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Malaria Transmission and Home-based Preventive Practices Amongst Women of Reproductive Age in South-South Nigeria

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Abstract

Malaria infection accounts for over 60% of outpatient visits to healthcare facilities, 30% of child fatalities, 25% of infant deaths, and 11% of maternal mortality in Nigeria. Notably, in Nigeria, children under five years of age and pregnant women are especially vulnerable to malaria-related illnesses and death. The study, therefore, seeks to assess the knowledge of malaria disease, transmission, and use of home-based prevention strategies amongst women of reproductive age group. A cross-sectional study design was employed to assess the knowledge of malaria transmission and home-based preventive practices amongst a multistage sample of 379 women of reproductive age seeking healthcare services at the public PHC facilities in South-South, Nigeria. Data was collected using an interviewer-administered semi-structured questionnaire. Descriptive and inferential analyses of data collected were carried out using the IBM SPSS version 20 software. The mean age of the respondents surveyed in this study was 32.2 (SD ± 6.9) years. Most respondents [88.4% (n = 335)] had good knowledge of malaria transmission and prevention; however, the majority were not knowledgeable about the period mosquitoes commonly enter the house (71.5%) and the peak mosquito biting period (63.1%). There were varied degrees of practice of the various malaria home-based prevention strategies. In conclusion, a significant proportion of the surveyed reproductive-age women had some grasp on the fundamental issues of malaria transmission and prevention with some identified gaps, and they used different home-based malaria prevention measures at varied degrees.

Keywords: Home-based malaria preventive practices, Knowledge, Malaria transmission and prevention, Primary healthcare, Reproductive age women, South-South Nigeria.

Introduction

In sub-Saharan Africa, malaria is rampant, with the heaviest burden of the disease globally [1]. Unfortunately, access to crucial interventions for mitigating this lifelong disease has remained a mirage due to the weakness of public health system in Africa [2,3].

Morbidity and mortality from the disease have remained a significant burden to the sub-region. Worst still is the high prevalence of this disease in Nigeria, where the disease risk of transmission exists throughout the country all year round [4]. Pregnant women, children under five (U5) years, and nonimmune visitors from non-endemic areas are particularly more

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susceptible than the general population [5]. An estimated 76% of the Nigerian population is at risk of malaria infection, with most living in high-transmission areas [6]. Nigeria accounts for 27% of all malaria cases worldwide, and the highest case fatality of 24% in 2019 [7]. Malaria puts colossal stress on Nigeria's public health and economy [8]. The disease causes thousands of deaths and stretches the health system, sometimes beyond its capacity [9]. It accounts for approximately 60% of outpatient visits to health facilities, 30% of child deaths, 25% of deaths in infants, and 11% of maternal mortalities [9]. Notably, pregnant women and children under five years of age are the most susceptible to illness and the resultant death from malaria in Nigeria [8,10].

To prevent, control, and eliminate malaria, the World Health Organization (WHO) recommended a multi-pronged strategy with critical intervention, including indoor residual spraying, insecticide-treated mosquito nets, diagnostic testing for malaria, and treatment of malaria-confirmed cases with effective antimalarial drugs [8]. Most of these malaria control measures require women to apply them in their various homes, while other measures are facility-based.

It is essential that health education on integrated vector management, including environmental management, should be taken into consideration, and be given to women, especially those of reproductive age group, as they are critical in the control of the disease during pregnancy and when nursing their vulnerable under five children. Their knowledge of the diseases, transmission, and prevention methods has a significant role in their application and use of the various prevention strategies, such as indoor residual spraying and insecticide-treated mosquito nets, amongst others that may be presented to them by healthcare practitioners. This study aims to determine the knowledge of women of reproductive age about malaria infection transmission and prevention strategies.

Methods

Study Area

The study was conducted in Edo State in the South-South geo-political zone of Nigeria. Edo State lies between longitudes 5⁰ and 6⁰45" East and latitudes 6⁰1" and 7⁰30" North, with a total land area of 19,281.93 square kilometers [11]. The State has 18 Local Government Areas, with its administrative headquarters in Benin City, Edo South Senatorial District. Edo State has both public and private Tertiary secondary and primary healthcare facilities. According to the Edo State Primary Healthcare Development Agency implementation committee report in 2021, there are 496 public primary healthcare in the State [12].

Study Design

This study used a descriptive cross-sectional design.

Inclusion Criteria

All consenting women of reproductive age who were either nursing an under-five child(ren), had childbirth in the last five years, or are currently pregnant attending any public PHC clinics or on admission at any public PHC in Edo State.

Exclusion Criteria

Women of reproductive age who are not nursing an under-five child(ren) or are not currently pregnant.

Study Duration

The study was conducted between December 2022 and February 2023 after obtaining institutional ethical approval for the research.

Sample Size Determination

The minimum sample size was determined using the formula for cross-sectional studies [13]. The minimum sample size for the number of women of reproductive age patients/clients on admission or clinics in public PHCs in Edo State.

$$n = \frac{Z^2 1 - \alpha / 2 P (1 - P)}{d^2} \quad [13]$$

Computing for the sample size (n) using the study results among pregnant women and female caregivers of under-five children in rural South-West Nigeria, where 42.3% of the women demonstrated good knowledge of malaria prevention [14]. P is the proportion of respondents (pregnant women) that demonstrated good knowledge, q is the complementary probability of P ($1 - p$), and d is the precision level 5% = 0.05. $P = 0.423$, while $q = 1 - 0.423 = 0.577$.

Using the formula for cross-sectional study sample size determination, where n is the calculated sample size, Z is the standard normal deviate at a 95% confidence interval of 1.96: therefore, using $p = 42.3\%$ [14] and $d = 5\%$, $q = 88\%$ (0.88).

By substitution,

$$n = \frac{1.96 \times 1.96 \times (0.423 \times 0.577)}{0.05 \times 0.05} = \frac{1.882 \times 0.2441}{0.0025} = \frac{0.4594}{0.0025} = 183.76$$

The minimum study population of women of reproductive age (participants) is 184.

Sampling Technique

The participants for this study were chosen using a multistage sampling process [15]. Stage one (1) was conducted using the senatorial (political) demarcations in Edo State, giving rise to three zones, namely, Edo South, Central, and North senatorial districts. Stage two (2) involved a simple random sampling technique (balloting) in each of the senatorial districts for one (1) LGA to give a total of three LGAs. In each LGA, women of reproductive age who met the selection criteria were sampled until the desired minimum sample size was achieved.

Study Instruments

A researcher designed and pretested semi-structured self-administered questionnaires administered to women of reproductive age. The questionnaire has three sections: the respondent's socio-demographic characteristics,

knowledge of malaria transmission and prevention, and home-based malaria prevention practices.

Data Collection

Trained research assistants assisted in distributing the pretested, semi-structured questionnaires to reproductive-aged group women seeking healthcare services at the public PHC facilities in Edo State to collect information on their socio-demographic characteristics and knowledge of malaria infection, transmission, and prevention.

Statistical Analysis

Survey data were coded, inputted, and cleaned for analysis in the SPSS version 20.0 spreadsheet [16]. The univariate data mean (standard deviation) was calculated and displayed in tables using non-parametric statistical analysis. Nominal, ordinal, and numerical variables were used to measure the variables. Frequencies and percentages were derived from the categorical variables. The alpha (α) significance threshold was set at 5%. Every p -value had two tails and was deemed statistically significant if it was lower than 0.05. The Chi-square test was used to evaluate the bivariate data (or Fisher's exact test where appropriate). The women's knowledge of malaria transmission and prevention was evaluated based on questions on the disease transmission, which include the disease mode of transmission [mode (mosquito bite = 1 point), vector agent (mosquito = 1 point)], period mosquitoes commonly enter the house or vector active time [period of mosquitoes commonly entering the house (evening time till around 10 PM and early morning = 1 point), the peak anopheles mosquito biting period in the day (midnights = 1 point)], and the vector breeding place/sites (stagnant water = 1 point). The questions on the disease transmission prevention methods were five points, (yes = 1 point each for 5 points) include keeping windows and doors closed, wearing garments that cover the body,

using mosquito nets, using indoor residual spray (IRS), and emptying nearby stagnant water bodies. Appropriate answers scored one, while incorrect answers scored zero. The aggregated score was converted to a percentage, and a score below 50% was regarded as poor, while those of at least 50% were regarded as good knowledge. Using an alpha (α) significance threshold of 5% in a two-tailed non-parametric statistical analysis, a Chi-Square test was conducted to

determine the relationships between the socio-demographic variables and knowledge of malaria infection transmission and preventive methods.

Results

Data were obtained from 379 women of reproductive age seeking PHC services in Edo State, Nigeria.

Table 1. Socio-demographic Characteristics of Clients (Women of Reproductive Age)

Variables	Frequency (N= 379)	Percentage (%)
Age (years)		
< 20	4	1.0
20 – 29	128	33.8
30 – 39	183	48.3
40 – 49	64	16.9
Mean \pm SD of age (years) = 32.2 \pm 6.9		
Religion		
Christianity	321	84.7
Islam	46	12.1
African traditional religion	9	2.4
None	3	0.8
Level of education		
No formal education	20	5.3
Primary education	44	11.6
Junior secondary education	44	11.6
Senior secondary education	144	38.0
Post-secondary education	127	33.5
Marital status		
Single	51	13.5
Married	308	81.3
Separated	16	4.2
Divorced	4	1.0
Tribe		
Esan	72	19.0
Akoko Edo	72	19.0
Bini	70	18.5
Igarra	48	12.7
Etsako	30	7.9
Igbo	26	6.9
Yoruba	20	5.3
Owan	15	4.0
Hausa	13	3.4

Okpe	9	2.4
Ikwerre	2	0.5
Urhobo	2	0.5

Legend: SD – Standard deviation

The mean (\pm SD) age of the 379 respondents surveyed in this study is 32.2 (\pm 6.9) years. One hundred and eighty-three (48.3%) of the respondents were aged 30 – 39 years, 128 (33.8%) between the age group of 20 – 29 years, and 64 (16.9%) were aged 40 – 49 years. A total of 321 (84.7%) practice Christianity, 46 (12.1%) practice Islam, 9 (2.4%) practice African traditional religion, and 3 (0.8%) do not belong to any religion. On the completed educational

level by the respondents, 144 (38.0%) had senior secondary school, 127 (33.5%) had post-secondary education, 44(11.6%), and 20(5.3%) had no formal education. On marital status, 308 (81.3%) were married, 51 (13.5%) were single, 16 (4.2%) were separated, and 4(1.0%) were divorced. Seventy-two (19.0%) were Esan by tribe, 72(19.0%) from Akoko Edo, 70 (18.5%) from Benin, and 48(12.7%) from Igarra, and 30(7.9%) from Etsako.

Table 2. Clients' Knowledge of Malaria Transmission and Prevention

Variable	Incorrect answer	Correct answer
	n (%)	n (%)
Disease transmission		
Mode of transmission	63 (16.6)	316 (83.4)
Agent vector	0 (0.0)	379 (100.0)
Period mosquitoes commonly enter the house	271 (71.5)	108 (28.5)
Peak mosquito biting period	239 (63.1)	140 (36.9)
Vector breeding place	67 (17.7)	312 (82.3)
Preventive measures		
Keeping windows and doors closed	124 (32.7)	255 (67.3)
Wearing garments that cover the body	184 (48.6)	195 (51.4)
Use of mosquito nets	47 (12.4)	332 (87.6)
Use of indoor residual spray (IRS)	112 (29.6)	267 (70.4)
Emptying nearby stagnant water bodies	97 (25.6)	282 (74.4)

The knowledge of malaria transmission was assessed, and all 379 respondents knew the correct vector of the malaria parasite. The majority were knowledgeable about the mode of transmission [316 (83.4%)] and vector breeding place [312 (82.3%)]. (However, only 108 (28.5%) could correctly answer the period mosquitoes commonly enter the house and 140 (36.9%) for the peak mosquito biting period. Regarding knowledge of the preventive measures against malaria transmission to humans, most respondents [332(87.6%)] knew

of mosquito nets as a preventive measure. Most respondents were also knowledgeable about other preventive measures such as the use of sprays [267 (70.4%)], emptying stagnant water bodies [282(74.4%)], and keeping windows and doors closed [255 (67.3%)]. Only about 195 (51.4%) were knowledgeable about wearing garments that cover the body as a preventive measure.

Most respondents, 335(88.4%), had good knowledge of malaria transmission and prevention.

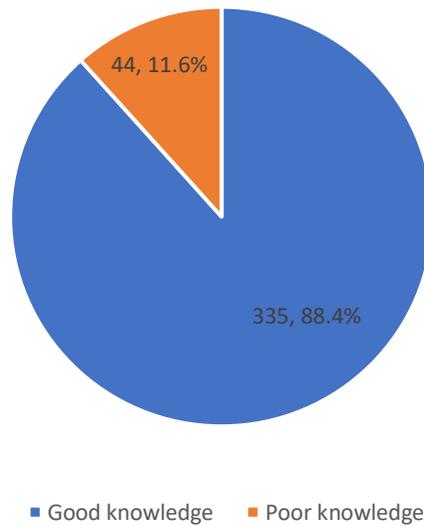


Figure 1. Clients' Overall Knowledge of Malaria Transmission and Prevention

Table 3. Association Between Clients' Socio-demographic Characteristics and Knowledge

Variable	Knowledge of malaria transmission		χ^2	p
	Good knowledge	Poor knowledge		
	n (%)	n (%)		
Age (years)				
< 30	111 (84.1)	21 (15.9)	4.505	0.105
30 – 39	168 (91.8)	15 (8.2)		
40 – 49	56 (87.5)	8 (12.5)		
Level of education				
No formal education	12 (60.0)	8 (40.0)	19.318	<0.001*
Primary education	79 (89.8)	9 (10.2)		
Secondary education	134 (93.1)	10 (6.9)		
Tertiary education	110 (86.6)	17 (13.4)		
Religion				
Christianity	293 (91.3)	28 (8.7)	24.441	<0.001*
Islam	36 (78.3)	10 (21.7)		
Others [#]	6 (50.0)	6 (50.0)		
Marital status				
Single	41 (80.4)	10 (19.6)	4.119	0.127
Married	277 (89.9)	31 (10.1)		
Divorced/separated	17 (85.0)	3 (15.0)		

*Statistically significant and #African traditional religion and no religious inclination/belief

There was a statistically significant association between reproductive-age women's educational status ($p < 0.001$) and religion ($p < 0.001$) with their knowledge of malaria transmission and prevention. The higher the

level of education attained by the women, the better their knowledge of malaria transmission and prevention. The respondents belonging to Christianity or Islamic faith tend to have better knowledge than those in other religious groups.

Table 4. Predictors of Clients' Knowledge of Malaria Transmission and Prevention

Variable	β -coefficient	Odds ratio	95% CI.	p-value
Age (years)				
< 30	Reference	-	-	-
30 – 39	-0.61	0.54	0.23 – 1.30	0.169
40 – 49	0.32	1.38	0.46 – 4.11	0.566
Level of education				
No formal education	Reference	-	-	-
Primary education	-2.12	0.12	0.03 – 0.57	0.008*
Secondary education	-2.08	0.13	0.03 – 0.50	0.003*
Tertiary education	-1.31	0.27	0.07 – 1.01	0.052
Religion				
Christianity	Reference	-	-	-
Islam	0.46	1.59	0.52 – 4.84	0.414
Others [#]	3.23	2.21	6.02 – 105.51	0.501
Marital status				
Married	Reference	-	-	-
Single	0.77	2.16	0.80 – 5.82	0.128
Divorced/separated	-0.07	0.93	0.21 – 4.19	0.925

*Statistically significant and #African traditional religion and no religious inclination/belief

Only the educational status of the respondents (reproductive-age women) was statistically significant for predicting their knowledge of malaria transmission and prevention (binary multivariate logistic regression model). Poor knowledge was less likely in educated

individuals than those without formal education. The odds ratios of poor knowledge in those with primary and secondary education were 0.12 (p=0.008 and 95% CI = 0.03–0.57) and 0.13 (p=0.003 and 95% CI = 0.03–0.50), respectively.

Table 5. Clients' Use of Malaria Prevention Methods in the Past One Year

Variable	Frequency	Percentage
	(N=379)	(%)
Use of malaria prevention methods		
Environmental method - clearing of stagnant water bodies	371	97.9
Door or window nets	346	91.3
Long sleeves	307	81.0
Insecticidal residual spray	274	72.3
Burning of insect coil	273	72.0
Insecticidal bed nets	256	67.6
Application of insect repellent cream	192	50.7

#Multiple responses for other methods

Regarding malaria prevention methods, the respondents were found to have used different methods, with 371(97.9%) using environmental methods - clearing of stagnant water bodies, 346(91.3%) for door nets, wearing long sleeves

307(81.0%), insecticidal residual spray 274(72.3%), burning of insect coil 273(72.0%), insecticidal bed nets 256(67.6%) and application of insect repellent cream 192 (50.7%).

Discussion

In this survey, the literacy level of the reproductive age women utilizing the PHC services in the study area was relatively high, with 71.5% of them reporting having completed their secondary education level. Such a level of educational status among the female gender is a positive one that could have a far-reaching effect on public health interventions requiring the understanding, knowledge, and practice adoption. This finding is higher than that reported in the national survey for women of reproductive ages in Nigeria in 2018, which had barely a little above half of the women with at least a secondary level of education. This difference may be accounted for by the fact that Edo State is one of the States with higher literacy levels in Nigeria. The State was ranked the 9th most literate State in Nigeria in 2023 with a score of 68.7%, while the country has an overall literacy rate of just a little above 50% [17]. Also of note is that the finding in this study is at variant with what was reported in Edo State in a 2018 study in which only about two-fifths of the women completed their secondary level of education [18].

In this study, the women demonstrated good knowledge of malaria transmission, as all 379 respondents knew the correct vector for the transmission of malaria parasite. The majority were knowledgeable about the mode of transmission and vector breeding place. The knowledge of the vector agent, mode of transmission, and their breeding places might not be enough to effectively achieve preventive measures even if there were good attitudes and practices. Knowing the peak time/period of the vector gaining entrance into the homes and their biting time would be vital to seeing an effective control with the application of measures like mosquito nets on the house doors and windows, use of bed nets, and IRS and other human activities during these periods. Unfortunately, this study showed that only 28.5% of the reproductive age women could correctly answer the period mosquitoes commonly get into the

living house, and 37% appropriately answer the peak mosquito biting period. The knowledge of these activities of the vectors is important because using insecticide-treated bed nets (ITNs) may be more effective in preventing malaria, where vectors primarily bite indoors and late at night [19]. Conversely, the effectiveness of ITNs in preventing mosquito bites is uncertain where vectors bite outdoors due to the activities of humans in the evening [19]. This knowledge gap could be a significant challenge in implementing integrated strategies for effectively preventing malaria by the clients and patients seeking healthcare services at the PHC facilities in Edo State.

Regarding clients' knowledge of the preventive measures against malaria transmission to humans, most respondents (87.6%) knew of insecticide-treated nets as a preventive measure. They were also knowledgeable about other preventive measures such as emptying stagnant water bodies (74.4%), use of indoor residual spray (70.4%), keeping windows and doors closed (67.3%), and wearing garments that cover the body (51.4%). These findings were quite commendable, showing the effect of several interventions and health education that have been used to impact the knowledge and positively change the attitude of the people of sub-Saharan Africans towards preventing malaria transmission. This was also reflected in the overall knowledge of malaria transmission and prevention, which was seen as high in most respondents, with 88.4% demonstrating good knowledge. This study revealed that 74.4% of the respondents were knowledgeable about emptying stagnant water bodies as a method of malaria control. The finding was, however, better than what was reported in the pre-intervention (50.4%) and post-intervention (64.5%) study carried out in Ogun State in 2013 [20].

In this study, women who attained a higher level of education demonstrated better knowledge, which was found to be statistically significant ($p=0.001$), and it is similar to what

was reported in the two studies conducted in South-West, Nigeria, and one in Tamale Teaching Hospital, Ghana [14,21,22]. It was not surprising that formal education has played a significant role in acquiring knowledge about other aspects of one's life and skills. Education for girls is much more important because it boosts economies and lessens inequality. It helps create more secure, resilient societies where everyone has the chance to reach their full potential, including boys and men. Beyond being a significant factor in the bivariate analysis, education was the only predictor of good knowledge of malaria transmission and prevention; in a multivariate logistic regression model comparing the educational status of those without formal education, poor knowledge was less likely in the educated individuals. The odds ratios of poor knowledge in those with primary education and secondary education were 0.12 ($p=0.008$ and 95% CI = 0.03–0.57) and 0.13 ($p=0.003$ and 95% CI = 0.03–0.50), respectively, in this study. Again, this was unsurprising because investing in girls' education profoundly impacts local communities, nations, and the entire planet. Education increases the likelihood that girls will live long, healthy lives. They often work to improve the futures of themselves and their family by taking an active role in the decisions that most directly affect family members.

In this study, religion was found to have a statistically significant relationship with the respondent's knowledge of malaria transmission and prevention, similar to what was reported in the study conducted in Tamale Teaching Hospital, Ghana [22].

More respondents with Christianity and Islam had better knowledge of malaria transmission and prevention than was obtainable for others (African traditional religion and no religious inclination/belief). This may result from the opportunity of public health-related teaching/education that is occasionally passed on to the congregations in these more organized settings or places of worship.

All the respondents were engaged in at least one form of malaria prevention methods. Some practiced the combination of different methods of malaria prevention in their homes. The two main methods that were commonly practiced by respondents in this study were clearing of stagnant water and the use of net as doors and windows screens. Almost all the respondents (97.9%) reported using environmental methods - clearing of stagnant water bodies and (91.3%) reported using door and window nets in their homes.

Most houses in the study area seem to have net covers for the windows and doors. To make this effective in controlling or preventing mosquito entrance into the dwelling houses, there must be deliberate efforts to ensure that the engineering design creates no opportunity for space between the door/windows and the frames. The nets should be changed as at when due, and in practice, the doors/windows with nets should have an inbuilt automated mechanisms to ensure closure almost immediately. Although, looking at the characteristics of these nets was not within the scope of this study.

Regarding the use of environmental methods - clearing stagnant water bodies in preventing mosquito breeding, it is one of the oldest malaria and other vector-borne disease control strategies that is still beneficial to date [23]. Surprisingly, this proportion practicing the method was more than those who knew the method was a strategy for reducing the population of mosquitoes by reducing their breeding sites. The reason for this better practice compared to their demonstrated knowledge might be because of the benefits the practice confers which are beyond its role in controlling the breeding of mosquitoes; it can help in the control of other disease vectors as well as give the environment an excellent aesthetic look. Unfortunately, in Nigeria, the strategy is gradually becoming abandoned and forgotten, as seen in this study in which respondents' knowledge about the strategy is not as popular as mosquito nets. This may result from recent publicities seen for mosquito nets

because of the attention and promotion by sponsors and donor agencies. Whereas the environmental sanitation that used to be routinely done by both the state through Local Government Authorities is gradually been abandoned.

Other methods commonly used by the respondents in this study were wearing long sleeves (81.0%), insecticidal residual spray (72.3%), and burning of insect coil 273(72.0%). The use of insecticidal bed nets (67.6%) and the application of insect repellent cream (50.7%) were amongst the least practiced methods by the respondents. This is not similar to the findings from Yaounde, Cameroon, where the most used method was mosquito bed nets, used by 82.5% of the respondents in the study [24]. The reason for these findings might be the ready availability of long-sleeved clothes for the respondents to purchase instead of short sleeves. Insecticidal residual spray and burning of insect coil give immediate effect. They are commonly used when the residents are likely doing some activities requiring frequent opening and closing of their house doors.

The possible inconvenience of applying cream on the body and the fact that the respondents may not be able to attest to the harmful effects of the cream could be why the practice of insect-repellent cream was low, especially with this study population of all females who have serious concerns for their skin would not likely apply any cream on it.

This study revealed that the different home prevention practices against malaria transmission were still observed at varied degrees amongst reproductive-age women in South-South Nigeria. It is important to emphasize that an integrated approach to vector control practices especially with consideration for human and vector activities in and around the homes will make a significant impact in malaria transmission prevention strategies in the endemic area like Nigeria. Therefore, efforts at having environmental control, IRS, and using

LLINs, among others, in a properly integrated approach will give a more synergistic effect.

Conclusion

A high proportion of women of reproductive age in Edo State generally demonstrated good overall knowledge of malaria transmission and prevention. However, there are lapses in knowledge of the period mosquitoes commonly enter the house and the peak mosquito biting period. Clearing stagnant water bodies, applying mosquito nets on doors and windows, and wearing long-sleeved clothing to prevent mosquito bites were the most commonly used strategies while applying insect repellent cream to the body was the least used. Health educational interventions should target the identified lapses in knowledge and re-enforce the benefits of a multi-prong home-based approach in controlling malaria amongst women of reproductive age in South-South Nigeria.

Declarations

This article was part of thesis work for the Doctor of Philosophy (Ph.D.) in Public Health award.

Ethical Consideration

Ethical clearance to conduct this research was sought and obtained from the Ethics and Research Committee of Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo State, Nigeria. Before being included in the study, each research participant was asked for their written, informed consent.

Consent for Publication

Not applicable.

Availability of Data and Material

The datasets used and analysed for this study are available from the corresponding author upon a reasonable request.

Competing Interests

The researchers assert that they have no competing interests.

Funding

The research was self-sponsored.

Authors' Contributions

EFO and PO conceived the study, and EFO developed the protocol with the guide of PO. EFO and ETO were involved in data collection,

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analysis, and all authors were involved in manuscript drafting.

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