Current Challenges and Opportunities in Molecular Diagnosis of Respiratory Viral Co-Infections in Low-Resource Settings: A Nigerian Perspective

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ABSTRACT

Respiratory viral infection continues to be a public health problem of great concern worldwide especially in low resource settings like Nigeria where there are a lot of infrastructural and diagnostic challenges in the health sector. Co-infections with respiratory viruses (e.g., influenza viruses, respiratory syncytial virus (RSV), adenoviruses, and SARS-CoV-2) have become more common and are complicating the diagnosis, monitoring, and clinical management of disease. Rapid and accurate detection of multiple respiratory pathogens has been enhanced with the use of molecular diagnostic technologies, such as reverse transcription polymerase chain reaction (RT-PCR), multiplex PCR assays, and next-generation sequencing. In Nigeria however, their use is limited due to their cost, poor laboratory facilities, insufficient expertise, inadequate surveillance system and policy issues.

This study used a qualitative systematic review design with contextual policy analysis to explore the molecular diagnosis of respiratory viral co-infections challenges and opportunities in Nigeria. The literature reviewed and analysed was themed and focused on the period 2015-2025.

The study identified that although there are barriers to molecular diagnostics that exist, there are also opportunities such as decentralised point-of-care diagnostics, digital health integration, local production of diagnostic materials, training of the workforce, and investment in post-COVID-19 diagnostics that provide strategic pathways to build molecular diagnostic capacity and enhance healthcare system preparedness for infectious diseases in low-resource settings.

Keywords: Molecular Diagnostics, Respiratory Viral Co-Infections, Public Health

INTRODUCTION

Respiratory viral infections are a significant health issue worldwide and are a significant cause of morbidity and mortality in all age groups. These infections are caused by a variety of viruses, including influenza viruses, respiratory syncytial virus (RSV), rhinoviruses, adenoviruses, parainfluenza viruses, human metapneumovirus, and coronaviruses. These can involve both the upper and lower respiratory tract and are frequently associated with acute respiratory illness. Throughout the world, respiratory viruses are a major cause of outpatient consultations, hospitalizations, and severe morbidity, especially in those with underlying comorbidities and in young children (Li et al., 2022; Wang et al., 2024).

Globally, lower respiratory tract infections are among the leading causes of mortality, accounting for millions of deaths annually and putting significant pressure on the healthcare systems and economies. This burden is borne particularly heavily by children under five years old. Vulnerable individuals often experience growth and cognitive growth delays from repeated infections in this age group. The disease severity and morbidity of co-infections with more than one respiratory virus (combined viral/bacterial infections) are further worsened, disease duration is increased, and the risk of hospitalization and mortality is increased. The COVID-19 pandemic exposed the systemic effects and very high transmission rates of new respiratory viruses and accelerated the development of global diagnostic and surveillance infrastructure (Troeger et al., 2018).

The epidemiology of respiratory viral infections shows unique characteristics in low- and middle-income countries associated with environmental, socioeconomic, and health-system factors. High-density, household air pollution due to biomass fuels, malnutrition, overcrowding, and below-average vaccination rates further aggravate transmission and clinical severity. Seasonal peaks are not uncommon in tropical countries like most of sub-Saharan Africa during rainy seasons or during the haramattan season, leading to predictable peaks in case numbers. In Africa, Nigeria is the most populous country with a high prevalence of these infections, accounting for a significant percentage of hospitalizations and deaths in children (Akanbi and Ukoli, 2009; Kabantiyok et al., 2022).

Acute respiratory infections continue to be a significant cause of visits to primary health care and hospital admissions in all age groups in Nigeria. Despite the generally high burden of childhood respiratory illness, pneumonia remains among the most important causes of child mortality in Nigeria, which also has one of the highest absolute numbers of respiratory deaths in under-fives in sub-Saharan Africa, often linked with viral aetiologies. The most common pathogens identified are respiratory syncytial virus, influenza viruses, and rhinoviruses, either as primary infections or in combination with other viruses and bacteria. HIV-infected children and other immunocompromised children are much more susceptible, may shed longer, have higher viral load, and have more rapid disease progression. With the emergence and widespread circulation of SARS-CoV-2, the resilience of Nigeria's health care infrastructure was tested, and a lot of critical gaps were uncovered, while at the same time, the momentum for strengthening molecular diagnostic and surveillance systems was gained (Odaibo et al., 2013; Okoror et al., 2025).

The traditional diagnosis of respiratory viral infections, such as viral culture, rapid antigen detection, and serological testing, has had limited sensitivity, specificity, and turnaround time. The reference standard for viral culture requires specialized biosafety facilities, well-trained personnel, and long incubation periods, making it impractical for timely clinical decision making and outbreak response. Rapid diagnostic tests based on antigen have the advantage of being faster, but they are less well-performing, especially in samples containing low viral loads and/or with specific virus types. These areas of weakness often lead to empiric or syndromic treatment using "broad-spectrum" antibiotics, leading to the development of antimicrobial resistance and delaying specific antibiotic or infection control treatment (Mahony, 2008).

The developments in molecular diagnosis – particularly nucleic acid amplification assays like reverse transcriptase-polymerase chain reaction (RT-PCR) and multiplex PCR platforms – have revolutionized the field of respiratory virus detection. The assays provide increased sensitivity and specificity and allow for the detection of multiple pathogens in a single specimen in hours, from an assortment of clinical samples. Multiplex formats in particular enable a complete aetiological diagnosis, differentiation of viral co-infections, and allow informed decisions to be made regarding antibiotic usage, isolation, and antiviral treatment. They have proven clinical value in achieving lower diagnostic uncertainty, shorter hospital stays in specific clinical settings, and improved infection prevention and control measures. RT-PCR testing has been essential for COVID-19 variant monitoring, public health response, and case identification and contact tracing during the COVID-19 pandemic (Fig. 3) (Zhang et al., 2011; Osaigbovo et al., 2021).

In addition to clinical management, molecular tools have significantly improved the understanding of the epidemiology of respiratory virus seasonality, transmission patterns and the under-recognised role of particular pathogens in the burden of disease. However, there are significant barriers to the broad-scale adoption of these technologies in the low-resource environment. These encompass reagent and instrumentation costs, reliance on continuous power supplies and cold-chain operations, severe constraints on qualified molecular lab staff, and a generally low level of laboratory infrastructure. Molecular testing is primarily restricted to a few urban tertiary hospitals and research institutes in Nigeria and similar low and middle-income country settings. Syndromic approach continues to be the mainstay of primary and secondary care facilities, leading to under-diagnosis of viral co-infections, delayed detection of outbreaks, and poor clinical outcomes (Okeke and Ihekweazu, 2021; Osaigbovo et al., 2021).

With the weak regulatory system, fragmented laboratory networks, and limited diagnostic capacity in the healthcare system, Nigeria has made some progress in enhancing the capacity of molecular diagnostics, especially in the era of the COVID-19 response. The Nigeria Centre for Disease Control and Prevention (NCDC)

and a few reference labs have acquired capacities in the testing of influenza, SARS-CoV-2, and other priority pathogens using RT-PCR. However, comprehensive, broad-range molecular testing of the full range of common respiratory viruses continues to be limited, and there is limited systematic data on viral co-infection. The diagnostic gap presents a challenge in developing policies based on evidence and effective and targeted public health interventions (Osaigbovo et al., 2021).

These obstacles require a holistic understanding of the technical characteristics of the molecular diagnostics as well as the systemic, infrastructural, and policy environment and factors affecting their uptake and scale-up. There are opportunities to leverage laboratory investments made during the pandemic, incorporate multiplex tests into national surveillance efforts, rapid development and validation of context-relevant point-of-care molecular diagnostic tests, and effective workforce development through structured training programmes. There are opportunities for increasing diagnostic equity and preparedness for future respiratory viral threats through cost-reduction, public-private partnerships, and intra-regional collaborations.

The present study thus explored the challenges and emerging opportunities of molecular diagnosis of respiratory viral co-infections in low-resource settings with a particular focus on the Nigerian setting. The study aims to synthesise existing evidence and to provide guidance on actionable measures to strengthen diagnostic systems, improve clinical management and ultimately decrease the significant burden of respiratory viral diseases in Nigeria and other epidemiological and economic settings.

Statement of the Problem

Viral infections, especially co-infections, have a significant impact on healthcare systems in LMICs. In Nigeria, acute respiratory infections (ARIs) are among the major causes of morbidity and mortality, particularly among children under five years of age, with pneumonia and other lower respiratory tract infections (LRTIs) accounting for a large proportion of under-five mortality. However, despite this high burden, there is a significant lack of accurate identification of viral aetiologies and co-infections. Traditional diagnostic techniques such as clinical syndromic management, viral culture, and antigen detection often fail to differentiate between single and multiple viral infections or between viral and bacterial infections. This limitation leads to the excessive empirical use of antibiotics, which contributes to the emergence of antimicrobial resistance.

Conventional molecular diagnostic methods such as multiplex RT-PCR and next-generation sequencing have greater sensitivity and the capability to detect several respiratory viruses simultaneously. However, they remain difficult to implement in low-income countries such as Nigeria. Challenges include poor laboratory infrastructure, unstable electricity supply, high costs of reagents and equipment, shortages of skilled personnel, and weak supply chains for laboratory consumables. As a result, molecular testing facilities are limited to urban centres, while the majority of primary and secondary healthcare providers continue to rely on syndromic methods. This diagnostic gap results in the under-detection of viral co-infections, delayed outbreak detection, inappropriate treatment, prolonged hospitalizations, and poorer clinical outcomes among vulnerable populations, including children living with HIV and malnourished individuals.

Moreover, the prevalence, distribution, and clinical effects of respiratory viral co-infections in Nigeria remain poorly and inconsistently documented, making evidence-informed policymaking and resource allocation difficult. Although post-COVID-19 investments have improved some molecular diagnostic infrastructure, the integration of multiplex testing into national surveillance systems and routine clinical practice remains uncommon. Without a comprehensive understanding of these challenges and the context in which they operate, Nigeria and other LMICs risk missing key opportunities to improve preparedness for seasonal epidemics and future pandemics.

Purpose of the Study

This study aims to explore the existing challenges and emerging opportunities associated with the molecular diagnosis of respiratory viral co-infections in low-resource settings, with a particular focus on Nigeria. The study is a qualitative systematic review complemented by contextual policy analysis. It combines existing empirical evidence to synthesize current molecular diagnostic techniques and assess the structural, institutional, and

operational realities of Nigeria's healthcare system. Specifically, the study seeks to identify recurring challenges affecting the effective use of molecular tools, examine the progress made since the COVID-19 pandemic, and identify scalable approaches for enhancing diagnostic capacity and equity.

Significance of the Study

This study is both theoretical and practical. It draws together global and regional evidence within the context of Nigeria's policy landscape and adds to the body of evidence on the process and implementation science of diagnostics in low-resource settings. The results will inform policymakers, public health officials, and laboratory partners on taking action to support the integration of multiplex molecular diagnostics into current surveillance and care delivery systems.

On a larger scale, improved molecular diagnosis of respiratory viral co-infections can help to reduce unnecessary antibiotic treatments, limit antimicrobial resistance, optimize patient care, and enhance outbreak response capacity. The study also identifies opportunities for innovation, workforce development, and public-private partnerships, all of which are critical in facilitating the diagnostic equity required to meet global health security agendas – in a cost-effective manner. Finally, the outputs of this work could be used to guide national strategic plans and help to lower the significant morbidity, mortality, and economic impact of respiratory viral diseases in Nigeria and similar low-resource countries.

Overview of Respiratory Viral Co-infections

Respiratory viral co-infections occur when two or more different viral illnesses of the respiratory tract are present in the same person at the same time or at a short time interval. Now these events are becoming well recognised, especially in children or when there is a high level of viral circulation. Of acute respiratory illnesses, co-infections can be responsible for 10% to 40% of cases, depending on the setting, the age group, and the method used for diagnosis (Babawale and Guerrero-Plata, 2024; Mandelia et al., 2021). Some common viral combinations are respiratory syncytial virus (RSV) and rhinovirus, influenza and respiratory syncytial virus (RSV), human metapneumovirus and parainfluenza viruses, and others. The clinical relevance of these co-infections is still being explored, and the effects they have on the severity, duration and immune responses of the host can be complex.

Respiratory viral co-infections are different in different regions, in different seasons, and in different population groups. Co-infections tend to be highest in temperate climates during the winter when several viruses are present during the same time period. In tropical and subtropical areas like sub-Saharan Africa, including Nigeria, the transmission patterns are influenced by rain, environmental conditions, and are year-round or bimodal. In low and middle-income countries, there are high detection rates of viruses in children with ALRI in children, with co-infection rates ranging from 20% to 57% in hospitalised children. Co-detection of multiple viruses - often the most common being human rhinovirus, then RSV, adenovirus and influenza - occur many times (Kaboré et al., 2025; Demboux Lyelet et al., 2025).

Co-infections with viruses can cause varying clinical effects, from mild upper respiratory illness to severe lower respiratory tract disease that may require hospitalisation or intensive care. There are some indications that certain combinations can produce synergistic effects, which include increased inflammation, longer virus shedding, and greater severity of disease. For example, there is an association of longer hospital stays and higher oxygen needs in young children with the co-infection with other viruses. Antagonistic interactions in which one virus suppresses the replication of another have also been reported, possibly due to the induction of interferon or competition for cell receptors. Such virus-virus interactions make it difficult to predict the clinical course and show the need for comprehensive diagnostic testing (Babawale and Guerrero-Plata, 2024; Deol et al., 2025).

Respiratory viral co-infections are especially prevalent in immunocompromised children, especially those with HIV infection. The increased susceptibility to multiple pathogens and poorer prognosis with increased shedding, increased viral load, and decreased CD4 count. Respiratory viral detection rates have been reported to exceed 50% among the HIV infected children, with frequent respiratory viral co-infections associated with poor immunological parameters in Nigeria (Okoror et al., 2025). People with comorbidities, the elderly, and

malnourished groups are at further increased risk. These trends were highlighted by the COVID-19 pandemic, which revealed that co-infections with SARS-CoV-2 and other respiratory viruses were linked to different severity outcomes, showcasing how different viruses can influence the severity of COVID-19. The COVID-19 pandemic further highlighted these trends, revealing that co-infections with SARS-CoV-2 and other respiratory viruses were linked to variable severity outcomes depending on the viral partners involved, demonstrating how different viruses can influence the severity of COVID-19.

Pathogenetically, co-infections may impair epithelial barriers, change innate and adaptive immunity, and promote secondary bacterial infections. Viral damage to the respiratory mucosa can facilitate bacterial adherence, while dysregulated cytokine responses may lead to immunopathology. These mechanisms explain why co-infected patients with pneumonia may have more severe disease, acute respiratory distress, or have longer recovery times than patients with mono-infections. Meta-analyses, however, reveal inconsistent links between clinical severity and coinfection status, indicating that there are critical modulating roles for host factors, viral loads, and specific combinations of pathogens (Babawale and Guerrero-Plata, 2024; Asner et al., 2014).

The introduction of multiplex polymerase chain reaction (PCR) panels and next-generation sequencing (NGS) has revolutionized the diagnosis and comprehension of the role of respiratory virus co-infections. These tools allow for the ability to detect multiple pathogens at the same time from a single specimen collected from the nasopharynx or lower respiratory tract, and identify polymicrobial patterns that have not been detected to date. However, the identification of causative agents from colonizers or bystanders is still a problem, especially in asymptomatic or mildly symptomatic patients. Under-recognition of co-infections persists in low-resource environments due to limited access to more sophisticated diagnostics and continued reliance on syndromic monitoring (Osaigbovo et al., 2021).

Therefore, respiratory viral co-infections are an important, but not fully characterized, component of the epidemiology of respiratory disease. They have the potential to affect individual patients, as well as healthcare systems and public health preparedness, and require ongoing research on virus-host interactions, better diagnostic approaches, and targeted interventions. The role of molecular surveillance for better diagnostic service through improved clinical outcomes and epidemic preparedness in contexts like Nigeria, where the burden of respiratory illnesses is high and diagnostic capacity is uneven, has the potential to create a strong way forward.

METHODOLOGY

Research Design

This study adopted a qualitative systematic review design with contextual policy analysis to explore the challenges and opportunities of molecular diagnosis of respiratory viral co-infections in low resource settings particularly Nigeria. It was deemed appropriate to use the systematic review approach as this allows the identification, synthesis and critical evaluation of existing evidence of molecular diagnostic technologies and their use in resource-limited healthcare settings. The addition of contextual policy analysis also gave insight into the institutional, regulatory and healthcare system issues that affect the implementation and effectiveness of molecular diagnostic services in Nigeria.

Data Sources

The sources for the study included peer-reviewed sources as well as grey literature. The articles were obtained from major scientific databases such as PubMed, Scopus, Web of Science, and Google Scholar. Grey literature sources such as publications and reports from the World Health Organization (WHO), Nigeria Centre for Disease Control (NCDC), Federal Ministry of Health, World Bank and other relevant international organisations in public health were also consulted to provide contextual and policy-related evidence. Peer-reviewed and grey literature were both included to enable a comprehensive assessment of all scientific evidence, policy developments and operational realities regarding molecular diagnostics in Nigeria and other low-resource settings.

Literature Search Strategy

A literature review was carried out to identify the relevant studies published from January 2015 to March 2025. By using keywords and the Boolean operators in search strategy, the maximum amount of relevant literature was retrieved. This included the terms: “respiratory viral co-infections”, “molecular diagnostics”, “RT-PCR”, “multiplex PCR”, “next-generation sequencing”, “point-of-care diagnostics”, “respiratory viruses”, “low-resource settings”, “LMICs”, “Nigeria”, and “sub-Saharan Africa”. The following terms were used in conjunction with each other - Boolean operators (AND and OR) were applied to widen or narrow the search results accordingly.

The first search resulted in a large number of publications from the selected databases. After eliminating duplicate records, titles and abstracts were reviewed for relevance to the goals of this study. Eligible studies were full-text reviewed and analyzed.

Inclusion and Exclusion Criteria

Studies were chosen according to the predetermined inclusion and exclusion criteria. Eligible studies were peer-reviewed journal articles, surveillance reports, policy documents, and technical reports in English from 2015 to 2025. The included studies included those relating to respiratory viral infections, viral co-infections, molecular diagnostic technologies, laboratory capacity, surveillance systems, and healthcare delivery in low- and middle-income countries, especially in sub-Saharan Africa.

Studies that were not about any infectious disease other than respiratory disease, that did not use non-molecular diagnostic testing methods, molecular testing was not done, or that did not have methodological clarity, that were not empirical studies (editorial articles, commentaries, conference abstracts, opinion papers), were not included. The review also did not include duplicate publications.

Study Selection Procedure

The article selection process was based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Records retrieved were first screened according to title and abstract to determine if there was relevance to the study goals. Full-text review of articles that were deemed potentially eligible was performed after this. Studies that were duplicated were excluded and publications that did not fulfill the inclusion criteria were excluded in the screening process.

The final list included studies that demonstrated evidence on molecular diagnostic methods, respiratory viral co-infections, challenges for the healthcare system and opportunities to improve diagnostic capacity of low resource settings. The structured selection process improved transparency and repeatability of the review.

Data Extraction Process

A common data extraction process was established to standardise the information extracted from the selected studies. The information retrieved comprised authors, publication year, country or setting of the study, diagnostic technology used, respiratory pathogens studied, co-infections reported, performance indicators of the diagnosis, infrastructure needs, challenges to the operation, policy implications, and recommendations for improving diagnostic systems.

Data extracted included technical and contextual data to achieve the study's objectives and to identify trends across various healthcare settings.

Data Analysis

Thematic analysis was used for analysing the extracted data. This analytical approach helped identify and interpret common trends and themes around the molecular diagnosis of respiratory viral co-infections.

The analysis was carried out in four phases.The analysis was performed in 4 phases. Firstly, the chosen research papers became familiar by repeated reading of the content of the papers. Secondly, the information was systematically coded to uncover common concepts and problems. Third, related codes were clustered together into thematic groups. Lastly, the themes identified in the study were translated in the light of Nigeria's healthcare system and the overall situation of low-resource countries.

Thematic categories that arose from the analysis were: infrastructural limitations, cost & affordability barriers, workforce capacity challenges, diagnostic accessibility, surveillance & data management weaknesses, policy & regulatory issues, and emerging opportunities for innovation and strengthening of the healthcare system. This allowed for a thorough understanding of factors affecting molecular diagnostic capacity and implementation.

Contextual Policy Analysis

The results of the systematic review were triangulated with national and international policy documents on infectious disease surveillance, laboratory services and molecular diagnostics to enhance the context of the study. Special attention was paid to the Nigeria Centre for Disease Control, the Federal Ministry of Health and the World Health Organization policies and strategic frameworks.

The policy analysis included an overview of current laboratory network organization, diagnostic guidelines, disease surveillance, workforce development, and investments in molecular diagnostic infrastructure, including post-COVID-19 pandemic. This allowed for the study to assess the policy gaps and opportunities to enhance diagnostic preparedness and response capacity.

Ethical Considerations

This study was conducted using only secondary data from publicly available sources and without human subjects, patient records, or confidential information. Thus, it was not necessary to obtain ethical approval. All sources of information were referenced appropriately, however, care was taken to interpret and represent the original findings correctly.

Limitations of the Methodology

There are some limitations of the study. First, there is a possibility of publication bias since studies that showed significant results are more likely to be published than studies that did not show significant results. Second, it was only English-language publications that were reviewed, so there may be studies in other languages that were not included. Third, available studies specific to Nigeria on respiratory viral co-infections and molecular diagnostics are limited and potentially could impact the extent to which contextual insights are captured. Despite these inherent constraints, the use of systematic evidence synthesis and policy analysis offered a solid base for understanding the opportunities and challenges of molecular diagnostics in low-resource settings.

Molecular Diagnostic Techniques For Respiratory Viral Infections

Respiratory molecular diagnostics has changed the way respiratory viruses are detected by allowing the rapid, sensitive and specific identification of respiratory viruses directly from clinical specimens. Molecular techniques detect viruses by targeting nucleic acids (DNA or RNA) and do not require viral culture or antigen detection, and can yield results within hours, while conventional methods take days, and allow detection of viruses which are hard to grow. They have turned out to be critical for the diagnosis of respiratory viral co-infections, clinical management, and public health surveillance, especially in the post-COVID-19 era (Alzahrani, 2026; Babawale and Guerrero-Plata, 2024).

Introduction to Molecular Diagnostics

Molecular diagnostic techniques are based on the detection and amplification of specific genetic material of a pathogen. They are more sensitive and specific than traditional methods and can detect non-viable viruses or low viral loads. These tools have been used in respiratory medicine to move from a syndromic to aetiological approach, which has enhanced patient outcomes and antimicrobial stewardship.

RT-PCR

The molecular diagnostics gold standard for RNA viruses like SARS-CoV-2, RSV and influenza continues to be reverse transcription polymerase chain reaction (RT-PCR). It involves reverse transcription of viral RNA to complementary DNA (cDNA) using reverse transcriptase, PCR amplification, and the real-time detection of cDNA using fluorescent probes. The analytical sensitivity of RT-PCR is very high and can detect as few as 10-100 viral copies per reaction and the turnaround time of the test may be as fast as 1-3 hours in standard lab settings (Alzahrani, 2026).

Multiplex PCR Assays

Multiplex PCR assays look for several respiratory pathogens in one reaction. Commercial panels can detect 10-22 viruses and bacteria, which makes them suitable for the study of co-infections. They will minimize reagents, specimen volume needed and the overall cost of testing and provide complete syndromic information. Multiplex PCR is shown to yield 30% to 50% more diagnostic results than singleplex and antigen-based testing and facilitate quicker clinical decisions (Clark et al., 2023; Huang et al., 2018).

Next-Generation Sequencing (NGS)

Metagenomic analysis is a next-generation sequencing method which allows for the detection of known and novel pathogens in a broad spectrum in an unbiased way. NGS is more resource-intensive but can be useful in outbreak investigations and for tracking variants, and in situations where a negative result was obtained through the routine panels, but with high clinical suspicion. It is still being used in respiratory diagnostics, and in reference laboratories (Vergara-Gómez et al., 2025).

Point-of-Care Diagnostic Tools

Point-of-care (POC) molecular platforms are cartridge-based platforms (e.g., Cepheid Xpert, BioFire FilmArray) that help to place testing closer to the patient. These instruments combine sample preparation, amplification and detection into a single, easy-to-use instrument, and provide results in as little as 15 to 60 minutes. POC molecular tests are especially promising in low-resource settings as many of these tests require minimal infrastructure and training (Alzahrani, 2026; Dhaini et al., 2024).

Benefits and Limitations

The major advantages of molecular diagnostics include rapid turnaround time, high sensitivity for detecting co-infections, reduced unnecessary antibiotic use, and improved infection control. Rapid multiplex testing in hospital settings has also been associated with shorter hospital stays and more appropriate antiviral prescribing (Clark et al., 2023).

However, several limitations remain, particularly in low-resource settings. The high cost of instruments and consumables, the requirement for stable electricity, the need for trained personnel, and the dependence on cold-chain storage for reagents make widespread adoption difficult in Nigeria and similar settings. Interpretation of results may also be challenging, particularly when distinguishing colonization from active infection, and over-detection of non-pathogenic viruses may occur. Although NGS is highly effective, it requires advanced bioinformatics expertise and remains inaccessible for routine use in many settings (Osaigbovo et al., 2021; Zu et al., 2025).

In conclusion, molecular diagnostic tools offer substantial aids in the diagnosis, management and treatment of respiratory viral infections and co-infections. To ensure their impact in low resource settings such as Nigeria, it is important they are implemented strategically, especially on platforms that are contextually relevant and capacity building.

Challenges Affecting the Molecular Diagnosis of Respiratory Viral Co-Infections in Nigeria

The molecular diagnostics, especially RT-PCR and multiplex assays, have revolutionized the detection of co-infections in the respiratory tract in all the world, providing a rapid, sensitive, and specific diagnosis. However, their use in Nigeria is greatly hampered by structural barriers that affect access, scalability and sustainability. These challenges complement the broader structural weaknesses of the country's healthcare and laboratory systems leading to under-diagnosis of viral co-infections even though the burden is high (Osaigbovo et al., 2021; Muoneke et al., 2025).

One of the most significant challenges is inadequate laboratory infrastructure. Molecular testing requires stable electricity, reliable cold-chain storage for reagents, biosafety level 2 or higher facilities, dedicated molecular laboratories equipped with thermocyclers and extraction systems, and robust quality control mechanisms. In Nigeria, such facilities are mainly limited to a few urban tertiary institutions and the Nigeria Centre for Disease Control and Prevention (NCDC) reference laboratory network. Most primary and secondary healthcare facilities across the 36 states lack these resources and therefore depend on syndromic management or referrals to distant urban centres. Poor electricity supply frequently disrupts testing processes, while inadequate equipment maintenance results in repeated equipment failures and prolonged downtime (Osaigbovo et al., 2021; Maduka et al., 2023).

Workforce shortages worsen the infrastructural shortages. There is a huge shortage of trained molecular biologists, medical laboratory scientists competent in nucleic acid amplification techniques and bioinformaticians with the ability to analyze multiplex or next-generation sequencing data in Nigeria. Skilled workers depart to find better wages and working environments, leading to brain drain, which worsens the situation. It is estimated that in the last few years, thousands of medical laboratory scientists have emigrated from the country, creating a shortage of resources among those remaining and restricting the growth of routine molecular testing for all respiratory viruses. Co-infections can further be under-detected and misreported due to the limited number of people who are proficient in the interpretation of multiplex PCR (Muoneke et al., 2025; Naidoo et al., 2020).

Another major obstacle is inadequate financing. The cost of molecular diagnostic reagents, consumables, and equipment maintenance makes these services inaccessible to many public health laboratories and patients paying out-of-pocket. Although the COVID-19 pandemic led to temporary government and donor support for SARS-CoV-2 testing, funding for routine respiratory viral panel testing remains inconsistent. Consequently, significant disparities exist between urban and rural areas, where multiplex testing remains largely unavailable. Patients in underserved regions often experience delayed or absent aetiological diagnoses, resulting in irrational antibiotic use and increased antimicrobial resistance (Kabantiyok et al., 2022).

Supply chain disruptions and logistical challenges also constrain diagnostic capacity. Nigeria depends heavily on imported reagents and diagnostic kits, making the system vulnerable to global shortages, currency fluctuations, and customs clearance delays. Transportation and storage problems, particularly cold-chain failures, can compromise reagent integrity, while inconsistent centralized procurement frequently causes stockouts, even in well-established laboratories. These issues are particularly severe for multiplex assays, which require specialized cartridges or panels that are often unavailable in low-resource settings (Osaigbovo et al., 2021).

There are also quite a few policy and regulatory constraints. Since the COVID-19 response molecular testing capacity has increased nationally, but multiplex respiratory virus diagnostics are not consistently integrated into routine clinical and surveillance programmes. Standardisation is hampered by weak coordination between the Federal and State levels, differences in quality assurance procedures throughout laboratories and poor oversight of point-of-care molecular devices. There is a low level of surveillance of non-COVID respiratory viruses, leading to limited information on co-infection and seasonality. This evidence gap is a hindrance to targeted public health interventions and resource allocation (Muoneke et al., 2025; Maduka et al., 2023).

Collectively, these challenges create a cycle of under-diagnosis and suboptimal treatment of respiratory viral co-infections. In a country where acute respiratory infections contribute substantially to childhood morbidity and

mortality, the lack of routine identification of viral aetiologies and co-infections delays clinical decision-making, prolongs hospitalization, and increases the risk of complications among vulnerable populations such as people living with HIV and individuals with comorbidities. Although some progress has been made through post-pandemic investments, molecular diagnosis of respiratory viral co-infections will continue to reach only a small proportion of those in need unless there is sustained commitment to strengthening healthcare systems, retaining skilled personnel, supporting domestic reagent production, and harmonizing policies (Osaigbovo et al., 2021; Kabantiyok et al., 2022).

Addressing these issues requires a coordinated national response that builds on the infrastructure gains achieved during the COVID-19 pandemic while simultaneously addressing longstanding systemic challenges. Until infrastructural, human resource, financial, logistical, and policy barriers are effectively resolved, the full potential of molecular diagnostics in Nigeria will remain unrealized.

Emerging Innovations in Molecular Diagnosis of Respiratory Viral Co-Infections

New innovations in molecular diagnostics are opening novel avenues to deal with some of the enduring challenges faced in low-resource communities. The advancements emphasize on the cost-effectiveness, mobility, ease of use, and integration with the existing health care systems, providing potential solutions to the timely identification of respiratory viral co-infections in Nigeria and other settings (Zu et al., 2025; Alzahrani, 2026).

Isothermal Amplification Techniques

Loop-mediated isothermal amplification (LAMP) and other isothermal methods are a paradigm change in PCR. These methods can amplify the nucleic acids at a fixed temperature, without requiring the use of costly thermal cyclers. The LAMP assays are highly sensitive and specific, and can be completed within a few hours, making it ideal for decentralised laboratories and field settings. Multiplex LAMP platforms that detect multiple respiratory viruses at a time have demonstrated good performance in tropical settings. They are also compatible with simple visual readouts or later flow devices, which also minimizes infrastructure needs (Koryukov et al., 2024; Juscamayta-López et al., 2021).

CRISPR-Based Diagnostics

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems are now being exploited as powerful diagnostic tool. CRISPR based assays like SHERLOCK and DETECTR use nucleic acid amplification and CRISPR programmable enzymes for highly specific detection. These platforms provide fast turnaround, low requirements for equipment and equipment personnel, and can differentiate between closely related viral strains. Multiplexed CRISPR assays have been recently developed for the simultaneous detection of SARS-CoV-2, influenza, and RSV. They have the potential to be printed on paper or integrated into a smartphone, making them a game-changer for point-of-care situations in resource-poor areas (Chen et al., 2023; Das et al., 2025).

Portable And Point-Of-Care Molecular Platforms

The next generation of point-of-care (POC) molecular devices combines the processes of sample processing, amplification, and detection in an integrated system that is small, portable, battery-powered, and user-friendly. There are platforms in place to facilitate testing at district hospitals or primary care levels, including improved GeneXpert modules and new cartridge-based multiplex panels. These innovations offer important benefits in terms of avoiding reliance on central laboratories, but with an accuracy of diagnosis. Concurrently, low-cost assays measuring host response along with pathogen detection are under development to assist with clinical decisions under conditions of limited trained personnel (Alzahrani, 2026; FIND, 2023).

Nanopore Sequencing Technologies

Portable sequencers such as Oxford Nanopore Technologies (ONT) MinION enable long-read sequencing in remote locations in real time. These devices will facilitate metagenomic analysis for a wide range of pathogen detection as well as identification of variants without the need of large laboratory infrastructure. ONT has been

applied in outbreak response and respiratory virus surveillance in Africa, including Nigeria, with success. It has the potential to provide actionable genomic information within hours, thus enabling the aid with co-infection pattern monitoring and emerging variants (Bastug et al., 2025; Mongan et al., 2020).

Artificial Intelligence and Digital Integration

Recent advances in artificial intelligence (AI) and machine learning algorithms are being leveraged more than ever with molecular diagnostics. AI-driven decision support systems help to interpret test results and make better treatment recommendations by analysing both clinical and epidemiological information. Mobile applications that are connected to POC devices allow sharing of results, monitoring of quality control, and aggregation of surveillance data. These digital innovations are assisting in addressing workforce gaps and enabling decentralised testing networks in Nigeria's multilingual states (Tillekeratne et al., 2024).

Open-Source Innovations

The goal of open-source molecular diagnostic platforms and local reagent manufacturing is to lower costs and improve sustainability. These are methods that promote technology transfer and adaptation to context. The implementation of affordable multiplex panels designed to African epidemiology is being validated and developed by public-private partnership and regional collaboration (Emperador et al., 2020).

Although these encouraging steps are observed, deliberate investment in regulatory processes, local training programmes, strengthening of the supply chain and integration into national diagnostic algorithms are required to achieve the goal of successful translation into routine use in Nigeria. Pilot implementations and cost-effectiveness studies will be crucial to support scalable adoption.

The innovative new developments in molecular diagnostics offer great promise to improve management of respiratory viral co-infections in low-resource countries. Through targeting innovative technologies that are durable, cost-effective, and easy-to-use, Nigeria can create resilient diagnostic systems that can meet existing demands and anticipate future risks. When properly utilized, these developments, coupled with continued policy commitment, can help to substantially close the diagnostic equity gap and enhance health outcomes nationally.

DISCUSSION

The review of evidence identified a high prevalence of respiratory viral co-infections in Nigeria, but these are under-detected due to weak molecular diagnostic services. The prevalence of co-infections in hospitalised children is usually 20% to 50%, and in many settings, testing is not routinely conducted for most children due to structural and financial challenges. The COVID-19 laboratory expansions have led to the ability to perform reference-level RT-PCR for SARS-CoV-2 and influenza, though this has not resulted in continued multiplex testing for common respiratory viruses like RSV, rhinovirus, and human metapneumovirus. This gap leads to persistent syndromic management, overuse of antibiotics, and unwarranted opportunities for specific antiviral treatments and infection control (Osaigbovo et al., 2021; Kabantiyok et al., 2022).

New developments like isothermal amplification, CRISPR-based testing, and point-of-care nanopore sequencing provide a tangible opportunity to decentralize testing. While these technologies do not require a reliable power supply and central laboratories, they are less utilized in Nigeria due to a lack of local validation research, time delay in regulatory processes, and purchase policies that incentivize imported platforms with high prices. The Nigeria Centre for Disease Control and Prevention policy documents recognise the need to scale up diagnostics, but implementation is limited, and mostly the integration of multiplex panels has not been incorporated into the national surveillance algorithms for non-COVID respiratory pathogens (Muoneke et al., 2025; Alzahrani, 2026).

RECOMMENDATIONS

The phased deployment of low-cost multiplex isothermal and cartridge-based molecular platforms to secondary-level hospitals in states of high disease burden should be encouraged in Nigeria. A tiered diagnostic algorithm to connect reference laboratories with district facilities needs to be developed within the framework of a

dedicated respiratory virus diagnostic network at the federal and state ministries of health. Cost of LAMP reagents and low-cost cartridges could be reduced and supply security enhanced through local manufacturing and/or regional procurement.

To build the workforce skills required for the implementation of molecular diagnostics, the inclusion of compulsory molecular diagnostics modules in medical laboratory science training programmes is required, and the continuous in-service training of the workforce should be supported by international partners. Regulatory authorities should enable a rapid approval process for WHO prequalified or local validation of point-of-care molecular devices, and implement quality assurance measures through all test levels.

Routine multiplex testing should be assessed for clinical impact, cost-effectiveness, and antimicrobial stewardship outcomes in a pilot implementation study to be funded by a public-private partnership. Implementing digital result reporting systems would further enhance real-time monitoring and support swift response to outbreaks or seasonal fluctuations in case of new variants. Finally, national health insurance plans should integrate multiplex respiratory panels in benefit packages, which would decrease OOP costs and contribute to equity (FIND, 2023; Dhaini et al., 2024).

Limitations of the Study

For this qualitative systematic review, there was a search of literature and policy documents that were publicly available and published between 2015 and 2025. This means that smaller facilities in Nigeria may not have had their literature published, resulting in publication bias. The molecular technologies are changing, and the policy changes in recent years due to the pandemic may not be fully reflected in the reviewed sources. Official documents were used as the basis for contextual policy analysis, where no primary data collection could be carried out to verify the implementation fidelity. The findings in this study are constrained by the specificities of Nigeria and other low-resource settings in Africa.

CONCLUSION

The molecular diagnosis of respiratory viral co-infections is at a crucial point in Nigeria. Although there is ample evidence of the clinical and public health value of sensitive multiplex testing, significant infrastructural, financial, and human resource barriers remain to accessing this technology. Deliberate policy initiatives, continued investments, and system-wide integration are necessary to make such opportunities a reality in everyday practice, but new innovative approaches for isothermal amplification, CRISPR diagnostics, and portable sequencing offer some feasible solutions to local realities.

Building up diagnostic capacity in the presence of respiratory viral co-infections will minimize diagnostic uncertainty, limit excess antibiotic prescribing, improve patient outcomes, and enhance preparedness for future epidemics. Nigeria already has the foundation post-COVID; now it is a matter of political will and coordination of execution to develop resilient and equitable molecular diagnostic systems for the masses.

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