Spatial-Temporal Distribution and Insecticide resistance Status of *Aedes Aegypti* in Ghana

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Abstract

Background: Vector control is the main intervention to control arboviral diseases transmitted by *Aedes* mosquitoes because for most there are no effective vaccines or treatment. This vector control relies heavily on the use of insecticides, effectiveness of which may be impacted by resistance. In addition, rational insecticide application requires detailed knowledge of vector distribution, dynamics, resting, and feeding behaviours, which are poorly understood for *Aedes* vectors in Africa. This study investigated the spatio-temporal distribution and insecticide resistance status of *Ae. aegypti* from across ecological extremes of Ghana

Methods: Immature mosquitoes were sampled from containers in and around human dwellings at each of seven study sites in urban, suburban, and rural areas of Ghana. Adult *Aedes* mosquitoes were sampled indoor and outdoor using Biogent sentinel-2 mosquito traps, human landing catches, and prokopack aspiration. Distributions of immatures and adult *Aedes* mosquitoes were determined indoors and outdoors during dry and rainy seasons at all sites. Phenotypic resistance status of *Aedes* mosquitoes to insecticides was determined using WHO bioassays. Host blood meal source was determined by PCR.

Results: A total of 16,711 immature *Aedes* were sampled, with over 70% found in car tires. Significantly more breeding containers had *Aedes* immatures during the rainy season 70.95% (11,856) compared to the dry season 29.05% (4,855). A total of 1,895 adult *Aedes* mosquitos were collected, including *Ae. aegypti* (97.8%), *Ae. africanus* (2.1%) and *Ae. Luteocephalus* (0.1%). Indoor sampling of adult *Aedes* mosquitoes yielded a total of 381 (20.1%) and outdoor a total of 1,514 (79.9%) ($z = -5.427$; $p = 0.0000$) over the entire sampling period. *Aedes aegypti* populations were resistant to DDT at all study sites. Vectors showed suspected resistance to Bendiocarb (96-97%), Permethrin (90-96%) and Deltamethrin (91-96%) and were susceptible to the organophosphate malathion from all study sites.

Blood meal analysis showed that the *Aedes* mosquitoes were mostly anthropophilic with HBI of 0.9 i.e. [(human = 90%), (human and dog = 5%), (dog and cow = 5%)].

Conclusion: *Aedes* mosquitoes were found at high densities in all ecological zones of Ghana. Resistance to pyrethroids and carbamates may limit control efficacy and requires careful monitoring.

Background

Aedes aegypti and *Ae. albopictus* are the most important vectors of several arboviruses, notably yellow fever, dengue, Zika, and chikungunya [1]. The importance of *Aedes* vectors in sub-Saharan Africa has increased recently because of outbreaks of arboviral diseases in multiple countries [2]. In West Africa, within the last five years, there have been outbreaks of dengue in Burkina Faso [3–5], Cote d'Ivoire [6, 7], Senegal [8], yellow fever in Cote d'Ivoire [9–11], and Nigeria [12–16] and recent confirmation of dengue cases and outbreaks of yellow fever have occurred in Ghana [17–21]. Therefore, the risk of dengue, yellow fever, and chikungunya outbreaks in Ghana appear high.

Aedes aegypti are highly anthropophilic and, in most of the world, typically endophilic [22]. Immature stages develop preferentially in artificial containers usually in close proximity to humans [23–26]. In sub-Saharan Africa two morphological subspecies (ecotypes) have been acknowledged: domestic *Aedes aegypti aegypti* and sylvan *Aedes aegypti formosus*. The presence/absence of white abdominal scaling patterns [27] is used to differentiate the ecotypes, but at present, clear genetic boundaries appear absent, probably as a result of recent historical gene flow [28, 29]. Breeding of *Ae. aegypti. formosus* occurs more frequently away from domestic environments and they feed readily on animals (zoophagy), so are less likely to be a threat to humans in the urban environments where *Ae. aegypti. aegypti* populations thrive [30]. Nevertheless, urbanisation of sylvatic environment could lead to contact with humans by these vectors, and or previously sylvatic species might adapt to new urban environments and hosts, or probably the introgression or hybridisation of *Ae. formosus* traits in domestic forms or vice versa may lead to variations, potentially increasing their

roles as vectors [29, 31]. Consistency of bionomic traits across ecozones remains poorly investigated. However, measures of abundance and distribution of *Aedes* infestation levels would give more reliable insights for both risk and mitigation strategies [32]. Several species of *Aedes* including *Aedes africanus*, readily feed on animals (both domestic and wild), as well as humans, hence their potential importance as bridging vectors and for zoonotic transmission [33]. Identification of the source of vector blood meals is critical to understand the degree of human-vector interaction (i.e. anthropophily) [34, 35], a crucial parameter in estimation of the capacity to transmit the disease [36].

Seasonal variations in population density are expected for *Aedes* with lower abundances in dry seasons, rising with increasing temperatures and potentially greater breeding site availability in the rainy season [37–39]. However, human activities involving water storage, and non-disposal of potentially water-holding containers greatly influence the breeding of *Aedes* in individual households and may provide breeding sites year-round [40]. Key factors that may influence productivity in different container types include the frequency of water replenishment, the availability of food for the larvae, the degree of sunlight exposure [41], and container covering [42]. The adaptation of the vectors to urban domestic habitats has led to the exploitation of a range of artificial containers and the capacity to exploit potential breeding water situated indoors or outdoors [43, 44].

Currently, and despite frequent concerns over efficacy of deployment methods [45], insecticidal interventions are the main tool to control *Aedes*-borne arboviral infections, since vaccines are either unavailable, ineffective or in limited supply [46–48]. To ensure that efficacy is maximised, correct insecticide choice is crucial, requiring surveillance for susceptibility of target populations, alongside the location of adults and immatures to be targeted [49]. Sustainability of effectiveness must also be considered: geographical variation in susceptibility may rapidly lead to the spread of resistance and require revision of a previously suitable insecticide choice.

Another important parameter when considering how to target vector control, especially for insecticidal spray deployment, is whether mosquitoes tend to rest indoors (endophily) or outdoors (exophily) after blood-feeding [50]. Insecticide-based interventions directed at the adult resting population represent a relevant approach for *Aedes* control and disease prevention. Targeted Indoor residual spraying (T-IRS) on *Aedes* resting locations can provide a significant protective effect against arboviral transmission, this control method also has the potential to control pyrethroid-resistant *Aedes* mosquitoes, as other classes of insecticides (non-pyrethroid) are available for residual application [51].

This study aimed to characterize the breeding habitats, seasonal abundance, and resting behaviour of *Ae. aegypti* and their insecticide susceptibility in rural, suburban, and urban sites in Ghana at different ecological zones. In addition to identifying targets and options for control, these results will also aid the development of a surveillance system for *Aedes* arbovirus vectors for disease control planning in Ghana [52, 53].

Methods

Study sites

This study was carried out in seven sites including rural, suburban, and urban communities within the three major ecological zones of Ghana i.e., coastal savannah, forest, and Sahel savannah, across wet and dry seasons, between May 2017 and May 2018. Sample sites are shown in Fig. 1. Sites in the coastal savannah found in southern Ghana consisted of Ada Foah (5°47' N, 0°38' E) a suburban tourist town, Accra (5°33'0" N, 0°12'0" W) an urban city, the capital of Ghana and the most populous city, and Tema (5°40'0" N, 0°0'0" E) an urban port-city where import of tires from Asia and America might facilitate the invasion of new *Aedes* genotypes or species. The coastal savannah has a tropical savannah climate, with an annual mean temperature of 26.5°C and average annual precipitation of 787 mm.

The site within the forest area was Konongo (06°37'00"N, 001°13'00"W), an urban town located in Asante-Akim central district in the middle of Ghana. In the forest zone, there is a high possibility that sylvan *Aedes* mosquitoes, which can serve as bridging vectors, might be present. The forest zone has a tropical rainforest climate, with an annual average temperature of 26.4°C and an annual average amount of precipitation of 1399.5 mm.

Sites in the Sahel savannah ecological zone consisted of Larabanga, Navrongo, and Paga. Larabanga (9°5'0" N, 1°49'0" W), a rural village, close to the Mole national park which harbours monkeys that could serve as reservoirs to arboviruses and which has experienced yellow fever outbreaks [54]. Navrongo (10°53'5"N, 01°05'25"W), an urban town close to the border between Ghana and Burkina Faso. Burkina Faso has reported a recent outbreak of dengue fever since 2016 [55]. The last site was Paga (10°59'32"N, 01°06'48"W), a suburban town located on the border of Burkina Faso and is 166 km south of Ouagadougou where a recent outbreak of dengue fever was recorded [55].

The climate in both the coastal savannah and forest area generally consists of a bimodal pattern of rainfall, with the long rainy season from April to June, and a short rainy season from October through November. Rainfall in the Sahel savannah is unimodal, with the rainy season between May to November and the dry season from December to April.

Distribution of immature Aedes mosquitoes

Exhaustive entomological surveys were carried out to identify water-holding containers in and around human dwellings, and inspected for *Aedes* immature in the dry and rainy seasons in each of the study sites and those positive for *Aedes* immatures were recorded. Sampling efforts were standardised with respect to number of habitats inspected and number of dips per habitat across sites and time points. All pupae and larvae (1st to 4th instars) from positive containers were collected using pipettes and ladles [40, 56], counted, and recorded on field-data forms. Water from large containers was first sieved and larval samples were placed in a white plastic tray with some water from which they were pipetted. Mosquito samples were placed immediately in labelled specimen bowls with water-filled from the water from which they were collected after collection and transported to the insectary. Immature mosquitoes were reared in the insectary in large white plastic trays at an average temperature of 28.15 ± 1.8°C (standard deviation) and relative humidity of 80.9 ± 6.3%, and larvae were fed on TetraMinbaby® fish food (Tetra Werke, Melle, Germany).

Adult mosquitoes that emerged from larval collections were used for the WHO susceptibility tests [57], and later identified morphologically using standard taxonomic keys [58]. Coordinates of all collection points were recorded using a GPSMAP® 60CSx geographical position system (GPS) instrument (Garmin International, Inc., Olathe, Kansas, USA).

Characterization of the breeding habitats and their productivity

In each entomological survey, the habitat type, its location in the household (indoor or outdoor), and its physical characteristics were recorded. Six container types were classified based on their use and material: car tires, air-condition saucers, discarded containers, drums, tanks, and buckets. Air-condition saucers are small (1-2 l) plastic containers positioned below the outlet of air conditioners to collect water. Discarded containers included broken jars, bottles, small plastic food containers, tins, plates, cans, cooking pots, and broken pots. Drums were defined as 100-500 l capacity plastic water storage containers. Tanks were defined as 100-500 l capacity water storage containers made of metal or concrete. Buckets included 10-25 l water storage containers made of metal or plastic. It is notable, because of potential for *Aedes* breeding site provision, that pipe-borne water was absent in Larabanga and Paga, with the consequence that households tend to have long-term water storage in tanks, drums, buckets, and pots, especially during the dry season.

Distribution of adult Aedes mosquitoes

The spatial distribution of adult *Aedes* mosquitoes was determined by sampling inside households and outdoors. Three sampling methods were used in the Sahel savannah zone, namely, BG traps, HLC, and prokopack aspiration (PPK) (John W. Hock Company, Gainesville, U.S.A.) [59], to collect vectors indoors and outdoors. The relative trap efficiency of the three

sampling methods was compared in the Sahel savannah zone. Two sampling methods were employed in the coastal and forest ecological zones due to logistical challenges, Biogent (BG traps) sentinel-2 mosquito traps (Biogents, Regensburg, Germany), and human landing catch (HLC).

The GPS coordinates of all collection points were recorded. Two cross-sectional surveys were undertaken, one in the dry season (January - March and December 2018) and one in the rainy season (May-June 2018).

Adult mosquito collection using Biogent sentinel 2 trap

Biogent sentinel traps were set both indoors (living room(s) and bedrooms) and outdoors (open, but secure verandas, granaries, or under a shed/tree where people sit to chat about 5 m from the house) during the times 5:00-8:00 am. and 3:00-7:00 pm. The BG traps were baited with carbon dioxide (CO_2) which was produced by either BG-lures and/or from a mixture of 17.5 g yeast (Angel Yeast (Egypt) Co. Ltd.), 250 g sugar in 1 litre of water [60]. At a 1-hour interval, the mosquito collection net of the BG-trap was changed. Mosquitoes trapped within the collection net were placed in a cooler box containing ice and then transported to the insectary. Sixteen houses were randomly selected at each site, with four houses sampled each day. Sampling using the BG trap was done on four different days. A written informed consent was sought from house leaders before the traps were set up for sampling.

Adult mosquito collection using human landing catches

The HLC is the standard method for measuring exposure of humans to mosquito bites, and it is essential to determine biting rate and biting time. During the dry and rainy seasons, sixteen houses were randomly selected at each of the study sites for adult mosquito collections using HLC. On each day, two trained volunteers were positioned to catch *Aedes* mosquitoes indoors and another two sat outdoors (open, but secure verandas, granaries, or under a shed/tree where people sit to chat about 5 m from the house) at four different houses. The houses used for sampling were changed each day (using a new house not yet sampled) on four different days during the season. The collection was done hourly from 5:00 -8:00 am. and then from 3:00 -7:00 pm. Collected *Aedes* were placed in well labelled paper-cups, placed in cool boxes with ice packs, and transported to the insectary for identification and further processing. Informed written consent was sought from volunteers involved in HLC sampling. Training on mosquito sampling was conducted and renumeration of volunteers after sampling.

Adult mosquito collection using prokopack aspirators

Prokopack aspiration was employed at the three sites in the Sahel savannah area, Larabanga, Navrongo, and Paga. Sixteen houses were randomly selected for *Aedes* collection per site. Sampling for *Aedes* mosquitoes was done indoors (bedroom and living rooms) and outdoors (including open, but secure verandas, granaries, under a shed/tree where people sit to chat about 5 m from the house). *Aedes* caught within the prokopack plastic collection cups were labelled and placed in a cooler box containing an ice pack and transported to the insectary for identification.

The height at which mosquitoes were caught by the PPK while resting was recorded using a tape measure. This was to determine whether there was heterogeneity in resting height among sites. Verbal consent was sought from house leaders before PPK aspiration was done in their houses.

Aedes **phenotypic resistance to insecticides**

Aedes larvae were collected from natural breeding sites or from oviposition traps that were set in each site. Larvae collected were brought to the insectary at the Department of Medical Microbiology, University of Ghana, and were raised to adults in the insectary under standard conditions (25 ± 2°C; 80% ± 4% Relative Humidity with a 12 h: 12 h light/dark cycle). Batches of 20-25 non-blood-fed 3-5-day-old females were used for susceptibility bioassays. Four replicates and two controls were used for each insecticide using the standard WHO tube assay procedure [57].

WHO test papers impregnated with; pyrethroids (0.05% deltamethrin or 0.75% permethrin); an organochloride (4% DDT); an organophosphate (5% malathion) or a carbamate (0.1% bendiocarb) insecticide were used. These pre-impregnated papers are supplied based on *Anopheles* diagnostic concentrations and for permethrin and malathion are 3x and approximately 6x the diagnostic concentration for *Aedes* mosquitoes. However, these papers are far more commonly used for assessment of *Aedes* susceptibility than those custom-produced at the recommended concentrations [61]. The knockdown time (KDT) was reported every 10 min during the 60 min exposure period. Mortality was recorded after the 24 h recovery period. Resistant mosquitoes were defined as mosquitoes that survived 24 h after the end of the bioassay, and susceptible mosquitoes as the mosquitoes that were knocked/dead down during the 60 min exposure time or that died within the 24 h recovery period.

Data analysis

Descriptive analysis was performed to compare larval and adult abundance between different population structures (urban, suburban and rural), indoor and outdoor and seasons.

The abundance of *Aedes* mosquito larvae and adults was compared among the seasons, indoor and outdoor study sites (ecozones) and sampling method (adults). Kruskal-Wallis, and Wilcoxon rank-sum test were used to test for association between continuous and categorical variables. The Fisher's Exact test was used to test the association between two categorical variables. Nested generalized linear mixed models with sites nested within ecological zones were used to model the effect of ecozone, season, population structure and sampling methods on larval and adult abundance. A regression analysis was done to test trap efficacy. Probability values less than 0.05 were interpreted as statistically significant.

Human blood index (HBI) was calculated as HBI = $\frac{Total number of positive for human blood}{Total number of specimen tested}$.

WHO insecticide susceptibility levels were classified using the WHO criteria [57]: 98-100% mortality, a test population is considered susceptible; 90-97% suggests possible resistance (requires confirmation); below 90% is considered resistant. Knockdown and mortality rates were compared between sites using Chi-square. Statistical analysis was performed using STATA® 16 (StataCorp LLC, 4905 Lakeway Drive College Station, Texas, USA).

Results

Larval breeding habitats and their productivity

A total of 81 positive breeding habitats were identified during the study period across the seven sites (only positive breeding habitats were recorded). Generalized linear model analysis revealed a significant interaction effect between ecozone and population on abundance. Compared to the other sites, the chances of getting *Aedes* larvae increased in the forest zone (unadj B = -204.12 [-306.01- -102.24], p = 0.000). The abundance of *Aedes* larvae increased in suburban areas (unadj B = -138.01 [-224.77- -51.26], p = 0.002) compared to urban areas, Table 2.

There were significantly more positive habitats during the rainy season than the dry season (N = 50 vs N = 31; df = 5; χ^2= 19.44; p = 0.001), Table 3. Within the 7 sites sampled, 78 (96.3 %) of larval breeding habitats were located outdoors and 3 (3.7%) located indoors (which were all found in Larabanga), Table 3, with larval abundance of 16,426 (98.3%) and 285 (1.7%) respectively (N = 78 vs N = 3; z = -0.138; p = 0.8903).

A total of 16,711 *Aedes* immatures were collected over the entire sampling period, of which 12,348 (73.9%) were from car tires, 3,138 (18.8%) from discarded containers, 730 (4.4%) from air-condition saucers, 230 (1.4%) from buckets, 210 (1.3%) from tanks and 55 (0.3%) from drums (χ^2 = 1.020, df = 5, p = 0.96), Table 4. In all the different sites, car tires had the

highest proportion 8,453 (71.3%) of immatures during the rainy season. During the dry season car tires were still very productive with the highest abundance for *Aedes* immatures 3,895 (80.2%) (χ^2 = 2.106, df = 2, p = 0.3490), Table 4.

From the different ecological zones, significantly higher numbers of *Aedes* immatures were collected from the coastal savannah 9,819 (58.8%), followed by the sahel savannah 5,794 (34.7%), then the forest zone 1,098 (6.6%) (χ^2 = 16.071, df = 2, p = 0.0003). A higher proportion of immature *Aedes* mosquitoes was found in urban areas with abundance of 10,876 (65.1%) [Accra = 3,670; Tema = 4,338; Konongo = 1,098; Navrongo = 1,770], followed by the suburban areas with a total of 3,890 (23.3%) [Ada Foah = 1,811; Paga = 2,079] and rural areas with a total abundance of 1945 (11.6%) [Larabanga = 1,945] (χ^2 = 10.040; df = 2; p = 0.0066). There were more *Aedes* larvae sampled outdoors, 16,426 (98.3%) immatures compared to indoors 285 (1.7%) (z = -0.138; p= 0.8903).

There were significantly more *Aedes* immatures during the rainy season 11,856 (70.9%) compared to the dry season 4,855 (29.1%) (z = -2.747; p = 0.0060, Table 4.

In the different study sites, the highest proportion of immatures were found in Tema 4,338 (26.0%) followed by Accra 3,670 (22.0%), Paga 2,079 (12.4%), then Larabanga 1,945 (11.6%), Ada Foah 1,811 (10.8%), Navrongo 1,770 (10.6%), Konongo 1,098 (6.6%) (χ^2 = 16.642, df = 6, p = 0.0107).

Seasonal distribution of adult Aedes mosquitoes

A total of 1,895 adult *Aedes* mosquitoes were collected from the study sites. Generalized linear model analysis revealed significant interaction effect between outdoor collection, ecozone and population structure on abundance. The chances of getting more adult *Aedes* mosquitoes increased in outdoor collection (adj B = 1.49 [1.0271- 1.9602], p = 0.000). There was a significant difference of adult *Aedes* mosquito abundance in suburban sites (adj B = -1.49 [-2.0433- -.9320], *p* = 0.000) compared to the urban sites, (Table 5). Adult *Aedes* were more abundant during the rainy season 1,257 (66.3%) compared to the dry season 638 (33.7%) (z = -1.433; p = 0.1519). Across the different ecological zones, the abundance of *Aedes* were high in the coastal savannah 955 (50.4%) [Accra (urban) = 718; Tema (urban) = 161; Ada Foah (suburban) = 76], followed by the sahel savannah 837 (44.2%) [Navrongo (urban) = 577; Paga (suburban) = 173; Larabanga (rural) = 87], and then the forest zone 103 (5.4%) [Konongo (urban) = 103] (χ^2 = 0.359, df = 2, p = 0.835). The urban sites had the highest abundance of *Aedes* mosquitoes; 1,559 (82.3%) [Accra = 718; Tema = 161; Konongo = 103; Navrongo = 577] followed by suburban sites; 249 (13.1%) [Ada Foah = 76; Paga = 173], then the rural sites 87 (4.6%) [Larabanga = 87] (χ^2 = 20.147; df = 2; p = 0.0001).

In the different sites during the dry season, the highest abundance of *Aedes* mosquitoes were found in Accra 178 (27.9%) (HLC= 163; BG= 15], followed by Navrongo 173 (27.1%) [HLC=157; BG=16], Tema 108 (16.9%) [HLC=102; BG=6], Konongo 103 (16.1%) [HLC=88; BG=15], Ada 60 (9.4%) [HLC=50; BG=10], Larabanga 15 (2.4%) [HLC=0; BG=15], then Paga 1 (0.2%) [HLC=1; BG= 0] (χ^2 = 20.500; df = 6; p = 0.0023).

During the rainy season, the highest abundance of *Aedes* mosquitoes were found in Accra 540 (43.0%) [HLC=499; BG=41] followed by, Navrongo 404 (32.1%) [HLC=354; BG=50], Paga 172 (13.7%) [HLC=168; BG=4], Larabanga 72 (5.7%) [HLC=54; BG=18], Tema 53 (4.2%) [HLC=31; BG=22], Ada Foah 16 (1.3%) [HLC=0; BG=16] then Konongo 0 (0%) [HLC=0; BG=0], (χ^2 = 132.896, df = 6, p = 0.0001).

Indoor and outdoor abundance of adult Aedes population

Overall mosquito abundance was highest outdoors as compared to indoors over the entire sampling period. Indoor sampling yielded a total of 381 (20.1%) and outdoor a total of 1,514 (79.9%) over the entire sampling period (z = -5.427; p = 0.0000). During the rainy season, a high proportion of *Aedes* mosquitoes were captured from outdoors 77.8% (978),

compared to indoors 22.2% (279) (z= –2.989; p = 0.0028). Similarly, a greater number of *Aedes* mosquitoes were sampled outdoors 84% (536) than indoors 16% (102) during the dry season (z= -5.021; p = 0.0000), Fig. 2.

A total of 1,140 *Aedes* mosquitoes were collected by HLC, BG traps and PPK in Larabanga, Navrongo, and Paga during the experiment. Overall, HLC (734) yielded 2.4 times higher adult abundance of *Aedes* mosquitoes compared to PPK (303), and yielded 7.1 times higher adult abundance of *Aedes* mosquitoes compared to BG (103), However regression analysis showed that there was a significant difference between HLC and BG trap (p = 0.000; CI = -2.180248 -.7245137), but no significant difference between HLC and PPK (p = 0.350; CI = -.4820909 1.359284), Table 6.

Generalized linear model analysis revealed significant interaction effect between outdoor collection, study site and sampling method on abundance. More adult *Aedes* mosquitoes were collected outdoor (adj B = 0. 87 [0.22, 1.52], p = 0.009). Adult *Aedes* mosquitoes were more abundant in Navrongo (adj B = 0.83 [0.07, 1.58], p = 0.032) and BG traps were the least efficient traps for the collection of *Aedes* mosquitoes (adj B = -1.39 (-2.14, -0.64), p < 0.001), Table 7.

Resting height of Aedes mosquitoes

The maximum height at which *Aedes* mosquitoes were caught resting was 5m whereas the lowest height for resting was 1m. The mean height that most of the *Aedes* mosquitoes caught preferred to rest ranged from 1.8m - 2.0m indoors and 1.3m - 2.8m outdoors (χ^2 = 1.408, df = 2, p = 0.4945). No mosquito was caught resting indoors in Navrongo, Table 8.

Aedes species composition in the study sites

Morphological identification of adult *Aedes* collected in all sites showed that *Aedes aegypti* 1,854 (97.8%) were the most abundant species present at all sites followed by *Aedes africanus* 40 (2.1%) and *Aedes luteocephalus* 1 (0.01%), Table 9. From the larvae collected in all sites that were allowed to grow to become adults in the insectary, all 11,506 *Aedes* mosquitoes that emerged and were identified morphologically were *Aedes aegypti*, Table 9.

Blood Meal Analysis Of The Vectors

Blood meal analysis was carried out on blood-fed mosquitoes that were sampled by the BG trap and Prokopack in Larabanga, Navrongo, and Paga. Out of 44 blood-fed mosquitoes analysed by PCR, 20 amplified. Out of the 20 that amplified, 18 (90%) had taken a human blood meal, 1 (5%) had fed on human and cow and 1 (5%) had taken a blood meal from dog and goat.

Phenotypic resistance of Aedes to insecticides

Phenotypic test results showed that *Aedes* mosquito populations from all study sites were resistant to DDT (0% - 88%). The highest DDT resistant site was in Tema, where none of the mosquitoes died on exposure to DDT. Vectors showed resistance to permethrin in Tema (21%), Accra (40.0%) and Larabanga (89%), suspected resistance in Navrongo (90%), Paga (96%) and Konongo (90%) (χ^2 = 1.331, df = 12, p = 0.0001). Vectors showed resistance to deltamethrin in Tema (68%) and suspected resistance in Accra (91.3%), Ada Foah (94%), Konongo (94%), Larabanga (93%), Navrongo (96%) and Paga (93%) (χ^2 = 560.000, df = 6, p = 0.0001). *Aedes* mosquitoes were resistant to bendiocarb in Larabanga (81%), suspected resistance in Tema (95.0%), Konongo (96%), Navrongo (96%) and Paga (97%) and susceptible in Accra and Ada Foah (χ^2 = 1.331, df = 12, p = 0.0001). *Aedes* mosquitoes were susceptible to organophosphates (malathion) at all sites, Fig. 3.

Discussion

Seasonal variation in their population density is common for *Aedes* mosquitoes due to their sensitivity to change in temperature and moisture [54, 62]. This study found a significantly higher abundance of *Aedes* immatures during the rainy season compared to the dry season. The rains may have resulted in increased aquatic habitats for *Aedes* breeding in car tires and other breeding containers encountered in this study [63], thus increasing the abundance of *Aedes* immatures due to an increase in the rate of oviposition. This finding was similar to what was observed in Kenya, with a higher abundance of immatures during the rainy season [40]. However, a study in Ghana showed more *Aedes* immatures during the dry than the rainy season [54].

Water collected in car tires, buckets, tanks, drums, discarded containers, and air-condition saucers were the main breeding sites and supported the development of *Aedes* immatures in all or some study sites. Distribution in container types varied between the dry and rainy seasons. In all, only car tires could be indicated as key breeding habitats in both seasons at all sites and contributed to over 70% of *Aedes* immatures collected during the study period, followed by discarded containers, air-condition saucers, buckets, tanks, and then drums. The abundance of *Aedes* immatures in car tires from our study was consistent with a study conducted in the Central African Republic, where used car tires were the most heavily colonized productive larval habitats for *Aedes* in both early and late wet seasons [64]. Car tires could therefore be targeted for vector control to eliminate most of the *Aedes* immatures.

The finding of this study shows *Ae. aegypti* rests at an average height of 1.8m - 2.0m indoors and 1.3m - 2.8m outdoors, this observation was quite different from previous findings from Iquitos, Peru, showing that of 56 [59] and in Acapulco, Mexico, 626 [65] *Ae. aegypti* collected indoors, 82% were found resting below 1.5 m. This may have major implication to IRS, due to the exophilic behaviours of some *Aedes* mosquitoes. Insecticide pressure indoor due to IRS, may also instigate exophagy and outdoor transmission of arboviruses.

In this study, *Aedes aegypti* was the predominant species in all study areas for both adult and larval sampling. The high number of *Ae. aegypti* caught from a geographically wide range of sites in different ecological zones, implies that yellow fever and dengue vector is well established in Ghana and the potential for arboviral disease transmission and an outbreak is high in the absence of effective vector control. This calls for constant monitoring to prevent an outbreak of arboviral disease. The zoonotic species: *Ae. africanus* and *Ae. luteocephalus* which transmit arboviruses between monkeys were found in this study; suggesting the potential for bridging of disease between sylvan and domestic environments because both are vectors of yellow fever in Ghana [66].

The WHO susceptibility tests showed that *Aedes* mosquito populations from all the study sites were resistant to DDT. This finding was similar to a previous study done in Accra, Ghana, which showed that vectors were resistant to DDT [66]. Deltamethrin, permethrin, and bendiocarb resistance were also recorded. These are some of the most widely used insecticides for vector control of *Aedes* species, [67, 68] and therefore could negatively affect the efficacy of vector control efforts in Ghana. Deltamethrin and permethrin resistance maybe as a result of the widespread use of pyrethroids for impregnating bednets and IRS against malaria vectors. However, pyrethroid resistance in *Aedes* population has been recorded worldwide [69, 70]. Cross-resistance between pyrethroids and DDT is also known to occur [30]. A previous study in Ghana [66] found *Aedes* to be susceptible to permethrin.

Conclusion

The results of this study indicate that *Aedes aegypti* breeding habitats are abundant outdoors and are diverse across Ghana. Car tires are responsible for the majority (>70%) of the *Aedes* immature production. Targeting source reduction efforts toward these productive container types may be a cost-effective way to reduce the risk of arboviral disease transmission in Ghana. Resistance to pyrethroids and carbamates may limit control efficacy and requires careful monitoring. Insecticide resistance management strategies are needed urgently.

Abbreviations

Ae. - Aedes

BG - Biogent

CO2 - Carbon dioxide

DDT - Dichlorodiphenyltrichloroethane

GPS - geographical position system

HBI - Human blood index

HLC - Human landing catch

KDT - Knockdown time

PCR - Polymerase chain reaction

PPK - Prokopack

WHO - World Health Organisation

Declarations

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Availability of data and materials

All datasets generated and/or analysed during this study are available on request.

Authors’ contribution

CMO-A, JAAM, DW, and YAA were responsible for the study design and supervised data collection and contributed to the writing of the manuscript whilst CMO-A performed data collection and analysis, laboratory work, and drafted the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval on the use of volunteers for mosquito sampling and protocols involved was sought from the Ethics and Protocol Review Committee of the College of Health Sciences of the University of Ghana with protocol identification number: CHS-Et/M.9 – P1.5/2017-2018. Permission to carry out the study at the various study sites was sought from community leaders, verbal consent was verbally sought from the heads of households before entrance into houses for inspection and sampling. Written informed consent was sought from volunteers who were involved in HLC sampling and were trained prior to sampling.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1
Study sites

Population structure	Major ecological zones of Ghana		
	Costal savannah	Forest zone	Sahel savannah
Urban	Accra (5°33'0" N, 0°12'0" W)	Konongo (06°37'00"N, 01°13'00"W)	Navrongo (10°53'5"N, 01°05'25"W)
	Tema (5°40'0" N, 0°0'0" E)		
Suburban	Ada Foah (5°47' N, 0°38' E)		Paga (10°59'32"N, 01°06'48"W)
Rural			Larabanga (9°5'0" N, 1°49'0" W)

Table 2
Factors associated with productivity and abundance.

Characteristics	Category	Unadjusted B (CI)	*p* - value	Adjusted B (CI)	*p* - value
Season	Dry	1		1	
	Rainy	80.51 (-2.26 – 163.27)	0.057	65.22 (-12.02 – 142.46)	0.098
Ecozone	Coastal Savanna	1		1	
	Forest	-204.12 (-306.01- -102.24)	0.000	-184.81 (-358.62- -10.99)	0.037
	Sahel Savanna	-72.9569 (-157.57- 11.65)	0.091	-61.23 (-158.68- 36.21)	0.218
Indoor/outdoor	Indoor	1		1	
	Outdoor	61.94 (-155.38- 279.25)	0.576	30.59 (-.194.75- 255.92)	0.790
Population	Urban	1		1	
	Suburban	8.62 (-96.67- 113.90)	0.873	13.89 (-88.91 – 116.71)	0.791
	Rural	-138.01 (-224.77- -51.26)	0.002	-9.43 (-156.49 – 137.63)	0.900

Table 3
Seasonal distribution of positive breeding habitats by location and season.

Container type	Season		Location		Total (%)
	Dry (%)	Rainy (%)	Indoor (%)	Outdoor (%)	
Tyre	26 (44.07)	33 (55.93)	0	59	59 (100.00)
Container	0	15 (100.00)	0	15	15 (100.00)
Bucket	2 (100.00)	0	2	0	2 (100.00)
Tank	0	1 (100.00)	0	1	1 (100.00)
Drum	0	1(100.00)	1	0	1 (100.00)
Air-condition saucer	3 (100.00)	0	0	3	3 (100.00)
Total	31 (38.27)	50 (61.73)	3 (3.7)	78 (96.3)	81 (100.00)

Table 4
Productivity profile of container type per site and season.

Container type	Seasons	Ada Foah	Tema	Accra	Konongo	Larabanga	Navrongo	Paga
Car tires	Dry	695	2260	535	0	405	0	0
	Rainy	1066	2078	487	558	505	1680	2079
Discarded containers	Dry	0	0	0	0	0	0	0
	Rainy	50	0	1918	540	540	90	0
Air-condition saucer	Dry	0	0	730	0	0	0	0
	Rainy	0	0	0	0	0	0	0
Bucket	Dry	0	0	0	0	230	0	0
	Rainy	0	0	0	0	0	0	0
Tank	Dry	0	0	0	0	0	0	0
	Rainy	0	0	0	0	210	0	0
Drum	Dry	0	0	0	0	0	0	0
	Rainy	0	0	0	0	55	0	0
Seasonal totals	Dry	695	2260	1265	0	635	0	0
	Rainy	1116	2078	2405	1098	1310	1770	2079
Total		1811	4338	3670	1098	1945	1770	2079

Table 5
Factors associated with adult *Aedes* mosquito abundance.

Characteristics	Category	Unadjusted B (CI)	*p* - value	Adjusted B (CI)	*p* - value
Season	Dry	1		1	
	Rainy	.34 (-.1487-.8221)	0.174	.49 (-.0109- .9931)	0.055
Ecozone	Coastal Savanna	1		1	
	Forest	-.44 (-1.3873-.5069)	0.362	-1.16 (-2.1213- -.2186)	0.016
	Sahel Savanna	-.16 (-.6561-.3312)	0.519	.19 (-.3609- .746917)	0.495
Indoor/outdoor	Indoor	1		1	
	Outdoor	1.45 (.9840- 1.9242)	0.000	1.49 (1.0271- 1.9602)	0.000
Population	Urban	1		1	
	Suburban	-1.25 (-1.8001- -.7042)	0.000	-1.49 (-2.0433- -.9320)	0.000
	Rural	-1.49 (-2.1720- -.8260_	0.000	-1.844 (-2.5952- -1.0935)	0.000

Table 6
Trap comparison in the Sahel ecological zone.

Study sites	Dry season						Rainy season					
	BG (%)		HLC (%)		PPK (%)		BG (%)		HLC (%)		PPK (%)	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
Larabanga	2 (13.3)	13 (86.7)	0	0	1 (4.4)	22 (95.6)	4 (22.2)	14 (77.8)	34 (63)	20 (37)	40 (32)	86 (68)
Navrongo	10 (62.5)	6 (37.5)	36 (22.9)	121 (77.1)	0	22 (100)	18 (36)	32 (64)	92 (26)	262 (74)	0	8 (100)
Paga	0	0	0	1 (100)	0	0	4 (100)	0	37 (22)	131 (78)	87 (70)	37 (30)
Total	12 (5.1)	19 (8.1)	36 (15.4)	122 (52.1)	1 (0.4)	44 (18.8)	26 (2.8)	46 (5.1)	163 (18)	413 (45.6)	127 (14)	131 (14.5)
Total per trap	31 (2.7)		158 (13.9)		45 (3.9)		72 (6.3)		576 (50.5)		258 (22.6)	

Table 7
Factors associated with trap efficiency.

Characteristics	Category	Unadjusted B (CI)	*p* - value	Adjusted B (CI)	*p* - value
Season	Dry	1		1	
	Rainy	0.31 (-0.45, 1.08)	0.425	0.09 (-0.71, 0.89)	0.821
In/out	Indoor	1		1	
	Outdoor	0.90 (0.24, 1.56)	0.007	0. 87 (0.22, 1.52)	0.009
Site	Larabanga	1		1	
	Navrongo	0.98 (0.22, 1.74)	0.011	0.83 (0.07, 1.58)	0.032
	Paga	0.65 (-0.22, 1.52)	0.142	0.34 (-0.54, 1.23)	0.446
Trap	HLC	1		1	
	BG	-1.45 (-2.18, -0.72)	0.0001	-1.39 (-2.14, -0.64)	0.000
	Prokopack	0.44 (-0.48, 1.36)	0.350	0.42 (-0.50, 1.34)	0.373

Table 8
Resting heights of *Aedes* per study site

Study Site	Total Number of Mosquitos (%)	Indoor	Outdoor	Highest height of the houses	Average resting height (indoor)	Average resting height (outdoor)
Paga	124 (48.1)	87	37	4m	1.8m	1.3m
Navrongo	8 (3.1)	0	8	5m	-	2.8m
Larabanga	126 (48.8)	40	86	5m	2.0m	2.4m

Table 9
Morphological species identification of *Aedes* mosquitoes per study site.

	Adults				**Larvae**
Study Site	Total per site (%)	*Aedes aegypti* (%)	*Aedes africanus* (%)	*Aedes luteocephalus* (%)	*Aedes aegypti* (%)
Ada Foah	76 (100.0)	64 (84.2)	12 (15.8)	0 (0.0)	981 (100.0)
Tema	161 (100.0)	161 (100.0)	0 (0.0)	0 (0.0)	3021 (100.0)
Accra	718 (100.0)	718 (100.0)	0 (0.0)	0 (0.0)	2650 (100.0)
Konongo	103 (100.0)	80 (77.7)	23 (22.3)	0 (0.0)	1098 (100.0)
Larabanga	87 (100.0)	83 (95.4)	3 (3.4)	1 (1.1)	1196 (100.0)
Navrongo	577 (100.0)	575 (99.7)	2 (0.3)	0 (0.0)	795 (100.0)
Paga	173 (100.0)	173 (100.0)	0 (0.0)	0 (0.0)	1765 (100.0)
Total	1895 (100)	1854 (97.8)	40 (2.1)	1 (0.1)	11506 (100.0)

Figures

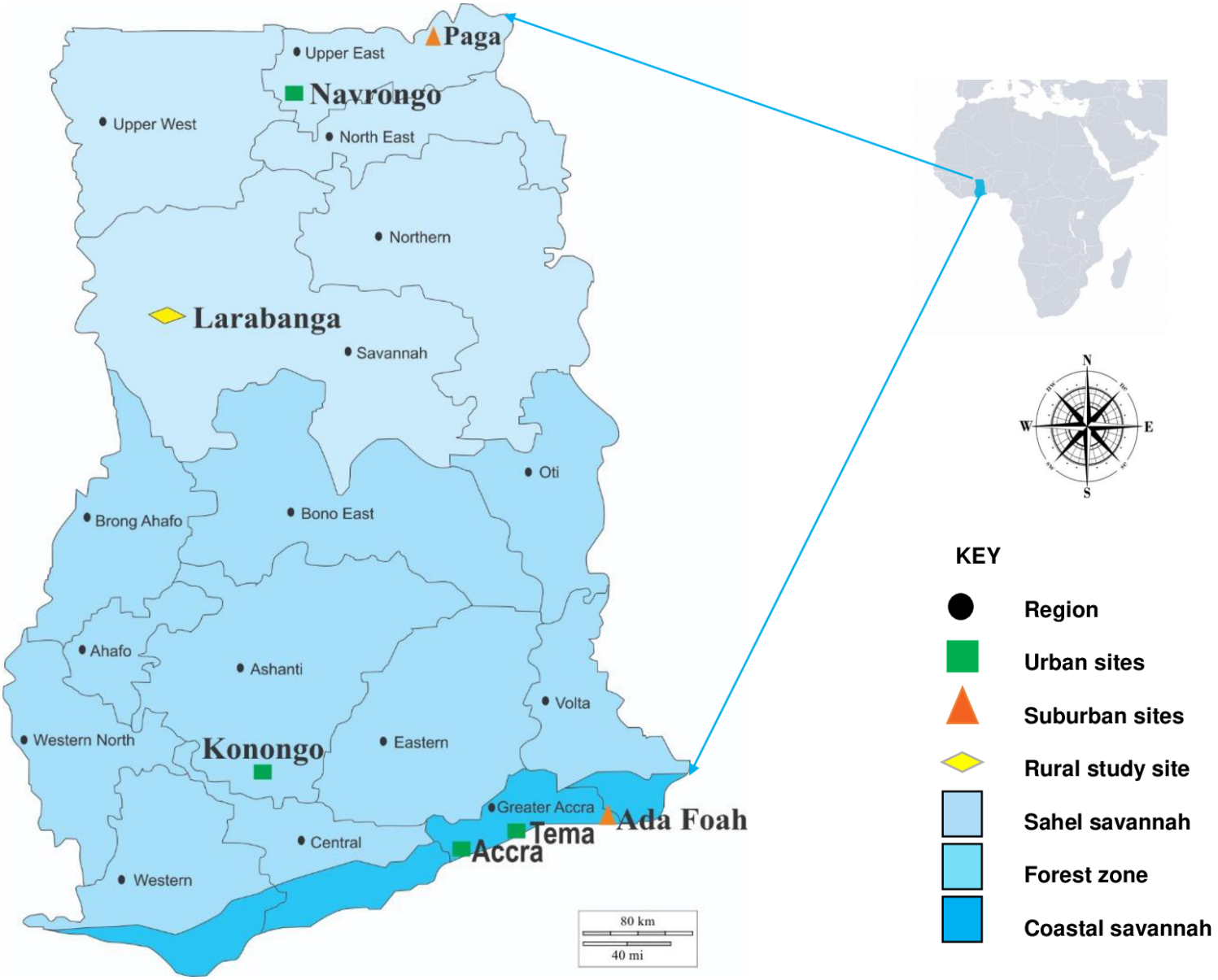

Figure 1

Map of Ghana showing the study sites.

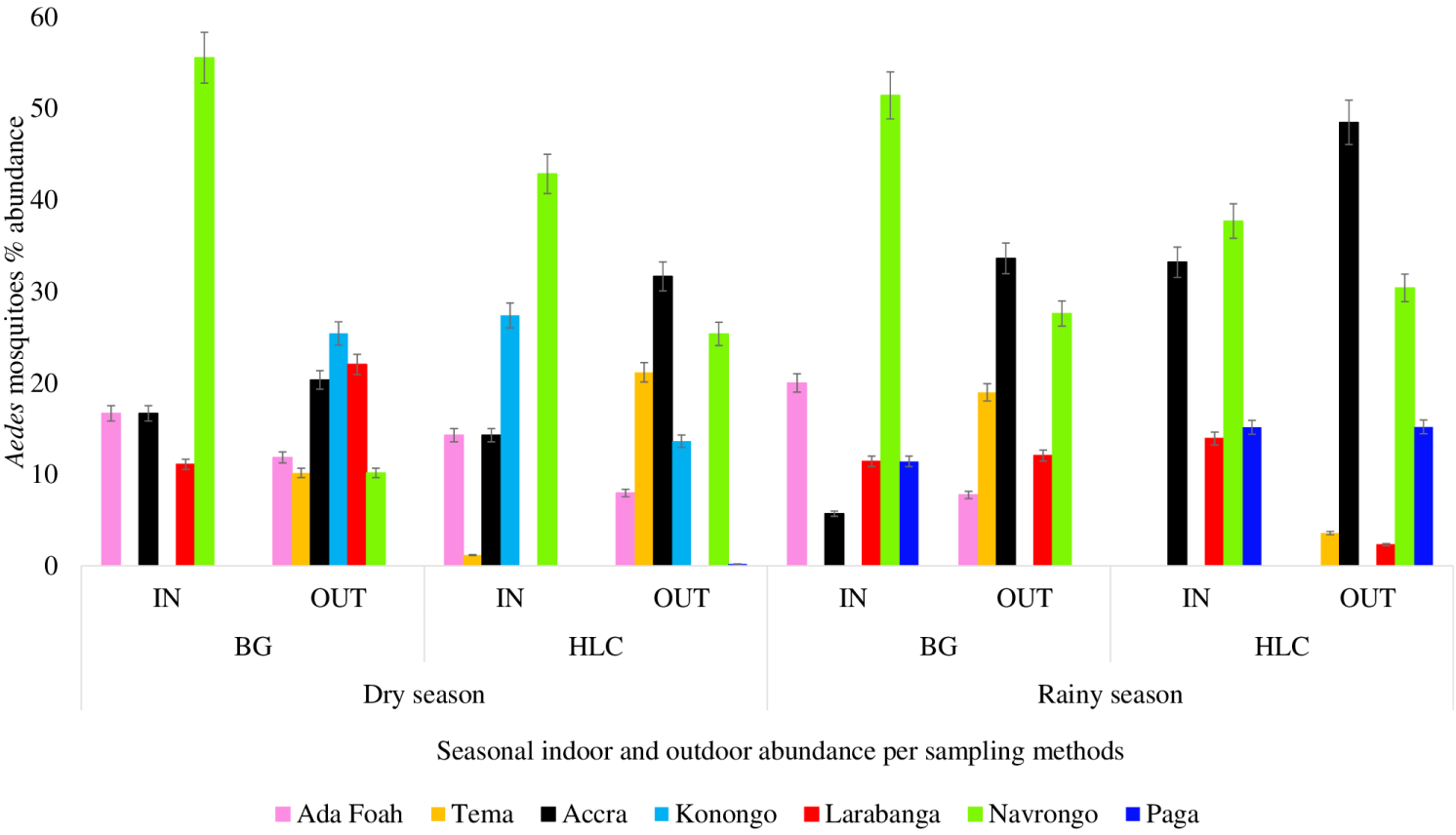

Figure 2

Seasonal distribution of indoor and outdoor adult Aedes mosquito abundance.

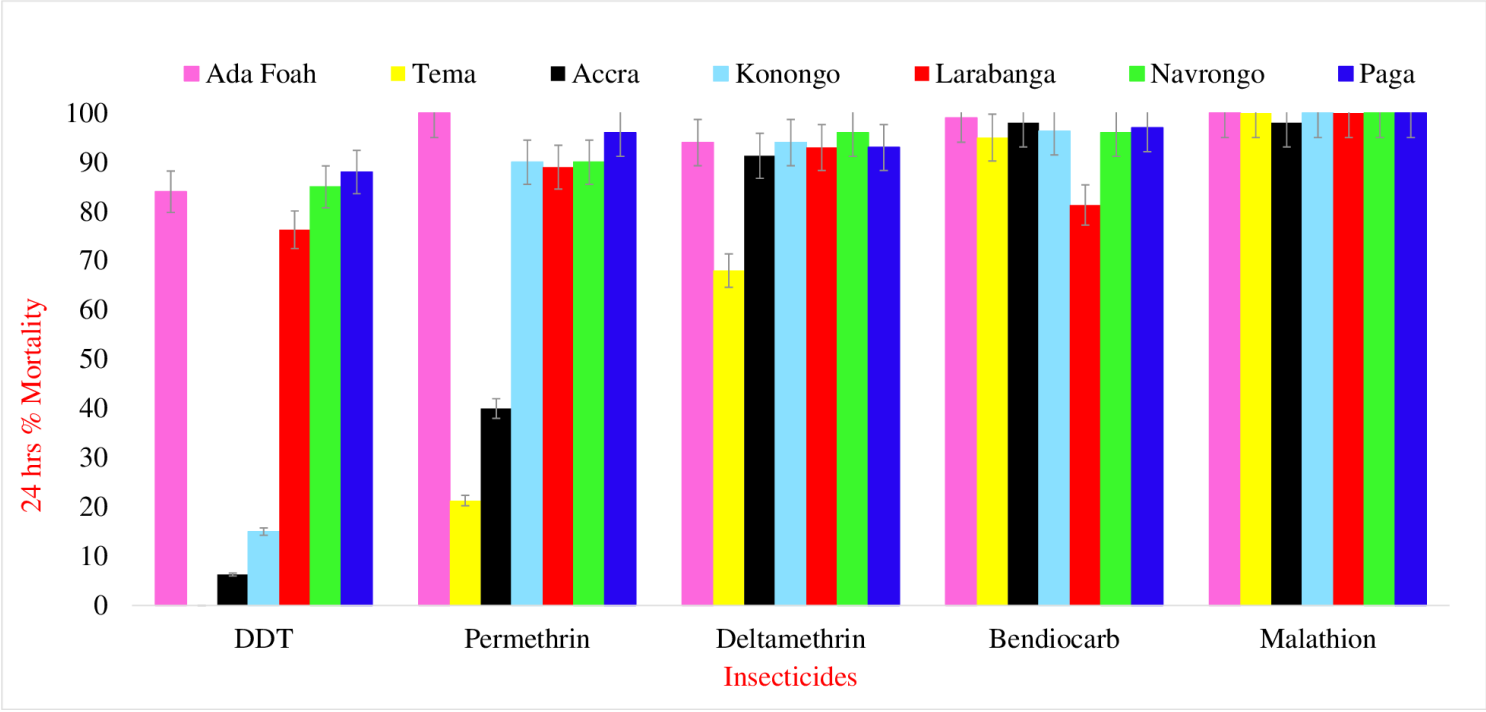

Figure 3

Phenotypic resistance status of Aedes mosquitoes to different insecticides.

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