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ORIGINAL RESEARCH

Influenza virus detected in Patients screened for *Mycobacterium tuberculosis* and HIV

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Abstract:

Introduction: A total of 1.3 million people died from tuberculosis (TB) in 2022 (including 167,000 individuals with HIV) with a large proportion of them from sub-Saharan Africa. Unfortunately, there are paucity of data on influenza virus in both tuberculosis and HIV patients in Nigeria.

Aims: Hence this study was designed to investigate influenza virus in patients undergoing screening for *Mycobacterium tuberculosis* and HIV in Lagos, Nigeria.

Materials and Methods: A total number of 400 samples were collected from four (4) different health facilities including three (3) general hospitals and one (1) tertiary institution health center. The population consists of 32.8% (131/400) males and 67.2% (269/400) females of which 11% (30/269) were pregnant. Their age ranged from 7 to > 65 years. Nasopharyngeal samples were extracted for influenza RNA with QIAamp mini kit followed by RT-PCR amplification. TB and HIV tests were performed as part of clinical management.

Results: Of the total patients tested, 26.8% (90/336) were HIV positive, 2.5% (10/400) had tuberculosis and 1.8% (7/400) were asthmatic. Furthermore, the result showed a 2.3% (9/400) low molecular prevalence of influenza A virus out of which 66.7% (6/9) were females. Interestingly, out of the nine (9) influenza A virus-positive patients, 66.7% (6/9) had HIV, 44.4% (4/9) had TB and 33.3% (3/9) were co-infected with TB and HIV. Ikorodu General Hospital accounted for 55.6% (5/9) of the molecular prevalence of influenza while 44.4% (4/9) was recorded in Badagry General Hospital.

Conclusion: In conclusion, this study is the first report on molecular detection of influenza virus in TB and HIV patients in Nigeria. The co-infection of influenza virus in TB and HIV patients underscores the need for national surveillance and provision of influenza vaccines and antiviral drugs for the affected individuals.

Keywords: Influenza, tuberculosis, HIV, RT-PCR, Molecular prevalence, Lagos.

1. INTRODUCTION

HIV/AIDS and tuberculosis remain the two (2) most endemic and killer infectious diseases ravaging the entire world. Despite exceeding a century of discovery, HIV/AIDS, tuberculosis (TB), and influenza remain formidable threats to global health, disproportionately impacting low- and middle-income countries (LMICs), particularly sub-Saharan Africa. While *Mycobacterium tuberculosis* was identified in 1882, more than a century before the discovery of human immunodeficiency virus (HIV) in 1983, the earliest documented influenza epidemic dates back to 412 BC, as recorded by Hippocrates. This significant gap in time underscores the extensive historical presence of tuberculosis and influenza before the isolation of the influenza virus from humans by Smith *et al*. [1].

Influenza virus, a respiratory aetiology primarily transmitted through airborne contact with virus-laden secretions from infected individuals is responsible for both seasonal epidemics and occasional pandemics, resulting in approximately one (1) billion infections annually across all demographics, with up to 650,000 associated deaths [2].

HIV infection profoundly compromises the immune system, rendering individuals susceptible to secondary infections such as influenza. This susceptibility is exacerbated by the observation that contracting influenza while HIV-positive can lead to a significant increase in HIV viral load, potentially affecting overall health and treatment efficacy [3].

The co-infection of tuberculosis (TB) and HIV remains a persistent public health challenge. In Nigeria, influenza virus has been associated with a considerable burden of morbidity in the general population, although the incidence of excess mortality remains unknown due to inadequate surveillance [4]. Nigeria carries one of the highest burdens of TB globally, with a prevalence rate of 318 per 100,000 population, and it is home to 9% of all people living with HIV worldwide [5]. However, the proportion of patients with TB/HIV co-infection in Nigeria is notably high, standing at 19.1% [6].

Influenza infection has the potential to accelerate the transition from latent *Mycobacterium tuberculosis* infection to active tuberculosis disease and may influence the clinical manifestation of tuberculosis. Additionally, there is a likelihood that influenza infection could worsen pulmonary tuberculosis [7]. Both influenza and tuberculosis impair host immune responses. In particular, influenza can compromise T-cell immunity and diminish innate immune defenses against subsequent bacterial infections [8]. Moreover, individuals diagnosed with pulmonary tuberculosis (PTB) might face heightened susceptibility to severe influenza illness and mortality owing to immunosuppression and chronic lung conditions including extensive pulmonary destruction, chronic lung abscess, and chronic obstructive pulmonary disease (COPD). Ecological investigations and mathematical modeling based on epidemiological data indicate a rise in influenza or severe influenza-related complications among individuals with PTB during influenza pandemics [9].

Tuberculosis (TB) and influenza embody significant global health challenges. Notably, influenza infections elevate the susceptibility to secondary bacterial infections, while diminished immunity associated with *Mycobacterium tuberculosis* infection heightens vulnerability to influenza [10]. In 2022 alone, approximately eleven (11) million individuals worldwide, comprising about 6 million men, 3.5 million women, and over one (1) million children were down with TB resulting in the death of 1.3 million individuals, including 167,000 people co-infected with HIV/AIDS, making it the second leading deadly aetiology after SARS-COV-2 and above HIV/AIDS [11].

Mycobacterium tuberculosis is carried by an estimated 2-3 billion individuals globally, with about 10% proportion progressing to active disease [12]. In 2009, the World Health Organization (WHO) raised concerns about the substantial number of deaths among patients with chronic respiratory conditions, highlighting the potential impact of influenza on individuals with active TB [13].

Furthermore, immune system activation in response to TB has been linked to increased HIV replication aggravating disease severity and transmissibility [14]. The co-occurrence of HIV infection and TB

constitutes a significant burden on public health systems worldwide particularly in sub-Saharan Africa where shared risk factors contribute to high rates of co-infection [11]. HIV infection in particular weakens the immune system substantially increasing the risk of TB infection and reactivation of latent TB infection into active disease [14].

By the end of 2014, an estimated 36.9 million people were living with HIV, with Sub-Saharan Africa bearing the highest burden of 25.8 million cases [5]. HIV infection also significantly elevates the risk of TB, with infected individuals being about 20 times more likely to develop TB compared to uninfected individuals [11]. Given these interconnected challenges, this study aims to investigate the molecular prevalence of influenza among patients undergoing screening for *Mycobacterium tuberculosis* and HIV in Lagos, Nigeria.

2. MATERIALS AND METHODS

2.1 Study design and location

A prospective and cross-sectional hospital-based epidemiological surveillance study was premeditated, involving chest clinics in public health centers managed by the Lagos State Government with support from NGOs including the AIDS Prevention Initiative of Nigeria (APIN) in different parts of the State cutting across three (3) divisions, comprising three (3) General hospitals and one (1) higher institution health center including Alimosho, Badagry, and Ikorodu General Hospitals, and the Lagos State University health center in Ojo from September 2019 to February, 2020. Both primary and secondary healthcare institutions were selected in different locations to promote community surveillance and rural engagement at all levels in line with the United Nations Sustainable Development Goals. Convenience sampling was carried out while a purposeful selection of centers was premised on the availability of diagnostic facilities where patients attending such chest clinics are screened for TB and HIV at no cost or a subsidized rate based on the availability of funds and resources provided by the sponsoring agency. At the same time, they are also enrolled for free treatment once tested positive for either TB or HIV. Socio-demographic data including age, gender, and underlying conditions were appropriately collected with questionnaires.

2.2 Inclusion and exclusion criteria

Patients having influenza-like symptoms of cough or sore throat reported cases of fever or pyrexia, and difficulty in breathing including suspected and laboratory-diagnosed outpatients attending chest clinics for respiratory tract complaints/pulmonary-related disorders willing to undergo tuberculosis and HIV testing were recruited for the study. The inclusion criteria also covered patients with underlying health and special conditions including pregnancy. Patients who did not meet the study inclusion criteria were excluded.

2.3 Sample collection and handling

Nasopharyngeal swabs using sterile swab sticks were aseptically and carefully collected into sterile cryovials containing approximately 1ml of PBS-glycerol viral transport medium (VTM) by purposive sampling from patients attending the different health facilities. The PBS-glycerol transport medium was prepared aseptically with precaution in a biological safety cabinet class II. Ten (10) PBS tablets were partially and evenly dissolved with distilled water before making up the PBS solution to 1L. The solution was then autoclaved at 121°C for 15 minutes. This was allowed to cool before mixing with 1L sterile glycerol to make up a 1:1 PBS-glycerol solution. Antibiotics were added to the PBS-glycerol solution including benzylpenicillin (2×10^6 IU/litre), gentamicin (250 mg/litre), nystatin (0.5×10^6 IU/litre), ofloxacin hydrochloride (60 mg/litre), polymyxin B (2×10^6 IU/litre), streptomycin (200 mg/litre), and sulfamethoxazole (0.2 g/litre). Each antibiotic was measured using a well-calibrated electrical weighing balance and properly mixed for homogeneity. Three (3) different 2 ml aliquots of the prepared PBS-glycerol VTM were dispensed into sterile cryovials for 24 hours to check for turbidity and growth of contaminants. About 1-2 mls were eventually distributed into new cryovials and stored in the refrigerator until sampling. Collected samples were kept in sample coolers stacked with ice packs before being conveyed to the laboratory for storage at -20 ^{o}C until analysis.

2.4 Laboratory Analyses

2.4.1 RNA extraction

Viral RNA was extracted from 140µl of the nasopharyngeal swab samples using QIAamp viral RNA extraction and purification kit (Qiagen Inc, Valencia, CA, USA). The extraction was performed following the manufacturer's instructions as previously described by [4].

2.4.2 Preparation of Master Mixture and PCR Amplification

The Nasopharyngeal samples' extracts were analysed at the Virology Research Laboratory, Central Research Laboratory, College of Medicine of the University of Lagos. A One-Step Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) was performed for the detection of the Influenza A matrix gene. SCRIPT One-Step RT-PCR kit designed for performing highly sensitive and specific RT-PCR supplied by Jena Bioscience (Germany) was used for the master mix preparation according to the manufacturer's guidelines and as earlier described by Anjorin *et al*. [15]. Briefly, the master mixture was prepared with 25 µl SCRIPT RT-PCR reaction mix, x µl RNA template, 1-2 µl forward primer, 1-2 µl reverse primer, 2 µl SCRIPT RT-PCR enzyme mix, while the mixture was filled up to 50 µl with RNase-free water. Primer sequence FLUA (F) 5'- AAGACCAATCCTGTCACCTCTGA 3' and FLUA (R) 5'- CAAAGCGTCTACGCTGCAGTCC 3' were used as synthesized from Macrogen, Korea. The PCR amplification was performed in a 96-well Gene Amp PCR system thermocycler for DNA amplification following reverse transcription at 50 °C for 30 min, initial denaturation at 95 °C for 5 mins, denaturation at 95 °C for 10 sec (35 cycles), annealing at 55-65 °C for 20 secs (35 cycles), elongation at 72 °C for 60 secs (35 cycles), and final elongation at 72 °C for 5 mins.

2.4.3 Gel electrophoresis: Amplicons can be defined as the products of the PCR amplification process in a thermocycler. The amplicons for this assay were identified by Gel electrophoresis and visualized with a safe imager transilluminator (Invitrogen) as previously described [4;16].

2.4.3 Determination of HIV

A rapid diagnostic test was used for the determination of HIV-1/2. Patients who tested positive in the initial screening with Determine HIV-1/2 kit were further tested using Uni-gold HIV 1/2 kit following the manufacturer's instructions.

2.4.5 Determination of Tuberculosis

Testing for M. *tuberculosis* included microscopy and Gene Xpert MTB/RIF test following the manufacturer's instructions. The Gene Xpert MTB/RIF is an automated sample processing and nucleic acid amplification test for the detection of *M. tuberculosis* while the positive specimens were confirmed using microscopy.

2.4.6 Statistical analyses

The study epidemiological data collected were appropriately computed in a Microsoft Excel sheet and analysed with either Chi-square or ANOVA using GraphPad Prism 9 for Windows Version 9.0.0 (121) (GraphPad Software Inc., San Diego, CA, USA). P-values were compared and determined among influenza, HIV, and tuberculosis patients with significant differences for each epidemiological parameter including sample locations, age, and gender. The significant difference was considered at P value <0.05.

3. RESULTS

A total of four hundred (400) samples were collected. Demographic data of the patients showed an age range of 7 to 65 years. Male patients' proportion of 32.8% (131/400) was doubled by females 67.2% (269/400), of which 11% (30/269) were pregnant. Approximately, a ratio of 1 to 4, (26.8%) of the total

patients tested (90/336) were HIV positive, 2.5% (10/400) had tuberculosis and 1.8% (7/400) were asthmatic.

Furthermore, the result showed a 2.3% (9/400) molecular prevalence of influenza A virus (Fig. 1), out of which 66.7% (6/9) were females while 33.3% (3/9) were males. Interestingly, out of the 9 patients positive for influenza A virus, 66.7% (6/9) had HIV, 44.4% (4/9) had TB and 33.3% (3/9) were co-infected with TB and HIV. Ikorodu General Hospital accounted for 55.6% (5/9) molecular prevalence of influenza while 44.4% (4/9) was recorded in Badagry General Hospital (Table 1).

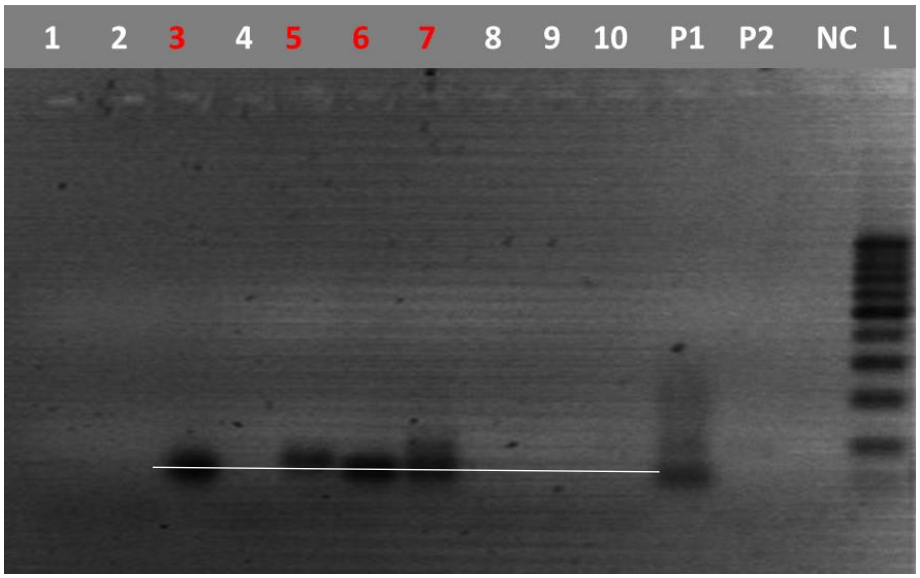

Figure 1. Agarose gel image showing RT-PCR amplicons' bands to Influenza A virus matrix-gene of some positive samples.

1-10 samples well, NC- Negative control, NTC- Negative test control, P- positive control, and L-Ladder of 50 base pair (bp).

Table 1. Epidemiological and clinical characteristics of Patients from different Chest Clinics in Lagos State, Nigeria

Epidemiological parameter	Total test	Flu positive	Tuberculosis positive	HIV positive	Asthma positive	χ2/ANOVA
	(n=400)	(n=9)	(n=10)	(n=90)	(n=7)	
Division						P=0.0174
Badagry	200	4(44.4)	7(70)	43(47.8)	2(28.6)	
Ikeja	100	0(0)	1(10)	32(35.6)	1(14.3)	
Ikorodu	100	5(55.6)	2(20)	15(16.7)	4(57.1)	
Location						P=0.0335

Alimosho	100	0(0)	1(10)	32(35.6)	1(14.3)	
Badagry	100	4(44.4)	5(50)	29(32.2)	2(28.6)	
Ikorodu	100	5(55.6)	2(20)	15(16.7)	4(4)	
Ojo	100	0(0)	2(20)	14(15.6)	0(0)	
Age group						P=0.0450
<15	3	0(0)	0(0)	0(0)	0(0)	
15-24	169	2(22.2)	2(20)	3(3.3)	2(28.6)	
25-34	76	1(33.3)	3(30)	14(15.5)	1(14.3)	
35-44	55	2(22.2)	4(40)	36(40)	2(28.6)	
45-54	57	4(44.4)	1(10)	23(25.6)	2(28.6)	
55-64	30	0(0)	0(0)	12(7.8)	0(0)	
=/>65	11	0(0)	0(0)	2(2.2)	0(0)	
Gender						P=0.3971
Female	269	6(66.7)	8(80)	76(84.4)	6(85.7)	
Male	131	3(33.3)	2(20)	14(15.6)	1(14.3)	

4. DISCUSSION

The findings from this study highlight several important observations regarding the molecular prevalence of the influenza virus in patients screened for *Mycobacterium tuberculosis* and HIV in different localities in Lagos. This study is the first report of molecular detection of influenza virus in TB and HIV-positive patients in Nigeria. The multi-site approach used was adopted to ensure representation from different locations within Lagos State and to facilitate community surveillance and rural engagement in line with the United Nations Sustainable Development Goals (SDGs) number 3 on good health and well-being through continuous surveillance.

Molecular method, particularly reverse transcription-polymerase chain reaction (RT-PCR) has emerged as a crucial tool for surveillance and accurate detection of pathogens [17]; [18], including emerging, re-emerging, and neglected influenza viruses. However, the high cost and limited availability of sophisticated equipment pose challenges for its widespread implementation, especially in resource-constrained settings. Nevertheless, conventional RT-PCR is a sensitive and rapid molecular technique for influenza surveillance, making it more feasible for smaller laboratories in such settings [19].

Although the low (2.3%) molecular prevalence of influenza virus in this research agrees with the work of Mhimbira *et al*. [20] who reported a prevalence of 3.1% in Tanzania and that of Roth *et al.* [21] in Thailand reported a much lower prevalence of <1%, it, however, suggests that influenza may not be a major contributing factor to debilitating conditions in this population compared to TB and HIV. This postulation, however, may be compared to the study by de Paus *et al*. [22] that an influenza virus infection is not a major

cause for developing clinically manifest tuberculosis but chronic tuberculosis is a predisposing factor for influenza. Furthermore, the co-infection rates of influenza with TB (44.4%) and HIV (66.7%) could give room to potential interactions between these pathogens, which may likely exacerbate disease severity and complicate clinical management.

Comparative analysis with previous studies revealed variations in molecular prevalence rates of influenza virus among different populations. For instance, Appiah *et al*. [23] reported a molecular prevalence of 1.7% while Dhakad *et al*. [19] reported a higher prevalence of 25%. The discrepancies in incidence rates could be attributed to differences in the study designs and characteristics of the sampled population. Interestingly, Anjorin *et al*. [24] detected 0.9% as against zero prevalence of influenza A virus among pig farmers in Lagos reported by Awosanya *et al*. [25], highlighting the importance of considering specific populations in influenza surveillance studies.

In an evaluative comparison, Cohen *et al*. [26] reported a molecular prevalence of 9% for influenza among patients living with HIV which contrasts with this current finding of a much higher prevalence of 66.7% in HIV-positive patients. Similarly, the study conducted by Walaza, *et al*. [10] indicated a prevalence of 10% for influenza among tuberculosis (TB) patients, whereas our study observed a significantly higher prevalence of 44.4% in TB patients. A major concern is the small cohorts affected which may not be generalized for the larger the population otherwise this calls for serious concern for the government at all levels.

The importance of demographic analysis in an epidemiological study like this cannot be overemphasized hence, the predominance of influenza virus among females (66.7%) raises questions about gender-specific susceptibility or differential healthcare-seeking behaviours. Additionally, the higher prevalence of influenza in Ikorodu General Hospital (55.6%) compared to Badagry General Hospital (44.4%) underscores the importance of geographic variations and local epidemiological factors in disease prevalence and transmission.

This study contributes valuable insights into the molecular prevalence of influenza virus among TB and HIV patients in Nigeria and sub-Saharan Africa where limited data currently exist on influenza virus in tuberculosis and HIV Patients. The significantly higher prevalence rates observed compared to previous findings underscore the importance of targeted surveillance and interventions in high-risk populations. Stakeholders including policymakers and government agencies should focus on expanding influenza surveillance programs, improving access to molecular diagnostic tools, and implementing preventive measures to mitigate the impact of influenza in vulnerable populations, particularly those co-infected with TB and HIV.

4. CONCLUSION

In conclusion, our investigation reveals the first report of molecular detection of influenza virus in tuberculosis and HIV patients in Nigeria. The co-infection of influenza virus in TB and HIV patients underscores the need for national surveillance to estimate the actual burden of influenza in HIV and TB patients for adequate planning and control. While the overall influenza A virus molecular prevalence of 2.3% is relatively low, its co-infection with TB and HIV further highlights the need for comprehensive approaches to respiratory infection control. Future investigations need to focus on genomic sequencing, underlying mechanisms of interaction between these pathogens, and evaluating the effectiveness of integrated interventions in reducing disease burden and improving public health outcomes.

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COMPETING INTERESTS

All authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

AAA- conceptualization, study design, experimentation, laboratory analysis, manuscript write up and served as the project supervisor; GAA- sample collection, experimentation, laboratory analysis, and manuscript draft; AMD- students' leader, sample collection, experimentation, laboratory analysis, and manuscript draft; SAS- result interpretation, manuscript draft; MTO- sample collection, experimentation, laboratory analysis, and manuscript draft; IOK- sample collection, experimentation, laboratory analysis, and manuscript draft; RA- experimentation, and laboratory analysis; SAO- resources, instrumentation, result interpretation, validation and laboratory director.

ETHICAL APPROVAL

ALL AUTHORS HEREBY DECLARE THAT ALL EXPERIMENTS HAVE BEEN EXAMINED AND APPROVED BY THE APPROPRIATE ETHICS COMMITTEE AND HAVE THEREFORE BEEN PERFORMED FOLLOWING THE ETHICAL STANDARDS LAID DOWN IN THe 1964 Declaration of Helsinki.

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