Original Article

Molecular Epidemiology of Dengue Virus Infection among Febrile Patients in Ilorin, Nigeria

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Abstract

Dengue is one of the viruses that has been established to be responsible for many febrile cases but most febrile illnesses are often misdiagnosed as malaria and typhoid in Nigeria due to the endemicity of malaria and typhoid. Most of the available data on the circulation of dengue virus in Nigeria were obtained from the less specific and less sensitive serological investigations. We assessed the prevalence of dengue virus and determined its association between socio-demographic factors in three health facilities in three local governments in Ilorin, Nigeria. This cross-sectional study therefore employed molecular technique to provide a specific and more sensitive data on the prevalence of dengue virus infection among febrile patients in Ilorin, Nigeria. We recruited 110 febrile patients from three health facilities in three local governments Areas within Ilorin Metropolis. The patients' blood were collected and transferred immediately to the Molecular Research and Diagnostic Laboratory, University of Ilorin for Molecular analysis. One-step RT-PCR was performed using Bosphore Dengue-Chikungunya Detection Kit to detect the presence of dengue virus RNA in the blood of the recruited patients.

The results showed 8.2% positive for RT -qPCR detection of dengue virus with no statistically significant relationship between all tested socio-demographic factors. Specifically, 12.5% of patients recruited from General Hospital, Ilorin were positive for RT-qPCR detection of dengue virus infection, 6.7% from Civil Service Hospital Ilorin and 3.13% from University of Ilorin Teaching Hospital. We recommend routine procedural investigations for Dengue virus infection among patients with febrile illness in our hospitals.

Keywords: Molecular Epidemiology, Dengue Virus, Infection, Febrile Patients, Nigeria

Introduction

Febrile illness, defined as a body temperature above 37.5°C, is a major global health concern, particularly in Africa.[1] It can result from infectious agents such as bacteria, fungi, parasites, and viruses, with dengue virus being a significant contributor.[2,3] Dengue virus, an arthropod-borne virus transmitted by Aedes aegypti and Aedes albopictus,[4] affects nearly half of the world's population and is the most common arthropod-borne virus, second only to Plasmodium among mosquito-borne pathogens.[5,6] Due to its error-prone RNA polymerase,[7,8] the virus mutates rapidly, increasing the risk of outbreaks. While many infections are mild, severe cases can lead to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which may be fatal.[9,10]

There are five closely related dengue virus serotypes with 62-67% sequence homology. The serotypes differ in immune interactions, and infection with one provides lifelong immunity to that serotype but only temporary immunity to others.[11] Secondary infections with different serotypes can trigger antibody-dependent enhancement increasing disease severity and the risk of dengue hemorrhagic fever and dengue shock syndrome can occur and may lead to death.[12]

Urbanization, migration, and climate change contribute to the spread of Aedes mosquitoes.[13] In Ilorin, poor hygiene, such as standing water and overgrown vegetation, supports vector proliferation.[14,15] Due to the endemicity of malaria and typhoid in Nigeria,[16] febrile illnesses are often misattributed to

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these diseases,[17] compromising diagnosis and treatment.[18] Fever is common in Sub-Saharan Africa, frequently linked to endemic diseases like malaria and typhoid,[19,20] but dengue virus and other Flaviviruses are also significant causes of febrile illness, especially in tropical regions.[21,22] Globally, dengue affects over 120 countries, with 50 million cases and 10,000 deaths reported annually, putting 3.9 billion people at risk.[23,24]

Limited data exist on dengue virus circulation in Ilorin, increasing the potential for future outbreaks.[24] Most Nigerian studies rely on serological testing, which is less specific than molecular techniques due to possible cross-reactions and poor antibody production in immunocompromised individuals.[9]

More data on dengue virus distribution in Ilorin is needed to assess outbreak risks and other vector-borne viruses.[25,26,27]

This study employs molecular methods to assess the prevalence of dengue virus in Ilorin and examines its association with socio-demographic factors. Findings will help raise public awareness and guide preventive measures against dengue outbreaks in the region.

Materials and Methods

Study Area

This study was carried out within Ilorin metropolis, Ilorin is the capital city of Kwara state, located in the North-central region of Nigeria. Ilorin is approximately on latitude $8^0$30'0.0000"N of the equator and longitude $4^0$32'60.0000"E of the Greenwich meridian with an area of about 100km.[2,15]

Study Site

Three health facilities in Ilorin were selected for this study: University of Ilorin Teaching Hospital (UITH) for Ilorin East, General Hospital, Ilorin (GHI) for Ilorin West, and Civil Service Hospital (CSH) for Ilorin South. UITH is a tertiary referral hospital in Kwara State with a 500-bed capacity, serving 10,000-12,000 in-patients and 120,000-126,000 out-patients annually, extending its services to neighboring states. CSH provides secondary healthcare for both civil servants and the general public. GHI, a 400-bed hospital, offers primary, secondary, and tertiary healthcare services in Kwara State and nearby areas.[15,24]

Study Design

This study adopted a cross-sectional study design.

Ethical Consideration

Ethical approval was sought and obtained from the Ethical Review Committee Ministry of Health, Ilorin with approval number ERC/MOH/2023/12/168, Institutional Review Committee of General Hospital, Ilorin with approval number GHI/IRC/246/Vol.1/134 to 587 (Appendix II) and Ethical Review Committee, University of Ilorin Teaching Hospital, Ilorin with approval number ERC PAN/2024/03/0469 before commencing this research. Permission was obtained from the Head of units of all the selected hospitals. Permission was also sought from the Head of Department, Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College Of Health Sciences, University of Ilorin.

Study Population

Febrile patients attending all the selected hospitals in Ilorin, Nigeria were recruited from February 2024 to May, 2024

Inclusion Criteria

Patients presenting with fever as diagnosed by the phlebotomist in all the selected hospital who consent to participate in this research were recruited irrespective of age and educational background. Guardians or relatives of children were consulted to give consent on behalf of their wards.

Exclusion Criteria

Patients who refused to give consent were excluded from this research. Unconscious patients who cannot grant consent were also exempted from this study

Sampling Technique

Purposive, non-probability sampling techniques were used to recruit participants for this study.

Sample Size Calculation

The sample size was determined using a modified method by Kolawole et al.[15], and a total of 110 samples were recruited for this study.

Sample Collection

The phlebotomist at the selected hospitals took 2ml of venous blood from febrile patients aseptically in an EDTA bottles. The samples were properly labeled and transferred to Molecular Diagnostic and Research Laboratory at the University of Ilorin for analysis.

RNA Extraction

Materials:

Norgen RNA purification kit, Blood sample, Micropipette, Pipette tips, columns, Nanodrop spectrophotometer, Centrifuge, Vortex mixer.

RNA Extraction Procedure

Viral RNA was extracted from blood samples using the NORGEN Total RNA Purification Kit following the manufacturer's instructions. Blood samples were left at room temperature to obtain serum. A 200 µL serum sample was mixed with 600 µL Buffer RL, vortexed for 10 seconds, and combined with 800 µL of 96–100% ethanol before vortexing again. The lysate was transferred to a spin column and centrifuged

at 14,000 RPM for 1 minute. After discarding the flow-through, the column was washed three times with 400 µL of ethanol, ensuring complete drying of the resin. The column was placed in a fresh elution tube, and 50 µL of Elution Solution A was added to the membrane and centrifuged at 2,000 RPM for 2 minutes, followed by 14,000 RPM for 1 minute.

Quantification of RNA Extracts and Storage

The concentration (ng/uL) and purity of extracted RNAs were determined using Nano drop spectrophotometer (produced by Thermo Fisher Scientific, Waltham, MA, USA) at 260/280 OD. The ratio of absorbance at 260/280 nm was observed. Those higher than 1.6 were considered pure but those lesser than or equal to 1.6 were considered to contain impurities. The purified RNA samples were stored at –80°C for further analysis.

One-step Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

One-step RT-qPCR was performed using Bosphore Dengue-Chikungunya Detection Kit.

Principle of Rt PCR

Polymerase chain reaction (PCR) amplifies DNA through repeated heating and cooling cycles using primers, dNTPs, Taq polymerase, buffer solution, and a DNA template. Primers anneal to specific template regions to initiate replication, while dNTPs serve as DNA building blocks. Taq polymerase synthesizes new DNA strands, and the buffer maintains optimal reaction conditions.[25]

RT-PCR enables real-time product detection.. It employs dual-labeled probes for increased sensitivity. Taq polymerase's 5' exonuclease activity cleaves the fluorescent hydrolysis probe during extension, separating the reporter from the quencher, generating detectable fluorescence.[25] Fluorescence intensity increases as PCR products accumulate. The threshold cycle (Ct) is the point where fluorescence surpasses background levels, showing a linear relationship with the log of the starting template quantity.[25]

Bosphore Dengue Detection Kit

Bosphore Dengue Detection Kit employs multiplex PCR and an internal control is incorporated into the system to check mistakes during the isolation procedure, to check for possible PCR inhibition and application mistakes. Dengue virus RNA and internal control are amplified in one PCR tube, using sequence-specific primers. The fluorescent signals generated by the amplification of the pathogens and internal controls, are detected by different probes (labeled at the 5' end with reporter molecules HEX/ROX).. Dengue virus is amplified and fluorescence detection is accomplished using the HEX filter. The amplification data of the internal control is detected with ROX filter.

Materials:

AriaMx PCR machine, Centrifuge, Vortex mixer, Micropipette, Nuclease free water, Master mix, RNA template and Controls.

PCR Procedure

PCR amplification followed the manufacturer's instructions. Kit components were thawed before use. For each reaction, 15µl of master mix, 10µl of RNA (sample/positive/negative control), and 0.2µl of internal control were added to PCR strips. The tubes were sealed, vortexed, and centrifuged.

In the AriaMx PCR machine (Agilent Technologies), filters (HEX and ROX) were selected, and samples and controls were identified. The manufacturer's thermal protocol for the Bosphore Dengue-Chikungunya Detection Kit was applied, including reverse transcription, initial denaturation, two-step amplification, and a terminal hold. Real-time data collection occurred during the second amplification step.

Result Interpretation

Results were interpreted as positive, negative or No Cq according to the manufacture's instructions.

Data Analysis

Data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive data are presented in tables, and Pearson's Chi-square test was used to assess relationships between categorical variables and prevalence rates, with statistical significance set at $p < 0.05$. Results were presented using Microsoft Word and Excel (version 2023).

Results

Socio-demographic Data of Recruited Participants

The socio-demographic characteristics of the 110 recruited febrile patients are summarized in Table 1. Participants were selected through purposive sampling: 30 from CSC, 48 from GHI, and 32 from UITH. Of these, 32 (29.1%) were male and 78 (70.9%) were female, with a mean age of 32.8 years. The majority (32.7%) were aged 20-29, with a median age of 29.7 years, a standard deviation of ±9.27 years, and an interquartile range of 19.74 years.

Regarding occupation, 35 (31.8%) were business persons, 32 (29.1%) students, 9 (8.2%) unemployed, 27 (24.5%) civil servants, and 7 (6.4%) farmers. Marital status distribution included 47 (42.7%) single, 59 (53.6%) married and 4 (3.6%) widowed. Religious affiliation comprised 47 (42.7%) Christians and 63 (57.3%) Muslims.

Table1: Socio-Demographic Characteristics of Study Participants

Socio-Demographic Data		Frequency	Percentage
Gender	Male	32	29.1
	Female	78	70.9
Age group (years)	0-9	11	10.0
	10-19	9	8.2
	20-29	36	32.7
	30-39	22	20.0
	40-49	9	8.2
	50-59	16	14.5
	60-69	2	1.8
	70-79	4	3.6
	Above 80	1	0.9
Location	CSC	30	27.3
	GHI	48	43.6
	UITH	32	29.1
Occupation	Business	35	31.8
	Student	32	29.1
	Unemployed	9	8.2
	Civil servant	27	24.5
	Farmer	7	6.4
Marital status	Single	47	42.7
	Married	59	53.6
	Widow	4	3.6
Religion	Christianity	47	42.7
	Islam	63	57.3
	Total	**110**	**100.0**

KEYS - CSC: - Civil Service Clinic
GHI: - General Hospital, Ilorin
UITH: - University of Ilorin Teaching Hospital

Table 2: Prevalence of Dengue Virus Infection by Gender

Gender	Negative n (%)	Positive n (%)	Subtotal n (%)	X^2	df	P value
Male	30 (27.3)	2 (1.8)	32 (29.1)	0.224	1	0.636
Female	71 (64.5)	7 (6.4)	78 (70.9)			
Total	**101 (91.8)**	**9 (8.2)**	**110 (100)**			

Prevalence of Dengue Virus Infection by Gender

Out of the 8.2% (9) positive samples, 6.4% (7) were female and 1.8% (2) were male. There was no statistically significant relationship between gender and the prevalence of dengue virus infection since the p-value was greater than 0.05 as shown in Table 2.

Prevalence of Dengue Infection by Age

Dengue virus was evenly detected in the age groups 0-9 (2.1%), 20-29 (2.1%), 30-39 (2.1%), 40-49 (1.1%), and 50-59 (2.1%). The virus was not detected in participants above 60 years old. The analysis established that there was no statistically significant relationship between age groups and the prevalence of

Table 3: Prevalence of Dengue Infection by Age

Age (years)	Negative n (%)	Positive n (%)	Subtotal n (%)	X^2	df	p value
0-9	9(8.2)	2 (1.8)	11 (10.0)	3.745	8	0.879
10-19	9 (8.2)	0 (0)	9 (8.2)			
20-29	34 (30.9)	2 (1.8)	36 (32.7)			
30-39	20 (18.2)	2 (1.8)	22 (20.0)			
40-49	8 (7.3)	1 (0.9)	9 (8.2)			
50-59	14 (12.7)	2 (1.8)	16 (14.5)			
60-69	2 (1.8)	0 (0)	2 (1.8)			
70-79	4 (3.6)	0 (0)	4 (3.6)			
Above 80	1 (0.9)	0 (0)	1 (0.9)			
Total	**101 (91.8)**	**9 (8.2)**	**110 (100)**			

Table 4: Prevalence of Dengue Virus Infection by Location

Location	Negative n (%)	Positive n (%)	Sub total n (%)	X^2	df	p value
CSC	28 (25.5)	2 (1.8)	30 (27.3)	2.372	2	0.305
GHI	42 (38.2)	6(**5.5**)	48 (43.6)			
UITH	31 (28.2)	1 (**0.9**)	32 (29.1)			
TOTAL	**101 (91.8)**	**9 (8.2)**	**110 (100)**			

Table 5: Prevalence of Dengue Virus Infection by Other Socio-Demographic Factors

Socio-demographic factors	Negative n (%)	Positive N (%)	Subtotal n (%)	X^2	df	p value
OCCUPATION						
Business	31 (28.2)	4 (**3.6**)	35 (31.8)	2.422	4	0.659
Student	29 (26.4)	3 (2.7)	32 (29.1)			
Unemployed	9 (8.2)	0 (**0**)	9 (8.2)			
Civil servant	26 (23.6)	1 (0.9)	27 (24.5)			
Farmer	6 (5.5)	1 (0.9)	7 (6.4)			
MARITAL STATUS						
Single	42 (38.2)	5 (4.5)	47 (42.7)	0.888	2	0.641
Married	55 (50.0)	4 (3.6)	59 (53.6)			
Widow	4 (3.6)	0 (**0**)	4 (3.6)			
RELIGION						
Christianity	44 (40.0)	3 (2.7)	47 (42.7)	0.353	1	0.552
Islam	57 (51.8)	6 (5.5)	63 (57.3)			
TOTAL	**101 (91.8)**	**9 (8.2)**	**110 (100)**			

KEYS - X^2 = Chi Square
df = degrees of freedom
P- Value = probability value at 95% confidence interval

dengue virus in Ilorin as shown in Table 3

Prevalence of Dengue Virus Infection in Each Study Site/location

Based on each study location, 6.7% (2/30)of patients recruited from CSC were positive for RT qPCR detection of dengue virus infection. 12.5% (6/48) were positive at GHI while 3.13% (1/32) were positive for RT qPCR detection of dengue virus infection at UITH as shown in Table 4.

Relationship between other socio-demographic factors and the prevalence of dengue virus Infection

Infection with dengue virus was detected in 4 (3.6)

business persons, 3(2.7%) students, 1 (0.9%) civil servant (1.1%), 1 (0.9%) farmer and zero (0%) unemployed patients. Based on marital status, 5 (4.5%) single patients, 4 (3.6%) married individuals and zero (0) widows were positive to RT qPCR detection of dengue virus. In terms of religion, 3 (2.7%) out of the dengue-positive patients were Christian while 6 (5.5%) were Muslims. The p values generated are above 0.05 as shown in Table 5 indicating that there is no statistically significant association between any of the tested Socio-demographic factors and the prevalence of dengue virus infection in Ilorin, Nigeria.

Discussion

Most people in Nigeria attribute all febrile illnesses to malaria or typhoid because these diseases are endemic in the country, resulting in the misidentification and misdiagnosis of many cases of dengue fever infection.[7] There is sparse information on the prevalence of dengue virus infection in Nigeria. However, in recent years, few researchers have employed serological techniques to report the prevalence of dengue virus infection in Nigeria.[6] This study employed a molecular method, which is more sensitive and specific to assess the prevalence of dengue infection among febrile patients in Ilorin, Nigeria

Prevalence of dengue virus infection in Ilorin

The prevalence reported in this study indicates active dengue virus transmission in Ilorin. The 8.2% prevalence observed represents an increase from the 6.25% reported by Kolawole *et al.*[5] This rise from 2016 to 2024 may be linked to rapid urbanization, inadequate waste management, and increased stagnant water, which create breeding sites for Aedes mosquitoes and enhance human-vector interaction. Human migration may also contribute to virus introduction in new areas. The 8.2% prevalence aligns with the 8.5% reported by Akinyemi *et al.*[13] among febrile patients in Lagos, Nigeria.

However, Islam *et al.*[4] reported zero dengue virus infections among 400 febrile patients in Kwara State, contrasting with this study's findings. This discrepancy may stem from differences in sample processing; while Shaibu *et al.*[26] used plasma, this study analyzed serum. Variations in preservation, storage, and transportation methods may have also influenced the results. Since dengue virus is an RNA virus prone to degradation, immediate processing of samples in this study may explain the detection difference compared to the delayed processing by Shaibu *et al.*[26]

A confirmed dengue virus case within a community is epidemiologically significant, as infected mosquitoes can spread the virus to a large susceptible population.[27] Although this study was conducted during the dry season (February–May 2024), stagnant water bodies persisted, supporting Aedes mosquito breeding and dengue virus transmission.

Relationship between Socio-demographic factors and the Prevalence of *Dengue virus* infection in Nigeria

A higher percentage of dengue-positive patients in this study were female, aligning with findings by Tizhe *et al.*[3], who also reported a higher prevalence among women. This may be due to increased exposure to Aedes mosquitoes, as women spend more time at home, where stagnant domestic water bodies serve as breeding sites.

The higher prevalence among women may also be influenced by the greater number of female participants recruited, despite the consecutive sampling of all febrile patients regardless of gender. Similar trends were observed in studies by Tizhe *et al.*[3] and Onyedibe *et al.*[18], where more female participants were enrolled, possibly reflecting a greater tendency for women to seek medical care.[26]

Prevalence of Dengue Virus infection in Each Study Site

The higher prevalence of dengue virus infection at GHI may be due to its role as a readily accessible primary healthcare facility. While CSC also serves as a primary healthcare center, its smaller capacity may explain the lower prevalence observed there.

UITH recorded the lowest prevalence, similar to the 2.9% reported by Onyedibe *et al.*[18] at another federal tertiary hospital in North Central Nigeria. This lower prevalence may be attributed to the referral-based nature of tertiary hospitals, where patients often seek care after visiting primary or secondary health facilities. By the time they reach these hospitals, their immune systems may have already cleared the virus.

Conclusion

This study has established that dengue virus is present in Ilorin, Nigeria, and is responsible for many febrile illnesses in Ilorin. We therefore recommend that febrile illness in Nigeria should not be limited to malaria and typhoid alone but Dengue virus should equally be considered. Diagnostic kits should be made available for routine hospital diagnostic procedures Dengue virus infections. Public enlightenment should be carried out to educate people about arthropod-borne infection and the need to eliminate environments that support mosquito breeding. Further studies in Ilorin should investigate the serotypes of dengue virus circulating in Ilorin, Nigeria.

Limitations of the Study

This study was conducted in selected health facilities; therefore, its findings may not fully represent

the broader perspective of the disease in the general population.

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