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Malaria and filariasis transmissions in endemic areas of Abakaliki Ebonyi State, Nigeria: use, misuse and challenges of insecticide treated net intervention strategy

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Abstract
Of the interventions, insecticide treated nets (ITNs) are least documented for concurrent malaria and lymphatic filariasis (LF). This study was conducted to assess the effectiveness of intervention on malaria and LF transmission indices in Abakaliki Ebonyi State, Nigeria. Preliminary survey identified 59 misusing households (MHs) and preceded cross sectional survey on ITN misuse. Thirty households from ITN misusing households (MH) and non-misusing households (NMH) served as permanent cohorts. The study also investigated the level of usage /non usage after mass distributed free ITN. Mosquitoes caught with pyrethrum spray catch (PSC) and aspirator were identified using standard morphological keys. Seventy five percent (75%) used ITN for other purposes than vector control while 25% didn't utilize it for mosquito control. About 68% didn't know the effect of using ITN for purposes other than malaria / LF while 32% were familiar with possible effects. Overall, 1,367 endophilic mosquitoes with 8.8 resting densities were caught; *Anopheles gambiae* sl (59.5%), An. *funestus* sl (0.8%), *Culex quiquefasciatus* (34.6%), *Aedes aegypti* and *Mansonia africana* (5.0%). Catches from MHs were significantly higher than NMH (65.0% versus 32.2%). The differences in filarial infection, infective and physiological status for the cohorts were insignificant (p>0.05). The calculated sporoziote infection rate (SR) for MH (8.9% and NMH (1.6%) differed (p<0.05). The entomological inoculation rates (EIR) recorded was 0.11 per person per night culminating in an annual 40 infective bites per person per year. Challenges regarding ITN use/nonuse were reported. The study highlights some malaria and LF risk indices in the context of on-going elimination using ITN intervention control strategy.

Keywords: malaria and lymphatic filariasis, transmission indices, vectors, insecticide treated bednets, misusing and non-misusing households, challenges

Introduction

Malaria and lymphatic filariasis (LF) are Africans most important vector-borne diseases. They are among the biggest health problems in sub Saharan Africa with contributions to morbidity and mortality among people in Africa as a subject of academic interest, political advocacy and thoughts (Snow et al., 2005) [38]. In sub Saharan Africa alone, 400 million persons are at risk and nearly all the 1 million death per annum from malaria in the world occur (Snow et al., 2005) [38] while over one-third of the 146 million people are infected with LF from Africa (Michael and Bundy, 1997) [24]. Malaria accounts for more cases and deaths in Nigeria than any other country in the world (US Embassy in Nigeria Malaria Fact Sheet, 2016) [1]. In addition, Nigeria is the third most endemic for LF worldwide with the largest population at risk in African continent (Eigege et al., 2002) [11]. In Africa including Nigeria, their transmissions are maintained by the presence of mosquito vectors mainly *Anopheles*, optimum local condition, scarce resources, poor environmental conditions, social political and economic instabilities (Elom et al., 2013) [12]. Both diseases co-exist in the same human populations with *Anopheles* playing dual role in their transmission suggestive of control strategy of integrated vector management targeting both diseases concurrently.

Of the interventions, insecticide treated nets (ITNs) which is a central component of current global malaria control initiatives has had overwhelming evidence as malaria control strategy. To demonstrate the success of vector control programme, careful evaluation of infection levels in human or vector following intervention is important. Due to ethical issues and reluctance to submit to regular blood examination, assessment of infection in vectors offer advantages to monitoring infection after implementation of intervention. Report exist on reduction in individual risk of morbidity and mortality associated with ITN use (Gambler et al., 2007; Lengeler, 2004), individuals' not sleeping under an ITN but living within an area of ITN on ITN coverage (Killeen et al., 2007; Howard et al., 2000) [21, 19]. Limited studies on evaluation of ITN on LF, nevertheless showed evidence for reduced morbidity associated with LF (Amaechi et al., 2017; Reimer et al., 2013; Pederson and Mukoko, 2002) [3, 35, 25]. Regrettably, this intervention has been used to assess each disease differentially. Consequently, misuse of nets has remained a growing concern that demands attention from researchers and policy makers (Honjo et al., 2013) [18].

Detailed knowledge of local determinants of malaria or LF transmissions is of primary importance in the development of area-specific control interventions that will effectively reduce the burden of the disease. Evaluation of the impact of ITN on these cohorts and possible challenges is crucial especially in sub Saharan Africa where there are inadequate medical facilities. Previous studies have not put these into considerations. Therefore, this study was aimed at assessing the impact of use, misuse and challenges of ITN in reducing malaria and LF transmissions.

Material and Methods

Study Area- The study was conducted in three sentinel villages (Obeagu Ibom, Mgbabeluzor and Okarie Echida) of Abakaliki Ebonyi State Nigeria (figure 1) between February 2016 and December 2017. The ecology, climate and topography together with the human activities which encouraged malaria and LF transmissions have been described in details (Amaechi et al., 2015) [2]. Their houses are predominantly constructed with mud and floors and thatched roofs. In these areas, both old and young people are constantly exposed to the bites of mosquitoes.

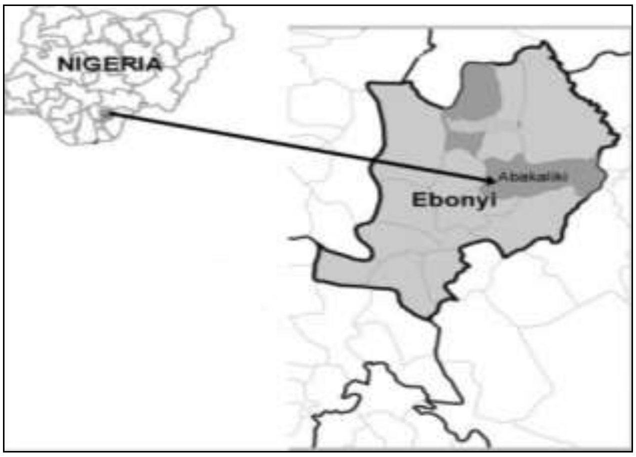

Fig 1: Map showing the location of the study area.

Ethical approval

The comprehensive protocol including the consent forms were approved by the Ebonyi State Ministry of Health and Post graduate board of Imo State University Owerri.

ITN study

The sentinel ITN study was conducted in the villages selected as a follow up to ITN impact surveys on LF (Emukah et al., 2009). ITN intervention was conducted in February 2016. The primary objective was to measure the impact of ITN on malaria and LF transmissions by the vectors to assist with certificate of elimination. Resource use was categorized in accordance with three phases of the project; start up, ITN distribution and entomological monitoring of ITN impact. In the first phase, meetings were held in each village to inform members about the study rationale and design and to provide opportunity for questions. In the distribution phase, researchers team distributed Perma Net 2.0, an insecticide treated bed net impregnated with deltamethrin per square meter (Vestergaard Frandsen). The ITNs were distributed by research team at central points in each village following a pre-arranged schedule. Each family was responsible for sending one representative to collect the nets and to receive training on how to ensure that ITNs were properly deployed. Preliminary survey on status of ITN misuse was undertaken and households misusing nets (MHs) for purposes other than malaria and LF control were identified. Inform consent of the household heads was obtained on the extent of net misuse. Thereafter, thirty households randomly selected from both misusing households (MHs) and non-misusing households (NMHs) served as permanent cohorts for entomological study. A pretested questionnaire was also used to obtain data on usage and reasons for non-usage.

Harvest of mosquitoes

Mosquitoes were collected by aspirators and pyrethrum spray catch (PSC) techniques according to WHO (1975) [42]. They were concurrently used to increase mosquito catch in terms of physiological status and differences in feeding and resting habits. Collected species were preserved and process individually for infection and infectivity status.

Laboratory analysis

The standard external and sex keys previously used by Gillet (1972) [17] was used for the identification of the harvested mosquitoes. After which all the parous and blood fed (for physiological conditions) were counted. The head, thorax and abdomen of mosquitoes caught in each household during the survey were dissected under the dissecting microscope and larval stages assessed (Nathan, 1981) [27]. Filarial infectivity rates were calculated (Kasili et al., 2009) while *Plasmodium* sporoziotes were assessed in randomly selected *Anopheles* species only. Dissection of the salivary glands for sporoziotes and ovary for parity was carried out according to the techniques of WHO (2002) [4]. Determination of Entomological Inoculation Rates (EIR) necessitated two other measurements; the sporoziote rate (SR) and human biting rate HBR, (WHO, 2013) [44].

SR=No of mosquitoes with sporoziotes / No of dissected mosquitoes

M=F/W F= total No of freshly fed mosquitoes, W= total No of human occupants in households used for collection, M= human biting rates

EIR= HBR X SR/100

Statistical Analysis

Data generated on infection / infectivity rates were subjected to analyses of variance Chi Square test of association using online statistical package (www physics csbsju edu/stats) while density was calculated as simple percentages. Probability level of 0.05 was considered significant. Data on questionnaires were entered into Microsoft Excel data sheet, cross checked and transferred and analysed using table and percentage distribution.

Results

Greater proportions of the respondents (74.6%) used ITN for other purposes than malaria and LF control. Cover of household problems (62.7%) was the major reason for misuse. About 67.9% didn't know the effect of using ITN for purposes other than malaria and LF control strategies while 32.2% were familiar with the possible effects of using ITN for purposes other than malaria and LF control programmes. Majority 66.1% responded that the frequency of utilizing ITN were limited to sometimes than utilizing on regular basis (33.9%) and knowledge of breeding habits of mosquitoes are summarized (Table 1). *Anopheles gambiae* (59.5%) was the predominant vector caught (Table 2), there was inter village variations ($p<0.05$). Catches from ITN misusing households were significantly higher than non ITN misusing households (65.0% versus 32.2%, Table 3). Tables 4 and 5 showed that the differences in filarial infection rates (0.0% versus 0.2%) as well as physiological status of the vectors from the two cohorts were insignificant ($p>0.05$).

Overall, 10.5% *Anopheles* were positive for the *Plasmodium* sporoziote (Table 6). The SR calculated for the cohorts (1.6% versus 8.9%) showed significant difference ($p<0.05$). The HBR of *Anopheles* ranged 1.0 to 2.2 bites per night per person and showed a significant difference ($p<0.05$, Figure 2). The EIR was calculated per person per night and had average of (0.11) culminating in 40 bites per year per person for the study community that had one infective mosquito. EIR for the ITN coverage cohorts showed no significant difference ($p>0.05$, Table 7). Challenges of using ITN are summarized in Table 8.

Table 1: Results of ITN misuse by the inhabitants (N = 59)

S/N	Variables	Frequency	Percentage (%)
1.	Status of misuse		
	Protecting houseflies	41	69.5
	Covering household materials	3	5.1
	Not used for mosquito control	15	25.4
2.	Reasons for misuse		
	Need to cover household problems	37	62.7
	Misunderstanding of its importance	7	11.9
	Technical limitation	10	16.9
	Others	5	8.5
3.	Perception on the effects		
	Spread of malaria	29	49.2
	Spread of filarial (LF)	0	0.0
	Economy decline	8	13.6
	No adverse effect	19	32.2
	Others	3	5.1
4.	ITN ownership		
	1	7	11.9
	2	21	35.6
	3	19	32.2
	>3	12	20.3
5.	Frequency of use		
	Regular	20	33.9
	Sometimes	39	66.1
6.	Time of advice and utilization		
	Susceptible malaria outbreak	21	35.6
	Prepardness for malaria program	11	18.6
	Reported cases from family members	9	15.2
	Others (public meeting and social affairs)	18	30.5
7.	Knowledge of breeding habitat of mosquitoes		
	Nearby garden/farm	31	52.5
	Dust bins	13	22.0
	Open/discarded containers	4	6.8
	Others	11	18.6

Table 2: LF and malaria vectors in the study area

Study communities	Species collected (%)				Total
	An. gambiae sl	An. funestus sl	Cx. quinquefasciatus	Others	
Obeagu Ibom	250 (65.1)	11 (2.9)	105 (27.5)	18 (4.7)	384 (28.1)
Mgbabeluzor	408 (57.3)	0 (0.0)	206 (36.8)	39 (5.5)	707 (51.7)
Okaria Echida	150 (56.5)	0 (0.0)	108 (39.1`)	12 (4.3)	276 (20.2)
Total	814 (59.5)	11 (0.8)	473 (34.6)	69 (5.0)	1,367

Note: Others = Ae. Aegypti and Mn. africana

Table 3: Overall composition and relative abundance of mosquitoes by households

Species	Insecticide treated bed nets	
	Non-misusing households	Misusing households
An. gambiae sl	297 (36.5)	517 (65.5)
An. funestus sl	2 (18.2)	9 (81.8)
Cx. quinquefasciatus	126 (26.6)	311 (65.8)
Others	17 (24.6)	52 (75.4)
Total	442 (32.2)	889 (65.0)

Table 4: Infection status of mosquitoes by households

LLN Coverage	Mosquito species	No (%) Caught/ Dissected	No containing larvae (L_1-L_3)	No infective (L_3 in head)	Infection rate (%)	Infective rate (%)
LLIN Non misusing households	*An. gambiae sl*	297 (36.5)	0	0	0	0.00
	An. funestus sl	2 (18.2)	0	0	0	0.00
	Cx. quinquefasciatus	126 (26.6)	0	0	0	0.00
	Others	17 (24.6)	0	0	0	0.00
	Total	442 (32.3)	0	0	0	0.4
LLIN Non misusing households	*An. gambiae sl*	517 (63.5)	5	2	1.0	0.00
	An. funestus sl	9 (81.8)	0	0	0	0.00
	Cx. quinquefasciatus	311 (65.8)	0	0	0	0.00
	Others	52 (75.4)	0	0	0	0.00
	Total	889 (65.0)	5	2	0.6	0.2

Table 5: Physiological status of mosquitoes in the study areas by households

Species	Parity Status (%)					
	Non misusing households			Misusing households		
	Fed	Unfed	Gravid	Fed	Unfed	Gravid
An. gambiae sl	57.6	23.2	16.2	37.7	40.4	4.9
An. funestus sl	50.0	50.0	0.0	22.2	0.0	77.8
Cx. quinquefasciatus	8.7	42.9	48.4	29.9	20.9	49.2
Others	0.0	64.7	35.3	17.3	5.8	76.9

Table 6: Sporozoites infection rates of salivary glands of *Anopheles* mosquitoes collected by PSC.

Study communities	No (%) caught	No (%) dissected	No infected PSC n (%)	Sporozoites (%) rates 124n (%)
Obeagu Ibom	327 (39.6)	46 (14.1)	4 (0.5)	4 (3.2)
Mgbabeluzor	471 (57.1)	51 (10.8)	9 (1.1)	9 (7.3)
Okaria Echida	27 (3.3)	27 (100.0)	0 (0.0)	0 (0.0)
Total	825	124 (15.0)	13 (1.6)	13 (10.5)
ITN Coverage				
Non misusing households	299(36.2)	62(20.7)	2(0.2)	2(1.6)
Misusing households	526(63.8)	62(11.8)	11(1.4)	11(8.9)

Table 7: Entomological inoculation rates of *Anopheles* species

ITN coverage	EIR
Non misusing households	0.19
Misusing households	0.02
Total	0.11

Table 8: Reported challenges of using ITN in the study area

S/N	Reported challenges	No sampled	Use (%)	Non-use (%)	
1.	Level of Usage (N= 90)	30	13 (43.3)	17 (56.7)	
	Obeagu Ibom	30	9 (30.0)	21 (70.0)	
	Mgbabeluzor	30	17 (56.7)	13 (43.3)	
	Okaria Echida	90	39 (43.3)	51 (56.7)	
	Total				
2.	Individuals using ITN (N= 39)				
	Study communities	Usage	Always	sometimes	
	Obeagu Ibom	13	5 (38.5)	8 (61.5)	
	Mgbabeluzor	9	4 (44.4)	5 (55.6)	
	Okaria Echida	17	7 (41.2)	10 (58.8)	
	Total	39	16 (41.0)	23 (59.0)	
3.	Study communities	Hot/heat	Phobia	Can't prevent LF and Malaria	In effective
	Obeagu Ibom	9 (50.0)	4 (22.2)	2 (11.1)	3 (16.7)

	Mgbabeluzor	11 (57.9)	3 (15.8)	1 (5.3)	4 (21.1)
	Okaria Echida	13 (61.9)	3 (14.3)	3 (14.3)	2 (9.5)
	Total	33 (56.9)	10 (17.2)	6 (10.3)	9 (15.5)
4.	Reasons for not hanging ITN (N= 48)				
	Low mosquito activities	17		(35.4)	
	Low night time temperature	7		(14.6)	
	Lack of sustainable place	13		(),27.1	
	Fear of chemical irritation	2		(4.2)	
	Smell of ITN	1		(2.1)	
5.	Pattern of deployment of ITN (N= 72)				
	Hung over bed or mat	54		(75.0)	
	Hung over the window	13		(18.1)	
	Hung over the door	3		(4.2)	
	Use of blanket	2		(2.8)	
6.	Reasons for non-usage of properly deployed nets (N= 71)				
	Hot night time temperature	53		(74.6)	
	Low mosquito activity	3		(4.2)	
	Forget to hang it	5		(7.0)	
	Too tired to hang it	3		(4.2)	
	Torn net	5		(7.0)	
	Disturbing sleep	2		(2.8)	

Discussion

Despite relationship between malaria and LF transmissions, there exists conspicuous lack of data on the simultaneous interruption by the vectors on integrated ITN, a fact which appear to suggest that this could only be assessed by parasitological evidence. An understanding of the local vectors, bionomics and transmission intensity are vital for successful control. The fact that catches from misusing households were twice higher than catches from non-misusing households (65% versus 32.2%) reflected how the inhabitants received bites from these vectors and contributions of ITN misuse. The proportions of *Anopheles gambiae* often decrease with ITN introduction (Bayoh et al., 2010) [8]. On the contrary, An. *gambiae* displayed plasticity in feeding behaviour and exhibited anthropophagy for both cohorts (Awolola et al., 2005; 2002) [6, 5]. This call for molecular assay for sibling species role, effective / efficient dominant vector species (DSV) of human malaria and LF.

The human blood index (HBI, represents the proportion of blood fed mosquitoes on people) and parity status of *Anopheles* species when judged from previous works in Nigeria (Okwa et al., 2006; Awolola et al., 2005) [28, 6], clearly expressed a high degree of human-vector contacts. The HBI of An. *gambiae* remained high in both cohorts indicating that they were unable to adapt to the intervention and shift its host preference. This preference for human blood meal is a specialization that allows for sustained high level of transmission (Lyimo et al., 2013) and the remarkable responsiveness could be attributed to human host cues such as carbon dioxide, odour and warmth (Omolo et al., 2013; Clement, 1999) [10]. Results from ITN misuse support poor utilization and identified potential factors included education status, colour and shape of net as well as sleeping arrangement. Often time ownership does not translate to utilization. The reported misuse of ITN for purposes other than LF and malaria control and challenges as prime factors have been reported elsewhere (Rissa, 2009) [37]. These together with misconceptions need to be addressed through educational campaign especially training and visit (TV).

No cross infection of the parasites were found in the vectors. Contrary to this, Awolola et al (2006) [7] found co-infection of *Plasmodium falciparum* and W. *bancrofti* in a single mosquito specimen and attributed it to behaviour of local vector species, asynchronous development or specific differences in the duration of parasite cycle. Further study is on-going to clarify this observation. Based on entomologic study, we found that ITN alone can reduce mosquito malaria and LF infection rates to a point where sporoziotes and infective larvae (L_3) are no longer detectable by dissection. The study identified *Anopheles gambiae* as the main vector and the infections as Anopheles-driven. Michael et al (2004) [25] and Pichon (2002) [33] had advocated for combination of mass drug administration (MDA) and net than MDA alone in LF Anopheles-driven condition. However, the result of this study demonstrated that net alone without misuse can halt LF transmission but without effect on malaria transmission. The transmission threshold below 0.65% mosquito larval infective rate has been established (Pederson et al., 2009) [32]. The study found this infective rate (0.0% versus 0.2%) which is less than values by Pederson and co. Comparison from these cohorts as well as previous reports on LF vectors infectivity rates seems to confirm that LF transmission has been halted by net in general and in ITN misusing and non-misusing households in particular.

The similarities in *Anopheles* infection rates could indicate that ITN stoppage of LF transmission may be by provision of barrier between the infected (microfilaremic) human carriers and the mosquito vectors. This stands out as convincing evidence that sustained education to regulate misuse and scale up of ITN distribution is imperative for control.

The findings herein indicated that entomological indices of malaria transmission (EIR and SR) are well established in the area, thus explaining endemicity. Beier et al (1999) [9] opined that the intensity of malaria parasite transmission is normally expressed as EIR and that in Africa it is highly variable ranging from <1 to >1,000 infective bites per person per year. The sporoziote rate 10.5%, 1.6% versus 8.9% and EIR 0.11, 0.19 versus 0.02 per person per night recorded are within available literatures (Aju-Ameh et al., 2016; Msugh-Tur et al., 2014; Massebo et al., 2013; Awolola et al.,2003) [1, 26, 23, 5]. However, sporoziote rates higher than these have been reported in parts of Nigeria (Omulu et al., 2015; Olayemi and Ande, 2008) [31, 30] and elsewhere in Africa (Fradin, 1988) [15]. This probably points to malaria vectorial system being more complex than expected. The comparable sporoziote rates for the cohorts may not be unconnected with human activities. This suggests that infective females that are compromised with *Plasmodium* parasites can put the inhabitants at risk of malaria disease.

Reddy et al (2011) [34] and Russel et al (2011) [36] posited that after ITN introduction, mosquitoes may change from feeding indoors to outdoors or from human to other animals (Takken and Verhulst, 2013) [40]. This may explain the density and infectivity status of opportunistic members of An. *funestus* and C. *quinquefasciatus.* Such behavioral shift could undermine the effectiveness of ITN intervention (Wanne et al., 2015) [41] and any control strategy aiming at control will factor in this heterogeneities. Continued human infections in the cohorts suggest the potential for resumption of transmission up on a drop in ITN ownership, use and continued misuse. Given these data, LF transmission would be said to have been suppressed rather than interrupted while malaria transmission remained unaffected. The challenge then remains to maintain sufficient net ownership and proper knowledge on usage.

The limitations of the study included first, the ITN arm (misusing and non-misusing) to which sentinel households belonged were obvious to the survey terms. Secondly, the villages were selected as a follow up to previous studies conducted and willingness to always participate. Obviously, this was needed for regular entomologic survey but may have introduced a bias. Finally, the frequent visitation of the research team to the same sentinel households may have encouraged the awareness about malaria, LF and link with mosquitoes, thus greater use of the nets and potential to assess misuse effect.

Conclusively, An. *gambiae* complex being the major vector implies targeting it with whatever method that is appropriate. Clear and comprehensive understanding of transmission dynamics of both diseases are crucially needed in the context of vector control which is an important component of integrated management.

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