Effect of *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance on the effectiveness of intermittent preventive therapy for malaria in pregnancy in Africa: a systematic review and meta-analysis

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Lancet Infect Dis 2019; 19: 546–56

Published Online March 25, 2019 http://dx.doi.org/10.1016/S1473-3099(18)30732-1

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Summary

Background **Resistance of *Plasmodium falciparum* to sulfadoxine-pyrimethamine threatens the antimalarial effectiveness of intermittent preventive treatment during pregnancy (IPTp) in sub-Saharan Africa. We aimed to assess the associations between markers of sulfadoxine-pyrimethamine resistance in *P falciparum* and the effectiveness of sulfadoxine-pyrimethamine IPTp for malaria-associated outcomes.**

Methods **For this systematic review and meta-analysis, we searched databases (from Jan 1, 1990 to March 1, 2018) for clinical studies (aggregated data) or surveys (individual participant data) that reported data on low birthweight (primary outcome) and malaria by sulfadoxine-pyrimethamine IPTp dose, and for studies that reported on molecular markers of sulfadoxine-pyrimethamine resistance. Studies that involved only HIV-infected women or combined interventions were excluded. We did a random-effects meta-analysis (clinical studies) or multivariate log-binomial regression (surveys) to obtain summarised dose-response data (relative risk reduction [RRR]) and multivariate meta-regression to explore the modifying effects of sulfadoxine-pyrimethamine resistance (as indicated by Ala437Gly, Lys540Glu, and Ala581Gly substitutions in the *dhps* gene). This study is registered with PROSPERO, number 42016035540.**

Findings **Of 1097 records screened, 57 studies were included in the aggregated-data meta-analysis (including 59 457 births). The RRR for low birthweight declined with increasing prevalence of *dhps* Lys540Glu (p_{trend}=0·0060) but not Ala437Gly (p_{trend}=0·35). The RRR was 7% (95% CI 0 to 13) in areas of high resistance to sulfadoxine-pyrimethamine (Lys540Glu ≥90% in east and southern Africa; n=11), 21% (14 to 29) in moderate-resistance areas (Ala437Gly ≥90% [central and west Africa], or Lys540Glu ≥30% to <90% [east and southern Africa]; n=16), and 27% (21 to 33) in low-resistance areas (Ala437Gly <90% [central and west Africa], or Lys540Glu <30% [east and southern Africa]; n=30; p_{trend}=0·0054 [univariate], I^2=69·5%). The overall RRR in all resistance strata was 21% (17 to 25). In the analysis of individual participant data from 13 surveys (42 394 births), sulfadoxine-pyrimethamine IPTp was associated with reduced prevalence of low birthweight in areas with a Lys540Glu prevalence of more than 90% and Ala581Gly prevalence of less than 10% (RRR 10% [7 to 12]), but not in those with an Ala581Gly prevalence of 10% or higher (pooled Ala581Gly prevalence 37% [range 29 to 46]; RRR 0·5% [–16 to 14]; 2326 births).**

Interpretation **The effectiveness of sulfadoxine-pyrimethamine IPTp is reduced in areas with high resistance to sulfadoxine-pyrimethamine among *P falciparum* parasites, but remains associated with reductions in low birthweight even in areas where *dhps* Lys540Glu prevalence exceeds 90% but where the sextuple-mutant parasite (harbouring the additional *dhps* Ala581Gly mutation) is uncommon. Therapeutic alternatives to sulfadoxine-pyrimethamine IPTp are needed in areas where the prevalence of the sextuple-mutant parasite exceeds 37%.**

Funding **US Centers for Disease Control and Prevention, the Malaria in Pregnancy Consortium (funded through a grant from the Bill & Melinda Gates Foundation to the Liverpool School of Tropical Medicine), Worldwide Antimalarial Resistance Network, European and Developing Countries Clinical Trials Partnership.**

Introduction

Without pregnancy-specific protection, an estimated 45% of 32 million pregnancies in malaria-endemic sub-Saharan Africa are exposed to *Plasmodium falciparum* malaria yearly,[1] leading to 900 000 malaria-associated low birthweight deliveries[2] and associated consequences for infant health.[3] In these areas, WHO recommends intermittent preventive treatment in pregnancy (IPTp) with antimalarials. IPTp with sulfadoxine-pyrimethamine, the only antimalarial currently recommended for this strategy, is associated with major reductions in maternal anaemia, low birthweight, and neonatal mortality.[4] However, the effectiveness of sulfadoxine-pyrimethamine IPTp is threatened by resistance to this

Research in context

Evidence before this study

We searched the Malaria in Pregnancy Library, PubMed, Web of Science, and Scopus for studies (published in English, up to March 1, 2018) in sub-Saharan Africa of the ecological relationship between molecular markers of sulfadoxine-pyrimethamine resistance and the effectiveness of sulfadoxine-pyrimethamine intermittent preventive treatment in pregnancy (IPTp) for preventing low birthweight, preterm birth, maternal malaria infection, and maternal anaemia. The following search terms were used: "Malaria AND pregnan* AND (intermittent OR IPT) AND Review". We found one prospective multi-country study (done in eight sites), two meta-analyses, and one modelling study. In the prospective study, prevalence of molecular markers of sulfadoxine-pyrimethamine resistance was strongly correlated with clearance of existing infections by the drug, and with duration of post-treatment prophylaxis, but showed no clear trend with regard to reductions in low birthweight, maternal anaemia, or plasmodium infections from this treatment. In this study, few areas with a high prevalence of the highly resistant sextuple-mutant *Plasmodium falciparum* parasite were investigated. One meta-analysis showed, based on three studies, no protective effect of sulfadoxine-pyrimethamine IPTp (*vs* placebo or no intervention) against low birthweight in areas with more than 50% *dhps* Lys540Glu mutation prevalence. By contrast, the other meta-analysis (nine studies) showed no reduced effectiveness of the treatment in areas with high sulfadoxine-pyrimethamine resistance. The modelling study did not directly investigate the relationship between the effect of sulfadoxine-pyrimethamine resistance and the effectiveness of sulfadoxine-pyrimethamine, but suggested that, even accounting for resistance, extending sulfadoxine-pyrimethamine IPTp to all women attending antenatal clinics would have a sizeable and cost-effective impact on maternal and infant health. Although this inference was valid in most malaria-endemic settings in sub-Saharan Africa, the single exception was highly resistant areas where sextuple-mutant parasites are common.

Added value of this study

This is the most comprehensive study of the effect of sulfadoxine-pyrimethamine resistance on the effectiveness of IPTp, involving 57 studies, 13 surveys, and more than 100 000 births. The aggregated data meta-analysis indicated substantial heterogeneity in effect size between studies, which might explain the contradictory findings between the two previous smaller reviews and the ongoing controversy about the continued use of sulfadoxine-pyrimethamine IPTp in areas of high resistance. We report for the first time a clear trend towards reduced effectiveness of sulfadoxine-pyrimethamine IPTp for low birthweight and *P falciparum* infection with increasing prevalence of molecular sulfadoxine-pyrimethamine resistance markers. Sulfadoxine-pyrimethamine was protective against low birthweight in areas of high resistance where parasites with the *dhfr* and *dhps* quintuple-mutant haplotype are essentially fixed. However, three observational cohort studies published elsewhere showed that these beneficial effects were not apparent in individuals infected with the highly resistant sextuple-mutant parasites (harbouring the quintuple mutant haplotype plus *dhps* Ala581Gly).

Implications of all the available evidence

Overall, evidence suggests a decline in the effectiveness of sulfadoxine-pyrimethamine IPTp for reducing malaria infection, anaemia, and low birthweight with increasing resistance. Nevertheless, use of sulfadoxine-pyrimethamine IPTp remains associated with reduced risks of low birthweight, even in areas where sulfadoxine-pyrimethamine fails to clear a third of asymptomatic infections in women receiving IPTp. These findings support WHO's recommendation to continue using sulfadoxine-pyrimethamine for IPTp in these high-resistance areas. However, an important exception is areas where sextuple mutant parasites are common (≥37% prevalence). In such areas, alternative preventive strategies are required now. The substantial heterogeneity between studies, even in areas with similar resistance levels, suggests that single observational studies of the relationship between sulfadoxine-pyrimethamine doses and low birthweight might not be informative as tools for making policy decisions. A decision tool using just two or three mutational markers in the *dhps* gene could be considered to guide sulfadoxine-pyrimethamine IPTp policy.

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drug combination, particularly in east and southern Africa.

In *P falciparum*, sulfadoxine-pyrimethamine resistance results from a series of single nucleotide polymorphisms in the parasite's dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes. At the ecological level, a high prevalence of quintuple-mutant *P falciparum* parasites, defined as those that harbour the five most common substitutions (*dhfr* substitutions Asn51Ile, Cys59Arg, and Ser108Asn, and *dhps* substitutions Ala437Gly and Lys540Glu), reduces the efficacy of sulfadoxine-pyrimethamine as an intermittent preventive treatment against malaria in infants and children,[5,6] undermines the ability of sulfadoxine-pyrimethamine to clear existing *P falciparum* infections in asymptomatic pregnant women,[7,8] and shortens the post-treatment prophylactic period following IPTp.[7] Sextuple-mutant *P falciparum* parasites, which harbour the additional *dhps* Ala581Gly mutation, are associated with enhanced sulfadoxine-pyrimethamine resistance in vitro, sulfadoxine-pyrimethamine treatment failure in patients with acute malaria,[9–11] and failure of the drug combination to inhibit parasite growth or prevent malaria-associated fetal growth restriction in pregnant women.[12–15]

Despite these effects, there are no guidelines on the use of molecular prevalence data to inform the use of sulfadoxine-pyrimethamine for IPTp.[16] The ecological

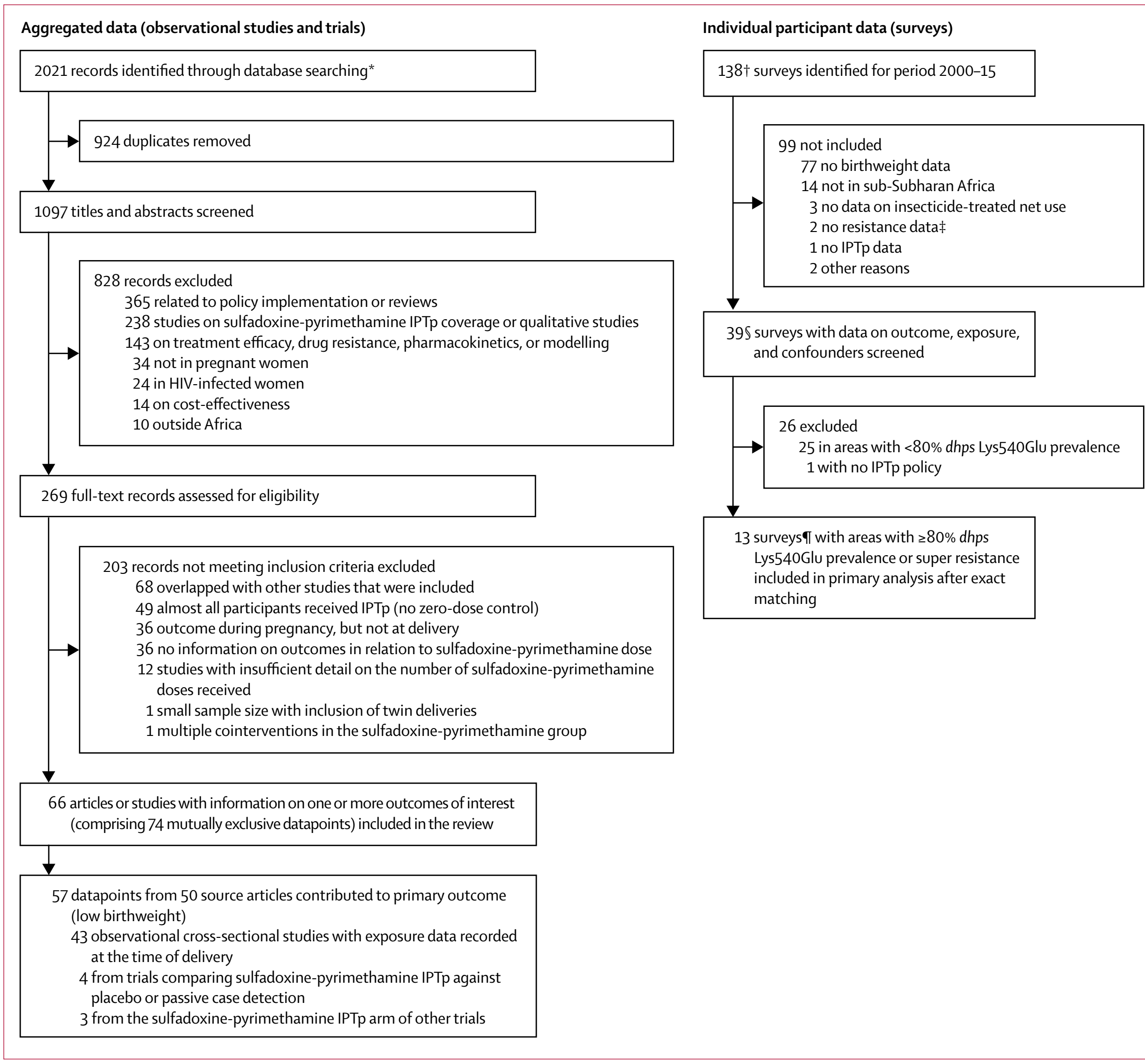


Figure 1: **Study profile**

dhps=dihydropteroate synthase. IPTp=intermittent preventive treatment in pregnancy. *561 from Malaria in Pregnancy Library, 440 from PubMed, 518 from Web of Science, and 502 from Scopus. †63 Demographic and Health Surveys, 13 Malaria Indicator Surveys, 54 Multiple Indicator Cluster Surveys (UNICEF), and eight AIDS indicator surveys. ‡Resistance data were not available for Comoros and São Tomé and Príncipe. §39 surveys with individual-level data available and information on outcomes, exposures, and potential confounders (276 383 single live births: 46% with measured birthweight available, 54% with perceived birthweight; mean birthweight 3217 g (SD 699), small birth size 14·1% of 276 383, low birthweight 9·4% of 128 347). ¶Comprising 49 481 births (of which 98·2% were singleton livebirths) before exact matching, and 42 394 singleton livebirths (19 429 with measured birthweight not available and 22 965 with measured birthweight available) after exact matching.

relationship between molecular measures of sulfadoxine-pyrimethamine resistance and the effect of sulfadoxine-pyrimethamine IPTp on clinically relevant birth outcomes, such as low birthweight, is not clear. Previous attempts to define these relationships reached conflicting conclusions,[17,18] possibly reflecting substantial between-study heterogeneity in the effect of sulfadoxine-pyrimethamine treatment on low birthweight.[14,18]

Using all available data derived from observational studies, clinical trials, and national surveys in sub-Saharan Africa, we did a meta-analysis of the ecological relationship between molecular markers of sulfadoxine-pyrimethamine resistance and the effect of sulfadoxine-pyrimethamine IPTp on low birthweight. We hypothesised that a higher prevalence of sulfadoxine-pyrimethamine resistance, as indicated by the prevalence of molecular markers of sulfadoxine resistance, would be associated with an attenuation of the sulfadoxine-pyrimethamine IPTp-associated reduction in low birthweight.

Methods

Search strategy and selection criteria

We did a systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses statement (appendix, p 41). Two main sources of data regarding IPTp effectiveness were used: aggregated data from observational studies and clinical trials (henceforth referred to collectively as clinical studies), and individual participant data from nationally representative surveys (referred to as surveys). Clinical studies were identified by two independent reviewers (AMvE and GK) by searching trial registries and electronic databases (Malaria in Pregnancy Library,[19] PubMed, Web of Science, and Scopus) for studies published between Jan 1, 1990, and March 1, 2018, without language restrictions, in addition to scanning reference lists of articles and consulting with experts in the field (appendix p 2). The search terms "Malaria AND pregnan* AND intermittent AND (prevent* OR prophyla* OR chemoprevent* OR chemoprophyla* OR IPT*) AND (sulfadoxine OR sulphadoxine OR pyrimethamine OR SP)" were used. Observational studies were included if they were done in sub-Saharan Africa, had information at delivery on the number of sulfadoxine-pyrimethamine doses received, and data on birthweight, maternal haemoglobin, or plasmodium infection at delivery. Trials were included if they were quasi-randomised or randomised trials done in sub-Saharan Africa, compared sulfadoxine-pyrimethamine IPTp against passive case detection or placebo, and otherwise fulfilled the same criteria as for the observational studies. Studies or study arms were excluded if they involved only HIV-infected women or if they combined sulfadoxine-pyrimethamine with other antimalarial drugs (such as artemisinin derivatives or azithromycin) or with other interventions (such as screening for malaria). Final study eligibility was agreed on by the reviewers. If no agreement could be reached, a third reviewer (FOtK) assessed the study and agreement was reached by consensus.

To identify surveys, one reviewer (DAL) searched all national-level datasets from surveys done in malaria-endemic countries in Africa after the year 2000 (when WHO introduced the sulfadoxine-pyrimethamine IPTp policy) and with datasets publicly available (as described in detail elsewhere;[4] search date May 31, 2015), including the Demographic and Health Surveys Program, UNICEF Multiple Indicator Cluster Surveys, and Malaria Indicator Surveys. Surveys were included if they contained data on low birthweight (perceived birth size and measured weight), measured IPTp use by number of doses among recently pregnant women, and measured insecticide-treated net coverage at the household level (appendix pp 2–3).

Data on molecular markers of sulfadoxine-pyrimethamine resistance were obtained from the clinical study reports or from the authors of those reports. If these data were not available, data were obtained from existing population prevalence maps of *P falciparum dhps* mutations by use of the molecular surveyor tool of the Worldwide Antimalarial Resistance Network (WWARN) and existing prediction surfaces of the prevalence of sulfadoxine-pyrimethamine resistance-associated mutations based on these data.[16,20–22] Malaria transmission intensity data were obtained from the Malaria Atlas Project.

See **Online** for appendix

For the **Malaria Atlas Project** see http://www.map.ox.ac.uk/

Extraction and quality assessment of IPTp effectiveness data

From clinical studies, extraction of summary data was done independently by two investigators (AMvE and GK or DECS). Authors of primary studies were contacted for missing information or if reported data did not fit the required format. The following information was extracted: first author, publication year, year of study start and end, study design, study and randomisation procedures (trials only), inclusion criteria (eg, any restrictions by gravidity), insecticide-treated net use, numerator and denominator per outcome per sulfadoxine-pyrimethamine dose, and details of control intervention (trials only). If available, sulfadoxine-pyrimethamine resistance data were extracted. Study quality was assessed by two reviewers (AMvE and GK or DECS) using an adaptation of the Newcastle-Ottawa Scale (appendix, p 3).[23]

From surveys, the following (individual patient-level) data were extracted: reported number of sulfadoxine-pyrimethamine doses received; composite of low birthweight (<2·5 kg) if measured birthweight was available, or perceived small birth size (very small or small) if birthweight was not available (the correlation between perceived and measured low birthweight has been described elsewhere[4]); and measured birthweight as a continuous variable.[4] Other data extracted included number of antenatal visits, tetanus vaccination, iron supplementation and insecticide-treated net ownership, household socioeconomic status, mother's education, mother's age and parity, birth spacing, newborn sex, season of birth, and whether it was a single or multiple birth.

Data on the prevalence of *dhps* Ala437Gly, Lys540Glu, and Ala581Gly mutations among *P falciparum* parasites were extracted from the clinical studies in pregnant women, the literature, and existing molecular surveyor databases (appendix p 4).[16,20–22] In areas where the prevalence of this quintuple mutant was more than 50%, the prevalence of the *dhps* Ala581Gly mutation served as a proxy for the sextuple mutant. Two areas were identified where the sextuple mutant was more than 10%: northeastern Tanzania, and the area crossing the borders of southwestern Uganda, eastern Rwanda, eastern Democratic Republic of the Congo, and northwestern Tanzania (appendix p 4). The prevalence of each point mutation and the *P falciparum* parasite prevalence in children aged 2–10 years ($Pf\mathrm{PR}_{2\text{–}10}$; using data from the Malaria Atlas Project) was matched to each study by time (the same year for $Pf\mathrm{PR}_{2\text{–}10}$ and within 2 years before or after for point mutations) and by location using latitude and longitude (within 300 km where possible).[24] For national surveys, these prevalence data were calculated for

For the **Demographic and Health Surveys Program** see http://dhsprogram.com/

For the **UNICEF Multiple Indicator Cluster Surveys** see http://mics.unicef.org/

For the **Malaria Indicator Surveys** see http://www.malariasurveys.org/

the administrative boundary of the given survey using Malaria Atlas Project data and WWARN's geospatial models (appendix p 4).[22]

Definition of resistance categories

To stratify resistance into low, moderate, and high levels, different combinations of threshold levels (at 5% step increases) of the resistance-associated mutations in *dhps* were explored in the aggregated-data meta-analysis. Because of distinct parasite populations and distributions of mutations in each region,[25] threshold analysis was done separately for central and west Africa and for east and southern Africa. Results were then combined to obtain a single categorical variable that represented the optimal thresholds based on the R^2 for each region.

Statistical analysis

The primary outcome was low birthweight. Secondary outcomes included anaemia, malaria, preterm delivery, birthweight, haemoglobin level, and gestational age. Analyses of clinical studies were done with Stata (version 14). A two-stage random-effects meta-analysis was done by use of a generalised least-squares regression for trend estimation of summarised dose-response data.[26,27] Effect sizes were expressed as relative risk reduction (RRR; 100×[1–relative risk]) for trend (appendix p 4), and were then combined across studies with use of a random-effects meta-analysis, with heterogeneity quantified using the I^2 statistic. Potential modifying effects of sulfadoxine-pyrimethamine resistance were examined with multivariate linear meta-regression, adjusting for the following prespecified covariates: malaria transmission, study quality, average number of sulfadoxine-pyrimethamine doses, and proportion of paucigravidae (defined as women in their first or second pregnancy).[28] The proportion of women using insecticide-treated nets was not found to be associated with resistance level in our analyses and was not included as covariate in the metaregression. Subgroup analyses by gravidity (paucigravidae *vs* multigravidae) were also done. For the assessment of the effect of sulfadoxine-pyrimethamine IPTp on continuous outcomes, only the no doses group versus the two or more doses group were compared. Further sensitivity analysis was done by excluding low-quality studies and exploring the presence and impact of potential small-study effects due to publication and other biases (appendix p 4).[29]

The survey analysis was done in R and restricted to the higher-resistance areas with more than 80% prevalence of *dhps* Lys540Glu. Only the most recent livebirth within the past 2 years was considered. To mitigate potential confounding of the effect of sulfadoxine-pyrimethamine dose on birthweight, exact matching was used (appendix p 4).[4] The modifying effect of sulfadoxine-pyrimethamine resistance was first assessed for each survey by use of random-effects log-binomial regression models for low birthweight and linear regression for birthweight with the matched birth strata included as a random intercept using the lme4 package in R.[30] IPTp exposures were considered as continuous variables similar to the aggregated meta-analysis. The effect measures were then further evaluated by resistance strata (quintiles) and compared by use of meta-regression.

This study is registered with PROSPERO, number 42016035540.

Role of the funding source

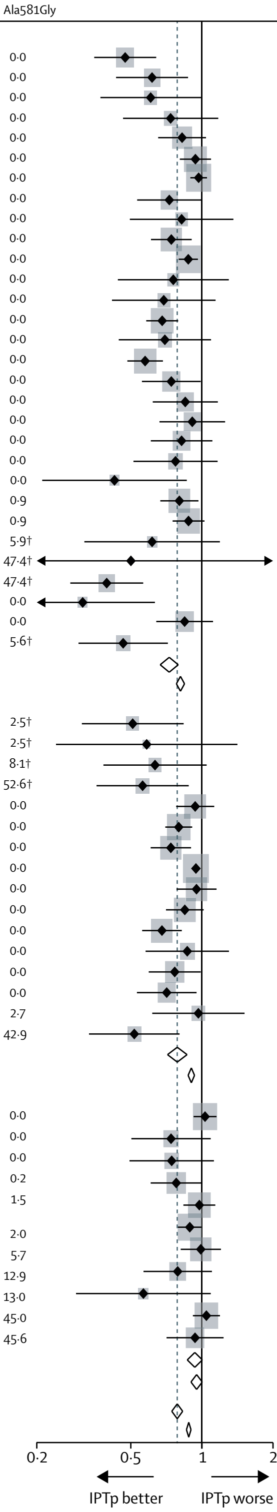
Except for the US Centers for Disease Control and Prevention (CDC) and WWARN, the funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. CDC and WWARN staff participated in the conduct of the study. AMvE, FOtK and DAL had full access to all the data in the study. AMvE and FOtK had final responsibility for the decision to submit for publication.

Results

For the aggregated-data meta-analysis, we identified 2021 records through database searching. After removal of duplicates, 1097 articles were assessed for eligibility, of which 66 were included in the review: 58 observational studies and eight trials (figure 1). A summary of the included studies is provided in the appendix (p 6). Of these, 50 source articles from 17 countries (appendix p 30) were included in the analysis of low birthweight, involving 57 datapoints (henceforth referred to as studies) and 59 457 births. The remaining 16 studies did not provide data on low birthweight, but contributed to the analysis of secondary outcomes. In central and west Africa (31 studies), the median prevalence of *dhps* Ala437Gly was 57·9% (IQR 39·3–77·6; range 15·2–100·0), despite a low prevalence of *dhps* Lys540Glu (0·1% [IQR 0·0–0·9;

Figure 2: **Relative risk of low birthweight associated with each incremental dose of sulfadoxine-pyrimethamine IPTp in all gravidae by resistance strata**
On the basis of the estimated prevalence of *dhps* mutations in the study areas (matched as described in text and appendix p 9), resistance was stratified into low (Ala437Gly <90% [central and west Africa], or Lys540Glu <30% [east and southern Africa]; 30 studies), moderate (Ala437Gly ≥90% [central and west Africa], or Lys540Glu ≥30% to <90% [east and southern Africa]; 16 studies), and high (Lys540Glu ≥90% in east and southern Africa; 11 studies). p values following the I^2 statistics represent the χ^2 test for heterogeneity. Weights are from random effects analysis. Data marker sizes indicate the weight applied to each study using random-effects meta-analysis. Diamonds represent summary effect of studies. CW=central and west Africa. *dhps*=dihydropteroate synthase. D+L=Dersimonian-Laird method for random effects models. ES=east and southern Africa. IPTp=intermittent preventive treatment in pregnancy. I-V=inverse variance method for fixed effects models. *Reference refers to the lowest sulfadoxine-pyrimethamine dose category (0 or 0–1 dose as indicated in the sulfadoxine-pyrimethamine dose category column), and the comparison column (included for illustration only) refers to all the other exposure groups pooled (eg, if the sulfadoxine-pyrimethamine categories were 0, 1, 2+, the comparison column would reflect the data in the 1 dose group and 2+ dose groups pooled; full sample sizes per dose group and average doses are shown in the appendix (p 13). †The high prevalence of *dhps* Ala581Gly in these studies was not accompanied by a high prevalence in *dhps* Lys540Glu, so this information was not interpreted as an indication of the presence of sextuple-mutant parasites.

	Site	Region	Study period	Sulfadoxine-pyrimethamine dose category	Low birthweight prevalence, n/N (%)*		Mutation prevalence, %			Risk ratio trend (95% CI)	Study weight, % (D+L)	Low birthweight relative risk reduction per dose, % (95% CI)
					Reference	Comparison	Ala437Gly	Lys540Glu	Ala581Gly			
Low-resistance areas												
Muhammad et al, 2016, Nigeria	Nguru, Yobe	CW	2014	0–1,2+	58/104 (55·8)	10/80 (12·5)	24·5	0·0	0·0	0·47 (0·35 to 0·64)	1·67	53 (36 to 65)
Kayentao et al, 2014, Mali	San	CW	2009–10	0,1,2+	18/110 (16·4)	22/320 (6·9)	27·5	0·0	0·0	0·62 (0·43 to 0·87)	1·41	38 (13 to 57)
Kayentao et al, 2014, Mali	San	CW	2006	0,1,2+	15/135 (11·1)	14/263 (5·3)	32·6	0·0	0·0	0·61 (0·37 to 0·99)	0·89	39 (1 to 63)
Kayentao et al, 2014, Mali	Djenne	CW	2006	0,1,2+	10/110 (9·1)	13/245 (5·3)	32·7	0·0	0·0	0·74 (0·46 to 1·17)	0·96	26 (–17 to 54)
Olliaro et al, 2008, Senegal	Mlomp	CW	2000–07	0,1,2+	57/532 (10·7)	29/372 (7·8)	39·3	0·0	0·0	0·82 (0·65 to 1·04)	2·14	18 (–4 to 35)
Mbaye et al, 2006, Gambia	Farafenni	CW	2002–04	0,2+	46/716 (6·4)	40/738 (5·4)	46·8	0·0	0·0	0·94 (0·81 to 1·09)	2·81	6 (–9 to 19)
Oduro et al, 2010, Ghana	Navrongo	CW	2006–07	0,1,2,3+	76/391 (19·4)	342/1886 (18·1)	53·8	0·0	0·0	0·97 (0·89 to 1·05)	3·36	3 (–5 to 11)
Falade et al, 2007, Nigeria	Ibadan	CW	2003–04	0,1+	16/171 (9·4)	31/595 (5·2)	63·0	0·0	0·0	0·73 (0·53 to 1·00)	1·60	27 (0 to 47)
Bouyou-Akotet et al, 2016, Gabon	Libreville, Melen	CW	2011	0,1,2+	5/58 (8·6)	14/241 (5·8)	66·7	0·0	0·0	0·82 (0·50 to 1·36)	0·85	18 (–36 to 50)
Coulibaly et al, 2014, Burkina Faso	Ziniare	CW	2011–12	0,1,2+	32/155 (20·6)	106/757 (14·0)	75·3	0·0	0·0	0·74 (0·61 to 0·91)	2·42	26 (9 to 39)
Tutu et al, 2011, Ghana	Offinso	CW	2005–07	0,1,2,3+	62/499 (12·4)	250/2084 (12·0)	77·6	0·0	0·0	0·88 (0·80 to 0·96)	3·28	12 (4 to 20)
Moleins et al, 2010, Senegal	Oussouye	CW	2007–08	0–1,2+	6/55 (10·9)	6/96 (6·3)	43·0	0·1	0·0	0·76 (0·44 to 1·30)	0·76	24 (–30 to 56)
Kayentao et al, 2014, Mali	Koro	CW	2006–07	0,1,2+	13/130 (10·0)	14/221 (6·3)	44·8	0·1	0·0	0·69 (0·42 to 1·14)	0·85	31 (–14 to 58)
Sirima et al, 2006, Burkina Faso	Koupela	CW	2004	0,1,2,3+	16/66 (24·2)	119/1054 (11·3)	48·1	0·1	0·0	0·68 (0·58 to 0·79)	2·78	32 (21 to 42)
Kayentao et al, 2014, Mali	Bougouni	CW	2006–07	0,1,2+	11/101 (10·9)	17/306 (5·6)	33·8	0·2	0·0	0·70 (0·44 to 1·09)	1·01	30 (–9 to 56)
Gies et al, 2009, Burkina Faso	Boromo	CW	2004–06	0,1,2+	19/52 (36·5)	204/1220 (16·7)	71·5	0·2	0·0	0·57 (0·48 to 0·68)	2·63	43 (32 to 52)
Kayentao et al, 2014, Mali	Kita	CW	2009–10	0,1,2+	18/124 (14·5)	38/420 (9·0)	15·2	0·7	0·0	0·74 (0·56 to 0·99)	1·77	26 (1 to 44)
Famanta et al, 2011, Mali	Bamako	CW	2009	0,1,2+	16/102 (15·7)	25/257 (9·7)	15·2	0·7	0·0	0·85 (0·62 to 1·17)	1·58	15 (–17 to 38)
Hommerich et al, 2007, Ghana	Agogo	CW	2006	0,1,2,3+	8/52 (15·4)	20/173 (11·6)	84·6	1·4	0·0	0·91 (0·66 to 1·25)	1·57	9 (–25 to 34)
Ramharter et al, 2007, Gabon	Lambarene	CW	2005–06	0,1,2+	11/97 (11·3)	60/596 (10·1)	57·9	3·3	0·0	0·82 (0·61 to 1·11)	1·69	18 (–11 to 39)
Bouyou-Akotet et al, 2010, Gabon	Libreville	CW	2005–06	0,1+	24/120 (20·0)	11/83 (13·3)	69·0	6·9	0·0	0·77 (0·51 to 1·17)	1·15	23 (–17 to 49)
Likwela et al, 2012, DR Congo	Mikalayi	CW	2007	0–1,2+	35/363 (9·6)	2/114 (1·8)	76·9	11·3	0·0	0·43 (0·21 to 0·86)	0·49	57 (14 to 79)
Toure et al, 2014, Côte d'Ivoire	Abidjan, Comoe	CW	2009–10	0,1,2,3+	50/436 (11·5)	61/876 (7·0)	52·1	0·9	0·9	0·80 (0·67 to 0·97)	2·52	20 (3 to 33)
Vanga-Bosson et al, 2011, Côte d'Ivoire	National	CW	2008	0,1,2,3+	35/309 (11·3)	172/1636 (10·5)	52·1	0·9	0·9	0·88 (0·75 to 1·03)	2·78	12 (–3 to 25)
Tonga et al, 2013, Cameroon	Sanaga–Maritime	CW	2011–12	0,1,2+	7/68 (10·3)	6/127 (4·7)	76·5	0·0	5·9†	0·62 (0·32 to 1·19)	0·55	38 (–19 to 68)
Alli et al, 2013, Nigeria	Kubwa	CW	2010–11	0,1+	4/158 (2·5)	0/42 (0·0)	84·2	0·0	47·4†	0·50 (0·05 to 4·78)	0·05	50 (–379 to 95)
Aziken et al, 2010, Nigeria	Benin City	CW	2009	0,1+	61/371 (16·4)	14/370 (3·8)	84·2	0·0	47·4†	0·40 (0·28 to 0·56)	1·39	60 (44 to 72)
Suleiman et al, 2003, Sudan	Wad Medani	ES	1999–2001	0,2+	19/53 (35·8)	2/57 (3·5)	13·3	0·0	0·0	0·31 (0·15 to 0·63)	0·49	69 (37 to 85)
Challis et al, 2004, Mozambique	Maputo	ES	2001–02	0,2+	27/203 (13·3)	19/200 (9·5)	26·1	25·4	0·0	0·85 (0·64 to 1·11)	1·83	15 (–11 to 36)
Likwela et al, 2012, DR Congo	Kisangani	ES	2007	0–1,2+	16/50 (32·0)	6/87 (6·9)	74·1	27·8	5·6†	0·46 (0·30 to 0·72)	1·06	54 (28 to 70)
D+L subtotal (I^2 70·5%, p<0·0001)										**0·73 (0·67 to 0·79)**	**48·32**	**27 (21 to 33)**
I-V subtotal										**0·81 (0·78 to 0·84)**		
Moderate-resistance areas												
Tongo et al, 2011, Nigeria	Ibadan	CW	2007–08	0–1,2+	68/649 (10·5)	4/147 (2·7)	92·4	1·0	2·5†	0·51 (0·31 to 0·84)	0·87	49 (16 to 69)
Olorunda et al, 2013, Nigeria	Ibadan	CW	2010	0,1+	22/246 (8·9)	4/84 (4·8)	92·4	1·0	2·5†	0·58 (0·24 to 1·41)	0·33	42 (–41 to 76)
Kilauzi et al, 2013, DR Congo	Kinshasa	CW	2011	0,1+	21/204 (10·3)	32/501 (6·4)	100·0	18·9	8·1†	0·63 (0·38 to 1·05)	0·85	37 (–5 to 62)
Igboeli et al, 2017, Nigeria	Enugu State	CW	2013	0,1+	8/101 (7·9)	7/315 (2·2)	96·8	0·0	52·6†	0·56 (0·36 to 0·88)	1·01	44 (12 to 64)
Njagi et al, 2002, Kenya	Bondo	ES	1997–99	0,2+	51/359 (14·2)	46/369 (12·5)	42·8	31·1	0·0	0·94 (0·78 to 1·13)	2·52	6 (–13 to 22)
Parise et al, 1998, Kenya	Kisumu	ES	1994–96	0,2,3+	52/340 (15·3)	53/656 (8·1)	42·8	31·1	0·0	0·80 (0·70 to 0·91)	2·98	20 (9 to 30)
van Eijk et al, 2004, Kenya	Kisumu	ES	1999–2000	0,1,2+	112/948 (11·8)	70/925 (7·6)	42·8	31·1	0·0	0·74 (0·61 to 0·90)	2·43	26 (10 to 39)
Cassam et al, 2007, Mozambique	Gaza	ES	2005–07	0,3+	756/8650 (8·7)	488/6645 (7·3)	53·2	47·6	0·0	0·94 (0·91 to 0·98)	3·59	6 (2 to 9)
Menendez et al, 2008, Mozambique	Manhica	ES	2003–05	0,2+	49/411 (11·9)	41/382 (10·7)	62·9	68·6	0·0	0·95 (0·78 to 1·15)	2·44	5 (–15 to 22)
Yussuf et al, 2010, Tanzania	Lindi	ES	2009–10	0,1,2+	55/123 (44·7)	44/123 (35·8)	79·7	72·7	0·0	0·85 (0·70 to 1·02)	2·54	15 (–2 to 30)
Feng et al, 2010, Malawi	Blantyre	ES	1997–99	0,1,2+	49/215 (22·8)	84/697 (12·1)	63·6	74·0	0·0	0·68 (0·56 to 0·82)	2·46	32 (18 to 44)
Ndeserua et al, 2015, Tanzania	Rufiji	ES	2012	0–1,2+	12/166 (7·2)	10/184 (5·4)	75·0	76·3	0·0	0·87 (0·58 to 1·30)	1·16	13 (–30 to 42)
Feng et al, 2010, Malawi	Blantyre	ES	1999–2001	0,1,2+	20/117 (17·1)	85/719 (11·8)	80·3	84·0	0·0	0·77 (0·60 to 0·99)	1·99	23 (1 to 40)
Mace et al, 2014, Zambia	Mansa	ES	2009–10	0–1,2,3+	17/157 (10·8)	13/266 (4·9)	83·7	84·0	0·0	0·71 (0·53 to 0·95)	1·74	29 (5 to 47)
Mosha et al, 2014, Tanzania	Rufiji, Moshi	ES	2012	0–1,2+	9/169 (5·3)	9/181 (5·0)	93·2	88·3	2·7	0·97 (0·62 to 1·52)	1·01	3 (–52 to 38)
Minja et al, 2013, Tanzania	Korogwe	ES	2008–10	0–1,2+	4/17 (23·5)	43/705 (6·1)	100·0	87·5	42·9	0·52 (0·33 to 0·80)	1·04	48 (20 to 67)
D+L subtotal (I^2 66·7%, p=0·0001)										**0·79 (0·72 to 0·87)**	**28·97**	**21 (14 to 29)**
I-V subtotal										**0·90 (0·87 to 0·93)**		
High-resistance areas												
Msyamboza et al, 2009, Malawi	Chikwawa	ES	2002–04	0–1,2,3+	65/427 (15·2)	157/891 (17·6)	87·0	92·7	0·0	1·03 (0·92 to 1·15)	3·14	–3 (–15 to 8)
Tetteh-Ashong et al, 2005, Malawi	Chikwawa	ES	2005	0–1,2,3+	6/42 (14·3)	13/186 (7·0)	94·1	94·8	0·0	0·74 (0·50 to 1·09)	1·24	26 (–9 to 50)
Namusoke et al, 2010, Uganda	Kampala	ES	2004–05	0,1,2+	28/162 (17·3)	19/159 (11·9)	93·5	95·1	0·0	0·74 (0·49 to 1·12)	1·14	26 (–12 to 51)
Arinaitwe et al, 2014, Uganda	Tororo	ES	2011	0–1,2+	29/227 (12·8)	25/325 (7·7)	97·3	97·5	0·2	0·78 (0·61 to 1·00)	2·02	22 (–0 to 39)
Gutman et al, 2013, and Kalilani et al, 2014, Malawi	Southern Malawi	ES	2009–11	0–1,2,3+	28/334 (8·4)	103/1498 (6·9)	94·4	99·6	1·5	0·98 (0·83 to 1·14)	2·78	2 (–14 to 17)
Feng et al, 2010, Malawi	Blantyre	ES	2002–06	0,1,2,3+	29/234 (12·4)	212/2137 (9·9)	93·5	94·7	2·0	0·89 (0·79 to 1·00)	3·11	11 (0 to 21)
Desai et al, 2014, Kenya	Nyanza	ES	2011–12	0–1,2,3+	10/135 (7·4)	59/734 (8·0)	93·0	95·6	5·7	0·99 (0·81 to 1·20)	2·44	1 (–20 to 19)
Braun et al, 2015, Uganda	Fort Portal	ES	2013	0,1,2+	8/56 (14·3)	52/552 (9·4)	100·0	100·0	12·9	0·79 (0·57 to 1·10)	1·49	21 (–10 to 43)
Harrington et al, 2011, Tanzania	Muheza	ES	2002–05	0,1,2+	6/80 (7·5)	11/292 (3·8)	100·0	90·2	13·0	0·57 (0·29 to 1·09)	0·55	43 (–9 to 71)
Ndyomugyenyi et al, 2011, Uganda	Kabale	ES	2004–07	0,2+	99/1577 (6·3)	107/1561 (6·9)	100·0	100·0	45·0	1·04 (0·92 to 1·19)	2·98	–4 (–19 to 8)
Likwela et al, 2012, DR Congo	Rutsuhuru	ES	2007	0–1,2+	16/177 (9·0)	39/493 (7·9)	88·1	91·2	45·6	0·94 (0·71 to 1·24)	1·82	6 (–24 to 29)
D+L subtotal (I^2 31·4%, p=0·15)										**0·93 (0·87 to 1·00)**	**22·71**	**7 (0 to 13)**
I-V subtotal										**0·95 (0·90 to 1·00)**		
D+L overall (I^2 69·5%, p<0·0001)										**0·79 (0·75 to 0·83)**	**100·00**	**21 (17 to 25)**
I-V overall										**0·88 (0·86 to 0·90)**		



	n	Univariate meta-regression					Multivariate meta-regression*				
		Coefficient (95% CI)	p value	τ^2	I^2, %	R^2, %	Coefficient (95% CI)	p value	τ^2	I^2, %	R^2, %
***dhps* Ala437Gly prevalence†**											
All studies	57	1·001 (0·999–1·004)	0·35	0·02596	69·9	1·8	1·001 (0·999–1·004)	0·25	0·01645	57·7	37·8
Excluding low-quality studies‡	50	1·002 (0·999–1·004)	0·13	0·02079	67·3	7·5	1·002 (1·000–1·004)	0·08	0·01323	54·1	41·1
Restricted to largest 50% of studies§	29	1·003 (1·000–1·005)	0·06	0·01615	73·9	13·3	1·002 (1·000–1·005)	0·09	0·01137	62·1	39·0
***dhps* Lys540Glu prevalence†**											
All studies	57	1·002 (1·001–1·003)	0·0060	0·02142	66·2	19·0	1·002 (1·001–1·003)	0·0031	0·01222	53·7	53·8
Excluding low-quality studies‡	50	1·002 (1·000–1·003)	0·0090	0·01732	61·9	22·9	1·002 (1·000–1·003)	0·0160	0·01133	51·1	49·6
Restricted to largest 50% of studies§	29	1·002 (1·000–1·003)	0·0223	0·01469	70·5	21·1	1·002 (1·000–1·003)	0·0132	0·00909	58·7	51·2
Resistance strata¶											
All studies	57	1·10 (1·03–1·18)	0·0054	0·02040	65·4	22·8	1·10 (1·03–1·17)	0·0043	0·01184	52·9	55·2
Excluding low-quality studies‡	50	1·10 (1·03–1·18)	0·0075	0·01687	61·6	24·9	1·09 (1·02–1·16)	0·0095	0·01067	49·7	52·5
Restricted to largest 50% of studies§	29	1·10 (1·02–1·18)	0·0122	0·01386	69·7	25·6	1·10 (1·03–1·17)	0·0067	0·00802	55·9	56·9

dhps=Plasmodium falciparum dihydropteroate synthase. *Adjusted for malaria transmission intensity, average number of sulfadoxine-pyrimethamine doses, study quality, and proportion of paucigravidae in study. †In the meta-regression, the sulfadoxine-pyrimethamine resistance variable was introduced as a linear continuous variable reflecting 1% stepped increases in prevalence of the resistance marker. ‡Excludes studies with less than three of six stars for quality. §To ascertain the effect of potential bias due to small-study effect, the analysis was restricted to the largest 50% of studies, based on their standard error of the log relative risk for low birthweight. ¶Sulfadoxine-pyrimethamine resistance, defined by the prevalence of molecular markers, stratified into low (*dhps* Ala437Gly <90% in central and west Africa or *dhps* Lys540Glu <30% in east and southern Africa), moderate (*dhps* Ala437Gly ≥90% in central and west Africa, or *dhps* Lys540Glu ≥30% and <90% in east and southern Africa), and high (*dhps* Lys540Glu ≥90% in east and southern Africa).

Table: **Effect of sulfadoxine-pyrimethamine resistance on the effectiveness of sulfadoxine-pyrimethamine IPTp to prevent low birthweight in women receiving intermittent preventive treatment during pregnancy (sub-Saharan Africa, 1997–2013, aggregated data)**

range 0·0–18·9]), whereas, in east and southern Africa (26 studies), the prevalence of *dhps* Ala437Gly (85·4% [IQR 62·9–94·1; range 13·3–100·0]) was similar to that of *dhps* Lys540Glu (85·8% [IQR 47·6–94·8; range 0·0–100·0]; appendix pp 27, 31). The *dhps* Ala581Gly mutation (used as a proxy for the sextuple mutant) mainly occurred in areas with a *dhps* Lys540Glu prevalence of more than 80% in east and southern Africa (figure 2, appendix p 27). Among sulfadoxine-pyrimethamine recipients, the median number of sulfadoxine-pyrimethamine doses received (study-level) was 1·7 (IQR 1·3–2·4; appendix p 13). The number of sulfadoxine-pyrimethamine doses received by study participants was not correlated with the prevalence of *dhps* Ala437Gly (r=–0·0295, p=0·83) or *dhps* Lys540Glu (r=0·1594, p=0·24).

Overall, per dose of sulfadoxine-pyrimethamine, sulfadoxine-pyrimethamine IPTp was associated with an RRR for low birthweight of 21% (95% CI 17–25; 57 studies; figure 2). RRR was 22% (17–27; 34 studies) in paucigravidae and 18% (11–24; 31 studies) in multigravidae (appendix p 17). There was substantial heterogeneity between studies (I^2=69·5%, p<0·0001; figure 2).

Univariate and multivariate meta-regression analyses showed a linear trend towards decreasing effectiveness of sulfadoxine-pyrimethamine IPTp (as indicated by the log relative risk of low birthweight moving closer to the null) with increasing prevalence of *dhps* Lys540Glu (table, figure 3). No differences were seen by gravidity (appendix p 16). No significant trend was observed for *dhps* Ala437Gly (table, figure 3). Of the different thresholds used to stratify resistance into low, medium, and high, the most predictive combination was low resistance defined as *dhps* Ala437Gly less than 90% in central and west Africa or *dhps* Lys540Glu less than 30% in east and southern Africa (RRR 27% [95% CI 21–33]); moderate resistance defined as *dhps* Ala437Gly 90% or higher (central and west Africa) or *dhps* Lys540Glu 30% or higher and less than 90% in east and southern Africa (RRR 21% [14–29]); and high resistance defined as *dhps* Lys540Glu 90% or higher in east and southern Africa (RRR 7% [0–13]; p=0·0054 for linear trend; table). These definitions of resistance strata explained 22·8% of the between-study variance (R^2) in univariate models and, combined with other covariates, 55·2% in multivariate models (table). Very similar results were obtained if alternative thresholds (<20% or <40%) for *dhps* Lys540Glu prevalence were used to define low resistance, or if a *dhps* Ala437Gly prevalence of less than 80% was used to define low resistance, or if the presence (≥1%) of *dhps* Ala581Gly (instead of *dhps* Lys540Glu ≥90%) was used to define high resistance (appendix p 18).

Meta-regression results were similar after the exclusion of low-quality studies (table). There was evidence for significant small-study effects (p<0·0001), but this effect was observed in all three resistance strata (appendix p 32), and restricting the analysis to the larger 50% of studies did not change the observed trend towards decreasing efficacy with increasing resistance (table). By region, multivariate meta-regression showed significant correlations between low birthweight and *dhps* Ala437Gly prevalence, *dhps* Lys540Glu prevalence, and resistance strata only in east and southern Africa (appendix p 16).

Only five studies were done in areas that had a more than 10% prevalence of sextuple-mutant *P falciparum* parasites (pooled *dhps* Ala581Gly prevalence 32% [95% CI 17 to 48]). Substantial heterogeneity in effect size was found among these studies (I^2=68·8%, p=0·012; appendix p 33): the three studies with a small sample size in the reference group had an RRR of 35% (14 to 51; pooled *dhps* Ala581Gly prevalence 21%), whereas the two remaining larger studies, both conducted in areas with the highest *dhps* Ala581Gly prevalence (pooled prevalence 46%) had an RRR of –2% (–15 to 9; p=0·0518 for subgroup difference).

When outcomes other than low birthweight were considered, we observed a linear trend towards decreasing effectiveness of IPTp with increasing prevalence of *dhps* Lys540Glu for maternal moderate-to-severe anaemia and for malaria infection (maternal, placental, or any malaria) at delivery. The RRRs at delivery for moderate-to-severe anaemia were 41% (28 to 51) in low-resistance, 20% (1 to 35) in moderate-resistance, and 13% (3 to 22) in high-resistance areas (p_{trend}=0·0049); and for any malaria infection were 20% (13 to 26) in low-resistance, 18% (10 to 26) in moderate-resistance, and 3% (–3 to 9) in high-resistance areas (p_{trend}=0·0164; appendix pp 21–26).

The analysis of individual participant data from surveys focused on areas with a more than 80% prevalence of the *dhps* Lys540Glu mutation, with the aim of ascertaining the effect of the sextuple-mutant *P falciparum* parasite in areas previously defined as super resistant (>10% *dhps* Ala581Gly prevalence).[16] Of 138 publicly available surveys, 39 met the inclusion criteria, and 13 surveys that included data from areas with a *dhps* Lys540Glu prevalence of more than 80% or with super resistance (all in east and southern Africa from 2008–15, and comprising 42 394 singleton livebirths) were included in the analysis after exact matching of probability of receiving IPTp, resistance, and malaria transmission intensity data (figure 1). Sulfadoxine-pyrimethamine IPTp in these areas was associated with an RRR of 11% (95% CI 8 to 13) for low birthweight. Even in areas with a *dhps* Lys540Glu prevalence of more than 90% and a *dhps* Ala581Gly prevalence of up to 10%, sulfadoxine-pyrimethamine IPTp was associated with significantly reduced risk of low birthweight (RRR 10% [7 to 12]; figure 4). However, in the two super-resistant areas, sulfadoxine-pyrimethamine IPTp did not protect against low birthweight (RRR 0·5% [–16 to 14]; figure 4). In these two areas, the pooled prevalence of the *dhps* Ala581Gly mutation across all contemporary molecular studies was 37% (29 to 46; appendix p 34).

Discussion

In our meta-analysis of aggregated data from 57 clinical studies, increases in the prevalence of two molecular markers of sulfadoxine resistance were associated with clear reductions in the effectiveness of sulfadoxine-pyrimethamine IPTp to avert low birthweight and other outcomes such as malaria infection at delivery and maternal anaemia. In our parallel analysis of individual participant data from nationally representative surveys, sulfadoxine-pyrimethamine IPTp was associated with a significant but modest protective effect against low birthweight in areas where the *P falciparum dhps* Lys540Glu mutation prevalence was 90% or higher and the prevalence of sextuple-mutant parasites was less than 10%.[16] However, these surveys also showed that, in areas where sextuple-mutant parasites are common (pooled prevalence estimate 37%), sulfadoxine-pyrimethamine IPTp did not protect against low birthweight. These findings are consistent with our understanding of the incremental increase in resistance to sulfadoxine-pyrimethamine with successive mutations in the *dhfr* and *dhps* genes, and with the previous studies

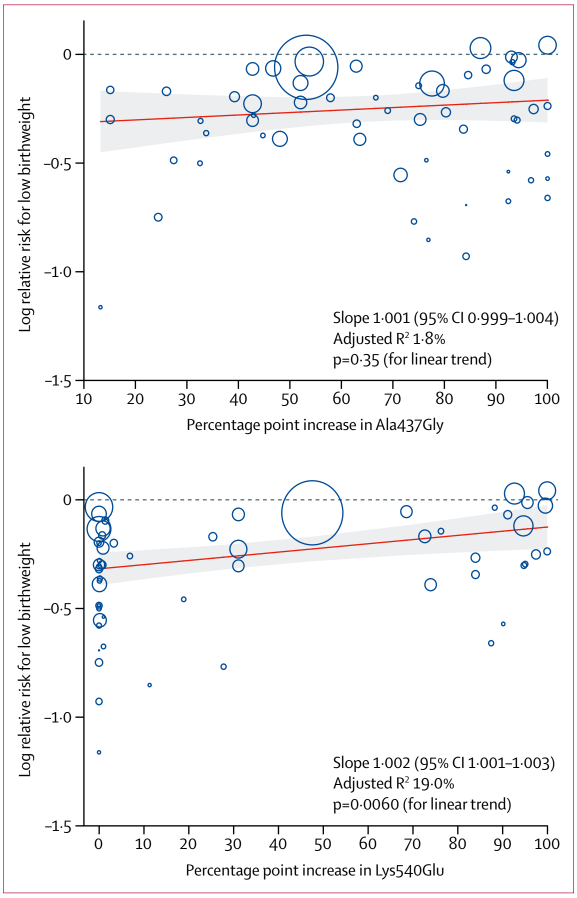
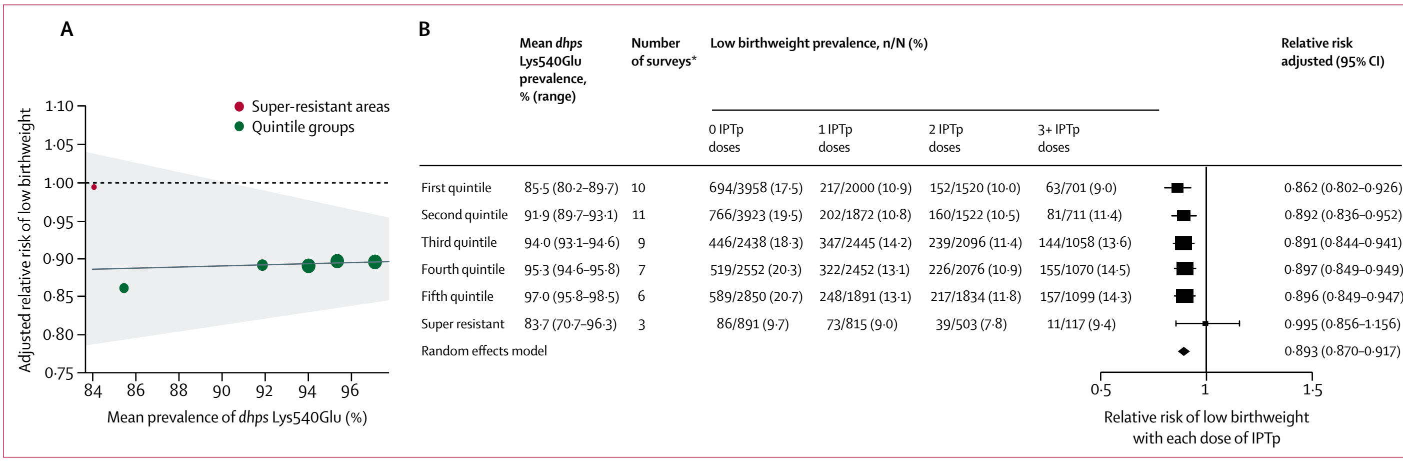


***Figure 3:* Correlation between relative risk for low birthweight in women receiving IPTp and the prevalence of *dhps* Ala437Gly or *dhps* Lys540Glu mutations in 57 studies**
Meta-regression bubble plots show the log of the relative risk estimates for low birthweight across each sulfadoxine-pyrimethamine dose category, obtained by use of generalised least-squares regression for trend estimation of summarised dose-response data, with 95% CI of the regression line represented by the shaded area. The size of the bubbles for individual studies is proportional to the random effects study weights. A positive slope indicates decreasing effectiveness of sulfadoxine-pyrimethamine IPTp for averting low birthweight with increasing mutation prevalence. *dhps*=dihydropteroate synthase. IPTp=intermittent preventive treatment in pregnancy.



	Mean *dhps* Lys540Glu prevalence, % (range)	Number of surveys*	Low birthweight prevalence, n/N (%)				Relative risk adjusted (95% CI)
			0 IPTp doses	1 IPTp doses	2 IPTp doses	3+ IPTp doses	
First quintile	85·5 (80·2–89·7)	10	694/3958 (17·5)	217/2000 (10·9)	152/1520 (10·0)	63/701 (9·0)	0·862 (0·802–0·926)
Second quintile	91·9 (89·7–93·1)	11	766/3923 (19·5)	202/1872 (10·8)	160/1522 (10·5)	81/711 (11·4)	0·892 (0·836–0·952)
Third quintile	94·0 (93·1–94·6)	9	446/2438 (18·3)	347/2445 (14·2)	239/2096 (11·4)	144/1058 (13·6)	0·891 (0·844–0·941)
Fourth quintile	95·3 (94·6–95·8)	7	519/2552 (20·3)	322/2452 (13·1)	226/2076 (10·9)	155/1070 (14·5)	0·897 (0·849–0·949)
Fifth quintile	97·0 (95·8–98·5)	6	589/2850 (20·7)	248/1891 (13·1)	217/1834 (11·8)	157/1099 (14·3)	0·896 (0·849–0·947)
Super resistant	83·7 (70·7–96·3)	3	86/891 (9·7)	73/815 (9·0)	39/503 (7·8)	11/117 (9·4)	0·995 (0·856–1·156)
Random effects model							0·893 (0·870–0·917)

***Figure 4:* Relative risk for low birthweight associated with number of sulfadoxine-pyrimethamine IPTp doses by resistance strata in areas with super resistance or *dhps* Lys540Glu of more than 80% in east and southern Africa**
(A) Linear meta-regression bubble plot with solid line representing the regression line and shaded area representing the 95% CI. (B) Forest plot. Individual observations in all areas with a *dhps* Lys540Glu prevalence of more than 80% were divided into quintiles after excluding the surveys in the super-resistant areas. Super-resistant areas were defined as those with a *dhps* Ala581Gly prevalence of more than 10% (southwestern Uganda, northern Tanzania, and eastern Democratic Republic of the Congo).[16] Overall, sulfadoxine-pyrimethamine IPTp was associated with an RRR of 11% (95% CI 8–13) for low birthweight. The RRR was 10% (7–12) in areas with a *dhps* Lys540Glu prevalence of more than 90% and Ala581Gly prevalence of less than 10%. *dhps*=dihydropteroate synthase. IPTp=intermittent preventive treatment in pregnancy. RRR=relative risk reduction. *Number of surveys (total 13) that contributed to each quintile group; surveys could contribute to more than one group.

that showed compromised efficacy of sulfadoxine-pyrimethamine in women infected with sextuple-mutant *P falciparum*.[12–15] This high resistance is currently restricted to a few foci in east Africa,[16] but its spread would have important implications for the continued use of sulfadoxine-pyrimethamine for IPTp.

Compared with other markers of sulfadoxine-pyrimethamine resistance, fewer data are available on the distribution of the *dhps* Ala581Gly mutation. Therefore, the aggregated-data meta-analysis was limited in its ability to define and validate different thresholds for the *dhps* Ala581Gly mutation. There were only five studies done in areas in east and southern Africa with a *dhps* Ala581Gly prevalence of more than 10%, and none were done in areas with a *dhps* Ala581Gly prevalence between 13% and 43%. Within these studies, there was also substantial between-study heterogeneity in the effect of treatment on low birthweight: the three smaller studies, with only four to eight low birthweight events in the reference groups,[12,15,31] showed a pooled effect size of 35% (95% CI 14 to 51), whereas the studies with larger reference groups reported an effect size of –2% (–15 to 9; appendix p 33).[32,33] The results of these larger two studies, which were done in areas with a *dhps* Ala581Gly prevalence of more than 45%, are consistent with the lack of effect on low birthweight in our analysis of survey data, which was based on much larger sample sizes and areas with an average *dhps* Ala581Gly prevalence of 37%.

Irrespective of sulfadoxine-pyrimethamine resistance, we observed large between-study heterogeneity in the treatment effect on low birthweight among the 57 clinical studies. This can be explained, in part, by the multicausal nature of low birthweight and the varying population-attributable fractions of malaria towards low birthweight, which depend on transmission intensity and uptake of interventions such as insecticide-treated nets. In the current study, insecticide-treated net use was not an effect modifier or confounder, but malaria transmission intensity was correlated with resistance (lower transmission levels were associated with higher resistance levels) and was thus a potential confounder, which is why it was important to adjust for malaria transmission in our models. Nevertheless, estimates of the effect of sulfadoxine-pyrimethamine resistance on the effectiveness of sulfadoxine-pyrimethamine IPTp for averting low birthweight (ie, the slope of the meta-regression lines) were largely unaffected by the inclusion of four covariates—malaria transmission, study quality, mean number of sulfadoxine-pyrimethamine doses, and proportion of paucigravidae—in the models, suggesting minimal confounding by these variables overall.

Although the effectiveness of IPTp for low birthweight decreased with increasing resistance, sulfadoxine-pyrimethamine IPTp remained associated with a 7–10% reduced risk of low birthweight even in areas where the resistant quintuple-mutant haplotype is fixed. This small but resilient effect on low birthweight contrasts with the lack of effect (RRR 3%) on malaria infection in high-resistance areas seen in the aggregated-data meta-analysis (appendix p 19), and with the previously observed unfavourable parasitological response in asymptomatic pregnant women receiving sulfadoxine-pyrimethamine IPTp in these areas, where clearance of parasites by day 42 was achieved in only 50% of paucigravidae.[7] That IPTp can decrease risk of low birthweight even in areas where its efficacy for clearance of infection is compromised might suggest that suppression, rather than radical clearance of parasites, is required to mitigate the adverse effects of malaria on placental function and growth, as observed in multigravidae (who acquire protective antimalarial immunity over successive pregnancies). Alternatively, sulfadoxine-pyrimethamine might have beneficial effects on birthweight that are independent of its antimalarial properties and are, therefore, unaffected by parasite resistance (eg, antimicrobial effects,[32–34] or effects related to immunomodulation, similar to those described for co-trimoxazole[35]).

The differences in *P falciparum* parasite populations (shown in the scatter plot of the prevalence of *dhps* Ala437Gly and Lys540Glu mutations in the appendix p 31) reflect the distinct geographical origins of two or three parasite populations in east and west Africa.[25] In east and southern Africa, the combination of the resistance alleles at *dhps* codons 540 and 581 could be considered to track sulfadoxine-pyrimethamine resistance. In central and west Africa, where the *dhps* Lys540Glu mutation is absent or rare, tracking *dhps* Ala437Gly might be informative. However, other mutations have started to emerge in west Africa, such as *dhps* Ile431Val, which has been reported on a haplotype bearing mutant alleles at codons 581 and 613 but a wild-type allele at codon 540.[36,37] The clinical implications of such new haplotypes require further study.

Our analyses have important limitations. First, the potential biases associated with observational data, in which the number of sulfadoxine-pyrimethamine doses is not determined by the study, have been discussed in detail elsewhere.[4] Although the use of exact matching and multivariate models will have reduced the potential for bias in the surveys, residual confounding cannot be excluded. Second, national surveys are subject to measurement error and information bias from respondent recall and self-report.[4] Similar limitations apply to the aggregated-data analysis, which could only adjust for study-level covariates. For some studies, time-matched local resistance data were not available and were obtained from other sources, which are less precise. Some studies were considered to be of poor quality, with a trend towards greater effectiveness with decreasing study quality, but sensitivity analysis showed that these low-quality studies were equally distributed across the resistance spectrum and did not affect the conclusions. Similarly, there was evidence of a small-study effect, but this effect was also observed in all three resistance strata, and restricting the analysis to the largest 50% of studies (which are least likely to be affected by publication bias) did not alter the conclusions. In addition, the meta-analysis suffered from design and reporting variation and small numbers in the extreme dose groups (zero doses and three or more doses). This limitation was partly mitigated by use of a dose-response analysis that placed less emphasis on the extreme dose groups.

This is the most comprehensive study of the effect of sulfadoxine-pyrimethamine resistance on the effectiveness of sulfadoxine-pyrimethamine IPTp, involving 57 clinical studies, 13 nationally representative surveys, and more than 100 000 births. The data show that, despite the substantial heterogeneity between studies with regard to the effectiveness of sulfadoxine-pyrimethamine IPTp on low birthweight, increasing prevalence of molecular markers of sulfadoxine resistance is correlated with a decrease in effectiveness of sulfadoxine-pyrimethamine to prevent low birthweight and malaria infections. These findings suggest that molecular monitoring of sulfadoxine-pyrimethamine resistance is a potential policy tool to guide the use of sulfadoxine-pyrimethamine IPTp. It is reassuring that a protective association of sulfadoxine-pyrimethamine IPTp with low birthweight can be detected even in high-resistance areas where quintuple-mutant *P falciparum* parasites are almost fixed. However, sulfadoxine-pyrimethamine IPTp is not likely to reduce malaria and malaria-associated low birthweight in areas where the prevalence of sextuple-mutant parasites, with the *dhps* Ala581Gly mutation, exceed 37% (the pooled estimate in the high-resistance areas). For these areas, the search for alternative strategies or drugs to replace sulfadoxine-pyrimethamine IPTp is a pressing research priority for the control of malaria in pregnancy.

Contributors

FOtK conceived the study. AMvE, DAL, and FOtK wrote the protocol. AMvE, DAL, GK, and DECS did the literature search, acquired the aggregated data, screened records, and extracted data. AMvE, GK, and DECS assessed the quality of included studies. CK and FOtK acquired and combined the individual participant data from different observational studies. AMvE, DAL, and FOtK did the statistical analysis. KK, MD, JG, SJR, SRM, SMT, CR, and LCO provided individual level participant clinical or molecular data. CR, LCO, and CHS set up and maintained the interactive maps of the distribution of molecular resistance markers used in the analysis. AMvE, DAL, and FOtK wrote the first draft of this manuscript. All authors provided critical conceptual input, interpreted the data analysis, and critically revised and approved the final version of the manuscript.

Declaration of interests

SJR received grants from the Wellcome Trust during the conduct of the study. LCO received grants from the UK Royal Society, the Bill & Melinda Gates Foundation, and the Medicines for Malaria Venture during the conduct of the study, as well as grants from WHO outside the submitted work. All other authors declare no competing interests.

Acknowledgments

This study was part of the Malaria in Pregnancy Scientific Group of The Worldwide Antimalarial Resistance Network (WWARN), and was co-funded by WWARN (which is funded by the Bill & Melinda Gates Foundation; grant numbers OPP1181807 [started October, 2017] and OPP1099191 [ended September, 2017]), and by the US Centers for Disease Control and Prevention (CDC) through a cooperative agreement between the Division of Parasitic Diseases and Malaria of the CDC Center for Global Health and the Malaria Epidemiology Unit of the Liverpool School of Tropical Medicine (award number 46099 [to FOtK]). Part of the earlier field work and molecular assays included in these analyses were supported by the European and Developing Countries Clinical Trials Partnership (grant number IP.2007.31080.003) and the Malaria in Pregnancy Consortium (funded through a grant from the Bill & Melinda Gates Foundation to the Liverpool School of Tropical Medicine; grant number 46099). The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the CDC. We thank the following people for providing us with additional information for this review:

Ikeoluwapo Ajayi, Dami Olorunda, Vera Braun, Frank Mockenhaupt, Harry Tagbor, Matthew Cairns, Brian Greenwood, Dominic Mosha, Mats Wahlgren, Fatuma Namusoke, Linda Kalilani-Phiri, Miriam Laufer, Mwayi Madanitsa, Kelias Msyamboza, Bernard Brabin, Daniel Minja, Gaoqian Feng, Sodiomon Sirima, Abraham Oduro, Peter Ouma, Scott Filler, Whitney Harrington, Patrick Duffy, Michal Fried, Aminata Famanta, Marielle Bouyou-Akotet, Kimberly Mace, Emmanuel Arinaitwe, Sheick Oumar Coulibaly, and Michael Alifrangis.

References

1 Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. *PLoS Med* 2010; **7:** e1000221.

2 Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC. Estimated risk of placental infection and low birthweight attributable to *Plasmodium falciparum* malaria in Africa in 2010: a modelling study. *Lancet Glob Health* 2014; **2:** e460–67.

3 Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007; **7:** 93–104.

4 Eisele TP, Larsen DA, Anglewicz PA, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. *Lancet Infect Dis* 2012; **12:** 942–49.

5 Gosling RD, Gesase S, Mosha JF, et al. Protective efficacy and safety of three antimalarial regimens for intermittent preventive treatment for malaria in infants: a randomised, double-blind, placebo-controlled trial. *Lancet* 2009; **374:** 1521–32.

6 Nankabirwa J, Cundill B, Clarke S, et al. Efficacy, safety, and tolerability of three regimens for prevention of malaria: a randomized, placebo-controlled trial in Ugandan schoolchildren. *PLoS One* 2010; **5:** e13438.

7 Desai M, Gutman J, Taylor SM, et al. Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth weight. *Clin Infect Dis* 2016; **62:** 323–33.

8 Kalilani L, Mofolo I, Chaponda M, et al. A randomized controlled pilot trial of azithromycin or artesunate added to sulfadoxine-pyrimethamine as treatment for malaria in pregnant women. *PLoS One* 2007; **2:** e1166.

9 Triglia T, Wang P, Sims PF, Hyde JE, Cowman AF. Allelic exchange at the endogenous genomic locus in *Plasmodium falciparum* proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. *EMBO J* 1998; **17:** 3807–15.

10 Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. *Pharmacol Rev* 2005; **57:** 117–45.

11 Picot S, Olliaro P, de Monbrison F, Bienvenu AL, Price RN, Ringwald P. A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in falciparum malaria. *Malar J* 2009; **8:** 89.

12 Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. *Clin Infect Dis* 2011; **53:** 224–30.

13 Harrington WE, Mutabingwa TK, Muehlenbachs A, et al. Competitive facilitation of drug-resistant *Plasmodium falciparum* malaria parasites in pregnant women who receive preventive treatment. *Proc Natl Acad Sci USA* 2009; **106:** 9027–32.

14 Gutman J, Kalilani L, Taylor S, et al. The A581G mutation in the gene encoding *Plasmodium falciparum* dihydropteroate synthetase reduces the effectiveness of sulfadoxine-pyrimethamine preventive therapy in Malawian pregnant women. *J Infect Dis* 2015; **211:** 1997–2005.

15 Minja DT, Schmiegelow C, Mmbando B, et al. *Plasmodium falciparum* mutant haplotype infection during pregnancy associated with reduced birthweight, Tanzania. *Emerg Infect Dis* 2013; **19:** 1446–54.

16 Naidoo I, Roper C. Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria. *Trends Parasitol* 2013; **29:** 505–15.

17 Muanda FT, Chaabane S, Boukhris T, et al. Antimalarial drugs for preventing malaria during pregnancy and the risk of low birth weight: a systematic review and meta-analysis of randomized and quasi-randomized trials. *BMC Med* 2015; **13:** 193.

18 Chico RM, Cano J, Ariti C, et al. Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis. *Trop Med Int Health* 2015; **20:** 1621–33.

19 van Eijk AM, Hill J, Povall S, Reynolds A, Wong H, ter Kuile FO. The Malaria in Pregnancy Library: a bibliometric review. *Malar J* 2012; **11:** 362.

20 World Wide Antimalarial Resistance Network (WWARN). Molecular surveyor. 2018. http://www.wwarn.org/dhfr-dhps-surveyor/#0 (accessed Sept 18, 2018).

21 London School of Hygiene & Tropical Medicine. Drug resistance maps: mapping the distribution of resistance genes of malaria in Africa. 2010. http://www.drugresistancemaps.org/ (accessed Sept 18, 2018).

22 Flegg JA, Patil AP, Venkatesan M, et al. Spatiotemporal mathematical modelling of mutations of the *dhps* gene in African *Plasmodium falciparum*. *Malar J* 2013; **12:** 249.

23 Wells G, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2014. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed Sept 18, 2018).

24 Okell LC, Griffin JT, Roper C. Mapping sulphadoxine-pyrimethamine-resistant *Plasmodium falciparum* malaria in infected humans and in parasite populations in Africa. *Sci Rep* 2017; **7:** 7389.

25 Pearce RJ, Pota H, Evehe MS, et al. Multiple origins and regional dispersal of resistant dhps in African *Plasmodium falciparum* malaria. *PLoS Med* 2009; **6:** e1000055.

26 Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 1992; **135:** 1301–09.

27 Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol* 2012; **175:** 66–73.

28 Higgins J, Green S. The Cochrane handbook for systematic reviews of interventions v5.1.0, 4th edn. Chichester, UK: John Wiley & Sons, 2011.

29 Borenstein M, Hedges LS, Higgens JPT, Rothstein HR. Chapter 30: Publication bias. In: Borenstein M, Hedges LS, Higgens JPT, Rothstein HR, eds. Introduction to meta-analysis. Chichester, UK: John Wiley & Sons, 2009.

30 Bates D, Maechler M, Bolker B. lme4: linear mixed-effects models using S4 classes. 2018. https://cran.r-project.org/web/packages/lme4/lme4.pdf (accessed Sept 18, 2018).

31 Braun V, Rempis E, Schnack A, et al. Lack of effect of intermittent preventive treatment for malaria in pregnancy and intense drug resistance in western Uganda. *Malar J* 2015; **14:** 372.

32 Desai M, Hill J, Fernandes S, et al. Prevention of malaria in pregnancy. *Lancet Infect Dis* 2018; **18:** e119–32.

33 Chico RM, Chaponda EB, Ariti C, Chandramohan D. sulfadoxine-pyrimethamine exhibits dose-response protection against adverse birth outcomes related to malaria and sexually transmitted and reproductive tract infections. *Clin Infect Dis* 2017; **64:** 1043–51.

34 Capan M, Mombo-Ngoma G, Makristathis A, Ramharter M. Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin. *Malar J* 2010; **9:** 303.

35 Church JA, Fitzgerald F, Walker AS, Gibb DM, Prendergast AJ. The expanding role of co-trimoxazole in developing countries. *Lancet Infect Dis* 2015; **15:** 327–39.

36 Oguike MC, Falade CO, Shu E, et al. Molecular determinants of sulfadoxine-pyrimethamine resistance in *Plasmodium falciparum* in Nigeria and the regional emergence of dhps 431V. *Int J Parasitol Drugs Drug Resist* 2016; **6:** 220–29.

37 Chauvin P, Menard S, Iriart X, et al. Prevalence of *Plasmodium falciparum* parasites resistant to sulfadoxine/pyrimethamine in pregnant women in Yaounde, Cameroon: emergence of highly resistant pfdhfr/pfdhps alleles. *J Antimicrob Chemother* 2015; **70:** 2566–71.