

Prospective surveillance of acute febrile illness in two tertiary health facilities in Nigeria, March 2021-September 2022

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Abstract

Background: Most infectious diseases present as acute febrile illness (AFI). Diagnostic gaps in most resource-constrained countries contribute to disproportionate disease burden, antimicrobial misuse, and poor epidemic preparedness. Understanding the etiology and epidemiology of AFI improves early warning systems for epidemic-prone pathogens and supports routine updates of the priority pathogen list. We describe the results of sentinel AFI surveillance in Nigeria.

Methods: Hospital-based sentinel surveillance was implemented at Asokoro District Hospital, Abuja, and Nnamdi Azikiwe University Teaching Hospital, Anambra, from 29 March 2021 to 29 September 2022. Blood specimens from patients ≥ 2 years old with a temperature $\geq 37.5^{\circ}\text{C}$ or self-reported fever for 2-7 days of unknown etiology were tested using AFI TaqMan® Array Card (TAC) assays to detect 36 pathogens.

Results: In this exploratory phase, only 393 of 2,155 specimens were tested due to limited funding and high TAC cost. The majority (62%) of participants tested were adults (246/393), and 52% were female (205/393). The top ten ranked pathogens of the 32 identified were West Nile virus (39%), Zika virus (17%), SARS-CoV-2 (16%), adenovirus (16%), Brucella (8%), Chikungunya virus (7%), Burkholderia (6%), Plasmodium vivax (6%), Plasmodium falciparum (6%), and Crimean Congo Hemorrhagic Fever virus (4%). Coinfections were detected in 45% (178/393) of patients; 24% (94/393) had two pathogens, 21% (84/393) had three or more, and one patient had eight.

Conclusion: This pilot study of AFI etiologies in Nigeria suggests emerging and re-emerging viruses are more common than malaria parasites. Co-infection in nearly half of cases warrants further investigation and supports investment in multiplex testing to address diagnostic gaps. The utility of TAC for exploratory studies in low-income settings may be limited by its prohibitive cost. These findings emphasize the need for continued investment in cost-effective diagnostic technologies and enhanced multi-pathogen surveillance systems, essential to early detection and response to epidemic-prone infections.

Background

Acute febrile illness (AFI) is often misdiagnosed in many low- and middle-income countries (LMIC) due to low diagnostic capacity.¹ In sub-Saharan Africa (SSA), severe-febrile-illness accounted for over sixteen million hospital admissions in 2014.¹ Since fever is a common manifestation of many infectious diseases, diagnostic limitations often lead to over-diagnosis of malaria and typhoid fever, and inappropriate antimicrobial use, contributing to antimicrobial resistance (AMR).^{2,3} Although AFIs may be caused by bacterial, viral, or protozoan pathogens, etiological patterns could vary across populations, geography and time.² Etiological and environmental factors further influence pathogen circulation and transmission. Routine surveillance of AFI provides a mechanism to monitor changing etiologic patterns, guide targeted public health interventions, and strengthen early warning systems for epidemic- and pandemic-prone infectious diseases through multi-pathogen screening. Profiling circulating pathogens among AFI also supports evidence-based updates to surveillance priorities and treatment guidelines, improving empirical antimicrobial use.

The integrated disease surveillance and response (IDSR) strategy was introduced in 1998 and became operational in Nigeria in 2002.⁴ IDSR relies on a priority list of pathogens for routine detection and reporting of

infectious diseases of public health importance.⁵ Samples are collected by the disease surveillance notification officers (DSNOs) from suspected cases and shipped to designated laboratories for testing, usually by single pathogen polymerase chain reaction (PCR). The National Reference Laboratory (NRL) of the Nigeria Centre for Disease Control and Prevention (NCDC) has grown its capacity to perform multi-pathogen PCR using advanced technologies such as the TaqMan Array Cards (TAC), customized to detect a select number of pathogens based on real-time quantitative PCR (qPCR).⁶ Multiple pathogens testing AFI cases enables detection of coinfections and pathogens not routinely tested for in clinical practice. Effective implementation of the IDSR role requires routine updates of the IDSR priority list to reflect current circulating pathogens.^{5,7} Sentinel AFI surveillance can therefore inform more responsive surveillance strategies and contribute to achieving Sustainable Development Goal 3.^{7,8} This study, therefore, describes the etiology and epidemiology of AFI in patients seeking care at two health facilities with wide catchment areas in two regions of the country, to guide effective infectious disease surveillance in Nigeria.

Methods

Study design and setting: The AFI study was a pilot prospective hospital-based active surveillance study. This study was carried out at the Asokoro District Hospital (ADH), Abuja, Federal Capital Territory in northcentral Nigeria, and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State in southeastern Nigeria which both serve as sentinel surveillance sites for influenza and are part of the established national sample referral network that delivers qualifying specimens to the NRL at the NCDC, Abuja. These hospitals serve a diverse population of Nigerians within large catchment areas. ADH serves a population within Abuja city and from neighboring states like Kaduna, Nasarawa, Kogi, Plateau and Niger. NAUTH has a catchment area covering Anambra and neighboring southern states like Enugu, Imo, Delta, Edo, Ebonyi, and Abia.

Sample size determination: Since the study did not involve hypothesis testing, a sample size calculation was not performed. However, for operational purposes, projections for the number of AFI cases were made using DHIS2 malaria testing data from similar public health facilities (with reported data) in the states from which sites were selected because DHIS2 malaria testing data for the selected hospitals were incomplete. Based on these projections, 1600 and 800 cases per month were estimated for the ADH and NAUTH, respectively. Given the existing laboratory capacity limits and understanding that not all AFI cases would meet the inclusion criteria or consent to the study, approximately 7% of the DHIS2 monthly estimate was used for a 15-month study period, i.e., 1,581 and 786 sample sizes for ADH and NAUTH respectively (total = 2,367 patients were expected to be recruited from both study locations).

Study population and participant recruitment: The study population was patients age two years or older who presented with acute fever at outpatient departments/clinics or were admitted to hospital wards in the participating hospitals during the surveillance study period. Inclusion criteria were all patients ≥ 2 years of age, with documented measured rectal or oral temperature ≥ 37.5 °C or a history of self-reported fever that had persisted for 2–7 days, and without an identified localized source of fever. Exclusion criteria were those returning for continuation of treatment of a known cause of fever; those previously enrolled in this AFI surveillance study within the preceding three months; or patients whose chief complaint was trauma or injury.

Data collection, protection, and management: Data collection lasted from March 2021 to September 2022. Demographic and clinical data collected from the two sites were transmitted in real-time to an open data kit (ODK) aggregate server hosted by the NCDC. In the laboratory, the barcode of the specimen was scanned to link the laboratory data to epidemiological data. Laboratory results were entered into a customized TAC platform-specific secure database and the Basic Laboratory Information System (BLIS). Raw data output from the TAC system was read and interpreted by subject matter experts for quality assurance. All data were backed up entirely and daily in a shared folder. Data submitted to the central server was downloaded using ODK briefcase for cleaning and then exported to Stata for analysis. Laboratory data from BLIS were integrated by electronic linkage with the clinical data into one central repository at NCDC. Data on the central servers and Android devices were password-protected and accessible only to relevant study personnel. The data were deidentified before data analysis. Data will be stored securely for a minimum of ten years before destruction.

Sample collection, transport, processing, and testing: Standard safety procedures were followed during the collection, transportation, and processing of all specimens. The samples were tested at the NRL. A specimen requisition form was used to document each enrolled participant's study-specific lab-related data (i.e., the participant's identification number [ID], name, sex, date of fever onset, date of specimen collection, and type of specimens) and accompanied the associated specimens. All collection media and forms were labelled with ID using barcoded, cryogenic stickers resistant to freezing and smearing. Venous whole blood was collected from all enrolled patients by venipuncture using vacutainer syringes and sterile techniques - 5mls in an EDTA container and another 5mls in a plain (serum) container (up to 2.5 ml for children and up to 10 ml for adults). Samples from the ADH were delivered to the NRL within 72 hours of sample collection and stored at 2 to -8°C. Samples from NAUTH were stored at -80°C within 12 hours of sample collection and transported to the NRL within four weeks of collection for testing. Whole blood samples were tested using the TAC with custom acute febrile illness assays to detect up to 36 pathogens (viral, bacterial, and parasitic). The sensitivity and specificity of TAC compared to conventional individual PCR has been shown to be 88% and 99% respectively.⁹ The pathogen list is included in the supplementary file Table S2. The standard operating procedure adhered to for the laboratory processes and TAC testing is included in the supplementary file Annex A.

Data Analysis: Cleaned data were analyzed using Stata 15 software (StataCorp LP, Texas, U.S.A). Descriptive statistics were computed with a focus on the number of specimens tested, laboratory results, and demographic and clinical characteristics of patients. To describe pathogen positivity among the enrolled AFI cases, frequencies and proportions were estimated for each pathogen or group of pathogens. This was done separately for each site, multiple age categories, and dichotomous child and adult categories (2-17 years and $\geq$ 18 years) across AFI TAC (blood-borne pathogens). Crosstabs for pathogens and age were conducted to show variations in frequencies and proportions across the two age categories (children and adults). Seasonal variations for the top ten pathogens were analyzed using data from November 1 to March 31 for dry season, and April 1 to October 31 for rainy season.

Results

Sociodemographic characteristics of participants

The study recruited 2,155 participants (1,282 from ADH (59.5%) and 873 (40.5%) from NAUTH, which was 91% of the target of 2,367 AFI cases from both sites. Of these, the first prospective 393 specimens collected from

February 2021 to February 2022 were tested using the TAC. Of these, 199 samples were from ADH and 194 from NAUTH. Therefore, the results presented here are based on the analysis of data from the first 393 samples.

The general characteristics of the participants tested are as shown in Table 1. Most (63%) participants from both sites were adults). A slight majority (58%, 85/147) of childhood participants were males, while a similar proportion (58%, 143/246) of adult participants were females. There were five pregnant participants from both sites. Approximately 60% of all participants had completed secondary school or higher education. Close to 70% described their occupation as either skilled or unskilled labor, with about 39% in the former category.

Pathogen Prevalence

A total of 32 pathogens were detected from both sites (Figure I). The most prevalent pathogen was West Nile virus (WNV), detected in 39.2% of the samples, followed by Zika virus (17%). Streptococci were the least prevalent at 0.3% (Figure II). The top ten pathogens are shown in Figure II.

Top ten pathogens by demographics

Table 2 shows pathogen positivity in children and adults for the top ten pathogens. All demographics data are shown in Supplementary file Table S1. All age groups were affected by the WNV, but children 2-12 years had the highest incidence, with over half testing positive. The average number of days from the onset of symptoms to sample collection for WNV was 3.16 (SD 2.32). Zika virus affected mainly children 2-12 years of age. Adults 36-59 years were the least affected. Chikungunya was detected in mostly adults 36-45 years and children 12 years or less; teenagers were unaffected. The incidence of *Plasmodium vivax* was highest (17%) among teenagers (13-19 years). No case of *Plasmodium vivax* was detected among those aged 60 years and above. Of the 6% that had the malaria parasite, teenagers were predominantly affected, with 17% of them testing positive for *Plasmodium falciparum*. SARS-CoV-2 was common across the various age groups, but positivity was highest among those 60 years and above, followed by the 46-59 years age category. The highest positivity rate for adenovirus was among the elderly 60 years and above. Though the incidence of Brucella across the age categories was low, about 14% of adults 46-59 years and 10% of teenagers were affected. The highest incidence of Burkholderia was among those aged 46-59 years and teenagers. Less than 10% rate of Crimean-Congo hemorrhagic fever (CCHF) positivity were seen in each of the age groups, with the highest incidence being among the age groups 36-45 years (9%) and 5-12 years (6%).

Sex disparities in positivity were highest for CCHF (male-to-female ratio 3:1), SARS-CoV-2 (male-to-female ratio 1:2), and Chikungunya (male-to-female ratio 2:1).. Two of the five pregnant women tested positive for WNV, one for Zika virus, and one for chikungunya.

Table 2 shows pathogen proportions in children and adults (2-17 and ≥ 18 years) for the top ten pathogens. Only four out of the top ten pathogens with the highest proportions showed appreciable differences in incidence between children and adults. While WNV (50% versus 33%) and Zika virus (20% versus 9%) showed higher rates among children 2-17 years, SARS-CoV-2 (13% versus 18%) and denovirus (8% versus 21%) had adult preponderance. The remaining six pathogens with the lowest cumulative proportions were similar between children and adults.

Table 2: Pathogens disaggregated by child and adult age categories in study sites

Pathogens	Total N (%)	Child (2-17 years)		Total N (%)	Adult (≥18 years)	
		ADH n (%)	NAUTH n (%)		ADH n (%)	NAUTH n (%)
Total	147 (37.4)	103 (70.1)	44 (29.9)	246 (62.6)	96 (39.0)	150 (61.0)
West Nile	74 (50.3)	53 (51.5)	21 (47.7)	80 (32.5)	30 (31.2)	50 (33.3)
Zika virus	29 (19.7)	25 (24.3)	4 (9.1)	23 (9.3)	6 (6.2)	17 (11.3)
SARS-CoV-2	19 (12.9)	8 (7.8)	11 (25.0)	45 (18.3)	5 (5.2)	40 (26.7)
Adenovirus	12 (8.2)	2 (1.9)	10 (22.7)	52 (21.1)	0 (0.0)	52 (34.7)
Brucella	13 (8.8)	7 (6.8)	6 (13.6)	18 (7.3)	8 (8.3)	10 (6.7)
Chikungunya	13 (8.8)	11 (10.7)	2 (4.5)	15 (6.1)	12 (12.5)	3 (2.0)
Burkholderia	11 (7.5)	6 (5.8)	5 (11.4)	14 (5.7)	7 (7.3)	7 (4.7)
P. vivax	8 (5.4)	5 (4.9)	3 (6.8)	11 (4.5)	6 (6.2)	5 (3.3)
P. falciparum	9 (6.1)	4 (3.9)	5 (11.4)	14 (5.7)	8 (8.3)	6 (4.0)
CCHF	6 (4.1)	4 (3.9)	2 (4.5)	11 (4.5)	9 (9.4)	2 (1.3)

Coinfections among AFI cases

Among the 70.7% (278/393) of AFI cases that had at least one pathogen detected, 64% (178/278) had coinfections (Table 3). There were no significant differences in symptoms and signs between cases with single infections and those with coinfections. While more infected cases were detected during the dry season (62%, 172/278), the prevalence of co-infections was significantly higher during the rainy season compared to the dry season (77.4% versus 55.8%; $p < 0.001$). The majority of those with coinfections had two pathogens. The highest pathogen burden consisted of eight pathogens detected in one sample. The most common co-infection combinations were WNV and Zika (11.5%), SARS-CoV-2 and Adenovirus (10.2%), Brucella and Burkholderia (5.6%), and Zika and Chikungunya (3.8%). There was no significant difference in co-infection status between adults (≥18 years) and children (<18 years).

Table 3: Coinfection status by symptom and season

Variable	Co-infection Status			p-value
	Total N (%)	Single n (%)	Co-infected n (%)	
Total	278 (100.0)	100 (36.0)	178 (64.0)	
Cough	98 (100.0)	32 (32.7)	66 (67.3)	0.39
Runny nose	39 (100.0)	17 (43.6)	22 (56.4)	0.28
Diarrhoea	20 (100.0)	7 (35.0)	13 (65.0)	0.93
Vomiting and Nausea	34 (100.0)	10 (29.4)	24 (70.6)	0.39
Abdominal pain	40 (100.0)	12 (30.0)	28 (70.0)	0.40
Rash	8 (100.0)	3 (37.5)	5 (62.5)	0.93
Headache	139 (100.0)	54 (38.8)	85 (61.2)	0.32
Arthralgia	19 (100.0)	7 (36.8)	12 (63.2)	0.93
Muscle pain	24 (100.0)	9 (37.5)	15 (62.5)	0.87
Jaundice	7 (100.0)	1 (14.3)	6 (85.7)	0.23
Dry season	172 (100.0)	76 (44.2)	96 (55.8)	<0.001
Rainy season	106 (100.0)	24 (22.6)	82 (77.4)	<0.001

Seasonal variations in the prevalence of top AFI pathogens

Combined data from both sites showed significant seasonal variation for all major pathogens except **Brucella** ($p=0.347$). **WNV** ($p<0.001$), **Zika virus** ($p=0.016$), **chikungunya** ($p<0.01$), **Burkholderia** ($p=0.011$), and **CCHF** ($p=0.014$) were more prevalent in the **dry season (November to March)**, whereas **adenovirus** ($p<0.001$), **SARS-CoV-2** ($p<0.001$), ***P. falciparum*** ($p<0.01$), and ***P. vivax*** ($p<0.001$) predominated in the **rainy season (April to October)**.

Season-specific patterns by site (Table 4) showed significant variation for only three pathogens in **NAUTH**, with **WNV** higher in the dry season and **SARS-CoV-2** and **adenovirus** higher in the rainy season. In **ADH**, all pathogens except **adenovirus**, **Brucella**, and **Burkholderia** varied significantly by season. **WNV**, **Zika**, **chikungunya**, and **CCHF** peaked in the dry season, while **SARS-CoV-2**, ***P. falciparum***, and ***P. vivax*** were more common in the rainy season. Overall co-infection prevalence among all cases was higher in the **rainy season** than the dry season (**51.9% vs. 40.9%; $p=0.001$**), though site-level analysis showed the opposite pattern in **ADH** (higher in the dry season).

Table 4: Seasonal variations in pathogen prevalence in study sites

Variables	ADH				NAUTH			
	Seasonality				Seasonality			
	Total N (%)	Dry Season n (%)	Rainy Season n (%)	c2 (P- value)	Total N (%)	Dry Season n (%)	Rainy Season n (%)	c2 (P- value)
Total	199 (100.0%)	107 (100.0%)	92 (100.0%)		194 (100.0%)	128 (100.0%)	66 (100.0%)	
West Nile	83 (41.7%)	60 (56.1%)	23 (25.0%)	<0.001	71 (36.6%)	65 (50.8%)	6 (9.1%)	<0.001
Zika Virus	31 (15.6%)	23 (21.5%)	8 (8.7%)	0.013	21 (10.8%)	16 (12.5%)	5 (7.6%)	0.30
SARS-CoV-2	13 (6.5%)	3 (2.8%)	10 (10.9%)	0.022	51 (26.3%)	10 (7.8%)	41 (62.1%)	<0.001
Adenovirus	2 (1.0%)	1 (0.9%)	1 (1.1%)	0.91	62 (32.0%)	9 (7.0%)	53 (80.3%)	<0.001
Brucella	15 (7.5%)	11 (10.3%)	4 (4.3%)	0.11	16 (8.2%)	10 (7.8%)	6 (9.1%)	0.76
Chikungunya	23 (11.6%)	22 (20.6%)	1 (1.1%)	<0.001	5 (2.6%)	2 (1.6%)	3 (4.5%)	0.21
Burkholderia	13 (6.5%)	10 (9.3%)	3 (3.3%)	0.083	12 (6.2%)	11 (8.6%)	1 (1.5%)	0.052
P-Vivax	11 (5.5%)	1 (0.9%)	10 (10.9%)	0.002	8 (4.1%)	3 (2.3%)	5 (7.6%)	0.082
P-Falciparum	12 (6.0%)	2 (1.9%)	10 (10.9%)	0.008	11 (5.7%)	5 (3.9%)	6 (9.1%)	0.14
CCHF	13 (6.5%)	12 (11.2%)	1 (1.1%)	0.004	4 (2.1%)	3 (2.3%)	1 (1.5%)	0.70
Co-infection	87 (43.7%)	56 (52.3%)	31 (33.7%)	<0.001	91 (46.9%)	40 (31.2%)	51 (77.3%)	<0.001

Clinical signs and symptoms of AFI patients in NAUTH and ADH

Symptoms and signs associated with AFI cases in NAUTH are shown in Figure V. Among these patients, headache (44%) and cough (31%) were the most common symptoms experienced. No case of unexplained bleeding occurred. Figure VI shows the distribution of signs and symptoms experienced by patients with AFI in ADH. Headache was the most common symptom. By systems, signs & symptoms were mainly respiratory (69%), neurological (59%), gastrointestinal (53%), and musculoskeletal (34%) (Figure VI). Similar to NAUTH, no case of unexplained bleeding was observed. No significant difference in clinical signs and symptoms was observed based on co-infection status. While jaundice was higher among patients with coinfections (, this was not statistically significant.

Discussion

Analysis of the first 393 samples detected 32 distinct pathogens. The ten most frequent pathogens were WNV, Zika virus, SARS-CoV-2 virus, adenovirus, Brucella, chikungunya virus, Burkholderia, *P. vivax*, *P. falciparum*, and CCHF virus. Coinfection were common, affecting nearly half of patients, with some harboring multiple.

This study revealed an unusual pattern of trending pathogens among AFI cases in Nigeria. The most frequent presumptive diagnosis for AFI in the clinical settings in Nigeria is malaria, a disease that is endemic in the country and caused by the female *Anopheles* mosquito-borne protozoa, *P. falciparum*.¹⁰ However, this study found approximately 6% prevalence of malaria. Irrational prescriptions of antimalarials for febrile cases in outpatient clinics, even in tertiary health facilities, remain prevalent in Nigeria.¹¹ WNV, a flavivirus transmitted by the *Culex pipiens* mosquito, had the highest prevalence of over 39% in this study. This was over six times that for malaria. This pattern may also reflect care-seeking behavior, with patients self-treating malaria prior to presentation.

WNV is not listed in Nigeria's IDSR priority disease list, which includes infectious diseases that are the principal causes of morbidity and mortality.⁵ Though only less than one percent of WNV infections are associated with severe disease, the AFI that they present with can cause significant hospital visits, school absenteeism, and loss of productive work days.³ It can also be associated with neurological diseases like the Guillain-Barré syndrome.¹² The higher prevalence observed in this PCR-based study compared with prior serologic studies may reflect the ability of PCR to detect active infections before antibody development..³ PCR is the gold standard for current infection and can detect pathogens even before antibodies are formed,. This could be the reason for the higher prevalence in our study. Nevertheless, the IgM is also a sensitive indicator of antibody response to a recent infection.¹³ The current rise in the prevalence of WNV could be a result of ecological transitions due to climate change that have led to the proliferation of arboviruses and their mosquito vectors.¹⁴ The tropical climate in Nigeria, with significant rainfall each year, provides a suitable environment for mosquitoes to breed and thrive, and could contribute to the spread of arboviruses. Entomological studies to ascertain the current distribution of *Culex* mosquitoes (and other mosquito species) could guide targeted One Health interventions against WNV.

Six out of the ten top pathogens in this study were viruses. This predominance of viral pathogens is consistent with prior studies of AFI in SSA..² In a prospective study in clinics in Uganda, the chikungunya virus constituted about 60% of non-malaria pathogens identified in AFI patients.¹⁵ Due to the self-limiting nature of most viruses, it is easy to misdiagnose viral infections as malaria or bacterial infections and for these to be validated when the fever stops following the administration of antimalarials or antibiotics. This could lead to the proliferation of AMR, particularly in African settings with limited diagnostic capabilities.

Zika virus prevalence was up to 17% in our study, placing the virus as the second most prevalent pathogen in AFI patients from both sites. This aligns with findings from studies in other Zika-endemic regions such as Brazil, where the prevalence of the disease during outbreak years ranged between 10% and 25%.¹⁶ This flavivirus transmitted by the *Aedes aegypti* mosquito has had wide geographic spread globally, even before the emergence of SARS-CoV-2.¹⁷ A higher proportion of children 2-17 years in this study tested positive for WNV and Zika virus compared to adults. While 50% and 20% of the children were affected by WNV and Zika virus, respectively, adult positivity rates for the viruses were 33% and 9%. A 2024 systematic review of Zika seroprevalence among asymptomatic individuals found a pooled global estimate of 21% (95% CI

16.1%-26.4%).¹⁸ This global estimate of Zika immunoglobulin G (IgG) includes endemic countries' values and represents past exposures to the virus.¹⁸ African countries have shown much lower rates. The seroprevalence of Zika antibodies among AFI cases in Uganda was less than 1%.¹⁹ Seroprevalence in our study population could be higher than the observed PCR results and should be investigated in future studies. Furthermore, Zika virus nucleic acid can remain in the blood for over three months.²⁰ It is, therefore, uncertain whether the etiology of AFI in this study was linked to Zika virus positivity. However, detecting the virus in up to 17% of the study population could imply that the virus is circulating in the country and demands sentinel surveillance to guide public health response, especially among women of reproductive age. Future serologic studies to determine the actual burden of Zika in Nigeria are recommended.

The indigenous transmission of *P. vivax* in Africa has been a subject of controversy. With *P. falciparum* being the predominant cause of malaria in SSA, much attention has not been paid to *P. vivax* due to the rare expression of the Duffy antigen in the red blood cells of indigenous African populations.²¹ The Duffy antigen is purported to be the only receptor for the protozoa. Recent studies, however, have challenged this notion, demonstrating transmission of *P. vivax* among Duffy positive and negative persons in SSA.²¹ While up to 10% of the global burden of *P. vivax* is attributed to southern Africa, less than 1% is said to occur in West and Central Africa.^{21,22} Microscopically, *P. vivax* has been misdiagnosed as *P. ovale*. Low parasitemia characteristic of *P. vivax* also limits diagnosis by microscopy, and PCR could be more specific in detecting the pathogen.²³ A prospective hospital-based study detected *P. vivax* infections by PCR in 8.1% of 259 febrile patients in southern Rwanda from 2018 to 2019, similar to the 6.1% positivity rate in our study.²⁴ All positive cases in Rwanda were Duffy positive. Despite having a similar clinical presentation, the infection is considered more benign than *P. falciparum*. Persistence of the parasite as hypnozoites in the liver, peculiar to *P. vivax*, promotes relapses of malaria that challenge eradication efforts and cause chronic anaemia.^{21,23} The parasite is not currently on Nigeria's IDSR disease priority list. A seroprevalence study of non-plasmodium malaria IgG in 31,234 children aged 0-14 years in Nigeria, conducted during the 2018 nationwide HIV survey showed zero sero-positivity for *P. vivax*.²⁵ This contradicts the 6% prevalence in our PCR-based study. While PCR is more likely to detect active infections, further studies of *P. vivax* infection in Nigeria are required to fully understand the epidemiology of this parasite and any emerging patterns in the country.

Coinfections were common in this study, affecting 64% of cases, and more prevalent during the rainy season. Seasonal variations likely reflect interactions among host immunity, behavior and environmental factors with heat and humidity increasing exposure to multiple vectors.²⁶ Clinical data did not include comorbidities or immune status, which may influence susceptibility to multiple infections. An increasing burden of coinfections, particularly in African populations, has been reported, including evidence of multiple-pathogen exposure among children.²⁷ The impact of coinfections on pathogen pathogenicity remains poorly understood. However, increased severity and hospitalization risk have been observed in pediatric dengue-influenza coinfections.²⁸ High pathogen burden is associated with poorer outcomes, including increased morbidity and mortality.^{29,30} The high rate of coinfections observed supports the need for multi-pathogen testing in infectious disease surveillance. However, more affordable approaches are required, as demonstrated by the inability to complete testing using TAC due to high costs.

The seasonal variations in pathogen prevalence observed in the study reflected common patterns for mosquito-borne protozoans, such as *P. falciparum* and *P. vivax*, with high transmission rates during warm, rainy seasons. However, the prevalence of arboviruses such as WNV, Zika, chikungunya, and CCHF during the dry season is contrary to the usual trends for the mosquito and tick vectors..³¹ These patterns may reflect temperature-driven transmission dynamics rather than rainfall alone, particularly across Nigeria's diverse climatic zones.^{32,33} A sub-analysis of site-specific data for the larger sample could provide more insight into the seasonal variations of the pathogens. The finding of higher coinfections in rainy season is not unexpected. Multiple arthropod breeding is highest in warm, humid climates, increasing the risk of multi-pathogen transmission.³¹

The clinical presentations of AFI in the study showed a constitutional non-specific pattern. This is a usual pattern for most AFI cases in the country. Besides headache, most AFI patients in both sites had respiratory symptoms. Antibiotic use for respiratory infections is common in SSA. A cross-sectional study of 856 under-five children with acute respiratory infection in northern Uganda showed over 60% antibiotic utilization.³⁴ Non-specific symptoms increase the possibility of misdiagnosis when clinical diagnosis is based solely on signs and symptoms. Laboratory diagnosis as part of the clinical care of patients with AFI is important to reduce misdiagnosis, improve rational antimicrobial use, and reduce AMR.

Study Limitations

The study provides valuable insights into the circulation of pathogens among patients from two tertiary health facilities with catchment areas spanning northern and southern Nigeria. Some limitations of this analysis are the small sample size and lack of national representativeness. Also, the observed test positives are yet to be confirmed by individual real-time PCR. Nevertheless, the 88% sensitivity and 99% specificity of TAC compared to individual PCR, as published in literature,⁹ improve confidence in the validity of the observed findings. The study does not provide insight into the time of exposure to these pathogens. While some detected pathogens may be responsible for the AFI at the time of enrolment, others may be chronic asymptomatic infections not implicated in the acute symptoms. This limits the ability to estimate the specific etiology of the AFI, especially in cases with coinfections. Further research enquiries are needed to understand risk factors for coinfections in AFI cases. Also, phylogenetic studies can reveal temporal characteristics and transmission dynamics of the detected pathogens.

Conclusions

Routine surveillance for pathogens implicated in the etiology of AFI could guide an evidence-based review of the IDSR pathogen list. Epidemic- and pandemic-prone infectious diseases can be tracked using effective AFI surveillance systems. This study has yielded some unexpected results in trending pathogens that could trigger a review and update of the IDSR pathogen list in Nigeria and guide clinical practice. Though the TAC technology is effective, its use in resource-scarce settings for routine AFI surveillance could be unrealistic due to relatively prohibitive costs. The top ten pathogens identified in this study could guide more targeted testing of the remaining samples using a more affordable technology like Multiplex PCR.

Abbreviations

AFI: Acute febrile illness

TAC: Taqman Array Card

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

LMIC: Low- and middle-income country

SSA: Sub-Saharan Africa

AMR: Antimicrobial resistance

IDSR: Integrated disease surveillance and response

DSNO: Disease surveillance and notification officer

PCR: Polymerase chain reaction

NRL: National Reference Laboratory

NCDC: Nigeria Centre for Disease Control and Prevention

ADH: Asokoro District Hospital

NAUTH: Nnamdi Azikiwe University Teaching Hospital

DHIS: Digital Health Information System

ODK: Open Data Kit

BLIS: Basic Laboratory Information System

ID: Participant's identification number

EDTA: Ethylenediaminetetraacetic acid

NHREC: National Health Research Ethics Committee

WNV: West Nile Virus

SD: Standard deviation

CCHF: Crimean Congo haemorrhagic fever

CI: Confidence interval

HIV: Human immunodeficiency syndrome

US CDC: United States Centers for Disease Control and Prevention

FMOH: Federal Ministry of Health

WHO: World Health Organization

RNA: Ribonucleic acid

Declarations

Ethics approval and consent to participate: The study protocol, informed consent and assent forms, and other study instruments administered to the participants were reviewed and approved by the National Health Research Ethics Committee (NHREC) of Nigeria (approval number: NHREC/01/01/2007-14/03/2023C). Modifications to the protocol were made only after obtaining written NHREC approval. This study upheld the confidentiality and privacy of the participants. Written informed consent and assent were obtained from the participants before recruitment in line with the approved study protocol. A token refund was provided to participants to support out-of-pocket payments for routine investigations. Participants received routine care for their presenting illnesses from clinicians at the study sites.

Consent for publication: Not applicable

Availability of data and materials: Data is available from the Nigeria Centre for Disease Control and Prevention upon reasonable request.

Competing interests: None declared

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Authors' contributions: Conceptualization: CLO & MK

Data collection: CN, AI, SCM, IO, CDU, EO, CLO

Specimen collection and laboratory testing: OA, MAA, IPA, CN, SK, AAO, OB

Data cleaning, analysis, visualization, and interpretation: NA, EO, CLO, FH

Initial draft of manuscript: CLO

Review of drafts and approval of final draft: All authors

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

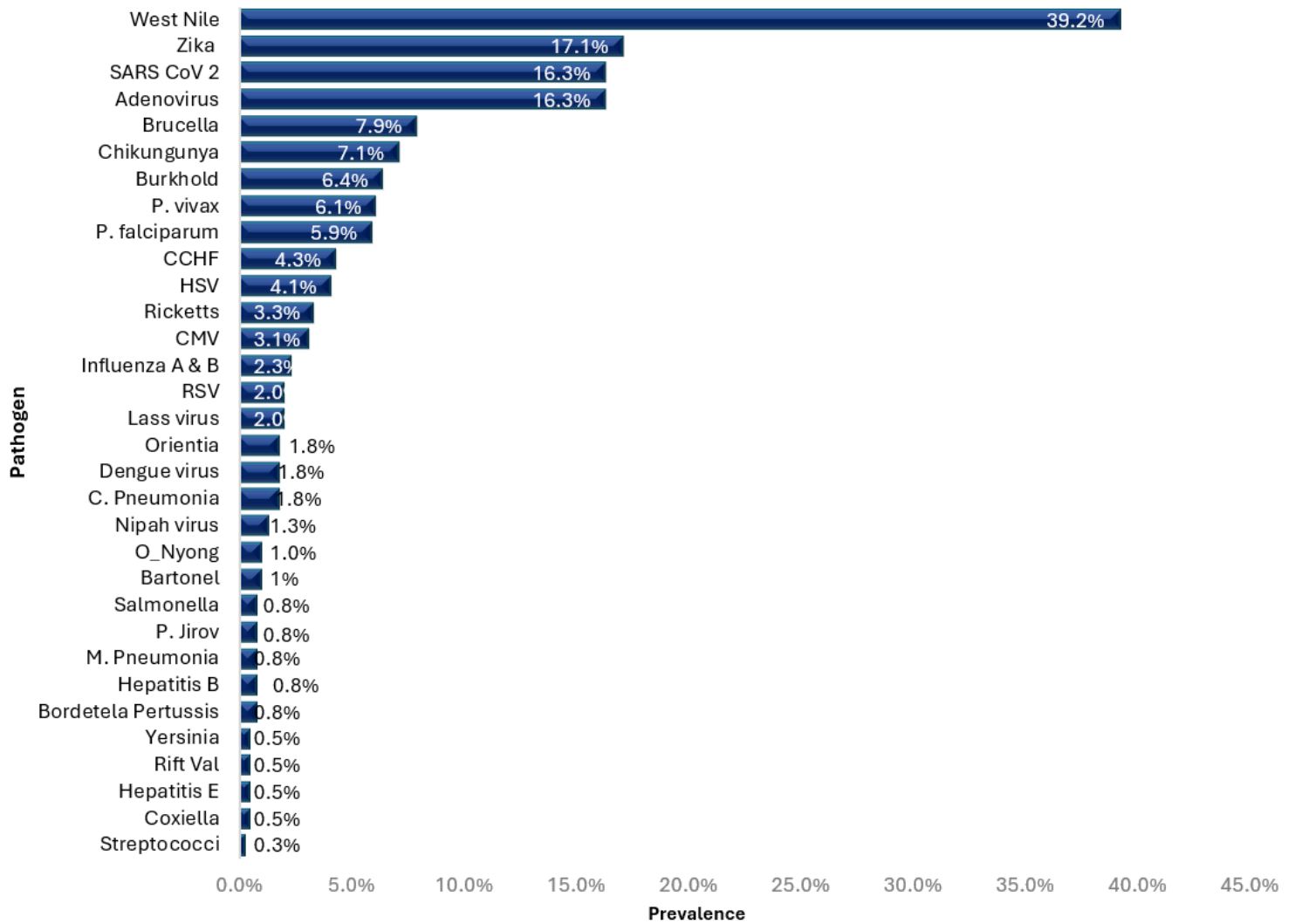


Figure 1

Prevalence of pathogens among patients with AFI in ADH and NAUTH, Nigeria, 2021-2022

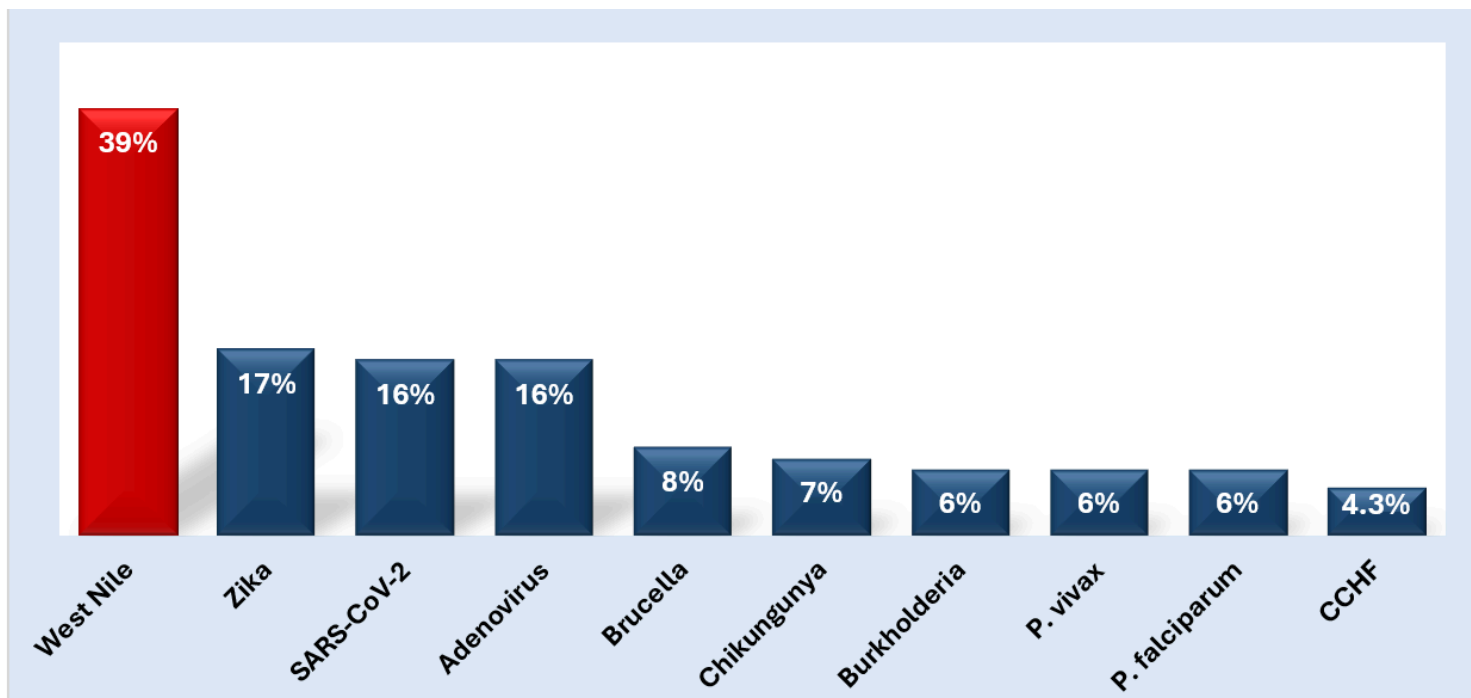


Figure 2

Top ten pathogens detected from both sites

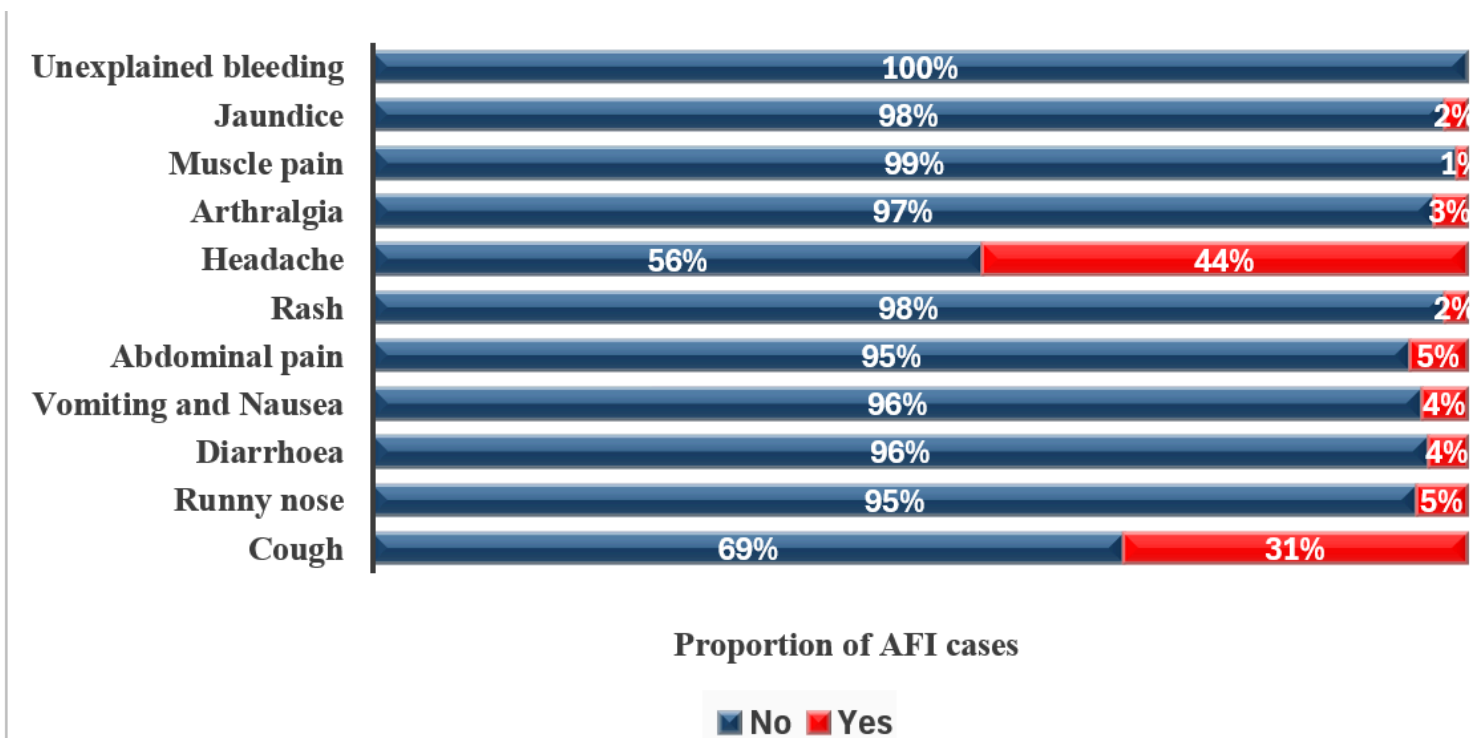


Figure 3

Clinical signs & symptoms of AFI patients in NAUTH, Nnewi, Nigeria, 2021-2022

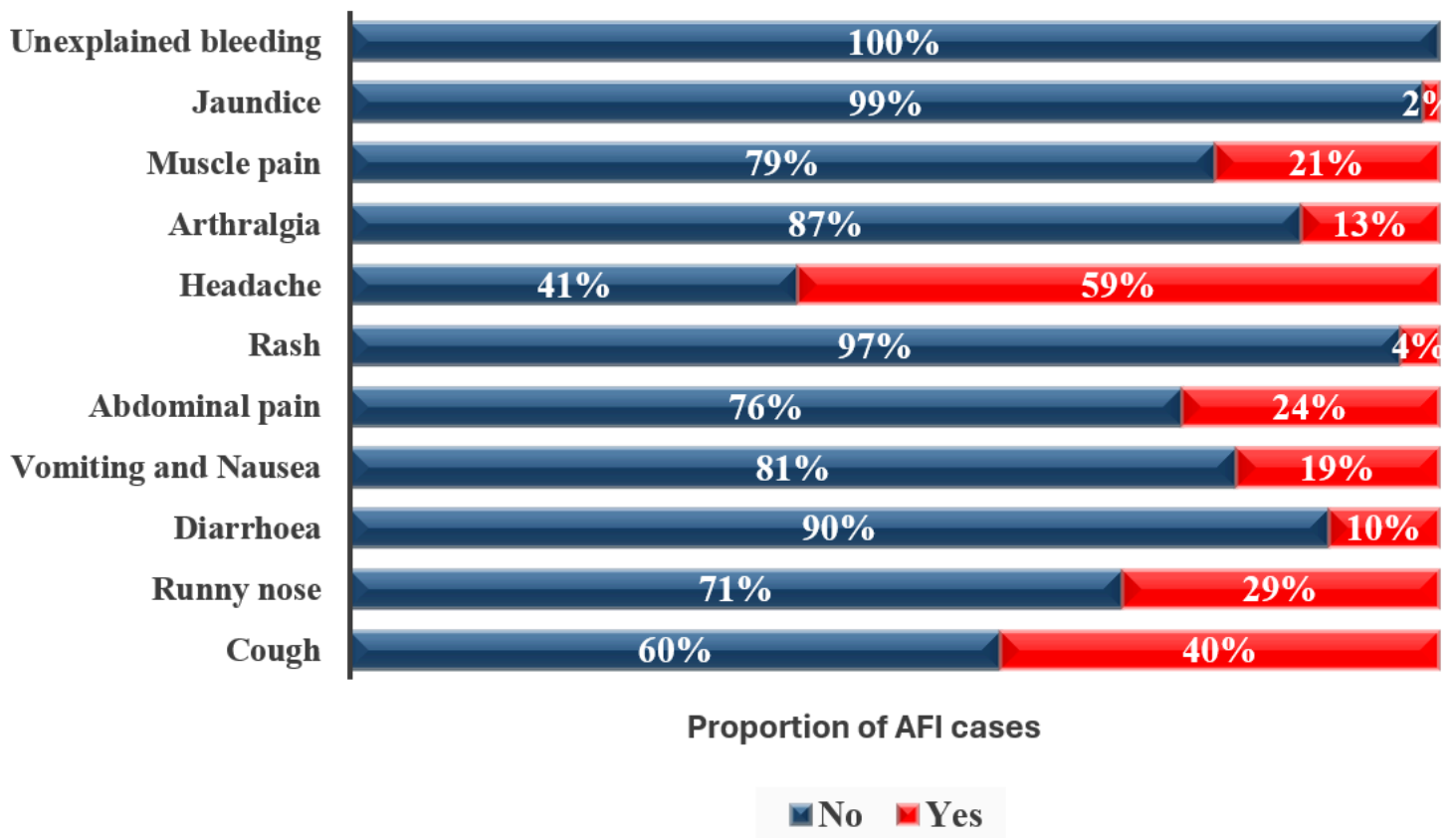


Figure 4

Clinical signs & symptoms experienced by patients with AFI in ADH, Abuja, Nigeria, 2021-2022

Supplementary Files

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