Surveillance assessment and morphological identification of Culex Mosquito found in Karachi, Pakistan

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Abstract- Mosquitoes are arthropods distributed throughout the world, and their requirement for blood meal for egg development makes them potential vectors of diseases, which pose a threat to humans. Whether for research or control measures, identification of adult female mosquitoes to species is essential. Morphology characteristics used to be the gold standard method for the identification of mosquito species but morphological identification has certain limitations including lack of expertise and damage of external features due to improper handling of samples. We identified 1601 adult Culex mosquitoes and the most infested area for Culex is the Shireen Jinnah Colony. Aedes species were also present in large number while Anopheles presence very low. The integrated taxonomy-based identification tool for mosquito species and its integration has the capacity to promote and enhance vector surveillance and control programs.

Index Terms- *Culex*, Microorganism, Mosquitoes, morphological

I. INTRODUCTION

Field evaluations for understanding the epidemiology of mosquito-borne diseases in urban environments are frequently carried out in places where mosquito concentrations and habitats are known to be high. Furthermore, during surveys, the selection of families and structures is frequently limited 1. To combat dengue fever, malaria, certain arboviral encephalitis, and mosquito-borne filariasis, almost all tropical and subtropical nations have mosquito vector research, monitoring, and control programs in place. Some of these diseases have resurfaced or experienced a significant increase in patient numbers during the last two decades 2. Similarly, Culex mosquitoes bite in a painful and persistent manner, causing lymphatic filariasis, a neglected tropical illness. When filariasis parasites are transmitted to humans by mosquitoes, the illness develops. Pakistan is a subtropical country and the main spot for many vector-borne diseases such as Dengue, Malaria, Leishmaniasis, West Nile virus disease, and Crimean Congo hemorrhagic fever 3,4.

Because its environment and temperature are considerably more suitable for vectors, Pakistan is suffering far more significant difficulties with vector-borne illnesses including leishmaniasis, Crimean Congo hemorrhagic fever (CCHF), chikungunya, dengue, and west Nile fever 5. In Pakistan, the arrival of migratory birds is every year and they arrive from Europe, Siberia, and Central Asia could be potentially one of the important natural reservoirs for the West Nile Virus infection. Hundreds of bird species both wild and domesticated are vulnerable to West Nile virus infection, most of which are subclinical. Birds that migrate are responsible for the propagation of West Nile fever virus during their journey because anti-West Nile virus antibodies are discovered less frequently in resident birds than in migratory birds. It is proposed that they can serve as a reservoir for West Nile virus and transport the virus over large distances, while resident wild birds serve as amplifiers for local West Nile virus strains 6. The West Nile Virus is an important member of the flavivirus genus. It has a mosquito-bird spread cycle, and a variety of mosquito species and bird species have been identified in the United States. Its dissemination is attributed to Culex mosquitoes 7. Maps for visualization of dengue surveillance data are particularly useful for public health professionals' advocating for increased resources, such as vector control.

To compensate for a specific drug for treatment and vaccine, vector-borne disease prevention is limited to vector control (chemical spraying, biological control, physical removal of breeding sites, and improving infrastructure) and Surveillance is essential to vector-borne disease management. Geographical Information Systems (GIS) allow further investigation of surveillance data through spatial statistical analysis and visualization of patterns and relationships between disease and the environment 8. The quantity and distribution of mosquito fauna are influenced by many factors including climate situations, specifically rainfall, humidity, temperature, human mobility and activities, etcetera but not limited to host availability 9. The accurate identification of vector mosquitoes and awareness of their geographical distribution, biology, and ecology are considered important factors for the control and surveillance of vectors 10. Morphological identification is a conventional and gold standard method for the identification of mosquitoes which depends on the external features. Polymerase chain reaction (PCR) is becoming a popular technique for mosquito identification based on DNA sequencing and the fact that every species has its own genetic identity 11.

Morphological identification requires expertise and is time-consuming as well. The mishandling of the samples might cause damage to some important features. Indeed the similarity of some

important characters by species complex members makes identification depend on taxonomic keys alone a tiresome work 12. In addition, new techniques of genomics are more reliable and shorter than the morphological identification technique 13. The current study has been undertaken to identify the prevalent mosquito species in Karachi and particularly the Culex species through morphological identification.

II. Materials and Methods

Study Areas and Period: The study was conducted in Karachi. This city is located at latitude of 24.54°N and longitude of 67.08°E and as a result, has a relatively mild climate. The average temperature for the year in Karachi is 26.7 °C and the annual percentage of humidity is 70%. The area receives rainfall during monsoons, with an annual rainfall of up to 146.5mm (5.77 in) the duration of study was summer 2023.

Sample collection: Collection of vector samples (*Culex, dengue* and *anopheles* mosquito) from specific areas of Karachi, Pakistan (Figure 1b). Mosquito vectors were collected more during and after rainfall.

Transportation of Sample: Transportation of Sample was done from field to lab by using the standard procedure

Environmental Parameters: All environmental parameters of mosquito habitat like temperature, humidity, and terrain features recorded on the Entomological Data Form and compiled. Additional locality data points, (longitude/latitude, elevation, etc.) were also filled out in data form (Figure 1a).

Geographical locations of Sampling Areas: Garmin GPS Map 62S was used to collect the geographical locations of sampling sites. Information about the latitude, longitude, and elevation of the site was taken through a GPS device. This information on sampling sites is always helpful to draw spatial and temporal patterns over maps. Spatial pattern helps to identify potential breeding sites of C*ulex* vectors while temporal pattern may reveal and explain population fluctuation over a period of time. Mapping of the location of the houses around the study region was done by the global positioning system (GPS) and the geographical coordinates were recorded.

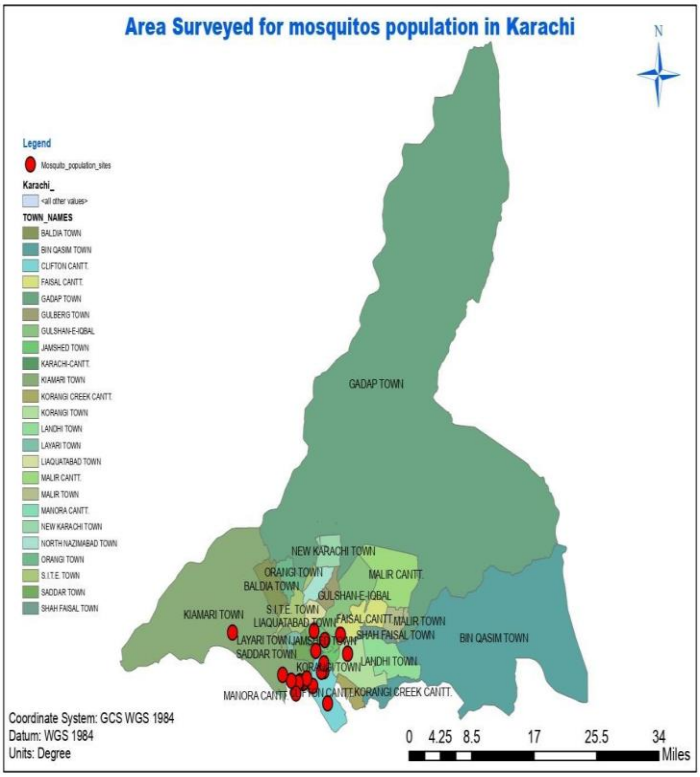

Figure 1a: GPS Surveillance spot

Morphological Identification: Mosquitoes collected were morphologically identified by using the key Zootaxa- 589 a Pictorial key for the identification of mosquitoes (Diptera: Culicidae) -Leopoldo M. Rueda under stereomicroscope (model No.1106) at 40x.Colony rearing was done in the insectary. The identified mosquitoes were preserved at -80°C for RNA/NA further experimentation.

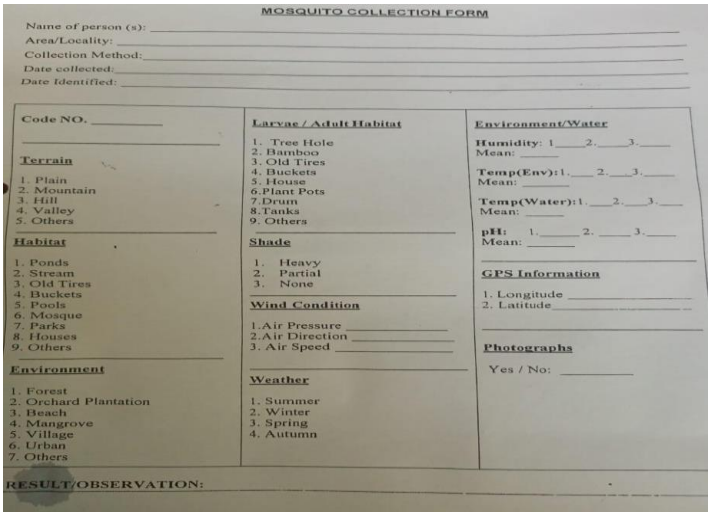

Figure 1b: Data form

Table 1a: Sample Collection Data

Site	Y	X	Aedes		Culex		Anopheles		Humidity	Temp	Total
			Male	Female	Male	Female	Male	Female			
Gurumandir	24.88	67.04		1	3	7	-	-	**38%**	**37.2**	
DHA Phase- IV Saad bin abiWaqas Masjid	24.83	67.06	1	1	2	6	1	--	**37%**	**39.8**	
DHA Phase IV Baithul Salam Masjid	24.83	67.05	51	66	2	2	--	--			**193**
DHA Phase II Masjid Ibrahim	24.84	67.06	6	3	30	11	--	--	**37%**	**38**	
New Cantt	24.85	67.04	--	--	49	16	--	1	**29%**	**38**	
PECHS	24.87	67.06	--	--	50	15			**37%**	**37**	**143**
Clifton Star Apartment Block-2	24.81	67.02	1	4	2	5	--	--	**50%**	**33.7**	
Jamia Masjid Abubakkar Siddique Clifton Block-2	24.81	67.01	79	148	29	120	--	--			
Masjid-e- Bilal Clifton Block-2	24.81	67.04	4	6	116	39	--	--	**42%**	**30**	
Baba Freed Ganj Bakhash Masjid Clifton	24.82	67.03	--	--	107	116	--	--	**42%**	**30**	**376**
Jamia Masjid Usman Ghani Clifton	24.81	67.01	1	3	77	62	2	--	**51%**	**29**	
Jamia Masjid Usman bin Affan Qayyumabad B area	24.88	67.09	4	4	24	61	--	--	**42**	**32.8**	**533**
Jamia Masjid Faizan Mustafa Korangi	24.85	67.11	--	2	140	28	--	--	**49%**	**36.5**	
Masjid Hamza Korangi	24.79	67.07	--	--	26	18	--	--	**38%**	**37**	**477**
Dar-ul-aalum Karachi korangi	24.85	67.11	1	2	43	123	--	1	**38%**	**37**	
Govt. boys & girls secondary school Shireen Jinnah Colony	24.82	67.00	39	61	142	69	--	--	**38%**	**37**	
Govt. boys & girls school kemari	24.88	66.88	--	--	12	10	--	--	**49%**	**32.2**	
Ali Ghaib shah mazar kemari Jackson	24.82	66.98	--	--	7	10	--	--	**47%**	**36.8**	
China port	24.80	67.00	--	--	-	22	--	--	42%	37.3	**372**
									42	38.1	
									54%	31.9	
			187	301	861	740	3	2			
										31.9	2094

III. RESULTs and Discussion

Culex mosquitoes showed various breeding habitats including fresh water, brackish, and salt containers in urban areas. Female Culex mosquitoes feed on wild, domestic birds and human beings. It is the concerned vector of Japanese encephalitis 14. The density of Culex mosquitoes is directly related to the temperature of an area. The peak season is the summer but it is highly in number in spring and it breeds throughout the year 15, which is in line with this study. Female mosquito feed primarily on pigs and birds, but will also bite men. It is an implicated vector of Japanese B encephalitis 14. The density of Culex was directly related to temperature. It also found full sunlight in stagnant water 15. This correlated with the temperature of Karachi.

Culex was by far the most abundant species (1601) among various sites (Table 1a) followed by Aedes (488), Anopheles presence was lowest in the sites visited. One Way ANOVA showed a significance value at 0.642 (Table 1b), which is greater than α value (0.05) meant that population varies within/ between the addresses (Figure 2 and 3), further confirmed by Tukey's Test. The highest population of both Aedes males and females is found in areas including Jamia Masjid Abubakkar Siddique Clifton Block-2, DHA Phase IV Baithul Salam Masjid, and Govt. boys & girls secondary school Shireen Jinnah Colony (Figure 4). The highest population of Culex male is found in areas including Govt. boys & girls secondary school Shireen Jinnah Colony and Jamia Masjid Faizan Mustafa Korangi. The highest population of Culex females is found in areas including Dar-ul-uloom Karachi Korangi, Jamia Masjid Abubakkar Siddique Clifton Block-2, and Baba Freed Ganj Bakhash Masjid Clifton (Figure 5). Anopheles existence was rare with males are more found in Jamia Masjid Usman Ghani Clifton area, while Anopheles females were almost negligible in numbers (Figure 6).

Morphological identification is the gold standard and the conventional method to identify mosquito species depending on their external characters 11. However, morphological identification is highly time consuming and requires experienced taxonomists. Additionally, the similarity of shared morphological features by species complexes members' makes identification based on taxonomic keys alone a difficult task 12. We also experienced the same challenges during our research work. Polymerase chain reaction (PCR) is becoming a popular technique for mosquitoes identification based on their DNA sequencing and the fact that every species has its own genetic identity 11. In addition, new techniques of genomics are reliable and shorter 13. The current study is the preliminary work and molecular identification would be executed for validation of results achieved so far.

IV. Conclusion

Morphology characteristics are still valuable and used as the gold standard method for the identification of Culex mosquito species. We identified 1601 adult Culex mosquitoes using taxonomic keys. Morphological identification is a complementary and integrated taxonomy-based identification tool for mosquito species and it has the capacity to promote and enhance vector surveillance and control programs as well as define the diversity of species in the region. Whether for research or control measures, identification of adult female Culex mosquitoes to species is essential. The identification of these vectors can be explored by probing in to morphological characteristics. Surveillance is essential to vector-borne disease management. Geographical Information Systems (GIS) allows further investigation of surveillance data through spatial statistical analysis and visualization of patterns and relationships between disease and the environment.

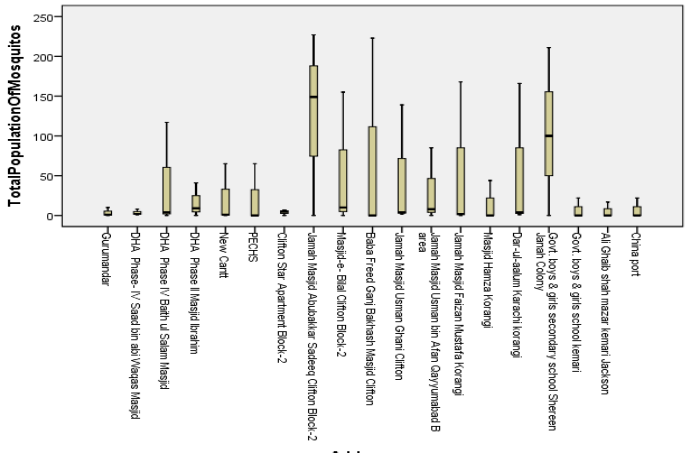

Figure 2: Population frequency in the sites studied

ANOVA

Total Population Of Mosquitoes

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	67055.228	18	3725.290	.843	.642
Within Groups	167835.333	38	4416.719		
Total	234890.561	56			

Table 1b: ANOVA results

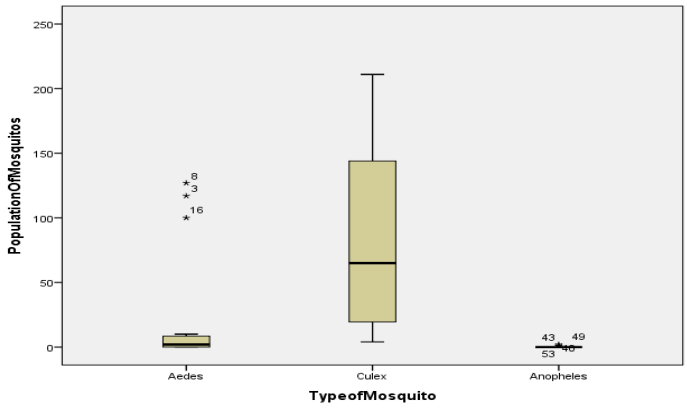

Figure 3: Mosquitos Population distribution

Table 2: Population of Aedes Male/Female in an Area

Address	*Aedes*	
	Male	Female
Gurumandir	0	1
DHA Phase- IV Saad bin abiWaqas Masjid	1	1
DHA Phase IV Baithul Salam Masjid	51	66
DHA Phase II Masjid Ibrahim	6	3
New Cantt	0	0
PECHS	0	0
Clifton Star Apartment Block-2	1	4
Jamia Masjid AbubakkarSideeq Clifton Block-2	79	148
Masjid-e- Bilal Clifton Block-2	4	6
Baba Freed Ganj Bakhash Masjid Clifton	0	0
Jamia Masjid Usman Ghani Clifton	1	3
Jamia Masjid Usman bin Affan Qayyumabad B area	4	4
Jamia Masjid Faizan Mustafa Korangi	0	2
Masjid Hamza Korangi	0	0
Dar-ul-aalum Karachi Korangi	1	2
Govt. boys & girls secondary school Shireen Jinnah Colony	39	61
Govt. boys & girls school kemari	0	0
Ali Ghaib shah mazar kemari JacksonS	0	0
China port	0	0

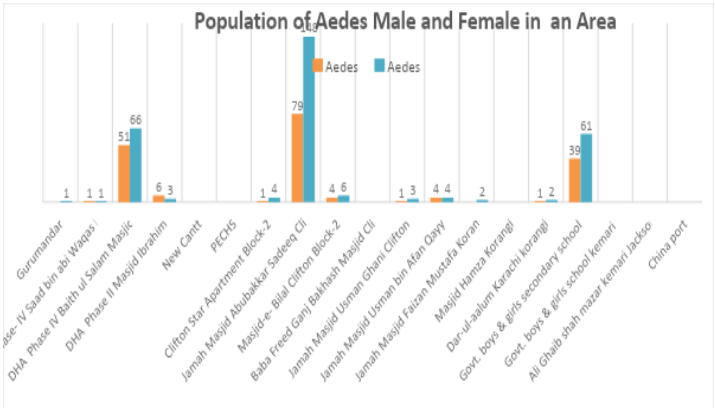

Figure 4: Population of Aedes in various sites

Table 3: Population of Culex Male/Female in an Area

Address	*Culex*	
	Male	Female
Gurumandir	3	7
DHA Phase- IV Saad bin abiWaqas Masjid	2	6
DHA Phase IV Baithul Salam Masjid	2	2
DHA Phase II Masjid Ibrahim	30	11
New Cantt	49	16
PECHS	50	15
Clifton Star Apartment Block-2	2	5
Jamia Masjid Abubakkar Siddique Clifton Block-2	29	120
Masjid-e- Bilal Clifton Block-2	116	39
Baba Freed Ganj Bakhash Masjid Clifton	107	116
Jamia Masjid Usman Ghani Clifton	77	62
Jamia Masjid Usman bin Affan Qayyumabad B area	24	61
Jamia Masjid Faizan Mustafa Korangi	140	28
Masjid Hamza Korangi	26	18
Dar-ul-aalum Karachi korangi	43	123
Govt. boys & girls secondary school Shireen Jinnah Colony	142	69
Govt. boys & girls school Kemari	12	10
Ali Ghaib shah mazar Kemari Jackson	7	10
China port	0	22

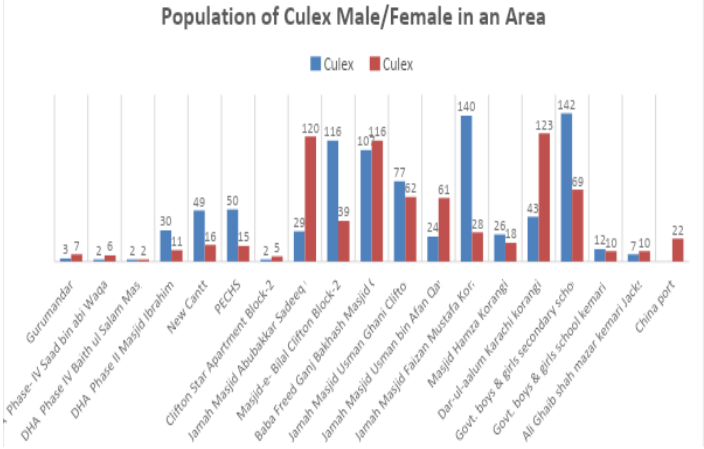

Figure 5: Population of Culex in various sites

Table 4: Population of Anopheles Male/Female in an Area

Address	*Anopheles*	
	Male	Female
Guru mandir	0	0
DHA Phase- IV Saad bin abiWaqas Masjid	1	0
DHA Phase IV Baithul Salam Masjid	0	0
DHA Phase II Masjid Ibrahim	0	0
New Cantt	0	1
PECHS	0	0
Clifton Star Apartment Block-2	0	0
Jamia Masjid Abubakkar Siddique Clifton Block-2	0	0
Masjid-e- Bilal Clifton Block-2	0	0
Baba Freed GanjBakhash Masjid Clifton	0	0
Jamia Masjid Usman Ghani Clifton	2	0
Jamia Masjid Usman bin Affan Qayyumabad B area	0	0
Jamia Masjid Faizan Mustafa Korangi	0	0
Masjid Hamza Korangi	0	0
Dar-ul-aalum Karachi korangi	0	1
Govt. boys & girls secondary school Shireen Jinnah Colony	0	0
Govt. boys & girls school Kemari	0	0
Ali Ghaib Shah mazar Kemari Jackson	0	0
China port	0	0

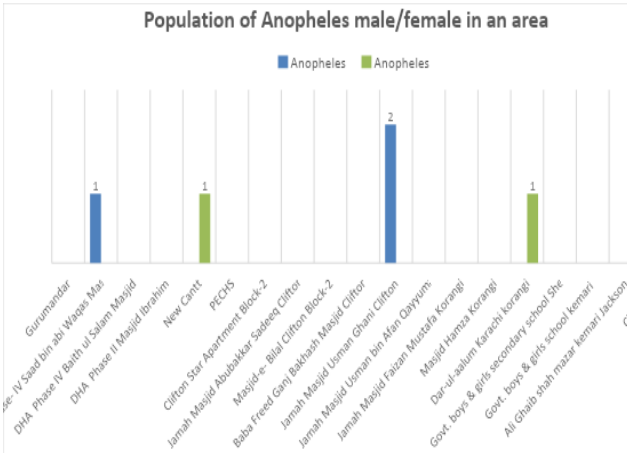

Figure 6: Population of anopheles in various sites

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