THE USEFUL LIFE OF BEDNETS FOR MALARIA CONTROL IN TANZANIA: ATTRITION, BIOEFFICACY, CHEMISTRY, DURABILITY AND INSECTICIDE RESISTANCE

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Contents

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2. Acronyms

ASTMH	American Society of Tropical Medicine and Hygiene
CRA-W	Walloon Agricultural Research Centre
GLOBVAC	Global Health and Vaccination Research Program of the RCN
HPLC	High Performance Liquid Chromatography
IHI	Ifakara Health Institute
ITT	Ifakara Tunnel Test
LLIN	Long-Lasting Insecticidal Net
LSHTM	London School of Hygiene & Tropical Medicine
NIMR	National Institute for Medical Research
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PMI	President's Malaria Initiative
RBM	Roll Back Malaria
RCN	Research Council of Norway
SOP	Standard Operating Procedure
Swiss TPH	Swiss Tropical and Public Health Institute
NMBU	Norwegian University of Life Sciences
VCWG	Vector Control Working Group
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme

3. Changes to the study protocol

- Drop follow-up time point at 30 months due to increased costs of running the field work. Households will be followed up after 10 (complete), 22 and 36 months.
- The experiments in the semi-field system (Ifakara Tunnel Test; ITT) were not performed on retrospective Olyset campaign nets due to a low recapture of mosquitoes within the tunnel chambers. This system has been optimized to be ready for testing prospective ABCDR LLINs.

4. Project result dissemination 2014/15

March 2014	**Oral presentation "Geographical variation of factors affecting durability of Long-Lasting Insecticidal Nets (LLINs) in Tanzania"** Presenter: Zawadi D. Mageni Venue: LSHTM Malaria Centre Retreat 2014, Brighton (UK)
Aug 2014	**Report "Retrospective net report for NMCP"** Author: Lena M. Lorenz Distribution: NMCP, NIMR, PMI
Oct 2014	**GLOBVAC donor visit in Bagamoyo, Tanzania** **Oral presentations:** – "Malaria situation in Tanzania" (Renata Mandike) – "Overview of the national insecticide treated nets (NATNETS) programme" (Karen Kramer) – "ABCDR project overview, collaborations and future of the project" (Hans J. Overgaard) – "ABCDR Field work report" (Zawadi D. Mageni) – "ABCDR Retrospective results" (Lena M. Lorenz) – "Insecticide resistance in Tanzania: A threat to malaria control" (Robert C. Malima)
Nov 2014	**Oral presentation "Long-lasting insecticidal nets (LLINs) for malaria control in Tanzania: Attrition, physical and chemical decay"** Presenter: Dennis J. Massue Venue: Health Intervention Unit Meeting, Swiss TPH, Basel (Switzerland) **Oral presentation "ABCDR Project – Field work report"** Presenter: Zawadi D. Mageni Venue: BCC Sub-Committee Meeting, PSI, Dar-es-Salaam (Tanzania) **Poster presentation "ABCDR - A retrospective evaluation of LLIN durability after 2-4 years in Tanzania"** Presenter: Lena M. Lorenz Venue: ASTMH 63rd Annual Meeting, New Orleans (USA)

Dec 2014	**Publication "Investigating mosquito net durability for malaria control in Tanzania - Attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): study protocol"** Lorenz, L.M. *et al* (2014) *BMC Public Health* 2014, **14**:1266 **Technical Report "Detection and monitoring of insecticide resistance to malaria vectors in Tanzania mainland"** Prepared by NIMR
Jan 2015	**Oral presentation "Updates on ABCDR study in Tanzania"** Presenter: Lena M. Lorenz Venue: 10th RBM-VCWG Meeting, LLIN Durability Workstream, Geneva (Switzerland)
Feb 2015	**Report "Prospective net report: Physical degradation of 10-month old LLINs based on data collected by ABCDR study in Aug – Oct 2014"** Author: Lena M. Lorenz Distribution: NMCP, NIMR, PMI, LLIN Taskforce committee
March 2015	**Oral presentation "ABCDR Study – First prospective results for BCC messaging"** Presenter: Lena M. Lorenz Venue: LLIN Taskforce Meeting, NMCP, Dar-es-Salaam (Tanzania) **Oral presentation "Updates of insecticide resistance to malaria vectors: A threat to malaria control in Tanzania"** Presenter: Robert Malima Venue: NMCP-PMI MOP2016 Consultative Meeting, Dar-es-Salaam (Tanzania) **Poster presentation "Factors affecting the durability (attrition, bio-efficacy, chemical residue and physical integrity) of Olyset® nets from national distribution campaigns in eight districts of Tanzania"** Presenter: Dennis J. Massue Venue: 9th Conference on Global health and Vaccination Research, Oslo (Norway)
April 2015	**Manuscript "Factors affecting the durability (attrition, bio-efficacy, chemical residue and physical integrity) of Olyset nets from national distribution campaigns in eight districts of Tanzania"** Submitted to Malaria Journal Massue *et al* (2015)

5. Completed Activities – March 2014 – March 2015

March 2014	– Study coordinator Lena Lorenz in Tanzania – Logistics planning of first prospective follow-up in August 2014 – Development of prospective questionnaire and informed consent
April 2014	– PhD candidate Dennis Massue successfully upgraded at Swiss TPH – Establishment of return net database - logging of all nets returned from the retrospective field work under supervision of Jason Moore – Random sub-sampling of 200 Olyset nets for physical, biological and chemical assessment
	– NIMR training of field implementers and malaria focal persons from 19 sentinel districts on detection and monitoring of malaria vectors resistance to insecticides
May-Jun 2014	– NIMR resistance fieldwork including mosquito larval collection and insecticide susceptibility testing in 18 sentinel districts
June 2014	– Zawadi Mageni in the Netherlands to attend course "Using GIS in disease control programmes" (2 weeks)
April – July 2014	– Retrospective laboratory analyses of Olyset nets (N=200) in Bagamoyo, Tanzania under supervision of Dennis Massue o D component: Hole index using a frame o B component: ▪ Sample knock-down and mortality of *An. gambiae* s.s. using WHO cone tests ▪ Sample blood-feeding inhibition and mortality using WHO tunnel tests on failed samples in Muheza
July 2014	– Shipment of 200 Olyset net samples to CRA-W for HPLC analysis – Recycling of all retrospective Olyset nets not used for the biological and chemical efficacy tests by A-Z Textiles Ltd – Prospective questionnaire programmed by IHI Data Central using ODK Collect – Lena Lorenz in Tanzania to prepare for field work logistics with Jason Moore – Development of metal frame with zoning for hole counts of nets in the field in a controlled and replicable manner. Development of SOP for building and use of frame for training purposes – Study PI Hans Overgaard in Tanzania for pre-training meetings – Informal ABCDR project meeting to plan field work, training and publication strategies attended by Hans Overgaard, Sarah Moore, Jason Moore, Dennis Massue, Zawadi Mageni and Lena Lorenz
Aug 2014	– 6^{th} – 9^{th} August: Training of field enumerators for prospective field work – Summary of net landscape in Tanzania based on retrospective results (questionnaire and return net database)
Jul– Aug 2014	– NIMR molecular laboratory work (PCR species identification and characterisation of insecticide resistance) to determine mosquito species and distribution of resistance mechanisms across the sentinel districts

Sept 2014	– WHOPES continue to uphold the withdrawal of Netprotect approval after re-evaluation
Oct 2014	– HPLC results returned from CRA-W – GLOBVAC committee visit to Bagamoyo. Lena Lorenz and Hans Overgaard travel to Bagamoyo to attend the site visit. – Analysis of retrospective results: physical degradation (hole counts), bio-efficacy (WHO cone tests) and chemical residue (HPLC)
Aug-Oct 2014	– Continuous prospective field work moving through 8 study districts (in order): Bagamoyo, Kinondoni, Kilosa, Iringa Urban, Mbozi, Kahama, Geita, Musoma Rural by field team comprising 15 field enumerators, 2 field managers and 2 drivers plus 1 technical support staff (based in Dar) and 1 logistics manager (based in Bagamoyo) – Real time data cleaning of survey data collected with tablet questionnaire continuously as every district is completed. – Regular communication between study coordinator and data manager Lena Lorenz and field management to optimise data collection and quality – Field work completed 1 day ahead of schedule – Total household completion rate: 3,143/3,398 households (92.5%) – Households temporarily unavailable, e.g. on farms: 74 (2.2%) – **Household loss to follow up**: 181/3,398 (5.3%); of which 23 households (12.7%) moved and 158 households (87.3%) withdrew consent – Information collected on 91.8% of nets distributed in 2013 (9,726/10,598), i.e. **net loss to follow up** = 8.2%
Oct–Dec 2014	– PhD candidate Dennis Massue at Swiss TPH in Basel for course work. Courses completed: 1. Epidemiological concepts 2. Epidemiological methods 3. Basic Biostatistics 4. Statistical Modelling 5. Interdisciplinary Seminar - Control of Infectious diseases and other issues 6. Methods in Qualitative Health Researches
Nov 2014	– Lena Lorenz attended ASTMH in New Orleans to present results and represent the ABCDR project
Nov-Dec 2014	– Random sub-sampling of households (48 per net product – 6 per net product per district) from net data: team of 2 technicians and 1 driver visited sub-sampled households and collected all the nets from the household. – Replaced collected nets with the same number of nets and remove households from study. – Net products replaced with the same product except for Netprotect nets, which are being replaced with Olyset (current best care in Tanzania) due to the withdrawal of WHOPES approval

Dec 2014	– Sarah Moore and Lena Lorenz together with Olivier Briët (Swiss TPH) obtain funding from PMI to investigate the relationship between holes and insecticide content in determining blood-feeding success of mosquitoes using a semi-field system
Jan 2015	– Data cleaning of whole data set – Initial data exploration and trouble-shooting – PhD candidate Zawadi Mageni's Upgrading at LSHTM, London – corrections to be submitted within 6 months – Lena Lorenz and Sarah Moore attend VCWG (Geneva) to present results and represent ABCDR project – Lena Lorenz and Zawadi Mageni submit an operational research concept note to PMI to fund a related net project, investigating the life of a net within the household using a mixed-methods approach
Jan – March 2015	– PhD candidate Zawadi Mageni in London, working with PhD supervisors Jo Lines and Lena Lorenz and attending 2 MSc courses. Courses completed: o Statistical Methods in Epidemiology o Medical Anthropology and Public Health – Jason Moore and Sarah Moore develop new methodologies for ITT
Feb–March 2015	– Jason Moore and Dennis Massue optimise the ITT for experiments to start late April 2015 – Lena Lorenz in Tanzania o pre-planning of field work Aug 2015 (22 month follow up) o analysis and publication plan of retrospective durability paper – Dennis Massue in Oslo to attend 9th Conference on Global Health and Vaccination Research – NIMR to prepare resistance field activities – purchase of reagents and equipment for insecticide resistance monitoring in 23 sentinel districts

7. Timeline 2015/16

April 2015	– Small changes in prospective questionnaire and translations
May 2015	– Annual ethics report due to NIMR and IHI – NIMR training of field implementers and malaria focal persons from 23 sentinel districts on malaria vector resistance surveillance
April – July 2015	– Analysis plan for prospective data (10 month time point) – Publication of net landscape in Tanzania paper in PLoS ONE (Mageni *et al*) – Logistics planning for field work – Small changes to submit to IHI Data Central for questionnaire programming
May – July 2015	– NIMR field work and data collection for insecticide resistance monitoring (mosquito larval collection, preservation and susceptibility testing) in 23 sentinel districts

April – Sept 2015	– Prospective laboratory analyses of 10 month old nets (N=48 per net product) in Bagamoyo, Tanzania o D component: Hole index using a frame. Also taking pictures on black background. o B component: ▪ Whole net blood-feeding inhibition and mortality of *An. gambiae* s.s. in the ITT ▪ Sample knock-down and mortality of *An. gambiae* s.s. using WHO cone tests ▪ Sample blood-feeding inhibition and mortality using WHO tunnel tests on failed samples
July 2015	– Set up logistics for second prospective census. – Training of field enumerators in Bagamoyo.
Aug – Oct 2015	– Second prospective census begins in Bagamoyo – Continuous field work for 3 months by field enumerators throughout the 8 study districts – Continuous quality assurance by monitoring of the survey data as it gets uploaded to the data server – Following field enumerators, a second IHI team will visit randomly sampled households and collect prospective LLINs (distributed 22 months ago) for further BCD testing in the laboratory in Bagamoyo
Jul - Sept 2015	– NIMR molecular analyses of preserved mosquitoes from the field for speciation and mechanisms of resistance
Oct 2015	– Ship prospective LLIN samples from Year 1 (10 month follow up) to CRA-W for HPLC analysis – Annual Progress Report to be submitted to GLOBVAC, RCN – Produce NIMR technical report on national monitoring of insecticide resistance
Nov 2015	– PhD student Dennis Massue to present results on LLIN durability at ASTMH 64th Annual Meeting, Philadelphia, USA
Dec 2015	– NIMR dissemination workshop on findings of insecticide resistance monitoring – Final technical report on insecticide resistance monitoring
Dec 2015 – March 2016	– Preliminary analysis and presentation of data to NMCP – Prospective laboratory analyses of sub-sampled LLINs (48 per LLIN product)

8. Preliminary Results from Prospective Survey

8.1. Net attrition

In October to December 2013, 10,598 new LLINs were distributed to the study households. After 10 months, we collected data on 9,726 nets (91.8%). The remainder of the nets were lost to follow up due to households moving away, withdrawing consent or field interviewers not correctly identifying ABCDR nets. Of the 9,726 nets, 77% were still in the households whereas 23% were no longer present in the households (Figure 1).

Nets no longer used for sleeping under include nets that were thrown away or used for something else. The main reason given for discarding and alternative use was that the net was too damaged to sleep under, mainly by wear and tear. Another reason for alternative use was that there were more useful things to do with net, mainly agricultural (i.e. protect animals, crops, fence off garden). Thus, net attrition, i.e. discarding nets or using them for alternative purposes, after 10 months in the field was 313/9,726 = 3.2%. Nets are discarded by being thrown away (54%), or by being burned outside (23%) and inside (13%) houses. These results have been reported to the NMCP as such activities may have significant adverse effects on human health and the environment.

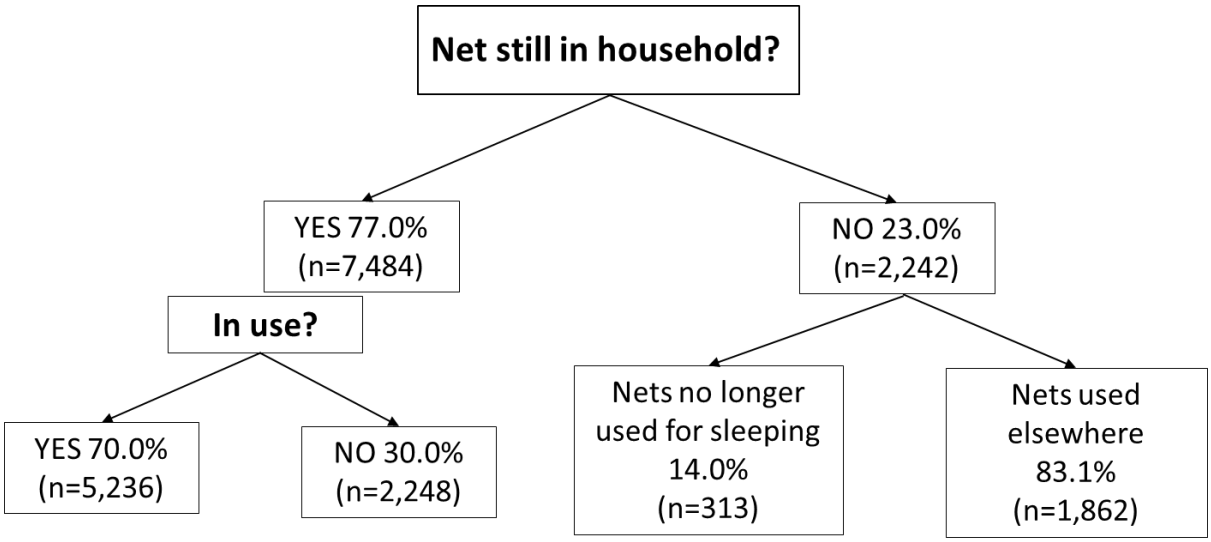

Figure 1 Net presence and use follow up of ABCDR nets (all three net products) after 10 months.

Nets given away (1,732 nets), sold (18 nets) and stolen (112 nets) were included in the category of "nets used elsewhere" (Figure 1). These are no longer traceable in the ABCDR study, but were assumed to be protective against mosquito bites elsewhere. Most of the nets were given away because "someone else needed it more", and more than half of the nets (52%) were given to 'other relatives'. 30% were given to children either going to school or starting their own family, 11% of nets were given to parents and only 3% were given to neighbours.

58% of the nets no longer in the households were lost more 6 months before the survey. The act of giving nets away mostly occurred shortly after receiving nets (67%) and only 17% of nets were given away in the 3 months before the survey. Of those nets that were discarded, 25% were thrown away more than 6 months ago and 48% in the three months immediately before the survey – presumably after they got too torn through frequent use.

8.2 Non-net use

There were many reasons given for why people did not use a mosquito net the night before the survey (Figure 2). Of the 2,248 nets not in use (Figure 1), most were saved for future use or visitors (28%). Reasons grouped in the "Other" category (14%) included: net full of bedbugs, net makes me sneeze, net not available for use (e.g. being washed), no place to hang the net. Only 2% of nets were reported not to have been used because they were too old or too torn.

Non-net use varied geographically by district (Figure 3). In Mbozi district, more than 50% of the general population and children under the age of five (U5) did not use a net the night before the survey, whereas in Bagamoyo district, the non-net use of both groups was below 20%. Given that all households were provided with enough LLINs to cover all sleeping spaces only 10 month prior to the survey, this may indicate a choice of not using a net rather than a lack of availability. Data analysis is underway to elucidate whether those households with decreased net use do not have enough access to nets, particularly in households where children under five are not using nets. These data will be reported to stakeholders as soon as they are available.

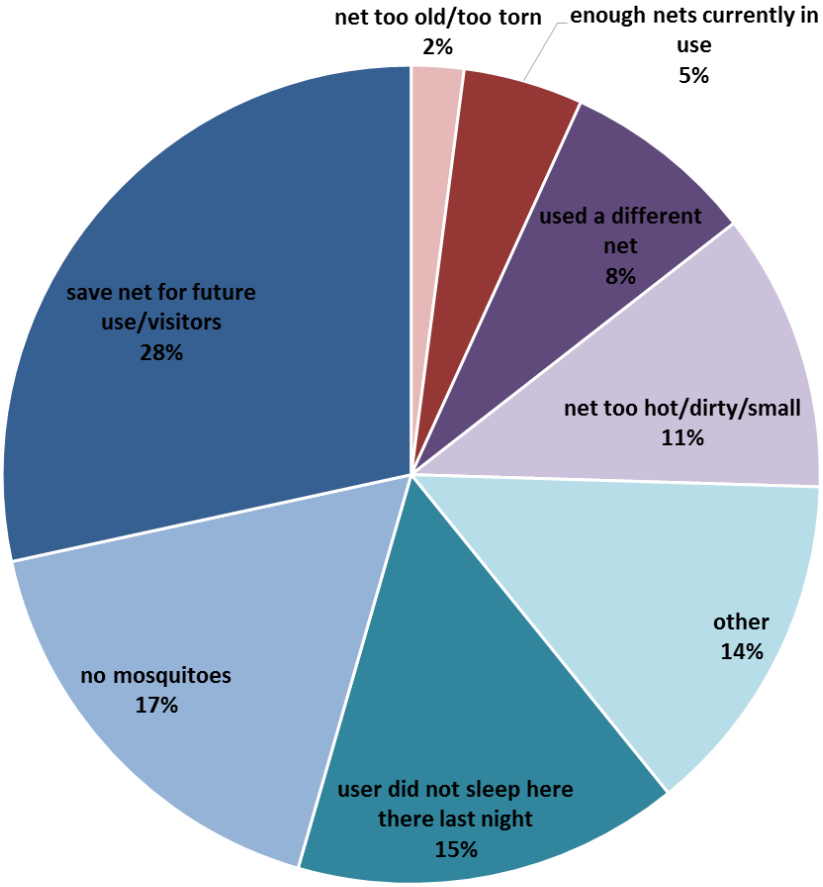

Figure 2 Reasons given for not using a mosquito net the night before the survey.

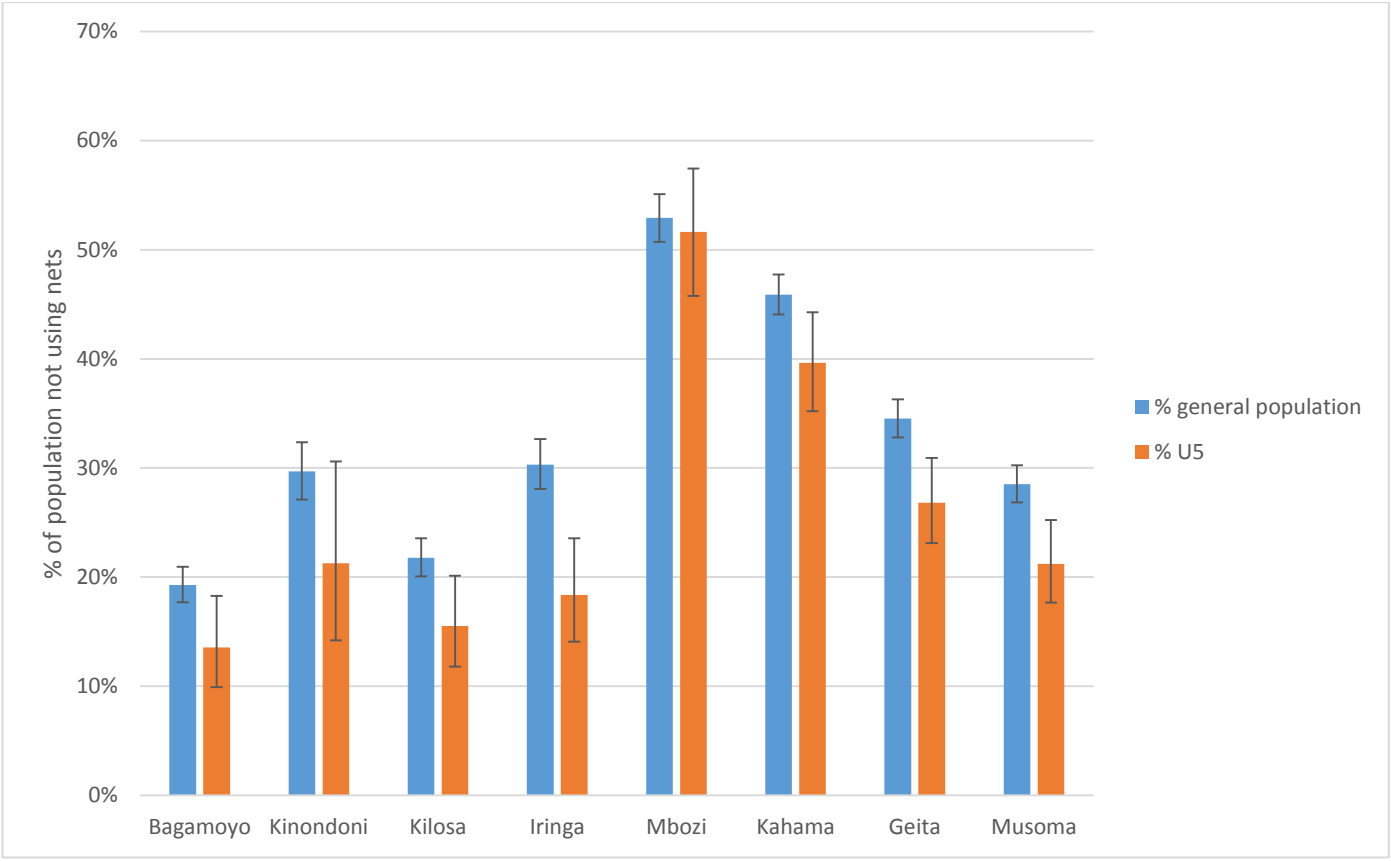

Figure 3 Percentage of population not using nets by district in Aug-Oct 2014. This bar chart shows the mean percentage (± 95% confidence intervals) of the general population (blue) and children under the age of five (U5, orange) not reporting to use a net the night before the survey by the eight ABCDR study districts. Each household had received enough nets to cover all sleeping spaces 10 month prior to this survey.

8.3 Physical damage of LLINs

A collapsible metal frame (Figure 4) was used to count holes in ABCDR nets still present within households. For logistical reasons, not more than three nets per household were assessed for hole counts. In total, holes were counted in 6,175 nets in the field, from which the proportionate Hole Index (pHI) was calculated as per WHO guidelines (Table 1). The two outcome measures reported in this report are 1) Are there any holes? (Yes/No) and 2) the three WHO categories: "good" (pHI < 64), "damaged" (pHI = 64 - 642), "too torn" (pHI > 643). These data were reported to NMCP and discussed at an LLIN Taskforce Meeting in March 2015.

Table 1 Hole size categories and their proportionate weights

Hole Category	Hole Size Description	Hole Size (cm)	Hole Diameter (cm)	Weight
Size 1	Smaller than a thumb (finger)	0.5-2	1.25	1
Size 2	Larger than a thumb but smaller than fist (hand)	2-10	6	23
Size 3	Larger than a fist but smaller than a head (head)	10-25	17.5	196
Size 4	Larger than a head	>25	30 (assumed)	578

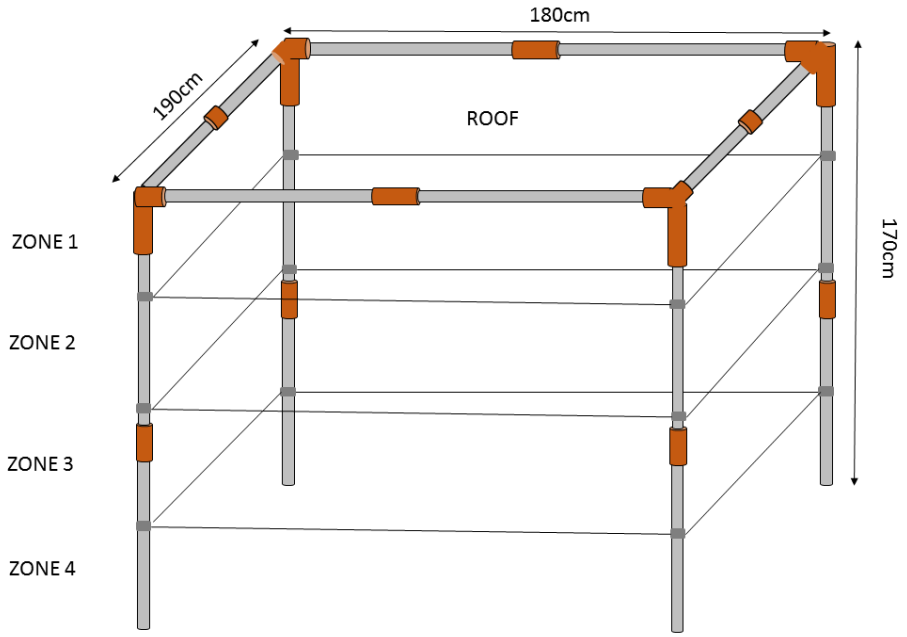

Figure 4 Mosquito net hole counting by zone. Collapsible metal frame (170cm x 180 cm x 190 cm) divided into four 37.5cm zones from the top to the bottom in order to count holes by zone under laboratory and field conditions.

Of the 6,175 nets in which holes were counted, 32% had no holes at all (Figure 5). Of those with holes, 51% were still deemed as "good" by WHO standards, 35% "damaged" and 14% as "too torn". Overall, 609 nets (10%) of nets distributed 10 months previously had too many holes to be deemed serviceable against malaria transmission as per WHO guidelines.

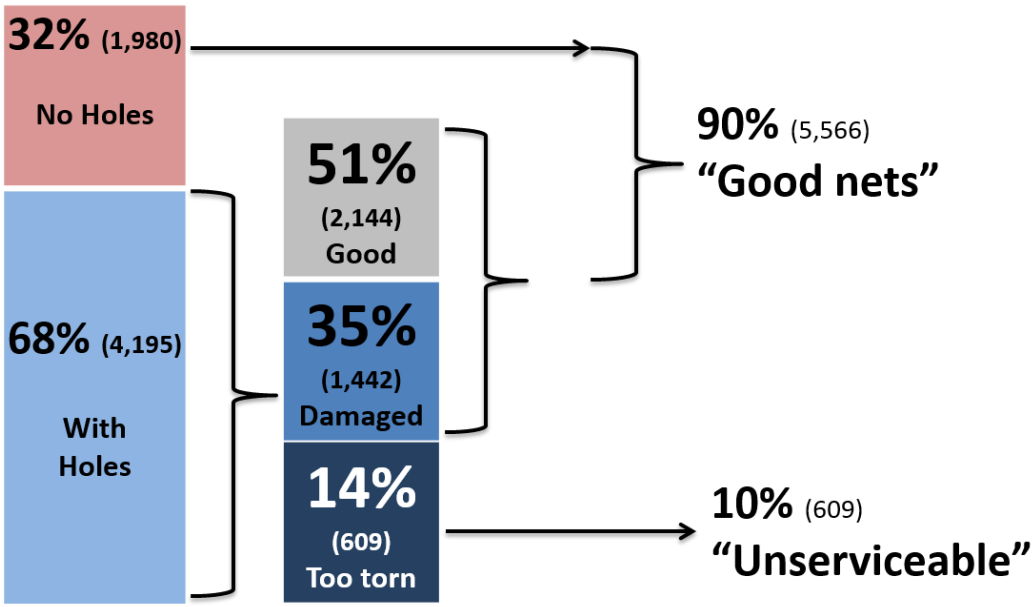

Figure 5 Physical degradation of nets following WHO protocol. Data from hole counts in the field of 6,175 nets. Categorization based on WHO cut-off points based on hole surface area in Table 1.

So far, we have identified the following risk factors that increase the likelihood of hole presence and reduce the likelihood of a net being categorized as "good" (according to WHO): room contains sources of cooking, heating or lighting such as open fire; presence of rats; tucking the net underneath a mat/mattress. These factors are not exhaustive and more will be further analysed.

Damage to nets that are tucked in under a mattress etc. are mainly from holes located in zone 4, i.e. the bottom 37.5cm of the net (Table 2a). The difference in net quality between tucking in the net or not is lessened when analysing without zone 4 (Table 2b).

Table 2a Nets tucked in by hole index from the whole net

	N (%) with holes	N (%) WHO "good"	N (%) WHO "damaged"	N (%) WHO "too torn"	TOTAL
Net tucked in	3,953	3,759	1,350	567	**5,676**
	70 (66 – 74%)	66 (62 – 69%)	24 (22 – 26%)	10 (9 – 12%)	
Net not tucked in	236	358	91	40	**489**
	42 (34 – 50%)	76 (71 – 81%)	15 (12 – 19%)	8 (6 – 12%)	

Table 2b Net tucked in by hole index from the net excluding zone 4 (bottom 37.5cm of a 150cm net)

	N (%) with holes	N (%) WHO "good"	N (%) WHO "damaged"	N (%) WHO "too torn"	TOTAL
Net tucked in	3,189	4,465	940	271	**5,676**
	57 (52 – 61%)	78 (76 – 81%)	17 (15 – 19%)	5 (4 – 6%)	
Net not tucked in	200	391	68	30	**489**
	35 (29 – 42%)	82 (78 – 86%)	12 (9 – 16%)	6 (3 – 9%)	

The presentation of results by net product will be produced in separate documents for each brand and emailed only to the net manufacturers of each particular brand. Comprehensive analysis and presentation of results will be performed by PhD candidates Dennis Massue and Zawadi Mageni, presented at national and international conferences and published in peer-reviewed publications as soon as possible.

9. Insecticide Resistance Monitoring (R-Component)

The malaria entomological surveillance was carried out between May and August 2014 in 19 districts for the detection and monitoring of malaria vectors resistance to insecticides of public health relevance in Tanzania mainland.

Anopheles mosquito larvae were collected and reared to adults for susceptibility tests and molecular analysis. Adult female mosquitoes of 2-5 days old *An. gambiae* s.l. were exposed to insecticides with discriminating dosages of 0.75% permethrin, 0.05% deltamethrin, 0.05% lambdacyhalothrin and 0.1% bendiocarb during susceptibility testing in the field following WHO protocol. The distribution of *An. gambiae* sub-species and pyrethroid target-site mutations (*kdr*) were investigated using molecular assays (standard and real-time PCR). Biochemical assays were used to detect the enzyme-based resistance mechanisms in mosquitoes across all the sentinel sites.

Susceptibility tests confirmed resistance to the tested insecticides. Mosquitoes showed highest resistance to lambdacyhalothrin with a 25% mortality after 1 hour exposure (Kilombero in Morogoro region) (Table 3). A mortality of 58.2% to bendiocarb was found in Arumeru sentinel site in Arusha region. Low mortalities were also detected in mosquitoes exposed to deltamethrin (59% in Kilombero) and permethrin (45% in Arumeru). Calculation of resistance ratios (RR50) for mosquitoes collected in Bagamoyo district showed that the 50% lethal dose (LD50) of lambdacyhalothrin were 9 times higher; permethrin 5.4 times higher, and deltamethrin 4.4 times higher in resistant than in susceptible mosquitoes.

A total of 1,936 *Anopheles* female adults from the 18 sampled populations were used for species identification by PCR. *Anopheles gambiae* s.s. were 28% mostly collected from rural sentinel sites, while the remaining 78% were *Anopheles arabiensis*.

The L1014S east-kdr-mutation was recorded in Kinondoni (20% frequency), Kyela (15%), Muheza (25%), Musoma (6%) and Ngara (6%) respectively. Elevated levels of non-specific esterases were significantly elevated in mosquitoes from Arumeru, and mosquitoes from Kilombero and Bagamoyo showed trends towards significance for elevated P450 oxidases, while Glutathione S-transferases were significantly elevated in mosquitoes from Arumeru only. Also mosquitoes from Arumeru, Kondoa, Kilombero and Bagamoyo showed trends towards significance for elevated levels of nonspecific esterases.

Table 3 Summary of susceptibility levels (mortality rates[1]) of *An. gambiae s.l* exposed to the WHO - recommended discriminating dosages of different insecticides

Sites	0.75% permethrin		0.05% Deltamethin		0.05% Lambdacyhalothrin		0.1% Bendiocarb	
	Mortality (%)	SE	Mortality (%)	SE	Mortality (%)	SE	Mortality (%)	SE
Arumeru	**45**	4.6	92	2.8	**49**	1.8	**58.2**	5.2
Babati	100	0.0	**74**	4.8	**38**	3.8	100	0.0
Bagamoyo	90	3.6	100	0.0	**86**	4.8	100	0.0
Geita	98.3	1.2	96.7	1.4	93.3	1.7	98.3	1.2
Iringa	100	0.0	100	0.0	100	0.0	100	0.0
Kahama	100	0.0	85.6	3.8	**51.4**	5.8	100	0.0
Kilombero	100	0.0	**59**	5.2	**25**	3.8	100	0.0
Kilosa	100	0.0	**72**	4.6	**63.8**	4.0	100	0.0
Kinondoni	92.5	1.8	**70**	4.0	**85**	3.6	92.5	1.8
Kondoa	100	0.0	100	0.0	100	0.0	100	0.0
Kyela	100	0.0	100	0.0	100	0.0	100	0.0
Magu	100	0.0	97.5	1.4	76.3	5.2	100	0.0
Mbozi	93.3	1.8	93.3	1.0	**88.9**	2.1	93.3	1.8
Muheza	**77.8**	4.8	97.8	0.8	**89**	3.8	100	0.0
Musoma	95	1.6	98.6	0.8	N/A		N/A	
Ngara	100	0.0	90	2.4	**83.3**	4.2	100	0.0
Ruangwa	100	0.0	100	0.0	100	0.0	100	0.0
Songea	100	0.0	100	0.0	100	0.0	100	0.0