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Original Article

Mosquito Species Breeding Within a Tertiary Hospital in South-East Nigeria: Are There Public Health Implications?

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Background: Mosquito-borne infections affect about 700 million people annually worldwide. We characterized mosquito species breeding within a Nigerian tertiary care hospital in order to highlight the potential public health risk faced by hospital communities in the tropics.

Methods: A cross-sectional study of mosquito species breeding within the premises of Nnamdi Azikiwe University Teaching Hospital Nnewi was carried out from November 2012 to October 2013. Immature stages of the mosquito were collected from stagnant water pools and water-holding containers within the hospital premises using modified U.S. Center for Disease Control and Prevention (CDC) ovitraps (for eggs) and ladles (for larva). Pyrethroid spray catch collection technique was used to collect endophagic and endophilic adult mosquitoes.

Results: A total of 2,504 mosquitoes comprising 226 (9.0%) eggs, 2,025 (80.9%) larvae and 253 (10.1%) adult vectors were collected. Three mosquito genera (Aedes, Culex and Toxorhynchites) comprising five species (Culex quinquefasciatus, Culex tigripes, Aedes aegypti, Aedes albopictus and Toxorhynchites species) were identified. Aedes albopictus 1,033 (41.3%) was predominant while C. tigripes 45 (1.8%) was the least frequent. Mosquitoes bred mostly in septic tanks; 499 (24.6%) and repurposed automobile tyres; 445 (22.5%) while they bred the least in plant leaf axils; 37 (1.0%).

Conclusions: This study found a large number of mosquitoes breeding within the premises of a tertiary care hospital in Nigeria, including species that are efficient vectors of arboviral and filarial diseases. Improved environmental sanitation including periodic fumigation is strongly recommended in healthcare facilities in the tropics to reduce the risk of vector-borne diseases in the hospital community.

Key words: Mosquitos, Breeding, Hospital, Nigeria, Vector

INTRODUCTION

Female mosquitoes are blood sucking gnats that have a worldwide distribution and are dreaded for their biting / noise nuisance, allergic reactions and disease transmission[1]. They are vectors of various viral, protozoal and helminthic diseases to man and his domestic animals[2]. Infectious diseases caused by mosquito vectors account for the highest number of vector-borne diseases as well as mortality and disability adjusted life years[3]. The *Culex* mosquitoes are transmitters of filariasis especially lymphatic filariasis which is a major public health problem in Africa and other tropical regions[4]. They also transmit several mosquito-borne encephalitis[1,2]. The *Aedes* group of mosquitoes are vectors of yellow fever, dengue haemorragic fever and other arboviral infections[2]. Arboviral infections including yellow fever transmitted by *Aedes* mosquitoes are very prevalent in the tropical zones of Africa and America and have been recognized as a devastating epidemic disease[5].

Numerous studies have been carried out on the ecology, burden, and distribution of mosquitoes in Nigeria and elsewhere[6-15]. An overwhelming majority of these studies have focused on residential settlements in the community. There is a paucity of literature on the distribution of mosquitoes on the premises of health facilities. Hospital environments in tropical climates may face similar risks as residential communities regarding the transmission of vector-borne diseases. Hence the need to study hospital environments to determine if they are sustaining the breeding of mosquitoes and the potential risk of transmission of mosquito-borne infections.

The main objective of this study was to investigate the mosquito species and their immature species within their breeding sites in the premises of a tertiary care hospital in South-eastern Nigeria. The findings of this study potentially provide baseline data for assessing public health risks in health facilities in the tropics. It also promotes evidence-based policy making regarding environmental and waste management in healthcare facilities.

MATERIAL AND METHODS

Study Site

The study was conducted at the temporary (old) site of the Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nnewi-North Local Government Area, Anambra State, Nigeria.

The old site of NAUTH is located along the old Nnewi-Onitsha road lying between latitude 6.025473º and longitude 6.916646º. It is located in close proximity to the famous Nkwo Nnewi Automobile Market.

A 24-hour water supply is made possible within NAUTH premises through properly reticulated pipe-borne water, sourced from four functional boreholes. This makes it unnecessary for the in-patients and other members of the hospital community to store water in containers. There are no streams or ground water within the hospital premises, but occasionally stagnant water pools may be found on the surrounding roads during the rainy season. Occasionally, the water drainage systems around or within the hospital premises become compromised especially during the dry season of the year. Despite routine environmental and waste management, solid objects or wastes that potentially hold water are seen within the premises.

Nnewi has a population of 391,227 inhabitants. The city experiences two seasons which are the dry season (November to March) and rainy season (April to October). During the dry season, it experiences a short spell of cold dry harmattan from December to early February. The inhabitants of Nnewi depend on agriculture and commerce for their daily livelihood. The city has numerous markets including the famous Nkwo Nnewi market that houses the largest automobile spare parts market in West Africa.

Study Design

It was a cross-sectional study of the hospital premises to collect mosquitoes from their breeding sites and to study the biting habits of adult mosquitoes in and around the hospital wards. The mosquitoes were sampled at three levels as follows: eggs, larvae and adult vectors using appropriate sampling methods. The study was conducted over a period of one year.

Ethical Considerations

Informed consent of the hospital community was obtained through proper explanations of the study purpose and gains. Physical protection from mosquitoes and other vectors was provided for the individuals who collected samples on the field.

Data/Sample Collection

Sampling of mosquito eggs: Modified CDC ovitraps were used for the collection of mosquito eggs. Each ovitrap consisted of a black plastic cup of about 250mls, lined internally with a strip of white calico cloth covering the internal perimeter of the cup. Each ovitrap was about three quarter filled with water and then placed within the hospital premises.

The ovitraps were left for 48 hours before collection. At the collection point, the white calico strips were examined for mosquito eggs. The positive calico strips were separated from the negative strips. The eggs were hatched and reared to the adult stages before identification.

Larva mosquito collection: Larva collections were done twice daily on the two chosen days of the week; between 10:00 am and 12:00 noon and between 4:00 pm and 6:00 pm local time. Mosquito larvae were collected with ladles from various water bodies including clean stagnant pools, gutters and septic tanks into plastic bucket or bowls. Water collections in repurposed automobile tyres, glass or ceramic wares, plastic and metal containers were equally sampled for mosquito larvae. Water-holding containers found with mosquito larvae were overturned into a plastic bowl. The collection was sieved into another plastic bowl to remove debris. With the aid of micropipette, the larvae were picked into specimen bottles containing water. The larvae and pupae were reared to adult stages for proper identification.

Adult mosquito collection: Indoor biting and resting adult mosquitoes were collected from the student hostels, house officers' quarters and doctors' call rooms in each ward using Pyrethrum spray catch (PSC) method.

The collections were made on a white sheet following a knock-down. Knocked down mosquitoes were carefully picked with forceps into Eppendorf's tubes. All the mosquito collections; eggs, larvae and adult vectors were later sent to the National Arbovirus and Vectors Research Centre Entomology Laboratory, Enugu, South-East Nigeria for proper identification.

Data Analysis

Data was analysed using the statistical Package for the Social Sciences (SPSS) Version 16. Categorical variables were represented as proportions and percentages while comparison between these variables was done with the Pearson's Chi-square test (χ^2) as appropriate. Statistical significance was set at p-value < 0.05.

RESULTS

A total of 2,504 mosquitoes were collected from NAUTH premises, including 226 (9.03%) eggs, 2025 (80.87%) larvae and 253 (10.10%) adult mosquitoes (***Table 1***). The collected mosquitoes consisted of three genera (*Aedes, Culex and Toxorhynchites*) and five species (*Culex quinquefasciatus, Culex tigripes, Aedes albopictus, Aedes aegypti and Toxorhynchites spp).*

Tables 1: The mosquito species collected within the premises of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, South-East Nigeria, November 2012- October 2013.

Mosquitoe Species	Developmental Stages			
	Eggs	**Larvae**	**Adults**	**Total**
Aedes aegypti	53 (23.45%)	328(16.20%)	0	381 (15.22%)
Aedes albopictus	173(76.55%)	860(42.47%)	0	1033 (41.34%)
Culex quinquefasciatus	0	742 (36.64%)	253	995 (39.74%)
Culex tigripes	0	45(2.22%)	0	45 (1.80%)
Toxorhynchites	0	50 (2.4%)	0	50 (2.4%)
Total	**226 (9.03%)**	**2025 (80.87%)**	**253 (10.10%)**	**2504**

The relative abundance of mosquitoes collected were; C *quinquefasciatus* 995 (39.7%), *Culex tigripes* 45(1.8%), *Aedes aegypti* 381(15.2%), *Aedes albopictus* 1,033 (41.3%) and *Toxorhynchites* species 50(2.5%). Only C. *quinquefasciatus* was collected as adult mosquitoes while only A. *aegypti* and A. *albopictus* were collected as eggs. A. *albopictus* was the most abundant mosquito 1, 033 (41.3%) and C *tigripes* was the least frequent 45 (1.8%).

The mosquito larvae collected from the different breeding sites located within the hospital premises are shown in Table 2. The highest number of mosquito larvae 499 (24.6%) was collected from septic tanks and the least 37 (1.8%) was from the plant axils. The numbers and percentages of mosquitoes from other breeding sites were 455(22.5%) from old tyres, 265 (13.1%) from drainage gutters, 296 (14.6%) from buckets around the hostels, 190 (9.4%) from empty cans, 174 (8.6%) from broken glass ware and 109 (5 .4%) from tree stumps. The differences in the mosquito larvae yield from the different habitats were found to be statistically significant ($p<0.05$). Septic tanks and the repurposed vehicle tyres were the most common breeding habitats for the mosquitoes.

Table 2: Relative abundance of mosquito larvae from the different breeding sites within the hospital premises

Season	Mosquito Species	BREEDING SITES								Total
		Old tyres	Plant Axil	Open Septic Tank	Broken Glass Wares	Empty Cans	Fallen paw paw tree	Drainage channels	Buckets around the hostels	
Raining season(April-October)	*Aedes albopictus*	228	-	-	103	109	-	-	147	587 (29%)
	Aedes aegypti	75	-	103	33	-	-	-	-	211 (10.4%)
	Culex quinquefasciatus	-	-	165	-	45	92	20	70	392 (19.4%)
	Culex tigripes	-	-	-	-	-	17	-	-	17 (0.8%)
	Toxorhynchytes	-	37	-	-	13	-	-	-	50 (2.5%)
	Sub total	**303**	**37**	**268**	**136**	**167**	**109**	**20**	**217**	**1257(62.1%)**
Dry season (Nov-March)	*Aedes albopictus*	133	-	-	38	23	-	-	79	273 (13.5%)
	Aedes aegypti	19	-	98	-	-	-	-	-	117 (5.8%)
	Culex quinquefasciatus	-	-	133	-	-	-	217	-	350 (17.3%)
	Culex tigripes	-	-	-	-	-	-	28	-	28(1.4%)
	Toxorhynchites	-	-	-	-	-	-	-	-	-
	Sub total	152	-	231	38	23	-	245	79	768 (37.9%)
	Grand total	**445**	**37**	**499**	**174**	**190**	**109**	**265**	**296**	**2025**
		(22.5%)	**(1.8%)**	**(24.6%)**	**(8.6%)**	**(9.4%)**	**(5.4%)**	**13.1%)**	**(14.6%)**	

The mosquitoes collected were separated into wet season (April-October) and dry season (November-March) collections. In the wet season, all the five species of mosquitoes identified in the area were collected while in the dry season, four species, except *Toxorhynchites* species, were collected. More mosquito larvae 1257 (62.10%) were collected in the wet season than in the dry season 768 (37.90%) and the difference was statistically significant ($p< 0.05$).

In both dry and wet seasons, no mosquito species was found breeding concurrently in all the sites sampled. Only *Culex* species (*C. quinquefasciatus* and *C. tigripes*) were collected from the fallen paw paw tree. No mosquito species was collected from plant axils and the stump of fallen paw paw tree in the dry season while tyres, open septic tanks, broken glass wares, empty cans, drainage gutters and buckets around the hostels remain viable breeding sites throughout the year. *A. albopictus, A. aegypti, C. quinquefasciatus and C. tigripes* were the hardiest mosquitoes that bred throughout the year. No *Anopheles* species was found in all the breeding sites sampled.

Indoor biting and resting mosquitoes numbering 253 were collected from the Doctors' call rooms and House Officers' quarters. Of this, 167(66.01%) were collected in the wet season and 86(33.99%) in the dry season. Of the 167 mosquitoes collected in the wet season, 77 (64.71%) were from the House Officers' quarters, while 90 (67.20%) were from the Doctors' call rooms. Of the 86 mosquitoes captured in the dry season, 42 (35.30%) were from House Officers' quarters, while 44 (32.84%) were from Doctors' call rooms. The mosquito collections from the Doctors' call rooms and House Officers' quarters in the wet season were slightly different from each other but the difference was not statistically significant ($P>0.05$). There was not statistically significant difference in the number of mosquitoes collected from the Doctors' call rooms and House officers' quarters (44 (32.84%) vs. 42 (35.30%), $p<0.05$).

In the pulled result of dry and wet season collections (***Table 3***), the number of mosquitoes from the Doctors' call room was significantly higher than that of the House Officers' quarters ($p<0.05$). Only *C. quinquefasciatus* adults were collected indoors.

Table 3: Indoor biting and resting adult mosquitoes collected from the House Officers' quarters and Doctor's call rooms.

Season of the year	Mosquito species collected	Site of collection		Total
		House officers' quarters	Doctors call rooms	
Wet season April to October	*Culex quinquefasciatus*	77(64.71%)	90(67.20%)	167(66.01%)
	Culex tigripes	-	-	-
	Aedes aegypti	-	-	-
	Aedes albopictus	-	-	-
	Toxorhynchytes spp	-	-	-
	Sub-Total	**77(64.71%)**	**90(67.20%)**	**167(66.01%)**
Dry season November to March	*Culex quinquefasciatus*	42(35.30%)	44(32.84%)	86(33.99%)
	Culex tigripes	-	-	-
	Aedes aegypti	-	-	-
	Aedes albopictus	-	-	-
	Toxorhynchytes spp	-	-	-
	Sub Total	**42(35.30%)**	**44(32.84%)**	**86(33.99%)**
	Grand Total	**119(47.04%)**	**134(52.96%)**	**253(100%)**

DISCUSSION

This study was aimed at determining the mosquito species breeding within the premises of a tertiary care hospital in South-East Nigeria. A total of 2,504 mosquitoes belonging to three mosquito genera that comprised five mosquito species; *Aedes albopictus*, *Aedes Aegyptii*, *Culex quinquifasciatus*, *Culex tigripes* and *Toxorhynchyte spp* were collected. Of these sampled mosquitoes, two- third were larvae, while the remaining third comprised indoor biting and resting adult mosquitoes and eggs. In a similar study in another tertiary hospital in Enugu, South-East Nigeria, a total of 399 mosquitoes consisting of both immature and adult stages belonging to three mosquito genera that comprised six species of *Aedes*, one species of *Culex* and one species of *Eretmapodites* mosquitoes were collected[18]. Also, another study at the University of Port Harcourt Teaching Hospital found two mosquito genera; Culex and Anopheles mosquito comprising three species; *Culex quinquefasciatus*, *Anopheles gambie and Anopheles mauchetti*[19]. These findings show the presence of actively breeding mosquitoes in the hospital premises which potentially constitutes a risk for transmission of mosquito-in the hospital community. This public health concern has been previously expressed[20].

The mosquito larvae were collected from eight different breeding sites found within the premises of the hospital and none of the five-mosquito species was found breeding in all the sites. This finding agrees with that of earlier studies that showed that mosquitoes usually breed in stagnant water pools found in blocked water drainage systems, unkept septic tanks, tin cans and broken utensils and even open water storage containers in our environments[1,15]. Also, another study found different mosquitoes from different breeding sites and opined that although all mosquitoes breed in stagnant water, they differ from each other in their microecological requirements[17]. Adeleke *et al* observed in Abeokuta Nigeria, that there were different larval habitats for *Culex quinquefasciatus*, *Aedes albopictus* and *A. eaegpti*[6]. In Orlu, Imo State, Nigeria, *Culex quinquefasciatus* and *C. tigripes* were sampled from blocked drainage system with decaying organic debris[21]. The findings of these studies show that the different established mosquito breeding sites exist for the different mosquito species in the hospital premises which support the prolific breeding of the mosquitoes. Also, the variation in the breeding sites for the different mosquitoes indicates that there might be ecological differences in the breeding requirement of the different mosquito species.

The predominance of immature stages of mosquitoes in this study did not only indicate that the mosquitoes were actively breeding in the hospital premises. . Mosquito larvae were collected in the hospital premises in both wet and dry seasons although there was a significant difference in wet season larval collections over those of dry season. This finding indicates that the water pools in the hospital premises continued to support the breeding of mosquitoes in both wet and dry seasons. The variation in the number of mosquitoes collected with season could be explained by the fact that the open drainage channels were frequently flushed by flood water during the raining season thereby preventing prolonged water stasis which is the basic requirement for the breeding of mosquitoes.

The public health implication of the prolific breeding of mosquitoes in the hospital premises as reported in various studies in the tropics include biting nuisance and allergic reactions to the mosquito bite as well as the increased risk of disease transmission to the hospital community as the mosquitoes seek for blood meals[22].

The low proportion of the adult mosquitoes found in the study could be attributed to the methods of collection which concentrated on only the adult mosquitoes that bite and rested indoors which agrees with the observations of Onyido *et al*[16]. Also, the collection of adult mosquitoes by the use of human bait was not done as this entails exposure of the human volunteers to the communicable public health mosquito-borne arboviral diseases in the hospital environment. Only *C. quinquefasciatus* was caught biting indoors. Unlike the *Aedes* mosquitoes which are diurnal in their biting activities, *C. quinquefasciatus* is a night biting mosquito which frequently enters the house to bite their victims at night while they are asleep. The result agrees with the findings of Onyido *et al* who observed that *C quinquefasciatus* and *Anopheles* species were the night biting mosquitoes that entered the student rooms to bite at the Nnamdi Azikiwe University hostel Awka[23]. Gordon and Lavoipierre observed that the more important vectors of mosquito-borne diseases are those which show close association with man and prefer him to other animals as source of food[24]. Thus, *C quinquefasciatus* seems to be the most medically important indoor biting mosquito within NAUTH premises.

Anopheles mosquitoes were not collected in this study. This agrees with the finding of Ezike *et al* in a similar study in Enugu[18] but disagrees with the finding of a similar study in Port Harcourt[19]. Also, another non-hospital-based study in Umudioka-Anambra State found *Anopheles gambiae* to be the most abundant indoor biting mosquito[13]. This may be due to the fact that NAUTH premises do not have ecological habitats that support the breeding of anopheles mosquitoes as the hospital premises are completely tarred except the bushy hedges. Also, the hospital has properly reticulated pipe-borne water from boreholes such that in-patients, students, staff and visitors have no need to store water thereby excluding the suspicion that Anopheles mosquitoes might be breeding in open water cisterns. In addition, there are no streams, lakes nor any sort of ground water within the hospital premises. The non-collection of adult *Aedes* mosquitoes in the study could be attributed to the methods of collection which excluded these species. *Aedes* mosquitoes are outdoor biters and diurnal in habit. They are best collected with human bait methods at crepuscular hours which was not done. It is likely that Aedes mosquitoes may be constituting serious biting nuisance to the patients care givers who usually sit-out along the passages of the hospital especially at dusk.

Five mosquito species namely *A. aegypti, A. albopictus, C quinquefasciatus, C tigripes and Toxorhynchites* species were collected in the study. Among these five species, *Culex quinquefasciatus* was the most abundant mosquito species collected. This is because the hospital has open septic tanks at various places and open water drainage gutter channels with stagnant foul-smelling water especially in the dry season. All these readily provide suitable breeding sites for *Culex quinquefasciatus* which breeds more in stagnant water with decaying organic materials. This agrees with the findings of Adeleke *et al* who reported large occurrence of *Culex quinquefasciatus* in gutters containing polluted water[6].

Aedes albopictus was the next in abundance to *Culex quinquefasciatus*. It was collected mostly in tyres, empty buckets, broken wares and every other breeding sites within the hospital environment that had polluted water pool. The abundance of *Aedes albopictus* in the hospital environment can be explained by the frequent use of old used-tyres as flower guides in the hospital premises and the proximity of the popular Nkwo, Nnewi spare parts market to the hospital. In this market, second hand tyres are brought in from South Eastern Asia and other European countries. Because of space, most of the tyres are left in the open where they collect water that forms breeding heaven for *Aedes albopictus*. Savage *et al* stated that *Aedes albopictus* was imported into Nigeria via second hand tyres importation[25] and these used tyres brought in for sale in Enugu metropolis have been found to provide suitable breeding site for *Aedes albopictus*[26]. This supports the opinion of Adeleke *et al* that the preponderance of *Aedes albopictus* is expected to be occurring in cities where tyre business booms or where there is improper management of used tyres[6]. Thus, the proximity of NAUTH to the famous Nkwo Nnewi motor spare parts market and the use of old automobile tyres as flower guides within the hospital environments partly explains the high number of *Aedes albopictus* within the hospital community.

Aedes aegypti were collected from repurposed automobile tyres, septic tanks and empty cans. This observation was in line with the findings of Nwoke *et al* who collected *Aedes aegypti* from used tyres, broken glass wares and open septic tanks[27].

The ability of *A. aegypti* to breed in open septic tanks ensures almost all year-round continuous breeding and maintenance of the population of the mosquito within the hospital environment. This also corroborates the reports of Okorie and Mafiana *et al* that *Aedes aegypti* breeds almost indiscriminately in nearly all available breeding habitats[28, 29]. Adeleke *et al* also reported in their study of mosquito larval habitats in Abeokuta Nigeria that *Aedes aegypti* was found in almost all larvae habitats except plant axils[6].

Among the five species of mosquitoes collected, *Aedes aegypti*, *A. Albopictus* and *C. quinquefasciatus* are proven vectors of public health diseases[25, 30]. *Aedes albopictus* is a competent vector of many viral diseases including dengue fever, eastern equine encephalitis, Chukungunya, Rift valley and West Nile viruses[2]. *Aedes aegypti* is a vector of yellow fever virus and has been involved in yellow fever outbreaks in Nigeria[1,14]. In South America, the urban yellow fever vectors are *A. aegypti* and *Haemogogus spp*[31]. Yellow fever is a viral disease that is transmitted by mosquitoes of *Aedes* group[5].

quinquefasciatus is known to transmit filariasis and various forms of of viral encephalitis[24,32]. It has been experimentally showed that *C. quinquefasciatus* is actively involved in transmission of urban lymphatic filariasis in Nigeria[32] and the principal vectors of bancroftian filariasis in Africa are *Anopheles gambiae, C. quinquefasciatus and Anopheles funestus*[27].

The breeding of mosquito vectors of public health importance in and around hospital premises poses a serious public health risk to the patients, caregivers, doctors, visitors and hospital staff. It may enhance the transmission of communicable diseases between the patients, staff and visitors. According to Ezike *et al*, hospitals form strong contact points for both the sick and healthy and the existence of flourishing populations of disease vectors in hospital premises will compound the problem of disease transmission in the community[18]. Akande observed that health institutions are supposed to be health friendly and should not support the transmission of diseases even when they accommodate patients with communicable and non-communicable diseases[20].

This study was not without limitations. Outdoor sampling of adult mosquitoes using human baits could not be done because of the likelihood of contracting arboviruses and other mosquito transmitted infections.

This is because the study area is a tertiary health facility; a pooling centre for many diseases. Secondly, indoor collection of adult mosquitoes was not undertaken in patients wards to avoid disturbing the patients. In conclusion, this study demonstrated active breeding of mosquitoes within the premises of a tertiary hospital in Nigeria, which potentially constitutes a public health risk. The findings of this study underscore the need to carefully consider the location of healthcare facilities since activities conducted in the surroundings could potentially increase breeding of arthropods such as mosquitoes. In addition to routine environmental sanitation and waste management, the hospital management of facilities situated in locations that make them more prone to the breeding of mosquitoes should take additional steps including targeted fumigation to discourage the breeding of mosquitoes within the hospital premises. .

DECLARATIONS

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Author Contributions: UCC, OAE and IMO conceived the study, UCC, AJU and EEK performed data collection, UUS, EOI, NHM, OCI, MJI and CKS performed literature search and review. All the authors reviewed the manuscript and approved the final version of the manuscript.

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