ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF BIOMEDICINE AND LABORATORY SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Molecular Detection of Respiratory Viruses and Bacterial Pathogens Causing Severe Acute Respiratory Infection and Influenza-like Illness in Under-Five Children at Health Facilities in Ethiopia

By: **Adimkewu Agunie (BSc, MSc candidate)**

Advisors: Dr. Kassu Desta (MSc, PhD)

Dr. Biruk Zerfu (MSc, PhD)

Adamu Tayachewu(MSc, PhD Fellow) From EPHI

ID GSR/039/17

A research Thesis submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of the requirements for the Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology)

July,2026

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

School of biomedicine and laboratory sciences

Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by **Adimkewu Agunie**, entitled **"Molecular Detection of Respiratory Virus and bacterial pathogens Causing Severe Respiratory Infection and Influenza-like Illness in under Five Children at Health Facilities in Ethiopia."** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

Table of Contents

List of tables v

List of figures vi

Acknowledgment vii

Abbreviation viii

Abstract ix

1. Introduction 1

1.1. Background 1

1.2. Statement of Problem 3

1.3. Significance of The Study 5

2. Literature Review 6

2.1. Literature review 6

3. Objectives 10

3.1. General objectives 10

3.2. Specific objective 10

4. Materials and Methods 11

4.1. Study design and Period 11

4.2. Study Setting 11

4.3. Population 11

4.3.1. Source population 11

4.3.2. Study population 11

4.4. Sample size determination 12

4.5. Sampling Methods and Procedures 12

4.5.1. Sampling method 12

4.6. Inclusion and exclusion criteria 13

4.6.1. Inclusion criteria 13
4.6.2. Exclusion criteria 14
4.7. Study variables 14
4.7.1. Dependent Variables 14
4.7.2. Independent Variables 14
4.8. Laboratory Methods 14
4.8.1. Viral nucleic acid extraction 14
4.8.2. Master mix preparation and PCR setup 15
4.9. Quality Assurance 15
4.9.1. Pre-analytical phase 16
4.9.2. Analytical phase 16
4.9.3. post-analytical phase 16
4.10. Data collection procedures 18
4.11. Data analysis and interpretation 18
4.12. Ethical considerations 19
4.13. Dissemination of the result 19
4.14. Operational definition 19
5. Results 20
5.1. Sociodemographic and Clinical Conditions of Study Participants 20
5.2. Respiratory Virus Distribution 23
5.3. Bacterial pathogen distribution 26
5.4 Viral co-detection 27
5.5. Viral and Bacterial pathogen co-detection with the average Ct value among 422 study participants in children under five years old. 28
5.6. Factors Associated with respiratory and bacterial infections 29

6. Discussion 31
7. Strengths and Limitations of the Study 36
7.1. Strength of the study 36
7.2. Limitation of the study 36
8. Conclusion 37
9. Recommendation 38
10. References 39
Annex I: Parental consent form for parents of children <5 years to participate in the study (English version) 44
Annex II: በጥናቱ ውስጥ ለመሳተፍ ከ5 ዓመት በታች ለሆኑ የልጆች የወላጅ ስምምነት ቅጽ (ለ አማርኛ ቋንቋ ተናጋሪ) 46
Annex III: Unka hayyama warraa Warri ijoollee isaanii <5 waggaa qorannicha irratti akka hirmaatan (Afaan Oromootiin) 48
Annex IV: Step of sample extraction 50
Annex V: Procedure for master mix preparation and RT-PCR set ups 51
Annex IV.I RNA/DNA addition room 53
Annex VI: SARI and ILI case-based format 54
SARI laboratory request and reporting form 54
ILI laboratory request and reporting form 56
Declaration 58

List of tables

Table 1: Demographics and clinical characteristics and viral and bacterial detection rates among ILI/SARI study participants in selected health facilities in Ethiopia, Jan to April 2026 (N=422). 20
Table 2: Distribution of ILI/SARI across the selected health facilities by sex and age group of Study participants in Ethiopia, Jan to April 2026 (N=422). 23
Table 3: Respiratory Virus Distribution with age, sex, and case category 24
Table 4: Bacterial pathogen Distribution with age, sex, and case category 26
Table 5:A summary of viral co-detection rate among study participants (N = 422) 27
Table 6: Multivariate logistic regression analysis of significant factors associated with viral and bacterial infection among the study population (N=422) 30

List of figures

Figure 1: Conceptual framework. 10
Figure 2; RT-PCR amplification plot A, Single detection B, Double detection C, Triple detection D, Quadruplex detection E, Internal control F, Positive control 18
Figure 3:Types of respiratory virus detected across different age groups 25
Figure 4: Types and subtypes of influenza virus detected among the study population 25
Figure 5: Viral and bacterial co-detection with two pathogens (A) and three or more pathogens (B) with an average CT-Value. 28
Figure 6: Viral and bacterial Co-detection 29

Acknowledgment

I extend my sincere gratitude to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, for permitting me to work on this thesis on a topic of great personal interest to me. My profound appreciation goes to my advisors, Dr. Kassu Desta, Dr. Biruk Zerfu, and Mr. Adamu Tayachew for their invaluable support and guidance.

I am also grateful to Mr. Feyissa Challa, the Ethiopian Public Health Institute National Referral and Reference Directorate director, for his open support, valuable advice, and for solving any difficulties by providing guidance and solutions.

I would like to extend my sincere appreciation to all my colleagues at the Influenza National Reference and Referral Laboratory at the Ethiopian Public Health Institute (EPHI) for their dedicated support.

Abbreviation

ARI	Acute Respiratory Infections
CDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
Dntp	Deoxynucleotide Triphosphate
HBOV	Human Bocavirus
HCoV	Human Coronavirus
HMPV	Human Metapneumovirus
HRSV	Human Respiratory Syncytial Virus
HRV	Human rhinovirus,
HPIV	Human parainfluenza virus
ILI	Influenza-Like Illness
PI	Principal Investigator
PIV	Parainfluenza virus
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SARI	Severe Acute Respiratory Infections
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
VTM	Viral Transport Media,
WHO	World Health Organization
HI	*Hemophilus Influenza*
LP	*Legionella pneumophila*
AB	*Acinetobacter baumanni*
SP	*Streptococcus pneumonia*
SA	*Staphylococcus aureus*
PA	*Pseudomonas aeruginosa*
CP	*Chlamydia pnemoniae*

Abstract

Background: Acute respiratory infections (ARIs) are a major public health concern globally. The cause of ARI in children under five includes viruses and bacteria. In Ethiopia less coverage of the influenza positivity rate among suspected cases from both severe acute respiratory illness (SARI) and influenza-like illness (ILI) sentinel sites. However, the role of other causative pathogens and bacterial agents has not been comprehensively investigated. This study aims to investigate viral and bacterial etiology from SARI and ILI cases in children under 5 years of age in Ethiopia.

Objective: To assess Common Respiratory viruses and bacterial pathogens Causing Severe Respiratory Infection and influenza-like Illness in Children under Five Years Old at health facilities serving as influenza sentinel sites in Ethiopia.

Methods: This study employed a cross-sectional study design. Epidemiological data and throat, nasal, and nasopharyngeal swab samples were collected from 14 influenza sentinel sites for influenza surveillance from January to April 2026. After testing for influenza, select all samples under 5, extract, and perform detection at the Influenza National Reference and Referral Laboratory of the Ethiopian Public Health Institute (EPHI). A Chi-squared test was used to compare differences, and a bivariate logistic regression analysis was used for associated factors with independent variables.

Result: A total of 422 children under five were tested across 14 Ethiopian influenza sentinel sites, which found that (131) 31% were positive for at least one viral pathogen and (156)37% for at least one bacterial pathogen, with the highest burden concentrated in infants under two years. Human Adenovirus (32) 7.5%, Influenza (27) 6.4%, and RSV (19) 4.5% were the leading viral pathogens, while *Haemophilus influenzae* (84) 20% and Streptococcus pneumoniae (58)13.8% dominated the bacterial pathogen findings. A viral co-detection rate of 20 of 131 (15.2%) was recorded, and a bacterial-viral co-detection rate of 4.6% of the total 64 co-detections; this finding underscores the remarkable complexity of pediatric respiratory illness in Ethiopia.

Conclusion: This study detected the circulation of viral and bacterial pathogens causing SARI and ILI in children under five, and it confirms that adenovirus, influenza, RSV, H. influenzae, and S. pneumoniae are the dominant etiological agents.

Key words: MULTI- CO- ARI, Co- MARI, CAR- MULTI, MARC- ARI.

1. Introduction

1.1.Background

Acute respiratory infection (ARI) can be described as an infectious process of the upper and lower respiratory tract caused by bacterial, viral, and Fungal agents. Globally, it's one of the leading causes of mortality and morbidity (1,2) According to the global burden of respiratory disease report in 2019, there were around 17.6 billion ARI cases reported globally, of which 2.5 million deaths were recorded. The maximum incidence and mortality rates are observed among children younger than 5 years of age. The mortality rate due to ARI in developing countries is 10 to 50 times higher than in developed countries (3,4) .

The World Health Organization (WHO) estimates that approximately 1.9 -2.2 million children die annually due to acute respiratory infections; of these deaths, 70% occur in Africa and Southeast Asia. Around 60% of the causative agent is Viral (5,6).

The causes of ARI in children under five include viruses, bacteria, and fungi. These pathogens can lead to infection in the upper respiratory tract, such as rhinitis and laryngotracheitis, as well as more serious infections in the lower respiratory tract, including bronchitis, bronchiolitis, and pneumonia (7). Especially, pneumonia is the most common complication and the major cause of mortality among children presenting with ARI; it accounts for 2 million deaths among children under 5 years of age annually (3).

Many pathogens are associated with ARI, and the signs and symptoms are similar across causative agents. Therefore, it makes it difficult to distinguish by clinical manifestation without a laboratory diagnosis (7).

Several viral agents such as respiratory syncytial virus (RSV), influenza virus type A and B, human rhinovirus(HRV), human adenovirus(HADV), human corona virus(HCOV) human parainfluenza virus(HPIV), human metapneumovirus(HMPV), human bocavirus (HBOV), and severe acute respiratory virus syndrome coronavirus 2(SARS-COV-2) are represent the most common viral causa of acute respiratory Infection(ARI) and The bacterial pathogen such as *Streptococcus pneumoniae(*SP*)*, *Mycoplasma pneumonias*(MP), *Staphylococcus aureus*(SA) and *Haemophiles influeanza* (HI) are frequent cause of ARI etiological agent (6,8–12).

Previous studies have shown that respiratory viral co-infection increases disease severity compared with single infections, and that children have a higher rate of co-infection than other age groups. Around 30.2% had co-infection with 2-4 viral pathogens. The primary pathogens detected were respiratory syncytial virus(RSV), rhinovirus, and influenza virus (13,14).

Several factors predisposing children under five years of age to ARI infection were studied in various parts of the world. The most common factors include demographic, preexisting medical conditions, socioeconomic conditions of individuals, geographic locations, co-morbid disease, Education status, immunocompetence, chronic lung disease, environmental factors, chronic heart disease, and asthma (15–20).

1.2. Statement of Problem

Globally, Acute lower respiratory infection (ALRI) remains highly impactful on the cause of childhood hospitalization and mortality in young children (under 5 years old), and In the 2021 report, there were an estimated 344 million incident episodes of lower respiratory infection (LRI) and 2.18 million (1.98-2.36) death globally and another global meta-analysis report showed that *Streptococcus pneumoniae* causes around 97.9 million lower respiratory infection episodes and 505,000 deaths globally. Other viral pathogens and Mycoplasma species caused about 46.4 million and 25.3 million cases, respectively, while *Staphylococcus aureus* and *Klebsiella pneumoniae* were responsible for around 424,000 and 176,000 deaths, respectively (21).

Data in sub-Saharan Africa have shown that upper respiratory tract infection and pneumonia are identified as the highest causes of morbidity and mortality, respectively. Each year, an estimated more than 900,000 pneumonia episodes occur in children under 5 years of age, leading to almost 8000 deaths annually (22).

In low-resource countries, including Ethiopia, detecting a broad range of respiratory pathogens remains a significant challenge due to limited diagnostic capacity. Consequently, many cases of severe acute respiratory infection (SARI) and influenza-like illness (ILI) are treated based on clinical symptoms alone, without laboratory confirmation of the actual causative agents. This practice results in empirical treatments, which can contribute to inappropriate medication use and poor disease management, a lack of early detection, and an inability to control any viral and bacterial outbreak. The lack of comprehensive pathogen identification challenges effective surveillance and public health response to respiratory infections in these settings (11,23,24).

In Ethiopia, previous studies have been conducted, mostly focused on specific viral pathogens, particularly influenza, COVID-19, and RSV (25–28).

Since 2008, Surveillance of severe acute respiratory infection (SARI) and Influenza-like illness (ILI) has been established in Ethiopia. Currently, the system includes a total of 21 ILI and SARI surveillance sites supported by four sub-national respiratory virus testing laboratories and national influenza reference laboratories (29). However, no study has been conducted on broad pathogens of bacterial and viral pathogens using this surveillance system to date. ARI due to bacterial infection has become a worldwide issue, particularly due to the increasing number of emergency

multidrug-resistant bacterial pathogens. Although some studies have examined the viral etiology of ARI in all age groups in Ethiopia, the information is lacking on bacterial pathogens (30,31).

Many of the viral and bacterial etiologies of ARI in Ethiopia remain unknown, and data are limited to broad information on the circulation of viral and bacterial pathogens. This study aims to address the existing Information gap by providing comprehensive information on the multiple pathogens responsible for SARI/ILI cases. It also sought to emphasize intervention strategies to control respiratory viral diseases.

1.3. Significance of The Study

The viral and bacterial pathogens that cause severe acute respiratory-like illness (SARI) and influenza-like illness (ILI) among children under 5 years of age are less understood in Ethiopia.

Therefore, this study: -

- To develop evidence-based national guidelines based on local data.
- Enhance diagnostic capacity to identify pathogens accurately and guide targeted treatments.
- Invest in robust surveillance to fill gaps on respiratory pathogens.
- Promote antimicrobial stewardship to reduce unnecessary antibiotic use and improve patient outcomes.

2. Literature Review

2.1.Literature review

A systematic analysis of the global burden of non-COVID-19 lower respiratory infections and their causes, based on a study of 204 countries from 1990 to 2021, showed that there are an estimated 344 million (95% uncertainty interval [UI] 325-364) LRI episodes and 2.18 million deaths. Of these, 502,000 deaths (406,000-611,000) occurred in children under 5 years old, and half of the deaths took place in countries with low socioeconomic development. Among 18 pathogens, *Streptococcus pneumoniae* was the most responsible pathogen for causing high ARI episodes, accounting for 97.9 million. followed by viral etiologies (46.4 million), and *Mycoplasma spp* (25.3 million), respectively (21).

A study conducted in the city of Colombia among 5083 nasopharyngeal samples of acute respiratory infections during the COVID-19 pandemic selected 101 typical symptomatic ILI cases for the detection of 18 respiratory viruses. 25.7% of independent cases were infected with a single virus, and co-infection involving two or more respiratory viruses represented 42% of the total of positive cases. Respiratory syncytial virus(RSV) was the most commonly identified virus, accounting for 30.7% of the positive cases, followed by human rhinovirus (HRV) at 15.4% (32).

A cross-sectional study conducted in Thailand between 2019 and 2020 collected nasopharyngeal samples from 169 children under five years old presenting with SARI cases. At least one pathogen was identified in 91.7 % of individuals, and the most frequent pathogen detected was rhino or enterovirus (45.2%), followed by RSV (31.6%), and parainfluenza virus (14.2%) (33).

A retrospective study conducted in China among 1266 SARI cases from January 2018 to December 2020 showed that 58.1% had at least one respiratory pathogen detected. The multiple detection rate was 28.5% (361/1266). The Viral detection rate (36.9%,468/1266) was less than the bacterial detection rate (39.0%,495/1266). The most frequent pathogen were Influenza virus (13.8%,175/1266) followed by *K.pnemonia*(10.0%,127/1266, *S.Pneumonia*(10.0%,127/1266), Adenovirus (8.2%, 105/1266), *P.aeruginosa* (8.2%, 105/12660, *M.Pneumonia* (7.8%, 100/1266), and RSV (7.7%, 98/1266) during different seasons (34).

Another study in China between 2019 to 2022, the detection of common respiratory pathogens from a total of 4804 collected children's throat samples showed that the total positive rate was 77,8%(3741/4804), 34.47%(1656/4804) were positive for a single pathogen and 43.40% (2085/4804) had identified more than two pathogens in different age groups and the highest overall positive detection rates occurred in spring (43.58) followed by Summer(38.64%), autumn (33.73%), and winter (29.7%). *Streptococcus pneumoniae* and *Haemophilus influenzae* were most prevalent in spring; *Mycoplasma pneumoniae* in summer; Rhinovirus, respiratory syncytial virus, and influenza virus in autumn; and *Chlamydia pneumonia,* Bocavirus, and influenza B virus in winter (35).

A prospective study in Bangladesh (Dhaka city) conducted bacterial and viral spectra in children under 5 years old with ARIs among 200 collected nasal swabs, 82.5% of specimens were positive, with 3%(6/200) single bacterial infections, while 43.5% (87/200) cases had only a single viral infection. The other 36% (72/200) cases were co-infections, and human rhinovirus was the most predominantly detected virus with a detection rate of 31.5%, subsequently by RSV (31%), HMPV (13%), HBOV (11%), HPIV-3(10.5%), and adenovirus (7%). *Streptococcus pneumoniae* was the most commonly isolated bacterial pathogen at a detection rate of 9%; similarly, *Klebsiella pneumoniae* (5.5%), *Streptococcus spp*(5%), *Enterobacter agglomerans*(2%), and *Haemophilus influenzae* (1.5%) were isolated from collected nasal swabs by culture methods (8).

A study held in West Bengal from 2021-2022 among 390 ILI cases (nasopharyngeal swab) of 0-2-year-old children by using multiplex PCR showed that 23.3% (91/390) of viruses were detected, 15% of the viruses were identified as single viral infection, and 1.3% of cases had co-infections. The most detected virus was RSV (12%, 49/390), followed by influenza (6.6%, 26/390), and SARS-CoV-2 (0.77%, 3/390). Among influenza subtypes, H3N2 (50%,13/26), H1N1 pdm2009 (34.6%.9/26), and Influenza B 15.4%,4/26) were detected. Other viruses, like parainfluenza virus 3(4.4%), bocavirus (3.7%), and adenovirus (3%) were identified. The major bacterial findings were *Streptococcus pneumoniae* (55.5%,75/135), *Haemophilus influenzae* (29%,39/135), and *Moraxella catarrhalis* (3.7%,5/135), and 20 % were identified as bacterial and viral co-detection (23).

In 2019/2020 there was a study in Italy to evaluate the co-infection of viral, bacterial, and viral bacterial infection from 2027 nasopharyngeal samples from ILI case there were found predominantly influenza A/H3N2 (14.40%) followed by rhino virus(14.30%), coronavirus (5.75%), and adenovirus (5.22%) and regard to bacterial infection the most frequent pathogen was *Heamophilus influenza*, *Streptococcus pneumonia*, and *Mycoplasma pneumonia* account 37.98%, 19.93%, and 3.60% respectively (36).

A systematic review on the burden of influenza virus and non-influenza respiratory virus contribution on SARI and ILI surveillance data conducted in Belgium, from 2015 to 2019 by using in-house multiplex qPCR result showed that from ILI, the most detected pathogen was picornavirus (5.1%;91/1791) and HCOV (4.3%;77/1791) followed by HMPV (2.3%;42/1791), and RSV (2.3%;41/1791). The contribution of other viruses like parainfluenza virus, adenovirus, and bocavirus was estimated at 1.4%,0.8%, and 0.1%, respectively; however, among SARI cases, the predominant pathogens were HMPV (6.1%;291/4744), HRSV (5.0%;238/4774), and coronavirus (3.4%;162/4,744), single detection of parainfluenza (1.4%. Adenovirus (1.7%), and bocavirus (0.9%) were detected (37).

A systematic review study conducted in sub-Saharan Africa identified RSV as the most common viral pathogen in children with respiratory infection, ranging from 0.6% to 59%, followed by Rhinovirus with a prevalence of 7.5% to 73%. Among bacterial pathogens, *Streptococcus pneumoniae* (1% to 96%) and *Haemophilus influenzae* (2.5% to 54%) were the predominant causes (38).

A Cross-sectional study held in South Africa from 2020-2021among 84 SARI cases of children under five age using RT-PCR detection methods showed that the total viral positivity rate was 88%(74/88) and from 21 target pathogen human rhinovirus/enterovirus (30%), RSV (26%), SARS-COV-2 (24%), and other viruses (20%) were the most frequently detected pathogens. The co-infection rate was 64.2%(54/84) (11).

Another prospective syndromic hospital surveillance study conducted in South Africa between 2018 and 2022 among 2595 children less than 1 year old who met the SARI case definition by taking nasopharyngeal samples for the detection of multiple pathogens using RT-PCR identified Rhinovirus (14.80%, 228/1540) and *Streptococcus pneumoniae* (28.50%, 439/1540) as the most

predominant pathogens detected from nasopharyngeal or oropharyngeal samples. Among the viral and bacterial pathogens associated with SARI cases were Influenza A and B, HRV, RSV, HCoV-229e, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* (15).

The study conducted in morocco on children under five years old from 2017 to 2019 collected nasopharyngeal samples. Among influenza-confirmed cases, 68.75%, 15.17%, and 16.04% were subtyped as influenza A (H1N1) pdm09, A (H3N2), and Influenza B, respectively. Among the positive cases, influenza affected children with SARI greatly (39).

A study conducted in Tanzania, sentinel surveillance for ILI and SARI cases between 2017 to 2019 using a multiplex RT-PCR kit among 255 samples showed that the most frequently detected viral pathogens were RSV(22.9%), Rhinovirus(21.8%), and Adenovirus(14.0%), and additionally, the prioritized bacteria were Klebsiella pneumoniae, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* (40).

A multicenter study conducted in Egypt in 2022 among 317 hospitalized children with SARI cases showed that the prevalence of 72.7% positive cases, the virus caused 92.1 % of infections, with RSV being the most common at 63.8% of infections, followed by RV at 10.0%, Influenza at 9.2%, Adenovirus at 5.2%, and 1.3% had co-infection with two viruses. Bacterial infection accounted for 3.5% of the cases, and 4.4% had mixed viral-bacterial infections (41).

The Study conducted in Kenya among children under 5 years of age living in refugee camps from 2010 to 2012, using SARI and ILI case data, showed that 40.3% of positive tests were positive for one or more viruses. Of these, the most frequently detected pathogen was Influenza A(36.9%), followed by Adenovirus (29.9%),Influenza B(20.2%), and para influenza 1,2, or 3(14.4%) (42).

The cross-sectional study conducted in northwest Ethiopia, ARI and its associated factors among children under five years of age showed that Age, location of residence, maternal factors, environmental-related factors, and education status were significant factors identified to be associated with an acute respiratory infection (43).The recently published influenza surveillance study data in Ethiopia from 2021-2022 among acute respiratory infection (both SARI and ILI) cases indicate that the influenza virus was detected in 11.1% of the tested samples(25).

3. Objectives

3.1.General objectives

- To identify viral and bacterial causative agents of respiratory tract infection in children <5 years with SARI/ILI at selected sentinel sites in Ethiopia, January to April 2026.

3.2.Specific objective

- Molecular detection and characterization of viral respiratory and bacterial etiologies among influenza-like illness and severe acute respiratory illness cases using real-time PCR.
- To assess the co-infections among acute respiratory illness cases.
- To identify factors associated with the occurrence of respiratory viral and bacterial infections among children under five years of age.

4. Materials and Methods

4.1. Study design and Period

The present cross-sectional study was conducted from January to April 2026 at the National Influenza Center, Ethiopian Public Health Institute (EPHI). Consecutive samples from children under 5 years of age were selected from those collected for the influenza test during the study period.

4.2. Study Setting

The study was conducted in selected health facilities found in 4 regions and 2 city administrations. In total, samples collected from 14 government health facilities (11 hospitals and 3 health centers) were analyzed. The hospitals were to serve as SARI surveillance sites, while the health centers were designated for ILI surveillance. All the selected health facilities, including the study sites, were purposively selected in accordance with the WHO protocol for routine influenza surveillance, based on geographical criteria, patient load, and staff profile. To ensure focused monitoring in the selected areas while aligning with national public health priorities for respiratory pathogen detection monitoring (44,45).

4.3. Population

4.3.1. Source population

The source population was all patients with acute respiratory illness cases from January to April 2026.

4.3.2. Study population

The study population was all patients weekly among under-5-year-olds from January to April 2026 with sufficient sample volume and complete data from all influenza sentinel sites.

4.4.Sample size determination

Sample size calculation was performed using the single population proportion formula, and since there is no published study data about the molecular detection of common respiratory viruses and bacterial pathogens causing SARI and ILI in Children under Five Years old in Ethiopia.

$$\boldsymbol{n = \frac{Z^2 p(1-p)}{D^2}}$$

Where n – minimum sample, P – Proportion, q = 1-p, Z – Significance level at confidence interval of 95%, Zα/2 = value of the standard normal distribution corresponding to the significance level of α 0.05, which is 1.96

$$\boldsymbol{n = \frac{1.96^2 0.5(1-0.5)}{0.05^2}}$$

n= 384, and so by considering insufficient sample volumes and incomplete demographic data, we calculated 10% contingency, and the total minimum number of samples to be tested will be 422.

4.5. Sampling Methods and Procedures

4.5.1. Sampling method

Weekly, the National Influenza Center (NIC) laboratory at the Ethiopian Public Health Institute (EPHI) received nasopharyngeal and/or oropharyngeal swab samples from each designated influenza sentinel surveillance site across Ethiopia. These samples were collected for routine molecular testing of influenza viruses (A and B) and SARS-CoV-2, following standardized national surveillance protocols established by EPHI in alignment with WHO guidelines for integrated respiratory pathogen monitoring (44,45).

For this study, which focused on severe acute respiratory infection (SARI) and influenza-like illness (ILI) in children under five years of age, all consecutive samples from children under five years of age were systematically selected each week from every participating sentinel site during the study period from January to April 2026. Selection continued weekly until the predetermined full sample size was achieved, ensuring unbiased, comprehensive enrollment that captured the

complete spectrum of pediatric respiratory cases without exclusion based on initial test results or other criteria.

4.5.2. **Specimen collection and transportation**

Nasopharyngeal or nasal swab samples were collected daily from suspected patients at 21 sentinel sites, based on the SARI/ILI case definition, by highly trained focal personnel. These sentinel sites enrolled patients according to the WHO SARI/IL case definition (45). If a site is designated as a SARI site. SARI case definition applied; Severe acute respiratory infection (SARI) is characterized by acute respiratory infection presenting with a history of fever or measured Fever of > 38 °C, cough with disease onset within the past 10 days and requiring hospitalization, on the other hand, if the site designated as an Influenza -Like Illness (ILI)site, ILI case definition used, Defined as an acute respiratory infection with a history of fever or measured fever of >38 °C, cough, with disease onset with in the past 10 days, but hospitalization is not criteria in this definition. Each sentinel site has a protocol for collecting a throat or nasopharyngeal swab specimen, and each focal person at the sentinel sites is well trained in specimen collection, management, and transportation. Before collecting the sample, the focal person obtained verbal consent from the first five ILI and all SARI cases who met the case definition (27). For this study, we were requested to provide a written parental consent form as detailed below in Annex (1). The collected specimen is placed in viral transport medium (VTM). All collected samples were stored at 4 °C until transported by postal service officers to the influenza reference laboratory at the Ethiopian Public Health Institute (EPHI). Shipment of specimens in viral transport media in a triple package using sufficient ice to keep the temperature in the refrigerator, with complete record information, and a weekly aggregate of SARI and ILI cases submitted to EPHI (44,46).

4.6. Inclusion and exclusion criteria

4.6.1. Inclusion criteria

All samples collected were from only children under five years of age from influenza sentinel sites from January to April 2026. With sufficient volume, complete case-based information was used for this study.

4.6.2. Exclusion criteria

Samples with insufficient volume, unlabeled samples, antibacterial drug use, and incomplete case-based information were excluded from this study.

4.7.Study variables

4.7.1. Dependent Variables

- Detection status of viral and bacterial pathogens and identification of co-detection using RT-PCR

4.7.2. Independent Variables

- Socio-demographic information (age, sex, place of residence)
- Clinical signs and symptoms
- Underlying medical condition

4.8.Laboratory Methods

4.8.1. Viral nucleic acid extraction

After receiving ethical consideration from the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, permission was obtained from EPHI. The selected under-five-year-old samples were extracted using the N32 extraction kit on automated nucleic acid extractors (MGISP-NE32, Wuhan, China), following the manufacturer's instructions. This extraction process is automated, with all necessary reagents prepared and sealed by the company. According to the SOP, see in detail below (Annex IV). We add only 300 μL of the swab samples into the designated sample well of the prepared reagent comb to wells 1 and 7 based on the printed sample ID and then insert it into the machine, which automatically performs four primary steps using magnetic bead principles: lysis, binding, washing, and elution. Finally, the machine discards the magnetic beads and delivers 60 μL of eluted sample from wells 5 and 11 (47).

4.8.2. Master mix preparation and PCR setup

For the detection of both viral and bacterial pathogens from extracted nucleic acid, we were use a multiple detection of nucleic acid kit for 18 respiratory pathogens (Fluorescence PCR), which helps to detected 9 respiratory viral pathogens and 9 bacterial pathogens from one sample at the same time from 6 different reaction tubes, performed according to standard operating procedure(SOP) for details in below (annex V). The master mix is supplied in six tubes labeled A -F, each containing reaction buffer, dNTPs, primers, probe, and internal controls. Each tube contains a mix of primer, probes, dNTPs, and internal controls of 3 different target pathogens. To prepare the reaction mix for one extracted RNA, add 18.5 µL of the reaction mix and 1.5 µL of the enzyme mix to each tube. After preparing the master mix, dispense 20 µL of the prepared mix into each designated well of the PCR plate. Then add 10 µL of the extracted RNA, Positive controls, and blank controls into their respective assigned wells. The master mix was prepared using the kit protocol. Then load the elute and reagent mix into the RT-PCR. The viral RNA is transcribed into cDNA using a specific primer-mediated reverse transcription step, followed immediately in the same tube by polymerase chain reaction (PCR) for RNA viruses. However, the DNA of different pathogens is amplified through denaturation steps simultaneously in the same tube by PCR. The presence of specific pathogen sequences in the reaction was detected by an increase in fluorescence observed from the relevant dual-labeled probe and was reported as a cycle threshold value (Ct) by the real-time thermocycler. Depending on the presence of exponential amplification, the result is interpreted as negative, invalid, or positive.

4.9.Quality Assurance

During the extraction and master mix preparation, we strictly follow all relevant guidelines, standard operating procedures (SOPs), and protocols. For each extraction run, a negative control using nucleus-free water was processed alongside the samples to monitor potential contamination. This control helps ensure reagent integrity and the accuracy of the procedures. Additionally, Positive and negative controls are included in each run to verify the validity and reliability of the entire process. The RT-PCR setup was always independently reviewed and verified by experienced and trained laboratory personnel to maintain quality and minimize errors.

4.9.1. Pre-analytical phase

A Socio-demographic characteristic of patients was rechecked from a case-based format. All samples were collected by well-trained laboratory personnel following the standard operating procedure. When specimens reach the laboratory, it was checked to ensure that the correct specimen has been sent and that the name on the specimen is the same as that on the specimen collection form. To avoid sample contamination, a leak-proof and sterile sample container was used. The extracted RNA was properly stored in -20 °C until performing the run and recording the refrigerator temperature daily. Expiry date of the extraction kit and RT-PCR reagents.

4.9.2. Analytical phase

All used RT-PCR assays incorporated positive controls (known viral templates to confirm reaction efficiency), negative controls (nucleic acid-free water to detect reagent contamination), and non-template controls (reaction mix without adding nucleic acid to identify carryover). At each extraction procedure, an extraction template (mock extraction) was used to validate the extraction processes. These measures minimize error, ensure assay reliability, and prevent contamination throughout the procedures. All PCR reagents were prepared according to the manufacturer's instructions. Pre-PCR setup and reagent mixing were conducted in a safety hood to prevent reagent contamination.

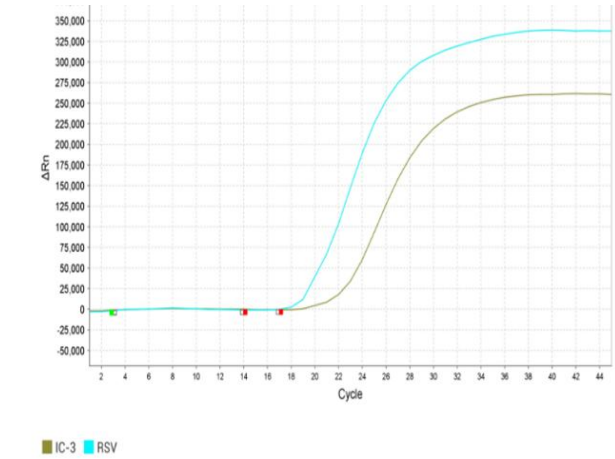
4.9.3. post-analytical phase

During the post-analytical phase, the results were recorded with the patients' identification numbers. The terminology and format used in reporting were standardized. All used reagents should be properly labeled and returned to their place according to storage conditions.

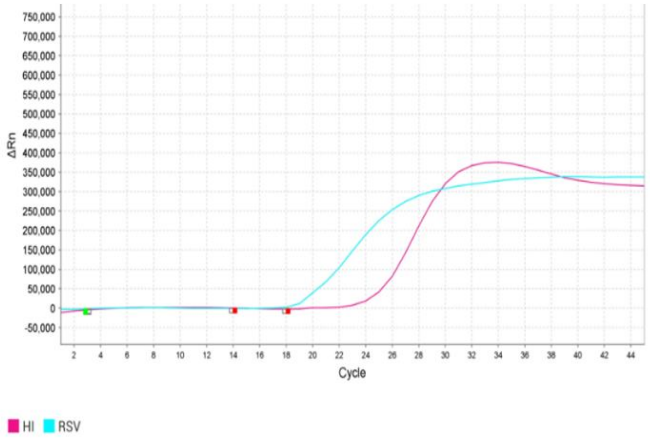
4.9.3.1.Result Interpretation

For samples to be considered as positive, all the following essential criteria should be fulfilled,

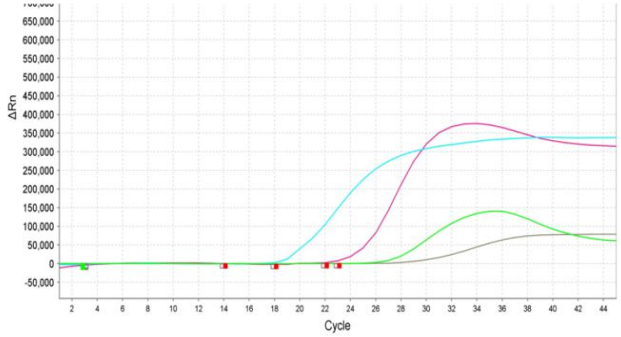
A. Internal control and positive control cycle threshold (CT) value less than 33
B. No amplified target at negative control
C. Standard amplification curve or sigmoidal shapes should be observed for samples; However, if A and B are fulfilled and a standard amplification curve was not observed for the samples, the result was interpreted as Negative.


ΔRn
Cycle
IC-3 RSV

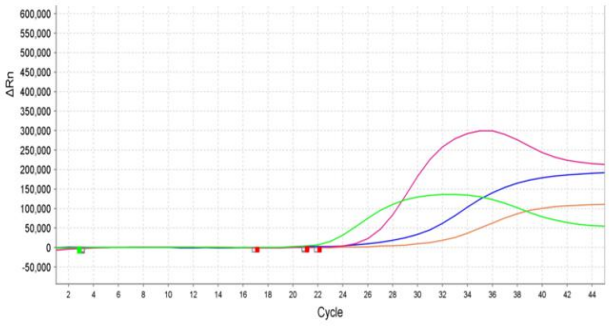
A


ΔRn
Cycle
HI RSV

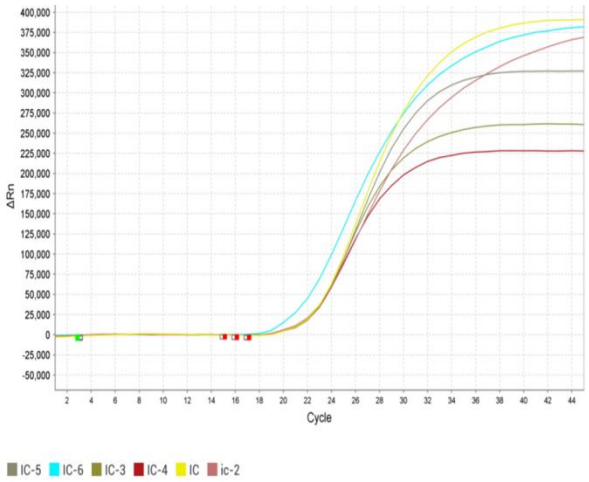
B


ΔRn
Cycle
IC-5 HI RSV SP

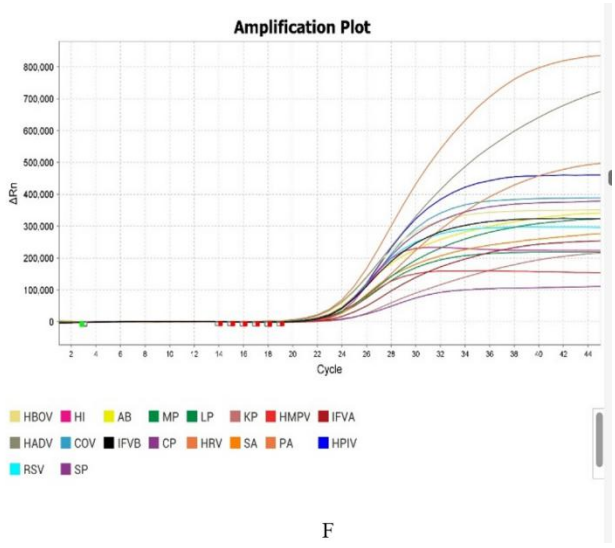
C


ΔRn
Cycle
HI HPIV SP HRV

D


ΔRn
Cycle
IC-5 IC-6 IC-3 IC-4 IC ic-2

E


Amplification Plot
ΔRn
Cycle
HBOV HI AB MP LP KP HMPV IFVA
HADV COV IFVB CP HRV SA PA HPIV
RSV SP

F

Figure 2; RT-PCR amplification plot A, Single detection B, Double detection C, Triple detection D, Quadruplex detection E, Internal control F, Positive control

4.10. Data collection procedures

For this study, we got permission from EPHI. For all selected patients' socio-demographic data, sample ID, location of the health facility, age, sex, and any other relevant information were taken from the existing completed case-based formats (SARI and ILI), shown in detail below (annex 5). To ensure the completeness of the data, it was cross-checked from DHIS-2, which is collected at the site level during surveillance. The Laboratory data quality was maintain by recording result of each sample run from the RT-PCR analysis after the end of every run using the printed PCR plate or worksheet and rechecking it before and after the data entered to the excel spread sheet and IBM SPSS software version 25 and also, The surveillance data quality were check the completeness and validity within a weekly by prepared checklist for key variables from line-listed data.

4.11. Data analysis and interpretation

All selected patient demographic data and laboratory results were entered into Excel version 16 to clear the data and were analyzed using IBM SPSS software version 27. The number of tested cases and proportionality categorical variables such as sex, ILI/SARI case classification, referring health facility, types of viral and bacterial pathogens, associated factors, and co-infection was be presented by percentage. A binomial logistic regression model was used to analyze the relationship of viral and bacterial pathogens and independent variables. Chi-square test to compare the differences in magnitudes of viral and bacterial positivity among independent variables. $P<0.05$ was considered statistically significant and analyzed by using a logistic regression model.

4.12. Ethical considerations

The proposal was approved by the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, and permission was also obtained from EPHI. The participants in this study had already given oral consent for influenza surveillance and other pathogen detection. No personal identifier is used at any stage of the study, and the data are archived securely.

4.13. Dissemination of the result

The results of this study were reported in a formal document and presented to the Department of Medical Laboratory Sciences, Addis Ababa University, and the Ethiopian Public Health Institute. Additionally, any test results were immediately reported to the site for patient treatment. Research findings were presented to and communicated to the international community through conferences and a peer-reviewed journal.

4.14. Operational definition

Acute respiratory infections (ARI); defined as an infection process of the upper and lower respiratory tract caused by bacterial and viral agents.

Severe acute respiratory infections (SARI) are characterized by acute respiratory infections presenting with a history or measured fever of > 38°C, cough, disease onset within the past 10 days, and requiring hospitalization.

Influenza-like illness (ILI): Defined as an acute respiratory infection with a history or measured fever of > 38°C, cough, with disease onset within the past 10 days, but hospitalization is not a criterion.

Co-detection: the condition where both viral and bacterial pathogens are detected simultaneously in the same patient.

Sentinel sites: Specific selected health facilities designated to systematically collect epidemiological data and virological specimens for influenza and respiratory pathogens surveillance in Ethiopia.

5. Results

5.1. Sociodemographic and Clinical Conditions of Study Participants

During the study period, 422 respiratory samples were collected from children under 5 years of age presenting with influenza-like illness (ILI) and severe acute respiratory illness (SARI) at 14 sites. The participants included 228 (54%) females and 194 (46%) males, indicating a slight male predominance. Most of the study participants were 1 -2 years of age, 194 (46%), followed by those aged less than 1 year, 139 (32.9%). The average age of participants was 1 year and 8 months and had an SD of 1,16. Most of the study participants had SARI cases, 304 (72%), while 118(28.0%) had ILI cases.

All patients presented with measured or historical fever 38 C and cough within 10 days of symptom onset, which was the main inclusion criterion to enroll the case. Among the symptoms reported, difficulty breathing, 257 (60.9%), and runny nose, 240(56.9%), were the most common clinical manifestations. However, sore throat ,101(23.1%), vomiting ,97(23.0%) and headache 17(4.0%) were also reported. Regarding specimen type, nasopharyngeal swabs were the most collected samples, 338(80.1%), followed by throat swabs, 58 (13.7%) (Table 1).

Table 1: Demographics and clinical characteristics and viral and bacterial detection rates among ILI/SARI study participants in selected health facilities in Ethiopia, Jan to

April 2026 (N=422).

Demographic Characteristic	Category	Frequency	Percentage
Sex	Male	228	54
	Female	194	46
Age, Mean (SD)	1.68 (1.164)		
Age group * (n=422)	< 1 yr	139	32.9
	1-2 yr	194	46.0
	3-5 yr	89	21.1
Admission type	SARI	304	72
	ILI	118	28
Specimen type	NP	338	80.1
	TS	58	13.7
	NS	26	6.2
Symptom	Arteritis (Joint Piane	95	22.5
	Runny nose	240	56.9
	Headache	17	4.0
	Sore throat	101	23.9
	Hemoptysis	3	0.7
	Difficulty of breathing	257	60.9

Abbreviation: - NP=Nasopharyngeal swab, TS=Throat swab, NS=Nasal Swab, ILI=Influenza like illness, SARI=Sever acute respiratory illness * Age group=mean, Standard deviation (1.68 (1.164)

The distribution of participants among facilities shows that some facilities contribute substantially more to participants than others. Facilities such as AKA, MEK, YEK, ADR, KOL, and SPH contributed relatively high numbers in catchment population size, patient flow, or strong surveillance practice among the sites, whereas ZMH, DUB, DFH, DCH, BGH, and ASO contributed very small numbers due to low under-5 patient flows. In more overall facilities, the highest cases were observed among children aged 1-2 years and those younger than 1 year, and both female and male participants were represented across all the study sites (**Table 2**)

Table 2: Distribution of ILI/SARI across the selected health facility by sex and age group of Study participants in Ethiopia, Jan to April 2026 (N=422).

	Female				**Male**				
	Age group in years			**Female**	Age group in years			**Male**	Ground Total
Facility	< 1	1-2	3 -5	**Total**	< 1	1-2	3 -5	**Total**	
ADR	6	16	3	25	7	15	8	30	55
AKA	6	15	20	41	3	9	18	30	71
ASO	0	1	1	2	0	0	0	0	2
BGH	0	0	1	1	2	3	0	5	6
DCH	2	0	0	2	1	0	1	2	4
DFH	0	1	1	2	0	0	0	0	2
DUB	3	5	1	9	4	4	0	8	17
FGH	4	0	0	4	4	9	0	13	17
KOL	5	13	3	21	9	11	4	24	45
MEK	13	15	6	34	14	12	4	30	64
SGH	3	5	3	11	3	9	3	15	26
SPH	2	7	1	10	17	6	5	28	38
YEK	10	12	2	24	12	19	3	34	58

ZMH	2	5	1	8	7	2	0	9	17
Total	56	95	43	194	83	99	46	228	422

Key: - ADR: Adari Hospital, AKA: Akaki health center, ASO: Asosa Hospital, BGH: Butajira Hospital, DCH: Dilchora Hospital, DFH: Dilfre health center, DUB: Dubti Hospital, FGH: Felegehiwot Hospital, KOL: Kolf Health Center, MEK: Mekelle Hospital, SGH: Shenengibe Hospitals, SPH: St. Petros Hospital, YEK: Yekatit 12 Hospital, ZMH: Zewuditu Memorial Hospital

5.2. Respiratory Virus Distribution

Among the 422 enrolled cases, the overall positivity rate was 31.0% (131/422), comprising 35% (67/194) and 28% (64/228) positive samples from females and males, respectively. showing that nearly one- third of participants are positive for at least one respiratory virus. HADV was the most frequently detected respiratory virus, which accounted for 7.5% (32), followed by Influenza 6.4% (27), RSV 4.5% (19), HCOV 3.8% (16), HBOV 4% (17), HRV 3% (13), HPIV 1.1% (5), and HMPV 0.4% (**Figure 3**).

Most respiratory viruses were observed in children <1 year (37%, 51/139), followed by 1-2 years (29%, 56/194), while a low positivity rate was observed at age 3-5 years (27%, 24/89), with different frequencies. This suggests that younger children, particularly infants, are more vulnerable to respiratory viral infection. In relation to admission type, the positivity rate was higher at ILI sites (40.7%,48/118) than at SARI sites (25%,77/304), even though sample volume was not evenly distributed across sites (Table 3). Most of the influenza viruses detected were type A; 26 out of 27 (96.3%), and 1out of 27 (3.7%) was type B (Figure 4).

Table 3: Respiratory Virus Distribution with age, sex, and case category

Demographic Data	Category	Respiratory Virus N (%)								
		HBOV	HMPV	HCOV	HPIV	HRV	RSV	HADV	Influenza	Positivity (%)
Sex	Female N=194	9(4.6)	2(1.0)	10(5.2)	3(1.5)	8(4.1)	7(3.6)	15(7.7)	13(6.7)	67(35)
	Male N=228	8(3.5)	0(0.0)	6(2.6)	2(0.9)	5(2.2)	12(5.3)	17(7.5)	14(6.1)	64(28)
Age category	<1 year N=139	8(6)	1(1)	5(4)	2(1)	8(6)	8(6)	12(9)	7(5)	51(37)
	1-2 years N=194	7(4)	1(1)	7(4)	2(1)	5(3)	10(5)	14(7)	10(5)	56(29)
	3 -5year N= 89	2(2)	0(0)	4(4)	1(1)	0(0)	1(1)	6(7)	10(11)	24(27)
Admission type	ILI N=118	6(5.0)	0(0.00)	14(11.9)	0(0.0)	1(0.8)	0(0.0)	15(12.5)	18(15.3)	54(45.7)
	SARI N=304	11(3.6)	2(0.7)	2(0.7)	5(1.6)	12(3.9)	19(6.3)	17(5.6)	9(3.0)	77(25.3)
Overall Positivity (%)		17(4)	2(0.4)	16(3,8)	5(1.1)	13(3.0)	19(4.5)	32(7.5)	27(6.4)	**131(31)**

HBOV=human boca virus, HMPV= Human metapneumovirus, HCOV=Human corona virus, HPIV=Human para influenza virus, HRV=Human rhinovirus, HRSV=Respiratory syncytial virus, HADV=Human adenovirus

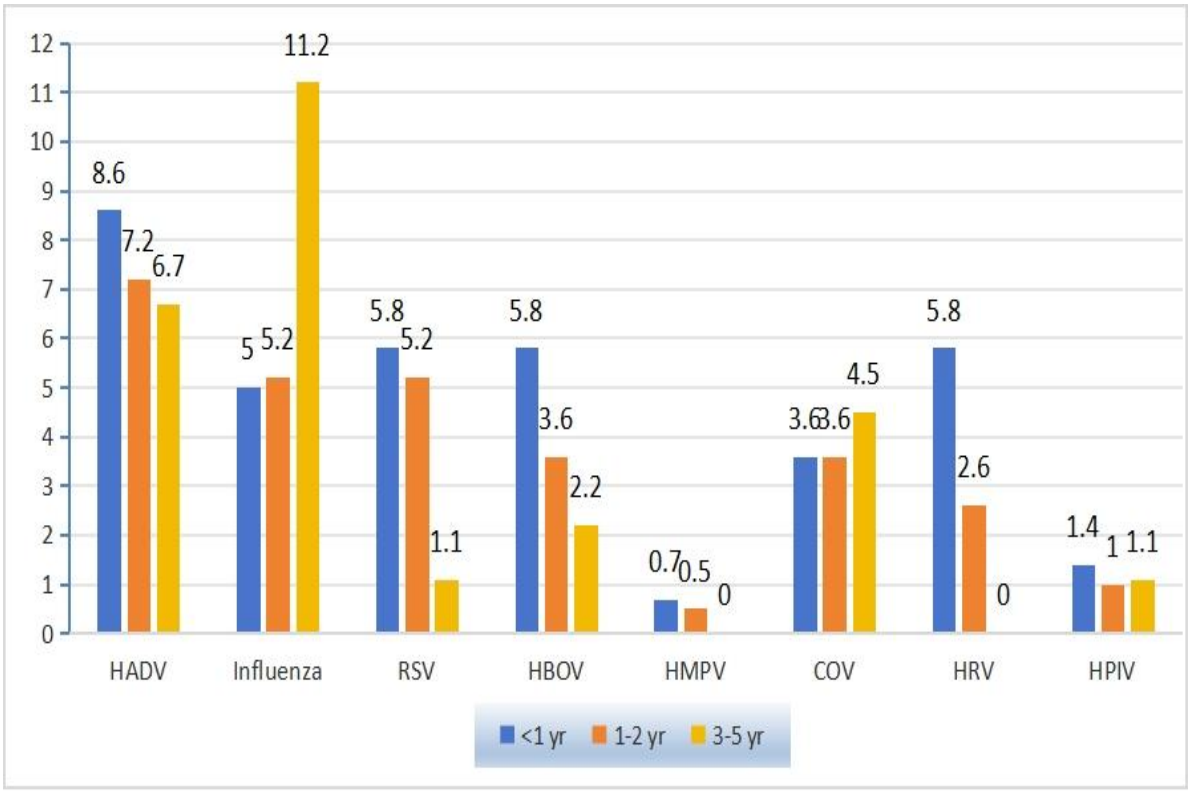


Figure 3:Types of respiratory virus detected across different age groups

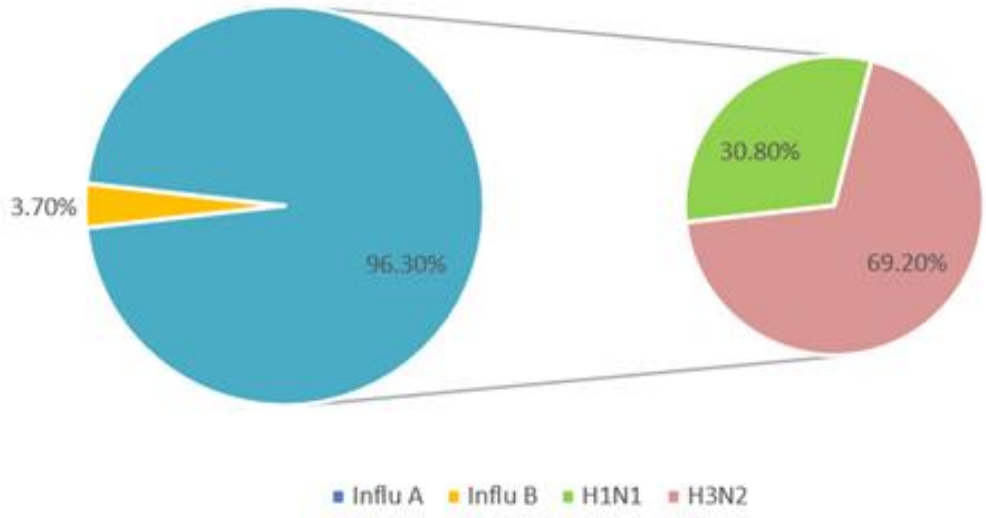


Figure 4: Types and subtypes of influenza virus detected among the study population

5.3. Bacterial pathogen distribution

The overall bacterial positivity was 37% (156), indicating that a bacterial pathogen detected more than one-third of participants. The most frequently detected bacterial pathogen was *Haemophilus influenzae,* 20% (84/422), followed by *Streptococcus pneumoniae,* 13.8% (58/422). other bacterial were detected at much lower frequencies, showing that the bacterial profile was dominated by these common respiratory pathogens. The bacterial positivity rate was relatively similar between females and males, although a slight difference was observed in females with 38.6% (75/194), compared to males with 36.8% (81/228). (**Table 4)**.

The highest bacterial positivity was recorded in the 1-2 age group with 39% (76/294), followed by the under-1-year group with 35% (49/139), while the 3-5 age group with 26% (23/89) had the lowest positivity. This indicates that bacterial respiratory infections were concentrated mainly in younger children under 2 years. In addition, ILI cases showed a higher positivity of bacterial infection than SARI cases.

Table 4: Bacterial pathogen Distribution with age, sex, and case category

Demographic Data	Category	Bacterial pathogen N (%)									
		HI	SP	AB	SA	LP	CP	PA	MP	KP	T.P (%)
Sex	Female N=194	42(21.6)	27(13.9)	4(2.1)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	75(38.6)
	Male N=228	42(18.4)	31(13.6)	4(1.8)	2(0.9)	0(0.0)	0(0.0)	2(0.9)	0(0.0)	0(0.0)	81(36.8)
Age category	< 1 year N=139	28(20.5)	19(4.5)	1(0.7)	2(1.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	50(36)
	1–2-year N=194	46(33.0)	27(14.0)	5(2.6)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	81(41.8)
	3- 5 years N= 89	10(11.2)	12(13.5)	2(2.2)	0(0.0)	0(0.0)	0(0.0)	1(1.1)	0(0.0)	0(0.0)	25(28)

Admission type	ILI N=118	29(24.6)	18(15.3)	3(2.5)	2(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	52(44)
	SARI N=304	55(18.1)	40(13.2)	5(1.6)	1(0.3)	0(0.0)	0(0.0)	2(0.7)	0(0.0)	1(0.3)	104(34.2)
Overall Positivity (%)		84(20)	58(13.8)	8(1.8)	3(0.7)	0(0.0)	0(0.0)	2(0.0)	0(0.0)	1(0.0)	**156(37)**

Abbreviation: T.P: Total positivity, N (%): Percentage, HI: *Heamophilus Influenza,* SP: *Streptococcus Pneumoniae,* MP*: Mycoplasma pneumoniae*, PA: *Pseudomonas aeruginosa, SA: Staphylococcus aureus, LP: Legionella pneumophila*, AB: *Acinetobacter baumannii*, CP: *Chlamydia pneumonia*, KP: *Klebsiella pneumoniae*

5.4 Viral co-detection

Among the 8 detected respiratory pathogens, the highest co-infection proportion was observed for HBOV 23.6% (4/17), followed closely by HRV 23.0% (3/13) and HPIV 20% (1/5), and HADV with 12.5% (4/32). suggesting that these viruses were more frequently detected among other respiratory pathogens. In contrast, influenza and COV showed relatively lower co- infection rates (**Table 5**).

Table 5: A summary of viral co-detection rate among study participants (N = 422)

Respiratory virus	**HRSV**	**hADV**	**HRV**	**hCOV**	**HBOV**	**HPIV**	**HMPV**	**Co-infection rate (%)**
Influenza	0	1	1	0	1	0	0	3/27(11.1)
RSV		1	1	1	0	0	0	3/19(15.79)
HADV			0	0	2	0	0	4/32(12.5)
HRV				0	0	1	0	3/13(23.0)
COV					1	0	0	2/16(12.5)
HBOV						0	0	4/17(23.6)
HPIV						0	0	1/5(20)
HMPV					0	0	0	0/2(0.00
Overall Co-infection								20/131(15.2)

Abbreviation: INF=Influenza, RSV: respiratory syncytial virus, HADV: Human adenovirus, HBOV=human boca virus, HPIV=Human parainfluenza virus, HMPV= Human metapneumovirus, HPIV= Human parainfluenza virus, HCOV=Human coronavirus

5.5. Viral and Bacterial pathogen co-detection with the average Ct value among 422 study participants in children under five years old.

Dual and multiple co-detection are also observed across viral and bacterial pathogens. Among the two pathogen infections, SP and HI were the most frequent combination, identified in 10 patients with average Ct values of 21 and 28, respectively. Other dual co-detection: Flu A and SP in 5 patients (Ct 24 and 30), HCOV and HI in 5 patients (Ct-26 and 27), SP and RSV in 5 patients (Ct 26.5 and 27), Flu A and HI in 4 patients (Ct 25 and 28.5), HI and AB in 3 patients (CT 29.5 and 29), HI and HADV in 3 patients (Ct-23 and 29), SP and HBOV in 2 patient (Ct 27 and 30.5), and HI and HRV in 1 patients (Ct 27 and 35) in table 4.

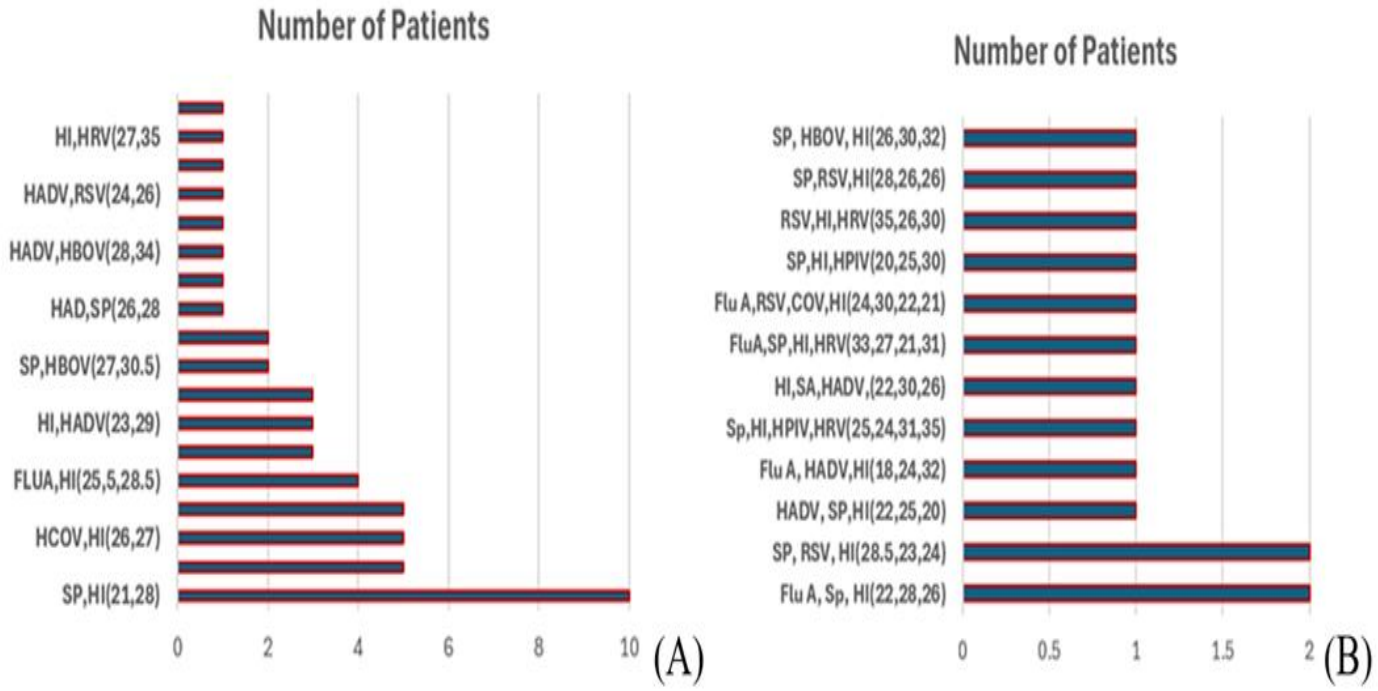


Figure 5: Viral and bacterial co-detection with two pathogens (A) and three or more pathogens (B) with an average CT-Value.

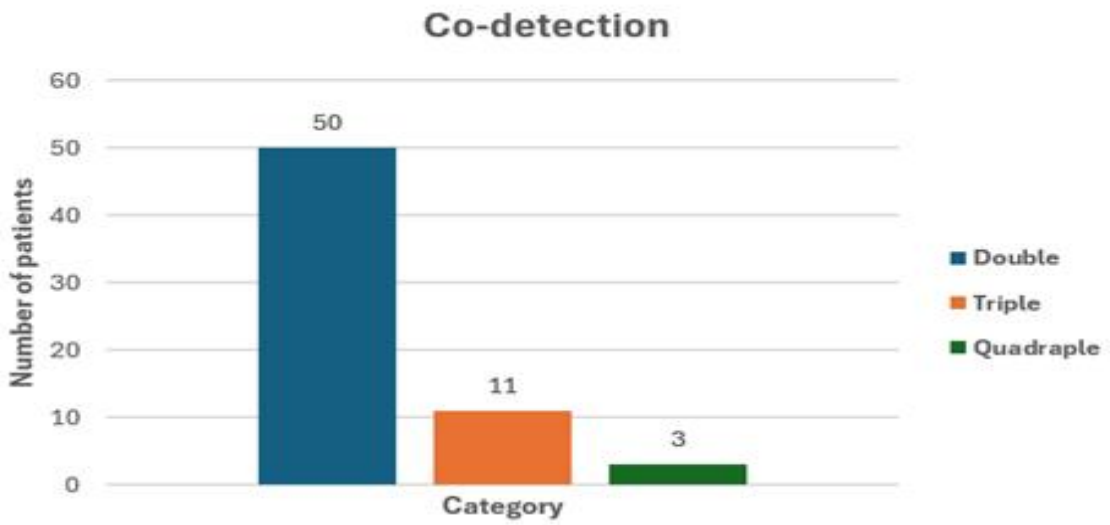


Figure 6: Viral and bacterial Co-detection

5.6. Factors Associated with respiratory and bacterial infections

During the chi-square association test, admission type, joint pain, and sore throat were significantly related to the occurrence of viral and bacterial pathogens. Among clinically significant factors, ILI admission was significantly associated with higher pathogen detection compared to SARI (56.8% vs 38.9%; AOR: 3.06; 95% CI: 1.098–8.490; p=0.018). Bivariate analysis was performed specifically to measure the association of viral infection with the type of specimen, where nasopharyngeal swab showed a higher odds of disease detection when compared to nasal swab (COR: 3.60; 95% CI: 1.06-12.3; p = 0.040). Furthermore, arthritis/joint pain was identified as a significant independent strong predictor of pathogen detection (AOR: 59.0; 95% CI: 20.8-167.5; P <0.01).

Table 6: Multivariate logistic regression analysis of significant factors associated with viral and bacterial infection among the study population (N=422)

Demographic data	Category	N (%)	Detected viral and bacterial pathogens			
			COR (95% CI)	P-Value	AOR (95% CI)	P-value
Admission Type	SARI	118(38.9)	1			
	ILI	304 (56.8)	2.071(1.35-3.19)	0.01**	3.06(1.098-8.490)	0.018**
Arteritis (Joint Pain)	No	330(78.2)	1		1	
	Yes	92(21.8)	56.4(20.14-157.89)	0.01**	59.0(20.7-167.5)	P<0.01
Sore throat	No	321(76.1)	1			
	Yes	101(23.9)	1.76(1.1-2.8)	0.037*	1.84(1.03-3.27)	0.022
Type of specimen (only for viral infection positivity)	NS	26(6.2)	1		-	
	NP	338(80.1)	3.60(1.06-12.3)	0.040*	-	
	TS	58(13.7)	1.60(0.40-6.37)	0.507	-	

Key. **: Indicate statically association (P<0.01) and * for P<0.05; 1: Reference, COR: Crude odds ratio, AOR: Adjusted odds ratio; N (%): Number in brackets are percentage

6. Discussion

This study demonstrates for the first time the simultaneous detection of viral and bacterial etiologies of acute respiratory infection in children under 5 with SARI and ILI within an established national sentinel framework within a three-month study period in Ethiopia. The data presented here are unique, to the best of our knowledge. It represents etiologies of 9 viral targets and 9 bacterial targets. The study used a multiplex PCR assay to detect common viral and bacterial pathogens as causes of acute respiratory infections.

The major findings of this study include: (a) the burden of viral and bacterial pathogens under 5 years of age, (b) co-detection of viral-viral and viral-bacterial, and bacterial-bacterial pathogens, (c) the age-dependent distribution pattern of pathogens was useful to identify the affected age group. (d) Clinical and operational association factors are present for viral and bacterial pathogens.

Of the 422 children tested, respiratory viral pathogens were detected in 131 cases (31.0%) and bacterial pathogens in 156 cases (37.0%), with both groups showing co-detection in a proportion of specimens. The overall viral detection rate of 31.0% observed in this study is broadly consistent with findings from several comparable settings. A study conducted in Kenya among children under five years living in refugee camps reported a viral positivity rate of 40.3% (42), and a study from Ghana documented a positivity rate of 34%(48). our bacterial positivity rate (37.0%) findings are consistent with other reports: Chinese SARI data (2018–2020) showed viral 36.9% and bacterial 38.0%, Colombian ILI viral 42%, and an Ethiopian study bacterial 51.5% (32,34,39,49).

However, both the viral and bacterial positivity rates in this study were lower than those reported from several other settings. Viral positivity rates exceeding 70% have been reported from Morocco (70%) (7), Thailand (91.7%) (33), China (77.8%) (35), South Africa (88%) (11), and Egypt (72.2%) (41). Similarly, bacterial detection rates were lower than those documented in South Africa, where multiplex PCR identified bacterial pathogens in 89% of SARI cases, with Haemophilus influenzae (61%) and Streptococcus pneumoniae (58%) as the most frequently detected organisms (50); and West Bengal, where bacterial pathogens were identified in 55% of nasopharyngeal specimens(32). Several methodological and contextual factors April account for these differences. Studies reporting higher detection rates tended to enroll participants during

peak epidemic seasons when pathogen circulation is most intense, using broader or more sensitive multiplex panels or other specimen types such as bronchoalveolar lavage or lower respiratory tract samples.

Human Adenovirus (HADV) was the most frequently detected viral pathogen in this study 32 cases, 7.4% of all enrolled children), which was similar to the study finding in Bangladesh (7%)(5),Italy (5.2%) (36), and Egypt (5.2%) (41). This respiratory virus was detected in all age categories, with a higher rate among those under two years old (81%,26/32). HADV has been documented as a significant cause of ARI across all age groups in Africa and the global setting (51,52).

Influenza virus was the second most frequently detected pathogen in this study (27 cases, 6.4% of all enrolled children). This result is similar to prior Ethiopian sentinel surveillance studies (2009-2015) (6.4%) (60), West Bengal, India (6.6%) (61), and Egypt (9.2%) (5,41). However, a higher detection rate was shown in China retrospective study (2028-2020) (13.8%)(34), Morocco (13.8%) (7), and Ethiopia (11.1%)(25). The reason is due to the large sample size used, and it includes all seasons.

In this study, the predominant influenza type was influenza A with a rate of (26/27,96.3%) and the dominant influenza subtype was H3N2 with a rate of (18/26,69.2%). In this regard, a study in West Bengal reported H3N2 (50%) dominance over H1N1pdm09(34.6%), and Influenza B (15.4%) (23), and a study done in Ethiopia from 2012 to 2017 found 65% influenza type A and influenza A (H3N2) with 60% (53) ,However, the Kenya refugee camp study found influenza B (20.2%) at a higher proportion (42). This indicates geographic variation in influenza subtype distribution.

Respiratory syncytial virus (RSV) was the third most detected virus, 4.5% (19) of the total samples, and 94.7%(18/19) of RSV-positive samples were from children under two years old, strongly supported by the West Bangladesh study (5). The positivity rate aligned with a systematic review study conducted in sub-Saharan Africa, identifying RSV as one of the leading lower respiratory tract infections in young children, with reported prevalence ranging from 0.6% to 59% (38), and also a systematic review on the burden of influenza and non-influenza virus contribution on SARI/ILI surveillance conducted in Belgium from2019 to 2025 using in-house multiplex

qPCR results that (5%) (40), and a China study(2018-2020) SARI case was detected at 7.7% (34). However, the lower detection rate than the study conducted in Ethiopia, South Africa(26%), and the Thailand study (2019-2020), which detected 31.6% of RSV cases (11,28,33).

The distribution of respiratory viruses regarding admission type was a good finding and clinically important. HADV and influenza demonstrate greater predominance in ILI sites than in SARI sites. HADV was detected at 15.3% among ILI versus 5.6% among SARI sites, and influenza virus also showed a similar ILI dominance pattern. This finding is supported by a study conducted in Ethiopia (2021-2022), which reported influenza positivity of 26.4% in ILI cases compared to only 3.8% in SARI cases (25), and the West Bengal study, which documented influenza at 6.6% in ILI cases (23).The result is nearly identical to the present finding. While Tanzania sentinel data similarly find adenovirus among the top three detected pathogens across ILI surveillance sites (40). Together, these findings reflect the classical ambulatory presentation of both hADV and influenza as self-limiting febrile respiratory illnesses driving outpatient consultation rather than hospitalization.

On the other hand, RSV, HRV, HBOV, and HPIV were detected predominantly among SARI cases, with detection rates of 100%,92.3%,64.7%, and 100%, respectively, among positive cases. These pathogen case sever hospitalized in young children. The finding aligned with South Africa's SARI surveillance, which documented rhinovirus and enterovirus as the single most detected pathogen at 30% among hospitalized children under five (11).

The overall bacterial positivity rate of 37% (156/422) in this study confirms that more than one-third of enrolled children had at least one detectable bacterial respiratory pathogen, positioning bacterial infection as a clinically critical dimension of pediatric ARI alongside viral etiology. *Haemophilus influenzae* (HI) was the dominant bacterial pathogen at 20% (84/422), followed by *Streptococcus pneumoniae* (SP) at 13.8% (58/422), while all other bacteria such as *Acinetobacter baumannii* (1.8%), *Staphylococcus aureus* (0.7%), *Pseudomonas aeruginosa* (0.4%), and *Klebsiella pneumoniae* (0.2%) were detected at substantially lower frequencies.

The findings of HI and SP are similar to the study done in a sub-Saharan African systematic review, with SP ranging from 1% to 96% and HI from 2.5% to 54% (38), and a 2021 global systematic analysis of 204 countries established SP as the single pathogen responsible for the

highest LRI burden worldwide at 97.9 million episodes and 505,000 deaths annually(21) , and also the West Bengal study similarly identified SP as the leading bacterial pathogen at 55.5%, followed by HI at 29% among 0–2-year-old ILI cases (23), while the Italian ILI surveillance study confirmed HI at 37.98% and SP at 19.93% from outpatient nasopharyngeal samples(36).

Age-stratified analysis revealed the highest bacterial positivity in the 1- 2-year group (41.8%), followed by infants under one year (36%), and the lowest in the 3–5-year group (28%). Sub-Saharan African data estimate more than 900,000 pneumonia episodes annually in children under five, leading to nearly 8,000 deaths per year (22), contextualizing the public health weight of the bacterial burden observed here. Notably, ILI cases showed higher bacterial positivity (44%) than SARI cases (34.2%), a finding consistent with the Italian ILI study documenting high HI and SP detection rates specifically from outpatient settings (36), and verified by the Chinese retrospective SARI study, where the bacterial detection rate (39.0%) actually exceeded the viral detection rate (36.9%) in hospitalized children (34). Collectively strengthening that bacteria are primary contributors across the full spectrum of pediatric ARI severity, not merely secondary opportunists.

The overall viral co-infection rate in this study was 15.5% (20/131), indicating that approximately one in six virus-positive children had infection with more than one respiratory pathogen simultaneously. This result is similar to a finding in a study done in Bangkok, Thailand, between 2019 and 2020, using a multiplex PCR panel, which found a co-infection rate of 15.4% (33). However, this rate is lower than the study finding in Colombia, where co-infection accounted for 42% of positive ILI cases (32), Bangladesh, where 36% of ARI cases in children under five were co-infected (5), South Africa, with a remarkably high rate of 64.2% (11), and Morocco, where 18.8% of positive specimens were co-infected, with 60.4% of the cases concentrated in children under five years old (7). The co-infection rate was low compared to other findings due to the multiplex panel's scope, predominantly SARI-based enrollment with later-stage sampling, the clinical case definition used, and the overall number of virus-positive cases.

Among the viruses detected, HBOV and HRV had the highest individual co-infection proportions, at 23.6% and 23.0%, respectively, while influenza had the lowest at 11.1%. The higher co-infection of HBOV aligns with findings from the China 2019-2022 study, which identified bocavirus as a common co-detected pathogen at different seasons (35). Similarly, in Belgium, in SARI surveillance, bocavirus was detected alongside other viruses, although at lower individual

rates of 0.9% in SARI cases (37), reflecting its persistent but variable co-detection pattern across geographic settings. Additionally, the HRV co-infection rate was found to be lower than the study done in Bangladesh. HRV was the most detected virus at 31.5%, with frequent co-detection alongside RSV and HBOV (8). The finding is aligned with the Sub-Saharan Africa study, which reported a rhinovirus co-detection rate of 7.5% to 73% (38), and, in contrast, the study in Colombia found that HRV accounted for 15.4% of positive ILI cases and was frequently involved in co-infections (32).

The most common bacterial-viral dual co-infection was Streptococcus pneumoniae and Haemophilus influenzae (Sp-HI), seen in 10 patients with low Ct values (21 and 28); this finding agrees with studies from South Africa, West Bengal, and Italy (23,36,54). Complex multi-pathogen combinations(Flu A-Sp-HI-HRV, Flu A-RSV-COV-HI and SP–HBOV–HI) highlight polymicrobial severity, with HI recurring most often, consistent with large cohorts from China and Bangladesh showing frequent multi-pathogen detection and worse illness with bacterial and viral codetection (8,35).

Our study identified ILI admission type, arthritis (joint pain), and nasopharyngeal specimens as significant independent factors of viral and bacterial pathogen detection in children under five, while sex and age category showed no significant association, consistent with previous studies reporting no gender or age-related difference in pathogen detection rate among homogeneous pediatric populations.

Regarding admission type, ILI cases yielded significantly higher pathogen detection than SARI (56,8% vs 38.9%; AOR: 3.06; P=0.018), likely reflecting higher nasopharyngeal viral load in the early phase of illness. This finding is similar to a retrospective Chinese study among 1266 SARI cases, reporting only 58.1% overall detection, attributed to complex mixed bacterial-viral pathogens (34). However, this contrasts sharply with a Thailand study among 169 children with SARI detecting at least one pathogen in 91.7% of cases, and a Bangladesh prospective study reporting 82.5% positivity (8,33). difference is likely driven by broader diagnostic panels, syndromic classification, and specimen type.

The most essential finding was the exceptionally strong association between arthritis (joint pain) and pathogen detection (AOR: 60.11, 95% CI: 21.7-170.65; $p<0.01$), indicating systemic

circulation of pathogens beyond the respiratory tract. This is highly supported by the pediatrics septic study on 171 children, which reported a 45% culture positive rate, with *Staphylococcus aureus* (67.5%), *Streptococcus pneumoniae(9.1%)*, and *Streptococcus pyogenes* (6.5%) as predominant causative agents(55). Furthermore, respiratory viruses, including influenza, RSV, rhinovirus, and coronavirus, are well-documented triggers of reactive arthritis as a systemic manifestation of active infection. Collectively, these findings established arthritis or joint pain as a powerful and underutilized clinical marker for predicting confirmed pathogen presence, with critical implications for triage, targeted diagnostics, and empirical treatment decisions in pediatric respiratory illness.

7. Strengths and Limitations of the Study

7.1.Strength of the study

To our knowledge, this study is the first laboratory finding that showed a wide range of viral and bacterial pathogens that can cause severe acute respiratory illness. We used a molecular test (multiplex RT-PCR), which is known for its high sensitivity and specificity. So that we can detect different viral and bacterial pathogens. The extraction kit was used automatically, which helps minimize the contamination that occurs during extraction procedures, and during each procedure, we used controls like an extraction template control. Negative template control, master mix template control, positive control, and negative control. All equipment used was calibrated.

7.2.Limitation of the study

- The study was limited to five months (January–April 2026), missing other seasons where pathogen circulation patterns April differ significantly.
- Only 14 of 21 sentinel sites contributed samples, with uneven distribution across sites.
- Bacterial culture was not performed for all samples, so antibiotic susceptibility data are limited.

8. Conclusion

This study provides the first comprehensive, simultaneous molecular detection of both viral and bacterial respiratory pathogens in Ethiopian children under five within a national sentinel surveillance framework in Ethiopia. It confirms that adenovirus, influenza, RSV, *H.influenzae*, and *S.pneumoniae* are the dominant etiological agents. A significant proportion of children had polymicrobial (mixed) infections, and ILI admission type, joint pain, and nasopharyngeal specimen type were independently associated with higher pathogen detection. Highlighted the viral and bacterial distribution, co-detection of viral and bacterial pathogens among under-five children tested.

The high prevalence of certain viral and bacterial pathogens highlights the need for increase multiplex detection for viral and bacterial pathogen rather than only detect influenza and covid-19 in Ethiopia to inform policies and interventions. Given high HADV and HI detection rate observed in this study.

9. Recommendation

- Multiplex PCR testing should be routinely integrated into all 21 national influenza sentinel sites for comprehensive pathogen surveillance beyond influenza alone.
- Nasopharyngeal swabs should be standardized across all sentinel sites to maximize diagnostic yield.
- Children presenting with joint pain/arthritis alongside respiratory symptoms should receive prioritized targeted diagnostics, as this is a strong independent predictor of confirmed infection.
- Bacterial culture should be performed by collecting representative samples from all sentinel sites to identify the bacterial causative pathogen for ARI.

10. References

1. Whitney CG. Measuring progress on preventing pneumonia deaths: are we there yet? Lancet Infect Dis. ;17(11):1100–1.

2. Charlton CL, Babady E, Ginocchio CC, Hatchette TF, Jerris RC, Li Y, et al. Practical Guidance for Clinical Microbiology Laboratories: Viruses Causing Acute Respiratory Tract Infections. Clin Microbiol Rev. ;32(1):e00042-18

3. Lucy Eberechukwu Yaguo Ide, Tochi Ada Uchenwa-Onyenegecha. Burden of Acute Respiratory Tract Infections as Seen in University of Port Harcourt Teaching Hospital Nigeria. J US-China Med Sci. ;12(4).

4. Jin X, Ren J, Li R, Gao Y, Zhang H, Li J, et al. Global burden of upper respiratory infections in 204 countries and territories, from 1990 to 2019. eClinicalMedicine. ;37.

5. Bhuyan GS, Hossain MA, Sarker SK, Rahat A, Islam MT, Haque TN, et al. Bacterial and viral pathogen spectra of acute respiratory infections in under-5 children in hospital settings in Dhaka city. Schildgen O, editor. PLOS ONE. 2017 Mar 27;12(3):4488

6. Bicer S, Giray T, Çöl D, Erdağ GÇ, Vitrinel A, Gürol Y, et al. Virological and clinical characterizations of respiratory infections in hospitalized children. Ital J Pediatr. 39(1):22.

7. Bimouhen A, Regragui Z, El Falaki F, Ihazmade H, Benkerroum S, Cherkaoui I, et al. Viral aetiology of influenza-like illnesses and severe acute respiratory illnesses in Morocco, September 2014 to December 2016. J Glob Health. 12:04062.

8. Bacterial and viral pathogen spectra of acute respiratory infections in under-5 children in hospital settings in Dhaka city | PLOS One10.1371

9. Adam K, Pangesti KNA, Setiawaty V. Multiple Viral Infection Detected from Influenza-Like Illness Cases in Indonesia. BioMed Res Int. (1):9541619.

10. Zoting A, Bhise S, Mategadikar P, Deshmukh P, Shrikhande S. Etiological Profile of Hospitalized Severe Acute Respiratory Infection (SARI) Patients During the COVID-19 Pandemic: A Cross-Sectional Study. Cureus.

11. Ogunbayo AE, Mogotsi MT, Sondlane H, Nkwadipo KR, Sabiu S, Nyaga MM. Pathogen Profile of Children Hospitalised with Severe Acute Respiratory Infections during COVID-19 Pandemic in the Free State Province, South Africa. Int J Environ Res Public Health.;19(16):10418.

12. Abdulkader RS, Chadwick J, Krupa NC, Prabakaran N, Madhavan V, Razik CA M, et al. Surveillance of severe acute respiratory infections (SARI) and influenza-like illness (ILI) in Tamil Nadu, India: a situation analysis. Discov Health Syst. ;4(1):75.

13. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single Versus Dual Respiratory Virus Infections in Hospitalized Infants: Impact on Clinical Course of Disease and Interferon-γ Response. Pediatr Infect Dis J. 2005;24(7):605.

14. Tabatabai J, Ihling CM, Manuel B, Rehbein RM, Schnee SV, Hoos J, et al. Viral Etiology and Clinical Characteristics of Acute Respiratory Tract Infections in Hospitalized Children in Southern Germany (2014–2018). Open Forum Infect Dis.;10(3).

15. Davids M, Johnstone S, Mendes A, Brecht G, Avenant T, du Plessis N, et al. Changes in Prevalence and Seasonality of Pathogens Identified in Acute Respiratory Tract Infections in Hospitalised Individuals in Rural and Urban Settings in South Africa; 2018–2022. Viruses.(3):404.

16. Amsalu ET, Akalu TY, Gelaye KA. Spatial distribution and determinants of acute respiratory infection among under-five children in Ethiopia: Ethiopian Demographic Health Survey 2016. PLOS ONE. 14(4):e0215572.

17. Tempia S, Walaza S, Moyes J, Cohen AL, von Mollendorf C, Treurnicht FK, et al. Risk Factors for Influenza-Associated Severe Acute Respiratory Illness Hospitalization in South Africa, 2012–2015. Open Forum Infect Dis. ;4(1):ofw262.

18. Ortiguerra RG. Risk Factors Associated With Severe Acute Respiratory Infections Cases. Public Health. 2004.

19. Seidu AA, Dickson KS, Ahinkorah BO, Amu H, Darteh EKM, Kumi-Kyereme A. Prevalence and determinants of Acute Lower Respiratory Infections among children under-five years in sub–Saharan Africa: Evidence from demographic and health surveys. SSM - Popul Health.;8:100443.

20. Alemayehu S, Kidanu K, Kahsay T, Kassa M. Risk factors of acute respiratory infections among under five children attending public hospitals in southern Tigray, Ethiopia, 2016/2017. BMC Pediatr. (1):1–8.

21. Bender RG, Sirota SB, Swetschinski LR, Dominguez RMV, Novotney A, Wool EE, et al. Global, regional, and national incidence and mortality burden of non-COVID-19 lower respiratory infections and aetiologies, 1990–2021: a systematic analysis from the Global Burden of Disease Study 2021. Lancet Infect Dis. ;24(9):974–1002.

22. Bénet T, Sylla M, Messaoudi M, Picot VS, Telles JN, Diakite AA, et al. Etiology and Factors Associated with Pneumonia in Children under 5 Years of Age in Mali: A Prospective Case-Control Study. PLOS ONE. ;10(12):e0145447.

23. Khan T, Halder S, Das RS, Jaiswal A, Leo PHS, Mahato A, et al. Molecular epidemiology of influenza, respiratory syncytial virus, SARS-CoV-2, other respiratory viruses and bacteria among children 0–2-year-olds in West Bengal: a one-year influenza-like illness surveillance study (2022–2023). Front Epidemiol.;5.

24. Umuhoza T, Bulimo WD, Oyugi J, Musabyimana JP, Kinengyere AA, Mancuso JD. Prevalence of human respiratory syncytial virus, parainfluenza and adenoviruses in East Africa Community

partner states of Kenya, Tanzania, and Uganda: A systematic review and meta-analysis (2007–2020). PLOS ONE. ;16(4):e0249992.

25. Biru M, Tayachew A, Teka G, Tigabu E, Eshetu F, Tadesse M, et al. Epidemiology of circulating influenza viruses in Ethiopia during the COVID-19 Pandemic: Evidence from National Severe Acute Respiratory Infection and Influenza-Like Illness Sentinel Surveillance (January 2021-August 2022).

26. Teka G, Woldeab A, Dereje N, Eshetu F, Gizachew L, Tazu Z, et al. Regional and temporal variations in COVID-19 cases and deaths in Ethiopia: Lessons learned from the COVID-19 enhanced surveillance and response. Chowdhury S, editor. PLOS Glob Public Health.;4(5):e0003175.

27. Shure W, Tayachew A, Berkessa T, Teka G, Biru M, Gebeyehu A, et al. SARS-CoV-2 co-detection with influenza and human respiratory syncytial virus in Ethiopia: Findings from the severe acute respiratory illness (SARI) and influenza-like illness (ILI) sentinel surveillance, January 01, 2021, to June 30, 2022. Nelson MI, editor. PLOS Glob Public Health. ;4(4):e0003093.

28. Tayachew A, Teka G, Gebeyehu A, Shure W, Biru M, Chekol L, et al. Prevalence of respiratory syncytial virus infection and associated factors in children aged under five years with severe acute respiratory illness and influenza-like illness in Ethiopia. IJID Reg. ;10:191–6.

29. Berkessa T, Tayachew A, Gebeyehu A, Teka G, Shure W, Agune A, et al. Evaluation of the sentinel surveillance system for influenza-like illness and severe acute respiratory infection in Ethiopia during the COVID-19 pandemic, 2021–2023. BMC Health Serv Res. ;25(1):759.

30. Mekuria S, Tolossa D, Abebe T, Nour TY, Tesfaye A, Roble AK. Prevalence, Antimicrobial Drug Resistance and Associated Risk Factors of Streptococcus Pneumoniae Bacteria Infection Among Under-Five Children With Acute Lower Respiratory Tract Infection Attending Sheik Hassan Yebere Referral Hospital, Jig-Jiga, Ethiopia. Infect Drug Resist. ;16:3511–23.

31. Mekonnen AT. Addis Ababa University College of Health Science School of Allied Health Sciences Department of Medical Laboratory Sciences.

32. Arrieta-Rangel L, Bello-Lemus Y, Luna-Rodriguez I, Guerra-Simanca M, Bermúdez V, Díaz-Olmos Y, et al. Characterization of Respiratory Viruses in Patients with Acute Respiratory Infection in the City of Barranquilla during the SARS-CoV-2/COVID-19 Pandemic. Diagnostics. (20):2269.

33. Wanlapakorn N, Thongpan I, Sarawanangkoor N, Vichaiwattana P, Auphimai C, Srimuan D, et al. Epidemiology and clinical characteristics of severe acute respiratory infections among hospitalized children under 5 years of age in a tertiary care center in Bangkok, Thailand, 2019–2020. Heliyon.;9(11).

34. Wang JZ, Yuan D, Yang XH, Sun CH, Hou L lin, Zhang Y, et al. Epidemiological and etiological characteristics of 1266 patients with severe acute respiratory infection in central China, 2018–2020: a retrospective survey. BMC Infect Dis. ;24(1):426.

35. Lv G, Shi L, Liu Y, Sun X, Mu K. Epidemiological characteristics of common respiratory pathogens in children. Sci Rep. ;14(1):1–10.

36. Ferrari A, Schiavetti I, Ogliastro M, Minet C, Sibilio R, Giberti I, et al. Co-detection of respiratory pathogens among ILI patients: characterization of samples collected during the 2018/19 and 2019/20 pre-pandemic seasons. BMC Infect Dis. ;24(1):881.

37. Subissi L, Bossuyt N, Reynders M, Gérard M, Dauby N, Lacor P, et al. Spotlight influenza: Extending influenza surveillance to detect non-influenza respiratory viruses of public health relevance: analysis of surveillance data, Belgium, 2015 to 2019. Eurosurveillance. ;26(38):2001104.

38. Demboux Lyelet JE, Mayengue PI, Koukouikila-Koussounda F, Leroy EM, Becquart P, Niama FR. Viral and Bacterial Etiology of Common Respiratory Infections in Children in Sub-Saharan Africa: A Systematic Review. Children. 12(9):1212.

39. Regragui Z, Bimouhen A, El Falaki F, Ihazmad H, Benkerroum S, Triki S, et al. Influenza-associated severe acute respiratory infections among children under five years old in Morocco, September 2017 to March 2019. Infez Med.;30(3):446–53.

40. Kelly ME, Gharpure R, Shivji S, Matonya M, Moshi S, Mwafulango A, et al. Etiologies of influenza-like illness and severe acute respiratory infections in Tanzania, 2017–2019. PLOS Glob Public Health. ;3(2):e0000906.

41. Kandeel A, Fahim M, Deghedy O, H. Roshdy W, K. Khalifa M, El Shesheny R, et al. Multicenter study to describe viral etiologies, clinical profiles, and outcomes of hospitalized children with severe acute respiratory infections, Egypt 2022. Sci Rep. ;13(1):21860.

42. Mohamed GA, Ahmed JA, Marano N, Mohamed A, Moturi E, Burton W, et al. Etiology and Incidence of Viral Acute Respiratory Infections among Refugees Aged 5 Years and Older in Hagadera Camp, Dadaab, Kenya. Am J Trop Med Hyg.;93(6):1371–6.

43. Dagne H, Andualem Z, Dagnew B, Taddese AA. Acute respiratory infection and its associated factors among children under-five years attending pediatrics ward at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia: institution-based cross-sectional study. BMC Pediatr. 2020 Dec;20(1):1–7.

44. Influenza Surveillance Protocol-Ethiopia-July 2022 - RVDSR Final and Signed | PDF | Influenza | Influenza Pandemic.

45. World Health Organization (WHO). Implementing the integrated sentinel surveillance of epidemic and pandemic potential respiratory viruses of influenza and other by the Global Influenza Surveillance and Response System: Standards and operational guidance.

46. Fitzner J, Qasmieh S, Mounts AW, Alexander B, Besselaar T, Briand S, et al. Revision of clinical case definitions: influenza-like illness and severe acute respiratory infection. Bull World Health Organ. ;96(2):122–8.

47. PDF Automated nucleic acid extractor MGISP-NE32 Machine 2025

48. Kwofie TB, Anane YA, Nkrumah B, Annan A, Nguah SB, Owusu M. Respiratory viruses in children hospitalized for acute lower respiratory tract infection in Ghana. Virol ;9(1):78.

49. Wada FW, Desta AF, Gebre M, Mihret W, Seyoum T, Melaku K, et al. Pneumococcal colonization and coinfecting respiratory viruses in children under 5 years in Addis Ababa, Ethiopia: a prospective case–control study.;14(1):4174.

50. Moleleki M, Reddy C, Ndlangisa K, du Plessis M, Hellferscee O, Mekgoe O, et al. Respiratory pathogens detected in children aged <5 years hospitalized with severe respiratory illness, South Africa, 2017. Front Pediatr. ;13:1498197.

51. Ukuli QA, Erima B, Mubiru A, Atim G, Tugume T, Kibuuka H, et al. Molecular characterisation of human adenoviruses associated with respiratory infections in Uganda. BMC Infect Dis.;23:435.

52. van der Zalm MM, Sam-Agudu NA, Verhagen LM. Respiratory adenovirus infections in children: a focus on Africa. Curr Opin Pediatr ;36(3):342–8.

53. Tadesse M, Mengesha M, Tayachew A, Belay D, Hassen A, Woyessa AB, et al. Burden and seasonality of medically attended influenza like illness (ILI) in Ethiopia, 2012 to 2017. BMC Infect Dis. ;20:148.

54. Davids M, Johnstone S, Mendes A, Brecht G, Avenant T, du Plessis N, et al. Changes in Prevalence and Seasonality of Pathogens Identified in Acute Respiratory Tract Infections in Hospitalised Individuals in Rural and Urban Settings in South Africa; 2018–2022. Viruses. ;16(3):404.

55. Li Z, Li G, Wu J, He B, Zhang Q. Clinical characteristics of children with septic arthritis caused by different pathogenic bacteria. Pediatr Discov;3(1):e2522.

Chapter 8. Annex

Annex I: Parental consent form for parents of children <5 years to participate in the study (English version)

Good morning/afternoon. My name is _____I am Addis Ababa University MSC student. Currently, I am researching "Molecular Detection of Common Respiratory Viruses and Bacterial Pathogens Causing Severe Respiratory Infections and Influenza-like Illness in Under-Five Children in Selected Health Facilities Serving as Influenza Sentinel Sites in Ethiopia". The main aim of this study is to determine the molecular epidemiology of Common viral and bacterial pathogens among acute respiratory illness cases in Ethiopia. The findings of this study will be an important input to better understand the causative agents of respiratory viral diseases and their burden, the prevention of respiratory viral disease outbreaks, and to guide better treatment and case management programs. We would like your child to be part of this study. You can choose for your child to participate in the study. Before you decide, we would like to tell you about the study and what it would be like to participate. We will answer any questions you have.

Purpose: We will collect nasopharyngeal/oropharyngeal swab samples from people with ARIs today in this facility and other selected facilities in Ethiopia. Associated sociodemographic and 24 clinical data will be collected to help the study better understand the causative agents of bacterial and viral pathogens of ILI/SARI cases.

Participation: I am asking you for your child to participate voluntarily in this study; if you agree for your child to participate, we will ask you to sign a parent consent form and respond to a short questionnaire interview. Risks associated: With this study, there are no risks associated during taking nasopharyngeal swabs or nasal swabs procedures. It will take only 15 minutes to complete the interview and to collect the samples.

Benefits: Any person involved in the study will not receive any incentives. There April not be a direct benefit to your child as a participant. The study will be conducted to identify the cause of patogen and further analysis, which we believe will help better understand the epidemiology, transmission, and genome diversity, which in turn will help to produce recommendations for policy makers and public health officials to design appropriate prevention and case management

programs important in preventing and reducing the health, social, and economic impacts of these deadly pathogens. Cost: There is no cost to you/your child. The work is free.

Confidentiality: All information you give and data obtained from laboratory analysis will be kept confidential. Formats containing data will be kept locked. Your/your child's name and other personal identifier/s will not appear if we present this survey or publish the results.

Sharing the result: Report will be written about the finding of the study, through either publication or any other means. The result will not bear any information relevant to your personality in anyway. Voluntary Participation: You are free to choose your child to be part of the study. You April refuse to answer any of the questions, and you April stop the questionnaire at any time. Nothing bad will happen to your child if you decide not to be in the project. Please take as much time as you need to decide whether or not you want to take part in this project. You can ask as many questions as you like to help you decide. If you have any questions, please contact the principal investigator of this study Mr. Adimkewu Agunie via +251-0909604116 or contact in person at the Ethiopian Public Health Institute located in Addis Ababa, Arbegnoch Street in Gulele Sub-city. Do you agree to participate and are you willing to spare 15 min some time to answer some questions? Yes, I am willing to participate in the study or No, I do not wish to participate in the study. Participant's parent/guardian statement: The above survey has been explained to me and I agree to take part. I understand that this is my choice and that if I change my mind, I can decide to end the interview at any time.

Participant's parent/guardian's name: _____Signature/Thumbprint: _____ Date (EC): _____ Witnesses: (If participant is not able to read or write) 1) Name: _____ Signature: _____ 2) Name: _____ Signature: _____

Annex II: በጥናቱ ውስጥ ለመሳተፍ ከ5 ዓመት በታች ለሆኑ የልጆች የወላጅ ስምምነት ቅጽ (ለ አማርኛ ቋንቋ ተናጋሪ)

እንደምን አደሩ/እድምን ዋሉ። ስሜ _____እኔ የአዲስ አበባ ዩኒቨርሲቲ የኤምኤስሲ ተማሪ ነኝ። በአሁኑ ጊዜ "በተለያዩ የመተንፈሻ አካላት ቫይረስ እና በባክቴሪያ በሽታ አምጪ ተህዋሲያን ላይ ከባድ የመተንፈሻ አካላት ኢንፌክሽን እና እንደ ኢንፍሉዌንዛ ያሉ ሕመሞችን የሚያስከትሉ በተመረጡ የጤና ተቋማት ውስጥ ባሉ ከአምስት ዓመት በታች በሆኑ ሕፃናት ላይ ጥናት እያካሄድኩ ነው። በኢትዮጵያ ለመተንፈሻ አካላት በተመርጡ ጤና ተቋማት ጥናት እያካሄድኩ ነው። የዚህ ጥናት ዋና ዓላማ በኢትዮጵያ ውስጥ ባሉ አጣዳፊ የመተንፈሻ አካላት በሽታዎች መካከል የተለመዱ የቫይረስ እና የባክቴሪያ በሽታ አምጪ ተህዋሲያን ሞለኪውላዊ ኤፒዲሚዮሎጂን በዝርዝር ማየት ነው።

የዚህ ጥናት ግኝቶች የመተንፈሻ አካላት የቫይረስ በሽታዎች መንስኤቸውን በተሻለ ለመረዳት፣ የመተንፈሻ አካላት የቫይረስ በሽታ ወረርሽኞችን ለመከላከል እና የተሻሉ የሕክምና እና የጉዳይ አስተዳደር ፕሮግራሞችን ለመምራት አስፈላጊ ግብዓት ይሆናሉ። ልጅዎ በዚህ ጥናት ውስጥ እንዲሳተፍ እንፈልጋለን። ልጅዎ በጥናቱ ውስጥ መሳተፍ ፈቃደኛ ከሆኑ? በጥናቱ ውስጥ መሳተፍ አለመሳተፉን እርሶ መምረጥ ይችላሉ። ከመወሰንዎ በፊት ግን ስለ ጥናቱ እና መሳተፍ ምን እንደሚመስል ልንነግርዎ እንፈልጋለን። ሊኖርዎት ለሚችሉ ማናቸውም ጥያቄዎች መልስ እንሰጣለን።

ዓላማ፡ ዛሬ በዚህ ተቋም እና በኢትዮጵያ ውስጥ በተመረጡ ሌሎች ተቋማት ውስጥ አጣዳፊ የመተንፈሻ አካላት ኢንፌክሽን ካለባቸው ሰዎች የአፍንጫ ወይም የአፍ ናሙናዎችን እንሰበስባለን። ጥናቱ ከባድ የመተንፈሻ አካላት ኢንፌክሽን እና እንደ ኢንፍሉዌንዛ ያሉ ሕመሞችን የባክቴሪያ እና የቫይረስ በሽታ አምጪ ተህዋሲያን መንስኤዎችን በተሻለ ሁኔታ ለመረዳት እንዲረዳ ተያያዥ ማህበራዊ እና 21 ክሊኒካዊ መረጃዎች ይሰበሰባሉ።

ተሳትፎ፡ ልጅዎ በዚህ ጥናት በፈቃደኝነት እንዲሳተፍ እጠይቃለሁ፤ ልጅዎ እንዲሳተፍ ከተስማሙ፣ የወላጅ ፈቃድ ቅጽ እንዲፈርሙ እና ለአጭር የጥያቄ ቃለ መጠይቅ ምላሽ እንዲሰጡ እንጠይቅዎታለን። ተያያዥ አደጋዎች፡ በዚህ ጥናት፣ የአፍንጫ/የአፍ ስዋብ ሂደቶችን በሚወስዱበት ጊዜ ምንም አይነት አደጋዎች የሉም። ቃለ መጠይቁን ለማጠናቀቅ እና ናሙናዎቹን ለመሰብሰብ 15 ደቂቃዎችን ብቻ ይወስዳል።

ጥቅሞች፡ በጥናቱ ውስጥ የተሳተፈ ማንኛውም ሰው ምንም አይነት ማበረታቻ አያገኝም። ልጅዎ እንደ ተሳታፊ ቀጥተኛ ጥቅም ላይኖረው ይችላል። ጥናቱ የሚካሄደው የበሽታውን መንስኤ ለመለየት እና ተጨማሪ ትንተና ለማድረግ ሲሆን ይህም ኤፒዲሚዮሎጂን፣ ስርጭትን እና በሽታ አምጪ ተህዋሲያን መንስኤዎችን በተሻለ ሁኔታ ለመረዳት ነው፣ ይህም በተራው የፖሊሲ አውጪዎች እና የህዝብ ጤና ባለስልጣናት የእነዚህን ገዳይ በሽታ አምጪ ተህዋሲያን የጤና፣ ማህበራዊ እና ኢኮኖሚያዊ ተፅእኖዎችን ለመከላከል እና ለመቀነስ አስፈላጊ የሆኑ ተገቢ የመከላከያ እና የጉዳይ አስተዳደር ፕሮግራሞችን እንዲነድፉ ምክሮችን ለማዘጋጀት ይረዳል። ወጪ፡ ለእርስዎ/ለልጅዎ ምንም ወጪ የለም። ስራው ነፃ ነው።

ሚስጥራዊነቱ፡ የሚሰጡት መረጃ እና ከላቦራቶሪ ትንተና የተገኘ መረጃ ሁሉ በሚስጥር ይጠበቃል። መረጃ የያዙ ቅርጸቶች ተቆልፈው ይቀመጣሉ። ይህንን ጥናት ካቀረብን ወይም ውጤቶቹን ካሳተምን የእርስዎ/የልጅዎ ስም እና ሌሎች የግል መለያዎች አይታዩም።

ውጤቱን ስለማጋራት፡- ስለ ጥናቱ ግኝት በህትመት ወይም በሌላ መንገድ ይፃፋል። ውጤቱ በማንኛውም መልኩ ከባህሪዎ ጋር ተዛማጅነት ያለው ማንኛውንም መረጃ አይይዝም። በፈቃደኝነት ተሳትፎ፡ ልጅዎ የጥናቱ አካል እንዲሆን የመምረጥ ነፃነት አለዎት። ለጥያቄዎቹ መልስ ለመስጠት ፈቃደኛ አለመሆን ይችላሉ፣ እና መጠይቁን በማንኛውም ጊዜ ማቆም ይችላሉ። በጥናቱ ውስጥ ላለመሳተፍ ከወሰኑ ልጅዎ ምንም መጥፎ ነገር አይደርስበትም። በዚህ ጥናት ውስጥ መሳተፍ ይፈልጉ እንደሆነ ወይም እንዳልሆነ ለመወሰን የሚያስፈልግዎትን ያህል ጊዜ ይውሰዱ። ውሳኔ ለማድረግ እንዲረዳዎት የሚፈልጉትን ያህል ጥያቄዎች መጠየቅ ይችላሉ። ማንኛውም ጥያቄ ካለዎት፣ እባክዎን የዚህን ጥናት ዋና መርማሪ አቶ አድምቀው አጉኔ በ+251-0909604116 ወይም በአዲስ አበባ በሚገኘው የኢትዮጵያ የህዝብ ጤና ተቋም፣ በጉለሌ ክፍለ ከተማ አርቢኞች ጎዳና በአካል ያግኙ። ለመሳተፍ ተስማምተዋል እና ለአንዳንድ ጥያቄዎች መልስ ለመስጠት 15 ደቂቃ ለማሳለፍ ፈቃደኛ ነዎት? አዎ፣ በጥናቱ ለመሳተፍ ፈቃደኛ ነኝ ወይም አይሆንም፣ በጥናቱ ውስጥ መሳተፍ አልፈልግም። የተሳታፊው የወላጅ/አሳዳጊ መግለጫ፡ ከላይ የተጠቀሰው ጥናት ለእኔ ተብራርቷል እና ለመሳተፍ ተስማምቻለሁ። ይህ የእኔ ምርጫ እንደሆነ እና ሀሳቤን ከቀየርኩ ቃለ መጠይቁን በማንኛውም ጊዜ ለማቆም መወሰን እንደምችል ተረድቻለሁ።

የተሳታፊው ወላጅ/አሳዳጊ ስም፡ _____ፊርማ/አጭር አሻራ፡ _____

ቀን (EC): _____

ምስክሮች፡ (ተሳታፊው ማንበብ ወይም መጻፍ የማይችል ከሆነ)

1. ስም፡ _____ ፊርማ፡ _____
2. ስም፡ _____ ፊርማ፡ _____

Annex III: Unka hayyama warraa Warri ijoollee isaanii <5 waggaa qorannicha irratti akka hirmaatan (Afaan Oromootiin)

Nagaa ganama/waaree booda. Maqaan koo _____ Ani barataa MSC Yuunivarsiitii Addis Ababaati. Yeroo ammaa kana "Molecular Detection of Common Respiratory Virus and Bacterial Pathogen of Severe Respiratory Virus and Influenza-like Infection in Ethiopia, Dhaabbilee Fayyaa Filatamoo Akka Iddoowwan Infuleenzaa Sentinel Itoophiyaatti Tajaajilan keessatti" irratti qorannoo gaggeessaa jira. Kaayyoon qorannoo kanaa inni guddaan Itoophiyaa keessatti namoota dhukkuba sirna hargansuu cimaan qabaman keessaa molakiyuulaa epidemiology paatojeenoota vaayirasii fi baakteeriyaa waliigalaa adda baasuudha. Argannoon qorannoo kanaa sababa dhukkuboota vaayirasii sirna hargansuu fi ba'aa, ittisa weerara dhukkuba vaayirasii sirna hargansuu, fi sagantaalee wal'aansaa fi bulchiinsa dhimmaa fooyya'aa qajeelchuuf galtee barbaachisaa ta'a. Mucaan keessan qaama qorannoo kanaa akka ta'u barbaanna. Hirmaannaan daa'imni keessan qorannicha irratti fedhiidhaan kan raawwatamudha. Qo'annaa sana keessa jiraachuu fi dhiisuu filachuu dandeessa. Murteessuu kee dura waa'ee qorannichaa fi hirmaachuun maal akka fakkaatu sitti himna. Gaaffii qabdan kamuu ni deebisna.

Kaayyoo: Namoota ARI qaban irraa saamuda nasopharyngeal/oropharyngeal swab har'a dhaabbata kanaa fi dhaabbilee filatamoo biroo Itoophiyaa keessatti ni sassaabna. Qorannoon kun sababoota paatojeenoota baakteeriyaa fi vaayirasii namoota ILI/SARI qaban caalaatti hubachuuf gargaaruuf ragaan hawaas-diimoogiraafii fi kilinikaa 21 walqabatee walitti qabama.

Hirmaannaa: Mucaan keessan qorannoo kana irratti fedhii isaatiin akka hirmaatu isin gaafachaa jira; Yoo daa'imni keessan akka hirmaatu walii galtan, unka hayyama warraa akka mallatteessitanii fi gaaffii fi deebii gaaffilee gabaabaaf deebii akka kennitan isin gaafanna. Balaawwan walqabatan: Qorannoon kun yeroo adeemsa qorannoo kanaa raawwatan balaan walqabatee jiru hin jiru. Af-gaaffii xumuruu fi saamuda walitti qabuuf daqiiqaa 15 qofa fudhata.

Faayidaa: Namni qorannoo kana irratti hirmaate kamiyyuu kaka'umsa tokkollee hin argatu. Faayidaan kallattiin daa'ima keessan akka hirmaataa tokkootti argachuu dhiisuu danda'a. Qorannoon kun sababa adda baasuu fi xiinxala dabalataa kan gaggeessu yoo ta'u, kunis garaagarummaa dhibee daddarbaa, daddarbiinsa, fi jiinoomii caalaatti hubachuuf gargaara jennee amanne, kunis dabaree isaatiin qaamolee imaammata baasan fi qondaaltonni fayyaa hawaasaa sagantaalee ittisaa fi bulchiinsa dhimmaa sirrii ta'an akka dizaayinii gochuuf yaada qopheessuuf

gargaara, kunis dhiibbaa fayyaa, hawaasummaa fi dinagdee paatojeenoota du'aa kana ittisuu fi hir'isuu keessatti barbaachisaa ta'a. Baasii: Baasiin siif/mucaa keetiif hin jiru. Hojiin bilisa.

Iccitii: Odeeffannoon ati kennituu fi daataa xiinxala laabraatoorii irraa argame hundi iccitii ta'ee ni eegama. Akkaataan odeeffannoo of keessaa qabu cufamee ni eegama. Qorannoon kana yoo dhiheessine ykn bu'aa isaa yoo maxxansinu maqaan/mucaa keessanii fi adda baastonni dhuunfaa biroo hin mul'atan.

Bu'aa isaa qooduu: Gabaasni waa'ee argannoo qorannichaa, karaa maxxansaa ykn karaa biraa kamiinuu ni barreeffama. Bu'aan odeeffannoo dhuunfaa kee wajjin walqabatu kamiyyuu nama biraaf dabarfamee hin kennamu. Tola Ooltummaadhaan Hirmaachuu: Mucaa keessan qaama qorannichaa akka ta'u filachuuf bilisa ta'uu dandeessu. Gaaffii kamiyyuu deebisuu diduu dandeessa, yeroo barbaaddetti gaafficha dhaabuu dandeessa. Pirojekticha keessatti akka hin argamne yoo murteessite mucaa keessan wanti hamaan hin mudatu. Mee yeroo hamma barbaachisu fudhadhuu pirojektii kana irratti hirmaachuu barbaadduu fi dhiisuu kee murteessi. Gaaffii hamma barbaadde gaafachuu dandeessa, kunis murteessuuf si gargaara. Gaaffii yoo qabaattan qorataa muummee qorannoo kanaa Obbo Admiqeewu Aguunee karaa +251-0909604116 qunnamaa ykn Inistiitiyuutii Fayyaa Hawaasaa Itiyoophiyaa Addis Ababa, Daandii Arbegnoch Magaalaa Guleletti argamutti qaamaan qunnamaa. Hirmaachuuf walii galtuu fi gaaffii tokko tokko deebisuuf yeroo muraasa daqiiqaa 15 qusachuuf fedhii qabduu? Eeyyee, qo'annicha irratti hirmaachuuf fedhii qaba ykn Lakki, qo'annicha irratti hirmaachuu hin barbaadu. Ibsa warra/guddistuu hirmaataa: Qorannoon armaan olii kun naaf ibsameera, anis qooda fudhachuuf walii gala. Kun filannoo koo akka ta'ee fi yaada koo yoo jijjiire yeroo barbaadetti gaaffii fi deebii xumuruuf murteessuu akkan danda'u nan hubadha.

Maqaa Maatii/Guddistuu: _____Mallattoo/Ashaaraa

qubaa: _____ Guyyaa (A.L.I): _____

Dhugaa Baatota: (Hirmaataan dubbisuu fi barreessuu yoo hin dandeenye)

1) Maqaa: _____ Mallattoo: _____

2) Maqaa: _____ Mallattoo: _____

Annex IV: Step of sample extraction

Procedure

1. Put the 96-well pre-packed reagents at room temperature.
2. Shake the 96-well plate upside down for three times, and tear off the plastic bag.
3. Centrifuge the pre-packed reagent for a few seconds (or swing by hand a few times).
4. Tear the aluminium foil film of the 96-well plate and identify the direction of the plate (magnetic beads in column 6 & 12).
5. Vortex the sample.
6. Add 300 μL sample to the 96 well plate columns 1 & 7
7. Place a 96 deep well plate in the instrument, install the 8-strip tips on the instrument correctly to its position.
8. Run the program according to the following procedures.

Machine set up

1. Press the run button.
2. Press the magabio plus folder on the screen, then insert the password.
3. Press the save and run button.
4. Press the run button, then must check to insert the 8-strip
5. Press run.
6. After the automatic purification is over, remove the comb and 96-well plate, transfer 50 μL Elution buffer to the centrifuge tube from columns 5 and 11

Annex V: Procedure for master mix preparation and RT-PCR set ups

Steps.

1. Thaw reagents for the reactions: primer, probes, Positive controls, and Negative controls at room temperature for 10-20 minutes
2. Estimate the number of reactions (N) in the test, which include blank control, positive control, and negative controls
3. Ready the RT-PCR program and set up(By setting the fluorescent channels based on the kit manufacturer's instructions). Show as below on table.
4. After assigning the target and fluorescent dye, configure the RT-PCR protocol

Table A 1: Pathogen target and Reporter

PCR Mix	**Reporter**	**Target**
PCR-Mix A	FAM	Influenza B virus(IFVB)
	ROX	Mycoplasma pneumonia (MP)
	CY5	Influenza A virus (IFVA)
	VIC/Hex	Internal reference
PCR-Mix B	FAM	Human Adenovirus (HADV)
	ROX	Pseudomonas aeruginosa (PA)
	CY5	Streptococcus pneumonia
	VIC/Hex	Internal reference
PCR-Mix C	FAM	Respiratory syncytial virus(RSV)
	ROX	Human bocavirus (HBOV)
	CY5	Staphylococcus aureus(SA)
	VIC/Hex	Internal reference
PCR-Mix D	FAM	Human metapneumovirus (HMPV)
	ROX	Coronavirus (Cov)
	CY5	Haemophilus influenza
	VIC/Hex	Internal reference
PCR-Mix E	FAM	Human parainfluenza virus (HPIV)

	ROX	Legionella pneumophila (LP)
	CY5	Acinetobacter baumanni(AB)
	VIC/Hex	Internal reference
PCR-Mix F	FAM	Human rhinovirus (HRV)
	ROX	Chlamydia pnemoniae (CP)
	CY5	Klebsiella pnemoniae (KP)
	VIC/Hex	Internal reference

Table A 2: RT-PCR protocol

Step	**Cycles**	**temperature**	**duration**	**Fluorescent signal collection**
1	1	50 °C	10 min	No
2	1	95°C	1 min	No
3	45	95 °C	5 sec	No
		55 °C	15 sec	Yes

Passive reference: None

Reaction volume:30 µL

5. Prepare 8 tubes of stripes for PCR-based on the estimated N of reactions for each PCR-mix, labelled from A-F
6. Pipet 18.5 reaction mix and 1.5 enzyme mix for one reaction in each 6 tubes
7. Pipette 10 µl of master mix (primer probe=PP) to the wells specified for each target group of pathogens
8. Take 96 96-well plate to RNA/DNA addition rooms

Annex IV.I RNA/DNA addition room

1. Take a 96-well plate that is compatible with the Quant Studio 5 RT-PCR machine.
2. Add 10 µl of the extracted samples, the extracted negative control and the positive control (which is not extracted; thaw at room temperature for 20 minutes and vortex thoroughly right before use). Each run must include a negative and a positive control.
3. Mix briefly by pipetting up and down.
4. Close the plate with the optical adhesive film.
5. Slightly vortex the plate and centrifuge briefly afterward.
6. Put the plate in the Quant Studio 5 RT-PCR machine plate holder.
7. Then check the RT-PCR instrument program carefully and start RUN

Annex VI: SARI and ILI case-based format

SARI laboratory request and reporting form

	Ethiopian Public Health Institute National Influenza, Arbovirus & VHF Reference Laboratory	**Document No: EPHI/NIAVRL/FO 5.4-004**	
		Version No: 1.0	
	SARI Laboratory Requesting and Reporting form	**Page: 54 of 1**	**Effective Date: Nov. 01, 2021**

Sentinel Site:_____ Epi-Week:_____ Date form Completed (dd/mm/yyyy)_____

CASE DEFINITION: Acute Respiratory Infection with ☐Onset within the last 10 days * ☐History of fever or Measured fever of ≥38ºC* ☐ Cough * ☐ **Hospitalization***

DATA COLLECTION FORM FOR SAMPLED CASES

Unique ID *	MRN	Date of symptom onset	Date of hospitalization	Date of specimen collection and Time

Client/Patient Information:

Full Name: _____ Sex __ Age (in years): _____Telephone: _
Address: Region: _____ Zone _____ woreda _____Kebele
Occupation: ☐ Office work ☐ Daily Labouré ☐ Farmer ☐Animal raring (production) ☐House wife ☐ Factory worker ☐ Student ☐ Unemployed ☐ Not applicable

Other Clinical Presentation (Symptoms)* Within the last 10 days, has the patient have any of the following symptoms*

▫Headache ☐Runny Nose ☐Hemoptysis (coughing out blood) ☐Arthritis (Joint Pain) ☐Vomiting
▫Skin Rash ☐Sort-throat ☐Difficulty of Breathing ☐Unusual Bleeding ☐Other, Specify ____

PRE-EXISTING MEDICAL CONDITION*

▫HD: Heart Disease ☐DB: Diabetes ☐NMD: Neuromuscular Dysfunction ☐AM: Asthma
▫CHD: Chronic Hematologic Disorder ☐IC: Immune Compromised ☐CLD: Chronic Lung Disease
▫CND: Chronic Neurological Disease ☐Pregnant., __trimester ☐LD: Liver Disease
▫CRD: Chronic Renal Disease ☐ Other, specify ____ ☐No known condition

VACCINE AND ANTIVIRAL

Exposure to influenza or other antiviral drugs during the last 14 days ☐ No ☐Yes, Patient ☐Unknown If Yes, Name
Vaccination for Influenza in current season ☐Yes ☐ No ☐ Unknown If Yes, Date___ Vaccination for SARS-

COV-2 ☐Yes ☐No ☐ Unknown If Yes Date___

TRAVEL AND EXPOSURE HISTORY*

Has the patient had close contact with person with SARI or COVID-19 symptoms? ☐ Yes ☐ No If yes please specify_ Has the patient had close contact with or handled sick or dead poultry? ☐ Yes ☐ No If yes please specify__

Has the patient any travel history in past two weeks around any epidemic suspected area? ☐ Yes ☐No If yes specify _____

PATIENT OUTCOME

Patient outcome: ☐ Discharged alive ☐ Died ☐ Unknown Date of discharge/death _____

Was the patient admitted to the ICU? ☐ Yes ☐ No ☐ Unknown

Did the patient require mechanical ventilation during hospitalization? ☐ Yes ☐ No ☐Unknown

Reporting Information

Type of specimen collected: ☐ Nasal swab ☐ Throat swab ☐ nasopharyngeal swab☐ other, _____ Name of person collecting specimen _Signature: ___

Name of person completing Form: _____Signature: ___

Name of reporting HCW(Requester) Telephone Number(s): _____

□ Specimen Received by___

□ Date of received, _ Time, _

□ Specimen: ☐ Acceptable ☐ Rejected

□ Reason for rejection----------

RT PCR Molecular Laboratory Results

Test Result Influenza ☐Positive ☐ Negative

SARS-COV-2 ☐Positive ☐ Negative **RSV** ☐Positive ☐ Negative

Other Viruses, specify _____☐Positive ☐ Negative

Other Viruses, Specify _____☐Positive ☐ Negative

Influenza type and Subtype ☐ IB: Influenza B ☐ IA: Influenza A ☐ IA/H1: Influenza IA/H1 □ IA/H3: Influenza A(H3) ☐IA/HIN1: Influenza A/(HIN1) pdm09 Unsub typeable

Extracted by(initial): _ Sign: _ Date: _Tested by(initial)_____Sign: _Date: _ Reviewed/approved by:_____Sign: _ Date :_

Comment____

ILI laboratory request and reporting form

	Ethiopian Public Health Institute National Influenza, Arbovirus & VHF Reference Laboratory	**Document No: EPHI/NIAVRL/F5.4-008**	
		Version No: 1.0	
	ILI Laboratory Requesting and Reporting Form	**Page: 56 of 1**	**Effective Date:Nov.01,2021**

Sentinel Site:_____ Epi-Week:_____

Date form Completed (dd/mm/yyyy)_____

CASE DEFINITION: Acute Respiratory Infection with ☐Onset within the last 10 days * ☐History of fever or Measured fever of ≥38ºC*

□ Cough *

DATA COLLECTION FORM FOR SAMPLED CASES

Unique ID *	MRN	Date of symptom onset	Date of hospitalization	Date of specimen collection and Time

Client/Patient Information:

Full Name: _____ Sex __ Age (in years): _____Telephone: _
Address: Region: _____ Zone _____ woreda _____Kebele _____

Occupation: ☐ Office work ☐ Daily Labouré ☐ Farmer ☐Animal raring (production) ☐House wife ☐ Factory worker ☐ Student ☐ Unemployed ☐ Not applicable

Other Clinical Presentation (Symptoms)* Within the last 10 days, has the patient have any of the following symptoms*

□Headache ☐Runny Nose ☐Hemoptysis (coughing out blood) ☐Arthritis (Joint Pain) ☐Vomiting
□Skin Rash ☐Sort-throat ☐Difficulty of Breathing ☐Unusual Bleeding ☐Other, Specify ____

PRE-EXISTING MEDICAL CONDITION*

□HD: Heart Disease ☐DB: Diabetes ☐NMD: Neuromuscular Dysfunction ☐AM: Asthma
□CHD: Chronic Hematologic Disorder ☐IC: Immune Compromised ☐CLD: Chronic Lung Disease
□CND: Chronic Neurological Disease ☐Pregnant., __trimester ☐LD: Liver Disease
□CRD: Chronic Renal Disease ☐ Other, specify ____ ☐No known condition

VACCINE AND ANTIVIRAL

Exposure to influenza or other antiviral drugs during the last 14 days ☐ No ☐Yes, Patient ☐Unknown If Yes, Name
Vaccination for Influenza in current season ☐Yes ☐ No ☐ Unknown If Yes, Date___ Vaccination for SARS-COV-2 ☐Yes ☐No ☐ Unknown If Yes Date___

TRAVEL AND EXPOSURE HISTORY*

Has the patient had close contact with person with SARI or COVID-19 symptoms? ☐ Yes ☐ No If yes please specify_ Has the patient had close contact with or handled sick or dead poultry? ☐ Yes ☐ No If yes please specify__

Has the patient travel history led past two weeks around any epidemic-suspected area? ☐ Yes ☐No If yes, specify_____

Reporting Information

Type of specimen collected: ☐ Nasal swab ☐ Throat swab ☐ nasopharyngeal swab☐ other, _____ Name of person collecting specimen _Signature: ___ Name of person completing Form: _____Signature: ___ Name of reporting HCW(Requester) .Telephone Number(s): _____	▫ Specimen Received by___ ▫ Date of received, _ Time, _ ▫ Specimen: ☐ Acceptable ☐ Rejected ▫ Reason for rejection---------- ----------

RT PCR Molecular Laboratory Results

Test Result Influenza ☐Positive ☐ Negative **SARS-COV-2** ☐Positive ☐ Negative **RSV** ☐Positive ☐ Negative	**Other Viruses, specify _____**☐Positive ☐ Negative **Other Viruses, Specify _____**☐Positive ☐ Negative

Influenza type and Subtype ☐ IB: Influenza B ☐ IA: Influenza A ☐ IA/H1: Influenza IA/H1 ▫ IA/H3: Influenza A(H3) ☐IA/HIN1: Influenza A/(HIN1) pdm09 Unsub typeable

Extracted by(initial): _ Sign: _ Date: _Tested by(initial)_____Sign: _Date: _ Reviewed/approved by:_____Sign: _ Date :_

Comment____

Declaration

I, the undersigned, agree to accept responsibility for the scientific, ethical, and technical conduct of the research project and for the provision of required progress reports as per the terms and conditions of the research publications office.

Name of Principal Investigator: Adimkewu Agunie (BSc, MSc Candidate)

Signature ……

Date of submission …….

This thesis project has been submitted with my approval as the university Advisor.

Advisor: Dr. Kassu Desta (PhD)

Signature…….

Date ……

Place Addis Ababa, Ethiopia

Advisor: Dr. Biruk Zerfu (PhD)

Signature ……

Date ……

Co-Advisor: Mr. Adamu Tayachew (PhD, Fellow)

Signature…….

Date ……

Place Addis Ababa, Ethiopia