PUBLIC HEALTH RESEARCH

The Effectiveness of the Long-Lasting Insecticidal Nets in Controlling Malaria Vector: A Meta-Analysis of Experimental Hut Studies

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ABSTRACT

Introduction Malaria is a life-threatening, preventable, and curable vector borne disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles*. The WHO Global Report 2010-2016 reported insecticide resistance in malaria. The main objective of this study is to determine the effectiveness of new generation Long-Lasting Insecticidal Nets (LLIN) compared to standard LLIN and untreated nets in terms of the mortality rate of adult female *Anopheles gambiae*.

Methods A comprehensive review of the literature was published in three databases (PubMed, Ovid, EBSCO Host) since 2010. Publications were searched with keywords including malaria, long-lasting treated bed net, long lasting insecticide-treated bed net, LLIN, and experimental hut. The search has identified 60 articles. Based on the PRISMA flowchart, 10 articles are qualified for data collection and analysis. The gathered data was analysed using Review Manager.

Results Following meta-analysis between subgroups, a risk difference of 0.31 between standard LLINs versus untreated net ($p<0.001$, $I^2=100\%$ 95% CI:0.01,0.60). A comparison of upgraded LLINs with the untreated net has shown a significant difference with a pooled risk difference of 0.54 favours upgraded LLINs ($p<0.001$, $I^2=100\%$ 95% CI: 0.54,0.84). Comparison between upgraded LLINs versus standard gave an overall risk difference of 0.24 ($p < 0.001$, $I^2 = 100\%$, 95% CI: 0.10–0.39).

Conclusion Upgraded LLINs significantly increase *Anopheles* mortality compared to standard LLINs and untreated nets, suggesting their potential for improved malaria control. Thus, using upgraded nets in the field and translating them into malaria preventive programs would help achieve the target and improve health outcomes for those living in endemic areas.

Keywords Malaria; Long-Lasting Insecticide Nets (LLINs); Experimental Hut; Insecticide

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INTRODUCTION

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. It is a preventable and curable vector borne disease and remains a disease of global health importance.[1] Globally, there were more than 219 million cases of malaria. Approximately 92% of all malaria cases in 2017 were diagnosed in the WHO African Region (200 million), followed by the WHO South-East Asia Region (5%) and the WHO Eastern Mediterranean Region (2%).[2]

An estimated 435 000 deaths from malaria were reported globally. Nearly 80% of the global malaria deaths in 2017 were concentrated in 17 countries within the WHO African Region and India; seven of these countries accounted for 53% of all global malaria deaths: Nigeria (19%), Democratic Republic of the Congo (11%), Burkina Faso (6%), United Republic of Tanzania (5%), Sierra Leone (4%), Niger (4%) and India (4%).[2] In 2017, an estimated US$ 3.1 billion was invested for malaria control and elimination efforts globally by the governments of malaria endemic countries and international partners, an amount slightly higher than the figure stated in 2016. Nearly three-quarters (US$ 2.2 billion) of investments in 2017 were spent in the WHO African Region, followed by the WHO regions of South-East Asia (US$ 300 million), the Americas (US$ 200 million), and the Eastern Mediterranean and the Western Pacific (US$ 100 million each).[2] Between 2015 and 2017, a total of 624 million insecticide-treated mosquito nets (ITNs), mainly long-lasting insecticidal nets (LLINs), were manufactured and delivered throughout the world. This represents a substantial increase compared to the previous period 2012–2014, whereby 465 million ITNs were delivered. Globally, 85% of the distributed ITNs were through free mass distribution campaigns, 8% in antenatal care facilities, and 4% as part of immunization programmes. Around half of the population was protected by this intervention, an increase from 29% back in 2010. Furthermore, the population with access to an ITN nearly doubled from 33% in 2010 to 56% in 2017.[2]

In 2017, Malaysia reported a total of 508 cases (local and imported) of the human type of malaria, substantially reduced from 6141 cases in 2010. Overall, malaria transmission in Malaysia is largely confined to Sabah and Sarawak, two states located on the island of Borneo, where a significant proportion of the population is at risk of the disease. About 85 indigenous human malaria cases and 423 imported human malaria cases were detected in 2017 with zero and 12 local human malaria and imported malaria deaths respectively.[3]

The WHO Global report on insecticide resistance in malaria vectors 2010 - 2016 showed widespread resistance to the four commonly used insecticide classes; pyrethroids, organochlorines, carbamates, and organophosphates in all major malaria vectors across the WHO regions of Africa, the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific. Resistance to at least one of the four insecticide classes in one malaria vector from one collection site was detected in 68 countries. In 57 countries, resistance to two or more insecticide classes was reported. Resistance to pyrethroids was detected in at least one malaria vector and highest in the WHO regions of Africa and the Eastern Mediterranean.[2] This may be the result of mutations in the target-site proteins (target-site resistance),[4] which led to a reduced sensitivity or increased activity of detoxification enzymes (metabolic resistance).[5] The evolution of insecticide resistance and its continuing spread threatens the operational success of malaria vector control interventions. The current impact of this resistance on malaria transmission is largely unquantified and will vary depending on the level of resistance, malaria endemicity, and proportion of the human population using LLINs (Churcher 2016)→ no reference stated in the reference section. However, it is generally accepted that the resistance will eventually erode the efficacy of pyrethroid-only LLINs and that further innovative approach in the LLIN market is essential to maintain the efficacy of this preventative measure.[6]

In 2011, WHO launched a large multi-country evaluation to assess the impact of insecticide resistance on core malaria vector control tools, primarily LLINs. The evaluation was conducted at 340 locations in five countries: Benin, Cameroon, India, Kenya and Sudan. According to the findings, LLINs continue to be an effective tool in the fight against malaria, even in areas where mosquitoes have developed resistance to pyrethroids.[7] Instead of using a non-pyrethroid insecticide to manage resistance, another valid approach for resistance management is the addition of synergists for LLIN treatment. These synergists can reduce resistance by inhibiting the enzymes responsible for resistance.[8]

We aim to systematically organize, review and determine established evidence on the effectiveness of new generation LLIN compared to standard LLIN and untreated net, highlighting the effectiveness in term of mortality rate of adult female of *Anopheles (An.) gambiae*.

METHODS

Using three databases (PubMed, Ovid, EBSCO Host), a comprehensive review of the literature published since 2010 was performed. Publications were searched for with keywords of ‘malaria’, ‘long-lasting treated bed net’, ‘long lasting insecticide-treated bed net’, ‘LLIN’, and ‘experimental hut’. This process identified 60 articles. Only experimental studies that reported on

the protective efficacy of LLINs or comparison between LLIN are included whereas studies that adopted cross-sectional and cohort study designs were excluded. Articles were also excluded if they met any of the following two criteria: review articles and original studies on non-malaria vector. Following screening based on these eligibility criteria, a total of 19 articles were identified for full review. The full texts of the 19 articles were read to confirm they were qualified for inclusion in the meta-analysis. Nine articles were excluded due to insufficient numerical information on the parameters that assess effectiveness of LLINs in experimental hut study for inclusion in the meta-analyses, such as the number of vector mortality in experiment and control hut. Finally, a total of 10 articles were shortlisted for data collection and analysis. (Figure 1).

Data Collection and Analysis

Data from the 10 articles were extracted and recorded with quantitative measures on the following covariates: total female caught, deterrence, exophilic, total female blood fed, blood fed inhibition, personal protection, total female dead and overall killing effect. We included studies that compared LLINs versus untreated bed net (UTN), or standard LLINs versus newer generation of LLINs in the market. The LLINs (which are factory-treated nets that are embedded with the insecticide, either within or bound around the net fibres) must have had either an interim or full recommendation from the WHO. The brands of treated nets were not recorded but classified according to the combination of chemical properties in the insecticide treated bed net used in the experimental hut study. The cost of the LLINs were not reported. This approach was undertaken as a means to promote and distribute information of the socially beneficial intervention rather than commercializing the product. As stated previously, nine articles were excluded due to the lack of quantitative data for at least one of the covariates listed above. The final sample for meta-analysis included 10 experimental hut studies on the effectiveness of LLINs.

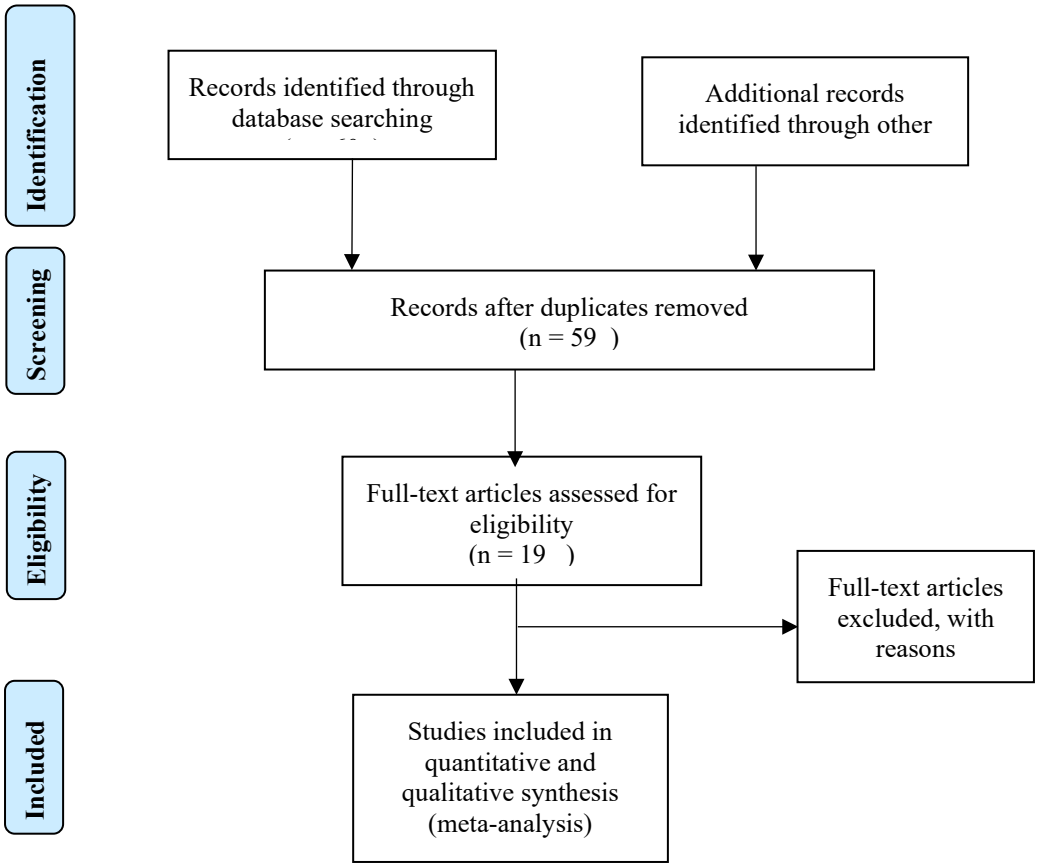

Figure 1 PRISMA flowchart

Study Selection

Two authors (M.S and I.F) independently screened the search results for potentially relevant studies and retrieved the corresponding full articles. M.F. and I.F independently assessed the articles for eligibility using a standardised form. Any discrepancies between the eligibility results were resolved through discussion. Multiple publications from the same study were identified, and if eligible, the original study was taken forward for inclusion.

Risk of Bias Assessment

We assessed the risk of bias of each included study in accordance with a quality assessment tool adopted from Clare et al for experimental hut trials.[9] Risk assessment is based on the seven criteria: comparability of mosquitoes in LLINs and untreated huts, collectors blinded, (3) sleepers blinded, (4) raw data reported for ITN and UTN groups, (5) ITNs randomly allocated to huts, (6) LLINs rotated, and (7) sleepers rotated. For all criteria, we made a judgement of high, low, or unclear risk of bias. For the hut trials, we followed an additional set of variables to assess the variability in the design and execution of the studies, called 'rigor of implementation'. This assessment included: nets being washed according to WHO protocol, cleaning of huts before the trial and between rotations to avoid cross-contamination of huts from the different treatment arms and to remove any insects that may have been missed during collections, and (3) whether male mosquitoes were excluded from the analysis.

Data Analysis

Analyses were carried out in Review Manager 5. Dichotomous outcomes were summarised using the risk difference; therefore, results are generalisable only to situations where the control group event rate is comparable to those observed here. When the same studies were compared, the event rate in the untreated group was split to ensure each mosquito was included in the analysis only once. The results of studies were pooled using meta-analysis when possible. Random effects models were used when heterogeneity was detected. It is worth noting that a random effects meta-analysis awards more weight to smaller studies than a fixed effects meta-analysis, and the weights for each study tend to reach equality as the between-trial variance increases.

Assessment of Heterogeneity

Heterogeneity was assessed by visually inspecting the forest plots to detect overlapping confidence intervals, applying the chi-squared test with a p-value < 0.05 to indicate statistical significance, and implementing the I^2 test statistic with a value of 50% that implies a moderate level of heterogeneity. Nevertheless, such assessments of heterogeneity are influenced by the number of included studies and should be interpreted with caution. Heterogeneity was noted to be high in all the analyses. Reporting biases were explored using funnel plots.

RESULTS

Characteristics of Included Studies and Risk of Bias

The 10 included hut studies were conducted in field sites located in Benin,[10-14] Ivory Coast,[15] Burkina Faso,[16] India[17] and Cameroon.[18] All comparisons were of *An. gambiae* mosquitoes. For the risk of bias assessment, rigor of implementation for each hut trial was focused on the study design characteristics (Table 1). It was unclear in all 10 studies whether the data collectors were blinded. Standardisation across studies was not consistent for both experimental design and reporting. Overall, 8 studies rotated LLINs and sleepers[11-17, 19] but 3 of these blinded the sleepers.[11-13] Of the 10 studies, 8 clearly demonstrated washing the net[11-14, 16-18, 20] in accordance with the WHO protocol of which 6 stated cleaning the huts before the study.[11-14, 17, 20] One study did not exclude male mosquitoes from the analysis.[19]

Four studies were comparing LLINs with cypermethrin (standard LLIN) versus cypermethrin + chlorfenapyr (upgraded LLIN),[11, 12, 15, 16] another 4 comparing permethrin (standard LLIN) versus permethrin + pyriproyfen (upgraded LLIN),[10, 13, 14, 19] and 2 comparing permethrin (standard LLIN) with permethrin + piperanyl butoxide (upgraded LLIN).[17, 18] All ten studies made comparison to untreated net as the control group, measured eight outcome parameters – total female caught, deterrence, total female blood fed, blood fed inhibition, personal protection, total female dead, mortality mosquito, and overall killing effect (Table 2). Meta-analysis was done for parameter measuring number of *Anopheles* mortality, comparing between standard LLINs versus untreated net, upgraded LLINs versus untreated net and between standard LLINs with upgraded LLINs (Table 3).

Table 1 Assessment of risk of bias for experimental hut trials

Study, Year	Comparability of mosquitoes in the hut	Collectors blinded	Sleepers blinded	Raw data reported	LLIN randomly allocated to hut	LLIN rotation	Sleepers rotation	Net washed according to WHO protocol	Cleaning of hut before and between rotation	Insecticide efficacy and residual activity test	Exclude male mosquitoes from analysis
N'Guessan, 2016 [10]	Y	Unclear	Y	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor, 2017 [11]	Y	Unclear	Y	Y	Unclear	Y	Y	Y	Y	Y	Y
Bayili, 2017 [12]	Y	Unclear	Y	Y	Y	Y	Y	Y	Y	Y	Y
Camara, 2018 [13]	Y	Unclear	Unclear	Y	Unclear	Y	Y	Y	Y	Y	Y
Ngufor, 2014 [14]	Y	Unclear	Unclear	Y	Unclear	Y	Y	Unclear	Unclear	Y	y
Djenontin, 2015 [15]	Y	Unclear	Unclear	Y	Unclear	Y	Y	Y	Unclear	Unclear	Y
Khoffi, 2015 [16]	Y	Unclear	Unclear	Y	Unclear	Y	Y	Y	Y	Y	Y
Ngufor, 2016 [17]	Y	Unclear	Unclear	Y	Unclear	Unclear	Unclear	Y	Unclear	Y	Y
Gunasekaran, 2016 [18]	Unclear	Unclear	Unclear	N	Y	Y	Y	Unclear	Unclear	Y	Unclear
Pennetier, 2013 [19]	Y	Unclear	Unclear	Y	Unclear	Unclear	Unclear	Y	Y	Y	Y

Note: Y: Yes, N: No

Table 2 Study characteristics of the included experimental hut trials

Study	Intervention	Chemical	ITN washed	Measure Outcome							
				TFC	D	TFV	TFBF	BFI	PP	MM	O
N'Guessan 2016	Interceptor LN	(Cypermethrin)	20	Y	Y	Y	Y	Y	Y	Y	Y
N'Guessan 2016	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	20	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor 2017	Interceptor LN	(Cypermethrin)	0	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor 2017	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	0	Y	Y	Y	Y	Y	Y	Y	Y
Bayili 2017	Interceptor LN	(Cypermethrin)	20	Y	Y	Y	Y	Y	Y	Y	Y
Bayili 2017	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	20	Y	Y	Y	Y	Y	Y	Y	Y
Camara 2018	Interceptor LN	(Cypermethrin)	20	Y	Y	Y	Y	Y	Y	Y	Y
Camara 2018	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	20	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor 2014	OlySet Net	Permethrin	0	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor 2014	Olyset Duo	Permethrin + Pyriproyfen	0	Y	Y	Y	Y	Y	Y	Y	Y
Djenontin 2015	OlySet Net	Permethrin	0	Y	Y	Y	Y	Y	Y	Y	Y
Djenontin 2015	Olyset Duo	Permethrin + Pyriproyfen	0	Y	Y	Y	Y	Y	Y	Y	Y
Khoffi 2015	OlySet Net	Permethrin	0	Y	Y	Y	Y	Y	Y	Y	Y
Khoffi 2015	Olyset Duo	Permethrin + Pyriproyfen	0	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor 2016	OlySet Net	Permethrin	0	Y	Y	Y	Y	Y	Y	Y	Y
Ngufor 2016	Olyset Duo	Permethrin + Pyriproyfen	0	Y	Y	Y	Y	Y	Y	Y	Y
Gunasekaran 2016	OlySet Net	Permethrin	20	Y	Y	Y	Y	Y	Y	Y	Y
Gunasekaran 2016	OlySet Plus	(Permethrin + Piperonyl Butoxide)	20	Y	Y	Y	Y	Y	Y	Y	Y
Pennetier 2013	OlySet Net	Permethrin	20	Y	Y	Y	Y	Y	Y	Y	Y
Pennetier 2013	OlySet Plus	(Permethrin + Piperonyl Butoxide)	20	Y	Y	Y	Y	Y	Y	Y	Y

Note: TFC:Total female caught (n), D:Deterrence (%), TFV:Total female in veranda (n), TFBF:Total female blood fed (n), BFI:Blood fed inhibition (%), PP:Personal protection (%), MM:Mortality Mosquito, O: Overall killing effect

Table 3 Results comparing Untreated Net vs standard LLIN vs upgraded LLIN for outcome measure

Study	Intervention	Chemical	ITN washed	Measure Outcome							
				TFC	D(%)	TFV(%)	TFBF	BFI(%)	PP(%)	MM	O (%)
N'Guessan 2016	Untreated Net	-	-	673	NA	NA	NA	NA	NA	377(56%)	NA
N'Guessan 2016	Interceptor LN	(Cypermethrin)	20	950	29.2	51.0	NA	47.0	22.0	675(71%)	44.4
N'Guessan 2016	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	20	929	27.6	56.0	NA	50.0	34.4	604(65%)	55.9
Ngufor 2017	Untreated Net	-		310	NA	NA	210	NA	NA	8(2.6%)	NA
Ngufor 2017	Interceptor LN	(Cypermethrin)	0	175*	44.0	NIL	65*	46.0	69*	20(8%)*	9.0
Ngufor 2017	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	0	251*	19.0	NIL	120*	30.0	43*	137(54%)*	55.0
Bayili 2017	Untreated Net	-		853	NA	NA	553	NA	NA	43(5%)	NA
Bayili 2017	Interceptor LN	(Cypermethrin)	20	1198	0.0	37.6	770	50.0	50.0	63(20%)	3.3
Bayili 2017	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	20	1028	0.0	51.5	531	90.0	90.0	151(81%)	92.6
Camara 2018	Untreated Net	-		611	NA	NA	300	NA	NA	55(9%)**	NA
Camara 2018	Interceptor LN	(Cypermethrin)	20	348	43.0	44.0	158	NA	47.0	35(10%)**	-2.7
Camara 2018	Interceptor G2 LN	(Cypermethrin + Chlorfenapyr)	20	369	40.0	51.0	120	34.0	60.0	303(82%)**	40.6
Ngufor 2014	Untreated Net	-		64	NA	NA	35	NA	NA	4(6.3%)	NA
Ngufor 2014	OlySet Net	Permethrin	0	76	0.0	53.0	34	15.0	53.0	21(27.6%)	12.0
Ngufor 2014	Olyset Duo	Permethrin + Pyriproyfen	0	72	0.0	56.0	10	75.0	92.0	36(50%)	13.0
Djenontin 2015	Untreated Net	-		152	NA	NA	140	NA	NA	1(0.6%)	NA
Djenontin 2015	OlySet Net	Permethrin	0	160	0.0	28.7	30	78.4	78.0	159(99.4%)	98.1
Djenontin 2015	Olyset Duo	Permethrin + Pyriproyfen	0	162	0.0	28.9	12	91.5	91.4	162(100%)	98.8
Khoffi 2015	Untreated Net	-		1399	NA	NA	482	NA	NA	110(7.9%)	NA
Khoffi 2015	OlySet Net	Permethrin	0	1431	0.0	43.4	744	-50.9	-54.4	177(14.7%)	13.6
Khoffi 2015	Olyset Duo	Permethrin + Pyriproyfen	0	1202	0.0	48.6	453	-6.0	6.0	125(8.7%)	60.9
Ngufor 2016	Untreated Net	-		2874	NA	NA	1004	NA	NA	159(5.5%)	NA
Ngufor 2016	OlySet Net	Permethrin	0	3804	0.0	65.0	444	66.0	58.0	1228(32.3%)	39.0
Ngufor 2016	Olyset Duo	Permethrin + Pyriproyfen	0	3840	0.0	64.0	281	79.0	72.0	1536(40%)	51.0
Gunasekaran 2016	Untreated Net	-		303	NA	NA	235	NA	NA	6(2%)	NA
Gunasekaran 2016	OlySet Net	Permethrin	20	54	82.2	3**	16	61.9	93.3	52(96.3%)	94.4
Gunasekaran 2016	OlySet Plus	(Permethrin + Piperonyl Butoxide)	20	36	88.1	4.4**	11	60.6	95.3	35(97.2%)	95.8
Pennetier 2013	Untreated Net	-		69	NA	NA	43	NA	NA	0(0%)	NA
Pennetier 2013	OlySet Net	Permethrin	20	124	0.0	0.0	31	60.0	27.9	45(35%)	44.9
Pennetier 2013	OlySet Plus	(Permethrin + Piperonyl Butoxide)	20	101	0.0	0.0	13	79.0	69.8	68(67%)	98.6

Forest Plots

A significant difference is detected between meta-analytic result for subgroup comparing standard LLINs versus untreated net with a risk difference of 0.31 (p<0.001, 12=100% 95% CI 0.01,0.60). There is high variability among the results from all studies although these studies significantly favour LLINs. Comparing upgraded LLINs with untreated net, a significant difference is detected with a pooled risk difference of 0.54 favouring upgraded LLINs (p<0.001, 12=100% 95% CI 0.54,0.84). A comparison between upgraded LLINs versus standard LLINs gave an overall risk difference of 0.24 (p<0.001 i2 =100 95% 0.10, 0.39). The mortality risk is increased by 24% using upgraded LLINs when compared to standard LLINs (without combination). (Figure 2-4).

Results of Subgroup Analyses, Sensitivity Analyses, and Funnel Plots

Considerable heterogeneity was found across all studies; therefore, sources of heterogeneity were explored using subgroup analyses. We carried out subgroup analyses by net type and insecticide used. Due to the wide variation between the studies in relation to these factors, the plots were numerous. We carried out analyses grouping in different ways, but these analyses failed to provide further explanation on the heterogeneity between studies. The funnel plots did not resemble symmetric funnels; and this may cause by the high level of variability between studies. For experimental hut trials, similar conclusions are drawn from the sensitivity analyses and primary analyses.

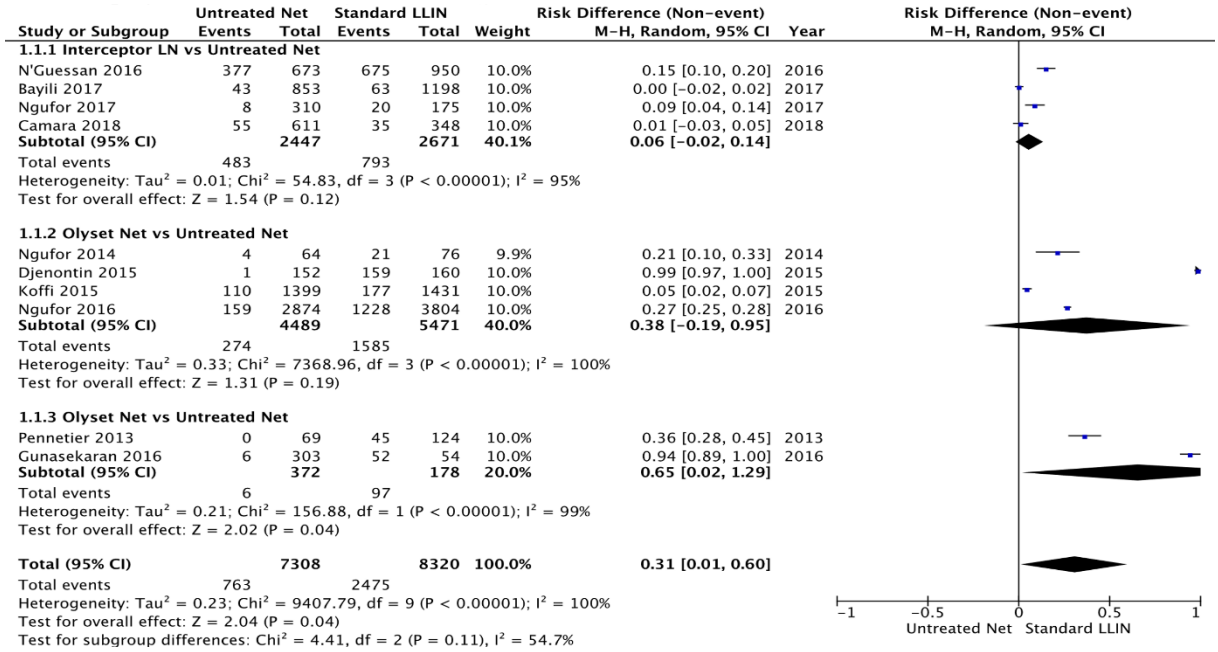

Figure 2 Forest plot for comparison between untreated net and standard LLIN

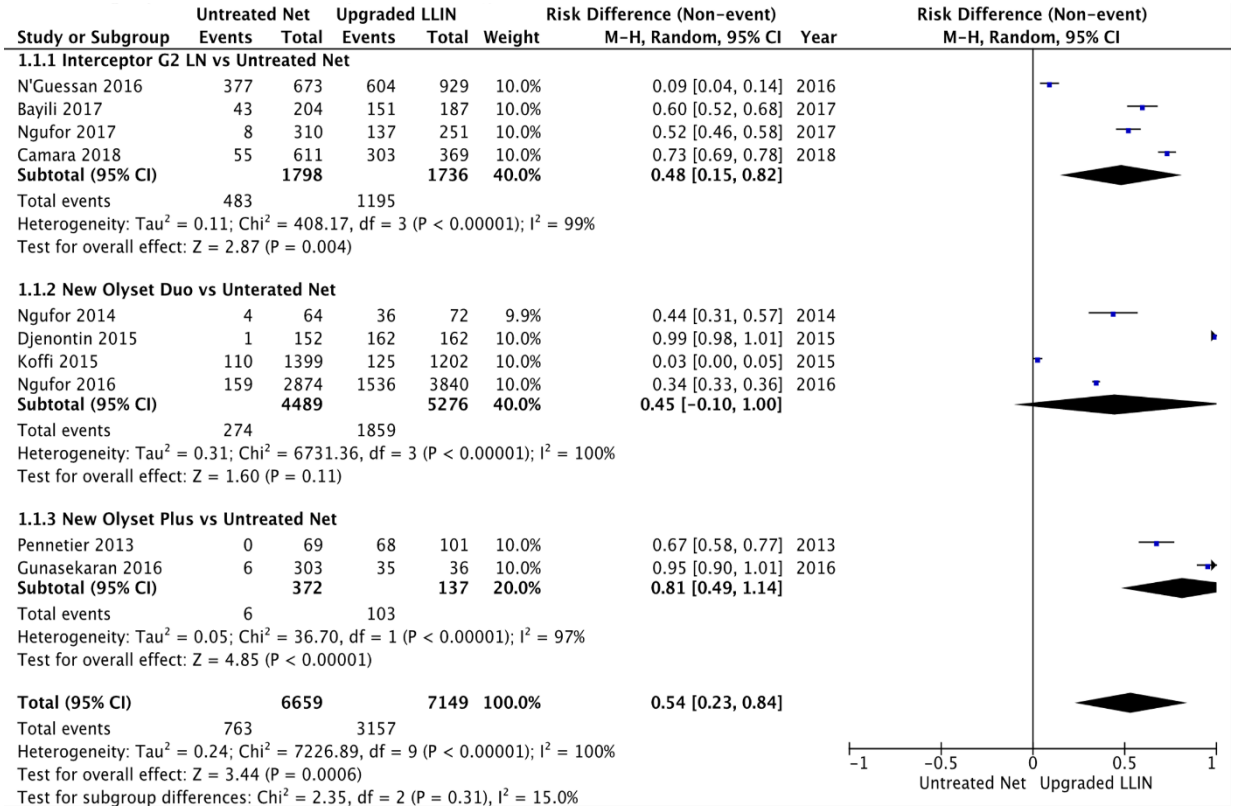

Figure 3 Forest plot for comparison between untreated net and upgraded LLIN

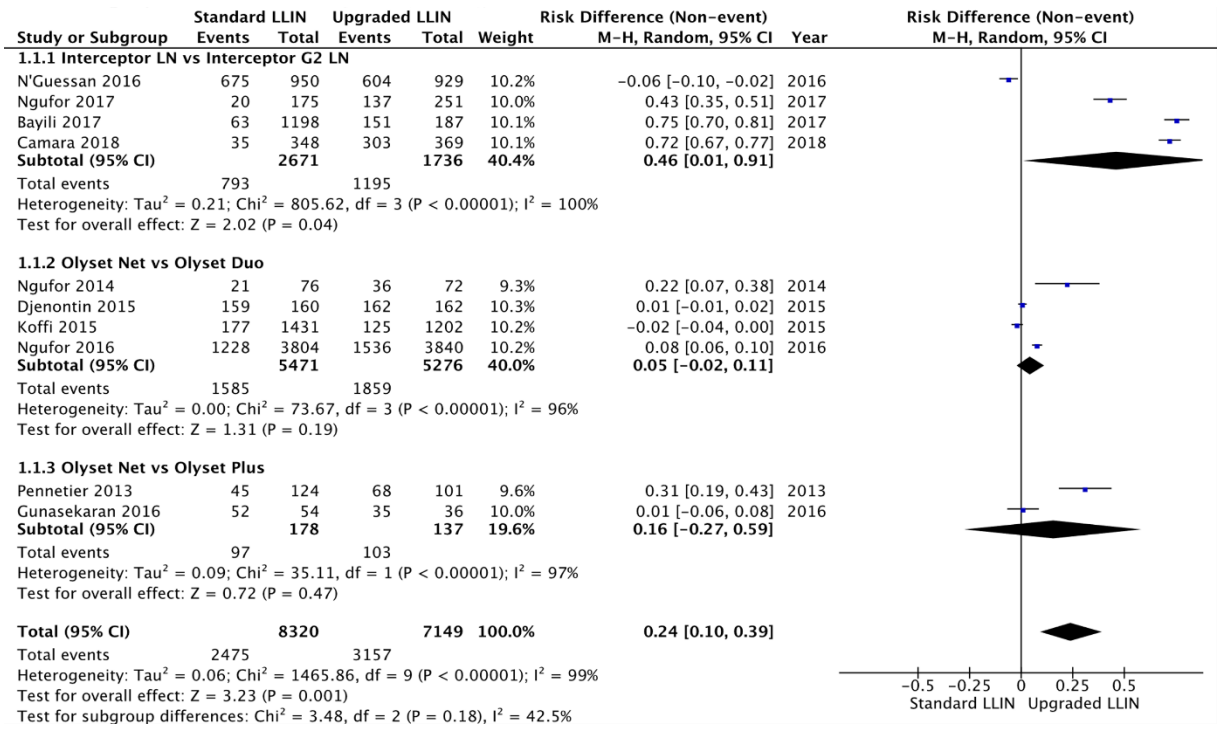

Figure 4 Forest plot for comparison between upgraded LLIN and standard LLIN

DISCUSSION

This study is to determine the effectiveness of standard LLIN and untreated net through experimental hut studies. The meta-analytic results showed that the difference in mortality female *Anopheles* risk using standard LLIN is increased by 0.31 (31%) compared to untreated net. The study also found high heterogeneity between studies. This could be due to the variability of *Anopheles* and the type or timing of outcome measurement (e.g.: LLIN rotation, sleeper rotation). Results from the meta-analysis has proved that the standard LLINs remain effective against female *Anopheles* vector in terms of killing effects. This finding is in line with the meta-analysis study by Clare Strode et al comparing mosquito mortality between insecticide-treated nets and untreated net with a risk difference of 0.28 (28%).[9]

Our meta-analysis also compared the effectiveness of upgraded LLINs and untreated net through experimental hut studies. The overall risk difference in the female *Anopheles* mortality is increased (0.54 (54%)) using upgraded LLINs versus untreated net. Similarly, high heterogeneity was observed in the comparison of standard LLINs with untreated nets. Inconsistency between studies is related to the study design, execution, reporting format across all experimental hut trials, and possible Recruitment bias such as mosquito density, geographical factor, type of hut, result bias.

In experimental hut trials, the risk difference of mosquito mortality for upgraded LLINs or nets with combination of additional insecticide showed an increase of 24% of anopheles' mortality risk when compared to standard LLINs. However, the high heterogeneity of the results from these studies may masked the real relationship between upgraded LLINs and mortality of the female *Anopheles* when compared with standard LLINs, thus the results need to be interpreted with caution. This may have stemmed from the different level of resistance of the vector studied towards standard LLINs, that contained only one type of insecticide (cypermethrin/permethrin). However, the results have clearly demonstrated that both standard and upgraded LLINs have substantive effect and are more favorable in causing female *Anopheles* mosquito mortality compared to untreated nets in all studies, despite the difficulties in explaining the heterogeneity between studies.

Based on the studies included in this meta-analysis, LLINs remain effective against female *Anopheles* vector about the killing effects although some studies did not clearly mention on the resistance status of the *Anopheles* population used in the study. Ideally, phenotypic resistance, target-site resistance, and metabolic resistance testing should be applied to mosquito populations in the vicinity of the hut trial. If this is not feasible, then a combination of either phenotypic and target-site resistance testing, or target-site and metabolic resistance testing should be performed. One area of concern is that mosquito resistance assessment is not standardized across studies. This might contribute to the high levels of heterogeneity. It is possible that the target-site and metabolic resistance exert a differential impact on LLINs' effectiveness, but most studies failed to accurately assess the presence of metabolic resistance.[20] Of note, phenotypic resistance, as measured by bioassays, is regarded as the first step in identifying resistance.[21]

Exploring Heterogeneity

There are factors that possibly contributed to the high percentage of heterogeneity; clinical and methodological diversity factors (Table 4). There is a discrepancy among the studies in terms of mosquito population, total Number of Adult Female Mosquitoes Caught, condition of the study area, and total duration of the trials. As for mosquito population, the predominant mosquito at trial sites was *Anopheles gambiae*, however, there were also presence of other species of Anopheles. *Anopheles coluzzii*, a member of *Anopheles gambiae* complex was found to share similar habitat (at the trial sites) with *Anopheles gambiae* in experimental hut studies.[11, 13-14, 17] In addition, there were a variety of mosquito's species that share similar habitat.[14-15, 17]

We also found a variety in the number of adult female mosquitoes caught among the trials. The lowest number of adult female mosquitoes caught was by Ngufor et al. 2014 (n=212), while the largest caught was in Ngufor et al. 2016 trial (n=10,518).[13,14] The condition of the study area could present as one of the factors that contributed towards the heterogeneity among the studies. Various study area conditions were observed: rice growing field [11, 12, 17], savanna, [13, 14] forested,[15, 19] and cultivation area.[16, 20] The duration of the trial differed between the studies, with the shortest and longest duration of 3 weeks[17] and 12 weeks[19, 20] respectively.

Table 4 Exploration of the reason for high heterogeneity among the included studies

Reason for Heterogeneity	N'Guessan 2016	Ngufor 2017	Bayili 2017	Camara 2018	Ngufor 2014	Djenontin 2015	Khoffi 2015	Ngufor 2016	Gunasekaran 2016	Pennetier 2013
Mosquitos population	An. Coluzzii An. gambiae	An. gambiae	An. gambiae An. coluzzii	An. coluzzii, An. gambiae, An. funestus, Culex sp., Mansonia sp.	An. Gambiae, Cx. quinquefasciatus	An. gambiae	An. Coluzzii, An. gambiae, An. funestus, Culex sp., Mansonia sp.	A. gambiae	An. fluviatilis	An. Gambiae, An. Funestus, An. arabiensis
Total Number of Adult Female Mosquitoes Caught	2552	736	3079	1328	212	474	4032	10,518	393	294
Condition of the study area	A rice irrigation zone	A large rice-growing field	Wooded savannah	Wet savannah	A village on the outskirt	A horticultural area	A huge rice growing area	A pyrethroid resistant malaria-endemic area	The terrain of the village is hilly and forested	One study area In an area of extensive cotton cultivation and another one near the Lake close to a vegetable farm
Raining Season	-	Rainy season extends from March to October	-	Single annual rainy season from April to October	-	-	One rainy season from April to October	Rainy season from March to October	Rainy season from July to October	-
Design of the hut	West-African style	West-African style	West-African style	West-African style	West-African style	-	-	West-African style	-	-
Wall	Concrete bricks	Brick plastered with cement on the inside	Concrete bricks	Concrete bricks	Brick plastered with cement on the inside	Concrete bricks	Concrete bricks	Cement-plastered brick	Brick walls with cement plaster	Concrete bricks
Roof	Corrugated iron roof	Corrugated iron roof	Corrugated iron roof	Corrugated iron roof	Corrugated iron roof	Corrugated iron roof	Corrugated iron roof	Corrugated iron roof	Tin-sheeted roofing	Corrugated iron roof

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Ceiling	Thick polyethylene sheeting	Palm thatch	Thick polyethylene sheeting	Thick polyethylene sheeting	Palm thatch	Thick polyethylene sheeting	Thick polyethylene sheeting	Palm thatch	Thatched roof	Thick polyethylene sheeting
Base	Concrete	Concrete	Concrete	Concrete	Concrete	Concrete	Concrete	Concrete	Brick and cement	Concrete
Yard	Surrounded by a water-filled channel	Surrounded by water-filled moats	Surrounded by a water-filled channel	Surrounded by a water-filled channel	Surrounded by water-filled moats	Surrounded by a water-filled channel	Surrounded by a water-filled channel	Surrounded by water-filled moats	Surrounded by water-filled moats	Surrounded by a water-filled channel
Windows	4 window slits constructed from pieces of metal, fixed at an angle to create an inverted funnel with a 1 cm wide gap	4 window slits (1cm gap) on the walls	4 window slits constructed from pieces of metal, fixed at an angle to create an inverted funnel with a 1 cm wide gap	4 window slits (1cm gap) on the walls	4 window slits	4 window slits constructed from pieces of metal, fixed at an angle to create an inverted funnel with a 1 cm wide gap	4 window slits constructed from pieces of metal, fixed at an angle to create an inverted funnel with a 1 cm wide gap	4 baffled window slits on the walls	4 windows; grilled with wooden planks fixed horizontally in tilted position one above the other	4 window slits constructed from pieces of metal, fixed at an angle to create an inverted funnel with a 1 cm wide gap
Veranda	A single veranda trap made of polyethylene sheeting and screening mesh	Veranda traps	A single veranda trap made of polyethylene sheeting and screening mesh	A single veranda trap made of polyethylene sheeting and screening mesh	Veranda Traps	A single veranda trap made of polyethylene sheeting and screening mesh	A single veranda trap made of polyethylene sheeting and screening mesh	A veranda trap on the fourth wall	Veranda trap	A single veranda trap made of polyethylene sheeting and screening mesh
Bioassay Procedure	Standard WHO cone and tunnel bioassays	Tunnel Test	Standard WHO cone bioassays	Standard WHO cone bioassays	Tunnel Test	-	Standard WHO cone bioassays	Standard WHO cone and tunnel bioassays	Standard WHO cone bioassays	Standard WHO cone bioassays
Random Allocation of the Sleeper	-	-	Yes, into 6 experimental huts	Randomized Greco-Latin square scheme	-	A Latin Square Design	-	Randomized Latin square design	A Latin Square Rotation Scheme	-
Sleeper Rotation	-	Rotated through the huts daily	Rotated on consecutive nights	Rotated each night	-	Rotated among huts each night	Rotated randomly each night	Rotated on successive night	Rotated daily	-

Treatment (Net) Rotation	-	-	Rotated weekly between huts	Rotated among the huts each week	-	Rotated treatments each night	-	Rotated every 2 days on each week	Rotated weekly	Rotated each week among the huts according to
Sleep Duration	Overnight until the next morning	9 pm until 5 am the next morning	Overnight until the next morning	Overnight until the next morning	Enter at 8 pm until 5 am the next morning	Enter at 9 pm and remained inside until dawn	Enter at dusk and slept until dawn	Overnight until the next morning	Enter at 7 pm until 5.30 am the next morning	Overnight until the next morning
Net Holes	Six holes (4cm x 4cm)	Six holes (4cm x 4cm)	Six holes (4cm x 4cm)	Six holes (4cm x 4cm)	Six holes (4cm x 4cm)	Variety of 6, 30, 150 holes (4cm x 4cm)	30 holes (4cm x 4cm)	Six holes (4cm x 4cm)	Six holes (4cm x 4cm)	Six holes (4cm x 4cm)
Active Ingredient Concentration for Standard LLIN	Cypermethrin 200 mg/m^2	Cypermethrin 200 mg/m^2	Cypermethrin 200 mg/m^2	Cypermethrin 200 mg/m^2	Permethrin 2% (w/w)	Permethrin 2% (w/w)	Permethrin 2% (w/w)	Permethrin 150mg/m^2	Permethrin 2% (w/w)	Permethrin 2% (w/w)
Active Ingredient Concentration for Advanced LLIN	(Cypermethrin 100 mg/m^2 + Chlorfenapyr 200 mg/m^2)	(Cypermethrin 100 mg/m^2 + Chlorfenapyr 200 mg/m^2)	(Cypermethrin 200 mg/m^2 + Chlorfenapyr 200 mg/m^2)	(Cypermethrin 100 mg/m^2 + Chlorfenapyr 200 mg/m^2)	Permethrin 2% (w/w) + pyriproxyfen 1% (w/w)	Permethrin 2% (w/w) + pyriproxyfen 1% (w/w)	Permethrin 2% (w/w) + pyriproxyfen 1% (w/w)	Permethrin 150mg/m^2 + pyriproxyfen 250mg/m^2	Permethrin 2% (w/w) + Piperonyl Butoxide 1% (w/w)	Permethrin 2% (w/w) + Piperonyl Butoxide 1% (w/w)
Net Wash for Standard LLIN	20 washes	0 wash	20 washes	20 washes	0 wash	0 wash	0 wash	0 wash	20 washes	20 washes
Net Wash for Advanced LLIN	20 washes	0 wash	20 washes	20 washes	0 wash	0 wash	0 wash	0 wash	20 washes	20 washes
Mosquitoes Collection Duration of the Trials	Collected in the morning 72 nights ~ 10 weeks	Collected in the morning 54 days ~ 8 weeks	Collected at 5:30 a.m. 8 weeks	Collected in the morning 36 nights ~5 weeks	Collected at 5 am -	Collected at 6 am 43 nights ~ 6 weeks	Collected in the morning 24 nights ~ 3 weeks	Collected in the morning 7 weeks	Collected in the morning 12 weeks	Collected in the morning 12 weeks
Raw Data Reported	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes

Subgroup Analysis

In a subgroup analysis, all included studies are split into subgroups and meta-analysis is performed on one or more of these subsets.[22] Such analyses are used to investigate the sources of heterogeneity and provide the estimates of effect (risk difference) for relevant subgroups of LLINs, i.e., the risk difference may vary among different subgroups of LLINs. If the trials are subgrouped and there is no heterogeneity within trials, then valid conclusions can be drawn using results from the subgroup analysis. To determine whether a statistically significant subgroup difference was detected, the p-value from the test for subgroup differences ought to be considered. Instead of a more traditional level of 0.05 as the significance level, in many practices, experts recommend a p-value < 0.10 as statistically significant subgroup effect due to the low power of heterogeneity while avoiding type II errors.[23]

In the presence of statistical heterogeneity, it is tempting to identify outlier studies and exclude them successively until the statistical test of heterogeneity is no longer statistically significant. However, this approach might be considered as a risky practice because excluding studies that appear to be accountable for the heterogeneity might be illuminating when it reaches to sensitivity analysis.[23]

CONCLUSION

In summary, the overall effect in terms of mortality of *Anopheles* favors the upgraded LLINs compared to the standard LLINs or untreated net. Thus, the utilization of these nets in the field for malaria prevention and program can help achieve the national and global target as well as better health outcomes for those living in the endemic areas. Worthy future research or review studies would be on exploring field research and analysis of cost effectiveness in long-term usage of upgraded LLINs that would help the policy makers and stakeholders for acquiring fund for mass distribution of nets to the public. Further study on field durability, user adherence and potential resistance development could also be considered in the future program.

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