2010). Surveys in 2004 reported prevalences of 6% and 11%. In 2005 this increased to 16% and in 2006 to 49%. By 2008, prevalence exceeded 50% in all sentinel sites and the average prevalence across the region was 65%. No *dhfr* 164L or *dhps* 581G was found in Maputo during 2004–2008 (Raman *et al*. 2010) and other studies found no 581G in other sites in Mozambique (Fernandes *et al*. 2007; Raman *et al*. 2008; Enosse *et al*. 2008). The resistant genotype is well established and surveillance at multiple sites confirms this to be highly consistent across a large geographical area.

In Madagascar, the *dhps* 540E was absent in 2006 (Andriantsoanirina *et al*. 2009) but an emerging focus of 164L mutant alleles was reported in the south of the country in annual surveys conducted in 2006, 2007 and 2008. Uniquely in Africa, the Malagasy 164L mutation is found as a single mutant and not in

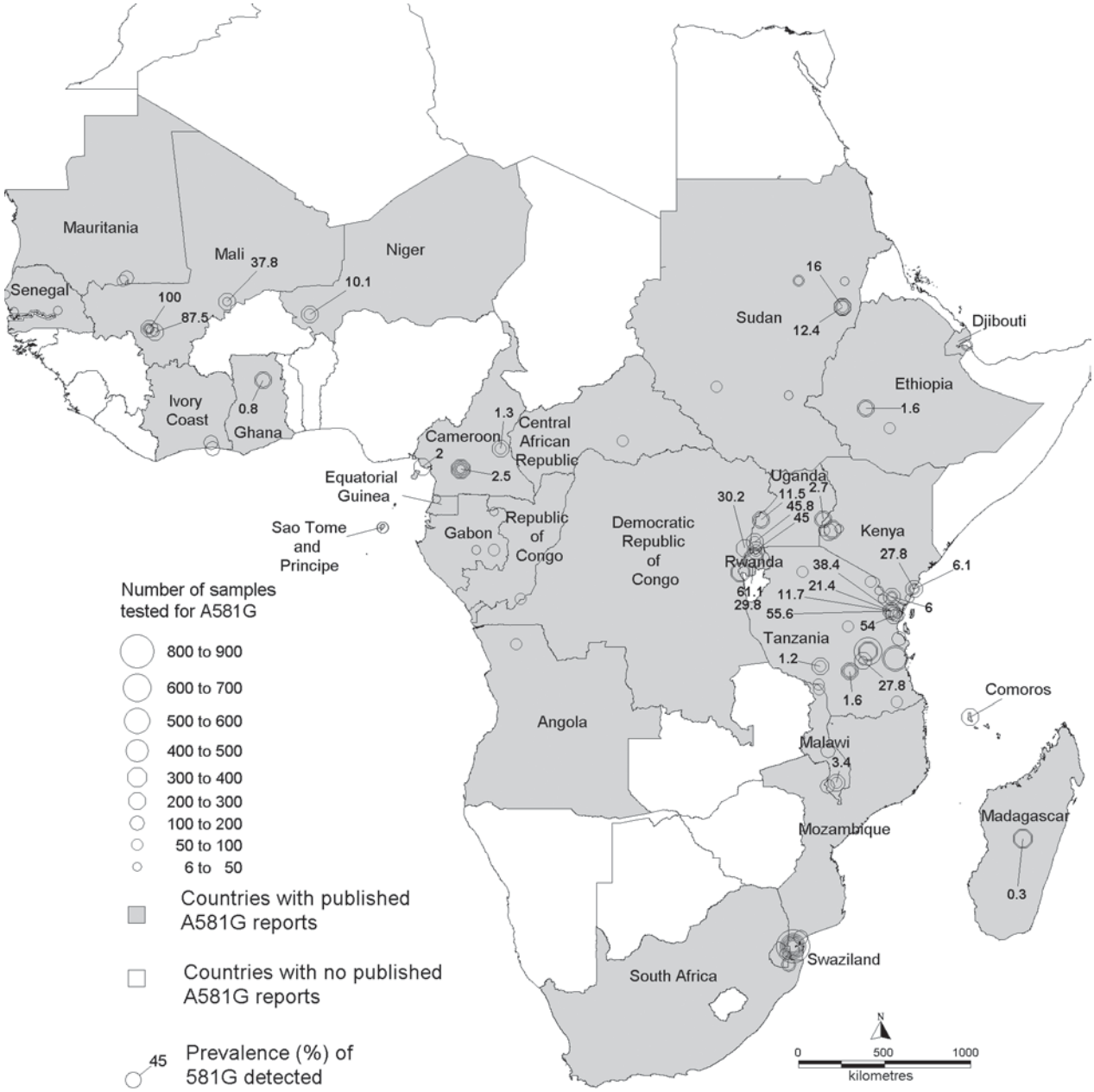


Fig 3. Map of *dhps* 581G survey sites. Countries where surveys were conducted are shaded and the survey sites are indicated by open circles. For positive surveys the prevalence of 581G is indicated.

combination with mutations at codons 51, 59 or 108. *In vitro* assays predict that the 164L on its own will not confer significant resistance to pyrimethamine (Lozovsky *et al*. 2009). Based on the criteria of the 50% 540E prevalence threshold there should be no impediment to implimentation of SP-IPTi in Madagascar. However, given the unique combination of resistance mutations and the indications of their evolution in isolation from the mainland, it is recommended that local assessments of SP efficacy be conducted.

In Mali, there were reports of *dhps* 581G, one from a survey in Bandiagara during 2000 (Thera *et al*. 2005) and another carried out prior to 1997 (Wang *et al*. 1997). The implications of this for efficacy of SP-IPTi are unknown and unfortunately recent surveys have not tested for 581G so the current situation with respect to this mutation in Mali is unknown. However the rarity of 540E in Mali was reconfirmed in recent large scale surveys (Tekete *et al*. 2009, Djimde *et al*. 2008, Dicko *et al*. 2010).

## CONCLUSION

High level SP resistance, as indicated by the prevalence of the 540E mutation, is advanced in sites all across East Africa. In Ethiopia, Kenya, Uganda, Rwanda, Tanzania, Malawi, Zambia and Mozambique measures of the prevalence of 540E were consistently 50% or higher since 2004. In addition, there has been significant intensification of resistance in parts of Uganda and Rwanda through

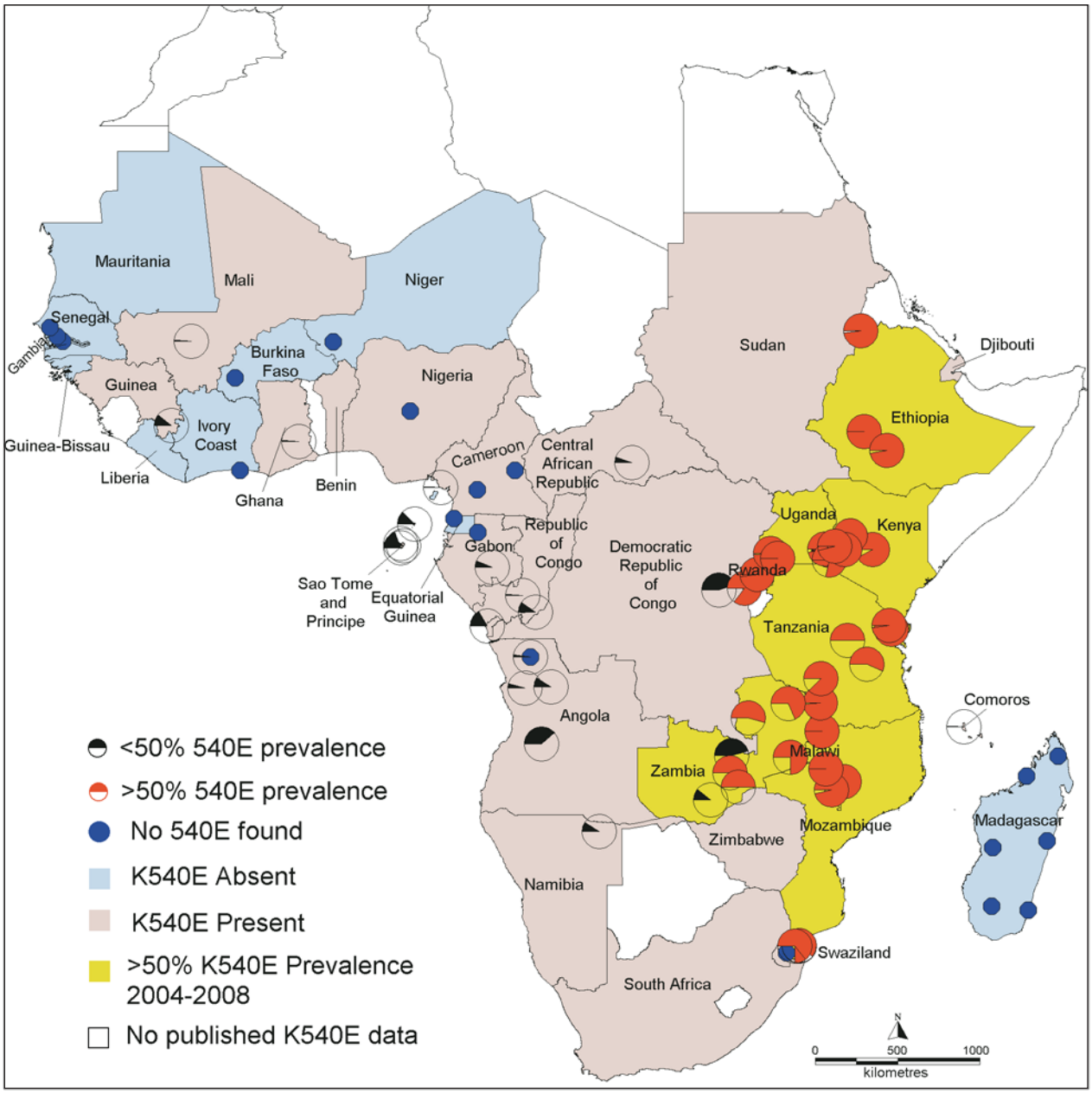


Fig 4. Map showing recent measures of the prevalence of 540E. All surveys conducted since 2004 are indicted by pie charts. The red pie charts indicate survey sites where prevalence exceeded 50% and these countries are shaded brown. The black pie charts indicate surveys with less than 50%. Blue circles indicate zero prevalence 540E. The countries shaded pink indicate that 540E has been detected at <50% at some stage whereas the blue ones are where it has never been detected. White indicates countries where no data was available.

acquisition of *dhps* 581G and *dhfr* 164L mutations. In northern Tanzania there is a well established focus of *dhps* 581G although 164L is absent. IPTi with SP is not recommended in these areas.

Elsewhere in Africa the 540E is encountered less frequently. Of the 36 countries for which data exists 27 have been confirmed to have 540E at low prevalence, leaving 10 countries: Burkina Faso, Gambia, Guinea-Bissau, Ivory Coast, Liberia, Madagascar, Mauritania, Niger, Senegal and Equatorial Guinea where recent surveys have been carried out but the 540E has still not been recorded. Based on this, SP-IPTi , is still practicable throughout much of Africa.

It will be important to maintain surveillance for markers of SP resistance to monitor changes over the next few years. SP has been withdrawn as a treatment for clinical malaria and is now recommended solely for use in intermittent preventive treatment in pregnant women and infants With this policy change there is expected to be a significant reduction in drug pressure (Malisa *et al*. 2010). The cumulative reports of 540E so far have shown rising prevalence in East Africa and a gradual spread westward (Naidoo and

Roper, 2010), it is likely these trends will slow or even discontinue now that SP drug pressure is reduced.

Surveillance in support of use of SP for IPTi or IPTp should be concentrated in areas where there is no data and those where resistance is currently borderline or heterogeneous. In the absence of local information the closest approximation is to look at resistance levels in sites in neighbouring states. For example, in Burundi it may be expected that high levels of SP resistance would occur because of measures made in neighbouring sites in Rwanda and Eastern Democratic Republic of Congo. In situations where resistance levels are borderline, such as the Democratic Republic of Congo and Sudan, it may be necessary to employ district level surveillance to inform IPTi policy at local administrative levels.

Supporting evidence of the region-wide circulation of drug resistance genotypes comes from molecular analysis of flanking microsatellite markers around *dhps* (Pearce *et al*. 2009) This study of resistant *dhps* alleles sampled in 20 African mainland countries showed that parasite populations are regional and probably are linked through networks of human circulation. Once a resistance mutation is established at a site it can rapidly be disseminated to other populations in the same regional genepool. Circulation of infected people between the major regions and between mainland and island populations is less frequent compared with their movement within regions and consequently exchange of resistance genes is more stochastic. Rogier (2005) reported that the Djibouti-Ethiopian railway was suspected to be an effective route for propagating malaria parasites. Both these countries lie in the Northeast region and it is expected (but as yet untested) that the SP resistance alleles will be common to both countries.

The importance of parasite circulation to shared resistance alleles among regional populations is an implicit assumption underlying the WHO recommendations. The presence of the *dhps* 540E is indicative of shared phenotype. In Madagascar, a picture of evolution of SP resistance determinants which was independent of the mainland emerged and a 164L mutation was found in a novel haplotype not recorded in mainland Africa. This illustrates an important caution in the application of a simple threshold prevalence of the 540E mutation. On the mainland, it serves as a valuable approximation of resistance levels among populations which share common resistant alleles and in which efficacy in relation to those alleles is well understood. They are not informative in areas where background genetics of *dhfr* and *dhps* are significantly different from areas where efficacy has been evaluated. In such cases, local evaluations of SP-IPTi efficacy are required.

Alternative treatments such as artemisinin-based combination therapies have not yet been sufficiently tested for safety, efficacy and interactions with the EPI to be advocated as alternatives to SP for IPTi. So use of IPTi rests upon the continuing efficacy of SP. Our recommendations for ongoing molecular surveillance of SP resistance markers in support of SP-IPTi policy in Africa are three fold. First, it is necessary that surveillance is introduced to the neglected geographical areas and coverage increased in countries like the Democratic Republic of Congo which have borderline levels of 540E. Secondly, ongoing molecular surveillance of *dhps* 540E should be maintained and expanded in sites throughout West and Central Africa in support of the continuing use of SP for intermittent preventive treatment there. Thirdly, monitoring of molecular markers in East Africa should be used to record the effect of reduction in drug pressure on the prevalence of SP resistance mutations.

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