Research Article

Surveillance of *Aedes aegypti* (L.) Mosquitoes in Mumbai International Seaport (India) to Monitor Potential Global Health Risks

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Aedes mosquitoes are highly invasive and can survive almost any climatic conditions. They transmit a number of major world's deadly diseases. Therefore, a study was undertaken during December 2010 to evaluate the entomo-epidemiological risk of *Aedes* mosquito borne diseases (VBD) in Mumbai international seaport areas to minimize potential global health risks and prevent introduction of new VBD in India. Surveys were undertaken in operational and residential areas of Mumbai Port Trust (MPT). All the entomological indices were found to be above the critical level, prescribed for seaports by International Health Regulations Act, 2005. The operational areas where large goods are handled from cargo ships were found to be more prone to mosquito breeding comparing to residential areas. High insecticide tolerance of *Aedes aegypti* population against temephos and fenthion from Mumbai port area is reported for the first time. A careful and regular invigilation of the international seaports to prevent building up of vector density of dengue/chikungunya and yellow fever is recommended.

1. Introduction

Aedes is a genus of mosquitoes originally found in tropical and subtropical zones. It is considered highly invasive in nature and can carry a variety of pathogens that can be transmitted to humans. The species *Aedes aegypti* L. and *Aedes albopictus* (Skuse) are the primary vectors of concern world-wide. *Ae. aegypti* mosquito is the main vector that transmits the viruses that cause dengue. It is also known to transmit filarial infections of *Wuchereria bancrofti* and *Dirofilaria immitis* and avian parasite *Plasmodium gallinaceum* [1]. *Ae. albopictus* is primarily a forest species that has become adapted to rural, suburban, and urban human environments. These species commonly transmit dengue virus (DENV) around the globe; an estimated 50–100 million new dengue fever infections occur each year, causing ~500,000 cases of dengue hemorrhagic fever (DHF) and >20,000 deaths [2–5]. It can also transmit yellow fever, chikungunya, and ross river viruses.

The incidences of vector-borne diseases are increasing alarmingly due to many factors including uncontrolled urban developments that support breeding of vector mosquitoes. World Health Organization (WHO) in 2010 stratified the current situation of dengue/DHF in India under category A, which means a major public health problem, leading cause of hospitalization and death among children. In India, National Vector Borne Disease Control Programme (NVBDCP) reported 28,055 dengue cases in 2010 from 31 out of 35 states in India (highest ever in a year) [6]. In 2013, a total of 22,092 dengue cases and 74 deaths were reported from the country (of which 23 deaths were from Maharashtra only) [6]. In Maharashtra state sporadic cases of dengue were reported in 1973, 1983, and 2000. Morbidity in Barsi, Maharashtra (1973), was about 37.5%. Recently the cases of Chikungunya are reported from villages Mungi, Balamtakli, and Madhi (district Ahamadnagar), Malegaon city (district Nashik), and all 8 districts of Marathwada region and in Vidharbh region 7 districts: Akola, Washim, Buldhana, Yeotmal, Nagpur, Wardha,

and Chandrapur [7]. Mumbai, too, had recorded 116, 416, 1008, and 250 dengue cases in 2010, 2011, 2012, and 2013, respectively [8]. Moreover, India shares sea route/connection with majority of yellow fever (YF) endemic countries. This raises more concern in India to control *Aedes* mosquito breeding in seaport areas to prevent any introduction of YF virus in the country. As per International Health Regulations Act, 2005 [9], all the international airports/seaports, ground crossings, and peripheral areas up to 400 meters should be kept free from *Aedes* mosquito breeding or indices should be kept less than one to eliminate the chance of spreading disease or vectors to any part of the world. Therefore, in view of the endemic situation in Maharashtra and as a member country of WHO, an entomo-epidemiological survey was undertaken to know the present scenario of entomological indices, presence of DENV in *Aedes* mosquitoes, insecticide susceptibility status, and so forth in and around Mumbai port area to assess potential global health risks.

2. Materials and Methods

2.1. Study Area. Present entomological surveillance was undertaken in and around the Mumbai seaport in December 2010. Mumbai the capital of Maharashtra states is the second most populated city in India. It is located towards west side of the Indian seacoast at 18°58' N and 72°50' E. Mumbai weather never gets "cold" since there is no real winter in the place. In fact when the minimum temperature goes below 15°C, it is considered "chilly." Most of the year the weather is hot and humid throughout the day and night. In December, humidity is low and the day highs hover around 32.0°C and nights do not fall below 18°C. Mumbai seaport has been the principal gateway to India and playing a pivotal role in the commercial shipping and connections with the major cities of different countries. Statutory autonomous corporation known as Mumbai Port Trust administrates Mumbai port. MPT is a major historical port established in 1873. This port is fully integrated multipurpose port handling container, dry bulk, liquid bulk, and break bulk cargos. During the present investigation, mosquito survey was undertaken in and around the seaport areas covering a perimeter of 500 m around operational areas of Mumbai seaport that includes three docks: Indira Dock, Victoria Dock, and Prince's Dock which comprises the administrative block, canteen sheds, and so forth. *Aedes* survey was also carried out into two residential colonies: Parichya colony and Carnac Bunder colony. Non-residential Butcher Island situated at a distance of 5 Km from seashore was also included in this study. Butcher Island has been included as premises under operational area for data analysis. In seaport, baygon and pyrethrum are being used for spraying against adult and temephos granules for larvae as vector control measures.

2.2. Entomological Surveillance. Entomological surveillance of *Aedes* mosquito has been standardized on different indices based on the simple determination of presence or absence of *Aedes* larvae either in each container or somewhat in each house [10, 11]. The house index (HI, percentage of houses

positive for larvae), container index (CI, percentage of containers positive for larvae), Breteau index (BI, number of positive containers per 100 houses), and Premises Index were calculated. All nonhermetically closed containers containing any volume of water were considered as potential breeding sites. All water-holding containers were examined. Either the name/type of the containers (namely, tire, cooler, fire extinguisher bucket, etc.) or construction materials (namely, cement tank, earthen pots, and plastic container) classified the breeding sites. The number of surveyed premises, houses, positive containers (with *Aedes* pupae or larvae), and houses with ≥ 1 positive container has been recorded in the study.

2.3. Detection of Dengue Virus (DENV) in Mosquitoes. Larvae and adults were collected from the MPT areas and brought to the Centre for Medical Entomology and Vector Management (National Centre for Disease Control, Delhi) for detection of DENV inside mosquitoes by antigen-capture enzyme linked immunosorbent assay (ELISA) followed by the protocol described by Gajanana [12]. Wild caught adult mosquitoes and adults reared from larvae were tested separately. Sex wise pool of 3–10 unfed *Ae. aegypti* mosquitoes were prepared and tested to see the presence of DENV. Mono-4 clonal antibody (MAb), D14G2 (1:1000), as capture antibody (broadly reactive against four serotypes of DENV) and Mab-peroxidase conjugate MAb 6B6C-1 as detector antibody (1:2000) were used. The ELISA plate contained known DENV infected suckling mouse brain homogenate as positive control and the homogenate of uninfected adult *Ae. aegypti* as negative controls. Mean [± 4 standard deviation (SD)] optical density (OD) of the normal laboratory reared mosquito pools (negative control) was taken as threshold level and any divergence of OD of the tested mosquito population that crosses this threshold level is considered as positive for DENV infection.

2.4. Insecticide Susceptibility Assays. Larvae were collected and brought to the laboratory to determine the susceptibility status. Multiple concentration tests were conducted in order to establish baseline data of susceptibility and resistance. Groups of 25 third and early fourth instar larvae of F1 were tested with different concentrations of temephos (dose 0.012 mg/L) and fenthion (dose 0.025 mg/L) in 250 mL of water with control having no insecticide solution but with acetone. Each bioassay tested a minimum of six concentrations with at least three replicates per concentration [13]. Larval mortality was recorded after 24 hours and lethal concentration (LC) values were calculated using dosage-mortality regression probit analysis [14].

2.5. Statistical Analysis. Kolmogorov-Smirnov (with Dallal-Wilkinson-Lilliefors *P* value), D'Agostino and Pearson Omnibus and Shapiro-Wilk normality test have been performed to see whether the data follows a Gaussian distribution. Appropriate statistical tests were implemented to calculate the corresponding *P* values. Larval survey data of different breeding sites based on the name/type of the containers (namely, tire, cooler, fire extinguisher bucket, etc.), construction materials (namely, tin, cement tank, clay pots, and plastic container)

TABLE 1: Entomological indices at operational and residential areas of Mumbai seaport area during December 2010.

Locality	Premises						Type of containers												Breteau index								
	Nonresidential operational area (MPT)						Plastic				Cemented		Fire extinguisher bucket		Cooler		Earthen pots				Tire		Other		Total		Container index
							S	P	PI	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	
Nonresidential operational area (MPT)	10	08	80	14	0	13	0	57	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84	25	29.76	
	05	04	80	0	0	10	0	31	3	0	0	0	0	0	0	0	0	0	0	0	0	0	41	3	07.32		
	05	01	20	4	0	9	0	36	1	0	0	0	0	0	0	0	0	0	0	0	0	0	49	1	02.04		
	06	00	00	2	0	7	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	13	0	00.00		
	26	13	50	20	0	39	0	124	29	0	0	0	0	4	0	0	0	0	0	0	0	0	187	29	15.50		
Residential areas			HI																						CI	Breteau index	
Parichya colony	30	05	16.67	36	3	0	0	0	0	1	1	3	1	0	0	1	1	41	6				41	6	12.50	16.67	
Carnak Bunder colony	29	04	13.79	77	5	0	0	0	0	8	0	1	0	3	0	0	0	89	5				89	5	05.61	17.24	
Total				113	8	0	0	0	0	9	1	4	1	3	0	1	1	130	11				130	11			
Grand total				133	8	39	0	124	29	9	1	4	1	7	0	1	1	317	40								

S: searched; P: positive; PI: premises index; HI: house index; CI: container index.

TABLE 2: Probability distribution showing positive mosquito (larvae) breeding among different types of containers at Mumbai seaport area during December 2010.

	Plastic	Cemented	Fire extinguisher bucket	Cooler	Earthen pot	Tire	Other	Total
Operational area (MPT)								
Indira Dock	0%	0%	44%	0%	0%	0%	0%	30%
Victoria Dock	0%	0%	10%	0%	0%	0%	0%	7%
Prince's Dock	0%	0%	3%	0%	0%	0%	0%	2%
Total	0%	0%	23%	0%	0%	0%	0%	16%
Residential areas								
Parichya colony	8%	0%	0%	100%	33%	0%	100%	15%
Carnak Bunder colony	6%	0%	0%	0%	0%	0%	0%	6%
Total	7%	0%	0%	11%	25%	0%	100%	8%
Grand total	6%	0%	23%	11%	25%	0%	100%	13%

TABLE 3: Insecticide susceptibility status of *Ae. aegypti* mosquitoes collected from Mumbai seaport area during December 2010.

Insecticides	LC ₅₀	95% CI	LC ₉₀	95% CI	Chi ² (DF)	Slope	Slope "t" (DF)
Temephos	0.029	0.017–0.049	0.107	0.062–0.189	13.983 (3) P = 0.0029	2.27	7.069 (3) P = 0.0058
Fenthion	0.018	0.011–0.032	0.043	0.025–0.076	15.755 (3) P = 0.0013	3.44	4.72 (3) P = 0.018

DF: degree of freedom; LC: lethal concentration; CI: confidence interval.

was analyzed. Plain vanilla probabilistic and chi-square frequency tests to judge the significance of chance of getting positive containers (i.e., containers contain single *Aedes* larvae) in different areas were applied. For all areas different entomological indices (namely, HI, CI, BI, and/or Premises Index) were calculated.

3. Results

3.1. Entomological Surveillance. In operational areas of MPT, out of the total 26 premises, 13 were found positive for *Ae. aegypti* breeding giving an overall premises index of 50% with high CI in Indira Dock (29.76%); however, no breeding in Butcher Island could be found at this time (Table 1).

Among residential colonies, Parichya colony showed higher HI and CI (16.7% and 12.5%, resp.) than Carnak Bunder colony, where BI (17.24) was relatively high (Table 1). The most of the breeding was detected in fire extinguisher bucket kept in the different sheds along with other containers. Various breeding containers searches were tires, coolers, tin containers, cement tanks, earthen pots, fire extinguisher bucket, and others. Distribution of mosquito breeding in different containers in nonresidential operational areas of MPT and two residential colonies adjacent to the seaport are shown in Table 1. Among these different containers, fire extinguisher bucket shows only breeding sites in operational areas with

overall probability of 23%, whereas in residential areas coolers, earthen pots, and other containers (mainly disposable plastic cups and glass) show maximum potential for breeding based on plain vanilla probability model (Table 2). No other genus of mosquito larvae was found during the surveillance.

3.2. Detection of Dengue Virus (DENV) in Mosquitoes. A total of 331 *Ae. aegypti* mosquitoes (99 males and 232 females) were tested for DENV infection, out of which 250 samples were reared from larvae collected from MPT and the rest were adult caught wild mosquitoes. However, all samples were found negative for DENV.

3.3. Insecticide Susceptibility Assays. Results of insecticide susceptibility assays are presented in Table 3. Baseline assay shows that LC₉₀ (0.107) against temephos is quite higher than the WHO recommended diagnostic dose. Overall, population was found to have high tolerance against both temephos and fenthion.

3.4. Statistical Analysis. Chi-square frequency test was used to understand whether any statistical dependency is present among breeding positivity in two types of areas (i.e., operational and residential) surveyed. Here, we found that operational areas of MPT are more prone to mosquito breeding comparing to residential areas (Chi-square statistics = 29.9,

degree of freedom = 6, alpha level = 0.05, and $\chi^2 = 12.6$; Chi-square statistics $> \chi^2$). We further analyzed that there is no significant dependency between types of positive container for breeding in two residential colonies (Chi-square statistics = 3.0, degree of freedom = 4, alpha level = 0.05, and $\chi^2 = 9.5$; Chi-square statistics $< \chi^2$).

4. Discussion

Vector borne diseases are on the rise in last few years with epidemics reported in many countries. In the present study, it was assessed how different types of containers contribute to the breeding of *Ae. aegypti* and how all this knowledge may help in establishing better control strategy for *Ae. aegypti* mosquitoes in MPT as well as other seaports in India. During investigation it was observed that MPT having limited infrastructural facilities and inadequate trained workforce to control the mosquitoes may be one of the factors for establishment and expansion of *Aedes* mosquito vectors in peak seasons.

During our survey, the highest percentage of positive containers for larvae has been shown in fire extinguisher buckets in operational areas (Table 1). Interestingly, these positive containers (fire extinguisher buckets) are emergency instrument and technically it should always be filled with water. However, the stored water should be changed regularly. Unfortunately, our findings revealed that the poor management of these buckets is the main cause for mosquito breeding. It is worth noting that in some areas we found that 100% of surveyed containers are positive for mosquito breeding.

We could not detect any mosquito sample positive with DENV, but this was perhaps due to the low viral load in the month of December (nonpeak season). However, we found that all the entomological indices are above the critical level, as prescribed in International Health Regulations (2005) [9]. The earlier studies conducted in seaport in India also showed high entomological indices and even during the dry months [15, 16]. Therefore, it is quite clear that seaport authorities need to give more attention to implementing International Health Regulations (2005) to counter the mosquito-breeding problem. Mosquito breeding surrounding seaport and/or international border is not just a simple local health problem or biting nuisance; it is a serious threat to global health security. Thus, a careful invigilation of the international airports and seaports by the trained scientists/vector control personnel is recommend to prevent breeding and unwanted export of vector species and identify any accidental and/or deliberate import of alien arthropod vectors as biosecurity and quarantine measures to prevent potential international health risks. Moreover, future research priorities should include regular surveillance of arthropod vectors, as well as molecular diagnostics of diseases, identification of vectors, and phylogenetic origin of the vector strains in seaport areas. A rapid action team of trained scientist and health workers, equipped with conventional and modern scientific instrumentations and suitable vector control tools, would be able to counter vector breeding during any epidemics and outbreaks situation.

Conflict of Interests

The authors do not have any conflict of interests.

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