Co-occurrence and distribution of East (L1014S) and West (L1014F) African knock-down resistance in Anopheles gambiae sensu lato population of Tanzania

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Abstract

Objective Insecticide resistance molecular markers can provide sensitive indicators of resistance development in Anopheles vector populations. Assaying these makers is of paramount importance in the resistance monitoring programme. We investigated the presence and distribution of knock-down resistance (kdr) mutations in Anopheles gambiae s.l. in Tanzania.

Methods Indoor-resting Anopheles mosquitoes were collected from 10 sites and tested for insecticide resistance using the standard WHO protocol. Polymerase chain reaction-based molecular diagnostics were used to genotype mosquitoes and detect kdr mutations.

Results The An. gambiae tested were resistance to lambdacyhalothrin in Muheza, Arumeru and Muleba. Out of 350 An. gambiae s.l. genotyped, 35% were An. gambiae s.s. and 65% An. arabiensis. L1014S and L1014F mutations were detected in both An. gambiae s.s. and An. arabiensis. L1014S point mutation was found at the allelic frequency of 4–33%, while L1014F was at the allelic frequency 6–41%. The L1014S mutation was much associated with An. gambiae s.s. (χ² = 23.41; P < 0.0001) and L1014F associated with An. arabiensis (χ² = 11.21; P = 0.0008). The occurrence of the L1014S allele was significantly associated with lambdacyhalothrin resistance mosquitoes (Fisher exact P < 0.001).

Conclusion The observed co-occurrence of L1014S and L1014F mutations coupled with reports of insecticide resistance in the country suggest that pyrethroid resistance is becoming a widespread phenomenon among our malaria vector populations. The presence of L1014F mutation in this East African mosquito population indicates the spreading of this gene across Africa. The potential operational implications of these findings on malaria control need further exploration.

Keywords kdr, L1014S, L1014F, insecticide resistance, Anopheles gambiae, Tanzania
by modifying their gating kinetics, resulting in the prolonged opening of individual channels leading to paralysis and death of the insect (Ranson et al. 2011).

Massive use of insecticides in agriculture (Yadouleton et al. 2010) and public health (Czeher et al. 2008; Trape et al. 2011) has resulted in increasing resistance among malaria vectors due to the selection pressure placed on resistance genes (Ranson et al. 2011). Reduced susceptibility of Anopheles mosquitoes to insecticides such as DDT (dichloro-diphenyl-trichloroethane), malathion, fenitrothion, propoxur and bendiocarb was first reported in 1950s (Brown 1958; Hamon et al. 1968). To date, resistance among Anopheles species to at least one of the four commonly used insecticide classes has been reported in 64 malaria-endemic countries worldwide, the vast majority reporting resistance to pyrethroids (WHO 2012a,b). Even in four insecticide classes available for IRS, resistance has been reported for all of them in some populations of Anopheles gambiae s.s. (Ranson et al. 2009). The increasing resistance of malaria vectors to available insecticides especially pyrethroids, puts current global control efforts at risk.

The major mechanisms by which insects acquire resistance to insecticides are elevated levels of detoxifying enzymes (metabolic resistance) and target-site insensitivity (Hemingway & Ranson 2000; Ranson et al. 2011). Metabolic resistance to pyrethroids is mostly associated with increased cytochrome P450 activity (Berge et al. 1998; Vulule et al. 1999). Recent studies have reported overexpression of cytochrome P450 genes: CYP6M2, CYP6P3 and CYP6Z2 in pyrethroid-resistant populations of An. gambiae (Muller et al. 2007, 2008; Djouaka et al. 2008; Mitchell et al. 2012).

Target-site insensitivity in An. gambiae is associated with two distinct mutations in the S6 transmembrane segment of domain II of the para-type sodium channel at position 1014. The mutations result in either a leucine–phenylalanine (L1014F) (Martinez-Torres et al. 1998) or a leucine–serine (L1014S) substitution (Ranson et al. 2000). The former mutation, which leads to the substitution of a leucine (TTA) for phenylalanine (TTT), was first detected in populations of the Savanna chromosomal form and S molecular form of An. gambiae s.s. in coastal Ivory Coast (Elissa et al. 1993). This was later found to be widespread in West Africa and reported to be strongly associated with pyrethroid resistance in An. gambiae (Martinez-Torres et al. 1998; Chandre et al. 1999a). The latter kdr mutation, with the same amino acid substituting the leucine (TTA) for serine (TCA), was first described in East African An. gambiae s.s. (Ranson et al. 2000). Both types of kdr mutations have been linked with DDT and pyrethroid-resistant phenotypes in wild An. gambiae s.l. populations (Martinez-Torres et al. 1998; Kolaczinski et al. 2000; Ranson et al. 2000; Donnelly et al. 2009).

Several studies with limited geographical sampling have attempted to detail the distribution of kdr mutations in An. gambiae. Most have either screened for the L1014F allele in West African countries (Martinez-Torres et al. 1998; Chandre et al. 1999b; Awolola et al. 2005; Coetzee et al. 2006), or the L1014S mutation in East Africa (Ranson et al. 2000; Kawada et al. 2011; Maweje et al. 2012; Protopopoff et al. 2013). However some studies have screened for the presence of both resistance alleles in several parts of Africa (Stump et al. 2004; Etang et al. 2006; Pinto et al. 2006; Verhaeghen et al. 2006; Awolola et al. 2007; Moreno et al. 2008). Studies have demonstrated the presence of L1014S point mutation in West Africa (Djegbe et al. 2011) and L1014F mutation in East Africa (Kulkarni et al. 2006), indicating that the two mutations do not follow the previously described geographical distribution. Although several studies have been carried out in Tanzania to investigate the insecticide resistance status of the malaria vectors (Kulkarni et al. 2006, 2007; Kabula et al. 2012; Protopopoff et al. 2013), there has been no detailed information on the presence and the distribution of both kdr mutations in the country. This is the first such study designed to investigate the presence and the distribution of the two kdr mutations (L1014F and L1014S) in local population of Anopheles gambiae s.l. of Tanzania.

Methods

Study sites

The study was a follow-up to the main insecticide resistance survey carried in 2011. This was carried out in 10 sentinel districts across Tanzania mainland (Figure 1), namely Muheza, Handeni, Lushoto, Arumeru, Uuyi, Kyela, Ilala, Muleba, Kilombero and Mvomero. Additionally, this study used mosquitoes (for molecular analysis) collected in the main insecticide resistance survey from Moshi, Dodoma, Magu and Babati whose results have been reported elsewhere (Kabula et al. 2013). The study districts were chosen to encompass previously described WHO-recommended criteria (Kabula et al. 2012, 2013). The detailed characteristics of these study districts are described elsewhere (Kabula et al. 2012, 2013) and are summarised in Table 1.

Mosquito sampling

Adult female Anopheles mosquitoes for susceptibility testing and molecular characterisation of insecticide
resistance were collected by the indoor-resting catch technique (WHO 1975) in June–July 2011. Indoor-resting catches were carried out between 0600 and 0900 h in all locations. Freshly blood-fed and unfed female Anopheles mosquitoes were collected. Captured mosquitoes were collected in paper cups and transported to a field laboratory for morphological identification (Gillies & Coetzee 1987) and susceptibility testing (WHO 1998). They were fed with 10% sugar solution embedded in cotton wool pads during transportation. In Tabora, Lushoto and Muleba, the number of adult Anopheles mosquitoes was not sufficient for the susceptibility test; therefore, larvae were collected and reared to adults under standard laboratory conditions (WHO 1975).

Insecticide susceptibility tests

The standard WHO susceptibility tests were conducted on field collected mosquitoes using test-kits and insecticide-impregnated filter papers supplied by the WHO (1975, 1998). Adult female Anopheles mosquitoes were exposed to 0.05% lambdacyhalothrin for 1 h. There were 4–9 replicates of 15–25 wild adult female mosquitoes per test. The controls were exposed to silicone oil impregnated paper. At this exposure time, the number of mosquitoes knocked down was recorded at 10, 15, 20, 30, 40, 50 and at 60 min (WHO 1998, 2013). Mosquitoes were then transferred into the holding tube and fed on 10% (w/v) sugar solution for 24 h. Final mortality was scored after a 24-h holding. Insecticide susceptibility was classified according to the WHO criterion, which considers mortality of 98–100% and below 90% representative of susceptible and resistant populations, respectively, while the intermediates (90–97%) need further investigation (WHO 2013). Estimates for 50% knock-down time (KDT50) were assessed using log-probit analysis (Finney 1971).

Mosquito identification

Mosquitoes were identified to species based on morphological characteristics (Gillies & Coetzee 1987) and stored individually over silica gel for molecular identification and detection of kdr variants. Surviving mosquitoes from susceptibility tests were killed by exposure to ether fumes or by freezing at -20°C prior to morphological identification and storage. All lambdacyhalothrin-resistant mosquitoes were picked from each sentinel site for molecular species identification and kdr analysis. Stored mosquito samples that were previously exposed to lambdacyhalothrin in the 2011 main insecticide resistance survey (Kabula et al. 2013) from Magu, Babati, Moshi and Dodoma were also used in this molecular analysis. In sites where the number of resistant mosquitoes was less than 25 or 0, An. gambiae s.l. were picked at random to make up the total number of 25 per site (Table 1). Genomic DNA was extracted from the whole mosquito of a proportion of females using standard methods (Collins et al. 1987) and amplified using specific diagnostic primers for An. gambiae s.l (Collins et al. 1987; Scott et al. 1993).
Detection of knock-down resistance (kdr) alleles in An. gambiae s.l.

Mutations associated with knock-down resistance (i.e. L1014S and L1014F) to pyrethroids were assayed using the standard PCR assays (Martinez-Torres et al. 1998; Ranson et al. 2000). The PCR products were electrophoresed through 2% agarose gel with ethidium bromide stain and visualised under UV light. Successful reactions had a band of 285 bp. Additionally, there was a 210-bp band for wild-type susceptible and 188 bp for resistant allele (Figures 2 and 3).

Results

Mean mortality rates of An. gambiae s.l. 24 h post-exposure (Figure 4) ranged from 72% to 100%. Full susceptibility to lambdacyhalothrin was observed in Mvomero, Lushoto, Handeni, Kilombero, Kyela and Uyui (mortality of 98–100%). Resistance to lambdacyhalothrin was recorded in Muheza, Arumeru and Muleba (mortality of 83.5%, 72%, and 85%, respectively), while Dar es Salaam recorded reduced susceptibility (mortality of 96.7%).

The median knock-down time (KDT50) of the wild mosquitoes ranged from 13.4 to 152.7 min. Highest KDT50 were recorded in Arumeru, Dar es Salaam and Muleba (KDT50 of 129, 42 and 39 min, respectively). The low KDT50 of 13.4, 20.9, 21.2, 25, 27.7 and 31.9 min were recorded in Kyela, Muheza, Mvomero, Lushoto, Uyui, Kilombero and Handeni, respectively. The proportion of KDT50 of the wild populations to that of susceptible laboratory Kisumu mosquitoes known as resistance ratio (RR) was also calculated. Muleba, Dar es Salaam and Arumeru had the highest RRs. The KDT50 in these sites was between 2.6, 2.8 and 8.5 times than that of the control susceptible Kisumu strain, respectively.
quitoes were also genotyped for the kdr mutations in Anopheles gambiae of Tanzania

Figure 2 Gel electrophoresis of East African knock-down (L1014S) resistance assay. All successful reactions contain a band of 285 bp, a band of 210 bp indicates the susceptible (wild-type) allele and one of 188 bp the resistant allele. The first lane contains a 100-kb ladder marker, lane 1 is the control for the L1014S homozygous resistant, lane 2 is control for the L1014S homozygous susceptible. Lanes 3 and 5 are samples from Muleba; Lanes 4 and 6 are samples from Dar es Salaam (Ilala); lane 7, sample from Handeni; and lane 8, negative control.

Figure 3 Gel electrophoresis of West African knock-down (L1014F) resistance assay. All successful reactions should contain a band of 285 bp, a band of 210 bp indicates the susceptible (wild-type) allele and one of 188 bp the resistant allele. The first lane contains a 100-kb ladder marker, lane 1 is the control for the L1014F homozygous resistant, lane 2 is a negative control, lanes 3–7 are samples from Muheza, Dar es Salaam (Ilala) and Muleba, respectively. Lanes 8 and 9 are samples from Babati (Magugu) and Mvomero respectively.

A total of 1563 mosquitoes were morphologically identified as An. gambiae s.l. and tested for their susceptibility to lambdacyhalothrin. Of these, 350 (22% of the total morphologically identified mosquitoes) were identified to species level using PCR-based techniques. Of the 350, 123 (35.1%) were identified as An. gambiae s.s. and 227 (64.9%) as An. arabiensis (Table 1). These 350 mosquitoes were also genotyped for kdr-east (L1014S) and kdr-west (L1014F) mutations. Of these, 341 were homozygous for the susceptible wild type and 9 were homozygous for L1014S genotype (Table 2). When genotyped for L1014F, 317 were homozygous for the susceptible wild type and 33 were heterozygous (Table 3). There was a significant difference in L1014S allele between lambdacyhalothrin-resistant and susceptible mosquitoes (Fisher exact P < 0.000001). However, there was no significant difference in L1014F allele between lambdacyhalothrin-resistant and susceptible mosquitoes (χ²=0.68; P = 0.409) (Table 4). No L1014S allele was identified among lambdacyhalothrin susceptible (Table 5).

The distribution of L1014S and L1014F mutations in An. gambiae s.s. and An. arabiensis in different parts of the country is shown in Tables 2 and 3 and in Figure 1. The L1014S mutation was detected in both An. gambiae s.s. and An. arabiensis. The L1014S mutation was found at the allelic frequency of 33.3% in Dar es Salaam (95% CI: 16–56%) and 23.8% in Muleba (95% CI: 13–38.3%) in An. gambiae s.s.; and 4.2% (95% CI: 1.1–13.9%) of An. arabiensis from Handeni. Similarity, the L1014F point mutation was detected in both An. gambiae s.s. and An. arabiensis. The L1014F mutation was found in An. gambiae s.s. from Muleba at the allelic frequency of 7.1% (95% CI: 2.5–19%). This L1014F mutation was found in An. arabiensis at the allelic frequency of 40.6% in Dar es Salaam (95% CI: 25.5–57.7%), 11.5% in Babati (95% CI: 4–28.9%), 20% in Muheza (95% CI: 10.5–34.8%), 37.5% in Muleba (95% CI: 13.7–69.4%) and 6% in Mvomero (95% CI: 2–16.2%). The L1014S and L1014F mutations occurred together in Muleba and Dar es Salaam (Figure 1). Although the two kdr mutations appeared in both An. gambiae s.s. and An. arabiensis, the L1014F was much associated with An. arabiensis (χ² = 11.21; P = 0.0008) while the L1014S was associated with An. gambiae s.s. (χ² = 23.41; P < 0.0001) (Table 5).

Discussion

Results from this study continued to demonstrate that the field population of An. gambiae s.l. are resistant to lambdacyhalothrin. Resistance of these malaria vectors to pyrethroids has previously been reported in Tanzania (Kabula et al. 2012, 2013; Protopopoff et al. 2013). The persistence of such resistance could be due the pressure created by the cumulative effect of insecticides used in malaria vector control and agriculture (Kabula et al. 2012, 2013). This study also reports the countrywide distribution of kdr mutations (L1014S and L1014F) in members of An. gambiae s.l. It reports the presence and wide distribution of the L1014S mutation in An. gambiae s.s. and An. arabiensis in Tanzania. It also further
confirms the presence of L1014F point mutation in An. gambiae s.s. and An. arabiensis. The L1014S and L1014F mutations were detected in both An. gambiae s.s. and An. arabiensis. However, L1014S mutation was frequently found in An. gambiae s.s. while L1014F was frequently found in An. arabiensis. Presence of L1014F mutation at very low frequency in An. gambiae s.s. had previously been reported in the country (Kulkarni et al. 2006) and in the neighbouring Kenya and Uganda (Stump et al. 2004; Kawada et al. 2011; Mawejje et al. 2012). The occurrence of both mutations in An. gambiae s.s. and An. arabiensis in this study may indicate that these mosquitoes have similar exposure to the sources which create selection pressure for knock-down resistance. The difference in their frequency of these mutations in the two members of An. gambiae s.l. may, however, be related to a different origin of the mutations in the two populations or linked to different ecological or behavioural characters between An. gambiae s.s. and An. arabiensis (Stump et al. 2004).

The L1014S mutation was detected in An. gambiae s.s. from Dar es Salaam (allelic frequency of 33%) and

| Site          | Anopheles gambiae s.s. | Anopheles arabiensis |
|---------------|------------------------|----------------------|
|               | Genotype count         | Allelic frequency    | Genotype count | Allelic frequency |
|               | RR RS SS               | R S                  | RR RS SS       | R S               |
| Handeni       | 1 0 1                  | 0.000 1.000          | 24             | 0.042 0.958       |
| Dar es Salaam | 9 3 6                  | 0.333 0.667          | 16             | 0.000 1.000       |
| Babati        | 12 0 12                | 0.000 1.000          | 13             | 0.000 1.000       |
| Muheza        | 5 0 5                  | 0.000 1.000          | 20             | 0.000 1.000       |
| Muleba        | 21 5 16                | 0.238 0.762          | 4              | 0.000 1.000       |
| Mvomero       | 0 * *                  | * *                  | 25             | 0.000 1.000       |
| Moshiri       | 0 * *                  | * *                  | 25             | 0.000 1.000       |
| Arumeru       | 0 * *                  | * *                  | 25             | 0.000 1.000       |
| Magu          | 0 * *                  | * *                  | 25             | 0.000 1.000       |
| Lushoto       | 25 0 25                | 0.000 1.000          | 0              | * * *            |
| Kilombero     | 0 * *                  | * *                  | 25             | 0.000 1.000       |
| Uyui          | 25 0 25                | 0.000 1.000          | 0              | * * *            |
| Kyela         | 0 * *                  | * *                  | 25             | 0.000 1.000       |
| Dodoma Rural  | 25 0 25                | 0.000 1.000          | 0              | * * *            |

RR, RS and SS are three possible kdr genotypes, where R represents the resistant L1014S allele and S represents the susceptible wild-type allele.

*No member of a particular species were found in molecular identification, that is, all were identified as either An. gambiae s.s. or An. arabiensis.

Figure 4 Mortality rates in field populations of Anopheles gambiae s.l. exposed to 0.05% lambdacyhalothrin for 60 min. 24-h mortalities <90% are indicative of resistance under WHO terminology and mortality of 90–97% indicates incipient resistance. N = number of mosquitoes exposed to lambdacyhalothrin. Mortality rates for Magu, Babati, Moshi and Dodoma were adapted from Kabula et al. (2013).
Muleba (allelic frequency of 24%) and in *An. arabiensis* from Handeni (allelic frequency of 4%). The L1014F mutation was found in *An. gambiae s.s.* from Muleba (allelic frequency of 7%) and in *Anopheles arabiensis* from Babati, Dar es Salaam, Muheza, Muleba and Mvomero. The L1014S and L1014F mutations co-occurred in Muleba and Dar es Salaam. The high frequency of kdr mutations in Muleba district, also previously reported (Protopopoff et al. 2013), may be a response to selection by recurrent IRS with lambdacyhalothrin since 2007.

| Table 3 Distribution of kdr-west (L1014F) mutation in *An. gambiae s.s.* and *An. arabiensis* mosquitoes |
| --- |
| Site | Genotype count | Allelic frequency | Site | Genotype count | Allelic frequency |
| --- | --- | --- | --- | --- | --- |
| Handeni | 1 | 0 | 0 | 1 | 0.000 | 1.000 | 24 | 0 | 0 | 24 | 0.000 | 1.000 |
| Dar es Salaam | 9 | 0 | 0 | 9 | 0.000 | 1.000 | 16 | 0 | 13 | 3 | 0.406 | 0.594 |
| Babati | 12 | 0 | 0 | 12 | 0.000 | 1.000 | 13 | 0 | 3 | 10 | 0.115 | 0.885 |
| Muheza | 5 | 0 | 0 | 5 | 0.000 | 1.000 | 20 | 0 | 8 | 12 | 0.200 | 0.800 |
| Muleba | 21 | 0 | 3 | 18 | 0.071 | 0.929 | 4 | 0 | 3 | 1 | 0.375 | 0.625 |
| Mvomero | 0 * | * | * | * | * | * | 25 | 0 | 3 | 22 | 0.060 | 0.940 |
| Moshi | 0 * | * | * | * | 25 | 0 | 0 | 25 | 0.000 | 1.000 |
| Arumeru | 0 * | * | * | * | * | 25 | 0 | 0 | 25 | 0.000 | 1.000 |
| Magu | 0 * | * | * | * | 25 | 0 | 0 | 25 | 0.000 | 1.000 |
| Lushoto | 25 | 0 | 0 | 25 | 0.000 | 1.000 | 0 * | * | * | 1.000 |
| Kilombero | 0 * | * | * | * | 25 | 0 | 0 | 25 | 0.000 | 1.000 |
| Uyui | 25 | 0 | 0 | 25 | 0.000 | 1.000 | 0 * | * | * | 1.000 |
| Kyela | 0 * | * | * | * | 25 | 0 | 0 | 25 | 0.000 | 1.000 |
| Dodoma Rural | 25 | 0 | 0 | 25 | 0.000 | 1.000 | 0 * | * | * | 1.000 |

RR, RS and SS are three possible kdr genotypes, where R represents the resistant L1014S allele and S represents the susceptible wild-type allele.

*No member of a particular species were found in molecular identification, that is, all were identified as either *An. gambiae s.s.* or *An. arabiensis*.|

| Table 4 Number of mosquitoes with kdr-east (L1014S) and kdr-west (L1014F) mutation genotypes among surviving (resistant) and dead (susceptible) mosquitoes after exposure to lambdacyhalothrin |
| --- |
| kdr-east genotype | kdr-west genotype |
| n | RR | RS | SS | Statistics | RR | RS | SS | Statistics |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Resistant (surviving) | 85 | 9 | 0 | 76 | Fisher’s exact test $P < 0.000001$ | 0 | 10 | 75 | $\chi^2 = 0.68; P = 0.409$ |
| Susceptible (dead) | 265 | 0 | 0 | 265 | 0 | 23 | 242 | |

RR, RS and SS are three possible kdr genotypes, where R represents the resistant L1014S or L1014F allele and S represents the susceptible wild-type allele.

| Table 5 Number of mosquitoes with kdr-east (L1014S) and kdr-west (L1014F) genotypes among *An. gambiae s.s.* and *An. arabiensis* |
| --- |
| kdr-east genotype | kdr-west genotype |
| n | RR | RS | SS | Statistics | RR | RS | SS | Statistics |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *An. gambiae s.s.* | 123 | 8 | 0 | 115 | $\chi^2 = 23.41; P < 0.0001$ | 0 | 3 | 120 | $\chi^2 = 11.21; P = 0.0008$ |
| *An. arabiensis* | 227 | 1 | 0 | 226 | 0 | 30 | 197 | |

RR, RS and SS are three possible kdr genotypes, where R represents the resistant L1014S or L1014F allele and S represents the susceptible wild-type allele.
permethrin LLINs in association with the extensive usage of pesticides in coffee plantations. However, kdr has been reported in some areas with no IRS pressure in Burundi (Protopopoff et al. 2008) – which explains the occurrence of kdr in Handeni. Low insecticide usage in Handeni for agriculture may also play a role in the occurrence of kdr mutation. High frequency of kdr mutation in Dar es Salaam may be attributed to increased selection pressure resulting from industrial waste/pollutants, high LLINs use (Kabula et al. 2013) and extensive local use of insecticides for fumigation and agricultural (mainly horticulture) purposes. The high kdr frequency in Dar es Salaam is supported by the previous report of high level of DDT resistance (Kabula et al. 2012). Occurrence of kdr mutations in Muheza, Babati and Mvomero may be attributed to the high LLINs use and use of pyrethroids in agriculture.

Selection of knock-down resistance has been attributed mainly to the use of DDT and pyrethroids in agriculture and public health (Elissa et al. 1993; Stump et al. 2004). For example, the use of pyrethroids in malaria vector control interventions such as ITNs and IRS is known to create the selection of kdr alleles (Stump et al. 2004; Protopopoff et al. 2013). Similarly, domestic use of insecticides (e.g. fumigation) may play an important role in selection of knock-down resistance (Elissa et al. 1993), and this may be the case for urban settings such as Dar es Salaam.

The L1014S allele occurred significantly more often in lambdacyhalothrin phenotypically resistant-selected samples than in susceptible ones. Apart from the association found in this study, some sites which previously reported pyrethroid and DDT resistance (Kabula et al. 2012, 2013; Protopopoff et al. 2013) were found with kdr mutations (e.g. Muheza, Muleba). Such resistance to pyrethroids and DDT in An. gambiae is known to associate closely with both L1014S and L1014F mutations (Williamson et al. 1996; Martinez-Torres et al. 1998; Ranson et al. 2000, 2004; Reimer et al. 2008). However, the association was not found in the case of L1014F mutation and the pyrethroid-resistant phenotypes. Similarly, this study could not establish such associations in some sites (e.g. Mvomero and Babati) where kdr mutations were recorded without obvious phenotypic resistance to pyrethroids being observed. The absence of pyrethroid phenotypic resistance in Mvomero and Babati may be explained by the recessiveness of the kdr allele. Henceforth, the occurrence of the genes in the heterozygous recessive form leads to their appearance at low frequencies in these two sites. This might explain the absence of phenotypic resistance to pyrethroids, as the conventional bioassay methods that measure phenotypic resistance cannot detect the heterozygous proportion of the population (Chandre et al. 2000). However, models of insecticide resistance show rapid increase in the frequency of resistance, especially when the frequency reaches levels as low as 0.1%, resulting in control failure (Roush & McKenzie 1987). Conversely, the presence of kdr mutation in Babati is strongly supported by KDT_{50} for lambdacyhalothrin. High values of KDT_{50} in the field mosquitoes gives early indication of the presence of kdr mutation (Chandre et al. 2000). A significant increase in knock-down time may be observed in some mosquito populations before any decrease in mortality, suggesting that knock-down time could also be a good indicator for the early detection of pyrethroid resistance (Chandre et al. 2000).

Mosquitoes from Moshi and Arumeru did not have kdr mutations despite having high levels of phenotypic pyrethroid resistance (Kabula et al. 2013). This suggests that other mechanisms are responsible for the observed phenotypic resistance in these sites. Possibly the main mechanisms involved in these sites might be biochemical resistance which had previously been reported in Moshi (Matowo et al. 2010). Even in the areas where the kdr mutations were found, the presence of other mechanisms cannot be ruled out. Both target-site insensitivity and metabolic resistance have been found in An. gambiae (Vulule et al. 1999; Stump et al. 2004; Mitchell et al. 2012). Therefore, there is a need to further investigate the presence and distribution of cytochrome P450-based metabolic resistance mechanisms in malaria vectors. Such information will help to explain the mechanism(s) of resistance responsible for the observed or even suspected resistance and thus facilitate planning for appropriate insecticide resistance management.

This study reports the countrywide distribution of L1014S and L1014F kdr mutations among members of An. gambiae s.l., and further confirms the presence of a typically West African L1014F kdr mutation in Tanzania. Therefore, we re-emphasise the need to test for both kdr mutations regardless of geographical location (Kulkarni et al. 2006). Sequencing analysis is required to provide further insights on the phylogenetic relations of the L1014F alleles found in East and West Africa. We also reported the presence and wide distribution of the L1014S mutation in An. gambiae s.s. and An. arabiensis in Tanzania. The presence of these kdr mutations in the mosquito populations has since been used as predictor for their susceptibility to DDT and pyrethroids (Ranson et al. 2004; Reimer et al. 2008). These findings coupled with previous reports on insecticide resistance in the country (Kabula et al. 2013; Protopopoff et al. 2013) suggest that pyrethroid resistance is a
widespread phenomenon among our malaria vector populations.

The implications of high kdr frequency on the malaria vector control interventions such as ITNs and IRS are uncertain. However, studies in Benin showed some reduced effectiveness of LLINs and IRS in areas where An. gambiae have high kdr frequency (N’guessan et al. 2007; Asidi et al. 2012). Thus, the potential operational impact of insecticide resistance on the effectiveness of vector control interventions such as ITNs and IRS needs to be properly evaluated. Meanwhile, periodic monitoring of the frequency of both L1014S and L1014F mutations and phenotypic pyrethroid resistance in An. gambiae s.l. is essential for the rational and effective control of these vectors.

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