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Dengue virus and *Plasmodium* coinfection among febrile patients in Osun State, Nigeria

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Abstract. *Ajayi BB, Olaitan KS, Owoyomi OF, Oni SE, Akinlabi AM, Aro EO, Ogbolu DO. 2025. Dengue virus and* Plasmodium *coinfection among febrile patients in Osun State, Nigeria. Nusantara Bioscience 17: 253-258.* Dengue and malaria are major co-endemic vector-borne diseases in Nigeria, presenting significant diagnostic challenges due to their overlapping clinical symptoms. In settings where malaria is hyperendemic, febrile illnesses are often presumptively treated as malaria, potentially leading to the misdiagnosis of other pathogens like dengue virus (DENV). This study aimed to determine the seroprevalence of DENV infection and the prevalence of DENV/*Plasmodium* coinfection among febrile patients in Osun State, Nigeria. A cross-sectional study was conducted from April to September 2024, involving 250 febrile patients from selected healthcare facilities. Blood samples were analyzed using rapid diagnostic tests (RDTs) for *Plasmodium* antigens and for DENV-specific Immunoglobulin M (IgM) and G (IgG) antibodies. Sociodemographic and risk factor data were collected via structured questionnaires. The prevalence of malaria was high at 53.6% (134/250), consistent with the region's hyperendemic status. Evidence of recent DENV infection (IgM positive) was found in 0.8% (2/250) of patients, while 6.4% (16/250) were IgG positive, indicating past exposure. Concurrent infection was detected in 0.8% (2/250) of patients for Malaria/DENV IgM and 3.2% (8/250) for Malaria/DENV IgG. Both malaria and DENV IgG seropositivity were significantly associated with age ($p<0.05$). This study confirms the endemic circulation of DENV in Osun State and highlights its contribution to the burden of non-malarial febrile illness. These findings underscore the critical need to revise existing diagnostic algorithms and integrate DENV surveillance into public health frameworks to mitigate misdiagnosis and improve patient outcomes.

Keywords: Coinfection, dengue virus, febrile patients, malaria, Nigeria, Osun State

INTRODUCTION

Vector-borne diseases, particularly dengue and malaria, impose a substantial burden on public health systems across tropical and subtropical regions (Salam et al. 2018; Osarumwense et al. 2022). While malaria has historically dominated the public health landscape in Africa, dengue is emerging as a significant and often under-recognized threat (Otu et al. 2019a). Recent meta-analyses confirm an increasing prevalence of acute dengue across the continent, with sporadic and epidemic cases reported from numerous West African nations, including Nigeria (Ayukekbong 2014; Gebremariam et al. 2023). This epidemiological shift creates a complex challenge, as both pathogens are transmitted by mosquitoes, thrive in similar ecological conditions, and, crucially, present with nearly indistinguishable symptoms in their early stages.

The epidemiological convergence of these pathogens is a pressing global health concern. Recent scholarship underscores the urgency of differentiating non-malarial febrile illnesses, which are frequently misdiagnosed. In regions across sub-Saharan Africa, studies increasingly demonstrate a significant prevalence of dengue among febrile populations, often mistaken for malaria (Gainor et al. 2022). This diagnostic confusion is critical, as standardized febrile illness protocols, heavily reliant on presumptive malaria treatment or malaria-specific rapid tests, systematically fail to identify dengue, leading to suboptimal patient management and unmonitored arboviral spread (Mbabazi et al. 2022). Furthermore, the clinical implications of simultaneous DENV-Plasmodium infection are a subject of intense investigation. While historically considered rare, recent meta-analyses confirm coinfection is geographically widespread. Although debate continues regarding whether coinfection mitigates or exacerbates disease severity, there is a consensus that the overlapping clinical presentation complicates diagnosis and may mask severe manifestations of either disease, posing a significant risk to patients (Rufai et al. 2022)

Nigeria bears one of the world's heaviest malaria burdens, accounting for a disproportionate share of global cases and mortality (World Health Organization 2021). This hyperendemicity has fostered a deep-seated clinical practice of presumptively diagnosing and treating most acute febrile illnesses as malaria, often without laboratory confirmation (Ayolabi et al. 2019). This practice, while understandable in a resource-limited context, casts a profound "diagnostic shadow" over other etiologies of fever. Consequently, pathogens like dengue virus (DENV) are systematically overlooked, leading to poor patient outcomes, as dengue requires specific supportive management distinct from antimalarial therapy, and contributing to the underestimation of its true public health impact (Otu et al. 2019b; Saidu and Okojie 2024).

The diagnostic dilemma is rooted in the clinical overlap between the two diseases. The initial presentation of uncomplicated malaria—fever, headache, myalgia, and fatigue—is virtually identical to that of classic dengue fever (Halsey et al. 2016; Kotepui and Kotepui 2019). This similarity makes differential diagnosis based on clinical grounds alone unreliable and hazardous (Rao et al. 2016; Nkenfou et al. 2021). Furthermore, concurrent infection with both pathogens, while once considered rare, is increasingly reported from endemic areas and may lead to more complex clinical presentations, potentially increasing the risk of severe outcomes such as deep bleeding or hepatic complications (Epelboin et al. 2012; Magalhães et al. 2014). Studies from various regions of Nigeria have confirmed the circulation of all four DENV serotypes and have documented coinfection rates with malaria, indicating that this is a widespread, not localized, phenomenon (Idoko et al. 2015; Moses et al. 2016; Mustapha et al. 2017; Ayolabi et al. 2019). The national average prevalence of dengue has been estimated at approximately 21%, suggesting that one in five Nigerians may be susceptible to infection (Otu et al. 2019a).

Despite this growing body of evidence, specific, localized data on the prevalence of DENV and its coinfection with malaria remain scarce for many parts of the country, including Osun State. This knowledge gap hinders the development of evidence-based clinical guidelines and targeted public health interventions. Therefore, this study was designed to investigate the seroprevalence of DENV infection, determine the prevalence of DENV/*Plasmodium* coinfection, and identify associated demographic and environmental risk factors among febrile patients seeking care in Osun State, Nigeria.

MATERIALS AND METHODS

Ethical considerations

Ethical approval for this study was obtained from the Ethical Research Committee of the Osun State Ministry of Health, Nigeria. Additional permission was secured from the medical officers in charge of the selected healthcare facilities. Written or verbal informed consent was obtained from all adult participants and from the parents or legal guardians of minors before enrollment. All patient data were anonymized to ensure confidentiality.

Study design, area, and period

A cross-sectional study was conducted between April and September 2024, coinciding with the rainy season, a period typically associated with increased mosquito vector activity. The study was carried out in two selected Local Government Areas (LGAs), Ede South and Oriade, within Osun State, southwestern Nigeria. Osun State is predominantly inhabited by the Yoruba people and is characterized by a tropical climate with distinct wet and dry seasons.

Study population and sampling

The study population consisted of 250 consenting febrile patients presenting at selected primary and secondary healthcare facilities in the study LGAs. Inclusion criteria included an acute febrile illness with a measured axillary body temperature of 37.5°C or higher, consistent with case definitions for both malaria and suspected dengue fever. Patients of all ages and both sexes who provided consent were recruited consecutively until the target sample size was reached.

Data and sample collection

A pre-tested, structured questionnaire was administered by trained interviewers to collect data on sociodemographic characteristics (age, sex, education, occupation) and potential risk factors for vector-borne diseases. These factors included history of blood transfusion, use of insecticide-treated nets (ITNs), presence of window nets, and environmental sanitation practices (e.g., presence of overgrown bushes, stagnant water). Following the questionnaire, a 5 mL venous blood sample was collected from each participant under strict aseptic conditions. The blood was transferred into an ethylene diamine tetraacetic acid (EDTA) anticoagulant tube for immediate processing and subsequent analysis.

Laboratory analysis

All laboratory procedures were performed at the Medical Microbiology Laboratory of Joseph Ayo Babalola University.

Malaria detection

Malaria parasites were detected using the First Response™ Malaria Ag *P. falciparum*/Pan Rapid Diagnostic Test (RDT) (Premier Medical Corporation Ltd., India). This qualitative, immunochromatographic assay detects *Plasmodium falciparum*-specific histidine-rich protein 2 (HRP2) and pan-*Plasmodium* lactate dehydrogenase (pLDH) in whole blood, allowing for the rapid diagnosis of *P. falciparum* and other *Plasmodium* species (Moses et al. 2016). The test was performed and interpreted according to the manufacturer's instructions.

Dengue serology

The remaining blood sample was centrifuged at 3,000 rpm for 10 minutes to separate plasma. The plasma was carefully harvested and stored in cryovials at -20°C until analysis. Dengue virus exposure was assessed using the EGENS® Dengue Virus IgM/IgG RDT kit (Egens Biotechnology, China). This solid-phase immunochromatographic assay qualitatively detects and differentiates between IgM and IgG antibodies to DENV in human plasma. The presence of IgM antibodies typically indicates a recent or acute primary infection, while the presence of IgG antibodies suggests a past or secondary infection (Adeleke et al. 2016; Moses et al. 2016). The assay was performed following the manufacturer's protocol, and results were read within 15 minutes.

Statistical analysis

Data from the questionnaires and laboratory tests were entered into Microsoft Excel and subsequently analyzed using the Statistical Package for Social Sciences (SPSS) version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the data; categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation. Associations between categorical variables (e.g., demographic factors, risk factors, and infection status) were assessed using the Pearson's Chi-square ($\chi2$) test. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to evaluate the strength of association between risk factors and disease occurrence. A p-value of less than 0.05 was considered statistically significant for all tests.

RESULTS AND DISCUSSION

Sociodemographic profile and overall prevalence

The study enrolled 250 febrile patients with a mean age of 24.94±16.76 years. The cohort was predominantly female (63.2%), single (62.4%), and had attained at least a secondary level of education (50.4%). Students constituted the largest occupational group (60.8%) (Table 1).

The burden of malaria in this febrile population was exceptionally high, with 53.6% (134/250) of participants testing positive via RDT. In contrast, serological evidence for dengue virus was less frequent but clearly present. The seroprevalence of recent DENV infection (IgM positive) was 0.8% (2/250), while the seroprevalence of past DENV exposure (IgG positive) was significantly higher at 6.4% (16/250). Critically, coinfection was identified in this population. The prevalence of concurrent malaria and recent dengue (MP/DENV IgM) was 0.8%, and the prevalence of malaria with past dengue exposure (MP/DENV IgG) was 3.2% (Table 2).

The serological landscape responsible for the endemic circulation of dengue virus

The serological profile observed in this study—a low DENV IgM rate (0.8%) coupled with a substantially higher DENV IgG rate (6.4%)—provides a critical snapshot of the local epidemiology. IgM antibodies are markers of a recent primary infection, typically appearing within days of symptom onset and waning over several months. In contrast, IgG antibodies develop later but persist for life, serving as a reliable indicator of past exposure (Khetarpal and Khanna 2016). The fact that IgG prevalence was eight times higher than that of IgM strongly suggests that DENV is not merely causing a transient, sporadic outbreak in Osun State. Instead, this pattern is characteristic of endemic circulation, where the virus is consistently present in the environment, leading to a significant cumulative history of exposure within the population. This finding aligns with broader seroprevalence studies across Nigeria and West Africa, which, despite showing varied prevalence rates, collectively confirm widespread DENV transmission (Ayolabi et al. 2019; Gebremariam et al. 2023; Saidu and Okojie 2024). The presence of a large IgG-seropositive population also raises public health concerns regarding the potential for more severe disease through antibody-dependent enhancement (ADE) if a new or different DENV serotype were to be introduced into the region (Khetarpal and Khanna 2016).

The dynamics of vector-specific risk factors

The risk factor analysis revealed a telling disparity between the two diseases (Tables 3 and 4). A single factor emerged as statistically significant: participants residing in homes with nets on the windows were significantly protected from malaria infection compared to those without (OR: 1.782, 95% CI: 1.067-2.975, p=0.027). This finding reinforces the value of physical barriers against the primary malaria vector, the *Anopheles* mosquito, which is predominantly nocturnal and endophagic (bites and rests indoors at night) (World Health Organization 2021).

Table 1. Sociodemographic characteristics of the participants (N=250)

Variables	Category	Frequency	Percent (%)
Age range (years) (Mean±SD = 24.94±16.76)	0-10	52	20.8
	11-20	62	24.8
	21-30	62	24.8
	31-40	38	15.2
	41-50	14	5.6
	51-60	12	4.8
	>60	10	4.0
Gender	Female	158	63.2
	Male	92	36.8
Marital status	Married	78	31.2
	Single	156	62.4
	Widow/Widower/Other	16	6.4
Highest level of education	Primary	48	19.2
	Secondary	126	50.4
	Tertiary	78	31.2
Occupation	Student	152	60.8
	Trader	40	16.0
	Civil servant	22	8.8
	Business	14	5.6
	Other	22	8.8

Table 2. Prevalence of dengue virus, malaria, and coinfections

Disease test	No. of Participants	Frequency	Percent (%)
Dengue virus IgG	250	16	6.4
Dengue virus IgM	250	2	0.8
Malaria Parasite (MP) RDT	250	134	53.6
Coinfections with Malaria			
MP RDT and DENV IgG	250	8	3.2
MP RDT and DENV IgM	250	2	0.8

Table 3. Comparative analysis of risk factors for malaria and dengue IgG seropositivity

Variables	Category	No. of Participants (%)	Malaria RDT Positive (%)	OR (95% CI)	p value	DENV IgG Positive (%)	OR (95% CI)	p value
Overgrown bushes	Yes	136 (54.4)	54.4	1.000 (Reference)	0.601	8.8	2.661 (0.834-8.492)	0.087
	No	114 (45.6)	52.6	0.931 (0.563-1.539)		3.5	1.000 (Reference)	
Windows with net	Yes	152 (60.8)	59.2	1.782 (1.067-2.975)	0.027	5.3	0.625 (0.227-1.724)	0.360
	No	98 (39.2)	44.9	1.000 (Reference)		8.2	1.000 (Reference)	
Water tank closed	Yes	166 (66.4)	55.4	1.243 (0.735-2.104)	0.417	4.8	0.481 (0.174-1.331)	0.151
	No	84 (33.6)	50.0	1.000 (Reference)		9.5	1.000 (Reference)	
Stagnant gutters	Yes	182 (72.8)	51.6	0.748 (0.426-1.314)	0.311	5.5	0.601 (0.210-1.722)	0.339
	No	68 (27.2)	58.8	1.000 (Reference)		8.8	1.000 (Reference)	

Table 4. Prevalence of dengue virus among participants by age and sex

		No. Examined	DENV Negative (%)	DENV IgG Positive (%)	DENV IgM Positive (%)	χ2	p value
Age range (years)	0-10	52	44 (84.6)	6 (11.5)	2 (3.8)	24.376	0.018
	11-20	62	62 (100.0)	0 (0.0)	0 (0.0)		
	21-30	62	54 (87.1)	8 (12.9)	0 (0.0)		
	31-40	38	38 (100.0)	0 (0.0)	0 (0.0)		
	41-50	14	12 (85.7)	2 (14.3)	0 (0.0)		
	51-60	12	12 (100.0)	0 (0.0)	0 (0.0)		
	>60	10	10 (100.0)	0 (0.0)	0 (0.0)		
Gender	Female	158	144 (91.1)	12 (7.6)	2 (1.3)	2.250	0.325
	Male	92	88 (95.7)	4 (4.3)	0 (0.0)		
Total		250	232 (92.8)	16 (6.4)	2 (0.8)		

Table 5. Prevalence of malaria and coinfections with Dengue virus among participants by age and sex

Variables	Category	No. Examined	Malaria RDT Positive (%)	Coinfections with DENV IgG (%)	Coinfections with DENV IgM (%)
Age range (Years)	0-10	52	32 (61.5)	4 (7.7)	2 (3.8)
	11-20	62	38 (61.3)	0 (0.0)	0 (0.0)
	21-30	62	22 (35.5)	2 (3.2)	0 (0.0)
	31-40	38	22 (57.9)	0 (0.0)	0 (0.0)
	41-50	14	8 (57.1)	2 (14.3)	0 (0.0)
	51-60	12	8 (66.7)	0 (0.0)	0 (0.0)
	>60	10	4 (40.0)	0 (0.0)	0 (0.0)
χ2, p value			12.894, 0.045	12.975, 0.043	7.677, 0.263
Gender	Female	158	86 (54.4)	6 (3.8)	2 (1.3)
	Male	92	48 (52.2)	2 (2.2)	0 (0.0)
χ2, p value			0.119, 0.730	1.174, 0.279	1.192, 0.275

Paradoxically, none of the assessed risk factors, including the use of insecticide-treated bed nets, showed any significant association with DENV IgG seropositivity. This "negative" result is not a methodological failure but rather a crucial epidemiological insight. It reflects the fundamental differences in the bionomics of the dengue vector, the *Aedes* mosquito. *Aedes aegypti* is primarily diurnal (bites during the day), often feeds outdoors, and breeds in small, artificial water containers around human dwellings (Khetarpal and Khanna 2016; World Health Organization 2024). Consequently, malaria-centric prevention questions about sleeping under a bed net are largely irrelevant to dengue risk. The study's findings inadvertently demonstrate that vector control strategies focused solely on *Anopheles* are insufficient for preventing dengue. This underscores the need for an integrated vector management (IVM) approach that combines nocturnal interventions like ITNs and indoor residual spraying for malaria with diurnal, source-reduction strategies for dengue control.

Age as a key determinant of infection

The stratified analysis revealed that age was a significant determinant for both infections, whereas gender was not (Table 5). The prevalence of DENV IgG antibodies was significantly associated with age (χ2=24.376, p=0.018), peaking at 14.3% in the 41-50 years age group. Similarly, malaria prevalence was significantly associated with age (χ2=12.894, p=0.045), with the highest rate (66.7%) observed among those aged 51-60 years.

This pattern, with prevalence increasing in older age brackets, points towards a model of cumulative exposure over a lifetime. An individual in their 40s or 50s has had decades more potential exposure to infectious mosquito bites than a child. The peak of DENV IgG—a marker of any past infection—in the 41-50 age group strongly supports this hypothesis. The even later peak for malaria prevalence could reflect a more complex interplay of factors, including lifelong exposure, potential waning of acquired immunity in older age, and occupational or behavioral patterns that increase risk (Saidu and Okojie 2024). These findings challenge the common perception of these diseases as primarily pediatric concerns and highlight the need for public health messaging and interventions that target all age groups, particularly adults who may have significant exposure.

Implications for clinical practice and public health

The confirmation of DENV circulation and its coinfection with malaria has profound implications. Clinically, it necessitates a paradigm shift in the management of acute febrile illness in Nigeria. A negative malaria test in a febrile patient should not be an endpoint but should instead trigger further investigation for other pathogens, with dengue being a primary consideration. Relying on presumptive malaria diagnoses is no longer tenable and risks poor outcomes for patients with dengue. Public health authorities must prioritize the development and dissemination of updated diagnostic algorithms for fever and ensure the availability of reliable dengue diagnostics at various levels of the healthcare system. Furthermore, enhanced surveillance, including molecular methods like RT-PCR for serotyping, is essential to monitor circulating DENV genotypes and detect the introduction of new strains that could precipitate more severe or widespread outbreaks (Ayolabi et al. 2019; Saidu and Okojie 2024).

Limitations of the study

This study, while providing valuable local data, has several limitations. First, the reliance on RDTs for diagnosis has constraints. While practical for field studies, RDTs for malaria can have lower sensitivity than expert microscopy, and serological RDTs for dengue cannot provide information on the specific viral serotype. Second, serological assays for flaviviruses are known to have cross-reactivity issues. It is possible that some of the DENV IgG positive results could be due to past infections with other co-circulating flaviviruses in Nigeria, such as Zika or Yellow Fever virus, potentially leading to an overestimation of dengue seroprevalence (Kotepui and Kotepui 2019; Siddig et al. 2023). Third, the cross-sectional design captures only a single point in time, preventing the establishment of causality between risk factors and disease or the observation of clinical progression. Finally, the risk factor data were self-reported and thus subject to recall bias. The questionnaire was also primarily designed with malaria in mind and may not have adequately captured key risk factors specific to *Aedes*-borne transmission, such as daytime activities and domestic water storage practices.

In conclusion, this study provides crucial, localized evidence for the co-circulation of dengue virus and *Plasmodium* parasites among febrile individuals in Osun State, Nigeria. The findings reveal a significant historical burden of dengue exposure, as indicated by IgG seroprevalence, and demonstrate age-specific patterns of infection that point toward endemic transmission for both pathogens. The high prevalence of malaria underscores its continued dominance as a cause of fever, yet the confirmed presence of dengue, both as a mono-infection and coinfection, highlights a critical gap in current diagnostic practices. These results strongly advocate for the integration of DENV testing into routine clinical algorithms for febrile illness and for the urgent development of dual-pronged, integrated vector management strategies that effectively target the distinct ecologies and behaviors of both *Aedes* and *Anopheles* mosquitoes.

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