EPIDEMIOLOGY OF SEASONAL INFLUENZA AND OTHER RESPIRATORY PATHOGENS DURING THE COVID-19 PANDEMIC IN ZAMBIA.

By

MARTIN NYAHODA

A Thesis Submitted to the University of Zambia in fulfilment of the Requirements for the Degree of Doctor of Philosophy in Epidemiology.

THE UNIVERSITY OF ZAMBIA

LUSAKA

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Date………………………………………………………………………………………………

CERTIFICATE OF APPROVAL

This Thesis of **Martin Nyahoda** has been approved as fulfilling the requirements for the award of a Degree of Doctor of Philosophy in Epidemiology by the University of Zambia.

Internal Examiner (I)

……………………….………………………………… **Date**……………………….......

Internal Examiner (II)

……………………………..…………………………… **Date**……………………….......

External Examiner

……………………………..…………………………… **Date**……………………….......

Chairperson, Board of Examiners

……………………………..…………………………… **Date**……………………….......

ABSTRACT

Respiratory infections are among the leading causes of morbidity and mortality globally especially in Sub-Saharan Africa. In 2021, there were an estimated 344 million global incident episodes attributable to lower respiratory infections translating to an estimated 4350 episodes per 100,000 population resulting in an estimated 2.18 million deaths which was about 27.7 deaths per 100,000 population. Sub-Saharan Africa accounts for about 66.4% of deaths attributable to lower respiratory tract infections per 100,000 persons. In Zambia, severe acute respiratory infections are estimated at 6181 and ranked between 5th and 8th on the top 10 leading causes of death. Between 2019 and 2021, non-pharmaceutical interventions (NPIs) to limit the spread of COVID-19 were implemented globally which included hand washing, face masking, school closures, limiting public gatherings, remote working, hand sanitizing, physical distancing and travel restrictions. This resulted in a decline of respiratory infections especially influenza and respiratory syncytial virus subsequently leading to a decline of all age mortality attributable to lower respiratory infections by 16%. While scientific studies to establish the impact of COVID-19 associated NPIs on circulation of respiratory pathogens have been conducted in several countries globally, there was little knowledge on how the COVID-19 pandemic and its associated public health and social measures changed the epidemiology of influenza and other respiratory infections in Zambia. Therefore, the aim of this study was to determine the broad range of respiratory pathogens that were circulating in Zambia during the COVID-19 pandemic and determine the effects of age and location (rural or urban) on the likelihood of infection. Additionally, through systematic review and meta-analysis, the study sought to ascertain the burden of respiratory infections in Africa and existing regional disparities.

This study was retrospective using nasopharyngeal specimens that were collected national wide to test for SARS-CoV-2. After testing for SARS-CoV-2, selected specimens were tested for influenza A virus (IAV), influenza A(H1N1) virus (swine lineage) (IAV(H1N1) swl), influenza B virus (IBV), influenza C virus (IVC), human coronaviruses (HCoV) NL63, 229E, OC43 and HKU1, human parainfluenza viruses (HPIV) 1, 2, 3 and 4, human metapneumoviruses (HMPV) A and B, human rhinovirus (HRV), human respiratory syncytial viruses (HRSV) A and B, human adenovirus (HAdV), enterovirus (EV), human parechovirus (HPeV), human bocavirus (HBoV), *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* B, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Bordetella* spp. (except *Bordetella parapertussis*), *Klebsiella pneumoniae*, *Legionella pneumophila* / *Legionella longbeachae*, *Salmonella* spp. and *Haemophilus influenzae* in order to understand the profile of pathogens that continued to circulate between July 2020 and July 2021. Proportional age stratified convenient sampling was used to select a total of 128 specimens from children, adolescents, adults and the elderly collected from both urban and rural areas. The final sample consisted 88 from urban and 40 from rural areas collected from Lusaka, Luapula, Northen and Muchinga provinces. Using multiplex rRT-PCR, 128 nasopharyngeal specimens were tested for influenza and other respiratory pathogens.

Overall, 71.1% (91/128) of samples tested positive for at least one respiratory pathogen. *Staphylococcus aureus* was the most prevalent respiratory pathogen detected accounting for 22.7% (29/128) followed by *Klebsiella pneumoniae* 20.3% (26/128). Influenza accounted for 13.3% (17/128). Of the 17 specimens testing positive for influenza, 16 were influenza A/(H1N1) while one specimen tested positive for influenza B. The prevalence of rhinovirus and respiratory

syncytial virus was estimated at 3.1% (4/128) and 2.3% (3/128), respectively. Children, adolescents and the elderly accounted for the most influenza positive specimens 76.5% (13/17) while 100% (3/3) specimens positive for *Moraxella catarrhalis* were all from children. All specimens that tested positive for *Haemophilus influenzae* (5/5) were from children and adolescents and 17 of the 29 specimens that tested positive for *Staphylococcus aureus* were also from the same age group. For co-infections, 52 specimens were found to be co-infected with at least 2 respiratory pathogens. Of the total co-infections, 11.5% (6/52) were virus-virus with adenovirus as the most co-infecting viral pathogen while virus-bacteria co-infections accounted for 48.1% (25/52) with *Staphylococcus aureus* as the most co-infecting bacterial pathogen.

Bacterial respiratory pathogens were more predominant than viral pathogens during the study period. Compared to prevalence prior to the COVID-19 pandemic, a decrease for both viral and bacterial respiratory pathogens was observed. The results seem to suggest that the emergence of COVID-19 and its associated public health interventions may have altered the epidemiology of influenza and other respiratory pathogens in Zambia. Increase in age was associated with an increased risk of infection with adenovirus and SARS-CoV-2 adjusted odds ratio Exp (B) 1.026, CI (0.999-1.053) and 1.048, CI (1.004-1.094) respectively. Although results suggested that rural areas were associated with an increased risk of infection for *Bordetella pertussis* adjusted odds ratio 3.10, CI (0.667-4.208) and *K. pneumoniae* adjusted odds ratio 1.588, CI (0.615-4.097) including *S. aureus* adjusted odds ratio 1.362, CI (0.561-3.303), the results were not statistically significant.

At the level of the African continent, overall pooled prevalence for viral respiratory pathogens was estimated at 56.6% (95% CI, 0.371-0.762, I^2 99.9%). Overall pooled prevalence for bacterial respiratory pathogens was estimated at 12.9% (95% CI, 0.122-0.137, I^2 99.8%). Rhinovirus and respiratory syncytial virus were the most prevalent pathogens with wide geographical spread across all African regions with 19.9% and 8.9% prevalence respectively. Generally, most studies in Africa were focused on viral respiratory pathogens. To fully understand the burden of respiratory infections in Africa, there is need for more studies combining both viral and bacterial respiratory pathogens especially with the advent of multiplex PCR.

Colonization and enhanced viral induced bacterial binding may have contributed to the persistence of bacterial respiratory infections more than viral pathogens. Co-infections were mostly observed in children because they may be still immunologically naïve and not able to take precautionary measures such as avoiding touching eyes and mouth including frequent hand washing. Although there were observed differences in the prevalence of infection between rural and urban areas, the differences were not statistically significant. Further, and location did not increase the likelihood of infection except for SARS-CoV-2 and adenovirus.

Overall, this study demonstrated that with sustained non-pharmaceutical interventions, it is possible to reduce the number of respiratory infections and contribute to improved public health outcomes. Additionally, the high number of bacterial respiratory pathogens detected in this study provides impetus to strengthen efforts to contain these pathogens as they are known to contribute to severe disease and death.

DEDICATION

This work is dedicated to all my family members, workmates and friends for their love and support during my period of study at the University of Zambia. I wish them God’s love and blessing.

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LIST OF ABBREVIATIONS AND ACRONYMS

AGID:	Agar gel immunodiffusion
CAP:	Community Acquired Pneumonia
CDC;	United States, Centers for Disease Control
COVID-19	Corona Virus Disease 2019
DNA:	Deoxyribonucleic acid
ELISA:	Enzyme Linked Immunosorbent Assay
HMPV:	Human Metapneumovirus
HIV:	Human Immuno-Deficiency Virus
IgM:	Immunoglobulin M Antibodies
IgG:	Immunoglobulin G Antibodies
ILI:	Influenza-Like Infections
LRTI:	Lower Respiratory Tract Infections
MERS:	Middle East Respiratory Syndrome
NHRA:	National Health Research Authority
NPIs:	Non-Pharmaceutical Interventions
OIE:	International Organization for Animal Health
PCR:	Polymerase chain reaction
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-analysis
PVC:	Pneumococcal Conjugate Vaccine
RNA:	Ribonucleic acid
RSV:	Respiratory Syncytial Virus
SARI:	Severe Acute Respiratory Infections
SARS:	Severe Acute Respiratory Syndrome
TB:	Tuberculosis
IRB:	Institutional Review Board for Research Ethics
µL:	Microliter
VTM:	Viral Transport Medium
WHO:	World Health Organization
ZSA:	Zambia Statistical Agency

CHAPTER ONE

1.1 Introduction

Respiratory infections are among the top ten leading causes of death in children aged 0-14 especially in low and middle-income countries (Mathers et al., 2006). In 2019, global morbidity associated with lower respiratory tract infections (LRTI) were estimated to be 488.9 million with about 2.4 million deaths (Safiri et al., 2023). In African children, estimates show that 38.6% of infectious disease deaths and 14.9% of all deaths are caused by LRTI (Safiri et al., 2023; Troeger et., 2017). Sub-Saharan Africa accounts for 66.4 deaths attributable to LRTI per 100,000 persons which is considered highest (Troeger et al., 2018). Epidemiological studies have further shown that co-infections of viral-bacterial pathogens increase severity of disease especially in immune compromised and TB patients leading to poor prognosis (Mhimbira et al., 2019). Incidence of LRTI remains high in HIV positive children even with the widespread of antiretroviral therapy (Cohen et al., 2015) thereby increasing the risk of severe disease and death.

Both viral and bacterial pathogens have been implicated in LRTI and are associated with community acquired pneumonia (CAP). Among viral pathogens responsible for LRTI causing influenza-like illnesses (ILI) and severe acute respiratory infections (SARI) include influenza, respiratory syncytial virus, Rhinovirus and Coronaviruses (Loevinsohn et al., 2021a) On the other hand, bacterial pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Bordetella pertussis* have been found to contribute to disease severity and poor patient outcomes in episodes of cases associated with LRTI especially in incidences of co-infections (Moore et al., 2021; Loevinsohn et al., 2021a).

Influenza virus infections are significant because they cause high morbidity and mortality. The World Health Organization estimates that influenza is responsible for causing close to 650,000 annual deaths globally with over 1 billion infections (WHO, 2020). Influenza spreads mainly through person to person by means of aerosolized secretions which are virus laden when coughing, sneezing and talking (Cox and Subbarao, 2000). Southern Africa is among the regions with the highest mortality rates from influenza and respiratory syncytial virus (RSV) because of challenges in health care delivery, high burden of underlying health conditions such as Human Immuno-deficiency Virus (HIV), Tuberculosis (TB) and malnutrition (Safiri et al., 2023; Walaza et al., 2015). In Zambia, the mean annual number of influenza-associated severe acute respiratory illness (SARI) hospitalizations were estimated at 6181 translating to about 43.2 per 100,000 population with children less than 5 years accounting for 4669 cases, about 187.7 per 100,000 population and 1521 translating to 13.1 per 100,000 population for persons above 5 years of age (Theo et al., 2017). The Zambia Statistics Agency estimates that influenza and pneumonia is ranked as the 4^{th} leading cause of death for children under the age of 5 and ranked between 4^{th} and 8^{th} among the top 20 underlying causes of death for all age groups (ZSA, 2020). It is estimated that influenza accounts for about 1.5% of total underlying causes of death in Zambia (ZSA, 2020).

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections globally especially in neonates and infants though it is also known to cause morbidity throughout childhood (WHO, 2017). In Zambia epidemiological studies conducted to detect respiratory pathogens using nasopharyngeal specimens from persons presenting with influenza like illnesses (ILI) and severe acute respiratory illnesses (SARI) have also shown RSV to be among the most common respiratory pathogens (Loevinsohn et al., 2021a; b; Mizuta et al., 1996). While RSV is a respiratory pathogen of concern, significant gaps exist in characterizing the virus including its long-term impact on respiratory health. Thus, one of WHO's consensus priority activities is for countries to conduct epidemiological studies to estimate the burden of disease, describe local seasonality patterns and co-infections in order to inform vaccination strategies (WHO, 2017).

Human parainfluenza virus (HPIV) is the most common cause of croup and second to RSV in causing lower respiratory tract infections (Henrickson et al., 2003). Human Parainfluenza Virus accounts for 30%-40% of all acute respiratory tract infections in infants and children (Parija et al., 2020). Based on genetic and antigenic characteristics, HPIV is divided into type 1-4 with type 4 being further subtyped into A and B (Henrickson et al., 2003). Studies conducted in Zambia to detect respiratory pathogens have found the presence of HPIV type 1-3 in respiratory specimens of persons presenting with ILI and ARI (Loevinsohn et al., 2021; Simusika et al., 2015). Interestingly, HPIV type 3 was also detected in baboons in Zambia showing that HPIV is also capable of infecting non-human primates (Sasaki et al., 2013). However, while genetic characterization was performed for HPIV 3 detected in non-human primates, no genetic characterization has been done for HPIV strains detected in humans to fully understand its molecular epidemiology.

Human rhinovirus (HRV) is responsible for more than 50% of cases of the common cold and results in billions of dollars of economic costs associated with medical visits and loss of man hours of work (Nichol et al., 2005). Based on viral genetic characteristics, HRV is divided into three distinct groups; A, B and C. Although HRV mostly results in mild symptoms, recent studies have shown that it is associated with exacerbation of chronic pulmonary disease, asthma development and severe bronchiolitis in infants and immune-compromised individuals (Jacobs et al., 2013). While studies to detect HRV as a respiratory pathogen have been conducted in Zambia (Loevinsohn et al., 2021; Simusika et al., 2015), no genetic characterization has been performed.

Consequently, there are gaps in fully understanding the molecular epidemiology of HRV in Zambia.

With the advent of COVID-19, non-pharmacologic interventions to limit the spread of the disease were globally implemented which included handwashing and sanitizing, respiratory etiquette, school closures, face masking and remote working (Olsen et al., 2020). Studies showed that these interventions proved effective in limiting the spread of other respiratory infections especially influenza (Cowling et al., 2020; Olsen et al., 2020). Against this background, this study sought to ascertain how these non-pharmaceutical interventions changed the epidemiology of influenza and other respiratory infections in Zambia.

Further, although several individual studies have been conducted to detect respiratory pathogens of concern at country level in Africa, there is need to understand the distribution of these respiratory pathogens across the African region in order to target interventions in countries and regions with the highest disease burden and ensure better resource utilization (Dowell et al., 2016). Therefore, besides understanding the distribution and diversity of respiratory pathogens that continued to circulate in Zambia during the COVID-19 pandemic in Zambia, this study sought to provide estimates of the prevalence, diversity and distribution of respiratory pathogens of concern across African regions.

1.2 Statement of the Problem

Lower respiratory infections highly contribute to morbidity and mortality. Globally, there is an estimated 344 million episodes attributable to lower respiratory infections translating to 4350 episodes per 100,000 population resulting in an estimated 2.18 million deaths or 27.7 deaths per 100,000 population each year. In Zambia, respiratory infections are ranked between 5th and 8th among the top 10 leading causes of death. While attempts have been made to provide incidences of LRTI, little information is available on the distribution of aetiological agents in specific regions across Africa. This makes it difficult to plan and implement interventions in specific areas of need, making interventions inefficient and costly. Further, there is little knowledge on the broad range and diversity of respiratory pathogens that continued to circulate despite non-pharmacological interventions implemented against COVID-19 in Zambia. Without this information, it difficult to make public health policy recommendations to limit the transmission of respiratory pathogens in order to reduce the burden of disease.

1.3 Justification of the Study

During the COVID-19 pandemic, several non-pharmaceutical interventions were implemented to limit the spread of the disease. These interventions had an impact on the epidemiology of other respiratory infections. Therefore, in order to inform future policy considerations on public health and social measures to help contain respiratory infections in Zambia, it was important to undertake this study. Further, this study provided a foundation for investigating association of respiratory infections and co-infections with severe disease outcomes and contribute to improved clinical care and management of respiratory infections. Mapping disease burden for respiratory pathogens across different regions in Africa may help to target interventions in countries and regions with the highest burden and ensure prudent resource utilization.

1.4 Research Questions

1. What was the broad range of respiratory pathogens that continued to circulate during the COVID-19 Pandemic in Zambia?
2. Did age or geographical location rural-urban have any effect on the likelihood of infection?
3. What regional disparities exist in the burden of respiratory infections across Africa?

1.5 Research Objectives

1.5.1 Overall Objective

The overall objective of the study was to investigate the impact of COVID-19 and associated non-pharmacologic interventions on the epidemiology of respiratory pathogens in Zambia and determine the effects of age and geographical location on the likelihood of infection. Further, the study sought to determine the burden of respiratory infections in Africa to understand regional disparities.

1.5.2 Specific Objectives

1. To determine the prevalence, broad range and diversity of respiratory pathogens that continued circulating during the COVID-19 pandemic despite implementation of non-pharmacologic interventions in Zambia and ascertain the impact these interventions had on the epidemiology of respiratory infections.
2. To understand the profile of co-infections for respiratory pathogens during the COVID-19 pandemic.
3. To elucidate the effect of age and geographical location on the likelihood of infection
4. To ascertain existing regional disparities in the burden of respiratory infections across African regions.

CHAPTER TWO: LITERATURE REVIEW

2.1 Aetiology and Clinical Signs for Influenza like (ILI) and Severe Acute Respiratory Illnesses (SARI)

Aetiological agents responsible for the largest proportion of acute respiratory infections in humans are viruses and bacteria, with viruses accounting for a greater proportion of these infections (Etemadi et al., 2019). The major viral etiological agents that have been implicated in causing acute respiratory infections in humans include; Coronaviruses; HKU1, NL63, 229E and OC43, influenza viruses, respiratory syncytial virus, parainfluenza viruses, adenovirus, rhinovirus, human metapneumovirus and human enterovirus (Simusika et al., 2015). More recently, human bocavirus, parvovirus and mimivirus have been implicated as etiological agents for acute lower respiratory tract infections (Calvo et al., 2010). On the other hand, bacterial pathogens that have been found to be etiological agents for respiratory illnesses include *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bordetella pertussis* and *Mycoplasma pneumoniae* (Troeger et al., 2018. While infection with one or more of these respiratory pathogens manifest with similar symptoms such as fever $\geq 38^{o}C$, myalgia, wheezing cough, headache and bronchiolitis, detecting specific etiological agents in acute lower respiratory tract infections is important in improving prognosis as it helps in making treatment decisions early in the course of the illness (Etimadi et al., 2019). Further, understanding morbidity of specific etiological agents helps in determining agent-specific interventions that help reduce morbidity and mortality such as vaccination (Rudan et al., 2008).

While substantial efforts have been made to identify etiological agents for acute respiratory infections, gaps still exist in the understanding and diagnosis of viruses that are responsible for acute lower respiratory tract infections (Rudan et al., 2008). For example, while respiratory syncytial virus and rhinovirus have been identified as pathogens causing most acute lower respiratory tract infections in the tropics and developing countries (Simusika et al., 2015; Calvo et al., 2010; Rudan et al., 2008; WHO, 2009), the epidemiology of these respiratory pathogens is not fully understood. In Zambia, epidemiological studies have been conducted to identify these respiratory pathogens in children and adults (Loevinsohn et al., 2021; Simusika et al., 2015; Theo et al., 2017). However, genetic characterization of respiratory pathogens of concern such as respiratory syncytial virus and rhinovirus causing a greater proportion of acute lower respiratory infections has not been done to fully understand their epidemiology.

The World Health Organization (WHO, 2013), provides case definitions for ILI and SARI to allow for standardization of systems for public health surveillance, monitoring geographical and temporal variations in prevalence, detecting increases that may signify outbreaks, provision of a basis for inclusion in laboratory testing and ensuring data comparability (Fitzner et al., 2018). Case definition for ILI is an acute respiratory illness with a measured fever of $\geq 38^oC$, cough and onset within the last 10 days (WHO, 2013). Case definitions for SARI is an acute respiratory illness with a measured fever of $\geq 38^oC$, onset within the last 10 days and requires hospitalization (WHO, 2013). Other signs and symptoms associated with ILI and SARI may include headache, cough, sore throat, myalgia, nasal congestion, weakness and loss of appetite (Monto et al., 2000).

2.2 Reservoir hosts of Influenza and Other Respiratory Infections.

Avian species, especially wild aquatic birds such as shorebirds and waterfowl, are a reservoir of influenza A viruses of different subtypes with interspecies transmission occurring among wild aquatic birds (Cox et al., 2000). Other aquatic birds known to be reservoirs of influenza A viruses include ducks, turkeys and gulls. High titers of influenza viruses have been found in droppings of these wild birds (Webster et al., 1992). Influenza infection in these avian hosts reaches prevalence levels of >20% (Latorre-Margalef et al., 2014). Influenza virus infections in these avian hosts has been found to generally be asymptomatic (WHO, 1981). Other than avian species being reservoirs, avian influenza A viruses have also been isolated from seals, whales, horses and pigs (Cox et al., 2000). Pigs and aquatic birds serve as major reservoirs of influenza A H1N1 and H3N2 (Wille, M. and Holmes, E.C. 2020; Stallknecht et al., 1990). Pigs can simultaneously be infected with avian and mammalian species thereby acting as intermediate hosts for genetic reassortment resulting in novel strains. Pigs are therefore thought to play a major role in generation of pandemic strains (Webster et al., 1992). Fruit bats have also been found to be a reservoir of diverse subtypes of influenza A viruses (Wille and Holmes, 2020). Influenza A viruses from bats are thought to possess biological features necessary to infect humans (Webster et al., 1992).

Influenza viruses that are perpetuated in wild aquatic birds can persist in lakes through bird droppings and remain infectious for up to 207 days at 17^{o}C and even longer at 4^{o}C (Stallknecht et al., 1990). Thus, other species susceptible to influenza viruses may be infected through water contact during the period which the virus may be viable. This is possible for influenza A viruses which are harbored by aquatic birds as reservoir hosts. Wild aquatic birds include water birds (waterfowl) such as ducks, geese, swans, gulls, and terns, and shorebirds, such as storks, plovers, and sandpipers.

Influenza B and C are thought to be exclusively human pathogens and are not found in avian hosts though these types have also been found in pigs giving rise to the idea that pigs may also serve as alternative reservoirs (Webster et al., 1992). Further, although influenza C is thought to be an exclusively human pathogen, antibodies found in pigs and dogs suggest that this pathogen could be maintained in these animals and may probably serve as intermediate hosts (Wolff et al., 2021)

Rhinoviruses are non-enveloped, positive-sense, single-stranded RNA viruses belonging to the genus *Enterovirus* of the family *Picornaviridae* (Lewis-Rodgers et al., 2017). Rhinoviruses were first discovered in monkey kidney cells (Ljubin-Sternak, S. and Mestrovic, T., 2023; Price, W.H., 1956), although it is considered mainly a human pathogen of the lower respiratory tract associated with respiratory clinical disease in humans. They replicate in the lower respiratory tract causing bronchiolitis and pneumonia requiring hospitalization. Based on nucleotide sequence identity, Rhinoviruses are classified into three distinct sub-types, A, B and C.

Coronaviruses belonging to the family *Coronaviridae* are responsible for causing mild to severe respiratory infections in humans (Fehr A.R and Perlman S., 2015), such as Severe Acute Respiratory Syndrome (SARS-CoV) and the Middle East Respiratory Syndrome (MERS-CoV). *Bats* and civets are considered hosts of most known coronaviruses with bats harboring a much wider diversity than any known animal species (Khrishna et al., 2007). Various horse shoe bats have been found to be natural reservoirs of genetically diverse SARS-like coronaviruses (Hu et al., 2015).

Human parainfluenza viruses (HPIVs) are single-stranded, enveloped RNA viruses of the *Paramyoviridaie* family (Branche AR and Falsey AR., 2016). Human Parainfluenza viruses (HPIV) are divided into those that are endemic in humans and those that are animal. There are four distinct sub-types of human parainfluenza viruses (PIV 1-4). Human Parainfluenza Viruses are known to have a broad host range which include hamsters, ferrets, guinea pigs, chimpanzees, macaques and monkeys (Henrickson et al., 2003). Infection with HPIV in humans may be asymptomatic or result in severe lower respiratory infections progressing to fatal disease (WHO, 2020) especially in infants, young children, immune compromised, those with chronic diseases and the elderly. Parainfluenza viruses are a major cause of croup, a high-pitched barking cough in children 2-4 years. The most common clinical manifestation of PIV infection include bronchiolitis and pneumonia (Branche AR and Falsey AR., 2016).

Although non-human primates like macaques may be infected with some bacteria like *Moraxella catarrhalis* (Embers et al., 2011), most known respiratory bacteria are part of the respiratory microbiome especially in the upper and lower respiratory tracts.

Bordetella pertussis, a gram-negative coccobacillus is a causative agent for pertussis commonly known as whooping cough (CDC, 2024; WHO, 2019). Infection with Bordetella pertussis is characterized by accumulation of mucus in the lungs leading to an extended period of coughing. Other characteristics may include nasal congestion, fever and runny nose. Severe pertussis in infants may induce forceful coughing resulting in rib fractures (Melvin et al., 2014). Pertussis may be diagnosed by polymerase chain reaction (PCR) in early stages and Enzyme-Linked Immunosorbent Assay (ELISA) in later stages.

Streptococcus pneumoniae is the most common cause of community acquired pneumonia (CAP). Besides causing pneumonia, infection with *S. pneumoniae* can cause meningitis,

bacteremia, otitis media, sinusitis sepsis (CDC, 2024). *S. pneumoniae* is a gram-positive alpha hemolic organism commonly found as part of the normal microbiota of the respiratory tract. Pneumococci initially colonize the bronchioles after which growth of the bacteria spreads to the alveoli. Infected individuals develop a reproductive cough with blood sputum in severe cases (Weiser et al., 2018). Pneumococcal Conjugate Vaccine (PCV 13) and Pneumococcal

Polysaccharide Vaccine are the vaccines which are available for prevention against *S. pneumoniae.*

Overall, a summary of bacterial respiratory infection aetiologies of public health concern, diagnostic methods, their signs and symptoms including transmission mechanism is presented in table 2.1

Table 2.1 Bacterial Infections of the Respiratory Tract

Disease	**Pathogen**	**Signs and Symptoms**	**Transmission**	**Diagnostic Methods**	**Antimicrobial Drugs**	**Vaccine**
Acute otitis media (AOM)	Haemophilus influenzae Streptococcus pneumoniae. Moraxella Catarrhalis	Earache. possible effusion; may cause fever, nausea, vomiting, diarrhea	Often a secondary Infection. bacteria from respiratory tract become trapped in Eustachian tube, cause infection	Identification of bacteria in ear swabs	Cephalosporins, fluoroquinolones, penincilin	None
Diphtheria	Corynebacterium diphtheriae	Pseudomembrane on throat, possibly leading to suffocation and death	Inhalation of respiratory droplets or aerosols from infected person	Identification of bacteria in throat swabs. PCM to detect diphtheria toxin in vitro	Erythromycin, penicillin, antitoxin produced in horses	DtaP. Tdap. DT. Td. DTP
Legionnaires disease	*Legionella pneumophila*	Cough, fever, muscle aches, headaches, nausea vomiting, confusion; sometimes fatal	Inhalation of aerosols from contaminated water reservoirs	Isolation, using Warthin-Starry procedure, of bacteria in sputum	Fluoroquinolones, macrolides	None
Pertussis (whooping cough)	Bordetella pertussis	Severe coughing with “whoop" sound chronic cough lasting several months;	Inhalation of respiratory droplets from Infected person	Direct culture of throat swab. PCR ELISA	Macrolides	DTaP. Tdap

		can be fatal in infants				
Q fever	Coxiella burnetii	High fever, coughing, pneumonia, malaise; in chronic cases, potentially fatal endocarditis	Inhalation of aerosols of urine, feces, milk, or amniotic fluid of infected cattle, sheep, goats	PCR ELISA	Doxycycline, hydroxychloroquine	None
Streptococcal pharyngitis, scarlet fever	Streptococcus pyogenes	Fever, sore throat, inflammation of pharynx and tonsils, petechiae, swollen lymph nodes; skin rash (scarlet fever), strawberry tongue	Direct contact, inhalation of respiratory droplets or aerosols from infected person	Direct culture of throat swab, rapid enzyme Immunoassay	B-lactams	
Tuberculosis	Mycobacterium tuberculosis	Formation of tubercles in lungs; rupture of tubercles, leading to chronic, bloody cough, healed tubercles (Ghon complexes) visible in	Inhalation of respiratory droplets or aerosols from infected person	Mantoux tuberculin skin test with chest radiograph to identify Ghon complexes	Isoniazid, rifampin, ethambutol. pyrazinamide	BCG

		radiographs; can be fatal				
Chlamydial pneumonia	Chlamydia pneumoniae	Bronchitis, mild to severe respiratory distress	Inhalation of respiratory droplets or aerosols from infected person (C. *pneumoni*ae); exposure to infected bird (C *psIttac C).* exposure In the birth canal (Chlamydia trachomatis)	Tissue culture. PCP	Tetracycline, macrolides	None
Haemophilus pneumonia	Haemophilus influenzae	Cough, fever or low body temperature, chills, chest pain, headache, fatigue	Inhalation of respiratory droplets or aerosols from infected person or asymptomatic carrier	Culture on chocolate agar, serotyping of blood or cerebrospinal fluid samples	Cephalosporins, fluoroquinolones	Hib
Klebsiella pneumonia	Klebsiella pneumoniae, others	Lung necrosis, "currant jelly" sputum; often fatal	Health care associated; bacteria introduced via contaminated ventilators, intubation, or other medical equipment	Culture, PCFR	Cephalosporins, fluoroquinolones antibiotic susceptibility testing necessary	None

Mycoplasma pneumonia (walking pneumonia)	Mycoplasma pneumoniae	Low fever, persistent cough	Inhalation of respiratory droplets or aerosols from infected person	Culture with penicillin, thallium acetate	Macrolides	None
Pneumococcal pneumonia	*Streptococcus pneumoniae*	Productive cough, bloody sputum, fever, chills, chest pain, respiratory distress	Direct contact with respiratory secretions	Gram stain, blood agar culture with optichin and sodium deoxycholate, quelling reaction	B-lactams, macrolides, fluoroquinolones	Pneumococcal conjugate vaccine (PCV13),pneumococcal polysaccharide vaccine (PPSV23)
Pseudomonas pneumonia	*Pseudomonas aeruginosa*	Viscous fluid and chronic inflammation of lungs; often fatal	Health care associated; bacteria introduced via contaminated ventilators; also frequently affects patients with cystic fibrosis	Culture from sputum or other body fluid	a beta-lactam antibiotic recommended antibiotic susceptibility testing necessary	None

2.3 Transmission of Influenza and other Respiratory Viruses

The modes of transmission for influenza and other respiratory viruses are similar. Generally, they spread from infectious individuals to susceptible persons through virus-laden droplets and aerosols that are expelled when coughing, talking and sneezing (WHO, 2011). Fomites and direct contact with infected individuals, for example through hand shaking, are also proven modes of transmission (Mubareka et al., 2009). Children have been found to be an important factor in the spread of influenza and other respiratory infections. Outbreaks among school children may mark the beginning of a community outbreak (WHO, 2011). Exposure to influenza in closed settings such as nursing homes, hospital wards, aircrafts and cruise ships is associated with high attack rates (Bridges et al., 2003; Mubareka et al., 2009; Arden et al., 1995). In summary, there are 3 major ways in which influenza and other respiratory viruses can be transmitted; direct contact with infected individuals, contact with objects that are virus contaminated and inhalation of virus-laden aerosols (Racaniello et al., 2021). These modes of transmission are common among other respiratory viruses as shown in table 2.1.

Table 2.2: Primary Routes of Transmission for Influenza and other Respiratory Viruses

(adapted from He at al. (2023).

Respiratory Virus	**Family**	**Primary Transmission Routes**
Influenza	*Orthomyxoviridae*	Droplets and aerosols, contact
Respiratory Syncytial Virus	*Paramyxoviridae*	Direct and indirect contact, droplets and aerosols
Human Metapneumovirus	*Paramyxoviridae*	Droplets and contact
Human Coronavirus	*Coronaviridae*	Droplet spray and aerosols, including contact
Human Bocavirus	*Parvoviridae*	Contact, droplet spray and aerosols
Rhinoviuses	*Picornaviridae*	Droplets and aerosols, contact
Adenoviruses	*Adenoviridae*	Contact, droplet spray and aerosols
Enterovirus	*Picornaviridae*	Contact
Parainfluenza viruses	*Paramyxoviridae*	Contact, droplet spray and aerosols

2.4 Co-Infections

Respiratory pathogen co-infection which is simultaneous infection of the host by two or more pathogens are common and associated with severe disease and poor patient outcomes (Trepat et al., 2024). Co-infections could be between viruses (Virus-Virus Co-infection), between bacteria (Bacteria-Bacteria Co-infection) or between viruses and bacteria (Virus-Bacteria Co-infection). Co-infections in viruses could be characterized in three interactions, homologous interactions which involve two viruses belonging to the same family such as RSV and HMPV, heterotypic which involves two viruses of the same species such as influenza H1N1 and H3N2 (Trepat et al.,2024). Heterologous co-infections involve virus interactions belonging to two different families such as SARS-CoV-2 with influenza. These co-infections can occur simultaneously or sequentially. Factors that influence viral co-infection include competition for receptors, competition for cellular resources and interference mediated by the host immune response (Nickbakhsh et al., 2019)

On the other hand, viral infections are known to enhance bacterial binding hence increase the likelihood of viral-bacterial co-infections (Martin-Loeches et al., 2017). Major bacteria associated with co-infections include *Staphylococcus aureus, Streptococcus pneumoniae and Haemophillus influenza*. Studies have shown that *S. pneumoniae* enhances influenza replication by producing neuraminidase, an influenza surface protein that allow the virus to be released from the host cell by breaking sialic acids from glycoproteins (Martin-Loeches et al., 2017)

2.4 Pathology of Influenza Virus Infections

2.4.1 Clinical Course of Disease for Influenza Virus Infections

The incubation period of influenza virus infections is short, ranging from 1 to 3 days, with the outcome ranging from subclinical upper respiratory tract disease to fatal lower respiratory tract disease (Kuiken et al., 2012). Infections are usually characterized by sudden onset of high fever, coryza, cough, headache, prostration, malaise and inflammation of the upper respiratory tract (Taubenberger and Morens., 2008). In acute stage, patients may present with these symptoms for 7-10 days with prostration lingering for weeks and complication of croup in small children (To et al., 2005). In persons with chronic pulmonary cardiac disease or diabetes mellitus, there is a high risk of developing severe complications such as hemorrhagic bronchitis, pneumonia and death (Gu et al., 2007). Severe cases may occasionally result in pulmonary edema and death may ensue within 48 hours after the onset of symptoms (Taubenberger and Morens., 2008).

2.4.2 Pathology of Influenza Virus Infections

Knowledge of the pathology of cases is important in helping to understand the differences in presenting signs and symptoms, duration of the disease and complications resulting from different influenza virus infections (Guarner and Falcon-Escobedo, 2009). It is also a basis for pathological presumptive diagnosis and a foundation for further diagnostic techniques such as PCR and viral culture that allow confirmation of the virus for specific cases (Uiprasertkul et al., 2007). Although the pathology caused by influenza viruses depend on the virulence of the specific infecting agent and the host response, all influenza viruses tend to infect the respiratory epithelium from the nasal passages to bronchioles with high virulent strains infecting pneumocytes and intra-alveolar macrophages (Guarner and Falcon-Escobedo, 2009). They also cause inflammation, edema and epithelial necrosis of the trachea, bronchi and bronchioles (Gu et al., 2007). Guarner and Falcon-Escobedo, (2009), summarizes pathological changes resulting from specific influenza A subtypes as shown in the table 2.3;

Table 2.3: Pathological Characteristics for Selected Influenza A Virus Infections

Influenza A Subtype	Pathological Changes
H3N2 (Fatal Cases)	• Mucosal desquamation and disorganization • Intra-alveolar hemorrhage • Submucosal congestion and edema
H5N1	• Diffuse alveolar damage • Fibroblast proliferation • Disposition of collagen • Intra-alveolar hemorrhage and hemophagocytosis
H1N1	• Diffuse alveolar damage • Intraalveolar edema • Alveolar wall necrosis • Denuded mucosa

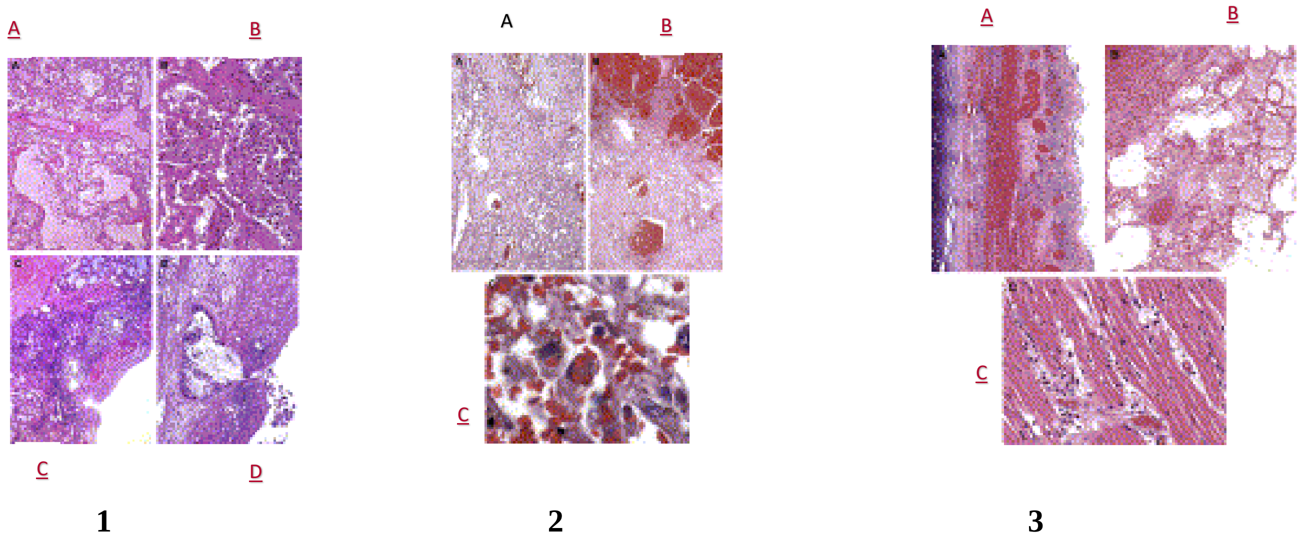

Figure 2.1: Pathological characteristics in the lower respiratory tract for influenza virus infections as described by Guarner and Falcon-Escobedo, 2009.

Notes;

1 (a) Histopathological characteristics showing intraalveolar edema in H1N1 2009pdm and;

(b) Alveolar wall necrosis

(c) Denuded mucosa of the treachea and bronchi with inflammation and necrosis

(d) Trachea and bronchi necrosis of the submucosa

2 (a) Histopathological characteristics of fatal cases with confirmed H5N1 virus infection showing diffuse alveolar damage in the later fibrous proliferative phase characterized by fibroblast proliferation and deposition of collagen

(b) Intra-alveolar hemorrhage seen in some cases

(c) Hemophagocytosis

3 (a) Histopathological characteristics of seasonal H3N2 virus infection showing mucosal desquamation and disorganization and submucosal congestion, hemorrhage, and inflammation in the trachea and;

(b) Lungs showing intra-alveolar hemorrhage and edema.

(c) Heart showing focal areas of mononuclear inflammatory infiltrate

2.5 Burden of Disease for Respiratory Infections

Estimates show that respiratory infections are among the top 10 leading causes of death in children aged 0-14 especially in low and middle-income countries (Mathers et al., 2006). In 2019, the global morbidity incidence associated with lower respiratory infections were estimated to be 488.9 million with about 2.4 million deaths (Safiri et al., 2023). Sub-Saharan Africa accounts for 66.4 deaths attributable to LRTI per 100,000 persons which is considered highest (Troeger et al., 2018). In African children, estimates show that 38.6% of infectious disease deaths and 14.9% of all deaths are caused by LRTI (Safiri et al., 2023; Troeger et al., 2015). Epidemiological studies have further shown that co-infections of viral-bacterial pathogens increase severity of disease especially in immune comprised and TB patients leading to poor prognosis (Mhimbira et al., 2019). Incidence of lower respiratory tract infections (LRTI) remains high in HIV positive children even with the widespread of antiretroviral therapy (Cohen et al., 2015) thereby increasing the risk of severe disease and death.

2.5.1 Morbidity Associated with Influenza Virus Infections

Influenza has spread globally with thousands of specimens testing positive in many countries since the first isolation of influenza A in 1933 and that of influenza B in 1940 as can be seen from Figure 2.2 below (Cox et al., 1992). The World Health Organization reports that globally, there are over 1 billion influenza infections annually (WHO, 2020). The highest burden of severe disease and hospitalizations for influenza is typically reported among persons with chronic health problems such as malnutrition, diabetes, asthma, compromised immune system such as HIV/AIDS and persons aged 65 years and older including children less than six months of age (Lafond et al., 2021; Poehling et al., 2012).

Globally, influenza accounts for over 14% of acute respiratory infection hospitalizations in adults with influenza A accounting for 10.6% while on the other hand influenza B is estimated to account for 3.5% of acute respiratory infection hospitalizations (Chaves et al., 2013). Overall, influenza-associated hospitalization in adults is estimated to reach over 5 million in persons 20 years and older with persons $\geq$ 65 years accounting for over 3 million hospitalizations translating to 437 hospitalizations per 100,000 persons for this age group (Lafond et al., 2021; GBD, 2017). Further estimates indicate that influenza A accounts for over 24 million lower respiratory infection episodes with influenza B accounting for over 7 million lower respiratory infection episodes worldwide (Lafond et al., 2016).

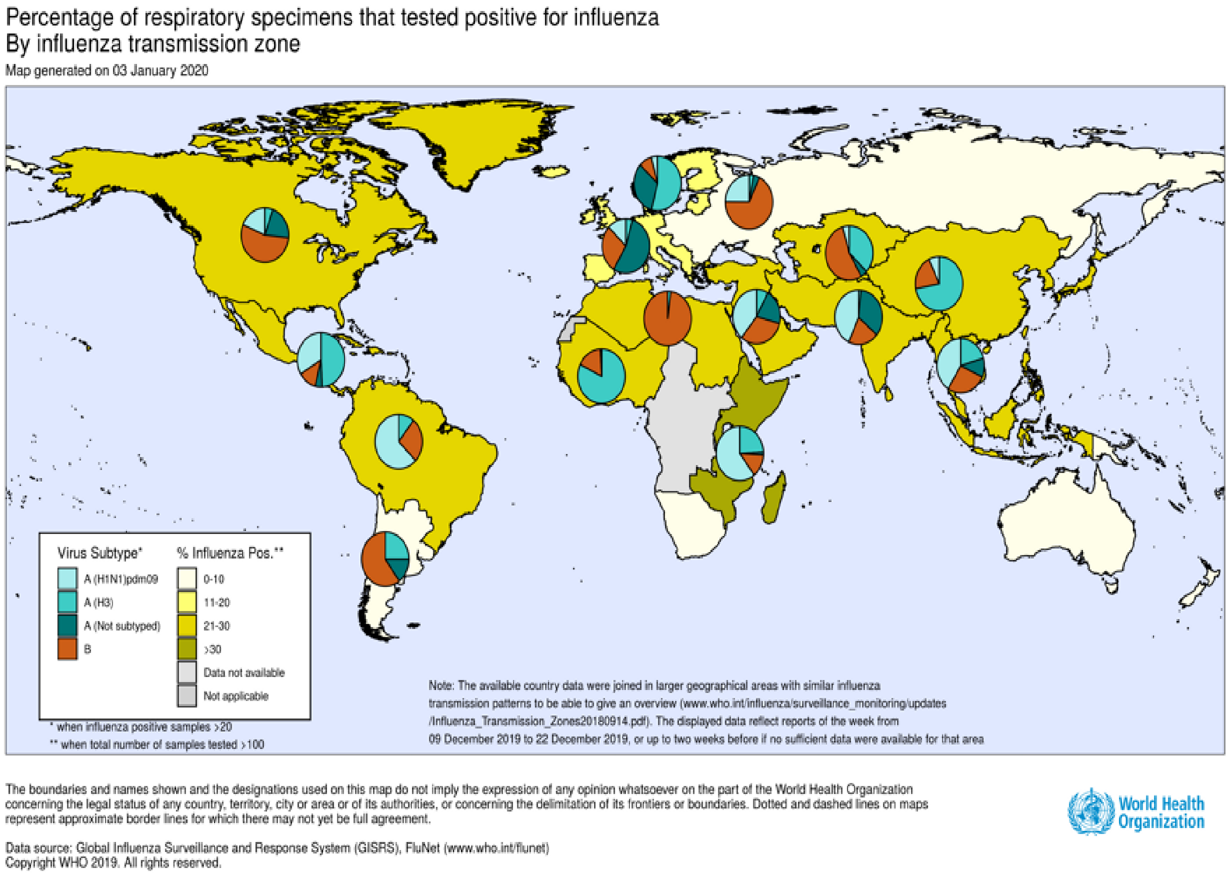

Figure 2.2: Percentage specimens tested positive for influenza. Source: WHO, 2021

In Zambia, the mean annual number of influenza associated SARI hospitalizations is estimated at 6,181 translating to about 43.2 per 100,000 population with the burden of disease being highest in children < 5 years having an estimated annual number of hospitalizations of about 4,669 translating to 187.7 per 100,000 (Theo et al., 2017).

2.5.2 Mortality Associated with Influenza Virus Infections

Recent mortality estimates indicate that globally, influenza is responsible for causing over 600,000 deaths per year with the majority of deaths occurring among older adults ≥ 65 years (WHO, 2020; Iuliano et al., 2018). In children aged < 5 years, influenza associated mortality is estimated to reach over 34,000 annually (Wang et al., 2020) with Eighty-two percent (82%) of influenza associated deaths in children < 5 years occurring in low income countries.

In Zambia, influenza is ranked between 5th and 8th among the top 10 underlying causes of death for all age groups and as the 4th leading cause of death for children < 1 year (ZSA, 2020). Overall, the Zambia Statistical Agency estimates that influenza may be responsible for over 400 deaths in Zambia annually (ZSA, 2020). Considering the number of estimated hospitalizations, this estimated mortality is about 6.5% of the annual estimated influenza-associated SARI hospitalizations in Zambia.

2.6 Burden of other Respiratory Infections

Respiratory syncytial virus (RSV) is the most common cause of LRTIs globally especially in neonates and infants though it is also known to cause morbidity throughout childhood (WHO, 2017). In 2019, the global burden of disease for RSV was estimated at 3.6 million hospitalizations in children less than 5 years (Li et al., 2022). In 2021, global episodes associated with RSV for all age groups were estimated to be 4.59 million with about 31,500 deaths (Bender et al., 2024). In Zambia, epidemiological studies conducted to detect respiratory pathogens using nasopharyngeal specimens from persons presenting with ILI and SARI have also shown RSV to be among the most common respiratory pathogens (Loevinsohn et al., 2021a;b; Mizuta et al., 1996). While RSV is a respiratory pathogen of concern, significant gaps exist in characterizing the virus including its long-term impact on respiratory health.

Human parainfluenza virus (HPIV) is the most common cause of croup and second to RSV in causing lower respiratory tract infections (Henrickson et al., 2003). Human Parainfluenza Virus accounts for 30%-40% of all acute respiratory tract infections in infants and children (Parija et al., 2020). Based on genetic and antigenic characteristics, HPIV is divided into type 1-4 with type 4 being further subtyped into A and B (Henrickson et al., 2003). Studies conducted in Zambia to detect respiratory pathogens have found the presence of HPIV type 1-3 in respiratory specimens of persons presenting with ILI and ARI (Loevinsohn et al., 2021; Simusika et al., 2015).

Human rhinovirus (HRV) is responsible for more than 50% of cases of the common cold and results in billions of dollars of economic costs associated with medical visits and loss of man hours of work (Nichol et al., 2005). While HRV mostly results in mild symptoms, recent studies have shown that it is associated with exacerbation of chronic pulmonary disease, asthma development and severe bronchiolitis in infants and the immune-compromised (Jacobs et al., 2013). Studies to detect respiratory pathogens conducted in Zambia (Loevinsohn et al., 2021; Simusika et al., 2015) have shown that rhinovirus is among the viral aetiologies causing respiratory illnesses in Zambia.

Streptococcus pneumoniae is responsible for the highest burden of aetiologies of LRIs with an estimated number of episodes reaching 97.9 million in 2021 with about 505,000 deaths across all age groups (Bender et al., 2024). It is also the most prevalent pathogen among community-acquired bacteria. Considering available global burden of disease estimates of LRIs since 1990, it can be noted that S.*pneumoniae* has overtaken *Haemophilus influenzae* in terms of incidences of LRIs episodes (Bender et al., 2014)

In 2021, it was estimated that *Staphyloccocus aureus* accounted for 424,000 episodes whereas *Klebssiella pneumoniae* had a contribution of 176,000 episodes (Bender et al., 2024). Because it is not vaccine preventable and its ability for antimicrobial resistance, infection with *S. aureus* usually results in complicated pneumonia with poor prognosis including sepsis and death (Troeger et al., 2018)

2.7 Influenza and Other Respiratory Infections during the COVID-19 Pandemic Era

2.7.1 Influenza Activity for Selected Countries during the COVID-19 Pandemic

With the advent of the COVID-19 pandemic, epidemiological investigations that were conducted revealed decreased influenza activity in several countries globally. For example, epidemiological studies conducted to investigate influenza infections in the United States revealed a decrease of 98% in terms of number of specimens testing positive for influenza from a median of 19.24% to 0.33% with a similar trend being reported in Australia, Chile and South Africa (Olsen et al., 2020). Results of the same study indicated that inter-seasonal influenza in the United States hit record low levels of 0.2% of specimens testing positive for the period May-August 2020 compared to 2.35% during the same period in 2019. A study conducted to assess the impact of non-pharmacological interventions against SARS-COV-2 on influenza in Hong Kong showed a 33% decrease in influenza community transmissibility based on pediatric hospitalizations (Cowling et al., 2020). The same study revealed that the effective reproduction number R_1 decreased from 1.28 before implementation of COVID-19 pandemic associated non-pharmacological interventions to 0.72 during the pandemic. An effective reproduction number $R_1 > 1$ gives an indication that the epidemic is growing while an effective reproduction number $R_1 <1$ shows a decreasing or slowing epidemic (Lim et al., 2020). Other countries that reported decreased influenza activity during the COVID-19 pandemic include Japan, where an overall decrease in influenza activity in all age groups was observed (Sakamoto et al., 2020). In South Africa, influenza activity was seen to decrease from 13.7% in the period April-July between 2017 and 2019 to 0.06% during the same period in 2020 (Olsen et al., 2020). The decrease in influenza infections was mainly attributed to public health and social measures instituted to curb the spread of COVID-19. Other studies have revealed that pathogenic competition exists between respiratory viruses including corona viruses and influenza such that infection with one virus may elicit immune responses that may diminish infection with the other (Nickbakhsh et al., 2019). Infection with influenza has been found to be associated with reduced risk of SARS-CoV-2 infection giving further evidence of pathogenic competition (Stowe et al., 2021). On the other hand, evidence exists that co-infection with influenza and SARS-CoV-2 results in more severe disease outcomes (Stowe et al., 2021).

2.7.2 Global Influenza Activity Outlook Before and during COVID-19 Pandemic

Global epidemiological data reported to the World Health Organization on influenza over the period 2017-2021 revealed a decreasing trend of influenza activity during the COVID-19 pandemic in terms of specimens testing positive. This can be seen by an analysis and comparison of year-by-year influenza charts extracted from FluNet presented below.

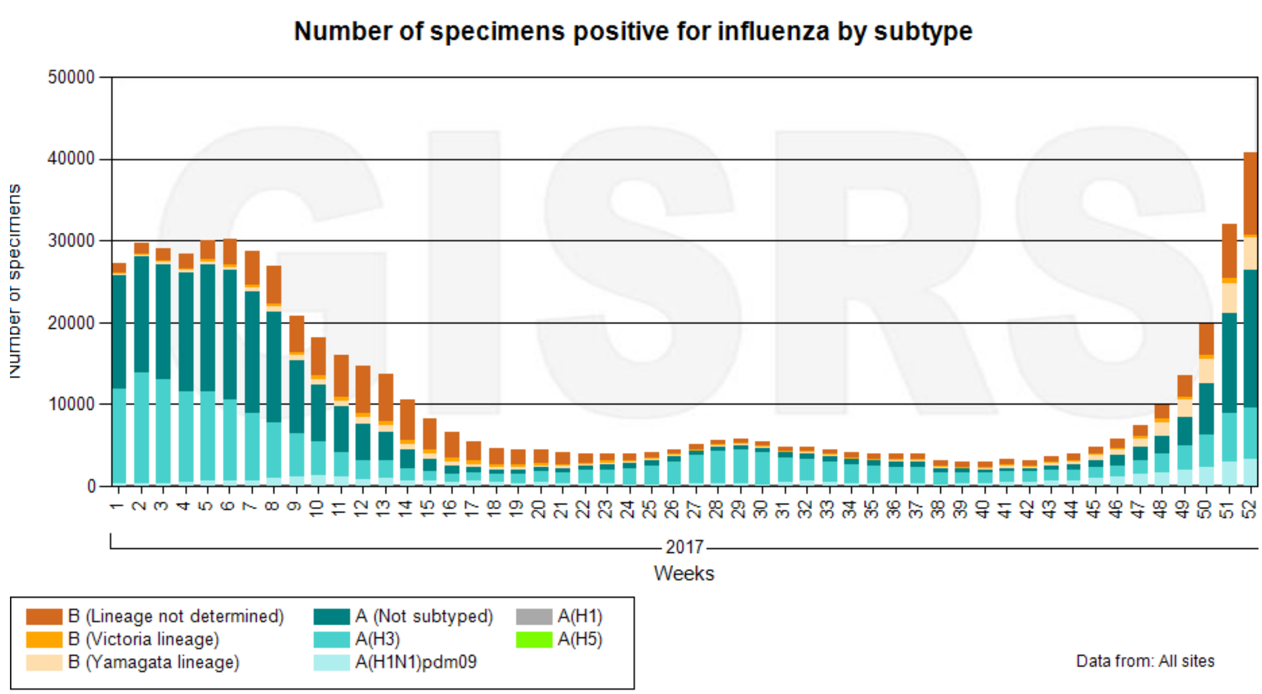

Figure 2.3: Number of specimens testing positive for influenza by subtype 2017. Source: FluNet (www.who.int/flunet)

Figure 2.3 shows a normal global influenza curve of specimens testing positive for influenza by subtype in 2017. It can be observed that influenza activity was high between week 1 and week 12 reaching a peak of 30, 000 specimens testing positive for influenza in week 5 and 6 and gradually declining from week 15 through to week 44 and gradually rising again from week 46 reaching over 40,000 specimens testing positive for influenza by subtype in week 52.

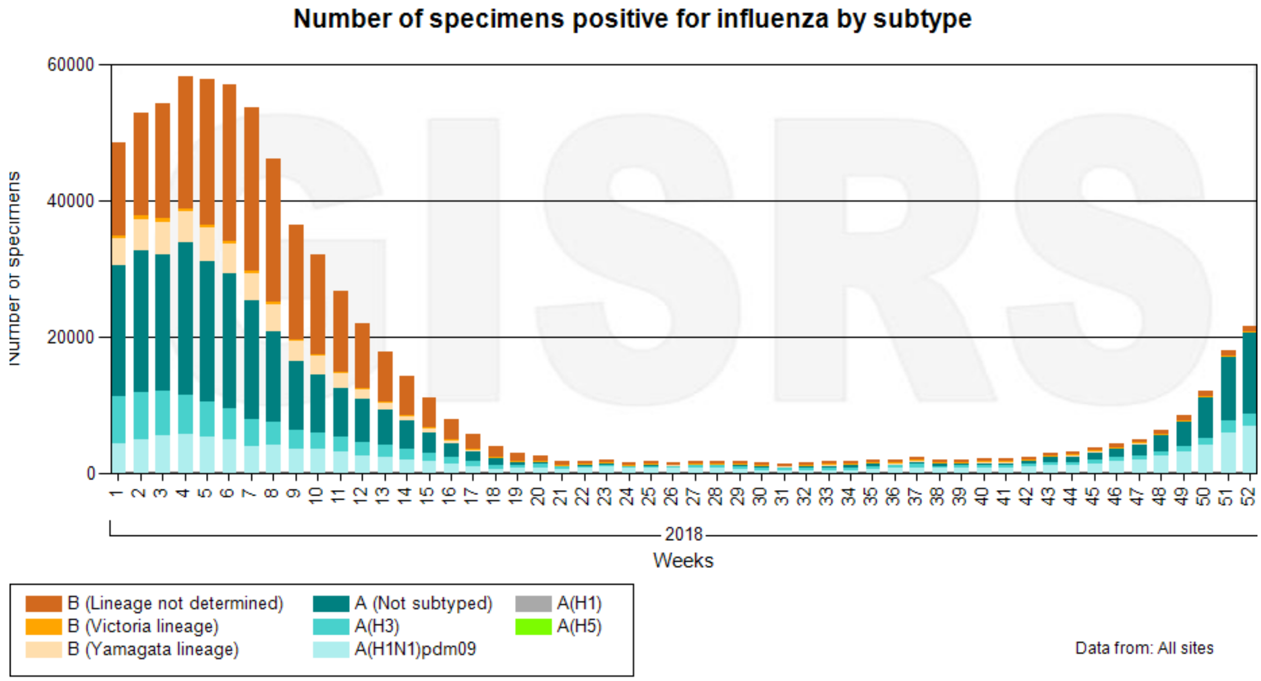

Figure 2.4: Number of specimens testing positive for influenza by subtype 2018. Source: *FluNet (*www.who.int/flunet*)*

Figure 2.4 shows a normal global influenza curve of specimens testing positive for influenza by subtype in 2018. It can be observed that influenza activity was high between week 1 and week 12 reaching a peak of close to 60, 000 specimens testing positive for influenza in week 4, 5 and 6 and gradually declining from week 13 through to week 44 and gradually rising again from week 46 reaching above 20,000 specimens testing positive in week 52.

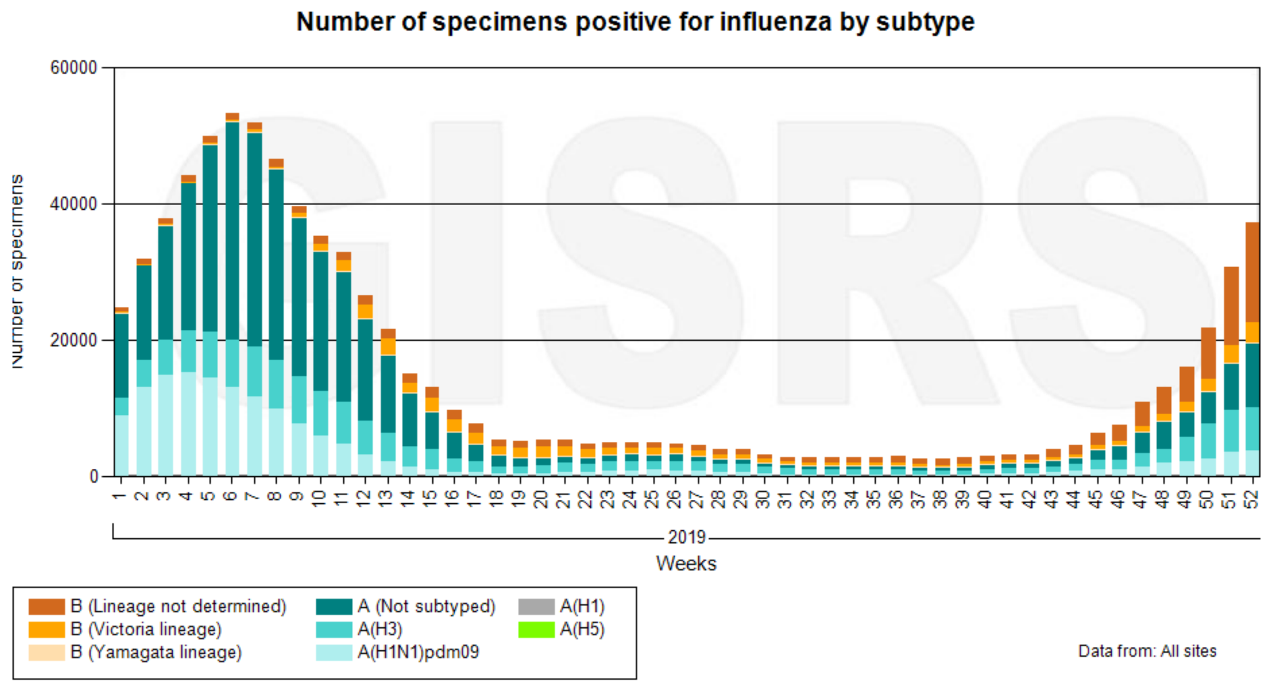

Figure 2.5: Number of specimens testing positive for influenza by subtype 2019. Source: *FluNet (www.who.int/flunet)*

Figure 2.5 shows a normal global influenza curve of specimens testing positive for influenza by subtype in 2019. It can be observed that influenza activity was high between week 1 and week 13 reaching a peak of over 50, 000 specimens testing positive for influenza in week 5, 6 and 7 and gradually declining from week 13 through week 44 and gradually rising again from week 46 reaching close to 40,000 specimens testing positive in week 52.

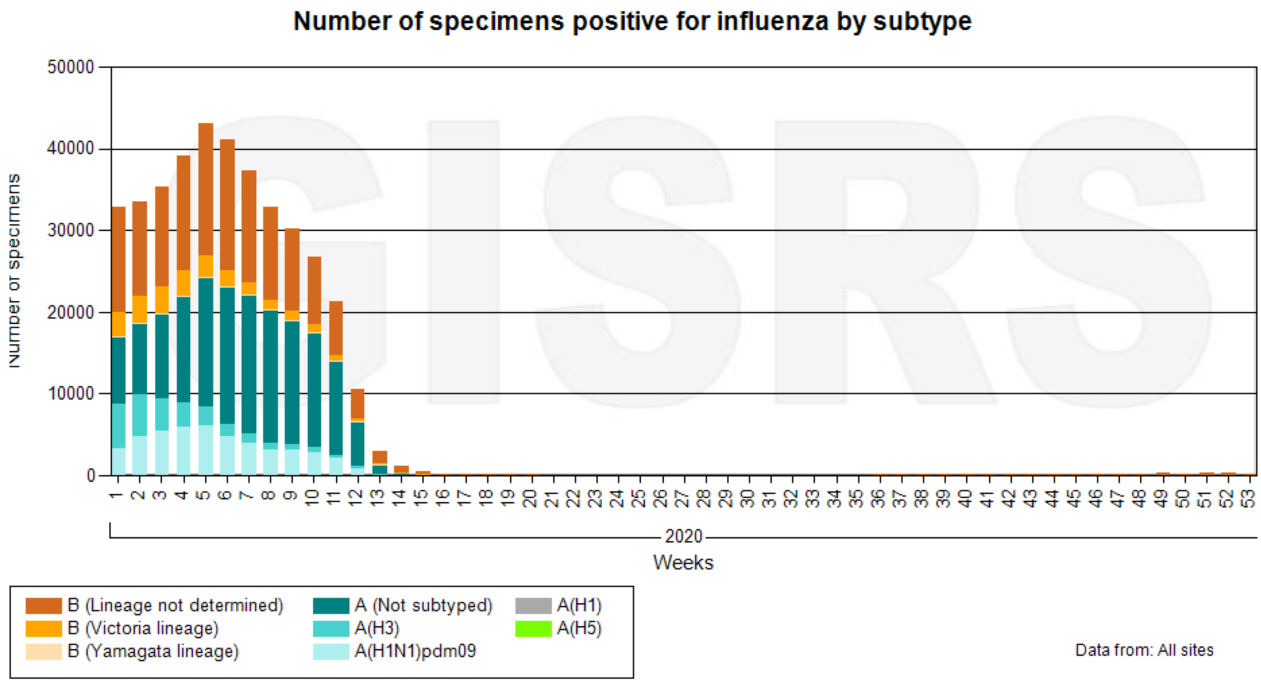

Figure 2.6: Number of specimens testing positive for influenza by subtype 2020. Source: FluNet (www.who.int/flunet)

In figure 2.6 above, it can be seen that in 2020, specimens testing positive for influenza by subtype was high between week 1 and week 12 reaching a peak of over 40,000 specimens testing positive for influenza in week 5 and 6. It can further be observed that after week 13 corresponding to March 2020, specimens testing positive suddenly dropped to unprecedented low levels through to week 52. Generally, normal influenza curves are U shaped as can be observed in the years prior to the COVID-19 pandemic. The trend curve observed in 2020 during the time of the COVID-19 pandemic departs from the normal influenza curve.

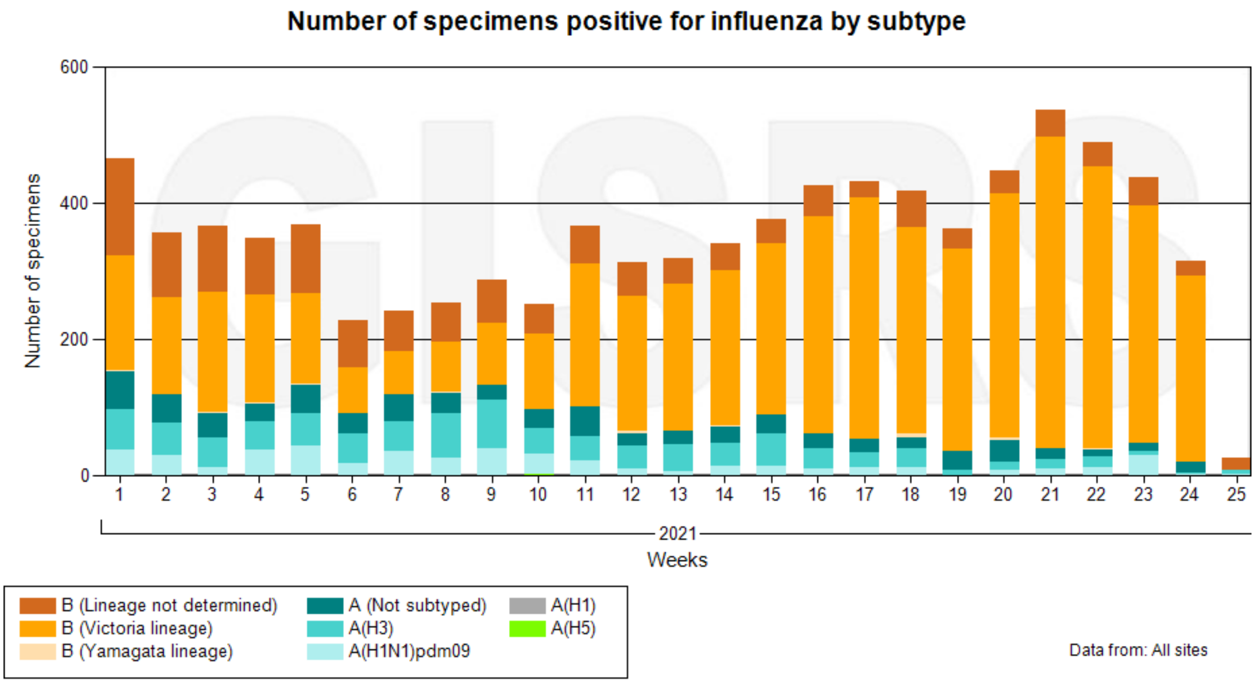

Figure 2.7: Number of specimens testing positive for influenza by subtype 2021. Source: FluNet *(*www.who.int/flunet*)*

In figure 2.7 above, it can be observed that the number of specimens testing positive for influenza by subtype across week 1 to week 25 in 2021 were at record low compared to the same period in the years before the COVID-19 pandemic. In 2021, the number of specimens testing positive for influenza by subtype was consistently below 600 from week 1 to week 12 while specimens testing positive for influenza by subtype over the same period was consistently over 20,000 reaching a peak of over 40, 000 specimens testing positive for most of the years before the pandemic.

2.7.3 Circulation of Influenza in Zambia before and during the COVID-19 Pandemic

An epidemiological study conducted by Theo et al, (2017) to estimate the burden of influenza-associated SARI hospitalizations in Zambia between 2011 and 2014 revealed an influenza prevalence of 5.5%. The prevalence was found to be 4.8% (48/998) in children aged < 5 years while in persons aged ≥ 5 years the prevalence was estimated at 6% (104/1736). The strains of influenza found to be circulating were distributed as A/H3N2 (38.7%), A/H1N1pdm09 (14.6%), A not subtyped (1.6%) and influenza B (45.2%). Most influenza viruses were detected between February and March with an all-time peak observed between June and August. Influenza curves were U shaped closely resembling those observed globally.

In a more recent study conducted by Loevinsohn et al, (2021), the estimated pathogen prevalence for influenza A was 12.07% while that of influenza B was 6.4%. This study further revealed that while influenza may have an all year circulation, the peak was observed between June and September for influenza A and July and December for influenza B.

A study by Sutcliffe et al., (2023), in rural Zambia revealed that influenza declined from about 14% in 2019 to 0% in 2020 before rebounding to 9.4% in 2021. This trend was similar with other respiratory pathogens such as RSV. This is the only published study at the time elucidating the impact of measures to limit the spread COVID-19 on other respiratory pathogens in Zambia.

Against this background, it was therefore important to investigate the impact of non-pharmacological interventions on influenza activity in Zambia from both a rural and urban perspective in order to fully understand the circulation of respiratory pathogens during the COVID-19 pandemic to inform public health policy.

2.7.4 Other Respiratory Infections during the COVID-19 Pandemic

Similar to influenza, infections with RSV were found to be lower than pre-pandemic periods in all countries in which comparative epidemiological studies were conducted (Principi et al., 2023). In England, confirmed cases of RSV, hospital admissions and emergency attendances were substantially reduced by over 80% in children aged five years and below (Britton et al., 2020). In Japan, no RSV outbreak was reported in 2020 at the peak of the pandemic (Ujiie et al., 2021) while there was a 93.4% reduction of RSV infections in Australia. This trend was similar with other non-enveloped respiratory viruses such as Rhinoviruses, Enteroviruses, Adenoviruses and human Bocavirus. Detection of these non-enveloped viruses declined as with enveloped respiratory viruses globally (Principi et al., 2023). The modifications in the circulation of bacterial respiratory pathogens was also observed during the pandemic as the case with viral respiratory pathogens (Principi et al., 2023; Juan et al., 2020).

While evidence shows considerable decreased activity for circulation of influenza and other respiratory pathogens during the COVID-19 pandemic especially in Western and Asian countries, the impact of interventions to limit the spread of COVID-19 has not been well elucidated in Africa resulting in limited information to support public health policy with regard to enforcing and sustaining these measures against influenza and other respiratory pathogens with similar transmission mechanisms post-COVID-19.

2.8 Diagnostic Techniques for Influenza and Other Viral Respiratory Pathogens

Since clinical manifestations associated with respiratory pathogens are non-specific and overlap significantly, laboratory confirmation of etiological agents is important to aid clinical management and vaccine strain selection (Mc Namara and Van Doorn, 2014). Laboratory techniques are based on the demonstration of the presence of the virus or viral components in specimens or the immune response to the virus (Ghebrehewet et al., 2016). The commonly used diagnostic techniques demonstrating presence of the virus or viral components include;

i. Demonstration of viral antigens by immunofluorescence
ii. Demonstration of viral infectivity by growth in cell culture
iii. Detecting presence of viral nucleic acid by polymerase chain reaction (PCR)

iv. Virus isolation using embryonated chicken eggs for influenza

On the other hand, serological techniques for influenza diagnosis may also include hemagglutinin inhibition test (WHO, 2011). From all the available diagnostic techniques, WHO recommends RT-PCR as the most sensitive method for detecting influenza and other respiratory pathogens (WHO, 2011). In addition to PCR, WHO also suggests the use of virus culture on a subset of specimens to allow detailed antigenic and genetic characterization of influenza viruses. For hemagglutinin and neuraminidase subtyping, the hemagglutinin and neuraminidase inhibition tests are recommended. In order to yield good diagnostic results, WHO further recommends taking specimens within 7 days after the onset of symptoms and keeping the samples that cannot be processed within 48-78 hours frozen below -70^{o}C.

2.9 Diagnostic Techniques for Bacterial Respiratory Pathogens

To detect bacterial respiratory infections, there are four methods at disposal that could be used which include bacterial culture, polymerase chain reaction (PCR), antigen detection assays which are also called rapid diagnostic tests (RDTs) and serological tests that detect IgM or IgG antibodies (Calderaro et al., 2022). Immunoglobulin M (IgM) antibodies are the first line of an immune response following an infection while immunoglobulin G (IgG) antibodies are produced in the acute stage of an infection and may persist during recovery (Das et al., 2018). It is important to understand that early and accurate diagnosis is critical in reducing the time to decision of treatment options. For this reason, bacterial culture for detection and identification of Chlamydophila and Mycoplasma species is not preferred as it requires weeks of growth. Therefore, molecular assays are preferred diagnostic alternatives for these species (Calderaro et al., 2022).

Usually, nasopharyngeal specimens are useful for performing bacterial culture in diagnosis of pertussis and pneumococcal respiratory infections. In performing bacterial culture, specimens are inoculated on sheep blood or chocolate agar and thereafter aerobically incubated 37^0C for 48 hours. Samples suspected to be infected with Staphylococcus aureus should be inoculated on Regan-Lowe charcoal agar with 10% horse blood and cephalexin after which they should be aerobically incubated at 35^0C for 5-7 days (Calderaro et al., 2022; Skevaki et al., 2012). Bacteria culture remains the gold standard for diagnosis of bacterial pathogens. However, because of the long time to decision and being labor intensive, molecular assays (PCR) and rapid tests are more preferred than bacterial culture for detection of bacterial respiratory infections.

2.10 Prevention, Control and Treatment of Respiratory Infections

In order to plan and implement effective prevention and control measures against respiratory infections, it is important to understand the modes of transmission (Seto et al., 2013). As literature has shown, mainly, respiratory infections are spread through respiratory droplets that may be produced when coughing or talking as well as contact transmission by direct contact with infectious persons, surfaces and other inanimate objects that may be contaminated (Wei and Norwood, 2001). With an understanding of these transmission methods, prevention and control strategies should include rigorous hand hygiene such as washing and alcohol based sanitizing to reduce the risk of self-inoculation of respiratory pathogens. Avoiding handshakes is also encouraged (Smith et al., 2023; Wei and Norwood, 2001). Respiratory hygiene such as covering the mouth when coughing and sneezing to block respiratory droplets is also important in infection prevention. Physical distancing which was previously known as social distancing, eye protection, face masking are also among key prevention strategies. Other interventions which have proved effective for prevention and control of respiratory infections and have been widely implemented include isolation and quarantine, regular cleaning and disinfection. Ventilation has been associated with increased risk of infection (Simth et al., 2023). Improved ventilation is known to provide higher dilution capabilities resulting in reduced risk of transmission (Seto et al., 2023). Besides these non-pharmaceutical interventions, vaccination against some viral and bacterial aetiologies such as influenza and pneumococci is available for prevention. Besides supportive therapies such as increased fluid intake and nasal saline sprays to enhance a moist environment, antibiotics and antivirals are available to treat viral and bacterial respiratory infections (Wei and Norwood, 2001). Antimicrobial resistance considerations should be taken into account when prescribing antibiotics for treatment against bacterial infections. Amoxicillin as the first line therapy and second line regimens such as moxifloxacin, azithromycin and levofloxacin are administered for treatment of bacterial respiratory infections (Smith et al., 2023; Wei and Norwood, 2001). Although they are frequently prescribed, antibiotics are not recommended for treatment of viral respiratory infections. This practice has contributed to antibiotic resistant bacterial strains. Oseltamivir (Tamiflu) and Zanamivir (Relenza) are prescribed for influenza treatment. Additionally, neuraminidase inhibitors such as amantadine and rimantadine can shorten duration and disease severity (Smith et al 2013).

It is evident from literature that while there have been studies to elucidate the impact of non-pharmaceutical interventions (NPIs) on influenza and other respiratory pathogens around the world, there is still very little knowledge on how these NPIs influenced the circulation of respiratory pathogens in Zambia. Further, there has been little efforts to ascertain the regions in Africa with the highest burden of respiratory infections.

Therefore, this study sought to ascertain the impact of NPIs on the epidemiology of respiratory infections and determine the diversity of circulating pathogens during the COVID-19 pandemic in Zambia.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Design and Setting

This was a retrospective study using COVID-19 nasopharyngeal diagnostic specimens collected across various provinces of Zambia from both rural and urban areas. The University of Zambia, School of Veterinary Medicine was involved as a key testing centre for SARS-CoV-2 throughout the period of the COVID-19 pandemic subsequently confirming the first case on March 18, 2020. Specimens collected through the Zambia National Public Health Institute (ZNPHI) from all provinces of the country were submitted to the School of Veterinary Medicine for testing. These specimens were collected during routine SARS-CoV-2 screening at various health facilities, ports of entry and during contact tracing efforts. Other specimens were collected during mass COVID-19 community screening and from persons seeking SARS-CoV-2 medical certifications for international travel. After testing for SARS-CoV-2, residual specimens were stored at -80 ^{o}C. From these stored specimens, 128 nasopharyngeal samples were selected from those collected in Northern, Muchinga, Lusaka and Luapula Provinces representing rural and urban areas. As defined by the Zambia Statistics Agency, a rural area has less than 5000 persons per square kilometer, without piped water and 75% of the population dependent on agriculture. On the other hand, an urban area has a population density of more than 5000 persons per square kilometer, piped water, electricity, flushable toilets and more than 75% of the population not dependent on agriculture. Systematic review and meta-analysis was undertaken following Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) for articles published between January 2013 and 28th September, 2023 to understand the burden of respiratory infections across the African region. Countries were clustered in regions as Southern, North, West, Central and East Africa.

3.2 Sampling

For purposes of this study, proportional age stratified sampling technique was used to select specimens to build a convenient sample size of 128 considering the reagents available for testing. The sample consisted of 88 from urban and 40 from rural areas drawn from stored residual specimens collected between July 2020 and July 2021. After specimens were first stratified by age, date, year and place of collection, simple random sampling was then used for specimen selection following different strata. Only specimens which were clearly identified with full demographic and geographic characteristics such as age, sex, date of collection, place of collection were selected. Specimens which did not meet this criteria were excluded. Nasopharyngeal specimens were collected in accordance with US Centers for Disease Control (CDC) and World Health Organization guidelines as follows; Dry nasal cotton swab was gently inserted into the nostril parallel to the palate and left in place for a few seconds before slowly being withdrawn using a rotation motion. The tip of the swab was then placed in a conical centrifuge tube containing 2ml viral transport medium (VTA) after which the stick was broken off. The samples were transported on ice using cooler boxes to the virology laboratory, department of disease control, School of Veterinary Medicine, University of Zambia and kept at -80^{o}C until required for processing.

3.3 Nucleic Acid Extraction

Nucleic acid extraction was performed using the NucliSENS® easyMAG® (bioMerieux, Durham, USA) automated extraction according to the manufacturer’s instructions. All specimens were first barcoded in readiness for automated nucleic acid extraction. After vortexing, 400 µL of each sample was pipetted into machine well plates. The machine sample well plates were then loaded on easyMAG 2.1 nucleic acid extraction machine for automated extraction using protocol B.2.0.1. One thousand (1000 µL) lysis buffer was added and run for 10 min. Thereafter, 70 µL of magnetic silica was added to each sample well by physically pipetting. After adding 4 µL of internal control, the extraction process was run for 61 minutes. Finally, a total of 110 µL nucleic acid was eluted and stored at -80^{0}C until required for multiplex real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) for detection of target respiratory pathogens. For SARS-CoV-2, nucleic acid extraction was performed as described by Simulundu et al, (2021) using QIAamp Viral RNA Mini Kit following the steps below:

Step 1: Pipetted 560 µL prepared Buffer AVL containing carrier RNA into a 1.5 mL microcentrifuge tube. Step 2: Added 140 µL of the sample to the Buffer AVL–carrier RNA in the microcentrifuge tube and mixed by pulse-vortexing for 15 seconds. To ensure efficient lysis, the sample was mixed thoroughly with Buffer AVL to yield a homogeneous solution. Step 3: The mixture was incubated at room temperature for 10 minutes. Step 4: The mixture was briefly centrifuged to remove drops from the inside of the lid. Step 5: Added 560 µL ethanol (96–100%) to the sample, and mixed by pulse-vortexing for 15 seconds. After mixing, the tube was briefly centrifuged to remove drops from inside the lid. Step 6: Carefully applied 630 µL of the solution from step 5 to the QIAamp Mini column (in a 2 mL collection tube) without wetting the rim and closed the cap, then centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini column was then placed into a clean 2 mL collection tube, and discarded the tube containing the filtrate. Note: Each spin column was closed to avoid cross-contamination during centrifugation. Step 7: The QIAamp Mini column was carefully opened and step 6 was repeated. Step 8: The QIAamp Mini column was carefully opened and added 500 µL Buffer AW1. The cap was closed and centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini column was placed in a clean 2 mL collection tube and discarded the tube containing the filtrate. Step 9: The QIAamp Mini column was carefully opened and added 500 µL Buffer AW2. The cap was closed and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 minutes. Step 10: The QIAamp Mini column was placed in a new 2 mL collection tube and discarded the old collection tube with the filtrate. The tube was then centrifuged at full speed for 1 min. Step 11: The QIAamp Mini column was then carefully opened and added 60 µL Buffer AVE equilibrated to room temperature. The cap was closed and incubated at room temperature for 1 minStep 12: The QIAamp Mini column was centrifuged at 6000 x g (8000 rpm) for 1 min and RNA was eluted and stored -80^{o}C until required for processing.

3.4 Detection of Respiratory Pathogens

Laboratory work for detection of respiratory pathogens was performed at University Teaching Hospital, Microbiology laboratory, Lusaka. To detect influenza and other respiratory pathogens, the FTD respiratory pathogens 33 (FastTrack Diagnostics, Luxembourg) multiplex rRT-PCR test kit was used on a QuantStudio® 5 (Thermo Fisher Scientific, Life Technologies, Singapore) thermocycler, following the manufacturer's instructions. The assay detects the following array of pathogens: influenza A virus (IAV), influenza A(H1N1) virus (swine lineage) (IAV(H1N1) swl), influenza B virus (IBV), influenza C virus (IVC), human coronaviruses (HCoV) NL63, 229E, OC43 and HKU1, human parainfluenza viruses (HPIV) 1, 2, 3 and 4, human metapneumoviruses (HMPV) A and B, human rhinovirus (HRV), human respiratory syncytial viruses (HRSV) A and B, human adenovirus (HAdV), enterovirus (EV), human parechovirus (HPeV), human bocavirus (HBoV), *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* B, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Bordetella* spp. (except *Bordetella parapertussis*), *Klebsiella pneumoniae*, *Legionella pneumophila* / *Legionella longbeachae*, *Salmonella* spp., *Haemophilus influenzae* and equine arteritis virus (EAV) which serves as an internal control (IC). A master mix reaction volume of 15 µL was used containing; Primer and probe mix 1.5 µL, buffer 12.5 µL and 1 µL enzyme. The following primers and probes were used; FluRhino for detection of influenza A and B, influenza A H1N1 and Rhinovirus; CoR for detection of Coronavirus NL63, Coronavirus OC43, Coronavirus 229E and Coronavirus HKU 1; ParaEAV for detection of HPIV 2-4; BoMpPf1 for detection of Human Bocavirus, Metapneumovirus, Human Parainfluenza virus 1; ResPA for detection of Respiratory sysncytial virus A and B, Adenovirus, Enterovirus and Human Parechovirus; KlePsa for detection of K. pneumoniae, Chlamydia pneumoniae, Streptococcus pneumoniae and Haemophilus influenza; MoBoCH for detection of Moraxella catarrhalis and Bordetella pertussis. ResP 21PC was the positive control plasmid pool used for respiratory viruses while ResP PC2 was used as the positive control plasmid pool for bacteria. Four reporter dyes (VIC, FAM, ROX and CY5) were used for viewing amplification. The thermo cycler was programmed and optimized for fast track master mix (FastTrack Diagnostics, Luxembourg) PCR according to the manufacturer's recommendations as follows: 50^{o}C for 15 minutes, 94^{o}C for 1 minute, 45 cycles of 94^{o}C for 8 seconds and 45 cycles of 60^{o}C for 1 minute. For SARS-CoV-2, nucleic acid detection was performed as described by Simulundu et al, (2021). Presence or absence of target pathogens was determined using fluorescence signals for reporter dyes. Amplification plots were considered positive for the target pathogen if cycle threshold (ct) values did not exceed 35.

3.5 Systematic Review and Meta-analysis

In undertaking this systematic review and meta-analysis, the guidelines for preferred reporting items for systematic reviews and meta-analysis (PRISMA) were followed.

3.5.1 Search Strategy and Eligibility Criteria

PubMed and Google Scholar were systematically searched for epidemiological studies published between January 2013 and 28th September, 2023 in English language. Search terms were a combination of three key concepts; respiratory pathogens, prevalence and co-infections. The search was performed on 6th July 2023 at 19:46. To bolster the search, the key concepts were coupled with similar or related words such as "co-infections" OR "dual infections", OR "multiple infections" OR "Lower respiratory infections" OR "upper respiratory infections".

3.5.2 Inclusion and Exclusion Criteria

Molecular studies with multiple detection of respiratory pathogens were included. Serological studies were excluded from the review. Only free articles were included. Only studies conducted in Africa were included, other regions were excluded. Studies on specimens collected from non-humans were all excluded. Only original articles on studies from human specimens were included, systematic reviews, conference proceedings and dissertations were excluded. Figure 3.1 shows the PRISMA flow diagram for search results and publications screened out.

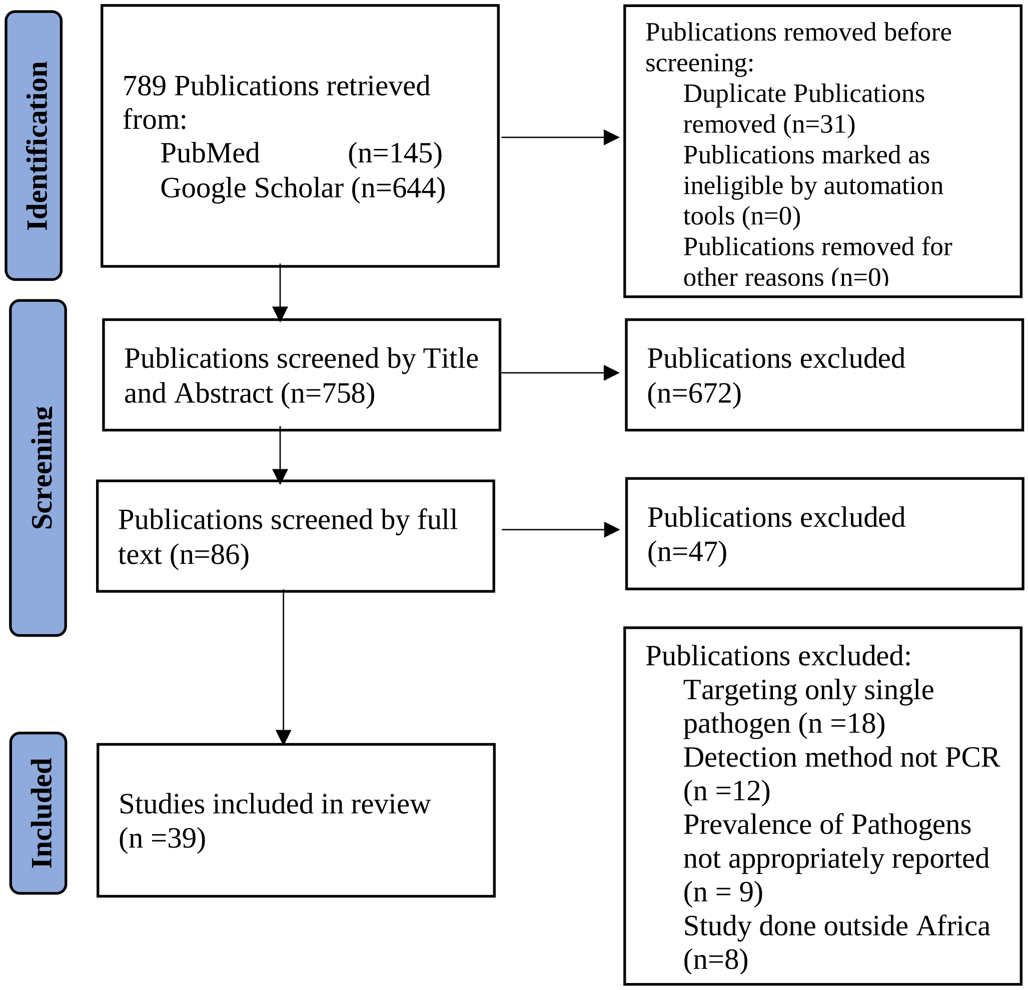

Figure 3.1: PRISMA flow diagram showing search results and studies screened at various stages

3.5.3 Data Abstraction

Using a predefined template in Microsoft excel, data was abstracted on author, study title, year of publication and country where the study was conducted. Sample size, method of detection, specimen type whether oral or nasopharyngeal swabs and age of participants were also abstracted from eligible studies. For both viral and bacterial respiratory pathogens, prevalence of infection for each the pathogens detected was recorded together with information on co-infections that were detected.

3.6 Data Analysis

QuantStudio® Design and Analysis software version 2.6.0 (Applied Biosystems, Thermo Fisher Scientific, USA) was used for analysis of presence and absence of target pathogens. The QuantStudio® design and analysis software was also used to assess the quality of amplification by comparing multicomponent and amplification plots against quantitative statistical amplification data. Data management for profiling of co-infections was done in Microsoft excel.

To determine the effects of age and geographical area, rural or urban on the likelihood of infection, logistic regression in SPSS (version 20, IBM Corp, 2011) was used. Regression covariates for prevalence of infection were age and geographical location, rural or urban for each selected respiratory pathogen. Data Analysis for systematic review and meta-analysis to estimate pooled prevalence and construction of forest plots was performed in OpenMeta (OSX: Yosemite 10.10, USA)

3.7 Ethical Approval

Ethical approval was granted by Macha Institutional Review Board (IRB 0007649), approval number (E2021.05) to utilize residual specimens to test for influenza and other respiratory pathogens. Authority to conduct the research was granted by National Health Research Authority approval number NHRA 000042/39/03/2022.

CHAPTER 4

RESULTS

4.1 Demographic and Geographic Characteristics of Specimens

After applying stratified simple random sampling, 128 specimens were selected across all age groups from urban and areas for screening respiratory pathogens as shown in table 4.1.

Table 4.1 Demographic and Geographic Characteristics of Selected Specimens

S/N	Age Group (Years)	Place of Collection (Town, Province)	Geographical Classification	Specimens Selected	Age Group Totals
1	0-9 Children	Lusaka, Lusaka	Urban	23	
		Isoka, Muchinga	Rural	1	26
		Mansa, Luapula	Rural	2	
2	10-19 Adolescents	Lusaka, Lusaka	Urban	19	
		Isoka, Muchinga	Rural	2	34
		Mansa, Luapula	Rural	13	
3	20-64 Adults	Mansa, Luapula	Rural	10	
		Isoka, Muchinga	Rural	4	
		Chinsali, Muchinga	Rural	3	44
		Lusaka, Lusaka	Urban	27	
4	≥65 Elderly	Mungwi, Northern	Rural	1	
		Chinsali Muchinga	Rural	1	
		Mansa, Luapula	Rural	1	24
		Lusaka, Lusaka	Urban	21	
	Totals			**128**	**128**

4.2 Prevalence of Influenza and other Respiration Pathogens

For all 128 specimens tested, 71.1% (91/128) were positive for at least one respiratory pathogen. Overall, bacterial respiratory pathogens were more prevalent than viral with 68.7% (88/128) testing positive for at least one bacterial pathogen while 50% (64/128) tested positive for at least one viral pathogen. Influenza A(H1N1) accounted for 13.3% (17/128), while influenza B accounted for only 0.8% (1/128). Coronaviruses excluding SARS-CoV-2 accounted for 3.9% (5/128) of the total specimens tested. Human adenovirus accounted for 10.9% (14/128), while Enterovirus had a prevalence of 3.9% (5/128). *Staphylococcus aureus* was the most prevalent respiratory pathogen with a prevalence of 22.7% (29/128), followed by *Klebsiella pneumoniae* which accounted for 20.3% (26/128). *Streptococcus pneumoniae* and *Bordetella pertussis* had a prevalence of 13.3% and 6.2% respectively. In total, there were 19 viral and bacterial respiratory pathogens detected, their percentage prevalence of which is summarized in Figure 4.1.

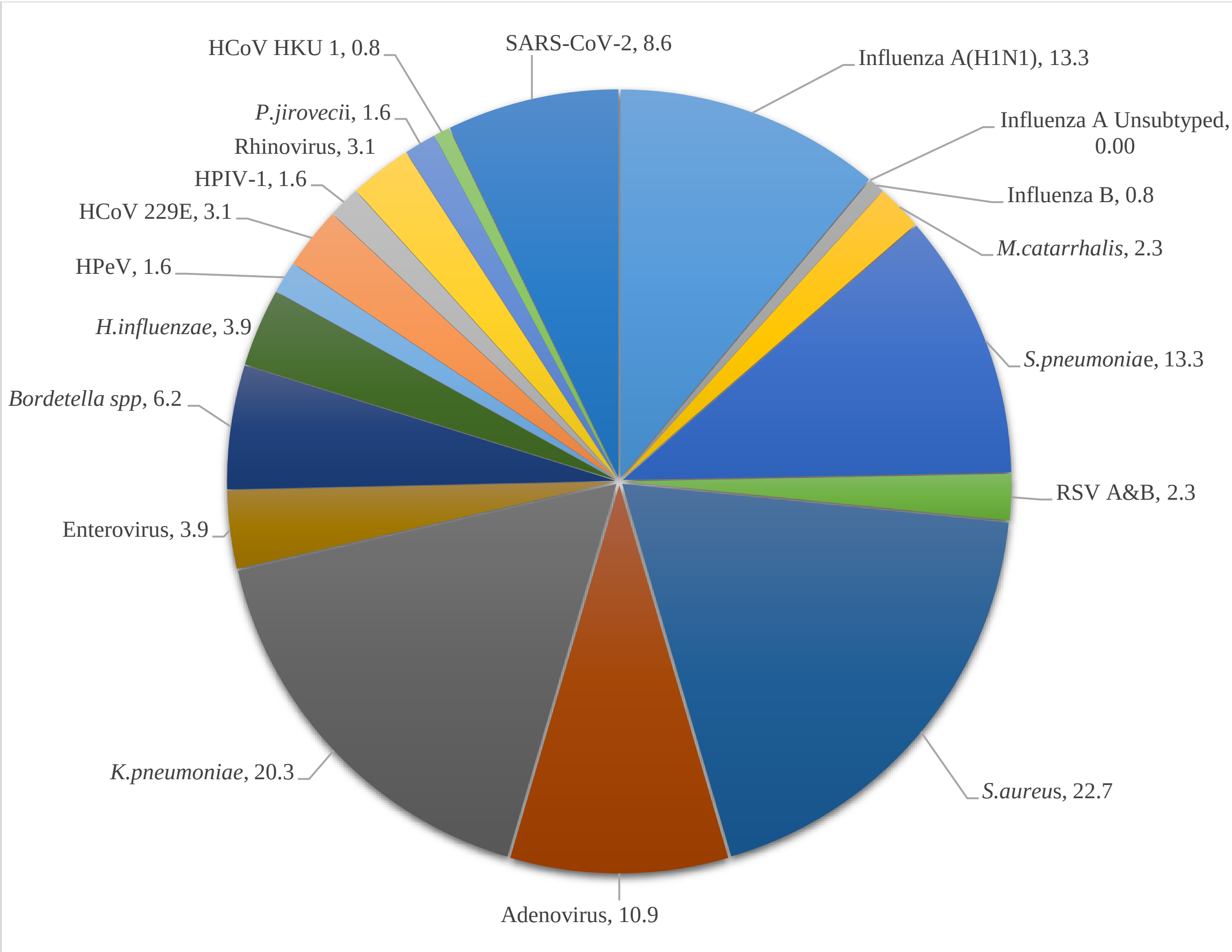

Figure 4.1: Percentage distribution of detected respiratory pathogens

4.3 Distribution of Pathogens by Rural-Urban Geographical Classification.

Of the total 17 specimens positive for influenza, 70.6% (12/17) were from urban areas while 29.4 % (5/17) were from rural areas. Of the total 11 specimens testing positive for SARS-CoV-2, only one (9.1) % was from a rural area while the rest (90.9%) were from urban areas. For the total 29 specimens positive for *Staphylococcus aureus,* 62.1% were from urban areas while 37.9% were from rural areas. All the specimens that tested positive for rhinovirus 100% (4/4) and HRSV 100% (3/3) were from urban areas. Rural Areas accounted for 50% (4/8) of the specimens that tested positive for *Bordetella pertussis.*

4.4 Distribution of Viral and Bacterial Infections by Age Group.

Influenza A(H1N1) was mostly detected in children (0-9), adolescents (10-19) and the elderly (>65 years) accounting for 76.5% (13/17) of the total 17 specimens that tested positive for influenza while adults accounted for 23.5% (4/17) of positive specimens. Only 1 specimen was positive for SARS-CoV-2 in children and adolescents representing 9.1% while 90.9% (10/11) were all from adults and the elderly. *Moraxella catarrhalis* was only detected in children and adolescents (3/3) with no specimens from adults and the elderly testing positive for the bacteria. *Staphylococcus aureus* was detected in specimens from all age groups with children and adolescents accounting for 65.5% (19/29) while adults and the elderly accounted for 24.1% and 10.3 % respectively. Of the total 8 specimens testing positive for *Bordetella pertussis*, only 2 representing 25% were from children and adolescents while 6 representing 75% were from adults and the elderly. *Klebsiella pneumoniae* was mostly detected in adults and the elderly accounting for 73.1% (19/26) while children and adolescents accounted for 26.9%. All specimens testing positive for *Haemophilus influenzae* 100% (5/5) were from children and adolescents. Overall infection by age group is depicted in in Figure 4.2.

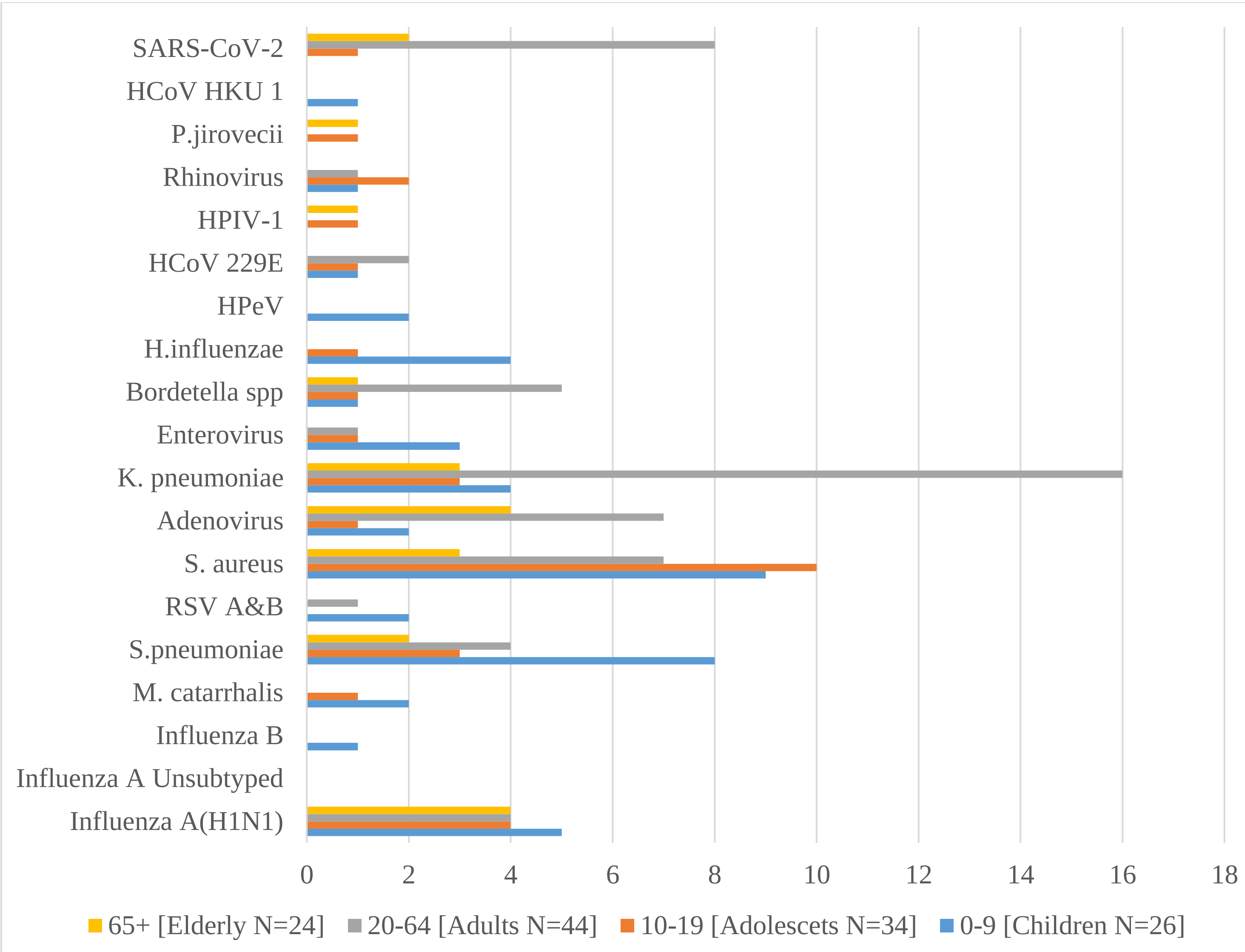

Figure 4.2: Distribution of respiratory pathogens by age group

4.5 Co-Infection Profile for detected Respiratory Pathogens

Overall, co-infections were observed in about 57.14% (52/91) of specimens testing positive for at least one respiratory pathogen. Of the co-infections observed, 11.5% (6/52) specimens had virus-virus co-infections with the most co-infecting pathogen being human adenovirus. On the other hand, bacteria-bacteria co-infections accounted for 26.9% (14/52). Virus-bacteria co-infections detected were 48.1% (25/52). SARS-CoV-2 co-infections were mainly with

Staphylococcus aureus 27.3% (3/11), *Klebsiella pneumoniae* 27.3%, (3/11) followed by co-infection with *Bordetella pertussis* 18.2% (2/11). Only 1 specimen positive for SARS-CoV-2 was co-infected with RSV while (2/11), were co-infected with Influenza A(H1N1). Five out of 17 (29.4%) specimens testing positive for influenza A(H1N1) were co-infected with *Staphylococcus aureus* while co-infection with *Streptococcus pneumoniae* was 23.5% (4/17). Co-infection of influenza A(H1N1) with *Klebsiella pneumoniae*, *Bordetella pertussis* and adenovirus was the same at 11.8% (2/17) for each the bacteria. Table 4.2 presents the overall co-infections observed from the study.

Table 4.2 Co-infection profile for detected respiratory Pathogens in Tested Samples (n=52)

	IAV	IBV	M.Cat	S.Pne	RSV	S.Aur	Aden	Enter	Bord	H.Inf	HPeV	CoV229	HPIV-1	Rhinov	P.Jir	HKU 1	S-CoV-2
IAV	X	1	0	1	0	4	5	2	1	1	0	1	0	0	2	0	2
IBV	--	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. Cat	--	--	X	1	0	0	0	0	0	0	0	0	0	0	0	0	0
S.Pne	--	--	--	X	1	5	1	0	0	2	1	2	0	2	0	0	1
RSV	--	--	--	--	X	1	0	0	0	0	1	0	0	0	0	0	1
S. Aur	--	--	--	--	--	X	2	2	3	3	0	0	1	1	1	0	3
Aden	--	--	--	--	--	--	X	1	2	1	0	0	0	0	0	0	1
Enter	--	--	--	--	--	--	--	X	0	0	0	0	0	0	0	0	0
Bord	--	--	--	--	--	--	--	--	X	1	0	0	0	1	0	0	2
H.Inf	--	--	--	--	--	--	--	--	--	X	0	0	0	1	0	0	0
HPeV	--	--	--	--	--	--	--	--	--	--	X	1	0	0	0	0	0
CoV 229	--	--	--	--	--	--	--	--	--	--	--	X	0	1	0	0	0
HPIV-1	--	--	--	--	--	--	--	--	--	--	--	--	X	0	0	0	0
Rhinov	--	--	--	--	--	--	--	--	--	--	--	--	--	X	0	0	0
P.Jir	--	--	--	--	--	--	--	--	--	--	--	--	--	--	X	0	0
HKU 1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	X	0
S-CoV-2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	X

Key:

Influenza A(H1N1)
M. Cat-*Moraxella catarrhalis*
S.Pne-*Streptococcus pneumoniae*
RSV-Respiratory Syncytial Virus
S.Aur-*Staphylococcus aureus*
Aden-Human Adenovirus
Enter-Enterovirus
Bord-*Bordetella pertussis*
H.Inf-*Haemophilus influenzae*
CoV 229-Human Coronavirus 229E
HPIV 1-Human Parainfluenza virus 1
Rhinov-Rhinovirus
P.Jir-P.Jirovecii
HKU 1-Human Coronavirus HKU 1
S-CoV-2-Novel SARS CoV-2

4.6 Effects of Age and Geographical Location on Prevalence of Infection for Selected Pathogens.

Generally, results for logistic regression model accuracy for all outputs were above 80%, a prediction accuracy of well over 50%. The *p values* for the Hosmer and Lemeshow test for data fit were > 0.5 for all respiratory pathogens except for SARS-CoV-2, *S. pneumoniae* and *K. pneumoniae*, table 4.3. The adjusted odds ratios for age were 1.026 for adenovirus CI (0.999-1.053) and 1.048 for SARS-CoV-2, CI (1.004-1.094) while influenza had an adjusted odds ratio of 0.996 CI (0.997-1.015). *Bordetella pertussis* had an adjusted odds ratio associated with age of 1.02, CI (0.986-1.055) while *Klebsiella pneumoniae* had an adjusted odds ratio associated with age of 1.021, CI (1.001-1.040). The adjusted odds ratios associated with location, rural or urban were 3.10 for *Bordetella pertussis* CI (0.667-14.208), 1.362 for *S. aureus* CI (0.561-3.303) and 1.588 for *K. pneumoniae* CI (0.615-4.097). The rest of the pathogens had adjusted odds ratios associated with location < 1.

Adults and the elderly were more likely to be infected with SARS-CoV-2 than children and adolescents (p=0.019). Similarly, adults and the elderly were more likely to be infected with adenovirus than children and adolescents (p=0.047). Although it seems persons living in rural areas were about 3 times more likely to be infected with Bordetella pertussis than those in urban areas, these results were not statistically significant, (p=0.391). While there were disparities in *K. Pneumoniae* infections among age groups the variations between children and adults were not significant statistically (p= 0.110). Generally, bacterial pathogens had greater odds of infection associated with age and geographical areas (rural or urban) than viral pathogens. The overall regression outputs for model accuracy, Omnibus tests for model coefficients, the Hosmer and

Lemeshow test for model data fit and the adjusted odds ratio for each selected respiratory pathogen was as shown in table 4.3.

Table 4.3: Regression output for effects of age and geographical location on prevalence of Infection for Selected Pathogens

Pathogen	Model Accuracy (%)	Omnibus Tests for Model Coefficients (*p-values*)	Hosmer and Lemeshow test for data fit (*p values*)	Adjusted Odds Ratio Exp(B)	95% CI
Adenovirus	89.1	0.047	0.595	Age: 1.026	[0.999-1.053]
				Loc: 0.544	[0.139-2.133]
Influenza	85.2	0.914	0.888	Age: 0.996	[0.997-1.015]
				Loc: 0.793	[0.254-2.470]
SARS-COV-2	91.4	0.019	0.197	Age: 1.048	[1.004-1.094]
				Loc: 0.389	[0.076-1.989]
Bordetella spp	93.8	0.391	0.885	Age: 1.02	[0.986-1.055]
				Loc: 3.10	[0.677-14.20]
S.aureus	74.2	0.377	0.926	Age: 0.992	[0.976-1.008]
				Loc: 1.362	[0.561-3.303]
S. Pneumoniae	86.7	0.261	0.143	Age: 0.981	[0.960-1.002]
				Loc: 0.705	[0.202-2.460]
K. pneumoniae	79.7	0.110	0.022	Age: 1.021	[1.001-1.040]
				Loc: 1.588	[0.615-4.097]

4.7 Regional Comparisons for the Burden of Respiratory Infections in Africa.

Studies were clustered in regions as Southern Africa, West Africa, East Africa, North Africa and Central Africa to enable regional comparisons on the distribution and prevalence of respiratory pathogens of interest. Southern Africa had the most publications with 20 followed by West Africa with 12. East Africa had 4 publications while North and Central Africa had the least number of publications with 2 each. The majority of studies (23/39) were conducted in children <15 years with children <5 years accounting for (18/23) studies. Although the majority of studies were in children, overall, all age groups were represented in the included studies. All studies except 2 had sample sizes of more than 100 specimens with a highest sample size of 18,282 in a single study.

At African continent level, overall pooled prevalence for viral respiratory pathogens was estimated at 56.6% (95% CI, 0.371-0.762, I^2 99.9%), Figure 4.3.

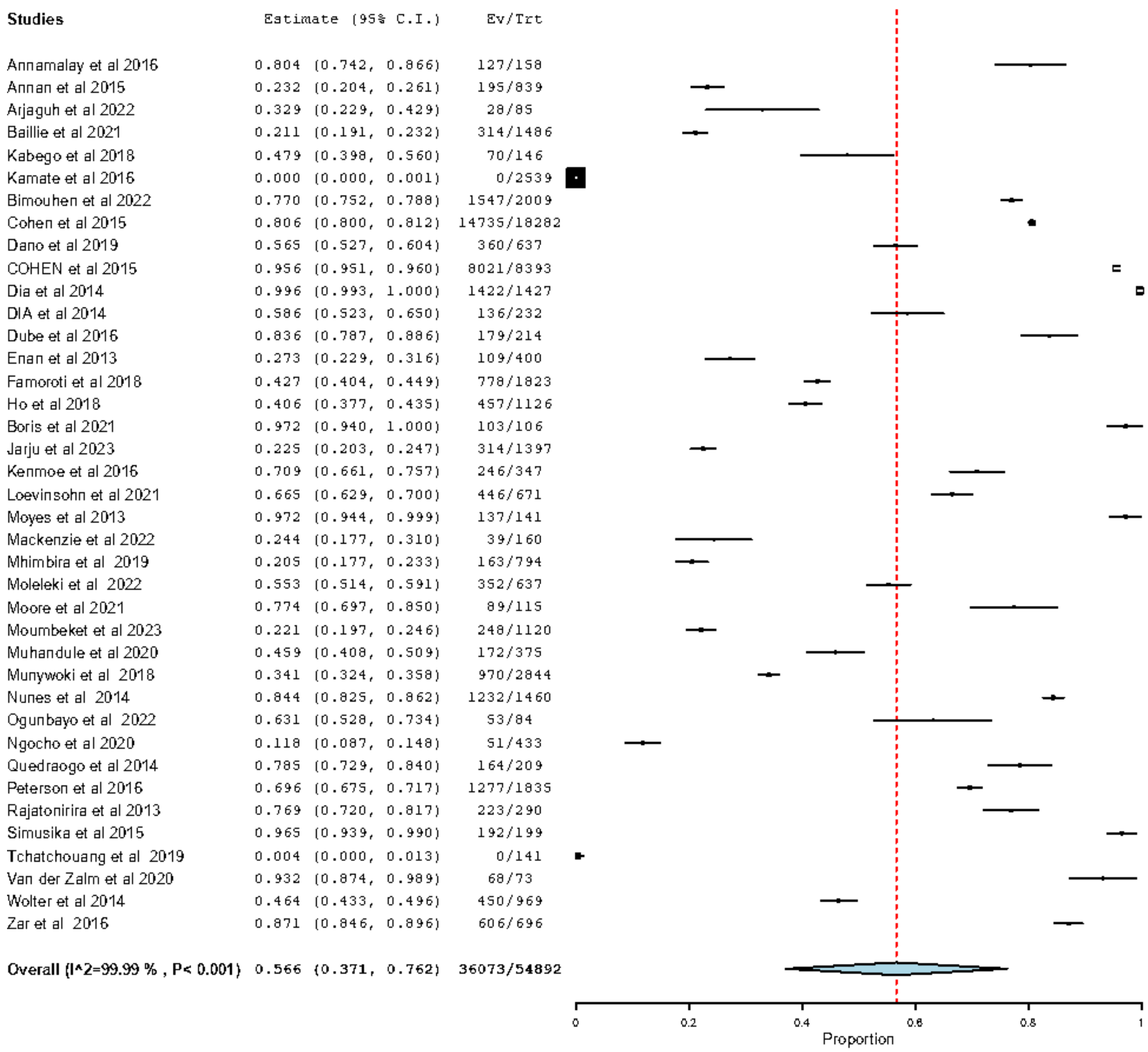

Figure 4.3: Pooled prevalence for viral respiratory infections in Africa.

Overall pooled prevalence for bacterial respiratory pathogens was estimated at 12.9% (95% CI, 0.122-0.137, I^2 99.8%). Regional sub-group analysis clustered as Southern, Central, North, East and West Africa revealed a highest influenza prevalence in North Africa with 28.2% followed by West Africa with 5.3%. Rhinovirus was most prevalent in Southern Africa with 25.9% followed by Central Africa with 26.3%. Rhinovirus was least recorded in East Africa with 8.1%. Respiratory syncytial virus was highest in North Africa with a prevalence of 16.1% followed by Southern Africa with 11.4%. Respiratory Syncytial Virus was least recorded in East Africa with an estimated prevalence of 2.4%. Human Adenovirus was highest in Southern Africa with an estimated prevalence of 16.8%. Generally, few bacterial respiratory infections were recorded in North Africa compared to the rest of the regions. Central Africa recorded the highest *Streptococcus pneumoniae* prevalence (55.5%) while the lowest was North Africa which did not record any *Streptococcus pneumoniae* infections. East Africa accounted for the highest proportion of *Moraxella catarrhalis* (2.8%) followed by Southern Africa (1.8%). The overall regional prevalence for both viral and bacterial respiratory infections is shown in Figure 4.4.

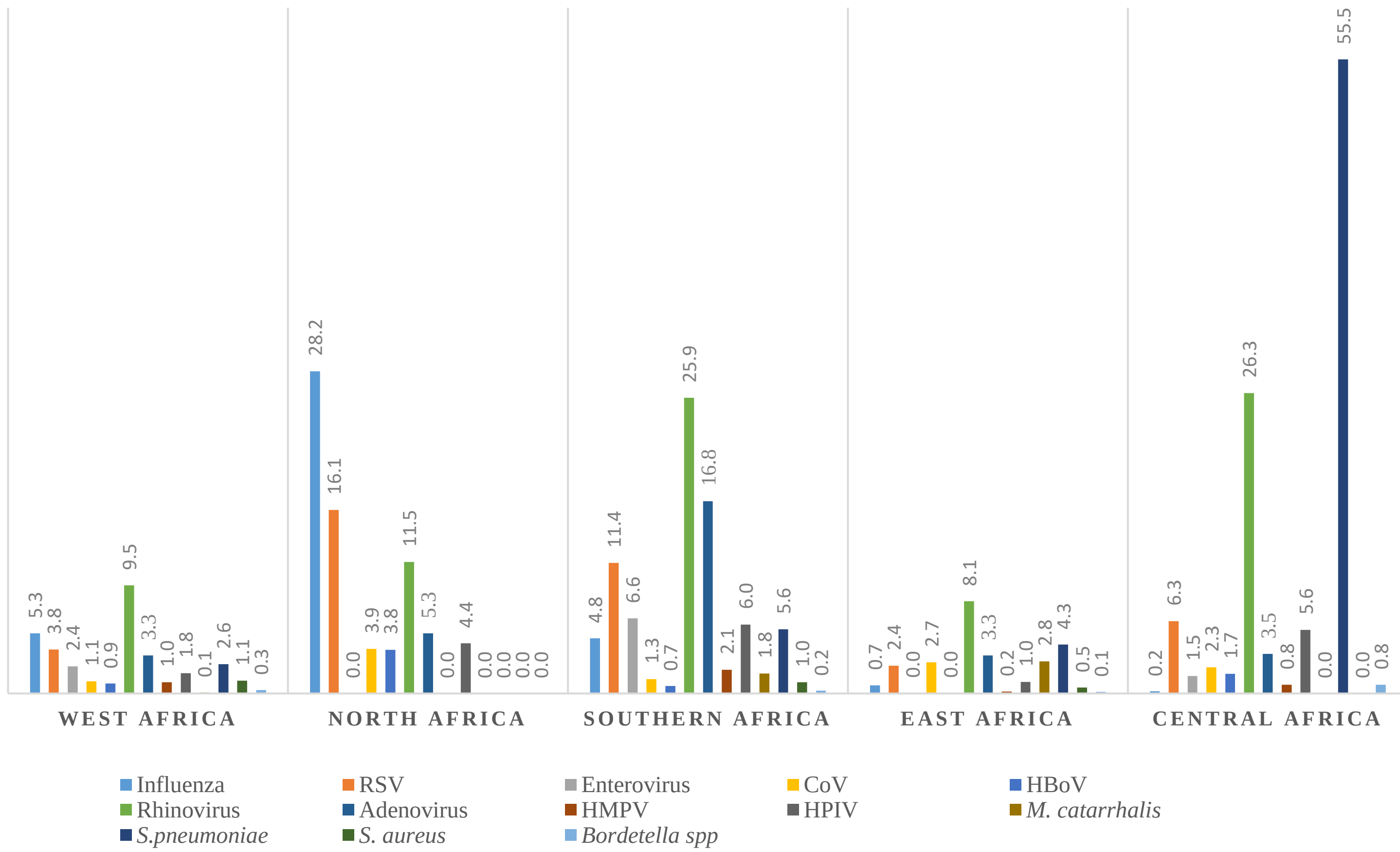

Figure 4.4: Regional comparisons for the burden of respiratory infections in Africa

CHAPTER 5: DISCUSSION

The heightened country-wide COVID-19 surveillance following the detection of the first SARS-CoV-2 case in Zambia on March, 18 2020 provided a great opportunity to understand the broad range and epidemiology of respiratory pathogens circulating in Zambia during the pandemic period. Previous studies were limited to urban areas especially Lusaka with a few in Macha in Choma. In this study, 128 specimens previously tested for SARS-CoV-2 at the University of Zambia, School of Veterinary Medicine virology laboratory were screened for other respiratory pathogens including influenza.

Generally, this study showed that bacterial respiratory pathogens were more prevalent than viral respiratory pathogens during the pandemic period. Bacterial respiratory pathogens seem to have persisted more than viral pathogens during the COVID-19 pandemic despite the implementation of public health and social measures. Bacterial colonization of the upper respiratory tract and increased bacterial binding resulting from viral infections may explain the predominance of bacterial pathogens detected in this study (Nickbakhsh et al., 2019). The results of this study are augmented by other studies (Liu et al., 2021; Cohen et al., 2022; Liu et al., 2023) that have shown high prevalence of bacterial respiratory infections among hospitalized children, adults and the elderly. Existing scientific literature shows that there has been much emphasis on respiratory viruses especially RSV and rhinovirus as the most etiological agents causing acute lower respiratory tract and influenza-like infections in developing countries neglecting bacterial pathogens (Anamalay et al., 2016; Kenmore et a., 2016; Loevinsohn et al., 2021). The results of this and other studies seem to suggest the need to re-evaluate this understanding and realign the bias. The role of bacterial respiratory pathogens cannot be overlooked in the pathogenesis of acute respiratory infections especially their role in disease severity in cases of co-infections

(Mackenzie et al., 2022). Moreover, besides causing respiratory infections, bacterial infections are a major cause of sepsis in pregnancy and childbirth leading to poor health outcomes for mothers and significantly contributing to neonatal mortality in Zambia (Tembo et al., 2024).

While there was persistence of other respiratory pathogens especially bacteria during the COVID-19 pandemic, it is significant to note that there was a general observed decline in prevalence of bacterial and viral respiratory pathogens from this study when compared to similar studies before the pandemic (Simusika et al., 2015). Results of this study reported an estimated prevalence of influenza at 13.3%, rhinovirus and RSV at 3.1% and 2.3% respectively, a significant reduction in prevalence from results of epidemiological studies conducted in years before the COVID-19 pandemic. Facility-based surveillance for influenza and RSV in rural Zambia conducted between 2018 and 2019 reported an estimated RSV prevalence of 11% and 26.4% for Rhinovirus while influenza prevalence was estimated at 18.2% (Loevinsohn et al., 2021a; Loevinsohn et al., 2021b). Similarly, another study conducted to identify viral and bacterial pathogens from hospitalized children with acute respiratory illness in Lusaka between 2011 and 2012 reported an estimated RSV prevalence of 33.7% and Rhinovirus at 11.4% (Simusika et al., 2015). On bacterial pathogens, this study reported a prevalence of *Streptococcus pneumoniae* at 13.3%, *Moraxella catarrhalis* at 2.3% and *Bordetella pertussis* at 6.2%. The study by Simusika et al., (2015) reported prevalence of *Streptococcus pneumoniae* at 54.8% and *Moraxella catarrhalis* at 46.2% showing a significant reduction in prevalence. Because of an observed general decline in respiratory infections of the same aetiologies in this study compared to those in pre-pandemic years, it is possible that public health and social measures implemented against COVID-19 may have altered the epidemiology of other respiratory pathogens besides helping to slow the transmission of SARS-CoV-2. With scientific evidence from this and other studies, it is possible to contain transmission of respiratory infections and reduce the burden disease by advocating and implementing non-pharmaceutical interventions.

Co-infections detected in this study were mostly virus-bacteria, followed by bacteria-bacteria. Of the total 52 co-infected specimens in this study, virus-bacteria co-infections accounted for 48.1% (25/52) while bacteria-bacteria co-infections accounted for 26.9% (14/52). Only 3 co-infections 5.8% (3/52) were virus-virus. These viral co-infections involved influenza, adenovirus and enterovirus. In a study of virus-virus interactions for respiratory pathogens by Nickbakhsh et al., (2019), it was found that virus-virus interactions may result in interaction induced effects such as cross-immunity and resource competition leading to attenuation. Additionally, infection with one respiratory pathogen may elicit immune response against the other. This may help to explain the small number of virus-virus co-infections detected in this study. Largely, co-infections were detected in children and adolescents who accounted for 44.2% (23/52) of the total co-infections detected. Children may be more susceptible to infection because they may still be immunologically naïve. These results agree with findings from other epidemiological studies in Zambia and globally that have demonstrated that co-infections for respiratory viruses occur largely in children (Loevinsohn et al., 2021; Zhang et al., 2014). The most co-infecting respiratory pathogens across all age groups were *Staphylococcus aureus, Streptococcus pneumoniae and Klebsiella pneumoniae*. This is the first study in Zambia to show *Staphylococcus aureus* among the most co-infecting respiratory pathogens in Zambia. It is important to note that recent studies have shown that co-infection with *Staphylococcus aureus* is associated with highest intensive care admission and death rates (Liu et al., 2021; Cohen et al., 2022). It was further found that interactions between viruses and bacteria could generally lead to enhanced bacteria binding, immune response dysregulation and slowed bacterial clearance. There may be need for epidemiological studies to explore the association between virus-bacteria co-infection with severe respiratory illnesses leading to hospitalization and death in Zambia.

Adjusted odds ratio of regression analysis for effects of age on the prevalence of infection for selected viral pathogens were 1.026 for adenovirus and 1.048 for SARS-CoV-2. The higher the age of persons the higher the likelihood of infection with adenovirus and SARS-CoV-2. These results are consistent with published scientific literature which has shown that susceptibility to infection with COVID-19 in children under the age of 10 years is significantly lower compared to adults (Goldstein et al., 2020). Therefore, interventions for limiting transmission and containing infections from SARS-CoV-2 and adenovirus should be targeted at adults rather than children. However, the increase in age reduced the likelihood of infection for other respiratory pathogens. It has been recognized that as persons progress in age, they develop immunity against most respiratory infections while children may be immunologically naïve and less likely to take precautions such as handwashing and hand sanitizing to prevent self-inoculation of respiratory infections (Kutter et al., 2018). Interestingly, geographical location, rural or urban seemed to be more associated with prevalence of infection for bacterial pathogens than viral pathogens. Adjusted odds ratio associated with geographical location for *Bordetella pertussis* was 3.10 and 1.362 for *S. aureus*, while it was 1.588 for *K. pneumoniae*. However, although these adjusted odds ratios seemed to suggest that rural areas increased the likelihood of infection with these bacterial respiratory pathogens by 3, 1.3 and 1.5 times respectively, these results were not statistically significant, $p>0.05$.

Notably, there were fewer studies undertaken during the review period to ascertain the profile of respiratory infections in Central Africa than the rest African regions. At the level of the African continent, Rhinovirus was the viral respiratory pathogen with the highest prevalence with 19.9%. Respiratory Syncytial Virus was second with a prevalence of 12%. . Among bacterial respiratory pathogens, *Streptococcus pneumoniae* had the highest prevalence with 5%. . Generally, there were more specimens testing positive for viral than bacterial respiratory pathogens. The results of this review showing Rhinovirus with the highest prevalence agree with other scientific literature which have shown that rhinoviruses are the leading cause of acute respiratory infections and represent a substantial proportion of the global burden of upper respiratory infections (Ljubin-Sternak, S. and Mestrovic, T., 2023; Jin, X., et al 2021). It is therefore evident that there is still urgent need to galvanize efforts to implement interventions to contain rhinovirus infections and reduce the burden of disease especially in Southern, North and Central Africa. While little attention has been paid to the role of adenovirus in both upper and lower respiratory tract infections and severe disease, this review seems to suggest that its prevalence may be significant in the pathogenesis of respiratory infections especially in cases of co-infections as demonstrated by other studies (Krumbein, H. et al., 2022).

After analysis for regional comparisons, North Africa had the highest prevalence of influenza virus infections with 28.2%, West and Southern Africa being second and third with 5.3% and 4.8% prevalence respectively. Central and East Africa were lowest with 0.2% and 0.7% respectively. These results were consistent with World Health Organization (WHO) epidemiological data on influenza for Africa which has shown highest influenza prevalence in North and Southern Africa (WHO, 2017). Since data shows that the burden of influenza virus infection is highest in North Africa, there is need to review WHO vaccine recommendations based on antigenic and genetic characteristics of influenza strains circulating in North Africa to help reduce the burden of influenza infections in this region. The prevalence of RSV was highest in North Africa with 16.1% followed by Southern Africa with 11.4%. Among all African regions, RSV prevalence was lowest in East Africa with 2.4%. Interestingly, in a systematic review and meta-analysis done by Regassa et al (2023), sub-regional analysis revealed that North and Southern Africa had the highest prevalence of RSV. These results may suggest that North and Southern Africa may be the regions with the highest burden of RSV in Africa. The prevalence of Rhinovirus was highest in Central Africa with 26.3% while it was 25.9% in Southern Africa. East Africa had the lowest Rhinovirus infections with 8.1%. Notably, all regions recorded Rhinovirus infections above 8% showing that it is a respiratory pathogen of concern across all African regions. These results show that there is still urgent need to mobilize resources and galvanize interventions in these African regions to implement interventions to reduce the burden of disease for Rhinovirus and improve public health outcomes.

Among bacterial pathogens, Central Africa had the highest *S. pneumoniae* prevalence with 55.5% while Southern Africa was second with 5.6%. East Africa had the highest *M. catarrhalis* prevalence with 2.8% while Southern Africa had a prevalence of 1.8% coming second. It is important to note that opportunistic bacterial infections especially *M. catarrhalis* have been implicated in causing otitis media and acute exacerbations of chronic obstructive pulmonary disease (COPD) in children and adults with co-infections resulting in adverse prognosis (Blakeway et al., 2017). Notably, North Africa did not record any infection with bacterial respiratory pathogens. This could be attributed to interventions such as high vaccine coverage for *H.influenzae* and pneumococcus in most Arab countries and the middle-high sociodevelopment index (SDI) of North African countries which is positively associated with low incidence of bacterial respiratory infections (Ashrafi et al., 2023; Mokdad et al., 2014). Therefore, with good vaccine coverage for *H. influenza and pneumococcus*, it is possible to reduce the burden of these bacterial infections in the rest of African regions and improve public health outcomes.

Generally, although bacterial respiratory pathogens play a major role in the pathogenesis of upper and lower respiratory tract infections especially in incidences of viral-bacterial co-infections resulting in poor patient outcomes (Krumbein et al., 2022; Brundage, 2006; Hendaus et al., 2015), it seems little attention is paid in undertaking epidemiological studies focusing on bacterial respiratory pathogens as the results of this review have shown. This study further revealed that there are few studies conducted targeting multiplex testing for both viral and bacterial respiratory pathogens especially in East and Central Africa. Most studies are focused on viral respiratory pathogens neglecting bacterial respiratory pathogens. This has the potential to limit understanding of the role of viral-bacteria respiratory co-infections in the pathogenesis of these infections. Consequently, efforts to control respiratory infections to achieve good public health outcomes may yield very little desired results.

With the advent of multiplex polymerase chain reaction, studies focusing on simultaneous detection of both viral and bacterial respiratory pathogens and elucidating their impact on disease severity should be encouraged in Africa.

5.1 Limitations of the Study

This study had limitations including being retrospective in nature and having a limited sample size. Lack of clinical data on participants contributed to inability of the study to elucidate associations between co-infections and disease outcomes. There were more specimens collected from urban than rural areas which made the sample size to have a bias towards urban areas. Additionally, most of the studies used to make comparisons on the impact of non-pharmaceutical interventions on the prevalence of infection were health facility based. On the other hand, specimens used in this study were a combination of those take from symptomatic persons in health facilities and asymptomatic persons during COIVD-19 screening in communities and ports of entry. The comparison was therefore not based on exclusively similar studies. Additionally, only free articles were included in the systematic review and meta-analysis. This may have contributed to not having all relevant articles included for a conclusive review.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

While both viral and bacterial respiratory pathogens were reported from this study, the latter were more predominant during the COVID-19 pandemic in Zambia. Respiratory infection aetiologies of public health importance such as influenza, RSV, rhinovirus, *Bordetella pertussis* and *staphylococcus aureus* continued to circulate during the pandemic period. Overall decreased circulation of respiratory pathogens observed in this study provides evidence that public health interventions against COVID-19 may have altered the epidemiology of respiratory infections. It is therefore possible to reduce the burden of respiratory infections of public health importance by implementing non-pharmaceutical interventions such as hand washing, face masking, hand sanitizing, physical distancing and observing respiratory etiquette.

Co-infections were largely virus-bacteria compared to virus-virus co-infections that were recorded. The most co-infecting bacterial respiratory pathogen was *Staphylococcus aureus* while the most co-infecting viral respiratory pathogen was adenovirus.

Adults and the elderly were more likely to be infected with SARS-CoV-2 and Adenovirus than children and adolescents. Although the prevalence of infection was greater in rural areas compared to urban for most respiratory pathogens, the observed differences were not statistically significant. Therefore, there should be no variations in implementing interventions to control respiratory infections in both urban and rural areas.

Results of the systematic review and meta-analysis provided evidence that the burden of viral respiratory infections was highest in North and Southern Africa. In terms of both viral and bacterial respiratory pathogens, Southern Africa had the highest burden of infection. North Africa had the lowest burden of bacterial respiratory infections.

6.2 Recommendations

- Considering the impact of viral-bacterial co-infections on the pathogenesis of respiratory infections and prognosis, there is need for more studies focusing on co-detection of viral and bacterial respiratory pathogens to clearly understand the burden of respiratory infections in Africa for effective public health interventions.
- The burden of viral and bacterial respiratory infections in Africa is highest in Southern Africa. Resources for public health interventions should be focused around this region to contain the burden of infection and improve public health outcomes. Further, it is imperative to review WHO recommendations for influenza vaccination based on antigenic and genetic characteristics of circulating strains in North Africa considering that it has the highest burden of influenza infections.
- Considering the fewer number of studies on respiratory infections compared to other African regions, it is important to conduct more studies to understand the burden of respiratory infections especially in Central and East Africa if the epidemiology of respiratory infections in Africa has to be fully understood.
- Reduced circulation of respiratory pathogens observed in this and other studies which may be attributed to public health and social measures implemented against COVID-19 is a basis for policy advocacy on sustaining these measures beyond COVID-19 to limit the spread of these infections, reduce the burden of disease and improve public health outcomes.

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APPENDICES

Appendix I: Manuscripts Accepted for Publication

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AOSIS

Page 1 of 7 Original Research

Respiratory pathogens detected in specimens collected for COVID-19 surveillance in Zambia

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Authors:
Martin Nyahoda[1]
Ngonda Saasa[1]
Katendi Changula[2]
Walter Muleya[1]
Zachariah Mupila[3]
Chilufya Chikoti[3]
[AQ1] Gift Moonga[3]
Catherine Sutcliffe[4]
Geoffrey Kwenda[5]
Edgar Simulundu[1]

Affiliations:
[1]Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

[2]Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

[3]Microbiology Laboratory, University Teaching Hospital, Lusaka, Zambia

[4]Department of Epidemiology, Bloomberg School of Public Health, John Hopkins University, Baltimore, United States of America

[5]Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka, Zambia

Corresponding author:
Martin Nyahoda,
martinny2@yahoo.co.uk

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Background: In Zambia, knowledge on the landscape of respiratory pathogens that circulated during the coronavirus disease 2019 (COVID-19) pandemic is limited.

Aim: This study investigated respiratory pathogens that circulated in Zambia during the COVID-19 pandemic.

Setting: Nasopharyngeal specimens collected between July 2020 and July 2021 for COVID-19 testing in hospitals, ports of entry, persons seeking certificates for international travel and in communities were used.

Methods: Proportional age-stratified sampling was used to select 128 specimens. The samples were screened for 33 other respiratory pathogens using the Fast Track Diagnostics multiplex molecular assay.

Results: Overall, 71.1% (n = 91/128) tested positive for at least one respiratory pathogen. Bacterial respiratory pathogens were more predominant (70.3%, n = 90/128) than viral (51.6%, n = 66/128). *Staphylococcus aureus* was the most prevalent, detected in 22.7% (n = 29/128). The prevalence of influenza was 13.3% (n = 17/128). Rhinovirus had a prevalence of 3.1% (n = 4/128), while it was 10.1% (n = 13/128) for adenovirus. Children, adolescents and the elderly accounted for most influenza-positive specimens, 76.5% (n = 13/17), while 100% (n = 3/3) of specimens positive for *Moraxella catarrhalis* were from children. All specimens testing positive for *Haemophilus influenzae*, 100% (n = 5/5) were from children and adolescents. Co-infections were detected in 57.1% (n = 52/91) of specimens testing positive for at least one pathogen.

Conclusion: Bacterial respiratory pathogens appeared to predominate circulation during the COVID-19 pandemic period.

Contribution: Bacterial respiratory pathogens should not be neglected when implementing public health mitigation measures.

Keywords: respiratory pathogens; COVID-19; co-infections; surveillance; Zambia.

Introduction

Zambia reported the first polymerase chain reaction (PCR)-confirmed coronavirus disease 2019 (COVID-19) case on 16 March 2020.[1] By 18 April 2020, at least 100 COVID-19 cases were reported in the country.[2]

With the emergence of COVID-19, the Zambia National Public Health Institute (ZNPHI) led the country's COVID-19 pandemic response with surveillance being a core activity. This included collecting nasopharyngeal (NP) specimens at ports of entry, contact tracing, health worker testing and health facility inpatient screening from both symptomatic and asymptomatic persons. Additionally, like in many other countries, a wide range of non-pharmaceutical interventions (NPIs) were implemented to limit the spread of the virus. These measures included physical distancing, handwashing, face masking, respiratory etiquette, travel restrictions, remote working and school closures.[3,4,5]

Studies have shown that NPIs implemented against COVID-19 have had an impact on the transmission dynamics of respiratory pathogens.[6] Epidemiological studies have shown a marked decrease in influenza and other respiratory infections during the COVID-19 pandemic. A study

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conducted in Japan using data from 2014 to 2020 revealed that influenza PCR-confirmed cases dropped in the 2019–2020 season by about 50% in persons less than 15 years of age with a similar trend being observed in other age groups.[4] In China, it was observed that the influenza season abruptly ended resulting in the season being shorter by 9 weeks.[7] The decrease in influenza activity was observed despite an increase in specimens tested from an average of 4104 to 5711 per week.[7] In Hong Kong, influenza community transmissions decreased by 44%, while paediatric hospitalisation decreased by 33% with the effective reproductive number dropping from 1.28 before the COVID-19 pandemic to 0.72 after emergence of the pandemic.[6] In the United States (US), samples testing positive for influenza decreased from a median of 19.24% to 0.33% with a similar trend being observed in Australia, Chile and South Africa.[8]

Similarly, a significant decrease in infections for other respiratory pathogens of concern such as respiratory syncytial virus (RSV), parainfluenza viruses, human rhinovirus (HRV) and enterovirus was observed during the pandemic period.[9] In England, confirmed cases of RSV hospital admissions and emergency attendances were substantially reduced by over 80% in children aged 5 years and below.[10] In Japan, no RSV outbreak was reported in 2020 at the peak of the pandemic, while there was a 93.4% reduction of RSV infections in Australia.[11] This trend was similar with other non-enveloped respiratory viruses such as enteroviruses, adenoviruses and human bocavirus. Detection of these non-enveloped viruses declined as with enveloped respiratory viruses globally.[12] The modifications in the circulation of bacterial respiratory pathogens were also observed during the pandemic as with viral respiratory pathogens.[12]

Information on changes in the epidemiology of respiratory pathogens in Zambia during the COVID-19 pandemic is limited, with only a few reports from rural Zambia.[13,14] In this study, we investigated the prevalence of a broad range of respiratory pathogens circulating in Zambia during the COVID-19 pandemic using specimens that were collected from both rural and urban areas for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing.

Research methods and design

Study design

This was a retrospective study using NP specimens collected for COVID-19 testing across various provinces of Zambia from both rural and urban areas. For the purpose of this study, proportional age-stratified sampling was used to select specimens to build a convenient sample (n = 128), consisting of 88 from urban and 40 from rural areas (Table 1). The samples were drawn from stored residual specimens collected between July 2020 and July 2021.

Study setting and data collection

The University of Zambia, School of Veterinary Medicine, Department of Disease Control served as a key testing centre for COVID-19 throughout the pandemic and provided PCR-based confirmation for the first case on 16 March 2020. Specimens collected by ZNPHI from all provinces of the country between 2020 and 2021 were submitted to the university for testing. These specimens were collected as part of surveillance at various health facilities, ports of entry, during contact tracing efforts and mass community screening as well as from persons seeking medical certificates for international travel. After testing for SARS-CoV-2, residual specimens were anonymised and stored at –80 °C.

TABLE 1: Demographic and geographic characteristics of selected specimens.

S/N	Age group (years)	Place of collection (town, province)	Geographical classification	Specimens selected	Age group (Total)
1	0–9	Lusaka, Lusaka	Urban	23	26
		Isoka, Muchinga	Rural	1	
		Mansa, Luapula	Rural	2	
2	10–19	Lusaka, Lusaka	Urban	19	34
		Isoka, Muchinga	Rural	2	
		Mansa, Luapula	Rural	13	
3	20–64	Mansa, Luapula	Rural	10	44
		Isoka, Muchinga	Rural	4	
		Chinsali, Muchinga	Rural	3	
		Lusaka, Lusaka	Urban	27	
4	≥ 65	Mungwi, Northern	Rural	1	24
		Chinsali Muchinga	Rural	1	
		Mansa, Luapula	Rural	1	
		Lusaka, Lusaka	Urban	21	
Totals	-	-	-	**128**	**128**

S/N, serial number.

Sampling strategy

The specimens were disaggregated by age, sex, year of collection, geographical location (rural or urban) and then stratified into age groups comprising of children 0–9 years, adolescents 10–19 years, adults 20–64 years and the elderly aged ≥ 65 years (Table 1). Specimens were then randomly selected from these age groups to make a total sample size of 128. The sample size was informed by the quantity of available testing reagents. Only specimens that were clearly identified with required demographic information such as age, date and place of collection were included.

Nucleic acid extraction

Nucleic acid extraction was performed using NucliSENS® easyMAG® (bioMerieux, Durham, US) according to the manufacturer's instructions. On completion of the automated extraction process, 110 µL of nucleic acid was eluted and stored at –80 °C until required for further analysis.

Detection of respiratory pathogens

For COVID-19 testing, Ribonucleic Acid (RNA) extraction and molecular detection were performed as previously described.[1] To detect influenza and other respiratory pathogens, the Fast Track Diagnostics (FTD) respiratory pathogens 33 (FastTrack Diagnostics, Luxembourg) multiplex rRT-PCR test kit was used on a QuantStudio® 5 (Life Technologies, Singapore) thermocycler, following the manufacturer's instructions. The assay detects 33 respiratory pathogens as follows: influenza A virus (IAV), influenza A(H1N1) virus (swine lineage) (IAV[H1N1] swl), influenza B virus (IBV), influenza C virus (IVC), human coronaviruses (HCoV) NL63, 229E, OC43 and HKU1, human parainfluenza viruses (HPIV) 1, 2, 3 and 4, human metapneumoviruses (HMPV) A and B, HRV, human respiratory syncytial viruses (HRSV) A and B, human adenovirus (HAdV), enterovirus (EV), human parechovirus (HPeV), human bocavirus (HBoV), *Pneumocystis jirovecii*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* B, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Bordetella* spp. (except *Bordetella parapertussis*), *Klebsiella pneumoniae*, *Legionella pneumophila*, *Legionella longbeachae*, *Salmonella* spp., *Haemophilus influenzae* and equine arteritis virus (EAV), which serves as an internal control (IC). The thermocycler was programmed and optimised for fast-track master mix PCR based on the manufacturer's recommendations as follows: 50 °C for 15 min, 94 °C for 1 min, 45 cycles of 94 °C for 8 s and 45 cycles of 60 °C for 1 min.

Data analysis

QuantStudio® Design and Analysis software version 2.6.0 (Thermo Fisher Scientific, Waltham, US) was used for analysis of the presence and absence of target pathogens. Presence or absence of target pathogens was determined using fluorescence signals for reporter dyes. Amplification plots were considered positive for the target pathogen when the cycle threshold (Ct) values did not exceed 35. Logistic regression in Statistical Package for Social Sciences (SPSS) (version 20, IBM Corp, 2011) was performed adjusting for age and location (rural or urban). Data management for profiling of co-infections was performed in Microsoft Excel.

Ethical considerations

Ethical clearance and waiver of consent was obtained from Macha Research Trust Institutional Review Board, approval number (E2021.05). Approval to conduct the study was granted by Zambia National Health Research Authority, approval number (NHRA000042/30/03/2022). To protect participant information, all specimens were de-identified by removing mobile telephone numbers, residential addresses and names.

Results

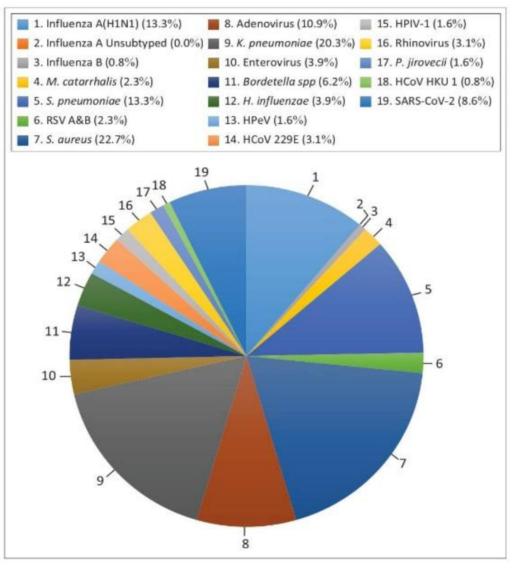
Prevalence of respiratory pathogens

From all 128 specimens tested, 71.1% (n = 91/128) were positive for at least one respiratory pathogen. Overall, bacterial respiratory pathogens were more prevalent in 70.3% (n = 90/128) than viral respiratory pathogens in 51.6% (n = 66/128). *Staphylococcus aureus* was the most detected respiratory pathogen with a prevalence of 22.7% (n = 29/128), followed by *Klebsiella pneumoniae* that accounted for 20.3% (n = 26/128) (Figure 1). *Streptococcus pneumoniae* and *Bordetella pertussis* had a prevalence of 13.3% and 6.2%, respectively. Influenza A(H1N1) accounted for 13.3% (n = 17/128), while influenza B accounted for only 0.8% (n = 1/128). Coronaviruses excluding SARS-CoV-2 accounted for 3.9% (n = 5/128). Human adenovirus accounted for 10.9% (n = 14/128), while enterovirus had a prevalence of 3.9% (n = 5/128).

Rural-urban geographical distribution of pathogens

Except for *S. pneumoniae,* the prevalence of bacterial pathogens was higher in rural areas than in urban areas (Table 2). *Staphylococcus aureus* had a rural prevalence of 27.5% compared to 20.5% in urban areas. *Bordetella pertussis* had a rural prevalence of 10% compared to 4.5% for urban.

Viral respiratory pathogens were more prevalent in urban areas compared to rural areas. All the specimens that tested positive for rhinovirus 100% (n = 4/4) and RSV 100% (n = 3/3) were from urban areas. Of the total 11 specimens testing positive for SARS-CoV-2, only 9.1% (n = 1/11) was from a rural area, while the rest 90.9% (n = 10/11) were from urban areas. Results of logistic regression showed that adjusted odds ratios associated with location, rural or urban were 3.10 for *Bordetella pertussis* confidence interval (CI) (0.667–14.208), 1.362 for *S. aureus* CI (0.561–3.303) and 1.588 for *K. pneumoniae* CI (0.615–4.097). The majority of other pathogens had odds ratios < 1.



M. catarrhalis, Moraxella catarrhalis; S. pneumoniae, Streptococcus pneumoniae; RSV, respiratory syncytial virus; *S. aureus, Staphylococcus aureus; K. pneumoniae, Klebsiella pneumoniae; H. influenzae, Haemophilus influenzae*; HPeV, human parechovirus; HCoV, human coronaviruses; HPIV-1, human parainfluenza virus 1; *P. jirovecii, Pneumocystis jirovecii*; HCoV HKU 1, human coronavirus HKU 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

FIGURE 1: Percentage distribution of detected respiratory pathogens.

Distribution of respiratory pathogens by age group

Bacterial respiratory pathogens were mostly detected in children and the elderly (Figure 2). *Moraxella catarrhalis* was only detected in children and adolescents (n = 3/3) with no specimens from adults and the elderly testing positive for the bacteria. *Staphylococcus aureus* was detected in specimens from all age groups with children and adolescents accounting for 65.5% (n = 19/29), while adults and the elderly accounted for 24.1% and 10.3%, respectively. Of the total eight specimens testing positive for *Bordetella pertussis*, 25% (n = 2/8) were from children and adolescents, while 75% (n = 6/8) were from adults and the elderly. *Klebsiella pneumoniae* was mostly detected in adults and the elderly accounting for 73.1% (n = 19/26), while children and adolescents accounted for 26.9%. All specimens testing positive for *Haemophilus influenzae* (100%, n = 5/5) were from children and adolescents. Similarly, except for SARS-CoV-2, viral respiratory pathogens were mostly detected in specimens from children and adolescents compared to other age groups. Influenza A(H1N1) was mostly detected in children, adolescents and the elderly accounting for 76.5% (n = 13/17) of the total 17 specimens testing positive for influenza, while adults accounted for 23.5% (n = 4/17) of positive specimens. Only one specimen was positive for SARS-CoV-2 in children and adolescents representing 9.1%, while the majority, that is, 90.9% (n = 10/11) were all from adults and the elderly.

TABLE 2: Distribution of pathogens by rural-urban geographic classifications.

Pathogen	Urban specimens positive	Rural specimens positive	% Prevalence urban (n = 88)	% Prevalence rural (n = 40)
Influenza A(H1N1)	12	5	13.6	12.5
Influenza B	1	0	1.1	0
M. catarrhalis	2	1	2.3	2.5
S. pneumoniae	10	3	11.4	7.5
RSV A&B	3	0	3.4	0
S. aureus	18	11	20.5	27.5
Adenovirus	11	3	12.5	7.5
K. pneumoniae	17	9	19.3	22.5
Enterovirus	5	0	5.7	0
Bordetella spp	4	4	4.5	10
H. influenzae	5	0	5.7	0
HPeV	1	1	1.1	2.5
HCoV 229E	5	0	5.7	0
HPIV-1	2	0	2.3	0
Rhinovirus	4	0	4.5	0
P. jirovecii	1	1	1.1	2.5
HCoV HKU 1	0	1	0.0	2.5
SARS-CoV-2	10	1	11.4	2.5

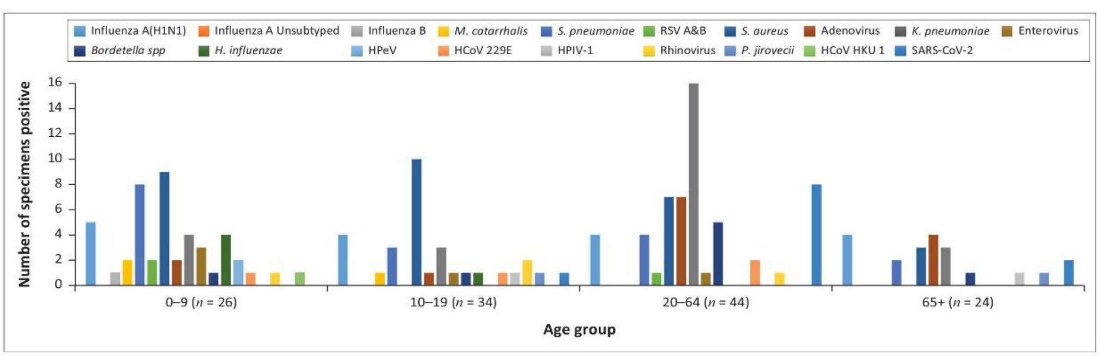
M. catarrhalis, Moraxella catarrhalis; S. pneumoniae, Streptococcus pneumoniae; RSV, respiratory syncytial virus; *S. aureus, Staphylococcus aureus; K. pneumoniae, Klebsiella pneumoniae*; HPeV, human parechovirus; HPIV-1, human parainfluenza virus 1; *P. jirovecii, Pneumocystis jirovecii*; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HCoV, human coronaviruses.

Co-infections of respiratory pathogens

Overall, co-infections were observed in 57.1% (n = 52/91) of specimens testing positive for at least one respiratory pathogen. The majority of co-infections were observed in children and adolescents (44.2%; n = 23/52). Of the co-infections observed, 11.5% (n = 6/52) specimens had virus-virus co-infections, with the most co-infecting virus being human adenovirus. On the other hand, bacteria-bacteria co-infections were 26.9% (n = 14/52) with the most co-infecting bacteria being *S. aureus* (Table 3). Virus-bacteria co-infections accounted for the remaining 48.1% (n = 25/52) of co-infections. Severe acute respiratory syndrome coronavirus 2 co-infections were mainly with *S. aureus* 27.3% (n = 3/11), *Klebsiella pneumoniae* 27.3% (n = 3/11) and *Bordetella pertussis* 18.2% (n = 2/11). Only one specimen positive for SARS-CoV-2 was co-infected with RSV, while 18.2% (n = 2/11) were co-infected with influenza A(H1N1). Five out of 17 specimens testing positive for influenza A(H1N1) representing 29.4% were co-infected with *S. aureus*, while co-infection with *Streptococcus pneumoniae* was 23.5% (n = 4/17). Co-infection of influenza A(H1N1) with *Klebsiella pneumoniae*, *B. pertussis* and adenovirus was at 11.8% (2/17) for each of these bacteria.

Discussion

The heightened country-wide COVID-19 surveillance efforts following the detection of the first SARS-CoV-2 case in Zambia provided a great opportunity to understand the



M. catarrhalis, Moraxella catarrhalis; S. pneumoniae, Streptococcus pneumoniae; RSV, respiratory syncytial virus; *S. aureus, Staphylococcus aureus; K. pneumoniae, Klebsiella pneumoniae; H. influenzae, Haemophilus influenzae*; HPeV, human parechovirus; HCoV, human coronaviruses; HPIV-1, human parainfluenza virus 1; *P. jirovecii, Pneumocystis jirovecii*; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

FIGURE 2: Distribution of respiratory pathogens by age group.

TABLE 3: Co-infection profile for respiratory pathogens during the COVID-19 pandemic.

Pathogen	IAV	IBV	*M. Cat*	*S. Pne*	RSV	*S. Aur*	Aden	Enter	*Bord*	*H. Inf*	HPeV	CoV229	HPIV-1	Rhinov	*P. Jir*	HKU 1	S-CoV-2
IAV	X	1	0	1	0	4	5	2	1	1	0	1	0	0	2	0	2
IBV	-	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. Cat	-	-	X	1	0	0	0	0	0	0	0	0	0	0	0	0	0
S. Pne	-	-	-	X	1	5	1	0	0	2	1	2	0	2	0	0	1
RSV	-	-	-	-	X	1	0	0	0	0	1	0	0	0	0	0	1
S. Aur	-	-	-	-	-	X	2	2	3	3	0	0	1	1	1	0	3
Aden	-	-	-	-	-	-	X	1	2	1	0	0	0	0	0	0	1
Enter	-	-	-	-	-	-	-	X	0	0	0	0	0	0	0	0	0
Bord	-	-	-	-	-	-	-	-	X	1	0	0	0	1	0	0	2
H. Inf	-	-	-	-	-	-	-	-	-	X	0	0	0	1	0	0	0
HPeV	-	-	-	-	-	-	-	-	-	-	X	1	0	0	0	0	0
CoV229	-	-	-	-	-	-	-	-	-	-	-	X	0	1	0	0	0
HPIV-1	-	-	-	-	-	-	-	-	-	-	-	-	X	0	0	0	0
Rhinov	-	-	-	-	-	-	-	-	-	-	-	-	-	X	0	0	0
P. Jir	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	0	0
HKU 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	0
S-CoV-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X

Note: X = co-infection already ascertained.

IAV, influenza A(H1N1) virus; IBV, influenza B virus; *H. inf*, *Haemophilus influenzae; M. cat*, *Moraxella catarrhalis;* CoV229, Human Coronavirus 229E; *Bord*, *Bordetella pertussis; S. Pne*, *Streptococcus pneumoniae;* HPIV 1, human parainfluenza virus 1; RSV, respiratory syncytial virus; Rhinov, rhinovirus; *S. aur*, *Staphylococcus aureus; P. Jir*, *P. jirovecii;* Aden, human adenovirus; HKU 1, human coronavirus HKU 1; Enter, enterovirus; S-CoV-2, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

broad range and epidemiology of respiratory pathogens circulating in Zambia during the pandemic period. Previous studies on respiratory infections were limited in geographic coverage, thus providing limited information on the distribution and burden of respiratory infections in the country.[15,16] Although most respiratory pathogens of public health concern continued to circulate, we found that bacterial respiratory pathogens were more predominant than viral pathogens during the pandemic period. *Staphylococcus aureus* was the highest detected bacteria pathogen followed by *Klebsiella pneumoniae*. Influenza A(H1N1) was the most prevalent viral respiratory pathogen detected followed by human adenovirus. Bacterial respiratory pathogens seem to have persisted more than viral counterparts during the COVID-19 pandemic despite the implementation of public health and social measures.

Although on observation, it seems that rural geographical areas increased the risk of infection for *Bordetella pertussis* with adjusted odds ratio of 3.10, *S. aureus* 1.362 and 1.588 for *K. pneumoniae*, the confidence intervals > 1 could not statistically support the significance of the observed differences between urban and rural areas.

The results of this study are comparable to other studies[17,18,19] that have shown a high prevalence of bacterial respiratory infections among hospitalised children, adults and the elderly because children may be immunologically naïve while the elderly may be progressing towards immunosenescence. Existing scientific literature shows that more emphasis has been placed on the role of viral pathogens as aetiological agents of acute lower respiratory tract infections in developing countries neglecting the role of bacterial pathogens.[20,21,22] While our study could not ascertain whether bacterial pathogens detected caused infections in the individuals tested, at the very least, it does show the presence or colonisation of NP areas with bacteria that could cause respiratory infections and progress to severe disease.

Although we have provided estimates of prevalence for a broad range of respiratory pathogens during the COVID-19 pandemic, it was difficult to determine the extent to which NPIs against COVID-19 had altered the epidemiology of these respiratory pathogens because studies before the pandemic[15,16] were based on specimens collected entirely from symptomatic individuals in health facilities, thus posing challenges in making comparisons. However, the percentage of specimens testing positive for bacterial and viral pathogens from this study was generally lower than those reported in pre-pandemic studies.[23] For example, we detected influenza virus, rhinovirus and RSV in 13.3%, 3.1% and 2.3% of the specimens, respectively, while influenza, rhinovirus and RSV among symptomatic outpatients in rural Zambia in 2019 were estimated at 21.5%, 24.0% and 16.5%, respectively.[12] Similarly, another study conducted to identify viral and bacterial pathogens from hospitalised children with acute respiratory illness in Lusaka between 2011 and 2012 reported an estimated RSV prevalence of 33.7% and rhinovirus at 11.4%.[13] With regard to bacterial pathogens, this study reported a prevalence of *Streptococcus pneumoniae* at 13.3%, *Moraxella catarrhalis* at 2.3% and *Bordetella pertussis* at 6.2%. On the other hand, a study conducted before the pandemic reported a higher prevalence of *Streptococcus pneumoniae* at 54.8% and *Moraxella catarrhalis* at 46.2% showing a considerable reduction in prevalence.[16] It is possible that public health and social measures implemented against COVID-19 may have altered the epidemiology of other respiratory pathogens besides helping to slow the transmission of SARS-CoV-2.[29,30,31,32]

Co-infections detected in this study were mostly virus-bacteria and bacteria-bacteria. In a study of virus-virus interactions for respiratory pathogens, it was found that virus-virus interactions may result in interaction-induced effects such as cross-immunity and resource competition leading to attenuation.[20,24] Additionally, infection with one respiratory pathogen may elicit immune response against the other. This may help to explain the small number of virus-virus co-infections and a higher bacterial co-infections observed in this study. Further, possible bacterial colonisation of the respiratory system may also have contributed to a higher bacteria detection. Largely, co-infections were detected in children and adolescents who accounted for 44.2% (n = 23/52) of the total co-infections detected. The results agree with findings of other epidemiological studies in Zambia and globally that have demonstrated that co-infections for respiratory viruses largely occur in children.[16,26,27,28] The most co-infecting respiratory pathogens across all age groups were *Staphylococcus aureus, Streptococcus pneumoniae* and *Klebsiella pneumoniae*. This is the first study in Zambia to show *Staphylococcus aureus* among the most co-infecting respiratory pathogens in Zambia.

It is important to note that recent studies have shown that co-infection with *Staphylococcus aureus* is associated with the highest intensive care admission and death rates.[17,18] It has further been found that interactions between viruses and bacteria could generally lead to enhanced bacteria binding, immune response dysregulation and slowed bacterial clearance.[17] Therefore, there is need for epidemiological studies to fully ascertain the association between virus-bacteria co-infection and severe respiratory illnesses leading to hospitalisation and death in Zambia given that previous studies have been more focused on viral pathogens.

This study had limitations including being retrospective study in nature and having a limited sample size. A lack of clinical data on participants contributed to the inability of the study to elucidate associations between co-infections and disease outcomes. There were more specimens collected from urban than rural areas that made the sample size to have a bias towards urban areas.

Conclusion

This is the first study combining both urban and rural diagnostic specimens to detect respiratory pathogens during the COVID-19 pandemic in Zambia. While both viral and bacterial respiratory pathogens of public health concern continued to circulate during the period, bacterial pathogens were more predominant. Overall, there was an observed decline in the prevalence of respiratory pathogens suggesting that public health interventions against COVID-19 may have altered the epidemiology of respiratory pathogens. Co-infections detected were mostly virus-bacteria with *S. aureus* being the most co-infecting pathogen. Considering the findings from this and other studies, sustaining public health and social measures instituted against COVID-19 may possibly help to limit the spread of other respiratory pathogens of public health concern.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

M.N., E.S. and N.S. conceptualised, collected data, performed data analysis and drafted the article. K.C., W.M. and C.S., substantially revised the draft article. G.K., Z.M., C.C. and G.M. performed laboratory work and data analysis and substantially revised the draft article.

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Data availability

Data associated with this article is available within the article and raw data can be made available upon request to the corresponding author, M.N.

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Burden of Viral and Bacterial Respiratory Infections in Africa: A Systematic Review and Meta-Analysis 2013-2023

Martin Nyahoda[1]; Annie Kalonda[2]; Katendi Changula[4]; Ngonda Saasa[1]; Edgar Simulundu[1,3]*

[1]Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka Zambia.

[2] Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka, Zambia.

[3]Macha Research Trust, Choma, Zambia.

[4]Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia.

***Corresponding Author:** Martin Nyahoda*

Email: martinny2@yahoo.co.uk

Abstract

Background: Respiratory infections are among the leading causes of morbidity and mortality globally especially in sub-Saharan Africa.

Objectives: The aim of this systematic review and meta-analysis was to compare the prevalence between viral and bacterial respiratory pathogens and determine the African regions with the highest burden of respiratory infections in order to help target public health interventions to African regions with the greatest need and achieve efficient utilization limited resources.

Methods: PubMed and Google Scholar were systematically searched for studies in Africa published between January 2013 and 28th September, 2023. Of the total 758 articles retrieved after removing duplicates, 39 studies were included in this review with a sample size of 72,196 with a representation of all age groups. Studies were grouped in 5 regions based on country of study, Southern, North, Central, East and West Africa.

Results: The majority of studies were from Southern Africa (20/39) while the Central Africa had the least with (2/39). With respect to age, children < 15 years accounted for the majority of studies with 59% (23/39) of the articles retrieved. Most studies (37/39, 94.9%) tested and reported viral respiratory aetiologies with (25/37), 67.6% of studies exclusively testing and reporting viral respiratory pathogens. Only (14/39), 35.9% studies tested and reported bacterial respiratory pathogens. Overall pooled prevalence for viral and bacterial respiratory aetiologies was estimated at 56.6% (95% CI, 0.371-0.762; I2 99.9%) and 21.5% (95% CI, 0.203-0.228; I2 99.96%) respectively. At continental level, rhinovirus and respiratory syncytial virus were the most predominant pathogens with a wide geographical spread across all African regions with 19.9% and 8.9% prevalence, respectively. North Africa was highest in RSV prevalence with 16.1% while Southern and Central Africa had the highest rhinovirus burden with 25.9% and 26.3% respectively. North Africa had the highest influenza burden with 28.2% with the least being Central Africa with 0.2% influenza prevalence. Streptococcus Pneumoniae was the commonly reported bacterial pathogen with the highest burden in Central Africa with 55.5% prevalence.

Conclusions: This study generated evidence that the burden of respiratory pathogens is highest in North and Southern Africa. Further, the majority of studies are focused on viral respiratory pathogens neglecting bacterial pathogens. To fully understand the burden of respiratory infections in Africa and improve prognosis, there is a need for more studies combining detection of both viral and bacterial respiratory pathogens.

Keywords: Respiratory infections; Burden; Africa; Viruses; Bacteria.

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Introduction

Estimates indicate that respiratory infections are among the top 10 leading causes of death in children aged 0-14 years especially in low and middle-income countries [1]. In 2019, global morbidity incidence associated with Lower Respiratory Tract Infections (LRTIs) was estimated to be 488.9 million with about 2.4 million deaths [2]. Sub-Saharan Africa accounts for 66.4 deaths attributable to LRTI per 100,000 persons which is considered the highest globally [3]. In African children, estimates show that 38.6% of infectious disease deaths and 14.9% of all deaths are caused by LRTI [1,4]. Epidemiological studies have further shown that co-infections of viral-bacterial pathogens may increase the severity of disease especially in immune compromised and TB patients leading to poor prognosis [5]. The incidence of LRTIs remains high in HIV-positive children even with the widespread use of antiretroviral therapy thereby increasing the risk of severe disease and death [6].

Both viral and bacterial pathogens have been implicated in LRTIs and associated with Community-Acquired Pneumonia (CAP). Among viral pathogens responsible for LRTI causing Influenza-Like Illnesses (ILI) and Severe Acute Respiratory Infections (SARI) include, Influenza, Parainfluenza Viruses (PIV), Respiratory Syncytial Virus (RSV), Rhinovirus, Human Bocavirus (HBoV) and Coronaviruses [7]. On the other hand, bacterial pathogens such as Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus and Bordetella pertussis have been found to contribute to disease severity and poor patient outcomes in incidences of co-infections [8,9]. However, even with the advent of multiplex Polymerase Chain Reaction (PCR), most scientific studies have focused on viral respiratory pathogens neglecting the interactions between viral and bacterial respiratory pathogens in causing severe respiratory illnesses and death. It is important to acknowledge that viral infections predispose to the growth of bacterial pathogens leading to a high risk of pneumonia.8 Therefore, studies on the aetiology of viral or bacterial respiratory infections do not need to be mutually exclusive but holistic encompassing both viral and bacterial pathogens to implement interventions that improve prognosis.

With interventions being implemented for prevention and treatment such as vaccination and antibiotic use in the management of LRTI, it is important to evaluate their impact and effectiveness in the light of well-documented scientific evidence and determine geographical disparities [10]. Tracking the geographical distribution of disease helps in planning targeted interventions resulting in better utilization of limited resources [10]. This systematic review and meta-analysis was therefore aimed at establishing the geographical distribution and prevalence of viral and bacterial respiratory infections in Africa.

Material and methods

In undertaking this systematic review and meta-analysis, the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) were followed. The systematic review was not registered to the International Prospective Register of Systematic Reviews (PROSPERO).

Search strategy and eligibility criteria

Pubmed and Google Scholar were systematically searched for epidemiological studies published between January 2013 and 28th September, 2023 in English language. Search terms were a combination of three key concepts; respiratory pathogens, prevalence and co-infections. To bolster the search, the key concepts were coupled with similar or related words such as "co-infections" OR "dual infections", OR "multiple infections" OR "Lower respiratory infections" OR "upper respiratory infections".

Molecular studies with multiple detections of respiratory pathogens were included. In terms of geographical coverage, only studies from Africa were included. Only studies with sample specimens collected from humans were included. Additionally, the review included only articles from original studies. Among studies excluded were serological studies, epidemiological studies done outside the African region, specimens collected from non-humans and systematic reviews, conference proceedings and dissertations.

Data abstraction and analysis

Using a predefined template in Microsoft Excel, data was extracted based on author, study title, year of publication and country where the study was conducted. Sample size, method of detection and specimen type (oral or nasopharyngeal swabs) were also abstracted from eligible studies. The prevalence of infection for each pathogen detected was recorded together with information on co-infections that were detected. Data Analysis to estimate pooled prevalence and construction of forest plots was performed in OpenMeta (OSX: Yosemite 10.10, USA). Heterogeneity was assessed as described by Krumbein et al, 2022 [11].

Outcomes

The main outcome of this systematic review and meta-analysis was a comparative prevalence of various aetiologies for respiratory infections in different African regions. Additionally, the review sought to determine the geographical distribution of both viral and bacterial respiratory pathogens across the African continent, revealing geographical disparities and regions with a high burden.

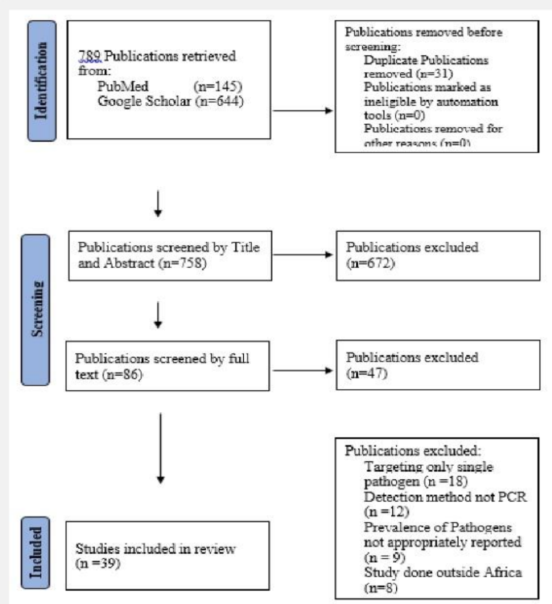

Figure 1: PRISMA flow diagram showing search results and studies screened at various stages.

Results

After a systematic search in PubMed and Google Scholar, 789 publications were retrieved as shown in Figure 1. After deduplication in endnote 21 version 21.2, seven hundred and fifty-eight publications were available for screening. After title screening, 613 publications were excluded remaining with 145 publications. After reviewing abstracts, 59 publications were excluded. After full-text screening, 39 publications were included in the systematic review and meta-analysis with a total sample size of 72,196.

Characteristics of included studies.

All studies included were conducted using either nasopharyngeal or oropharyngeal specimens with multiplex RT-PCR as the detection method. Studies were clustered in 5 regions as Southern Africa, West Africa, East Africa, North Africa and Central Africa to enable regional comparisons on the distribution and prevalence for respiratory pathogens of interest. Southern Africa had the most publications with 20 followed by West Africa with 12. East Africa had 4 publications while North Africa and Central Africa had the least number of publications with 2 each. The majority of studies (23/39), 59% were conducted in children <15 years with children <5 years accounting for the majority (18/23), 78% of studies. Although the majority of studies were in children, overall, all age groups were represented in the included studies. All studies except 2 had sample sizes of more than 100 specimens with a highest sample size of 18,282 in a single study.

Overall prevalence of respiratory pathogens

After analysis of a total sample of 72,196 specimens, viral respiratory pathogens accounted for a larger proportion of positive specimens than the bacterial. The pooled prevalence for respiratory viral infections was 56.6%, (CI, 0.371-0.762, I2 99.9%) for specimens testing positive for at least one pathogen (Figure 3). Bacterial respiratory pathogens had an estimated pooled prevalence of 21.5% (CI 0.203-0.223, I2 99.96%) of specimens testing positive for at least one pathogen, Figure 4. Among viral respiratory pathogens, Rhinovirus had the highest proportion of specimens positive accounting for 29.9% (Figure 2) with a prevalence of 19.9% (Table 1). Adenovirus and Respiratory Syncytial Virus (RSV) were second and third with 18% and 13.4% of the total specimens respectively and a prevalence of 12% and 8.9% respectively. Human Bocavirus was the least prevalent viral respiratory pathogen with only 1.1% proportion of the total specimens testing positive and a prevalence of 0.7%. For bacterial pathogens, Streptococcus pneumonia had the highest prevalence with a 7.5% proportion of specimens testing positive and a prevalence of 5% followed by Moraxella catarrhalis with 2.2% and a prevalence of 1.5%.

Regional prevalence comparisons for detected respiratory pathogens

After analyzing regional prevalence, clustered as Southern, Central, North, East and West Africa, the prevalence of influenza was predominant in North Africa with 28.2% followed by West Africa with 5.3% (Figure 5). Rhinovirus was most prevalent in Southern Africa with 25.9% followed by Central Africa with 26.3% (Figure 5). East Africa recorded the least Rhinovirus prevalence with 8.1%. Respiratory Syncytial Virus (RSV) was highest in North Africa with a prevalence of 16.1% followed by Southern Africa with 11.4%. East Africa recorded the least RSV prevalence estimated at 2.4%. Human Adenovirus was highest in South-

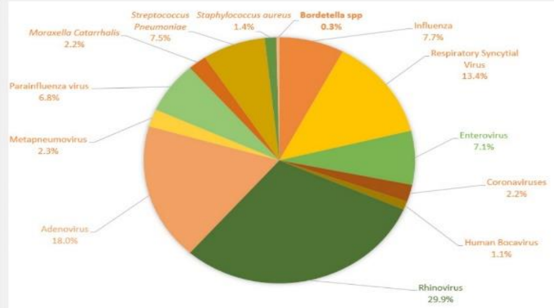

Figure 2: Proportions of various respiratory pathogens detected. (Specimens positive: n=48158).

Table 1: Prevalence for specific aetiologies of respiratory infections - All African regions.

Pathogen	Specimens positive	%Prevalence (N=72196)
Influenza	3730	5.2
RSV	6447	8.9
Enterovirus	3407	4.7
CoV	1080	1.5
HBoV	533	0.7
Rhinovirus	14401	19.9
Adenovirus	8664	12.0
HMPV	1118	1.5
HPIV	3263	4.5
M. catarrhalis	1062	1.5
S.pneumoniae	3619	5.0
S. aureus	666	0.9
Bordetella spp	168	0.2
Total	**48158**	

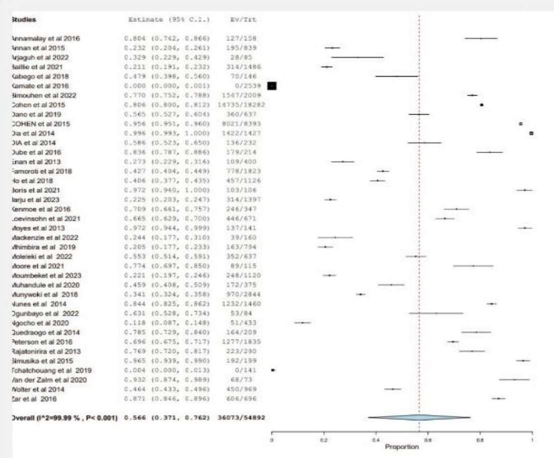

Figure 3: Forest plot of overall pooled prevalence for viral respiratory infections using the random effects model.

ern Africa with an estimated prevalence of 16.8%. Overall, few bacterial respiratory infections were recorded in North Africa compared to the rest of the regions. Central Africa recorded the highest Streptococcus pneumoniae prevalence with 55.5% while the lowest was North Africa which did not record any streptococcus pneumoniae infections. East Africa accounted for the highest proportion of Moraxella catarrhalis with 2.8% followed by Southern Africa with 1.8%.

Age group comparisons for detected respiratory pathogens

Results for age group comparisons (Figure 6), revealed that children accounted for a greater proportion of both viral and bacterial respiratory infections than adults. More specifically, Rhinovirus had an estimated prevalence of 16.4% in children while it was 9.9% in adults. RSV had an estimated prevalence of 9.3% in children and 2.5% in adults. For bacteria, the prevalence of M. catarrhalis was estimated to be 2.6% in children while it was 0.3% in adults. S. pneumoniae infection was higher in adults with 6.7% prevalence than in children with 4.6%.

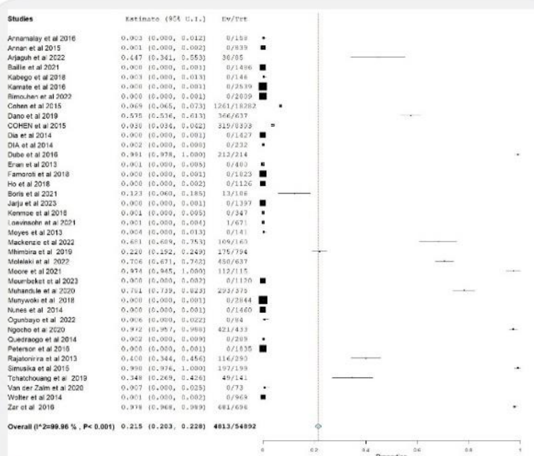

Figure 4: Forest plot of overall pooled prevalence for bacterial respiratory infections using the random effects model.

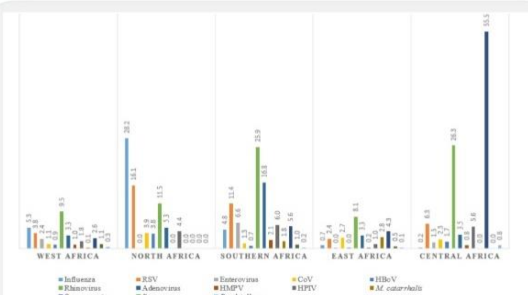

Figure 5: Regional prevalence for various respiratory aetiologies.

Discussion

This systematic review and meta-analysis was aimed at estimating the prevalence of viral and bacterial respiratory pathogens and making regional geographical comparisons to determine African regions with high a burden of respiratory infections between 2013 and 2023. This information is important in order

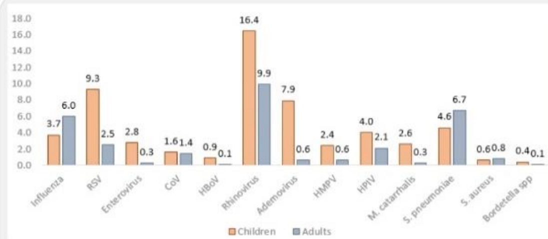

Figure 6: Prevalence of respiratory aetiologies in children and adults.

to target public health interventions to most needed areas and ensure better utilization of resources [10]. This study revealed that epidemiological studies have to a large extent focused on elucidating viral aetiologies for respiratory infections in Africa with little attention paid to bacterial pathogens. This could help explain the high prevalence of viral respiratory aetiologies compared to bacterial respiratory aetiologies observed in the review. The high prevalence of Rhinovirus observed in the studies in this review agrees with other scientific literature which have shown that Rhinoviruses are the leading cause of acute respiratory infections and represent a substantial proportion of the global burden of upper respiratory infections [12,13]. While little attention has been paid to the role of adenovirus in both upper and lower respiratory tract infections and severe disease, this review seems to suggest that its prevalence may be significant in the pathogenesis of respiratory infections especially in cases of co-infections as demonstrated by other studies [11].

Comparing the results of this review on influenza prevalence with the World Health Organization (WHO) epidemiological data on influenza in Africa revealed that the results of this study were similar. Influenza prevalence was highest in North and Southern Africa with 28.2% and 5.3% respectively, consistent with WHO epidemiological data which has shown highest influenza prevalence in North and Southern Africa [14].

In this study, the prevalence of Respiratory Syncytial Virus (RSV) was highest in North Africa with 16.1% followed by Southern Africa with 11.4% consistent with results of a systematic review and meta-analysis by Regassa et al 2023 [15], on the molecular epidemiology of respiratory syncytial virus in children with acute respiratory illnesses in Africa in which sub-regional analysis revealed that North and Southern Africa had the highest prevalence of RSV.

These results show that North and Southern Africa may be the regions with the highest burden of RSV in Africa. Further, this study revealed that Central and Southern Africa have the highest burden of Rhinovirus with 26.3% and 25.9% prevalence respectively. Public health interventions targeting reduction of the burden of disease from these respiratory infections should be more considered in these regions of Africa.

Epidemiological studies have revealed that opportunistic bacterial infections especially M. catarrhalis have been implicated in causing otitis media and acute exacerbations of Chronic Obstructive Pulmonary Disease (COPD) in children and adults with co-infections resulting in adverse prognosis [16]. With this study showing a high burden of S. pneumoniae and M. catarrhalis in Central and Southern Africa, improving patient outcomes from these respiratory infections especially in cases of co-infections with viral pathogens may require preventive programs that limit exposure to these respiratory infections in these regions.

Generally, although bacterial respiratory pathogens play a major role in the pathogenesis of upper and lower respiratory tract infections especially in incidences of viral-bacterial co-infections resulting in poor patient outcomes, results of this study have shown that little attention is paid in undertaking epidemiological studies focusing on bacterial respiratory pathogens [11,17,18]. With the advent of multiplex polymerase chain reaction, studies focusing on simultaneous detection of both viral and bacterial respiratory pathogens and elucidating their impact on disease severity should be encouraged especially in Africa. This is necessary in designing intervention efforts for prevention and mitigating effects of viral-bacterial interactions that may result in severe disease outcomes.

As other studies have shown this review demonstrated that the burden of both viral and bacterial respiratory pathogens is higher in children than in adults [7,11].

Conclusions

This systematic review and meta-analysis provides evidence that the burden of respiratory viral infections is highest in North and Southern Africa. In terms of both viral and bacterial respiratory pathogens considered together, Southern Africa has the highest burden of infection. For bacterial respiratory pathogens alone, North Africa has the lowest burden of infection. This review provides further evidence that there are few studies conducted targeting multiplex testing for co-detection of viral and bacterial respiratory pathogens especially in East and Central Africa.

Most studies are focused on viral respiratory pathogens. Considering the impact of viral-bacterial co-infections on the pathogenesis of respiratory infections and prognosis, there is need for more studies focusing on co-detection of viral and bacterial respiratory pathogens to clearly understand the burden of respiratory infections in Africa and help target interventions to improve patient outcomes.

Declarations

Competing interests: The authors declare no competing interests.

Author contributions: MN and ES conceptualized the study. MN and AN performed the search, analyzed the data and drafted the manuscript. NS, ES and KC substantially revised the draft manuscript.

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Appendix III: FTD Respiratory Pathogens 33 Optimized PCR Assay Plate Layout (64 Reactions)

Plate 1: Viral

PP mix 1 Flu-Rhino	PP mix 2 ParaEAV	PP mix 3 Cor	PP mix 4 Bo-Mp-pf1

S1 Flu-Rhino	**S8 Flu-Rhino**		**S1 ParaEAV**	**S8 ParaEAV**		**S1 Cor**	**S8 Cor**		**S1 Bo-Mp-pf1**	**S8 Bo-Mp-pf1**	
S2 Flu-Rhino	**S9 Flu-Rhino**		**S2 ParaEAV**	**S9 ParaEAV**		**S2 Cor**	**S9 Cor**		**S2 Bo-Mp-pf1**	**S9 Bo-Mp-pf1**	
S3 Flu-Rhino	**S10 Flu-Rhino**		**S3 ParaEAV**	**S10 ParaEAV**		**S3 Cor**	**S10 Cor**		**S3 Bo-Mp-pf1**	**S10 Bo-Mp-pf1**	
S4 Flu-Rhino	**S11 Flu-Rhino**		**S4 ParaEAV**	**S11 ParaEAV**		**S4 Cor**	**S11 Cor**		**S4 Bo-Mp-pf1**	**S11 Bo-Mp-pf1**	
S5 Flu-Rhino	**S12 Flu-Rhino**		**S5 ParaEAV**	**S12 ParaEAV**		**S5 Cor**	**S12 Cor**		**S5 Bo-Mp-pf1**	**S12 Bo-Mp-pf1**	
S6 Flu-Rhino	**S13 Flu-Rhino**		**S6 ParaEAV**	**S13 ParaEAV**		**S6 Cor**	**S13 Cor**		**S6 Bo-Mp-pf1**	**S13 Bo-Mp-pf1**	
S7 Flu-Rhino	**S14 Flu-Rhino**		**S7 ParaEAV**	**S14 ParaEAV**		**S7 Cor**	**S14 Cor**		**S7 Bo-Mp-pf1**	**S14 Bo-Mp-pf1**	
PC Flu-Rhino	**NC Flu-Rhino**		**PC ParaEAV**	**NC ParaEAV**		**PC Cor**	**NC Cor**		**PC Bo-Mp-pf1**	**NC Bo-Mp-pf1**	

Key: S = Sample

NB: The Positive Control (PC) sample should be added at the end (ie after the addition of the Negative Control). Contamination of the other wells by the PC sample should be completely avoided. It might be a good idea to add all the PC samples on the plate at the same time.

Plate 2: Bacteria

PP mix 5 RsEPA			PP mix 6 RespBac2			PP mix 7 KLePSa			PP mix 8 MoBoCH		
S1 RsEPA	S8 RsEPA		S1 RespBac2	S8 RespBac2		S1 KlePSa	S8 KlePSa		S1 MoBoCH	S8 MoBoCH	
S2 RsEPA	9 RsEPA		S2 RespBac2	S9 RespBac2		S2 KlePSa	S9 KlePSa		S2 MoBoCH	S9 MoBoCH	
S3 RsEPA	S10 RsEPA		S3 RespBac2	S10 RespBac2		S3 KlePSa	S10 KlePSa		S3 MoBoCH	S10 MoBoCH	
S4 RsEPA	S11 RsEPA		S4 RespBac2	S11 RespBac2		S4 KlePSa	S11 KlePSa		S4 MoBoCH	S11 MoBoCH	
S5 RsEPA	S12 RsEPA		S5 RespBac2	S12 RespBac2		S5 KlePSa	S12 KlePSa		S5 MoBoCH	S12 MoBoCH	
S6 RsEPA	S13 RsEPA		S6 RespBac2	S13 RespBac2		S6 KlePSa	S13 KlePSa		S6 MoBoCH	S13 MoBoCH	
S7 RsEPA	S14 RsEPA		S7 RespBac2	S14 RespBac2		S7 KlePSa	S14 KlePSa		S7 MoBoCH	S14 MoBoCH	
PC RsEPA	NC RsEPA		PC RespBac2	NC RespBac2		PC KlePSa	NC KlePSa		PC MoBoCH	NC MoBoCH	

Appendix IV: Programming of the Thermocycler for ABI 7500 or Quant Studio[5]

PPmix	Pathogen	Reporter	Dye	Excitation wavelength (nm)*	Detection wavelength (nm)*
Flu-Rhino PPmix	IAV	FAM	Green	520	
	HRV	VIC	Yellow	550	
	BV	ROX	Orange	610	
	IAV(H1N1)	CY5	Red	670	
ParaEAV PPmix	hCoV 229	FAM	Green	520	
	HCoV NL63	VIC	Yellow	550	
	HCoV HKU1	ROX	Orange	610	
	HCoV OC43	CY5	Red	670	
Cor PPmix	HPIV3	FAM	Green	520	
	HPIV2	VIC	Yellow	550	
	HPIV4	ROX	Orange	610	
	IC(EAV)	CY5	Red	670	
RsEPA PPmix	HPIV1	FAM	Green	520	
	HMPV A/B	VIC	Yellow	550	
	HBoV	ROX	Orange	610	
	M. pneumoniae	CY5	Red	670	
Bo-Mp-pf1 PPmix	HRSV A/B	FAM	Green	520	
	HPeV	VIC	Yellow	550	
	EV	ROX	Orange	610	
	HAdV	CY5	Red	670	
RespBac2 PPmix	*S. aureus*	FAM	Green	520	
	C. pneumoniae	VIC	Yellow	550	
	**H. influenzae B*	ROX	Orange	610	
	S. pneumoniae	CY5	Red	670	
KlePSa PPmix	*P. jirovecii*	FAM	Green	520	
	L. pneumophila/ L. longbeachae	VIC	Yellow	550	
	K. pneumoniae	ROX	Orange	610	
	Salmonella spp	CY5	Red	670	
MoBoCH PPmix	*M. catarrhalis*	FAM	Green	520	
	ICV	VIC	Yellow	550	
	Bordetella	ROX	Orange	610	
	**H. influenzae*	CY5	Red	670	

*Please note there are two *H. influenzae* strains

Appendix V: Selected Regression Outputs on Effects of Age, Gender and Location on Infection

```
GET DATA /TYPE=XLSX
  /FILE='C:\Users\Martin Nyahoda\Desktop\Regression Workbook.xlsx'
  /SHEET=name 'Enterovirus'
  /CELLRANGE=full
  /READNAMES=on
  /ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet2 WINDOW=FRONT.
LOGISTIC REGRESSION VARIABLES DiseaseStatus
  /METHOD=ENTER Age Gender Location
  /CONTRAST (Gender)=Indicator
  /CONTRAST (Location)=Indicator
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20) CUT(.5).
```

Logistic Regression

Notes

Output Created		12-AUG-2024 11:46:25
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	128
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing

Syntax		LOGISTIC REGRESSION VARIABLES DiseaseStatus /METHOD=ENTER Age Gender Location /CONTRAST (Gender)=Indicator /CONTRAST (Location)=Indicator /CRITERIA=PIN(.05) POUT(.10) ITERATE(20) CUT(.5).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.02

[DataSet2]

Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	128	100.0
	Missing Cases	0	.0
	Total	128	100.0
Unselected Cases		0	.0
Total		128	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
0	0
1	1

Categorical Variables Codings

	Frequency	Parameter coding
		(1)

Location	Rural	40	1.000
	Urban	88	.000
Gender	Female	59	1.000
	Male	69	.000

Block 0: Beginning Block

Classification Table[a,b]

	Observed		Predicted		
			Disease Status		Percentage Correct
			0	1	
Step 0	Disease Status	0	123	0	100.0
		1	5	0	.0
	Overall Percentage				96.1

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-3.203	.456	49.284	1	.000	.041

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	2.974	1	.085
		Gender(1)	.405	1	.525
		Location(1)	2.365	1	.124
	Overall Statistics		6.323	3	.097

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	7.964	3	.047
	Block	7.964	3	.047
	Model	7.964	3	.047

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	34.264[a]	.060	.215

a. Estimation terminated at iteration number 20 because maximum iterations has been reached. Final solution cannot be found.

Classification Table[a]

	Observed		Predicted		
			Disease Status		Percentage Correct
			0	1	
Step 1	Disease Status	0	123	0	100.0
		1	5	0	.0
	Overall Percentage				96.1

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Age	-.034	.022	2.411	1	.120	.967
	Gender(1)	1.121	.960	1.362	1	.243	3.067
	Location(1)	-18.535	6057.504	.000	1	.998	.000
	Constant	-2.298	.864	7.070	1	.008	.100

a. Variable(s) entered on step 1: Age, Gender, Location.

```
GET DATA /TYPE=XLSX
  /FILE='C:\Users\Martin Nyahoda\Desktop\Regression Workbook.xlsx'
  /SHEET=name 'SARS-CoV-2'
  /CELLRANGE=full
  /READNAMES=on
  /ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet1 WINDOW=FRONT.
LOGISTIC REGRESSION VARIABLES DiseaseStatus
  /METHOD=ENTER Age Gender Location
  /CONTRAST (Gender)=Indicator
  /CONTRAST (Location)=Indicator
  /CLASSPLOT
  /PRINT=GOODFIT CORR ITER(1) CI(95)
  /CRITERIA=PIN(0.05) POUT(0.10) ITERATE(20) CUT(0.5).
```

Logistic Regression

Notes

Output Created		10-JAN-2024 11:14:42
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	128
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing

Syntax		LOGISTIC REGRESSION VARIABLES DiseaseStatus /METHOD=ENTER Age Gender Location /CONTRAST (Gender)=Indicator /CONTRAST (Location)=Indicator /CLASSPLOT /PRINT=GOODFIT CORR ITER(1) CI(95) /CRITERIA=PIN(0.05) POUT(0.10) ITERATE(20) CUT(0.5).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.02

[DataSet1]

Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	128	100.0
	Missing Cases	0	.0
	Total	128	100.0
Unselected Cases		0	.0
Total		128	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
0	0
1	1

Categorical Variables Codings

		Frequency	Parameter coding
			(1)
Location	Rural	40	1.000
	Urban	88	.000
Gender	Female	59	1.000
	Male	69	.000

Block 0: Beginning Block

Iteration History[a,b,c]

Iteration		-2 Log likelihood	Coefficients
			Constant
Step 0	1	81.153	-1.656
	2	75.272	-2.209
	3	75.018	-2.355
	4	75.017	-2.364
	5	75.017	-2.364

a. Constant is included in the model.

b. Initial -2 Log Likelihood: 75.017

c. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.

Classification Table[a,b]

	Observed		Predicted		
			Disease Status		Percentage Correct
			0	1	
Step 0	Disease Status	0	117	0	100.0
		1	11	0	.0
	Overall Percentage				91.4

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-2.364	.315	56.204	1	.000	.094

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	6.966	1	.008
		Gender(1)	.346	1	.556
		Location(1)	.957	1	.328
	Overall Statistics		8.832	3	.032

Block 1: Method = Enter

Iteration History[a,b,c,d]

Iteration		-2 Log likelihood	Coefficients			
			Constant	Age	Gender(1)	Location(1)
Step 1	1	77.100	-2.073	.011	.158	-.285
	2	67.287	-3.257	.024	.333	-.630
	3	65.300	-4.165	.038	.430	-.880
	4	65.048	-4.653	.045	.443	-.942
	5	65.039	-4.771	.047	.443	-.945
	6	65.039	-4.776	.047	.443	-.945
	7	65.039	-4.776	.047	.443	-.945

a. Method: Enter

b. Constant is included in the model.

c. Initial -2 Log Likelihood: 75.017

d. Estimation terminated at iteration number 7 because parameter estimates changed by less than .001.

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	9.979	3	.019
	Block	9.979	3	.019
	Model	9.979	3	.019

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	65.039[a]	.075	.169

a. Estimation terminated at iteration number 7 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	11.080	8	.197

Contingency Table for Hosmer and Lemeshow Test

		Disease Status = 0		Disease Status = 1		Total
		Observed	Expected	Observed	Expected	
Step 1	1	17	17.810	1	.190	18
	2	13	12.835	0	.165	13
	3	11	10.783	0	.217	11
	4	16	15.676	0	.324	16
	5	11	10.365	0	.635	11
	6	12	11.763	1	1.237	13
	7	10	12.786	5	2.214	15
	8	11	9.310	0	1.690	11
	9	8	7.876	2	2.124	10
	10	8	7.796	2	2.204	10

Classification Table[a]

	Observed		Predicted		
			Disease Status		Percentage Correct
			0	1	
Step 1	Disease Status	0	117	0	100.0
		1	11	0	.0
	Overall Percentage				91.4

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age	.047	.022	4.685	1	.030	1.048	1.004	1.094
	Gender(1)	.443	.666	.443	1	.506	1.558	.422	5.747
	Location(1)	-.945	.833	1.287	1	.257	.389	.076	1.989
	Constant	-4.776	1.371	12.131	1	.000	.008		

a. Variable(s) entered on step 1: Age, Gender, Location.

Correlation Matrix

		Constant	Age	Gender(1)	Location(1)
Step 1	Constant	1.000	-.934	-.195	-.085
	Age	-.934	1.000	-.046	.006
	Gender(1)	-.195	-.046	1.000	-.160
	Location(1)	-.085	.006	-.160	1.000

```
GET DATA /TYPE=XLSX
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  /SHEET=name 'Adenovirus'
```

```
  /CELLRANGE=full
  /READNAMES=on
  /ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet1 WINDOW=FRONT.
LOGISTIC REGRESSION VARIABLES DiseaseStatus
  /METHOD=ENTER Age Gender Location
  /CONTRAST (Gender)=Indicator
  /CONTRAST (Location)=Indicator
  /SAVE=PRED
  /PRINT=GOODFIT ITER(1) CI(95)
  /CRITERIA=PIN(0.05) POUT(0.10) ITERATE(20) CUT(0.5).
```

Logistic Regression

Notes

Output Created		11-SEP-2023 08:57:21
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	128
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing

Syntax		LOGISTIC REGRESSION VARIABLES DiseaseStatus /METHOD=ENTER Age Gender Location /CONTRAST (Gender)=Indicator /CONTRAST (Location)=Indicator /SAVE=PRED /PRINT=GOODFIT ITER(1) CI(95) /CRITERIA=PIN(0.05) POUT(0.10) ITERATE(20) CUT(0.5).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.01
Variables Created or Modified	PRE_1	Predicted probability

[DataSet1]

Case Processing Summary

Unweighted Cases[a]		N	Percent
Selected Cases	Included in Analysis	128	100.0
	Missing Cases	0	.0
	Total	128	100.0
Unselected Cases		0	.0
Total		128	100.0

a. If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
0	0
1	1

Categorical Variables Codings

		Frequency	Parameter coding
			(1)
Location	Rural	40	1.000
	Urban	88	.000
Gender	Female	59	1.000
	Male	69	.000

Block 0: Beginning Block

Iteration History[a,b,c]

Iteration		-2 Log likelihood	Coefficients
			Constant
Step 0	1	92.467	-1.563
	2	88.473	-2.009
	3	88.373	-2.094
	4	88.373	-2.097
	5	88.373	-2.097

a. Constant is included in the model.

b. Initial -2 Log Likelihood: 88.373

c. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.

Classification Table[a,b]

	Observed		Predicted		
			Disease Status		Percentage Correct
			0	1	
Step 0	Disease Status	0	114	0	100.0
		1	14	0	.0
	Overall Percentage				89.1

a. Constant is included in the model.

b. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-2.097	.283	54.838	1	.000	.123

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Age	3.728	1	.053
		Gender(1)	.066	1	.797
		Location(1)	.706	1	.401
	Overall Statistics		4.653	3	.199

Block 1: Method = Enter

Iteration History[a,b,c,d]

Iteration		-2 Log likelihood	Coefficients			
			Constant	Age	Gender(1)	Location(1)
Step 1	1	89.875	-1.837	.009	-.034	-.219
	2	83.967	-2.633	.018	-.081	-.455
	3	83.441	-2.993	.024	-.119	-.588
	4	83.430	-3.058	.025	-.126	-.608
	5	83.430	-3.060	.025	-.126	-.608
	6	83.430	-3.060	.025	-.126	-.608

a. Method: Enter

b. Constant is included in the model.

c. Initial -2 Log Likelihood: 88.373

d. Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	4.943	3	.176
	Block	4.943	3	.176
	Model	4.943	3	.176

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	83.430[a]	.038	.076

a. Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	6.471	8	.595

Contingency Table for Hosmer and Lemeshow Test

		Disease Status = 0		Disease Status = 1		Total
		Observed	Expected	Observed	Expected	
Step 1	1	15	14.489	0	.511	15
	2	14	13.338	0	.662	14
	3	11	12.277	2	.723	13
	4	17	16.747	1	1.253	18
	5	11	10.775	1	1.225	12
	6	8	8.855	2	1.145	10
	7	9	9.103	2	1.897	11
	8	7	8.239	3	1.761	10
	9	12	11.323	2	2.677	14
	10	10	8.854	1	2.146	11

Classification Table[a]

	Observed		Predicted		
			Disease Status		Percentage Correct
			0	1	
Step 1	Disease Status	0	114	0	100.0
		1	14	0	.0
	Overall Percentage				89.1

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age	.025	.013	3.551	1	.050	1.026	.999	1.053
	Gender(1)	-.126	.590	.046	1	.831	.882	.277	2.804
	Location(1)	-.608	.697	.762	1	.383	.544	.139	2.133
	Constant	-3.060	.785	15.173	1	.000	.047		

a. Variable(s) entered on step 1: Age, Gender, Location.