

Original Research

Risk factors for *Anopheles* mosquitoes in rural and urban areas of Blantyre District, southern Malawi

Themba Mzilahowa¹, Madalitso Luka-Banda¹, Veronica Uzalili¹, Don P. Mathanga¹, Carl H. Campbell Jr², Mavuto Mukaka³, John E. Gimnig⁴

1. Malaria Alert Centre, College of Medicine, University of Malawi, Blantyre, Malawi

2. Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, USA

3. Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

4. Entomology Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Correspondence: Dr Themba Mzilahowa (tmzilahowa@mac.medcol.mw)

Abstract

Background

Although urban malaria transmission is low and seasonal, it remains a major public health problem. This study aimed at demonstrating the presence of *Anopheles* mosquitoes and their potential to transmit malaria in urban settings.

Methods

Two cross-sectional surveys were carried out in Blantyre District, Malawi, during the dry and wet seasons of 2008 and 2010, respectively. A map of Blantyre was divided into a grid of 400 cells, of which 60 cells were randomly selected. Five households located within 100 m from the centre of each selected cell were enrolled, a standard questionnaire was administered, and indoor resting mosquitoes were sampled.

Results

In 2008 and 2010, a total of 960 and 1045 mosquitoes were collected, respectively. *Anopheles funestus* comprised 9.9% (n = 95) and 10.3% (n = 108) during the two surveys, respectively. *Anopheles gambiae sensu lato* (s.l.) was rarely detected during the second survey (n = 6; 0.6%). Molecular identification was performed on samples collected during the first survey, and *An. funestus sensu stricto* (s.s.) was the only sibling species detected. All the *Anopheles* mosquitoes were collected from households located in rural areas of Blantyre and none from urban areas.

In univariate analysis, the presence of open eaves was associated with increased *Anopheles* prevalence, both during the dry (incidence rate ratio, IRR = 4.3; 95% CI 2.4 – 7.6) and wet (IRR = 2.47; 95% CI 1.7 – 3.59) seasons. Chances of detecting *Anopheles* spp. decreased with increasing altitude (IRR = 0.996; 95% CI 0.995 – 0.997) and during the dry season, but increased during the wet season (IRR = 1.0017; 95% CI 1.0012 – 1.0023). These factors remained significant following a multiple Poisson regression analysis. No association was found between insecticide-treated bednet ownership and the number of *Anopheles* mosquitoes detected.

Conclusions

The presence of *An. funestus* s.s and *An. gambiae* s.l. in the periphery of Blantyre city was an indication that malaria transmission was potentially taking place in these areas.

Introduction

In most urban settings across sub-Saharan Africa, malaria transmission and parasite prevalence are generally low and seasonal, with most of the transmission taking place in the wet season.^{1–5} Even with such low and seasonal disease transmission, malaria continues to pose a serious challenge to urban-dwelling communities, both in terms of disease severity and the number of reported cases. The sources of malaria infections observed among urban-dwelling populations likely varies from city to city. In some areas, vector breeding has been documented in urban settings with measurable entomological inoculation rates (EIRs).^{6–8} Other studies have suggested that urban–rural migration is a major source of the malaria cases reported or detected in urban areas.^{9–11} In either case, it is important for malaria control programmes to know the contribution of each potential source of malaria infection. Such knowledge is vital to designing better and more suitable control strategies.

Malaria is a common disease in Blantyre District in southern Malawi, including urban areas of Blantyre, which account for nearly 60% of the population of the district. Like the rest of sub-Saharan Africa, malaria transmission in Malawi is largely sustained by two efficient vectors: *Anopheles gambiae* sensu lato (s.l.) and *Anopheles funestus*. There are, however, notable differences in endemicity of the disease across the country, with lakeshore and low-lying areas experiencing intense and perennial malaria transmission (holoendemic), while upland areas are characterised by seasonal transmission coinciding with the wet season.^{12,13} Epidemiological studies have suggested that the strongest risk factor for malaria

acquisition among urban residents in Blantyre is a history of travel to rural areas,⁹ but there is potential for local malaria transmission due to mosquito breeding in isolated pockets across the city. On the other hand, if most transmission is occurring among travellers outside the city, then vector control targeting urban areas would have limited impact. This study was carried out, therefore, to assess the presence and number of *Anopheles* vector species in urban areas of Blantyre, in comparison to the rural areas, as a proxy indicator for malaria transmission.

Methods

Study setting

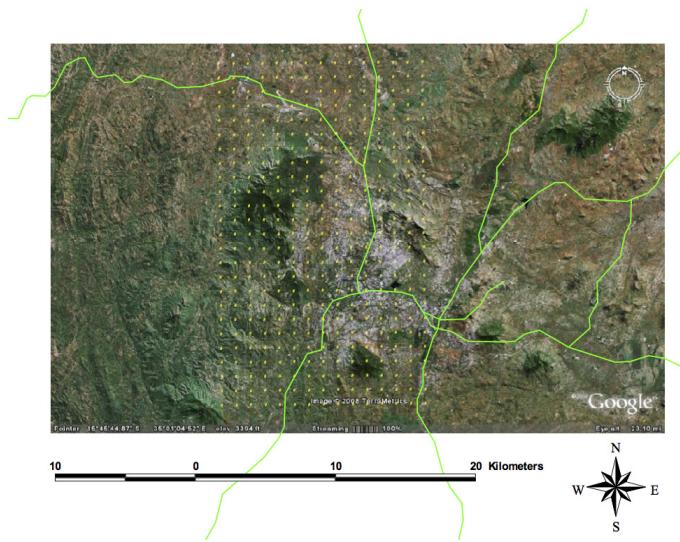
Blantyre District has an estimated total population of 1,001,984 people, with 661,256 people residing in urban Blantyre city. The district is located in the Shire Highlands and lies at 15° 42' S and 35° E. The city is surrounded by four mountains with varying topography, ranging from about 780 m to 1612 m above sea level.¹⁴

Blantyre has a tropical continental climate, with two distinct seasons in the year. The wet season is from November to April, with continuing light cold showers from May to July. The dry season is from May to October. The mean annual rainfall is 1122 mm, of which about 80% falls between November and March. The city is generally cool with mean annual temperatures ranging from 13 °C (55 °F) during the cold season (May to July) to 21 °C (70 °F) during the hot season (September to November).¹⁴ Blantyre is served by a number of health facilities, both private and government-owned. It has one major referral hospital (Queen Elizabeth Central Hospital) and two major private hospitals. Besides these, there are additional health facilities in all the major townships within the city and rural areas in the district.

Study sites selection

A grid with 500 m x 500 m cells was drawn over a map of Blantyre city and the surrounding area to generate a sampling frame (Figure 1). The total area of the grid was approximately 100 km², with a total of 400 grid cells. Many of the cells were in completely unpopulated areas and were therefore ineligible for sampling. Sixty cells were randomly selected and teams went to the approximate centre of each cell using geographical positioning system (GPS) navigation. In each cell, 5 houses nearest to the centre and within 100 m were selected for household characteristic data collection and mosquito sampling. Urban areas were defined as areas located within the city boundaries, while all households located outside these official boundaries were classified as rural.

Figure 1: Map of Blantyre district overlaid with a grid containing cells measuring 500 m x 500 m as sampling points



Field data collection

There were two teams of five people in each cell. Each team had two personal digital assistants (PDAs) with GPS units (Dell Axim X50s, Dell Inc., Austin, TX, USA), a backpack aspirator (John W. Hock Company; Florida, USA) powered by a 12 V rechargeable battery (Power SP Sonic, Model PS - 12180 NB), a set of petri dishes, and field notebooks. Using PDAs and GPS sample software,¹⁵ each team navigated to a preselected cell until they reached the approximate centre. Five houses nearest to the centre in each of the randomly selected cells were sampled for the presence of *Anopheles* mosquitoes using a backpack aspirator.

At the time of sampling, housing characteristics, including roof type, wall type, house eaves, and windows were also recorded, along with the number of people sleeping in the house and the number of insecticide-treated bednets (ITNs) in the house.

Before mosquito collection was carried out, written informed consent was obtained from the house owners for permission to enter their houses for mosquito collection and to conduct interviews using a standard questionnaire. The first survey was carried out during the dry season of November 2008 and the second one at the end of the wet season, in April 2010. This study was approved by the Malawi College of Medicine Research and Ethics Committee (COMREC).

Mosquito collection

Motorised backpack aspirators were used to collect mosquitoes. All mosquitoes collected from individual houses

were put in separate petri dishes, labelled and transported in cooler boxes to the Malaria Alert Centre (MAC) laboratory, in Blantyre, where specimens were counted and morphologically identified using standard morphological identification keys.¹⁶ A subset of *Anopheles funestus* s.l. specimens collected from the first (2008) survey were submitted for further molecular identification of the individual member species by polymerase chain reaction (PCR).

Molecular identification of *Anopheles* spp.

Molecular analyses were carried out on a sample of 48 mosquitoes morphologically identified as *An. funestus* s.l.. PCR was run for *An. gambiae* s.l., as well, to rule out any misidentifications.¹⁷⁻¹⁹ Briefly, a multiplex PCR was run to discriminate members of the *An. funestus* group in 25 µL reactions. The cocktail comprised a forward universal primer and six reverse primers capable of identifying only five of the ten members of the *An. funestus* group of mosquitoes, namely, *An. funestus sensu stricto* (s.s.), *An. parensis*, *An. rivulorum*, *An. leesonii*, and *An. vaneedeni*. Primers for the other members of this species complex were not included because they are not important in disease transmission and are scarce in the local environment. All reactions were run with both positive and negative controls. The PCR cycle conditions were: an initial denaturation at 94°C for 4 minutes for 1 cycle, followed by 30 cycles at 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds, and a final annealing cycle at 72°C for 7 minutes.

Data analysis

All mosquitoes collected were recorded as counts. The various species detected were summarised as proportions. A multiple Poisson regression model was carried out to assess if the various factors (open eaves, altitude, window type, and presence of ITNs) included in the data set were independently associated with *Anopheles* abundance.

Results

Household characteristics

Household characteristics from the first (2008) and second (2010) surveys are summarised in Table 1. A total of 250 and 206 households were sampled during the respective surveys. In both surveys, more rural households were sampled (70.8%; 68.9%) and fewer (29.2%; 31.1%) from urban areas. Slightly more than half (53.6%; 52.8%) of the sampled rural houses had a thatched roof during the first and second surveys, respectively. Although more houses in urban areas had iron roofs (97.6%; 89.1%), a few (2.9%; 10.9%) had thatched roofs. Overall, more than half of the houses sampled had sealed eaves ($n = 122$, 55.2%; $n = 130$, 63.1%) during the two surveys. In both surveys (2008; 2010), a significantly higher proportion of houses ($n = 63$, 92.7%; $n = 52$, 81.3%)

Table 1: Household characteristics from 2008 and 2010 surveys

Characteristics	2008 n (%)			2010 n (%)		
	Rural	Urban	Total	Rural	Urban	Total
Households	177 (70.8)	73 (29.2)	250 (100)	142 (68.9)	64 (31.1)	206 (100)
Roof type						
Thatched	82 (53.6)	2 (2.9)	84 (38)	75 (52.8)	7 (10.9)	82 (39.8)
Iron sheet	71 (46.4)	66 (97.6)	137 (62)	67 (47.2)	57 (89.1)	124 (60.2)
Eaves						
Open	94 (61.4)	5 (7.4)	99 (44.8)	64 (45.1)	12 (18.8)	76 (36.9)
Sealed	59 (38.6)	63 (92.7)	122 (55.2)	78 (54.9)	52 (81.3)	130 (63.1)
ITN ownership	106 (69.3)	47 (69.1)	153 (69.2)	121 (85.2)	50 (78.1)	171 (83.0)
Net retreatment	98 (92.3)	44 (93.6)	142 (92.8)	83 (68.6)	46 (92.0)	129 (75.4)

Table 2: Total number of mosquitoes captured in each survey and mosquito density per house

Mosquito species	2008 n (%)			2010 n (%)		
	Rural	Urban	Total	Rural	Urban	Total
<i>An. gambiae</i> s.l.	0 (0.0)	0 (0.0)	0 (0.0)	6 (0.9)	0 (0.0)	6 (0.6)
<i>An. funestus</i> s.l.	95 (29.9)	0 (0.0)	95 (10.3)	108 (16.1)	0 (0.0)	108 (10.3)
<i>Culex</i> spp.	223 (70.1)	602 (100)	825 (89.7)	542 (80.7)	373 (100)	915 (87.6)
<i>Mansonia</i> spp.	0 (0.0)	0 (0.0)	0 (0.0)	16 (2.4)	0 (0.0)	16 (1.5)
<i>Aedes</i> spp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	318	602	920	672	373	1045
No. of houses	197	53	250	142	64	206
Mosquitoes/house	1.6	11.4	3.7	4.7	5.8	5.1

located in urban areas had sealed eaves. More houses located in rural areas characteristically had open eaves both in 2008 ($n = 94$, 61.4%) and in 2010 ($n = 64$, 45.1%).

ITN ownership was generally high ($n = 153$, 69.2%; $n = 154$, 83.0%) during the first and second surveys, respectively, and the majority of the nets owned had been retreated with an insecticide (92.2%; 75.4%), following the routine net retreatment campaigns implemented at the time.

Mosquito species abundance

The number of mosquitoes captured in rural and urban households in 2008 and 2010 is summarised in Table 2. The two surveys (2008; 2010) yielded a total of 920 and 1045 mosquitoes, respectively. In both surveys, the bulk of the mosquitoes ($n = 825$, 89.7%; $n = 915$, 87.6%) found resting in people's homes were *Culex* spp., which were equally abundant in both rural and urban areas. Of the anophelines collected, *An. funestus* was the most common species ($n = 95$, 10.3%; $n = 108$, 10.3%) detected during both surveys. *An. gambiae* s.l. was only detected in the 2010 survey ($n = 6$, 0.6%). Of the mosquitoes identified morphologically as *An. funestus*, 14/48 (29.2%) specimens were identified as *An. funestus* s.s. by PCR from the first survey collections. However, the majority of the specimens ($n = 34$, 70.8%) did not amplify. No further molecular identifications were carried out on the specimens collected during the second survey.

Factors associated with Anopheles presence

By univariate analysis, several factors (presence of ITNs, type of windows [open or closed], house eaves [open or sealed], and elevation) were analysed for association with presence of *Anopheles* mosquitoes by Poisson regression (Table 3). ITN ownership was not associated with the number of *Anopheles* mosquitoes in either survey, while having closed windows was significantly associated with fewer *Anopheles* mosquitoes (incidence rate ratio, $IRR_1 = 0.53$, 95% confidence interval, $CI = 0.33$ to 0.85 ; $IRR_2 = 0.60$, 95% $CI = 0.37$ to 0.98). In both surveys (2008; 2010), houses with open eaves were more likely to harbour *Anopheles* spp. ($IRR_1 = 4.3$, 95% $CI = 2.4$ to 7.6 ; $IRR_2 = 2.47$, 95% $CI = 1.7$ to 3.59 ; $P < 0.001$) compared to houses with closed eaves. Although altitude was significantly associated with incidence of *Anopheles* mosquitoes in both surveys, the direction of association was different in the two surveys. Decreasing altitude was associated with increasing number of *Anopheles* ($IRR_1 = 0.996$, 95% $CI = 0.995$ to 0.997) during the first survey while in the follow-up survey, increasing altitude was associated with increased number of *Anopheles* mosquitoes ($IRR_2 = 1.0017$, 95% $CI = 1.0012$ to 1.0023).

In a multivariate Poisson regression, two factors (house eaves and altitude) remained significantly associated with

incidence of *Anopheles* spp. after performing multiple Poisson regression and adjusting for other factors (Table 3). Open eaves were significantly associated with increased numbers of *Anopheles* mosquitoes during the two surveys ($IRR_1 = 3.5$, 95% $CI = 1.5$ to 8.1 ; $IRR_2 = 2.08$, 95% $CI = 1.48$ to 3.04), while increasing elevation was associated with fewer mosquitoes in 2008 but more mosquitoes in 2010 ($RR_1 = 0.997$, 95% $CI = 0.996$ to 0.998 ; $IRR_2 = 1.002$, 95% $CI = 1.001$ to 1.002).

Discussion

The results of this study show that two known malaria vectors, *An. funestus* and *An. gambiae* s.l., exist in Blantyre District in southern Malawi. The former was found during both surveys (dry and wet seasons), while the latter was only detected in low numbers in the second survey (at the end of the wet season). In this study, the two *Anopheles* species were observed only in rural areas. These findings indicate that people living in rural or peripheral areas in Blantyre are more exposed to *Anopheles* mosquito bites than urban inhabitants. The scarceness of *Anopheles* mosquitoes in urban areas of Blantyre has previously been reported—using pyrethrum space spray catches (PSCs), investigators collected a combined total of more than 900 *An. gambiae* s.l. and *An. funestus* from two rural sites and only 2 *An. funestus* mosquitoes from an urban site.²⁰

This study also supports the findings of the 2010 Demographic and Health Survey (DHS),²¹ those reported generally from several major cities in sub-Saharan Africa,^{11,22} as well as the findings of Mathanga and others,^{9,23} who demonstrated that malaria cases in urban Blantyre were more likely to have a history of recent travel to rural areas.

It was not surprising to observe that anophelines were present in the study area at very low densities of 10.3% and 12.4% in 2008 and 2010, respectively. The majority of mosquitoes found inside study households were culicines, as these mosquitoes are adapted to urban environments characterised by organically polluted water habitats. Although molecular identifications by PCR were unsuccessful on a large proportion of the specimens ($n = 34$, 70.8%), the detection of *An. funestus* s.s., an important and efficient malaria vector in sub-Saharan Africa (including Malawi), was an important finding in the study, as it was primary evidence of the potential for malaria disease transmission in the study area. The excess PCR failures could have been attributable to two factors. The primary reason may have been that DNA quality was compromised by DNA degradation, as specimens were

Table 3: Multivariate analysis of factors associated with Anopheles abundance using Poisson regression

Variable	2008		2010	
	IRR (95% CI)	P-value	IRR (95% CI)	P-value
ITNs (Yes)	0.78 (0.39 to 1.53)	0.47	0.97 (0.82 to 1.15)	0.73
ITNs (No)	Reference		Reference	
Windows (Yes)	0.78 (0.39 to 1.53)	0.099	1.1 (0.64 to 1.79)	0.80
Windows (No)	Reference		Reference	
Eaves (Yes)	3.5 (1.5 to 8.1)	0.004	2.08 (1.43 to 3.04)	< 0.001
Eaves (no)	Reference		Reference	
Altitude	0.997 (0.996 to 0.998)	< 0.001	1.002 (1.001 to 1.002)	< 0.001

IRR = incidence rate ratio; CI = confidence interval; ITN = insecticide-treated net

stored dry, using a desiccant (silica gel), and samples were not processed immediately. It is also possible that some of the specimens that did not amplify were sibling species whose primers were not included in the primer cocktail used in the PCR identifications. For instance, a study carried out in Karonga, northern Malawi, showed the presence of an *An. funestus*-like mosquito, described as a new species within this group of mosquitoes.²⁴

Several factors (presence of open house eaves, altitude, window type, and ITN ownership) were associated with the incidence of *Anopheles* mosquitoes. In univariate analyses, houses with open eaves and open windows were associated with an increased incidence of indoor resting *Anopheles* mosquitoes, corroborating previous studies that have shown that houses with open eaves permit mosquito entry.^{25,26} We did not assess the presence of ceilings, but these have also been shown to reduce mosquito entry in houses in urban areas²⁷ and could have contributed to the absence of anopheline mosquitoes in urban areas of Blantyre as well.

Ownership of ITNs was not associated with a reduced number of mosquitoes in either survey. The use of ITNs has repeatedly been shown to reduce the number of mosquitoes inside houses²⁸⁻³⁰, so the lack of a statistically significant impact was somewhat surprising. It is possible that net use was so high in both urban and rural areas that there was limited statistical power to detect a difference between households with and without bednets. Furthermore, we did not assess the condition and insecticide levels of the nets. Both surveys were conducted before long-lasting nets were widely available, and the nets may therefore have been inadequately treated. It is also possible that *An. funestus* populations sampled in the study area were resistant to the deltamethrin (a pyrethroid insecticide) used to treat ITNs, and that could partly explain the lack of association between ITN ownership and vector density in the study households. Unfortunately, this study did not assess the resistance status of vector populations in the study area.

Our study showed conflicting results when elevation was analysed in the two surveys. The number of *An. funestus* increased at lower elevations during the first survey, while in the second survey, numbers increased with increasing elevation. The finding of increased altitude being associated with increased incidence of *An. funestus* during the second survey was unexpected. *Anopheles* mosquitoes are generally more prevalent in lower altitudes, where standing water is more abundant. It is possible that there was more standing water at lower elevations during the wet season but more standing water at high elevations in the dry season as streams dry up and leave pools of water. However, because we did not assess the availability of potential larval habitats in this study, it is difficult to conclusively explain the observations and the ecological parameters that governed mosquito breeding and presence.

It was not surprising to find *Culex* spp. constituting the bulk of mosquitoes sampled in the study area. This mosquito species is adapted to urban environments, as it proliferates in breeding habitats rich in organic matter. However, the presence of *Mansonia* spp., though in very low numbers, is worth highlighting. *Mansonia* mosquitoes breed in swampy environments, where water plants are prevalent, and therefore their presence in the study area pointed to the existence of such localised habitats. Both *Culex* spp. and *Mansonia* spp.

have been implicated in the transmission of lymphatic filariasis, caused by *Wuchereria bancrofti*, in Chikwawa District, southern Malawi.³¹

The absence of anopheline mosquitoes in urban areas of this study contrasts with observations from other urban settings, where transmission was present in urban areas, even if it was indeed lower than transmission in rural areas. In most of these studies, transmission was generally linked to clearly defined breeding sites and declined at increasing distances from these sites. In a study carried out in urban Dakar, vectors decreased with increasing distance from a large breeding site.³² In Accra, Ghana,⁸ breeding of *An. gambiae* s.s. was found to be associated with urban agriculture, with an EIR of 19.2 infectious bites per person per year (ibppy) near agricultural areas, compared to 6.6 ibppy at sites further from agricultural areas. In Lagos, Nigeria,³³ and in Dar es Salaam, Tanzania,³⁴ *An. gambiae* s.l. larvae have been found breeding in polluted water bodies within urban environments, suggesting that mosquitoes have adapted to the changing conditions in these environments. These studies highlight the diversity of urban environments and the diversity of responses of *Anopheles* mosquitoes to the anthropogenic changes related to urbanisation.

Failure to detect indoor resting *Anopheles* mosquitoes from houses located in urban areas in the present study could be due to a number of factors. First, it is possible that there is no *Anopheles* breeding within urban areas of Blantyre inside city boundaries. The lack of breeding could result from unfavourable breeding habitats due to organic pollution, although this study did not assess the availability or water quality of potential larval habitats. Furthermore, many houses had closed windows and eaves, meaning that even if breeding habitats were available, human hosts may not have been available, or mosquitoes may have been resting outdoors because of the changes in house construction. Additionally, this study was carried out over a relatively short period of time. In an environment where *Anopheles* mosquitoes are rare, more effort may be required over time and space to detect the few and sparse mosquitoes present. Finally, all efforts to collect *Anopheles* mosquitoes in the study area relied on a single collection technique (the backpack aspirator) to collect indoor resting mosquitoes. Multiple collection techniques, targeting the various species with different resting behaviour characteristics, may be required to detect low densities of *Anopheles* mosquitoes, particularly if there are behavioural changes associated with differences in housing construction. Further studies targeting areas that may likely hold potential breeding sites may provide further insight into the potential for malaria transmission within urban Blantyre.

Conclusions

The detection of *An. funestus* s.s. and, to a lesser extent, *An. gambiae* s.l. within the periphery of Blantyre city was indicative of malaria transmission taking place in these rural areas. However, we were unable to detect either species within urban Blantyre. This study confirms previous findings suggesting that much of the malaria burden in Blantyre city results from movement of urban residents to and from rural areas. There is strong need to fully understand the ecological and environmental factors that support breeding of *Anopheles* mosquitoes found in the peripheral areas surrounding the city boundaries.

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Conflicts of interest

The authors declare that they have no conflicts of interest related to this study.

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