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Molecular Epidemiology of Dengue Fever Virus Infection Among Patients with Febrile Illness Attending Clinics at Primary and Tertiary Hospitals in Ebonyi State, Nigeria

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Abstract: Dengue virus infection is a global threat to public health due to its widespread morbidity and mortality. The incidence of dengue has grown dramatically around the world in recent decades. The aim of this study is to detect and characterize dengue fever virus infection among patients with febrile illness attending healthcare facilities in Ebonyi State, Nigeria. Those reporting with febrile conditions were recruited for the study excluding patients under two years of age. The study was carried out at the major primary health care centres and hospitals within Ebonyi State and the phlebotomy unit of each of the hospitals was used as the sample collection point. The study adopted a cross-sectional survey and a well-structured questionnaire was used to obtain information from the patients. ELISA technique was used to presumptively detect the presence of dengue virus in the patient's serum sample and then subsequently analysed molecularly using nested PCR technique to identify the different serotypes of the dengue virus. Other major febrile causing agents were also assessed to determine the level of co-infection with dengue virus. The other febrile agents assessed in this study are, *Plasmodium falciparum*, hepatitis C virus (HCV), hepatitis B virus (HBV) and enteric fever. A total of 376 individuals with febrile illness were enrolled in this study comprising of 41.5% male and 58.5% female. The age range of the subjects are between 12 to 71 year-olds with a mean age of 37.6 ±14.4 years old. A prevalence rate of 11.7% of the subjects were seropositive to only IgM, 19.7% had IgG and 11.2% had both IgM and IgG circulating in their system. Considering other causes of febrile conditions, Immunoglobulin M (IgM) co-existed with *Plasmodium spp* (13.1%), HBV (2.5%), HCV (0.6%) and Enteric fever (11.3%) while IgG immunoglobulin co-existed with *Plasmodium spp* (25.6%), HBV (5%), HCV (2.5%) and Enteric fever (13.1%). Similarly, subjects that are seropositive to both IgG and IgM also had *Plasmodium spp* (9.4%), HBV (1.9%), HCV (0.6%) and Enteric fever (13.8%) co-infections. Four serotypes were detected but the most prevalent serotypes are DENV-3 and DENV-4. The findings in this study showed that dengue fever virus infection is present in Ebonyi State.

Keywords: *Immunoglobulin, DENV, ELISA, IgM, IgG, HCV, HBV, and Plasmodium falciparum.*

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I. INTRODUCTION

Fever is one of the most frequently reported clinical symptoms by patients seeking health care in most areas of the tropics, where it may occur either solely or in association with other common symptoms such as joint pains, cough, diarrhea etc. (WHO 2012). Fever without localizing features presents a particular challenge to health care workers (Prasad *et al.*, 2015) and health systems because it may be caused by a wide range of bacterial, fungal, parasitic, and/or viral infections (Petti *et al.*, 2006), as well as by non-infectious conditions (Mayxay *et al.*, 2013). Clinical prognosis has limited accuracy both for identifying the likely cause and for the early recognition of patients who will progress to serious or fatal disease. Compounding the limitations of clinical assessment is the paucity of available epidemiologic data on common causes of fever (Crump 2014) and inadequate clinical laboratory services in many areas (Archibald and Reller 2001).

Dengue fever virus infection is recently considered a global threat to public health. Arboviruses, mainly dengue viruses, have caused widespread morbidity and mortality in the world, especially in Africa (WHO, 2012). The incidence of dengue has grown dramatically around the world in recent decades, with cases reported to WHO increased from 505, 430 cases in year 2000 to about 5.2 million in 2019 (WHO 2019). A vast majority of cases may be asymptomatic, mild and/or self-limiting, and hence the actual numbers of dengue cases are under-reported. Many cases are also misdiagnosed as other febrile illnesses (Bhatt *et al.*, 2013). The largest number of dengue cases ever reported globally was in 2019. All regions were affected, and dengue transmission was recorded in Afghanistan for the first time (WHO, 2019). Recent modelling estimates indicated 390 million dengue virus infections per year of which 96 million manifest clinically (Brady *et al.*, 2012). In 2023 (from January-December), over 6 million dengue cases and over 6000 dengue related deaths were reported from 92 countries/territories (WHO, 2023).

The disease is now endemic in over 90 countries in the WHO Regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. The Americas, South-East Asia and Western Pacific regions are the most seriously affected, with Asia representing around 70% of the global disease burden (WHO, 2023). There are four major strains of the virus, referred to as DENV-1, DENV-2, DENV-3 and DENV-4 (Chen and Wilson, 2010) and more recently DENV-5 (WHO, 2015). The vectors responsible for Dengue virus transmission are infected female *Aedes* mosquitoes (Chiefly, *Ae. aegypti* and *Ae. albopictus*) and are principally diurnal and outdoor biters (Lwande *et al*., 2020).

These fever causing diseases have become significant public health problem worldwide, especially in Africa due to their endemicity and similarities in signs and symptoms. (Baba *et al.*, 2009). Owing to these similarities in clinical presentations, there is possibility that persons infected with any of these microbial agents could be misdiagnosed when using clinical considerations alone without recourse to laboratory investigations. Management of febrile illnesses may be complicated if several disease causing agents co-exist in a patient, which may prolong treatment or cause drug resistance due to drug interactions (Domingues, 2009).

- *Aim of the Study*

The aim of this study was to determine the occurrence of dengue fever virus and strains circulating in Ebonyi State, Nigeria.

II. MATERIALS AND METHODS

- *Study Area:*

The study was carried out at the major hospitals within Ebonyi State. The phlebotomy unit of each of the hospitals was used as the sample collection point. Ebonyi State lies within the coordinates of 6º15^{I}N and 8º05^{I}N. It has a total land area of 6,400 km^2. Ebonyi State is a state in the South-East geopolitical zone of Nigeria, bordered to the north and northeast by Benue State, Enugu State to the west, Cross River State to the east and southeast, and Abia State to the southwest.

- *Study Population and Design:*

The study adopted a cross-sectional survey among which involves a one time collection of sera samples from the enrolled individuals. The people recruited were patients reporting to the out-patient department with elevated body temperature and/or those with other illness with fluctuations of body temperature. The participants were issued with structured questionnaire to obtain information on demographic characteristics of such as; Age, Gender, and Risk factors that may predispose them to mosquito bites especially in the evening hours. The self-use of antibiotics by the participants was part of the questionnaire. Since the vectors for dengue virus are majorly outdoor biters, children below the ages of 2 years were excluded from the study because there are naturally meant to spend most of their times indoors.

- *Sample Size*

A total of 376 blood samples was collected for the study. The total sample size was calculated using the formula:

$$n = \frac{N}{1+N\,(e)2}$$

Where;

n = Sample size;

N = Population size;

e = level of precision at 0.05.

- *Ethical Consideration:*

Ethical approval was obtained from Ebonyi State health management board, Abakaliki. Also, an informed consent form was issued to the participants and was duly filled. The

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informed consent form contained all the information concerning the study and participation was purely voluntary.

III. BLOOD SAMPLE COLLECTION

Five millilitres (5ml) of whole blood was collected aseptically through venipuncture using sterile 5 ml syringe and part transferred into a sterile plain tube container already properly labeled and the other part into EDTA container. The serum was harvested from the samples in the plain tube for ELISA and molecular analysis and the samples in EDTA was used for malaria microscopy, rapid diagnostic testing and typhoid and paratyphoid Widal detection. The samples was then transferred to National Arbovirus and Vectors Research Centre (NAVRC) Enugu for molecular analysis. In case of any delay the serum was kept frozen at -20ºC until time for molecular analysis.

➢ *Detection of Malaria Parasite*

Malaria parasite was evaluated from the whole blood by Giemsa and Leishman staining of thick and thin films respectively. There were examined under the light microscope using oil immersion objective lens.

➢ *Detection of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) Infections.*

Rapid detection kits for HBV and HCV was used to rapidly detect the presence of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in the extracted serum of the subjects following the manufacturers' instructions.

➢ *Detection of Salmonella spp Infection*

Widal test technique (Slide agglutination test) was carried out in accordance with the manufacturers' guide to detect both the *Salmonella* somatic and flagella antigens in the sera of the subjects.

➢ *Detection of Dengue Fever Virus*

Enzyme Linked ImmunoSorbent Assay (ELISA) technique was used to detect the presence of dengue immunoglobulin M (IgM) and immunoglobulin G (IgG) in the serum of the recruited subjects Molecular Analysis:

➢ *Primer Design and Synthesis:*

The under listed set of primers was designed using NCBI bioinformatics tool and synthesized at Inqaba Biotec West Africa. *D1---- TCA ATA TGC TGA AAC GCG CGA GAA ACC G, D2 ---TTG CAC CAA CAG TCA ATG TCT TCA GGT TC, TS1 --- CGT CTC AGT GAT CCG GGG G, TS2—CGC CAC AAG GGC CAT GAA CAG, TS3--- TAA CAT CAT CAT GAG ACA GAG C-3, TS4 --- CTC TGT TGT CTT AAA CAA GAG A-*. The primers were used for nested PCR to detect the dengue virus and the various serotype of dengue virus.

➢ *RNA Extraction:*

Viral RNA was extracted from the blood samples using Qiamp RNA extraction (from Qiagen Company, Germany). AVL buffer containing carrier RNA was mixed thoroughly with the serum in a 1.5ml of clean Eppendorf tube by vortexing. After brief centrifugation, absolute ethanol was added to the mixture and mixed by vortexing. The mixture was allowed to stand at room temperature for one minute and then transferred into a QiaAmp Mini spin column and centrifuged at 10000rpm for one minute. The filtrate was discarded as the column must have trapped back the genomic RNA. AW_1 and AW_2 was used to wash the RNA successively to ensure complete washing off of debris from the extract. AVE buffer was used to elute 50ul of the RNA. The concentration of the extracted RNA was quantified using Nanodrop spectrophotometer.

➢ *RNA Amplification*

- *Amplification of dengue virus RNA by Nested PCR*

The constituents were added as follows; 10 µl of 5X buffer, 2 µl of 10mM of dNTPs, 1 µl each of forward primer (D_1) and reverse primer (D_2), 2 µl of Enzyme mix (Polymerase enzyme and 12.5mM of $MgCl_2$), 5 µl of RNA template and the total reaction volume was made up to 50µl with nuclease free water. One-step amplification protocol was adopted during the amplification process. The protocol involved Hold @50ºC for 30 min (for reverse transcription), initial hold @95ºC for 15min, total of 35 cycles involving denaturation @94ºC for 30 seconds, annealing @55ºC for 60 seconds, Elongation @72ºC for 2 min and a final hold @72ºC for 7 minutes. During the second amplification process, the primer D_1 will be substituted with the Dengue virus type-specific primers, TS_1, TS_2, TS_3 and TS_4. This protocol was adopted from Lanciotti *et al.,* (1992) and slightly modified.

- *Gel Electrophoresis*

After amplification, the amplicons were stained with ethidium bromide and run on 1.5% agarose gel for 1hr30mins at 120V. The stained DNA bands was visualized under an ultraviolet transilluminator.

IV. RESULTS

A total of 376 individuals with febrile illness were enrolled in this study comprising of 41.5% male and 58.5% female. The age range of the subjects was between 12 to 71 year-olds with a mean age of 37.6 ±14.44 and year-olds. The age group of 41 and above had the highest number of participants accounting for 29.3% (110/376) while those of 21 – 30 and 31 – 40 year olds accounted for 26.1% each. The age groups of ≤20 had the least number of participants with 18.6%. The educational status of the subjects indicated that majority (39.6%) had tertiary education whereas 21% had no education (Table 1). Those with elevated body temperature represented 71.3% of the subjects and 37% of them presented with headache, 19.5% presented with cough and 17.8% had diarrhea while very few had had dyspnea 7% and pneumoniae 8..7%. Majority 79% agreed to have experienced fever after treating malaria and 24.7% of them usually resort to antibiotics while 24.2% do retake another antimalarial drugs when the fever persists. A reasonable number 83% agreed that they usually notice mosquito bites during evening period (Table 2). A total of, 11.7% of the subjects were seropositive to dengue fever virus IgM, 19.7% had IgG and 11.2% had both IgM and IgG circulating in their system (Table 3). In this study, Ebonyi State was partitioned into two major zones;

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Afikpo and Abakaliki zones. A total of 63.3% subjects participated in the study within Afikpo zone and dengue immunoglobulins distributed as IgG (12%), IgM (5.1%) and IgG/IgM (4%) while 36.7% participated within Abakaliki zone and had dengue immunoglobulins distributed as IgM (6.6%), IgG (7.7%) and IgG/IgM (7.2%) (Fig 1). Amongst dengue IgG positive subjects, it comprises of male (12.5%) and female (7.2%) P-value 0.026; that of IgM, male (7.2%) and female (4.3%), P-value 0.978 while that of IgG/IgM is male (6.9%) and female (4.3%), P-value 0.826. Out of the 71.3% of the subjects that presented with febrile conditions, 12.7% were seropositive to dengue Immunoglobulins distributed as IgM (2.9%), IgG (6.9%) and IgM/IgG (2.9%). Only cough, headache and dyspnea were clinical symptoms observed amongst dengue seropositive subjects. Those who were seropositive to only IgM presented with headache (2.4%) and dyspnea (0.3%); those seropositive to only IgG had cough (2.4%) and headache (2.7%) while those that showed seropositivity to both IgG and IgM had cough (0.3%) and headache (2.7%). 9.3% of the 12.7% dengue seropositive subjects agreed to still have fever even after taking anti-malarial chemotherapy but 4.8% normally continue with another dosage of anti-malaria drugs while 7.7% do consider taking antibiotics instead and minority 0.3% resort to herbal drugs. Considering other causes of febrile conditions, Immunoglobulin M (IgM) co-existed with *Plasmodium spp* (13.1%), HBV (2.5%), HCV (0.6%) and Enteric fever (11.3%) while IgG immunoglobulin co-existed with *Plasmodium spp* (25.6%), HBV (5%), HCV (2.5%) and Enteric fever (13.1%). Similarly, subjects that are seropositive to both IgG and IgM also had *Plasmodium spp* (9.4%), HBV (1.9%), HCV (0.6%) and Enteric fever (13.8%) co-infections (Table 5).

Table 1 General Demographic Characteristics of the Study Subjects

Variables	**F**	**Percentage**	
Gender			
Male	156	41.5	
Female	220	58.5	
Age	Range 12 - 71		SD±Mean 37.60±14.44
≤ 20	70	18.6	
21-30	98	26.1	
31-40	98	26.1	
41 & Above	110	29.3	
Educational status			
No education	79	21.0	
Primary only	57	15.2	
Secondary	93	24.7	
Tertiary	147	39.1	
Total			
Occupation			
Farmer	48	12.8	
Civil Servant	64	17.0	
Student	76	20.2	
Trader	58	15.4	
Unemployed	56	14.9	
Drivers	36	9.6	
Other	38	10.1	
Types of Residence			
Rural	114	30.3	
Semi-urban	182	48.4	
Urban	80	21.3	
Types of Living home			
Multi yard	124	33	
Block of flats	194	51.6	
Private yard	58	15.4	
When did you travel outside Nigeria			
Less than 3 months	4	1.1	
Above 3 months	14	3.7	
Less than 6 months	20	5.3	
Above 6 months	72	19.1	
Over a year ago	100	26.6	
Never traveled out	166	44.2	

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Table 2 Observed Clinical Features of the Study Subjects.

Variables	F	Percentage (%)
Body Temperature		
36.5ºC – 37.4ºC	108	28.7
≥37.5ºC	268	71.3
Clinical Symptoms		
Cough	90	19.5
Headache	170	37.0
Dyspnea	32	7.0
Vomiting	46	10
Pneumonia	40	8.7
Diarrhea	82	17.8
Period of Pyrexia		
Both seasons	180	47.9
Rainy season	120	31.9
Dry season	76	20.2
Fever after Malaria Drugs		
Yes	297	79
No	79	21
How do you handle the fever		
Continue with Malaria drugs	91	24.2
Take antibiotics	93	24.7
Take herbal medications	56	14.9
Take no medications	57	15.2
Days of fever if it occurs		
2 days	30	8
3 days	63	16.8
4 days	55	14.6
5 days	61	16.2
6 days	43	11.4
7 days	45	12
Do you Notice Mosquito bites during evenings		
Yes	312	83
No	64	17

Table 3 Detection of Dengue Virus Immunoglobulins (IgG and IgM) Among Febrile Patients

Antiobdies	Number positive (%)
IgM	44 (11.7)
IgG	74 (19.7)
IgM/IgG	42 (11.2)

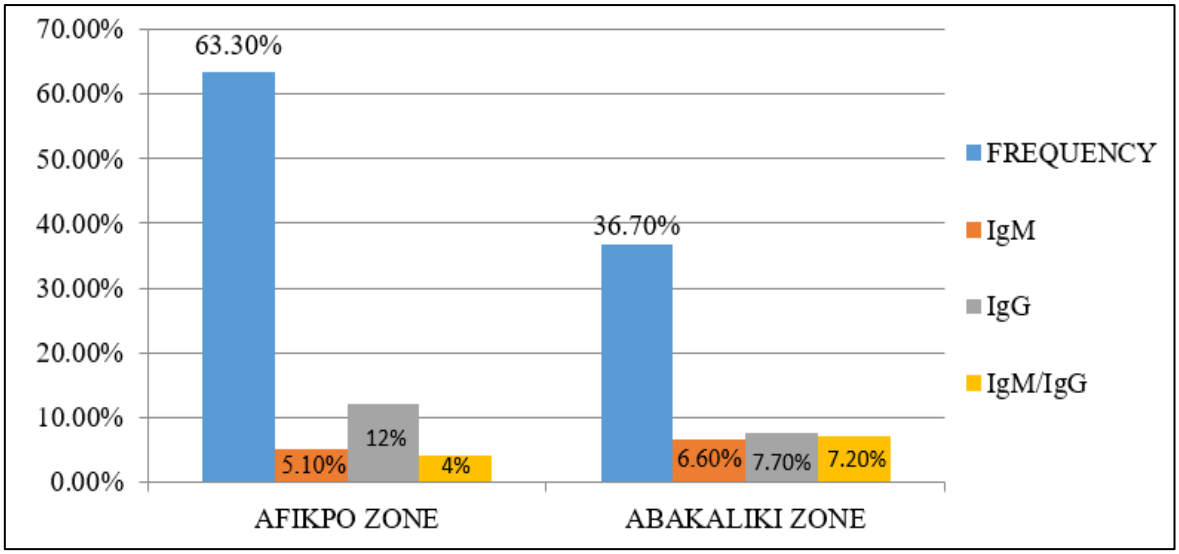

Fig 1 The Distribution of Dengue Virus Infection Across the Major Zones of Ebonyi State.

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Table 4 Molecular Characterization of Dengue Virus Serotypes in Ebonyi State

Sample Code	DEN-1	DEN-2	DEN-3	DEN-4
Abakaliki Zone				
Ab221	-	-	+	+
Ab242	-	-	+	+
Ab273	+	-	+	+
Ab204	+	+	+	+
Ab305	+	+	+	+
Ab316	+	+	+	+
Afikpo zone				
Af011	+	-	+	+
Af72	+	-	+	+
Af113	-	-	+	+
Af178	+	+	+	+
Af189	+	+	+	+
Af198	+	-	+	+
Af199	+	-	+	+
TOTAL Positive	10(76.9%)	5(38.5%)	13(100%)	13(100%)

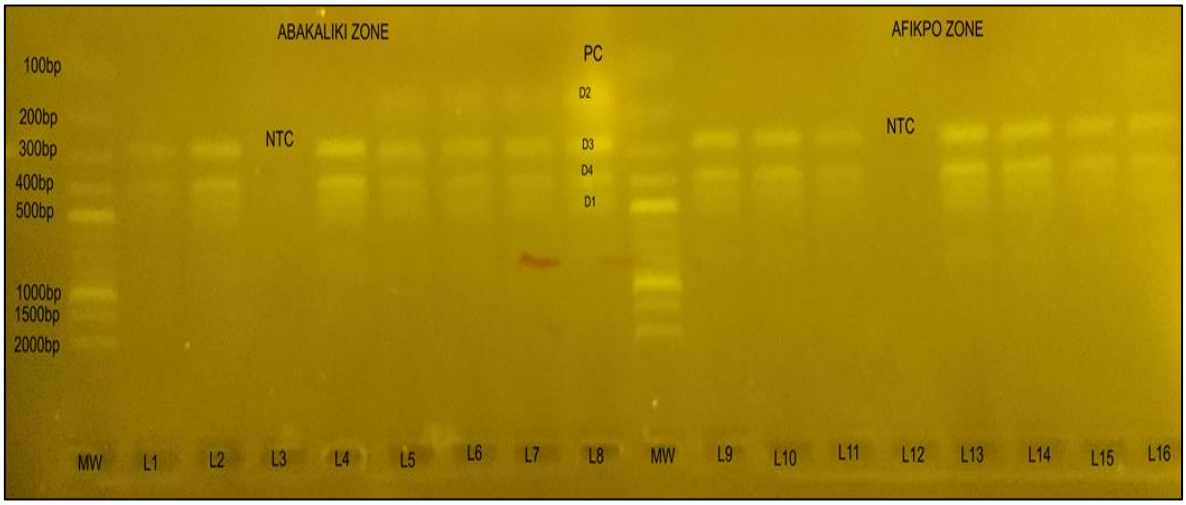

Fig 2 Molecular detection of dengue fever virus serotypes. MW is the 100bp Molecular weight marker. NTC is the Non template control (L3 and L12), PC is the positive control L8. L1 to L7 are the positive samples collected within Abakaliki zone, while L9 to L15 are the positive samples within Afikpo zone. D1, D2, D3, and D4 resolved at 492bp, 123bp, 246bp and 369bp respectively.

Table 5 Co-infection of Dengue Virus and some Other Causes of Febrile Conditions.

	Malaria (%)	HBV (%)	HCV (%)	Enteric fever (%)	Total
IgG	41(25.6)	8 (5)	4 (2.5)	21 (13.1)	74 (46.5)
P-value	0.028	0.737	1.000	0.056	
IgM	21 (13.1)	4 (2.5)	1 (0.6)	18 (11.3)	44 (27.5)
P-value	0.049	0.690	1.000	0.053	
IgG/IgM	15 (9.4)	3 (1.9)	2 (1.3)	22 (13.8)	42 (26.3)
p-value	0.014	0.462	0.924	0.095	

V. DISCUSSION

Dengue fever is a re-emerged arboviral infection that poses a serious public health concern in the past decade and it's documented to have affected millions of individuals worldwide, especially those living within the tropical and subtropical regions (Sameer *et al.*, 2017). The present study evaluated the occurrence of dengue fever virus amongst individuals presenting with febrile cases in Ebonyi State primary and tertiary hospitals. The study enrolled 58.5% females and 41.5% males, 42.6% of the subjects had dengue Immunoglobulins representing 26.8% male and 15.8% females. The *Aedes* mosquitoes responsible for the transmission of dengue virus are diurnal in their biting habit (day bitters) and have a peak biting period around late evenings 5pm to 7pm. This possibly explains why male subjects were more affected because they stay mostly outdoors during this biting peak of the mosquito and are more exposed to the mosquito bite. This present finding agrees with the previous report by Power *et al.*, 2022, they also reported a higher dengue Immunoglobulins in males than in their

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female counterparts. Another separate study reported equal distribution of dengue virus among gender (Bhatt *et al.*, 2013)

Exposure of an individual to the particular serotype of the dengue virus doesn't confer any immunity against exposure to another dengue serotype, this could be the reason why some individuals had both Immunoglobulins G and M concurrently. The major risk factors for the transmission of dengue virus identified in the study were level of education, occupation, type of residence and type of living home. Students especially those at the tertiary level were more exposed to Aedes mosquito bites which accounted to the higher dengue Immunoglobulin level detected among this category of subjects. These students are usually seen wondering about the school premises during late evenings due to some fellowship or other social activities that keep them exposed to the mosquito bite during this *Aedes* biting peak. This present finding slightly disagrees with previous reports by Lwande *et al.*, 2020, they reported that traders were more affected by dengue virus. There is need to educate the students about the transmission mode of dengue virus and best means of avoiding mosquito bites during the day which includes but not limited to proper covering of the anatomical body extremities. Also wearing of dark clothes has been noted as an attractant to *Aedes* mosquitoes (WHO, 2012), therefore students should be adequately informed about that to reduce the biting indices of these mosquitoes. Currently, dengue fever do not have a vaccine and every effort to control the spread of the virus lies heavily on vector control and mosquito bite prevention. The subjects who reside in rural areas and those living in multi yards were more exposed to mosquito bites resulting in the elevated percentage that were infected with dengue virus. This is probably because of indiscriminate disposal of tires and containers which are both favorable breeding habitats for *Aedes* mosquitoes. There is need to address the manner many yards disposes their domestic waste containers and used tires around the environment as they contribute greatly to the proliferation of dengue virus vectors within our communities.

Dengue virus is currently being noted as one of the lead causes of febrile condition of viral origin (WHO, 2023). Although other viruses like hepatitis viruses, bacteria and parasites are also principal causes of febrile condition, this present study equally evaluated the co-existence of some of this agents with dengue virus. The study revealed that *Plasmodium species*, Hepatitis B virus, Hepatitis C virus and *Salmonella species* were also detected in the serum of some dengue seropositive subjects which could result in some complications due to the pathophysiology of the different pathogens. In another separate study by Epelboin *et al.*, 2012, they also found that there was Malaria co-infection among dengue seropositive patients *Baba.et al.*, 2009 reported enteric fever co-infection in dengue seropositive subjects.

VI. CONCLUSION

Dengue virus is actually in existence throughout Ebonyi State and spread of the virus is possible due to the abundance of the potential vectors. Different serotypes were detected but the most prevalent serotypes are DENV-3 and DENV-4.

REFERENCES

[1]. Archibald Lennox K. and Barth Reller L. (2001) "Clinical Microbiology in Developing Countries" Journal of Emerging infectious disease, Vol. 7(2) p.302-305

[2]. Baba M.M., Marie-Francois S., Vorndam A.V., Adeniji J.A., Diop O., Olaleye D. (2009). Dengue virus infections in patients suspected of malaria/ typhoid in Nigeria. Journal of American Science. 5(5):129-134.

[3]. Bhatt Samir, Peter W Gething, Oliver J Brady, Jane P Messina, Andrew W Farlow, Catherine L, Moyes, John M Drake, John S Brownstein, Anne G Hoen, Osman Sankoh, Monica F Myers, Dylan B George, Thomas Jaenisch, G R William Wint, Cameron P Simmons, Thomas W Scott, Jeremy J Farrar, Simon I Hay "The global distribution and burden of dengue". Nature, 2013. 496(7446):p. 504–507.

[4]. Brady, O.J., Peter W. Gething,Samir Bhatt,Jane P. Messina,John S. Brownstein,Anne G. Hoen, Catherine L. Moyes,Andrew W. Farlow,Thomas W. Scott, and Simon I. Hay Refining the global spatial limits of dengue virus transmission by evidence- based consensus. PLOS Neglected Tropical Diseases, 2012. 6(8): p. e1760

[5]. Chen, L.H and Wilson, M.E. (2010). "Dengue and chikungunya infections in travelers". Current Opinion in Infectious Diseases. 23(5): 438– 44.

[6]. Petti Cathy A., Christopher R. Polage, Thomas C. Quinn, Allan R. Ronald and Merle A. Sande (2006). "Laboratory Medicine in Africa: A Barrier to Effective Health Care" Journal of Clinical infectious disease Vol 42 p.377-82.

[7]. Lanciotti R.S, Charles H.C, Duane J.G, Gwong-Jen C and Vance V.A (1992). Rapid detection and typing of Dengue viruses from clinical samples by using reverse Transcriptase-Polymerase Chain Reaction. Journal of Clinical Microbiology Vol 30 (3). p.545-551

[8]. Lwande, O.W., Obanda, V., Lindström, A., Ahlm, C., Evander, M., Näslund, J. and Bucht G. (2020). Globe –Trotting Aedes aegypti and Aedes albopictus: Risk Factors for Arbovirus Pandemics. Vector Borne Zoonotic Disease, 20(2):71-81.

[9]. Mayxay Mayfong, Josée Castonguay-Vanier, Vilada Chansamouth, Audrey Dubot-Pérès, Daniel H Paris, Rattanaphone Phetsouvanh, Jarasporn Tangkhabuanbutra, Phouvieng Douangdala, Saythong Inthalath, Phoutthalavanh Souvannasing, Günther Slesak, Narongchai Tongyoo, Anisone Chanthongthip, Phonepasith Panyanouvong, Bountoy Sibounheuang, Koukeo Phommasone, Michael Dohnt, Darouny Phonekeo, Bouasy Hongvanthong, Sinakhone Xayadeth, Pakapak Ketmayoon, Stuart D Blacksell, Catrin E Moore, Scott B Craig, Mary-Anne Burns, Frank von Sonnenburg, Andrew Corwin, Xavier de Lamballerie, Iveth J González, Eva Maria Christophel, Amy Cawthorne, David Bell, and Paul N Newton (2013), "Causes of non-malarial fever in Laos: a prospective study" Articles Vol 1 p.e42-54

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26mar1698

[10]. Domingues R.B. (2009). Treatment of viral encephalitis. Central Nervous System Agents Medical Chempathology. 9:56-62
[11]. Epelboin L., Hanf M., Dussart P., Ouar-Epelboin S., Djossou F., Nacher M., Carme B. (2012). Is dengue and malaria co-infection more severe than single infections? A -retrospective matchec-pair study in French Guiana. Malaria Journal. 11:142.
[12]. World Health Organization (2024). Dengue global situation.
[13]. World Health Organization (2012). Handbook for clinical management of dengue, Geneva: