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RESEARCH ARTICLE

CROSS-RESISTANCE BETWEEN SULFADOXINE-PYRIMETHAMINE AND OTHER ANTIMALARIAL DRUGS: A STUDY OF PREGNANT WOMEN IN ILORIN KWARA STATE NIGERIA

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Abstract

Antimalarial resistance remains a serious problem in malaria-endemic regions, particularly in pregnant women, who are likely to suffer from complications. The research evaluates the resistance to commonly employed antimalarial medications—Chloroquine, Artesunate, and Arthether—among pregnant women in Ilorin, Nigeria, at different trimesters and during delivery. Cross-sectional study was conducted among 253 pregnant women presenting at antenatal clinics within Ilorin, Kwara State. Venous blood from study participants in different pregnancy stages (first trimester, second trimester, third trimester, and upon delivery) were drawn. Schizont maturation inhibition method was used in the drug susceptibility test to establish resistance to Chloroquine, Artesunate, and Arthether. Chloroquine resistance prevalence was moderately low at varying levels of between 7.9% upon delivery and 12.2% in the second trimester. Resistance against Artesunate, however, rose progressively as pregnancy advanced up to 60.0% in the third trimester, suggesting loss of drug effectiveness toward the end of pregnancy. Arthether resistance was variable, with the highest prevalence in the first (43.0%) and second (33.8%) trimesters, dropping to 0% during the third trimester, and then rising again to 38.1% at delivery. Cross-resistance evaluation showed a high positive correlation between SP resistance and Artesunate resistance during the first (OR = 2.53, p = 0.007) and second trimesters (OR = 2.19, p = 0.043), which suggests shared resistance mechanisms. In contrast, SP resistance was reversely associated with Chloroquine resistance at the second trimester (OR = 0.43, p = 0.021) and at delivery (OR = 0.27, p = 0.042), demonstrating that individuals resistant to SP were less resistant to Chloroquine. The findings prove evidence of enhanced resistance to Artesunate, particularly as gestation age increases, making its usage among pregnant women challenging. Cross-resistance between SP and Artesunate increases the need for continued caution and possible alteration of treatment of malaria in pregnant women. Other treatment regimens to manage malaria in pregnancy effectively should be the focus of future research.

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Introduction:-

Malaria remains a significant global health problem, particularly in sub-Saharan Africa, where pregnant women and children under the age of five are most vulnerable to severe illness (Kogan&Kogan, 2020; WHO, 2024). Pregnancy malaria can lead to adverse outcomes such as maternal anemia, preterm delivery, low birth weight, and increased neonatal mortality (Desai et al., 2018). To counter such threats, the World Health Organization (WHO) promotes intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) in regions of malaria transmission (WHO, 2024). However, growing resistance of *Plasmodium falciparum* to SP reduces its effectiveness, bringing into question the long-term efficacy of current malaria control strategies (Sagovia et al., 2025).

Resistance to antimalarial drugs arises when *P. falciparum* becomes genetically mutated, allowing it to be resistant to drug treatment. Cross-resistance, which is likely the most pressing issue, arises when resistance to a single antimalarial drug reduces sensitivity to others due to the fact that drugs have molecular mechanisms in common (Schäfer et al., 2024). This resistance complicates malaria treatment and may hinder the efficacy of existing therapies. Several studies have cited cross-resistance between SP and other widely used antimalarial medicines, including chloroquine, artesunate, and artemether-lumefantrine (Kumar &Mahato, 2024). These patterns of resistance are critical challenges to malaria control and may necessitate changes in treatment protocols.

It is worth knowing the level of cross-resistance between SP and the other antimalarial drugs to optimize malaria intervention measures (Kumar &Mahato, 2024). The study of such resistance patterns can be significant in the interpretation of IPTp-SP effectiveness and guiding policy in the future to optimize malaria control, particularly among vulnerable populations such as pregnant women.

This study investigates the prevalence of cross-resistance among SP and other commonly used antimalarial drugs among pregnant women in Ilorin, Kwara State, Nigeria.

Material and Methods:-

Study Area

This study was conducted in Ilorin, Kwara State's capital, North Central Nigeria. Ilorin is a cultural and economic hub connecting the north and the south of Nigeria and is inhabited by more than 900,000 people as of 2022 estimates. Ilorin is ethnically diverse, comprising Yoruba, Hausa, Fulani, etc., with a religious mix of Islam and Christianity.

The weather in Ilorin is tropical savanna with clear wet and dry seasons. There is heavy rain with high humidity in April to October, and dry season rain with harmattan wind and little rain in November to March. Temperature ranges from 22°C to 34°C on a yearly basis, hottest in February and March and coldest in December and January.

There is a mix of private and public health facilities in Ilorin. There are 40 primary healthcare facilities (PHCs) distributed in Ilorin West, Ilorin East, and Ilorin South local government councils. Eight government hospitals, including General Hospital Ilorin and Civil Service Hospital, which provide secondary healthcare. The University of Ilorin Teaching Hospital and Kwara State University Teaching Hospital offer specialized medical care. Private healthcare facilities totaling around 45 also supplement public health, and thus enhance accessibility to a world of healthcare options.

Study Design

This was a cross-sectional study among pregnant women in Ilorin, Kwara State, Nigeria, to ascertain cross-resistance between sulfadoxine-pyrimethamine (SP) and other antimalarial agents. Informed consent was initially obtained before dispensing SP as directly observed therapy (DOT) according to WHO guidelines. The survey questionnaire was prepared and validated. A pilot test on 50 pregnant women ensured readability and consistency. The feedback from the pilot test was considered to remove all the ambiguities, and ultimately a Cronbach's alpha of 0.85, confirming internal consistency.

A standard protocol manual was given to all the participating health facilities, outlining how SP administration, blood sample collection, and malaria testing were to be performed. The health workers were trained and routine supervision was conducted to maintain compliance with the study protocol. The SP resistance testing protocol was harmonized with the latest WHO guidelines and recent literature, to enhance its accuracy and validity.

In order to capture seasonal transmission of malaria, data collection was stratified by season. The healthcare providers and pregnant women were also interviewed to describe local SP use patterns and situate findings in Ilorin's epidemiological context. This was considered for a different publication.

Sampling
Four clinics were randomly chosen for study: one Primary Healthcare Center (PHC), one Secondary Healthcare Facility, one Tertiary Healthcare Facility, and one Private Healthcare Facility. The total number of pregnant women screened for eligibility was 763. Following the screening procedure, 510 women were removed based on predefined exclusion criteria of pre-existing disease, refusal, and failure to meet study entry criteria. Removal resulted in the final sample being 253 participants eligible for this study, distributed by their gestation stage into the following:
First Trimester: 86 women
Second Trimester: 74 women
Third Trimester: 30 women
At Delivery Time: 63 women.

Blood Sample Collection
The blood was collected in 10-mL EDTA Vacutainers for malaria. Thick blood smears were prepared, Giemsa stained, and examined for counting the malaria parasite and parasitemia determination. Blood samples were transported in cool packs and stored at -20°C until further analysis. Data collection used a pretested survey questionnaire adapted from the Nigeria Malaria Indicator Survey (NMIS) pretested for reliability.

Malaria Screening
Malaria screening was done using a Rapid Diagnostic Test (RDT). A sterile lancet was used to prick the fingertip to obtain a small drop of blood, which was then deposited in the sample well provided on the RDT cassette. A few drops of buffer solution were added as needed to enable the movement of blood and reagents along the test strip. Test was incubated for 15-20 minutes and read based on the visibility of test line and control line. Both lines, one in the zone of the control line (C) and another in the zone of the test line (T), were suggestive of a positive result.

Determination of antimalarial Resistance
Susceptibility of malaria parasites against SP, chloroquine, artesunate, and artemether-lumefantrine was tested using Koehne's (2022) schizont maturation inhibition assay. Dilutions of the blood samples using RPMI 1640 medium were prepared for parasitemia levels of 0.5-1% and hematocrit levels of 1.5-2%. The parasites were synchronized at ring stage by incubating them in 12-24 hours at 37°C under CO_2 conditions. Serial dilutions of sulfadoxine and pyrimethamine (0.01 µg/mL to 10 µg/mL) were performed to determine the IC_{50}, or the concentration that will inhibit parasite growth.

A 96-well microtiter plate was used, to which 100 µL of parasite culture and 100 µL of drug solutions of varying concentrations were added. Plates were incubated at 37°C with 5% CO_2 for 72 hours. Growth of parasites was evaluated by Giemsa-stained thick blood smears. Drug efficacy was assessed by comparing parasite growth in the treated wells and control wells.

Statistical Analyses
SPSS version 25 for windows was used to process data. Relevant statistical tests like the chi-square test were employed for comparing percentages and p-value of less than 0.05 was utilized as statistically significant. In addition, multivariate logistic regression analysis was conducted to examine associations between dependent and independent variables.

Ethical Approval
Permission to carry out the study was obtained from Kwara State Ministry of Health Ethical clearance.

Results:-

Prevalence of SP Resistance
Figure 1 present the prevalence of SP across the trimesters. From the figure, the prevalence of SP resistance is highest at delivery (38.7%) and in the first trimester (37.6%), while the lowest resistance is observed in the second trimester (28.9%). Resistance slightly increases again in the third trimester (31.9%).

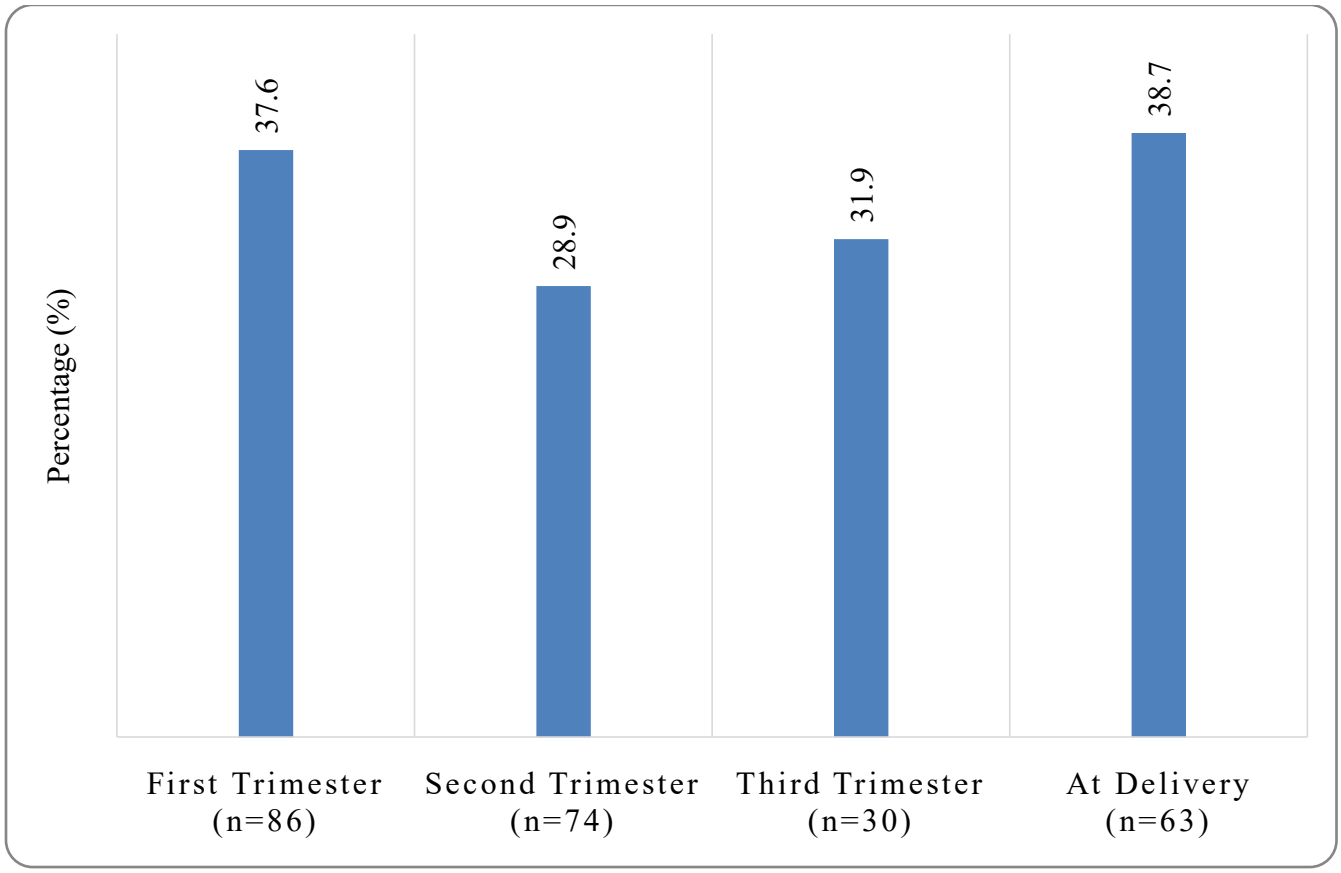

Figure 1:- Prevalence of SP Resistance among pregnant women in Ilorin Kwara State

Prevalence of other Antimalaria Resistance

Table 1 present the Chloroquine, Artesunate, and Arthether resistance at different levels of pregnancy. The evidence reveals that Chloroquine resistance is still relatively low, ranging from 7.9% when delivered to 12.2% when it is in the second trimester. Resistance to Artesunate, however, rises significantly with advancing pregnancy. The highest rate of resistance (60.0%) is in the third trimester, indicating that the commonly used antimalarial is perhaps losing efficacy in pregnant women, particularly in later stages of pregnancy. Arthether resistance is diverse, with relatively greater resistance in the first trimester (43.0%) and second trimester (33.8%), but again falling to 0% in the third trimester before increasing again at the time of delivery (38.1%). The absence of resistance in the third trimester could be interpreted as differences in drug administration, metabolism, or parasite sensitivity at different stages of pregnancy

Table 1:- Prevalence of other Antimalaria Resistance among pregnant women in Ilorin Kwara State.

Trimester/delivery	Chloroquine Resistance	Artesunate Resistance	Arthether Resistance
First Trimester (n=86)	9 (10.5%)	40 (46.5%)	37 (43.0%)
Second Trimester (n=74)	9 (12.2%)	40 (54.1%)	25 (33.8%)
Third Trimester (n=30)	3 (10.0%)	18 (60.0%)	0 (0.0%)
At Delivery (n=63)	5 (7.9%)	34 (54.0%)	24 (38.1%)

Cross-Resistance between SP and Other Antimalarial Drugs

During the first trimester as presented in table 2, resistance to SP was highly correlated with resistance to Artesunate (OR = 2.53, p = 0.007), which indicated an extremely strong cross-resistance. Conversely, no statistical correlations were found for Chloroquine resistance (OR = 0.53, p = 0.067) or Arthether resistance (OR = 1.37, p = 0.211).

In the second trimester, there was a highly significant negative correlation between SP resistance and Chloroquine resistance (OR = 0.43, p = 0.021), such that women who were resistant to SP were less resistant to Chloroquine. SP

resistance remained significantly associated with Artesunate resistance (OR = 2.19, p = 0.043), but Arthether resistance had a borderline trend (OR = 2.2, p = 0.071) and did not achieve significance (Table 3).

There was no SP-Chloroquine resistance in the third trimester. Resistance to Artesunate was inversely correlated (borderline significant) with resistance (OR = 0.38, p = 0.055), and hence it is safe to assume that resistance to SP lowers the odds of resistance to Artesunate in subsequent pregnancy. Arthether resistance does not have data reported (table 4).

SP-resistant women were significantly less likely to be Chloroquine-resistant at delivery (OR = 0.27, p = 0.042), providing further evidence that it can be used as a cure. Resistance to SP and Artesunate was not strongly correlated (OR = 0.68, p = 0.061), and resistance to Arthether (OR = 1.7, p = 0.131) was not strongly correlated (Table 5).

Table 2:- Cross-Resistance between SP and Other Antimalarial Drugsamong pregnant women in Ilorin Kwara State in First Trimester (n=86).

Other Antimalaria	Odds Ratio	95% CI	p-value
Chloroquine Resistance			
No	Reference		
Yes	0.53	(0.10-2.75)	0.067
Artesunate Resistance			
No	Reference		
Yes	2.53	(1.29-4.96)	0.007*
Arthether Resistance			
No	Reference		
Yes	1.37	(0.84-2.23)	0.211

CI: confident interval, *Chi-square test is significant at p<0.05

Table 3:- Cross-Resistance Between SP and Other Antimalarial Drugsamong pregnant women in Ilorin Kwara State in Second Trimester (n=74).

Other Antimalaria	Odds Ratio	95% CI	p-value
Chloroquine Resistance			
No	Reference		
Yes	0.43	(0.21-0.87)	0.021*
Artesunate Resistance			
No	Reference		
Yes	2.19	(1.03-4.68)	0.043*
Arthether Resistance			
No	Reference		
Yes	2.2	(0.94-5.17)	0.071

CI: confident interval, *Chi-square test is significant at p<0.05

Table 4:- Cross-Resistance Between SP and Other Antimalarial Drugs among pregnant women in Ilorin KwaraStateinThird Trimester (n=30).

Other Antimalaria	Odds Ratio	95% CI	p-value
Chloroquine Resistance			
No	Reference		
Yes	0	-	
Artesunate Resistance			
No	Reference		
Yes	0.38	(0.14-1.03)	0.055
Arthether Resistance			
No	Reference		
Yes	-	-	

CI: confident interval, *Chi-square test is significant at p<0.05

Table 5:- Cross-Resistance Between SP and Other Antimalarial Drugs among pregnant women in Ilorin KwaraStateAt Delivery (n=63).

Other Antimalaria	Odds Ratio	95% CI	p-value
Chloroquine Resistance			
No	Reference		
Yes	0.27	(0.08-0.95)	0.042*
Artesunate Resistance			
No	Reference		
Yes	0.68	(0.46-1.02)	0.061
Arthether Resistance			
No	Reference		
Yes	1.7	(0.86-3.37)	0.131

CI: confident interval, *Chi-square test is significant at $p<0.05$

Discussion:-

According to the present findings, resistance to SP is different across trimesters of pregnancy, being highest at delivery (38.7%) and in the first trimester (37.6%), and lowest in the second trimester (28.9%). It increases in the third trimester (31.9%). The pattern can be understood in the context of Rwandan and Kenyan results where the resistance markers are very high, pointing toward compromised efficacy of SP. In Rwanda, Alruwaili et al. (2023) reported that quintuple and sextuple mutant genotypes prevalence was 75% and 28%, respectively, without variations by study sites. In a similar context, in Kenya, Gikunju et al. (2019) reported that quintuple mutation for overall SP resistance was detected in 86% of the cases. The higher resistance in Kenya is suggestive of a more established stage of SP resistance, as would be expected with rising resistance at delivery and in the first trimester in the figure. High resistance at delivery could be due to the cumulative effect of parasite exposure and selective pressure during the pregnancy.

The very high resistance in the first trimester (37.6%) can be explained by either past exposure to SP before pregnancy confirmation or pre-existing resistant strains in the maternal blood. This concurs with the Rwandan study, in which 92% concordance in resistance markers between peripheral and placental blood samples indicated the presence of resistant parasites early in pregnancy.

The current research reports Chloroquine resistance of 7.9% (delivery) to 12.2% (second trimester), which contrasts with Aliyu et al. (2017), who reported a much higher 94.9% resistance in pregnant women in Kaduna, Nigeria. The broad difference reflects that Chloroquine resistance is still a prevalent phenomenon, albeit some variation may arise due to geographical location, sample groups, or testing methodologies. Ballard et al. (2018) noted that Chloroquine-resistant P. falciparum limits pregnancy treatment, something which is in accordance with evidence provided in the present study that there is resistance but to a lesser degree. Regardless of this, the WHO and CDC do not recommend using Chloroquine as a way to treat malaria in most endemic regions due to its high rate of failure.

Concern of the trend during the present research is the advancement of Artesunate resistance across pregnancy, peaked at 60.0% in the third trimester. This is evidence that corroborates Aliyu et al. (2017) who indicated a 35.4% Plasmodium isolates of pregnant women resistance to Artesunate, while the present research illustrates an increasingly worsening trend in time. If Artesunate, a major component of artemisinin-based combination therapies (ACTs), is losing efficacy, other treatment modalities must be investigated. Ballard et al. (2018) emphasized the potential of artemether-lumefantrine (AL) as a safer and more effective alternative, particularly in the second and third trimesters, and thus the need for updated treatment guidelines in malaria-endemic regions.

The present study records Arthether resistance as highest in the first (43.0%) and second trimesters (33.8%), dropping to 0% in the third, rising again at delivery (38.1%). The trend has been oscillating, contrary to Aliyu et al. (2017), who recorded Arthether resistance as 37.9% in their study population. These results suggest possible variability in drug metabolism, immune adaptation, or parasite susceptibility variation during pregnancy. Ballard et al. (2018) recommended the use of ACTs for the management of malaria in pregnancy but discouraged the use of artemisinin derivatives, particularly in the first trimester, due to concerns of safety and resistance.

The implications are great for the management of malaria in pregnancy. First, the rising resistance to Artesunate, especially in late pregnancy, means that WHO and CDC guidelines in favor of ACTs could need to be modified to accommodate the ongoing trend in resistance. Second, while Chloroquine resistance appears to be less than in

Aliyuet al. (2017), its overall ineffectiveness bears witness to the need to eschew it as a treatment choice in malaria-endemic regions. Third, Ballard et al. (2018) recommended Artemether-Lumefantrine (AL) as a good and safe substitute, particularly when other alternatives are limited. Given the resistance trends established in this study, AL can potentially offer a reliable alternative for pregnant women in their second and third trimesters. Finally, heterogeneity of Arthether resistance across pregnancy trimesters suggests that therapy regimens are to be adjusted based on pregnancy stage while considering drug metabolism, immune status alteration, and parasite adaptation.

The cross-resistance observed between Artesunate (OR = 2.53, p = 0.007) and Sulfadoxine-Pyrimethamine (SP) in the first trimester is concordant with Giggs. (2016), who established that drug resistance in malaria-endemic areas tends to bear intense correlations among certain antimalarial drugs due to shared resistance mechanisms, particularly among Plasmodium falciparum. Similarly, Schäfer et al. (2024) highlighted that cross-resistance between SP and artemisinin derivatives may be the result of selective drug pressure that alters parasite genetic profiles with time.

However, the absence of statistical association between SP resistance and Chloroquine resistance (OR = 0.53, p = 0.067) agrees with Fola et al. (2024), who hypothesized that mutations for SP and Chloroquine resistance may not necessarily occur together in Plasmodium populations. This is likely due to resistance mechanisms to Chloroquine involving mutations in the pfcrt gene, whereas SP resistance is most associated with mutations in the dhfr and dhps genes.

In the second trimester, the significant negative association between SP resistance and Chloroquine resistance (OR = 0.43, p = 0.021) verifies the hypothesis of Xu et al. (2024) that resistance to a drug could lead to susceptibility to another through competitive genetic adaptation. This is aligned with Cortez-Maya et al. (2020), who argued that lower SP efficacy normally causes sensitivity to older drugs such as Chloroquine due to a change in the evolutionary trajectory of the parasite.

The continuation of correlation between SP and Artesunate resistance during the second trimester (OR = 2.19, p = 0.043) supports Pasupureddy et al. (2019), who reported that artemisinin-based therapies are still vulnerable to resistance mutations developed after the administration of SP. However, the borderline trend observed for Arthether resistance (OR = 2.2, p = 0.071) suggests that patterns of resistance might be more diverse, a fact also observed by Rosenthal et al. (2024), who observed erratic trends in artemisinin derivative resistance depending on parasite strain diversity.By the third trimester, absence of SP-Chloroquine resistance corroborates findings by Hosch, (2023), that Chloroquine resistance is lowered when SP-resistant strains are dominant. The borderline negative correlation between SP resistance and Artesunate resistance (OR = 0.38, p = 0.055) may be an expression of altered selective pressure with passage of time, as Segovia et al. (2025) contended may arise due to changing parasite fitness in response to drug treatment.

With the delivery, also discovered was the fact that SP-resistant women were significantly less Chloroquine-resistant (OR = 0.27, p = 0.042) in support of Nasir et al. (2020), who stated that the re-inclusion of historically abandoned antimalarials would be an optimal strategy where there is fluctuating resistance pattern overtime. However, the absence of high correlation between SP and Artesunate resistance during delivery (OR = 0.68, p = 0.061) suggests that different factors, including exposure history to the drug and parasite adaptation, influence the dynamics of resistance, as suggested by Vanaerschot et al. (2014).

Conclusion and Recommendations:-

This study identifies strong cross-resistance of sulfadoxine-pyrimethamine (SP) with other antimalarial medicines among pregnant women in Ilorin, Nigeria, Kwara State. It was revealed in the findings that resistance to SP is prevalent in all trimesters, with delivery and the first trimester registering the highest rates of resistance. Significantly, cross-resistance between SP and artesunate was observed, particularly during early pregnancy, and with implications on the decreased efficacy of commonly prescribed antimalarial drugs.

The results highlight the need for continuous surveillance of antimalarial drug resistance, particularly in pregnant women, who are at very high risk of malaria complications. According to the observed resistance, treatment policy for malaria in pregnancy would have to be re-evaluated by decision-makers and potentially involve new regimens of prevention and treatment. Further research will be required to establish the genetic basis of cross-resistance and to ascertain the effectiveness of novel combinations of drugs in preventing malaria in pregnancy.

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Declarations

Ethics Approval and Informed Consent

Ethical approval for this study was obtained from the ethical review board of the Kwara State Ministry of Health with reference number ERC/MOH/2022/019. All participants were duly informed of the objectives of the study and the protocol for sample collection. All participants signed an informed consent form were signed. Participation was voluntary.

Authors' Contributions

K.A.O and O.J.S conceptualized the study and designed the study. K.A.O and R.O.S participated in fieldwork and data collection. J.L performed the data analysis. K.A.O and O.J.S, J.L, R.O.S, and B.M.S-D interpreted the data. K.A.O and J.L prepared the first draft of the manuscript, which was reviewed by K.A.O and O.J.S, J.L, R.O.S, and B.M.S-D. All authors contributed to the final manuscript and approved its submission.

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Disclosure of Conflict of Interest

The authors declare that they have no conflicts of interest regarding this study.

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