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Distribution and spread of pyrethroid and DDT resistance among the *Anopheles gambiae* complex in Tanzania

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Abstract

The development of insecticide resistance is a threat to the control of malaria in Africa. We report the findings of a national survey carried out in Tanzania in 2011 to monitor the susceptibility of malaria vectors to pyrethroid, organophosphate, carbamate and DDT insecticides, and compare these findings with those identified in 2004 and 2010. Standard World Health Organization (WHO) methods were used to detect knock-down and mortality rates in wild female *Anopheles gambiae s.l.* (Diptera: Culicidae) collected from 14 sentinel districts. Diagnostic doses of the pyrethroids deltamethrin, lambdacyhalothrin and permethrin, the carbamate propoxur, the organophosphate fenitrothion and the organochlorine DDT were used. *Anopheles gambiae s.l.* was resistant to permethrin in Muleba, where a mortality rate of 11% [95% confidence interval (CI) 6–19%] was recorded, Muheza (mortality rate of 75%, 95% CI 66–83%), Moshi and Arumeru (mortality rates of 74% in both). Similarly, resistance was reported to lambdacyhalothrin in Muleba, Muheza, Moshi and Arumeru (mortality rates of 31–82%), and to deltamethrin in Muleba, Moshi and Muheza (mortality rates of 28–75%). Resistance to DDT was reported in Muleba. No resistance to the carbamate propoxur or the organophosphate fenitrothion was observed. *Anopheles gambiae s.l.* is becoming resistant to pyrethoids and DDT in several parts of Tanzania. This has coincided with the scaling up of vector control measures. Resistance may impair the effectiveness of these interventions and therefore demands close monitoring and the adoption of a resistance management strategy.

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Keywords

Anopheles gambiae ; DDT resistance; insecticides; malaria vectors; pyrethroid resistance; Tanzania

Introduction

The application of insecticide-based malaria control measures in Africa is expanding with rapid increases in both the distribution of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) in many endemic countries. The longlasting insecticidal net (LLIN), the modern form of the ITN, and IRS are the cornerstones of malaria control programmes (Roberts & Enserink, 2007). Vector control interventions are dependent on a limited number of insecticides from four chemical classes, namely, the organochlorines, organophosphates, carbamates and pyrethroids (World Health Organization, 2012a). The World Health Organization (WHO) has approved 12 insecticides belonging to these classes for use in IRS. The chemical arsenal for use with ITNs and LLINs is more limited and only six insecticides, all of which are pyrethroids, are available (WHO, 2012a). The pyrethroids are the only class approved for use on ITNs as a result of their low human toxicity, excito-repellent properties, rapid rate of knock-down and killing effects (Zaim et al., 2000).

These four insecticide classes are also widely used for the control of agricultural pests in Africa (Santolamazza et al., 2008). This can pose additional selection pressure on malaria vectors when insecticide-contaminated ground water permeates mosquito larval habitats (Ranson et al., 2009). Exposure of mosquitoes to insecticides from multiple sources may combine to accelerate resistance and compromise malaria control efforts (Ranson et al., 2011). Resistance to the organochlorines DDT (dichlorodiphenyltrichloroethane) and the now obsolete dieldrin was first reported in African malaria vectors in the 1950s and 1960s (Brown, 1958; Hamon et al., 1968). Pyrethroid resistance was first detected in West African malaria vectors in 1993 (Elissa et al., 1993), since when there has been an increasing number of reports of pyrethroid-resistance in *Anopheles gambiae s.l.* in countries in west, central, east and southern Africa (Vulule et al., 1996; Chandre et al., 1999; Stump et al., 2004; Roberts & Enserink, 2007; Munhenga et al., 2008; Protopopoff et al., 2008; Ndjemai et al., 2009; Hunt et al., 2010; Chanda et al., 2011) and in *Anopheles funestus* in Ghana, Mozambique, Malawi, Zambia and South Africa (Hargreaves et al., 2000; Okoye et al., 2008; Chanda et al., 2011). Carbamate- and organophosphate-resistant populations of *An. gambiae* have also been reported in West Africa (Corbel et al., 2007). The continuing success of IRS and LLIN interventions against the rising tide of insecticide resistance depends on the acquiring of comprehensive knowledge of the factors underlying selection in order to support the development of effective resistance management strategies. This, in turn, raises the need for regular surveys to monitor the insecticide susceptibility of vector species and to assess their impact on malaria control activities (Kelly-Hope et al., 2008).

The use of ITNs in Tanzania started in the early 1990s as projects in the Muheza and Korogwe districts in Tanga, the Kilombero and Ulanga districts in Morogoro, and the Bagamoyo district in the coastal region (Schellenberg et al., 1999, 2001; Abdulla et al.,

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2001; Maxwell et al., 2002; Erlanger et al., 2004; Magesa et al., 2005). These projects were then expanded to cover larger areas in 1998 through the Social Marketing of Insecticide-Treated Nets (SMITN) project and from 2002 onwards through the Strategic Social Marketing for Expanding the Commercial Market of ITNs (SMARNET) programme (Magesa et al., 2005). This was followed by the rolling out of vouchers for ITNs, which were offered to pregnant women through the Tanzania National Voucher Scheme (TNVS) in 2004 (Magesa et al., 2005; Hanson et al., 2009). To accelerate coverage and address an equity gap, Tanzania adopted the free distribution of LLINs in 2008. The ITNs were distributed through an 'Under-5 Catch-Up' campaign, which was followed by various 'universal coverage' campaigns (WHO, 2012b). All the LLINs distributed recently are Olyset™ nets (Sumitomo Chemical Co., Ltd, Tokyo, Japan), impregnated with permethrin. Indoor residual spraying was reintroduced in the country in 2007 as a way to control malaria outbreaks in selected areas of two districts (Muleba and Karagwe) in northwest Tanzania (WHO, 2012b). By March 2011, IRS operations with lambdacyhalothrin had expanded to 18 districts in the Lake Victoria Zone and reached approximately 94% of the targeted structures in those districts (WHO, 2012b). Through these campaigns, national coverage with ITNs and LLINs rose dramatically from 38% in 2007–2008 to 91% in 2011–2012 and that of IRS reached 13% in 2011–2012 (National Bureau of Statistics, 2012). Therefore, it is imperative to regularly monitor the susceptibility status of local malaria vectors to the insecticides used in these interventions. This will enable the timely deployment of resistance mitigation tactics when resistance is recognized.

Earlier large-scale surveys in Tanzania demonstrated the efficacy of all four classes against malaria vectors (Kulkarni et al., 2007) with focal points of phenotypic resistance (Kabula et al., 2012). This survey was carried out as part of continued monitoring of the susceptibility status of local malaria vectors to insecticides in use in the country. The present study reports the findings of a national survey carried out in 2011 at 14 sentinel sites to monitor the susceptibility of malaria vectors to pyrethroid, organophosphate, carbamate and DDT insecticides and to compare current trends with the situations existing in 2004 and 2010. Some of the 2004 comparison data were novel and some were published (Kulkarni et al., 2007). All comparison data for 2010 have been previously reported elsewhere (Kabula et al., 2012).

Materials and methods

Study sites

The study was carried out in 14 sentinel districts selected from across the country. The selection of sentinel districts was based on WHO-recommended selection criteria, which include: a history of insecticide use by communities living in the areas (in agricultural and public health contexts); malaria endemicity (moderate to high transmission); high coverage by ITNs and LLINs; demographic setting (urban/rural), and easy site accessibility. The characteristics of these districts have been previously described (Kabula et al., 2012). Additional characteristics and the distribution of the study districts are shown in Table 1 and Fig. 1, respectively. These sentinel districts and insecticide resistance monitoring sites were initially established in 1999 with funding from the Ministry of Health and Social

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Welfare (MoHSW). This was followed by further monitoring and the detection of insecticide resistance in the context of the scaling up of ITN distribution in 2004, at which point the survey was carried out in 11 sentinel sites. In response to the concern of the global malaria community over the threat implied by insecticide resistance, the country continued with the periodic monitoring of resistance. In 2008, funding from the Bill & Melinda Gates Foundation through the WHO enabled the number of surveillance sites to be expanded to the current 14.

Mosquito collections and identification

At each study site, indoor-resting *Anopheles* mosquitoes were collected from human habitations, using torches, mechanical aspirators and paper cups, between 06.00 hours and 09.00 hours (WHO, 1975). The collections were made in the rainy season during May and June 2011 in all but one of the sites; this is the optimal time to capture adequate numbers of mosquitoes for susceptibility testing as per guidelines issued by the WHO (1998). An exception was the Muleba district, where collections were made during April to June and in November and December 2011 in 11 villages described by Protopopoff et al. (2013). The collected mosquitoes were maintained on a fresh 10% glucose solution before being tested for insecticide susceptibility. In Ilala and Dodoma districts adult collections were insufficient. Therefore larvae were collected using dippers and transferred in cool boxes to the laboratory, where they were reared on ground Tetramin® fish food at 27–30 °C. The geographical coordinates of each sampling site were determined by GPS (Trimble Geoexplorer II; Trimble Navigation Ltd, Sunnyvale, CA, U.S.A.). Adult *Anopheles* mosquitoes were carefully sorted for susceptibility tests, as recommended by the WHO (1998), and morphologically identified using a standard morphological key according to Gillies and Coetzee (1987). Technical and logistical constraints prevent the inclusion of the results of the molecular identification of *An. gambiae* complex specimens in this analysis.

Insecticide susceptibility/resistance bioassays

Insecticide susceptibility/resistance bioassays were carried out using WHO insecticide susceptibility test kits (WHO, 1975) according to standard procedures (WHO, 1998) with four or five replicates of 15–25 wild-caught adult female mosquitoes per test. For convenience purposes this study used the unfed wild-caught adult female anopheles mosquitoes to test their susceptibility to insecticides in most of the sites. Therefore the age of the mosquitoes tested might be less certain. Mosquitoes were exposed to papers impregnated with the WHO-recommended discriminating concentrations (w/v) of 0.05% deltamethrin, 0.05% lambdacyhalothrin, 0.75% permethrin, 4% DDT, 0.1% propoxur and 1% fenitrothion; the papers were prepared at University Sains Malaysia (WHO, 1998). The controls were exposed to control papers impregnated with silicone oil (pyrethroid control), risella oil (DDT control) or olive oil (organophosphate/carbamate control). During exposure, time to knock-down (KDT) rates were recorded after 10, 15, 20, 30, 40, 50 and 60min for pyrethroid and organochlorine insecticides. Mosquitoes were then transferred to holding tubes and supplied with glucose solution. In instances of knock-down of less than 80% during the 60-min exposure period, knock-down was monitored for a further 20 min in the holding tube. Mortality was scored after a 24-h holding period (WHO, 1998).

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Statistical analyses

Mortality percentages and 95% confidence intervals (CIs) in WHO susceptibility tests were calculated using the binomial exact method in SPSS for Windows Version 16.0 (SPSS, Inc., Chicago, IL, U.S.A.) and VassarStats, a web-based statistical package (http://www.vassarstats.net/). Abbott's formula was used to correct observed mortality when mortality in the control samples was between 5% and 20% (Abbott, 1925). The KDT_{50} and KDT_{95} (time required to knock down 50% and 95%, respectively, of the test mosquitoes) were estimated by probit analysis (Finney, 1971) using SPSS Version 16.0. Resistance ratios (RRs) at KDT_{50} were estimated by comparing the KDT_{50} of field-collected mosquitoes with that of the *An.gambiae* Kisumu susceptible strain.

Results

Mortality

The highest percentages of mosquitoes resistant to permethrin were observed in samples collected from Arumeru (26%), Moshi (26%) and Muheza (25%), followed by those from Kilombero (15%) and Ilala (10%). Lower levels of survival and hence resistance to permethrin (1–5%) were found in populations from Babati, Magu and Handeni districts. Complete susceptibility (mortality of 100%) following exposure to permethrin was observed in populations collected from the five districts of Lushoto, Dodoma, Tabora, Mvomero and Kyela (Table 2).

Following exposure to WHO-recommended diagnostic doses of deltamethrin, highest survival was observed in populations from Moshi (34%), Muheza (26%), Arumeru (10%) and Handeni (7%), but low survival (<4%) was observed in mosquitoes collected from Ilala, Kilombero and Babati. None of the mosquitoes collected from Magu, Lushoto, Dodoma, Tabora, Mvomero and Kyela districts survived.

Following exposure to lambdacyhalothrin, highest survival rate was recorded in mosquitoes collected from Moshi (42%), Arumeru (30%), Muheza (18%) and Handeni (2%) (Table 2).

Conversely, following exposure to DDT, no mosquitoes from any of the sentinel districts survived, except those from Ilala and Magu, where survival rates were 12% and 5%, respectively (Table 3). Samples of mosquitoes from the 14 sentinel sites exposed to either fenitrothion or propoxur exhibited 99–100% mortality. Only 1% of tested mosquitoes from the Kilombero, Kyela and Mvomero districts survived exposure to propoxur. Similarly, 1% of mosquitoes survived exposure to fenitrothion in Kyela district (Table 4).

Mortality trends in An. gambiae s.l. *exposed to insecticides*

The 2004 surveys found no clear evidence of pyrethroid resistance in any part of the country; the only exceptions were Arumeru and Moshi, where mosquitoes showed some resistance to permethrin (mortality rates of 91% and 97%, respectively). By 2010, however, there was growing evidence of resistance in selected localities. In Moshi, for example, permethrin-induced mortality had decreased to 77% and there was some indication of permethrin resistance in Kilombero, Arumeru, Ilala and Handeni, where mortality rates

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ranged from 91% to 96%. This trend continued in 2011: mortality achieved by permethrin in Moshi was 74% and there was growing evidence of resistance in Arumeru (mortality: 74%), Ilala (mortality: 90%), Kilombero (mortality: 85%), Handeni (mortality: 95%), Muheza (mortality: 75%) and Magu (mortality: 96%).

Decreased test mortality to the alphacyano pyrethroid deltamethrin in 2009/2010 (relative to 2004) was observed in Moshi (mortality: 94%), Arumeru (mortality: 97%), Ilala (mortality: 88%) and Kilombero (mortality: 90%). The trend of decreasing mortality continued in 2011 in Moshi (mortality: 66%) and Arumeru (mortality: 90%), and resistance appeared in Muheza (mortality: 75%) and Muleba (mortality: 28%). In Ilala (mortality: 97%) and Kilombero (mortality: 96%), percentage mortalities in 2011 were similar to those in 2010. Trends in mortality rates in *An. gambiae s.l.* exposed to diagnostic concentrations of permethrin, deltamethrin and lambdacyhalothrin, including 95% CIs, are shown in Table 2.

Mortality in response to lambdacyhalothrin in 2011 (relative to 2010) was consistent with results observed in response to permethrin and deltamethrin. Resistance was evident in Moshi (mortality: 58%), Arumeru (mortality: 70%), Ilala (mortality: 95%), Muheza (mortality: 82%) and Muleba (mortality: 31%). Generally, the highest frequencies of pyrethroid resistance appear to be in Moshi, Arumeru and Muleba, followed by Ilala, Kilombero, Muheza and Handeni.

Resistance to DDT appears not to parallel that to pyrethroids. Test survival in 2011 was observed in Ilala (mortality: 88%), Muleba (mortality: 67%) and Magu (mortality: 95%), but other sites showed full susceptibility (Table 3). The reduced susceptibilities to DDT observed in Ilala and Magu were formerly recorded in 2010 and 2004, respectively.

Knock-down

The median knock-down times (KDT_{50}) obtained from time–mortality probit regression analysis ranged from 14min to 84 min for permethrin, from 13min to 92 min for deltamethrin, from 12min to 114 min for lambdacyhalothrin, and from 19 min to 38 min for DDT (Table 5). It took longer for mosquitoes to be knocked down by lambdacyhalothrin than by other insecticides. Arumeru registered the highest KDT_{50} across all pyrethroids, followed by Moshi, Babati and Ilala (Table 5).

Data for KDT_{50} in the field-caught samples were compared with those in the susceptible Kisumu strain to obtain RRs. Resistance ratios ranged from 1.1 to 6.8 for permethrin, from 1.1 to 6.7 for deltamethrin, from 0.8 to 7.5 for lambdacyhalothrin and from 0.9 to 2.0 for DDT (Table 5). Significantly high RRs were recorded in Babati, Arumeru, Moshi and Ilala.

Discussion

The nationwide surveys conducted in 2011 demonstrate the wide spreading of resistance to pyrethroids in *An. gambiae s.l.* across Tanzania. The occurrence and spreading of resistance was not uniform, but was clearly evident in pockets in the northwest (Muleba), north (Moshi, Babati, Arumeru), northeast (Muheza, Handeni), east (Ilala) and central

(Kilombero) districts of the country. The resistance extended to all three pyrethroids tested (permethrin, deltamethrin, lambdacyhalothrin). Resistance to DDT was evident in Muleba and Ilala, but not in the other districts in which resistance to pyrethroids was recorded. Mosquito samples were not characterized to species and the mechanism of resistance was not identified in every location as a result of differences in survey intensity, objectives and resources between the national survey and the groups working in the Muleba and Moshi districts. The resistance surveys in Muleba constituted part of a 2-year vector control trial; high-level resistance was present in *An. gambiae s.s.*, but absent from *Anopheles arabiensis* (Protopopoff et al., 2013). Resistance in Moshi was restricted to *An. arabiensis* because *An. gambiae* was absent from collections from this region (Matowo et al., 2010). Findings of incipient pyrethroid resistance in *An. gambiae s.l.* from Moshi, Arumeru, Ilala, Kilombero and Handeni were strongly supported by increased median knock-down times (KDT_{50}) because higher KDT_{50} values in field populations give an early indication of the involvement of the *kdr* mechanism of resistance (Chandre et al., 1999). Resistance in *An. gambiae s.s*. in Muleba is associated with a high frequency of *kdr*-east variant and with metabolic mechanisms (Protopopoff et al., 2013). The *kdr*-east variant shows cross-resistance to DDT (Ranson et al., 2000) and therefore the absence of DDT resistance in several districts in other parts of the country means that a mechanism other than *kdr* may be contributing to pyrethroid resistance in these places. The pyrethroid resistance in *An. arabiensis* in Moshi is attributed to elevated levels of mixed-function oxidases (Matowo et al., 2010) and to *kdr*-west being present in very low frequencies (Kulkarni et al., 2006). This calls for further investigation to elucidate the mechanisms involved in pyrethroid resistance in *An. gambiae* and *An. arabiensis* populations across Tanzania. The failure to perform species identification or resistance characterization (e.g. *kdr* genotyping) in the national survey, which is attributable to logistical constraints, limits the interpretation of phenotypic resistance, as recently emphasized by the WHO (2013). There was no evidence of resistance to the organophosphate fenitrothion or the carbamate propoxur. However, a recent study showed resistance to the carbamate bendiocarb in Muleba (Protopopoff et al., 2013).

The first indication of pyrethroid resistance in Tanzania was present in surveys in Moshi and Arumeru in 2004, but it took several years, until 2010 and 2011, for resistance to reach serious levels in *An. gambiae s.l.* from Moshi, Arumeru, Ilala, Kilombero and Handeni. *Anopheles gambiae s.l.* from Muheza, Muleba and Magu showed evidence of resistance for the first time in 2011 in comparison with the full susceptibility reported previously (Kulkarni et al., 2007; Kabula et al., 2012). The highest frequencies of pyrethroid resistance were observed in Moshi, Arumeru and Muleba, and were particularly high in Muleba (Protopopoff et al., 2013). Populations in the districts of Dodoma, Kyela, Lushoto, Mvomero and Tabora continued to show full susceptibility (Kabula et al., 2012).

As previously documented, the potential contribution of agricultural insecticide use to resistance in areas with large-scale agricultural production and floriculture continues in places such as Arumeru and Moshi (Kabula et al., 2012). In other countries, the intensive use of insecticides in agriculture has been implicated in the development of insecticide resistance in malaria vectors (Diabate et al., 2002). Overall, there has been a rapid decrease in susceptibility status across Tanzania compared with that identified in the last nationwide insecticide resistance surveys (Kulkarni et al., 2007; Kabula et al., 2012). This may indicate

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the cumulative effects of increases in the use of ITNs (such as in the Under-5 Catch-Up and universal coverage campaigns), which have been ongoing since the 1990s and have shown relative increases since 2010. Similar observations have been made in Niger, Equatorial Guinea (Bioko) and Kenya (Stump et al., 2004; Sharp et al., 2007; Czeher et al., 2008). Recent studies in Senegal and Liberia have also demonstrated increased frequencies of pyrethroid resistance after high LLIN usage (Ndiath et al., 2012; Temu et al., 2013). The rapid rise of pyrethroid resistance in Muleba also coincides with the intensive use of IRS in the district (since 2007) for the control of malaria transmission and was recorded a few years earlier in the neighbouring country of Uganda (Protopopoff et al., 2013). Indoor residual spraying is commonly associated with the selection of pyrethroid resistance (Sharp et al., 2007; WHO, 2010).

Resistance as measured in WHO susceptibility tests does not imply that ITNs/LLINs are no longer protective against local vector populations. The operational significance of resistance can be demonstrated in experimental hut trials against wild host-seeking resistant mosquitoes and through careful surveillance for increasing malaria transmission. The present level of resistance in Lower Moshi appears not to impair the effectiveness of permethrin-treated nets. Field trials in Moshi show that although such treated nets kill relatively few host-seeking *An. arabiensis* that enter local houses, the nets continue to provide personal protection through the strong excito-repellent activity of permethrin (Mosha et al., 2008). However, high pyrethroid resistance in *An. gambiae* can impair the effectiveness of ITNs/LLINs and IRS as reported in Benin (N'Guessan et al., 2007; Asidi et al., 2012). The operational failure of IRS with deltamethrin was noted previously in South Africa, where the malaria vector *An.funestus* was found to have developed resistance to this insecticide (Hargreaves et al., 2000).

In the present context, it is of paramount importance that levels of surveillance and the monitoring of resistance are increased across the country and that the operational implications of resistance on the effectiveness of pyrethroid-based LLIN and IRS interventions are assessed. This can partially be ensured by strengthening systems for the surveillance of human malaria cases in the areas in which ongoing insecticide resistance is monitored. Without the capacity to overlay insecticide resistance data and human case surveillance data, the full implications of reductions in vector susceptibility will remain uncertain.

Conclusions

Resistance to pyrethroids and DDT is emerging across Tanzania at unprecedented rates that have not been seen previously. This has coincided with the scaling up of malaria vector control interventions and may have serious implications for the future of the national malaria control programme, which largely depends on the use of pyrethroids in IRS and LLINs/ITNs. A resistance management strategy that develops the rotational use of insecticides, especially in areas in which IRS is used, is recommended. For example, mosquito populations remain susceptible to the organophosphate and carbamate classes, which indicates that these can be used to good effect in IRS as part of a resistance management strategy. Currently, only pyrethroids are recommended for LLINs/ITNs, which

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is problematic. Regular monitoring of the susceptibility status of malaria vectors to insecticides commonly used in IRS and LLIN interventions, and the characterization of resistance are paramount in the management of resistance and the planning of mosquito control measures.

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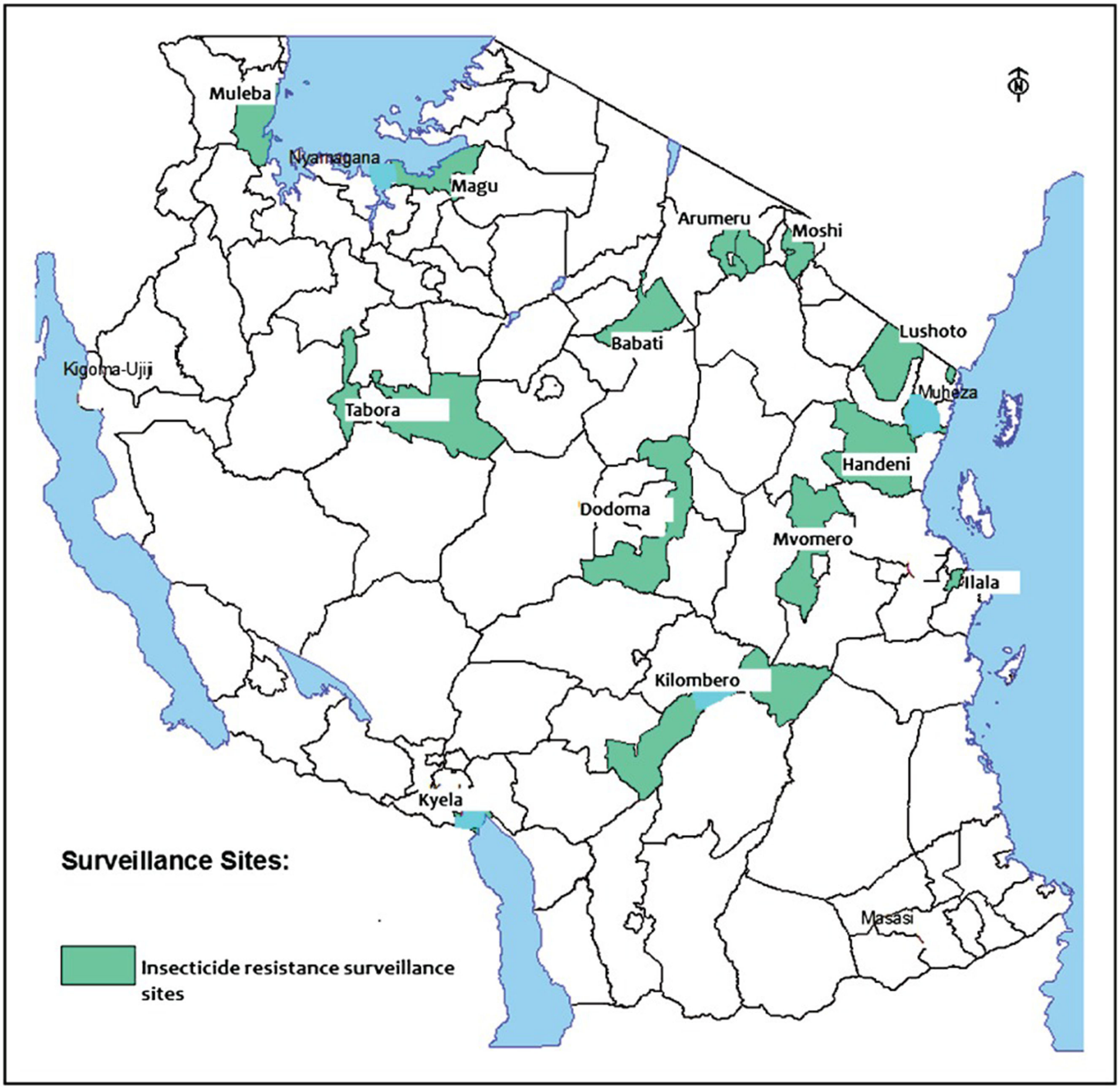

Fig. 1.
Map of Tanzania showing the distribution of insecticide resistance surveillance districts.

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Table 1.

Geographical coordinates and characteristics of the sentinel districts.

		ITN coverage trend (at least one ITN)					
Site	**Coordinates**	**2004**[*]	**2009**[†]	**2011**[‡]	**Malaria prevalence**[‡]	**Other interventions using insecticides**	**Demographic settings**
Arumeru	03°08′ S, 36°52′ E	23.7%	32.4%	84.5%	0.1%	Floriculture, agriculture, livestock	Semi-urban
Babati	04° 13′ S, 35°45′ E	8.1%	22.1%	88.6%	0.1%	Agriculture, livestock	Rural
Dodoma	07°00′ S, 36°30′ E	13.4%	28.2%	92.8%	2.6%	Agriculture, livestock	Rural
Handeni	05°43′ S, 38°01′ E	17.3%	38.6%	90.5%	5.5%	Agriculture, livestock	Rural
Ilala	06°49′ S, 39° 14′ E	60.8%	70.7%	78.6%	3.6%	Horticulture	Urban
Kilombero	08°31′ S, 37°22′ E	32.6%	44.1%	91.2%	13.0%	Agriculture	Rural
Kyela	09°35′ S, 33°55′ E	13.6%	29.9%	91.4%	0.5%	Agriculture	Rural
Lushoto	04°78′ S, 38°28′ E	17.3%	38.6%	90.5%	5.5%	Horticulture	Rural
Magu	02°30′ S, 33°30′ E	28.1%	48.4%	96.1%	19.1%	IRS since 2011, agriculture, livestock	Rural
Moshi	03°21′ S, 37°20′ E	13.1%	29.9%	94.8%	0.1%	Agriculture	Rural
Muheza	05° 10′ S, 38°46′ E	17.3%	38.6%	90.5%	5.5%	Agriculture	Rural
Muleba	01°45′ S, 31°40′ E	13.9%	29.5%	91.9%	8.5%	IRS since 2006, agriculture	Rural
Mvomero	06°30′ S, 37°30′ E	32.6%	44.1%	91.2%	13.0%	Agriculture, livestock	Rural
Tabora	04°55′ S, 32°49′ E	18.4%	39.8%	94.5%	9.2%	Agriculture, livestock	Rural

[*] Tanzania Demographic and Health Survey 2004–2005.

[†] Tanzania HIV/AIDS and Malaria Indicator Survey 2007–2008.

[‡] Tanzania HIV/AIDS and Malaria Indicator Survey 2011–2012.

IRS, indoor residual spraying.

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Table 2.

Susceptibility status of *Anopheles gambiae s.l.* to diagnostic concentrations of permethrin, deltamethrin and lambdacyhalothrin in 2011 compared with 2004 and 2010. Mean percentage mortality in World Health Organization susceptibility tests and number of mosquitoes exposed.

		Mortality, % (mosquitoes exposed, *n*)		
Insecticide	**Site**	**2004**	**2009/2010**[*]	**2011**
Permethrin	Arumeru	91% (150)[†]	93% (150)	74% (125)[‡]
	Babati	–	98% (200)	99% (123)
	Dodoma	–	–	100% (80)
	Handeni	–	91% (80)	95% (100)
	Ilala	–	92% (64)	90.3% (75)
	Kilombero	99% (543)	96% (180)	85% (80)[‡]
	Kyela	99% (219)	100% (81)	100% (86)
	Lushoto	–	100% (100)	100% (100)
	Magu	100% (79)[†]	–	96% (80)
	Mvomero	100% (225)	91% (160)	100% (82)
	Moshi	97% (675)	77% (555)	74% (542)
	Muheza	100% (180)	100% (100)	75% (95)[‡]
	Muleba	–	100% (100)	11% (98)[‡§]
	Tabora	100% (160)	100% (75)	100% (80)
Deltamethrin	Arumeru	100% (150)[†]	96.7% (150)	90.4% (125)
	Babati	100% (245)	98% (200)	96% (125)
	Dodoma	–	–	100% (80)
	Handeni	–	–	93% (99)
	Ilala	–	88% (60)	97% (85)
	Kilombero	100% (100)[†]	90% (180)	96% (80)
	Kyela	99% (198)	100% (80)	100% (78)
	Lushoto	–	100% (100)	100% (100)
	Magu	100% (79)[†]	–	100% (40)
	Mvomero	100% (66)[†]	94% (180)	100% (80)
	Moshi	100% (453)	94% (482)	66% (533)[†]
	Muheza	100% (100)	100% (80)	75% (95)[†]
	Muleba	–	100% (100)	28% (106)[*‡]
	Tabora	100% (80)	100% (80)	100% (80)
Lambdacyhalothrin	Arumeru	–	87% (150)	70% (125)[†]
	Babati	–	98% (200)	100% (125)
	Dodoma	–	–	100% (80)
	Handeni	–	84% (80)	98% (92)
	Ilala	–	94% (94)	95% (79)
	Kilombero	–	94% (180)	100% (80)

		Mortality, % (mosquitoes exposed, *n*)		
Insecticide	**Site**	**2004**	**2009/2010**[*]	**2011**
	Kyela	–	90% (88)	100% (83)
	Lushoto	–	100% (100)	100% (100)
	Magu	–	–	100% (80)
	Mvomero	–	88% (160)	100% (83)
	Moshi	–	73% (515)	58% (531)[†]
	Muheza	–	95% (80)	82% (95)[†]
	Muleba	–	100% (100)	31% (1099)[*‡]
	Tabora	–	100% (76)	100% (80)

[*] Kabula et al. (2012).

[†] Kulkarni et al. (2007).

[‡] Significant reduction in mortality rate at 95% confidence interval.

[§] Protopopoff et al. (2013).

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Table 3.

Susceptibility status of *Anopheles gambiae s.l.* to diagnostic concentrations of DDT in 2011 compared with 2004 and 2010. Mean percentage mortality in World Health Organization susceptibility tests and number of mosquitoes exposed.

		Mortality, % (mosquitoes exposed, *n*)		
Insecticide	**Site**	**2004**	**2009/2010**[*]	**2011**
DDT	Arumeru	100% (150)[†]	100% (150)	100% (125)
	Babati	99% (299)	99% (200)	100% (100)
	Dodoma	–	–	100% (80)
	Handeni	–	–	100% (100)
	Ilala	–	65% (88)	88% (520)
	Kilombero	93% (702)	100% (100)	100% (125)
	Kyela	100% (281)	90% (84)	100% (85)
	Lushoto	–	92% (92)	100% (100)
	Magu	98% (79)[†]	–	95% (80)
	Mvomero	100% (128)	100% (160)	100% (83)
	Moshi	100% (518)	99% (491)	99% (648)
	Muheza	100% (100)	99% (100)	100% (100)
	Muleba	–	100% (100)	67% (234)[‡§]
	Tabora	95% (20)[†]	100% (79)	100% (80)

[*] Kabula et al. (2012).

[†] Kulkarni et al. (2007).

[‡] Protopopoff et al. (2013).

[§] Significant reduction in mortality rate at 95% confidence interval.

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Table 4.

Response of wild-caught *Anopheles gambiae s.l.* local populations to discriminatory dosages of 0.1% propoxur and 1% fenitrothion in treated papers in 14 sentinel districts of Tanzania in 2011. Mean percentage mortality in World Health Organization susceptibility tests and number of mosquitoes exposed.

	Mortality, % (mosquitoes exposed, *n*)	
Site	**Propoxur**	**Fenitrothion**
Arumeru	100% (125)	100% (125)
Babati	100% (125)	100% (100)
Dodoma	100% (80)	100% (100)
Handeni	100% (100)	100% (100)
Ilala	100% (68)	100% (60)
Kilombero	99% (80)	100% (123)
Kyela	99% (79)	99% (80)
Lushoto	100% (100)	100% (100)
Magu	100% (90)	100% (80)
Moshi	99% (338)	99% (249)
Muheza	100% (100)	100% (100)
Muleba	100% (80)	100% (60)
Mvomero	99% (80)	100% (81)
Tabora	100% (80)	100% (100)

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Table 5.

Knock-down times in wild-caught *Anopheles gambiae s.l.* local populations exposed to discriminatory dosages of 0.05% deltamethrin, 0.75% permethrin, 0.05% lambdacyhalothrin and 4% DDT in 2011.

	Permethrin		Deltamethrin		Lambdacyhalothrin		DDT	
Site (sentinel district)	KDT_{50}, min, mean (95% CI)	KDT_{50} ratio	KDT_{50}, min, mean (95% CI)	KDT_{50} ratio	KDT_{50}, min, mean (95% CI)	KDT_{50} ratio	KDT_{50}, min, mean (95% CI)	KDT_{50} ratio
Kisumu strain *	12.5	–	13.8	–	15.2	–	21.5 (18.7–24.4)	–
Arumeru	84.4 (67.2–151.0)	6.8	92.2 (84.0–118.5)	6.7	114 (91.7–171.7)	7.5	29.0 (27.9–30.1)	1.3
Babati	15.5 (13.4–17.7)	1.2	34.4 (31.6–37.3)	2.5	118.0 (96.0–179.0)	7.8	38.1 (36.1–40.2)	1.8
Dodoma	17.3 (16.3–18.3)	1.4	22.2 (19.6–24.9)	1.6	21.8 (19.4–24.2)	1.4	26.4 (24.1–28.8)	1.2
Handeni	33.4 (27.3–42.2)	2.7	26.7 (25.1–28.5)	1.9	33.6 (31.8–35.4)	2.2	22.1 (12.9–31.2)	1.0
Ilala	37.9 (36.1–39.7)	3.0	32.5 (28.6–36.5)	2.4	41.5 (37.7–45.9)	2.7	31.2 (28.6–33.8)	1.5
Kilombero	31.3 (28.8–33.9)	2.5	22.3 (19.3–25.3)	1.6	31.5 (28.7–34.4)	2.1	19.2 (15.4–23.3)	0.9
Kyela	15.7 (14.5–16.8)	1.3	13.3 (11.6–14.9)	1.0	11.9 (10.9–12.9)	0.8	31.1 (28.1–34.4)	2.0
Lushoto	15.8 (13.2–18.2)	1.3	23.8 (21.4–26.4)	1.7	28.9 (22.7–35.1)	1.9	36.1 (34.7–37.4)	1.7
Magu	23.8 (19.2–27.9)	1.9	34.2 (29.3–39.4)	2.5	41.7 (35.8–49.6)	2.7	33.3 (24.7–40.2)	1.5
Moshi	47.8 (46.0–49.8)	3.8	47.6 (46.3–48.9)	3.4	59.3 (50.2–82.0)	3.9	36.2 (34.3–38.1)	1.7
Muheza	28.3 (25.8–30.8)	2.3	25.2 (23.7–26.9)	1.8	29.6 (25.9–33.5)	1.9	32.7 (29.9–35.4)	1.5
Mvomero	17.9 (16.4–19.5)	1.4	15.3 (12.9–17.4)	1.1	20.6 (18.5–22.8)	1.4	27.3 (22.7–32.7)	1.3
Tabora	23.5 (20.0–27.1)	1.9	20.9 (16.6–25.4)	1.5	26.7 (24.2–29.4)	1.8	30.5 (24.1–38.8)	1.4

*Kisumu strain, *Anopheles gambiae s.s.* susceptible Kisumu strain was used as susceptible control.

KDT_{50}, time required for 50% of test mosquitoes to be knocked down; KDT_{50} ratio, KDT_{50} of the tested population/KDT_{50} of the susceptible Kisumu strain.

95% CI, 95% confidence interval.