# The Impact of HIV Infection on the Burden and Severity of Influenza Illness in Malawian Adults

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of

**Doctor of Philosophy**

by


## Antonia Ying Wai Ho

BSc, MBChB, MRCP (Infectious Diseases), DTMH, MSc, MRes


**April 2016**

## Declaration

I declare that this thesis was composed by me, and that the work contained therein is my own, except where explicitly stated otherwise. I was not involved in the design of the adult influenza surveillance study, or in the first two years of data collection.

The work within this thesis has not been submitted for any other degree or professional qualification.

## Funding

This study was funded by a Wellcome Trust Clinical PhD fellowship (097464).

## Acknowledgements

This work would not have been possible without the enthusiastic input from a great number of people. First of all, I am extremely grateful to the patients and their guardians who took part in this study, and willingly returning for repeated reviews and nasopharyngeal specimens.

I wish to thank Dr Jane Mallewa, Dr Joep van Oosterhout, Dr Mulinda Nyirenda, Sister Rose Malamba, Sister Molly Lungu. and Christine Hamuza at the Department of Medicine, Adult Emergency Trauma Centre, and HIV services at the Queen Elizabeth Central Hospital (QECH) for their helpful advice and input when I was planning and setting up the project and for their support throughout the study.

Special thanks to my study team: Nyasha Gutukunuhwa, Sitithana Muyaso, Mike Magola, Chimwemwe Mtawali, Blessings Mkwaila, Dan Chunda, Collins Chiliwawa, Hannah Masangwi, Emily Lifa, and Rosaleen N'goma, for their hard work, dedication and for maintaining their enthusiasm throughout this project.

I would also like to thank Naor Bar-Zeev and Ingrid Peterson for their epidemiological input. Thanks also to Marc-Allain Widdowson and Meredith McMorrow, from the Influenza Division of the Centers for Disease Control and Prevention, for their expert advice on influenza surveillance and epidemiology.

I am particularly indebted to the molecular laboratory team for generating all the PCR results for my study, with special thanks extended to Dean Everett, Brigitte Denis, Maaike Alaerts, and Mavis Menyere. Thanks also go to Mavuto Mukaka and Nico Nagelkerke for their statistical advice.

I am grateful to Kate Jones, John Spafford, Carolyn O'Leary and Matt Hanlon from the Wellcome Trust Liverpool Glasgow Centre for Global Health Research for helping me relocate to and from Malawi, and their logistical assistance in all aspects of my study.

The Malawi-Liverpool-Wellcome Trust (MLW) Clinical Research Programme was a stimulating and supporting research environment to study in. I received excellent support from the operational departments at MLW. This study also benefited enormously from the input of fellow students and senior researchers, who challenged and encouraged me in equal measures. I am also grateful to the Wellcome Trust for funding my Clinical PhD Fellowship.

Patients in the case control study were recruited with my co-investigators, Stephen Aston and Hannah Jary (since July 2014). Heartfelt thanks go to them for their invaluable support through the ups and downs of this study, for being my 'work family', and most of all for the great fun we have had in the last three years.

I would like to express my sincere gratitude to my supervisors Professor Neil French and Professor Rob Heyderman. I really appreciate the time they have generously given me, as well as their continuous support, sage advice, motivation, and encouragement to develop as an independent researcher. Their expert guidance has helped me throughout the study and writing of this thesis.

Most of all, I would like to thank my family for their unrelenting support and belief in everything I do. Lastly, I would like to express my deepest love and gratitude to my boyfriend Jamie for his emotional support, encouragement, and patience during the most difficult times of thesis writing.

# Abstract

**Background.** Influenza infection and its complications cause substantial morbidity and mortality worldwide. However, the epidemiology of influenza in sub-Saharan Africa (SSA) is not well known. Furthermore, the effects of HIV infection on influenza burden and severity in adults in a region with high HIV prevalence are also poorly characterised. Although seasonal influenza vaccination is recommended for HIV-infected persons in developed settings, it is not part of routine HIV care in Africa. The primary aim of this thesis was to examine the impact of HIV on the incidence and severity of influenza infection among adults in urban Malawi, to determine whether HIV-infected adults should be prioritised for influenza preventative measures.

**Methods.** At the Queen Elizabeth Central Hospital in Blantyre, Malawi, we characterised the epidemiology, seasonality and risk factors for influenza infection in Malawian adults enrolled in a sentinel surveillance study. Secondly, we established a prospective cohort to compare the incidence of laboratory-confirmed influenza illness between HIV-infected and HIV-uninfected adults over two years. Thirdly, we conducted a case control study of adults with mild (influenza-like illness (ILI)) and severe (hospitalised lower respiratory tract infection (LRTI)) influenza illness to explore risk factors for severe influenza presentation, with HIV as the primary exposure of interest. Lastly, as a potential mechanism through which influenza and other respiratory viral infections predispose to secondary bacterial pneumonia, we investigated the association between respiratory viral infections and nasopharyngeal carriage density of *Streptococcus pneumoniae* in a cross-sectional and case control analysis nested within the prospective cohort.

**Results.** Influenza was a common cause of mild and severe acute respiratory infections in Malawian adults in the hospital-based sentinel surveillance. **P**regnant women were found to have nearly double the risk of hospital-attended influenza infection compared to non-gravid women of childbearing age. In the prospective cohort of 608 adults (59% HIV-reactive), 24/229 (10.5%) ILI episodes in HIV-infected and 5/119 (4.2%) in HIV-uninfected adults were influenza PCR-positive (incidence rates 46.0 vs. 14.5 per 1000 person years). After accounting for potential confounders, HIV-infected adults had an approximately three times higher incidence of influenza infection compared to HIV-uninfected adults. In the case control study, HIV was the single most important risk factor for severe influenza-related presentation; HIV-infected adults experienced a five-fold greater risk of severe influenza disease compared to HIV-uninfected adults, and over 50% of influenza-related hospitalised LRTI were attributable to HIV. Influenza infection was associated with pneumococcal carriage, but respiratory

viral infection was not associated with pneumococcal acquisition or an increase in pneumococcal colonisation density.

**Conclusion.** We have clearly demonstrated a high burden of influenza disease among Malawian adults. Moreover, HIV-infected adults have increased susceptibility and severity of influenza presentations, thus should be considered a priority group for targeted vaccination in the region. Influenza vaccine policies that maximise health benefit through efficient use of limited resources are urgently needed.

# List of Abbreviations

| | |
|---|---|
| AETC | Adult emergency trauma centre |
| AFB | Acid-fast bacilli |
| ARI | Acute respiratory infection |
| ART | Antiretroviral therapy |
| BMI | Body mass index |
| CAP | Community-acquired pneumonia |
| CBA | Childbearing age |
| CDC | Centers for Disease Control and Prevention |
| CFP | Case fatality proportion |
| CFU | Colony-forming unit |
| CI | Confidence interval |
| COM | College of Medicine |
| COMREC | College of Medicine Research Ethics Committee |
| COPD | Chronic obstructive pulmonary disease |
| CRF | Case record form |
| CRT | Cluster-randomised trial |
| Ct | Cycle threshold |
| EPV | Events per variable |
| GAVI | Global Alliance for Vaccines and Immunisation |
| GBS | Guillain-Barré syndrome |
| GDP | Gross domestic product |
| GEE | Generalised estimating equations |
| GISRS | Global Influenza Surveillance and Response System |
| HA | Haemagglutinin |

| | |
|---|---|
| HAI | Haemagglutination inhibition |
| HAS | Health surveillance assistant |
| HDU | High dependency unit |
| Hib | *Haemophilus influenzae* type b |
| HIV | Human immunodefiency virus |
| hMPV | Human metapneumovirus |
| HPAI | Highly pathogenic avian influenza |
| IAP | Indoor air pollution |
| ICR | Intelligent scanning recognition |
| ICU | Intensive care unit |
| IFA | Immunofluorescence assay |
| IHD | Ischaemic heart disease |
| IIV | Inactivated influenza vaccine |
| ILI | Influenza-like illness |
| IMCI | Integrated management of childhood illnesses |
| IPD | Invasive pneumococcal disease |
| IRR | Incidence rate ratio |
| LAIV | Live-attenuated influenza vaccine |
| LRTI | Lower respiratory tract infection |
| LSTM | Liverpool School of Tropical Medicine |
| MDG | Millennium Development Goal |
| MDHS | Malawi Demographic and Health Survey |
| MERS | Middle East respiratory syndrome |
| MEWS | Modified Early Warning Score |
| MGIT | Mycobacteria Growth Indicator Tube |
| MLW | Malawi-Liverpool-Wellcome Trust Clinical Research Programme |

| | |
|---|---|
| MUAC | Mean upper arm circumference |
| NA | Neuraminidase |
| NAAT | Nucleic acid amplification tests |
| NEQAS | National External Quality Assessment Scheme |
| NI | Neuraminidase inhibitor |
| NNV | Number needed to vaccinate |
| NP | Nasopharyngeal |
| OI | Opportunistic infection |
| OP | Oropharyngeal |
| OR | Odds ratio |
| PAF | Population attributable fraction |
| PCR | Polymerase chain reaction |
| PITC | Provider-initiated testing and counselling |
| PIV | Parainfluenza virus |
| PMTCT | Prevention of mother-to-child transmission |
| QECH | Queen Elizabeth Central Hospital |
| RCTs | Randomised controlled trials |
| RDT | Rapid diagnostic test |
| RR | Risk ratio |
| rRT-PCR | Real-time reverse transcriptase polymerase chain reaction |
| RSV | Respiratory syncytial virus |
| SAGE | Strategic Advisory Group of Experts |
| SARI | Severe acute respiratory infection |
| SARS | Severe acute respiratory syndrome |
| SGA | Small for gestational age |
| SPINE | Survellance programme of inpatients and epidemiology |

| | |
|---|---|
| SSA | Sub-Saharan Africa |
| STGG | Skim-milk triptone glucose glycerol |
| TB | Tuberculosis |
| TLR | Toll-like receptor |
| UK | United Kingdom |
| US | United States |
| VCT | Voluntary counselling and testing |
| VE | Vaccine efficacy or effectiveness |
| VPDI | Vaccine preventable disease incidence |
| WHO | World Health Organization |

# Table of Contents

# List of Figures

# List of Tables

# CHAPTER 1. INTRODUCTION

## 1.1 Overview of Influenza

### 1.1.1 Characteristics of influenza viruses

Human influenza viruses are enveloped single-stranded RNA viruses of the Orthomyxoviridae family. It was first isolated from ferrets that had been infected by throat swabs from patients with clinical influenza in 1933 (Smith et al., 1933). There are three types of influenza viruses – A, B and C, classified on the basis of their nucleoprotein (NP) (Figure 1.1). Influenza A and B account for most cases of the disease, and thus will be the focus of this thesis. In contrast, influenza C viruses cause a mild respiratory illness and are not thought to cause epidemics. Influenza A viruses are divided into subtypes based on the antigenic properties of the haemagglutinin (HA) and neuraminidase (NA) surface glycoproteins (figure 1.1) (Lamb and Krug, 2001). To date 18 haemagglutinin and 11 neuraminidase subtypes have been identified (H1-H18 and N1-N11 respectively) (Centers for Disease Control and Prevention, 2014), with H1-H3 and N1-N2 causing the majority of human disease. The predominant circulating strains are currently H1N1 and H3N2.

Influenza A viruses circulate in many animal hosts, such as birds, pigs, horses, and sea mammals. Phylogenetic studies have suggested that aquatic birds are the source of all influenza A viruses in other animals (Webster et al., 1992). All influenza A virus subtypes except H17N10 and H18N11 are found in the digestive and respiratory tracts of aquatic bird populations, but the majority do not produce disease (Centers for Disease Control and Prevention, 2015b). However, a number of avian influenza strains are highly virulent (highly pathogenic avian influenza (HPAI)). Direct avian-to-human transmission of A(H5N1) in Southeast Asia, Africa, and the Middle East, and A(H7N9) in Asia, have been reported (Qin et al., 2015). Although human-to-human transmission is rare (Nguyen et al., 2008), avian influenza is of public health concern as the reported sporadic infections have been associated with severe morbidity and mortality (World Health Organization, 2015a; Qin et al., 2015).

In contrast, influenza B viruses only circulate among humans. They are divided into lineages and strains, rather than subtypes. Two distinct influenza B lineages have co-circulated since the mid-1980s: B/Yamagata and B/Victoria.

The nomenclature for influenza virus isolates follows a World Health Organization (WHO)-accepted convention (1980), and comprises:

i) the influenza virus type (e.g. A,B or C)

ii) the host of origin (for non-human-origin viruses)

iii) Geographical origin

iv) Strain number

v) Year of isolation

vi) For influenza A viruses, the haemagglutinin and neuraminidase variants in brackets

For example: A/Wisconsin/15/2009 (H3N2)

B/Brisbane/9/2014

The unique ability of influenza viruses to cause recurrent epidemics and pandemics is largely due to two types of antigenic variations: minor changes (*antigenic drift*) due to frequent point mutations in the RNA gene segments coding for HA or NA in both influenza A and B viruses, result in new strains that can evade host immune response, thus causing seasonal outbreaks or epidemics. Major changes (*antigenic shift*) occur only in influenza A viruses, either from the genetic reassortment between a human and a non-human virus or from direct transmission of an animal strain to humans, resulting in a novel strain in human population that is immunologically distinct from circulating influenza viruses. Rarely, person-to-person transmission of the novel virus in a large and immunologically susceptible population results in a pandemic.

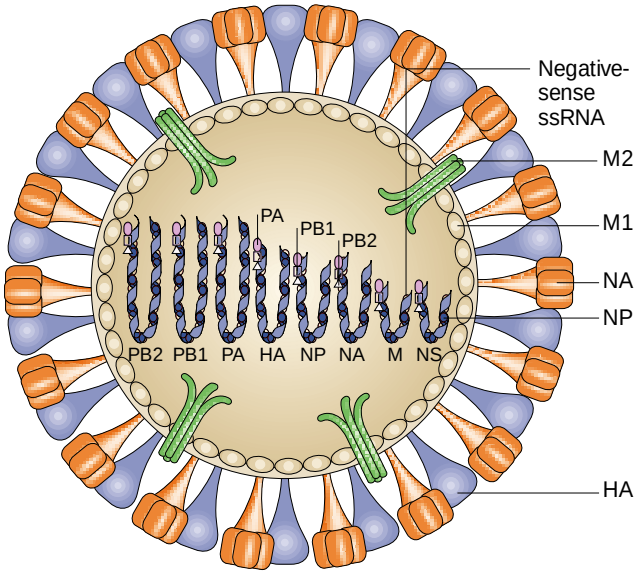



**Figure 1.1 Diagram of an influenza A virion**

An influenza A virion consists of 2 surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), and M2 ion channel (M2) that are embedded in the viral envelope; the ribonucleoprotein complex, which consist of a viral RNA segment associated with the core viral nucleoprotein (NP) and 3 polymerase proteins (PA, PB1, PB2); and the matrix protein (M1), which is associated with both the viral envelope and ribonucleoprotein. Figure reproduced from Horimoto *et al.* (Horimoto and Kawaoka, 2005).

### 1.1.2 Transmission

Influenza virus is highly infectious, associated with a basic reproduction number[i] ($R_0$) of 1.6-3.0 (Truscott et al., 2012). The main mode of transmission is through large respiratory droplets (>5μm) at close distance (Brankston et al., 2007), though influenza may spread through aerosolised particles expelled through sneezing or coughing (Tellier, 2009; Cowling et al., 2013) or direct contact with respiratory secretions through contaminated fomites (Morens and Rash, 1995). The typical incubation period is 2 days (range 1-4 days) (Cox and Subbarao, 1999).

The risk of influenza transmission varies according to the setting. Increased mixing and contact among individuals, poor hygiene and individual factors such as susceptibility to infection and immunity from previous exposure may contribute to transmission (World Health Organization, 2010a). Influenza transmission has been evaluated in households, schools, hospitals, and confined environments such as nursing homes and military facilities (World Health Organization, 2010a). In observational studies of influenza transmission within households, the secondary attack rate (i.e. the probability that infection occurs among susceptible individuals after known contact with an infectious contact or source (Halloran, 2005)) ranged from 13 to 32% (Longini et al., 1982; Viboud et al., 2004; Hall et al., 1973; Philip et al., 1961) for seasonal influenza, and 3 to 38% during the 2009 influenza A(H1N1) pandemic (Lau et al., 2012). The countries, setting and study design varied substantially among these studies. Increased risk of transmission have been observed in young children (Casado et al., 2014; Petrie et al., 2013; Viboud et al., 2004), contacts of infected young children (Viboud et al., 2004), and high number of susceptible occupants in the household (Longini et al., 1982). In a household cohort study in Vietnam, 25% (95% CI 21-30%) of influenza infection was thought to be attributable to household transmission (Cauchemez et al., 2014). Since children have a much higher attack rates than adults (World Health Organization, 2014c), schools are thought to be important settings for the spread of influenza. A Taiwanese study found that the $R_0$ (the average number of secondary cases that result from one infected person) in schools were between 2.8 (aged 25-45 years) and 16.9 (aged 4-6 years), compared to an $R_0$ of 1.2 to 2.4 in the community (Chen and Liao, 2008). Closed environments are associated with some of the highest rates of influenza transmission; attack rates of >40% have been reported in boarding schools (Smith et al., 2009; Rajatonirina et al., 2011), military facilities (Earhart et al., 2001), and nursing homes (Patriarca et al., 1987; Morens and Rash, 1995). Lastly, influenza outbreaks due to nosocomial transmission between healthcare workers and hospitalized patients have been reported (Balkovic et al., 1980; Horcajada et al., 2003; Taylor et al., 2014), thus highlighting the

[i] The number of secondary cases which one case would produce in a totally susceptible population [Dietz K. (1993) The estimation of the basic reproduction number for infectious diseases. *Stat Methods Med Res* 2: 23-41.

importance of compliance with infection control measures such as handwashing and use of face masks, in addition to vaccination in healthcare workers.

### 1.1.3 Clinical Presentation

In adults, influenza infection is characterised by sudden onset of fever, myalgia, malaise, headache, respiratory tract symptoms such as cough, sore throat, and rhinorrhoea, and occasionally vomiting and diarrhoea. In most people, it manifests as a self-limiting illness that resolves within a week. However, influenza can cause severe illness or death, particularly in certain at-risk populations (see Section 1.1.4.2). Pneumonia is by far the commonest complication, which can be categorised into primary viral pneumonia, secondary bacterial pneumonia, or mixed viral/bacterial pneumonia (Chertow and Memoli, 2013; Metersky et al., 2012) (see also Chapter 6). *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Streptococcus pyogenes* are the most frequently identified bacterial co-pathogens (2009a; Martin-Loeches et al., 2011; Rice et al., 2012). Concomitant or subsequent bacterial infection causes a substantial proportion of influenza-related morbidity and mortality (Morens et al., 2008). Rarely, influenza has been associated with neurological (encephalitis (Santini et al., 2012; Cunha et al., 2012), transverse myelitis (Salonen et al., 1997), Guillain-Barre syndrome (Vellozzi et al., 2014), and Parkinsonism (Toovey et al., 2011)), musculoskeletal (e.g. myositis (Oba et al., 2000), rhabdomyolysis (Ayala et al., 2009)) and cardiac (e.g. myocarditis, pericarditis) (Estabragh and Mamas, 2013) complications. Moreover, influenza can exacerbate existing chronic medical conditions, such as chronic obstructive pulmonary disease (COPD) (De Serres et al., 2009; Mohan et al., 2010) or ischaemic heart disease (Lichenstein et al., 2012; Warren-Gash et al., 2011).

### 1.1.4 Epidemiology of influenza in developed settings

#### *1.1.4.1 Characteristics of influenza outbreaks*

In temperate regions, distinct outbreaks occur predominantly in the winter months (Figure 1.2). The seasonality of influenza is less well defined in tropical and subtropical regions; influenza activity may occur throughout the year (Chapter 1.2) (Azziz Baumgartner et al., 2012). However, even in regions where annual winter epidemics occur, the timing, severity and distribution of viruses are highly variable. A myriad of factors are likely to contribute to this variability; these are summarized in Figure 1.3.

The WHO Global Influenza Surveillance and Response System (GISRS) is a network consisting of six WHO Collaborating Centres for Reference and Research on Influenza (US x2, UK, China, Japan, and Australia), in addition to 142 institutions from 112 countries (World Health Organization, 2014 ). GISRS

monitors the evolution of influenza viruses through collated surveillance data, and provides recommendations on vaccine composition twice yearly. Additionally, it acts as an alert mechanism for the emergence of influenza viruses with pandemic potential. The WHO estimates annual attack rates at 5–10% in adults and 20-30% in children (World Health Organization, 2014c), and annual epidemics are thought to result in around 3 to 5 million cases of severe illness, and around 250 000 to 500 000 deaths worldwide (World Health Organization, 2014c).

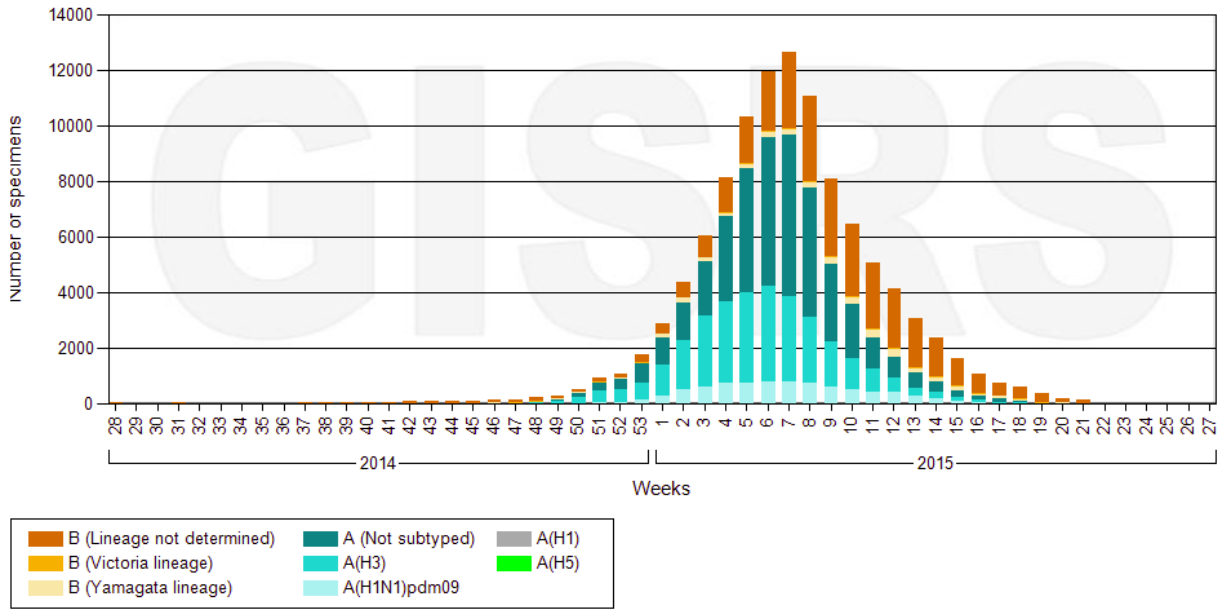



**Figure 1.2 Influenza subtypes detected in the 2014-2015 influenza season**

From the WHO European Region, Global Influenza Surveillance and Response System (GISRS) (**http://www.who.int/influenza/gisrs_laboratory/updates/summaryreport/en/**). Note multiple influenza strains typically circulate concurrently in an influenza season.

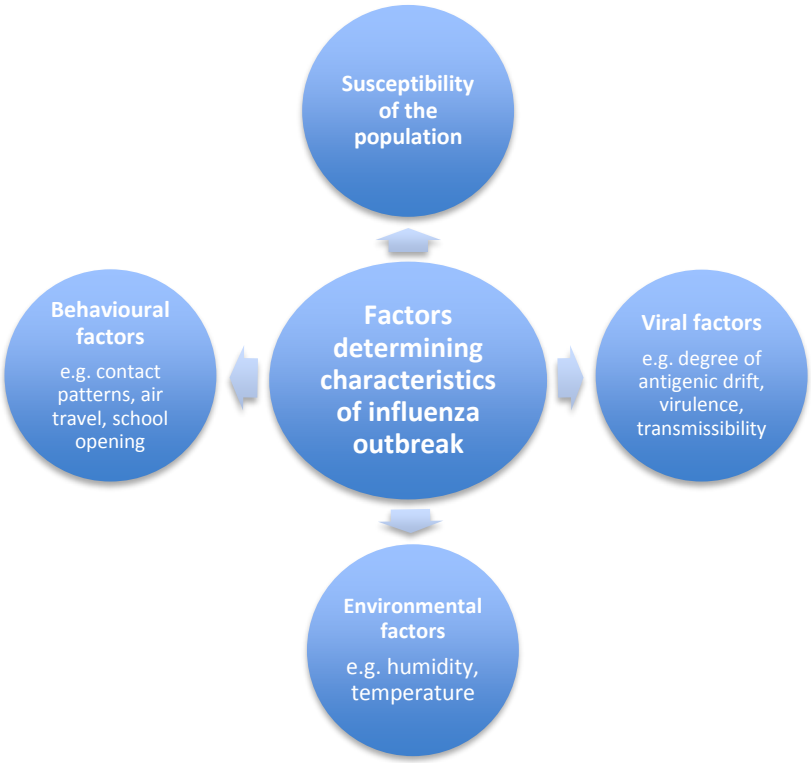



**Figure 1.3 Factors that determine the extent and severity of influenza outbreaks**

#### *1.1.4.2 Risk groups for influenza*

Risk groups for influenza include individuals at increased risk of exposure, e.g. healthcare (Weber et al., 2010) or childcare workers (Cordell et al., 2004), in addition to those at higher risk of severe disease (e.g. hospitalisation or death), or complications. The latter include children < 5years (Poehling et al., 2006; Ampofo et al., 2006; Coffin et al., 2007; Zhou et al., 2012b; Chaves et al., 2014), the elderly (≥65 years) (Zhou et al., 2012b; Matias et al., 2014; Thompson et al., 2003; Quandelacy et al., 2014; Van Kerkhove et al., 2011b), pregnant women (Neuzil et al., 1998; Dodds et al., 2007; Mosby et al., 2011) and those with underlying medical conditions, including chronic respiratory disease, significant cardiovascular disease, chronic renal or liver disease, diabetes mellitus and immunodeficiency (Nicoll et al., 2008; Pebody et al., 2010; Quandelacy et al., 2014; Van Kerkhove et al., 2011b; Kunisaki and Janoff, 2009; Memoli et al., 2014). In many developed settings, influenza vaccines (see Section 1.1.5) are targeted at these vulnerable populations (England, 2013; Nokleby and Nicoll, 2010). In the US, the influenza vaccination policy has expanded to include all individuals aged 6 months and over since 2010 (Fiore et al., 2010). In 2012, the World Health Organization (WHO) Strategic Advisory Group of Experts on Immunisation (SAGE) designated pregnant women as the highest priority group (2012).

#### *1.1.4.3 Previous pandemics*

As discussed in Section 1.1.2, antigenic shift in an influenza A virus may give rise to a pandemic. Pandemics are characterised by the rapid dissemination of a new influenza A subtype, to which human populations have minimal or no immunity, has capacity for human-to-human transmission and causes serious disease (2012; Doshi, 2011). The first recognized influenza pandemic was in 1510 (Morens et al., 2010), thought to have originated from Asia, then rapidly spread to Africa and along trade routes to Europe. A first-hand account described "a 'gasping oppression' with cough, fever, and a sensation of constriction of the heart and lungs began to rage, seemingly everywhere at once" (Fernal, 2005). Major pandemics have occurred sporadically every 10-50 years since then (Morens et al., 2010). Over the past century, the most severe and extensive pandemic of 1918-19 was associated with up to 50 million deaths (Patterson and Pyle, 1991; Johnson and Mueller, 2002). Less severe pandemics occurred in 1957, 1968, and most recently in 2009 (Table 1.1).

The first reports of the influenza activity in 1918 came from Spanish wire news services, largely due to media blackout in allied countries and Germany during the First World War. As a result, the virus was erroneously believed to be the origin of the pandemic (hence "Spanish flu") (Trilla et al., 2008). This pandemic was unique in many respects: firstly, case fatality rate was very high (>2.5% vs. <0.1% in other pandemics) (Marks and Beatty, 1976); one of the most striking aspects was the unusually high mortality in adults aged 20 to 40 years (~1000 per 100,000 persons), in addition to infants and the elderly ('W'-shaped mortality curve) (Taubenberger and Morens, 2006). Secondly, most deaths were due to secondary bacterial pneumonia caused by common respiratory tract organisms, such as *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus* (see also Chapter 1.6.2.1) (1920; Jordan, 1923). Thirdly, three distinct waves of influenza activity occurred between 1918 and 1919; the second of which caused widespread disease and high mortality rates (Taubenberger and Morens, 2006). Fourthly, most pandemics developed in Asia and spread to the rest of the world (Taubenberger and Morens, 2006), but the geographical origin of the 1918 virus remains unknown (Reid et al., 2004). Finally, recently published viral sequencing data suggest that the 1918 virus was derived from an avian-like influenza virus that was novel to humans, and not produced by reassortment from an existing strain that acquired new genes (Reid et al., 2004; Taubenberger et al., 2005). To date, it remains unclear why the 1918 influenza pandemic was so fatal (Morens and Taubenberger, 2012).

**Table 1.1 Influenza pandemics over the past century**

| Time period | Name | Subtype | How strain was derived | Reproduction number (R) (Biggerstaff et al., 2014) Median (IQR) | Numbers of Deaths |
|---|---|---|---|---|---|
| 1918-19 | Spanish flu | H1N1 | An avian virus with HA(H1) that adapted to humans? (Taubenberger et al., 2005) | 1.80 (1.47-2.27) | ~50 million |
| 1957-58 | Asian flu | H2N2 | Reassortment of an avian HA(H2), NA(N2), and PB1 viral segments + human viral genes (Subbe et al., 2006) | 1.65 (1.53-1.70) | ~1-2million |
| 1968-70 | Hong Kong flu | H3N2 | Reassortment of avian HA(H3) + human viral genes (Subbe et al., 2006) | 1.47 (1.30-1.70) | ~ 1 million |
| 2009-10 | Swine flu | H1N1 | Triple reassortment of human, swine, and avian viral genes (Garten et al., 2009) | 1.28 (1.19-1.37) | ~284,000 |

HA, haemagglutinin; NA, neuraminidase

#### *1.1.4.4 Influenza surveillance*

Influenza activity is monitored globally by surveillance programmes, either in defined populations or in a limited number of sentinel sites. It involves systematically testing individuals that present to healthcare facilities with mild and severe acute respiratory symptoms for influenza (± other respiratory viruses). The purpose of surveillance is to estimate disease burden, describe influenza seasonality, determine circulating strains, monitor changes in antigenicity of viruses, guide the selection of strains for the annual influenza vaccine, as well as providing candidate viruses for vaccine production (World Health Organization, 2013b). The following standardised case definitions, as recommended by the WHO and the United States Centers for Disease Control and Prevention (CDC), allow comparisons between surveillance sites and countries (Pan American Health Organization & Centers for Disease Control and Prevention (PAHO-CDC), 2006):

1) **Influenza-like illness (ILI)**
    - Sudden onset fever >38°c AND
    - Cough OR sore throat
    - Onset with 7 days
2) **Severe acute respiratory infection (SARI)**

    *Age <5 years:*
    - Clinically suspected of having pneumonia or severe/very severe pneumonia

- Requiring hospital admission
- Onset within 7 days

(adapted from the programme for Integrated Management of Childhood illness (IMCI) (World Health Organization))

*Age ≥5 years:*

- Sudden onset fever >38°c AND
- Cough OR sore throat AND
- Shortness of breath OR difficulty breathing AND
- Requiring hospital admission

ILI surveillance monitors persons seeking care in outpatient facilities, whereas SARI surveillance captures persons with more severe illness that may require hospitalisation. As described in Section 1.1.3, influenza causes a non-specific febrile illness that is difficult to distinguish from other respiratory infections. Clinical case definitions have to achieve a balance between sensitivity and specificity; a more sensitive definition will capture a larger proportion of all cases, but at the expense of testing a large number of cases caused by pathogens other than influenza. Conversely, a more specific definition will improve accuracy in capturing cases, but may miss a larger proportion of the total. The ILI and SARI definitions above demonstrated low sensitivity and specificity for influenza (Murray et al., 2013). Following a global technical consultation by the WHO in 2011, sore throat was removed as a qualifying symptom, as several studies have suggested that it is negatively associated with influenza. In 2013, the case definitions were revised to (World Health Organization, 2013b):

1) **ILI**

   **An acute respiratory infection with:**

   - Measured fever ≥38°c AND
   - Cough
   - Onset within 10 days

2) **SARI**

   **An acute respiratory infection with:**

   - Measured fever ≥38°c AND
   - Cough
   - Onset within 10 days
   - Requires hospitalization

### 1.1.5 Influenza vaccination in adults

#### *1.1.5.1 Types of influenza vaccines*

Vaccination is the most effective way to prevent influenza disease and/or severe outcomes (World Health Organization, 2014c). Safe and effective influenza vaccines are available, in the form of inactivated influenza vaccine (IIV) and live-attenuated vaccines (LAIV). There are three types of inactivated vaccines: whole cell, split virus and subunit vaccines. 'Split virus' vaccines are prepared by disrupting whole viruses with organic solvents or detergents; 'subunit' vaccines contain highly purified HA and NA antigens prepared from disrupted virus particles. The purified viruses are then grown in chick embryos, chemically inactivated and further purified to produce the vaccine. IIV can be given as a single dose by intramuscular or intradermal injection to individuals aged 6 months or over (England, 2013).

LAIV is prepared using cold-adapted, live attenuated viruses from which reassortants with vaccine strain HA and NA antigens are generated. Also grown in embryonated hens' eggs, LAIV is given intranasally to healthy children aged 2 to <17 years in the UK (Public Health England, 2015) (2 to 49 years In the US (Grohskopf et al., 2015)). The decision for the preferential use of LAIV in children is based on superior efficacy of LAIV compared to IIV3 in young children <6 years (Belshe et al., 2007), with weaker evidence of superior efficacy in older children (Fleming et al., 2006). On the contrary, most comparative studies in adults have demonstrated either that LAIV and IIV were of similar efficacy (Treanor et al., 1999; Forrest et al., 2011) or that IIV was more efficacious (Ohmit et al., 2006; Ohmit et al., 2008; Monto et al., 2009).

Current vaccines are trivalent (IIV3) or quadrivalent (IIV4) – the trivalent vaccine contains two strains of influenza A (A/H3N2 and A/H1N1) and one influenza B strain; the quadrivalent preparation contain an additional B virus (both B/Victoria and B/Yamagata lineages). The latter has been licenced in the UK since April 2013 (England, 2013).

Due to antigenic drift, influenza vaccine formulation is updated twice-yearly (for the Northern and Southern Hemisphere) using virus strains predicted to circulate in the forthcoming winter from the global epidemiological surveillance (Grohskopf et al., 2015). Vaccine production takes around six months. Mismatches between vaccine strains and circulating strains occur occasionally, resulting in reduced efficacy (Bridges et al., 2000; Flannery et al., 2015), though a recent systematic review suggested that both IIV and LAIV can provide a degree of cross protection (vaccine efficacy (VE) 42-75%) against non-matching circulating strains (Tricco et al., 2013).

#### *1.1.5.2 Safety of influenza vaccines*

IIVs are considered safe in healthy adults (Vellozzi et al., 2009). Local injection site reactions are frequently reported, but systemic side effects such as fever, malaise and myalgia are uncommon (Jefferson et al., 2014a). A minor increase in the risk of Guillain-Barré syndrome (GBS) has been reported in older adults (estimated at 1 to 1.6 additional case per million persons vaccinated) (Juurlink et al., 2006; Salmon et al., 2013). IIVs have also been found to be safe in pregnancy; no study to date has demonstrated an increased risk of maternal complications, or adverse foetal outcomes (Englund et al., 1993; Zaman et al., 2008; Naleway et al., 2014; Tamma et al., 2009). IIVs are contraindicated in individuals who have had a severe allergic reaction to eggs or a previous influenza vaccine, or GBS within 6 weeks of receiving an influenza vaccine (England, 2013).

LAIV have been associated with mild transient upper respiratory tract symptoms, but no serious adverse events (Rudenko et al., 1996; Jefferson et al., 2014a). This vaccine is not recommended for pregnant women or adults with immunosuppression (England, 2013; Grohskopf et al., 2015), but is licensed for children aged 2-17 years with stable HIV infection receiving antiretroviral therapy (ART) in the UK (England, 2013; Children's HIV Association, 2015).

#### *1.1.5.3 Vaccine efficacy in healthy adults*

Previous studies of influenza vaccines in healthy adults have estimated vaccine efficacy at between 70 and 90% (Fiore et al., 2008). However, a comprehensive review in 2014 suggested that efficacy may be much lower (Jefferson et al., 2014a). The meta-analysis of randomised trials and observational studies found that IIV3 provided around 60% (95% confidence interval (CI) 53-66%] protection against virologically-proven influenza infection, with a number needed to vaccinate[ii] (NNV) of 71 (95%CI 65-80) (Jefferson et al., 2014a). The VE was similar when there was mismatch between the vaccine strains and circulating viruses (VE 55% (95% CI 41-66%), NNV 60 (95%CI 50-80) (Jefferson et al., 2014a). IIV4s are as effective as IIV3 based on non-inferior immunogenicity for the three shared antigens (Beran et al., 2013; Greenberg et al., 2013; Kieninger et al., 2013; Pepin et al., 2013; Tinoco et al., 2014). Additional benefit through protection against the additional influenza B strain has also been demonstrated (Beran et al., 2013; Kieninger et al., 2013; Pepin et al., 2013; Tinoco et al., 2014). However, the magnitude of this additional benefit is dependent on the circulating influenza B strain and the degree of cross protection between the B strains.

Immune responses are often attenuated in the elderly and those with underlying conditions. However, vaccination can still protect against severe disease, complications such as bronchopneumonia, hospital admission and death (Mangtani et al., 2004; Baxter et al., 2010; Puig-Barbera et al., 2012; Ridenhour

[ii] This expresses the number of adults needed to be vaccinated to prevent one case of influenza.

et al., 2013; Kwong et al., 2013). As for LAIV, overall efficacy of 53% (95% CI 32-54%) has been reported, corresponding to a NNV of 39 (95%CI 32-54) (Jefferson et al., 2014a). As stated above, although LAIV appears to have better clinical efficacy than IIIV in children (Ambrose et al., 2008), studies in adults have shown that IIV is equivalent or more effective than LAIV (Edwards et al., 1994; Ohmit et al., 2006; Monto et al., 2009; Wang et al., 2009; Phillips et al., 2013).

### 1.1.6 Treatment of seasonal influenza in healthy adults

There are 2 classes of antiviral drugs for influenza: M2 protein inhibitors (amantadine, rimantadine), which are only active against influenza A, and neuraminidase inhibitors (NIs) (oral oseltamivir and inhaled zanamivir), which are active against influenza A and B. NIs are recommended as first-line antiviral treatment (World Health Organization, 2010d), as most of the currently circulating influenza A viruses are resistant to M2 inhibitors (2010).

Oseltamivir given as post-exposure prophylaxis has been shown to be ~55% effective (95%CI 33-70%) in preventing symptomatic influenza, but had no effect on hospitalisation (Jefferson et al., 2014b). However, recent reviews on the effects of NI used as treatment on symptom duration and outcomes are conflicting. An updated Cochrane review of 46 randomised controlled trials (RCTs) challenged the value of antiviral use for influenza prevention and treatment as it reported only a modest reduction of illness duration (0.7 and 0.6 days with oseltamivir and zanamivir respectively), no effect on hospitalisation (relative risk 0.92, 95%CI 0.57-1.50), but an increased risk of gastrointestinal symptoms (Jefferson et al., 2014b). A more recent meta-analysis of RCTs (n=9) on oseltamivir use in adults with Influenza-like illness (ILI) demonstrated a similar reduction in symptom duration in those who received oseltamivir, but noted fewer lower respiratory tract complications requiring antibiotics (risk ratio (RR) 0.56, 95%CI 0.52-0.75) and hospitalisations (RR 0.37, 95%CI 0.17-0.81) (Dobson et al., 2015). A separate meta-analysis of 78 studies on >29,000 hospitalised patients with 2009 H1N1 influenza infection reported 19% reduction of death in adults treated with NI at any time in hospital with influenza (adjusted odds ratio (aOR) 0.81, 95%CI 0.70-0.93), and if given within 48 hours of symptom onset, halved the risk of death compared to no treatment (aOR 0.50, 95%CI 0.37-0.67) (Muthuri et al., 2014).

There are numerous methodological reasons for the observed differences. Firstly, the Cochrane Review included studies that were statistically underpowered and were not designed to assess the effects of antivirals on severe influenza outcomes. On the other hand, the latter two studies were industry-funded, which may have introduced bias in study selection. Although Dobson *et al*. (Dobson et al., 2015) utilised individual patient data from all trials rather than aggregated study results, which

is the preferred approach for a meta-analysis (Lyman and Kuderer, 2005), only Roche-funded RCTs were included. With regards to the meta-analysis by Munthuri *et al*. (Muthuri et al., 2014), concerns have been raised regarding the inadequate handling of time-dependent bias (e.g. timing of treatment in relation to death, time between illness onset and presentation to care, and time between hospital admission and treatment initiation); the small proportion (19%) of available data included; and the lack of quality assessment of data (Kmietowicz, 2014; Jones et al., 2014; Antes and Meerpohl, 2014). At present, NIs are recommended in individuals with severe disease (e.g. requiring hospitalisation) or those at risk of complications (defined in Section 1.1.4.2, in addition to morbidly obese individuals) (World Health Organization, 2010d; National Institute for Health and Care Excellence, 2009).

Oseltamivir can cause mild nausea and vomiting, and occasionally psychiatric and renal side effects. Bronchospasm and decline in respiratory function have been reported with zanamivir use in patients with asthma and COPD, hence it is contraindicated in this group.

A promising agent on the horizon is nitazoxanide. Currently used as an anti-parasitic agent, it appears to have anti-influenza activity by blocking the maturation of viral HA at the post-translational level (Rossignol et al., 2009). In a recent randomized Phase 2b/3 trial, nitazoxanide was associated with a significant reduction in the median duration of symptom from 117 to 96 hours in patients with acute uncomplicated influenza (Haffizulla et al., 2014).

## 1.2 The Epidemiology of Influenza in sub-Saharan Africa

As evident from Chapter 1.1, the substantial contribution of influenza to morbidity and mortality in the developed world is well described (World Health Organization, 2014c). In contrast, the epidemiology and burden of influenza in sub-Saharan Africa (SSA) are less well characterized. Lack of awareness of the disease, similarity of clinical presentation with other febrile illnesses such as malaria and bacterial pneumonia, inadequate diagnostic capacity, and prioritisation towards other high-burden public health problems are likely contributory factors. As a result, the lack of epidemiological data was often interpreted as an absence of influenza disease (Yazdanbakhsh and Kremsner, 2009). However, since ~920 million people reside in SSA (Population Reference Bureau, 2014), and many countries in the region have high prevalence of co-morbidities, such as HIV, malaria and tuberculosis (TB), in addition to other factors common to low-resource settings, e.g. malnutrition and scarce health resources (Schoub, 2010), the illness burden is likely to be far greater than assumed.

### 1.2.1 The impact of 1918 and 1968 influenza pandemics on SSA

Published evidence suggests that the 1918 A(H1N1) and 1968 A(H3N2) pandemics had a significant impact on SSA. Sub-Saharan Africa missed the mild initial wave of the 1918 pandemic influenza A(H1N1) (Patterson, 1981). However, the severe second wave arrived in Freetown, Sierra Leone by an English ship, and also in East African coastal cities (Mombasa and Beira) by ships from Asia between August and September 1918, and rapidly spread from these locations through the continent via ship, river boat and railway (Patterson and Pyle, 1983). The rapidity of spread was attributed to wartime mobilisation of soldiers and labourers, and more importantly, the newly created colonial railway network (Patterson and Pyle, 1983). A death toll of 2.2 million in SSA was estimated, with a corresponding mortality rate of 23 per 1000 population (Johnson and Mueller, 2002), which is substantially higher than the global rate of 2.5 to 5 per 1000 population. This data also support the belief that the pandemic did not originate in Africa, since East and West Africa were infected separately, and the disease appeared to have been introduced at major ports (Patterson and Pyle, 1983).

A systematic review of published articles on human infection with the 1968 influenza A(H3N2) pandemic also reported high level of morbidity in all African regions (Ortiz et al., 2012). Community attack rates of ~20% were reported, comparable to studies from North America, Europe and Australia (Jackson et al., 2010). 22–64% of clinic patients and 8–72% of hospitalized patients tested by viral culture or serology had evidence of 1968 H3N2 virus infection (Ortiz et al., 2012).

### 1.2.2 Seasonal influenza epidemiology of SSA prior to 2009 Influenza A(H1N1) pandemic

A systematic review of seasonal influenza epidemiology in SSA illustrated the scarcity of published data in the region (Gessner et al., 2011). It included 49 studies from 14 countries between 1980 and 2009; the main findings are summarised in Table 1.2. Over half of the studies were from South Africa and Madagascar; both countries have served as WHO National Influenza Centres for over 30 years (McAnerney et al., 2012; Orelle et al., 2012). Seasonality trends, particularly outwith the southern African region, remain unclear. Eight studies described seasonal influenza outbreaks, several of which reported alarmingly high case fatality rates (1.5-2.5%) (2003; 2002). Influenza diagnosis was made by viral culture, serology or immunofluorescence assay (IFA) by most studies; only two used molecular tests, such as polymerase chain reaction (PCR) (2003; Besselaar et al., 2004), which have superior sensitivity and are now considered the gold standard for respiratory viral diagnosis (Mahony, 2010). The review concluded that most countries in SSA had insufficient data to develop rational strategies for influenza control. Additionally, a number of important data gaps were highlighted, including influenza incidence and mortality; seasonality over a number of years; its contribution to adults hospitalised with ARI; interaction of influenza with malaria, tuberculosis and malnutrition; school and workplace absenteeism related to influenza; vaccine interventions; and economic burden of influenza (Gessner et al., 2011). Of note, a WHO-led consultation on the 'Public Health Research Needs for Influenza in Africa' in 2012 identified similar research objectives to the review authors (World Health Organization, 2012a).

### 1.2.3 The impact of 2009 Influenza A(H1N1) pandemic on SSA

During the initial months of the pandemic, a number of reports emerged from South Africa (Archer et al., 2009; Koegelenberg et al., 2010) and Madagascar (Rajatonirina et al., 2011; Rajatonirina et al., 2012a; Rajatonirina et al., 2012b; Orelle et al., 2012), where the first imported cases of influenza A(H1N1)pdm09 were described on 14 June 2009 and 12 August 2009, respectively.

In South Africa, 12,331 cases and 91 deaths were reported in the first 3 months of the pandemic (Archer et al., 2009). Amongst the deceased cases, the most common underlying conditions included HIV infection (17/32, 53%), pregnancy (25/45, 56% women of reproductive age), and active tuberculosis (TB) (7/72, 10%). Fatal cases had a significantly higher median age compared to non-fatal cases (34 vs. 15 years, $p<0.01$). A case series of 19 intensive care unit (ICU) admissions in Cape Town with confirmed influenza A(H1N1)pdm09 also found a high prevalence of risk factors for severe disease, including pregnancy (n = 6), type 2 diabetes mellitus (n = 6), obesity (n = 4), HIV infection (n = 3), immunosuppressive therapy (n = 3) and active pulmonary TB (n = 2) (Koegelenberg et al., 2010). 13

**Table 1.2 Summary of studies included in the systematic review of seasonal influenza epidemiology in SSA - 1980-2009** (Gessner et al., 2011)

| Characteristic | Studies (n) | Countries (ref) | Findings/Comments |
|---|---|---|---|
| Seasonality | 11 | Kenya (Hazlett et al., 1988)<br>Madagascar (Ravaoarinoro et al., 1982; Morvan et al., 1990; Rakoto-Andrianarivelo et al., 1993)<br>Nigeria (Olaleye et al., 1989)<br>Senegal (Dosseh et al., 2000)<br>South Africa (McAnerney et al., 1994; Karstaedt et al., 2009)<br>The Gambia (Forgie et al., 1992; Mulholland et al., 1999)<br>Zambia (Mizuta et al., 1997) | - Strong seasonal trend seen in Southern African countries (South Africa, Zambia and Madagascar) in cool, dry months [June to August], but year-round activity in Senegal.<br>- 3 studies had <20 influenza-positive cases. |
| Outbreak | 8 | DRC (2003)<br>Madagascar (Clerc et al., 1980; Cassel-Beraud et al., 1988; 2002; Soares et al., 2003)<br>Reunion island (Lassalle et al., 1998)<br>South Africa (Besselaar et al., 2004)<br>Zambia (Mizuta et al., 1995) | - 2 studies were in schoolchildren (Besselaar et al., 2004; Mizuta et al., 1995); the others were of all ages.<br>- Proportion of samples influenza-positive 6 to 95% (mean 41%; median 28%).<br>- High case fatality rates (CFR) were reported in DRC (1.5%) (2003) and Madagascar (2.5%) (2002). |
| Proportion of outpatient respiratory illness associated with confirmed influenza (all ages) | 12 | Cote D'Ivoire (Nichol et al., 1995)<br>Kenya (Gachara et al., 2006)<br>Madagascar (Ravaoarinoro et al., 1982; Morvan et al., 1990; Rakoto-Andrianarivelo et al., 1993; United Nations Development Programme, 2014)<br>Nigeria (Olaleye et al., 1989)<br>Senegal (Dosseh and Rogier, 1996; Dosseh et al., 2000)<br>South Africa (Schoub et al., 1986; McAnerney et al., 1994) | - 1-25% patients with ARI were influenza positive (mean 9.5%; median 10%).<br>- Surveillance period <12 months (n=3). |

| | | | |
|---|---|---|---|
| | | Zambia (Mizuta et al., 1997) | |
| Proportion of inpatient adult respiratory illness associated with confirmed influenza | 2 | Ethiopia (Aderaye, 1994)<br>Kenya (Scott et al., 2000) | - 7/104 (7%) (Aderaye, 1994) and 14/281 (5%) (Scott et al., 2000) were positive for influenza. |
| Incidence (children only) | 1 | South Africa (Madhi et al., 2000) | - Influenza-associated hospitalisation 1268/100,000 in HIV-infected and 148/100,000 in HIV-uninfected children <2 years. |

of the 19 patients died. Another case series of ICU admissions with A(H1N1) in Reunion Island between 2009 and 2010 also reported a high prevalence of co-morbidities (22/25, 88%) and mortality (12/25, 49%) (Gauzere et al., 2011). In Madagascar, 20% more deaths than expected were seen following the first wave of 2009 A(H1N1) pandemic, particularly from respiratory disease, with excess mortality in those aged >50 years (Rajatonirina et al., 2012b).

As expected, very few laboratory-confirmed deaths due to influenza A(H1N1)pdm09 were reported to the WHO from the African region (n=168, April 2009 - August 2010) (World Health Organization, 2010b). However, a modelling study of global mortality associated with the first 12 months of the pandemic estimated that 29% of the 201,200 respiratory deaths occurred in the region. The estimated mortality rate in the region (~7 per 100,000 population) was around 2 to 4 times higher than other regions (1.7-3.0 per 100,000) (Dawood et al., 2012).

On the milder end of the disease spectrum, sero-epidemiological studies on influenza A(H1N1)pdm09 were performed on household cohorts in the Reunion Island (Dellagi et al., 2011), Mali (Koita et al., 2012) and Djibouti (Andayi et al., 2014), as part of the Cohorts for Pandemic Influenza (CoPanFlu) programme (Table 1.3). Seroprevalence was high across all three studies (27-73%). Dellagi et al. (Dellagi et al., 2011) reported a 3-fold higher seroincidence of A(H1N1)pdm09 infection than that estimated from clinical surveillance in the Reunion island cohort, indicating that around two thirds of infections in the community escaped medical detection.

In all age groups, pre-pandemic HAI titres of $\geq$1/40 prevented seroconversion, and likely protected against infection. In all three studies, individuals aged <20 years were the most affected; the highest seroconversion rates were observed in this age group.

**Table 1.3 Sero-epidemiological studies in SSA during the 2009 influenza A(H1N1) pandemic**

| Study, year | Country | Study period | Study population | Seroprevalence HAI > 1:40 | Seroconversion[a] |
|---|---|---|---|---|---|
| Dellagi et al., 2011 | Reunion Island | July-Dec 2009 | 1687 individuals, 772 households | <20yrs 29.8%[b]<br>20-59yrs 35.6% [b]<br>>60yrs 73.3% [b] | <20yrs 63.2%<br>20-59 39.4%<br>>60yrs 16.7% |
| Koita et al., 2012 | Rural Mali | | 202 individuals | 4-19yrs 31.0%<br>20-53yrs 27.8% | 4-19yrs 18.4%<br>20-53yrs 12.2% |
| Andayi et al., 2014 | Djibouti | Oct 2010-Feb 2011 | 1045 individuals, 324 households | <25yrs 35.9%<br>>65yrs 29.5% | |

HAI, haemagglutination inhibition assay

[a]Defined as seronegative (HAI <1:40) to seropositive, or a 4-fold increase in HI titre between first and second paired serum sample, if first sample was seropositive ((HAI ≥1:40)

[b]Serum sample obtained within the first two weeks of A(H1N1)pdm09 activity on Reunion Island, therefore represents baseline seroprevalence

### 1.2.4 SSA since the 2009 Influenza A(H1N1) pandemic

#### *1.2.4.1 Influenza surveillance: 2010-2015*

The emergence of the influenza A(H1N1) pandemic in 2009, and to an extent the highly pathogenic avian influenza A(H5N1) in 2003 (World Health Organization, 2014a), led international organisations, such as the Centers for Disease Control and Prevention (CDC), WHO, and Institut Pasteur, to galvanise efforts with local health authorities and governments to strengthen epidemiological and laboratory surveillance capacity for influenza and other respiratory viruses in SSA (World Health Organization, 2012a; Steffen et al., 2011; World Health Organization, 2009c; Steffen et al., 2012). As of 2015, 22 of the 47 countries in SSA contribute routine influenza surveillance data to the WHO Global Influenza Surveillance and Response system (GISRS) (Figure 1.4) (World Health Organization, 2015b).

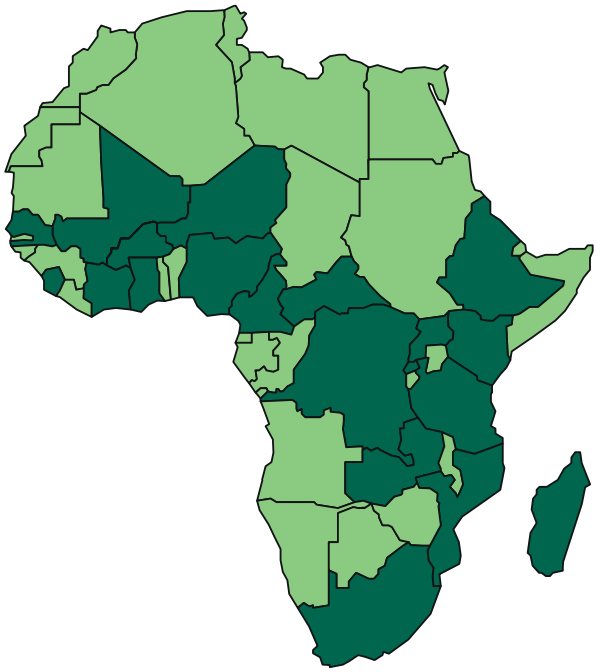


**Figure 1.4 The 22 SSA countries (dark green) that contribute influenza surveillance data ot the Global Influenza Surveillance and Response System (GISRS) in 2015**

To identify publications on influenza epidemiology in SSA published since 2009, a literature search was conducted using the same search terms as the review (Gessner et al., 2011): the National Library of Medicine was searched through PubMed for ("influenza" AND "Africa") OR ("Africa" AND ("pneumonia" OR "acute respiratory infection")) OR ("influenza" AND each individual sub-Saharan African country). The search was limited to human studies, studies that included adults, and studies published between 1 January 2010 and 31 July 2015.

Over the 5.5-year period, 37 influenza surveillance studies from 25 countries were identified (Table 1.4), reflecting the rapid expansion in surveillance activity as well as growing interest in influenza epidemiology in the region. A complete supplement was devoted to 'Influenza in Africa' in the *Journal of Infectious Diseases* in December 2012. *The* highest number of publications came from Kenya (n=8), South Africa (n=5), and Senegal (n=5), all of whom had surveillance platforms in place prior to the 2009 A(H1N1) pandemic.

Most studies used the WHO- and CDC-recommended case definitions for ILI and SARI (Pan American Health Organization & Centers for Disease Control and Prevention (PAHO-CDC), 2006). However, the maximum time from symptom onset to recruitment varied from 3 (Njouom et al., 2010; Razanajatovo et al., 2011; McAnerney et al., 2012; Niang et al., 2012; Theo et al., 2012; Dia et al., 2014b; Majanja et al., 2013) to 14 (Ahmed et al., 2012; Feikin et al., 2012c; Emukule et al., 2014) days, and hospitalization was not a requirement for some SARI definitions. A number of Kenyan studies used 'acute respiratory infection' (ARI), a variation of the SARI case definition, which

**Table 1.4 Summary of influenza surveillance studies in SSA published since 2009**

| Country | Study | Surveillance period | Full years | ILI sites | SARI sites | No. of specimens tested for influenza | | No. of influenza-positive specimens | | Peak season |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ILI (N) | SARI (N) | Influenza + ILI (N,%) | Influenza + SARI (N,%) | |
| ***North*** | | | | | | | | | | |
| Sudan | Enan et al., 2013 | 2010-2011[a] | N | | 1[b] | | 400 | | 64 (16) | Jan-Mar |
| ***Central*** | | | | | | | | | | |
| Cameroon | Njouom et al., 2010 | 2007-2008 | Y | 7 | - | 238 | - | 70 (29) | - | Nov-Dec |
| Cameroon | Njouom et al., 2012◆ | 2009 | Y | 14 | - | 561 | - | 158 (28) | - | Jun-Jul; Oct-Nov |
| DRC | Muyembe Tamfum et al., 2012◆ | 2009-2011 | N | 5 | 2 | 3217 | 939 | 502 (16) | 103 (11) | Jan-May |
| Gabon | Lekani-Douki et al., 2013 (Lekana-Douki et al., 2013) | 2010-2011 | Y | 6 | - | 966 | - | 131 (14) | - | Mar-May, Nov-Jan |
| Gabon | Lekani-Douki et al., 2014 | 2010-2011 | N | 6 | - | 1041 | - | 119 (11) | - | Oct-Mar |
| Rwanda | Nyatanyi et al., 2012◆ | 2008-2010 | Y | 6 | 6 | 945 | 971 | 276 (29) | 101 (10) | Oct-Nov, Feb-Mar |
| ***West*** | | | | | | | | | | |
| Senegal | Niang et al., 2012◆ | 1996-2009 | Y | 2-13 | - | 9176 | - | 1233 (13) | - | Jul-Sep |
| Senegal | Dia et al., 2014b | 2009-2011 | Y | NS | - | 232 | - | 67 (29) | | Sep-Nov |
| Senegal | Dia et al., 2014a | 2012-2013 | N | 11 | - | 1427 | - | 317 (22) | | Sept-Oct (Flu A); Jan-Aug (FluB) |
| Cote d'Ivoire | Kadjo et al., 2012 | 2003-2010 | Y | 26 | - | 5074 | - | 969 (19) | - | Mar-Sep |
| Nigeria | Dalhatu et al., 2012◆ | 2009-2010 | N | 4 | 4 | 2053 | 338 | 167 (8) | 17 (5) | Year-round |
| Senegal, Cape Verde, Mauritania, Guinea | Dia et al., 2013 | 2009-2010 | N | NS | - | Senegal 2264<br>Cape Verde 498<br>Mauritania 227<br>Guinea 166 | - | Senegal 717 (32)<br>Cape Verde 132 (27)<br>Mauritania 42 (19)<br>Guinea 20 (12) | - | NS |
| Niger | Jusot et al., 2012 | 2009-2010 | Y | 10 | 10 | 320[c] | - | 76[c](24) | - | Mar-May |
| Niger | Mainassara et al., 2015 | 2009-2013 | Y | 5 | 4 | 1176 | 952 | 146 (12) | 54 (6) | Nov-Apr |

| Country | Study | Surveillance period | Full years | ILI sites | SARI sites | No. of specimens tested for influenza | | No. of influenza-positive specimens | | Peak season |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ILI (N) | SARI (N) | Flu+ ILI (N,%) | Flu+ SARI (N,%) | |
| ***West (continued)*** | | | | | | | | | | |
| Togo | Maman et al.,2014 (Maman et al., 2014) | 2010-2012 | N | 2 | - | 955 | - | 236 (25) | - | Year-round |
| Burkina Faso | Tarnagda et al., 2014 | 2010-2012 | N | 6 | - | 881 | - | 58 (7) | - | Oct-Mar (2010-11) Aug-Apr (2011-12) |
| ***East*** | | | | | | | | | | |
| Ethiopia | Ayele et al., 2012◆ | 2008-2010 | N | 3 | 2 | 59 | 117 | 7 (12) | 5 (4) | May-Jul |
| Kenya | Feikin et al., 2012c | 2007-2009 | Y | - | 7 | - | 2079 | - | 204 (10) | May-Oct |
| Kenya | Feikin et al., 2012b | 2007-2010 | Y | | | | | | | |
| Kenya | Katz et al., 2012◆ | 2007-2010 | Y | Biweekly HH visits | 2 | Urban 35652 Rural 25799 | Urban 1197 Rural 1641 | - | Urban 319 (27) Rural 359 (22) | Year-round |
| Kenya | Ahmed et al., 2012 | 2007-2010 | Y | 2 | 2 | 1815 | 4449 | 252 (14) | 516 (12) | May-Nov |
| Kenya | Katz et al., 2014 (Katz et al., 2014) | 2007-2013 | Y | 11 | 11 | 14013 | 24762 | 2041 (15) | 237 (10) | Year-round |
| Kenya | Majanja et al., 2013 | 2008-2011 | N | 8 | - | 11592 | - | 2073 (18) | - | Year round |
| Kenya | Pretorius et al., 2012◆ | 2009-2010 | N | - | 6 | - | 8173 | - | 704 (9) | Jun-Oct |
| Kenya | Emukule et al., 2014 | 2009-2012 | Y | 1 | 1 | 1508 | 4387 | 206 (14) | 348 (8) | Year-round |
| Madagascar | Razanajatovo et al., 2011 | 2008-2009 | Y | 9 | - | 313 | - | 97 (31) | - | Year-round |
| Tanzania | Mmbaga et al., 2012◆ | 2008-2010 | N | 5 | 5 | 1083 | 695 | 89 (8) | 51 (7) | Year-round |
| Uganda | Lutwama et al., 2012◆ | 2007-2010 | N | 10 | 5 | 2656 | 102 | 353 (13) | 6 (6) | Jun-Oct |
| ***South*** | | | | | | | | | | |
| South Africa | (McAnerney et al., 2012)◆ | 1984-2008 | Y | 10-170 | - | (2005-08) 5211 | - | (2005-08) 1811 (35) | - | May-Aug |
| South Africa | Cohen et al., 2013 | 2009-2011 | N | - | 6 | - | 11925 | - | 1056 (9) | May-Sept |
| South Africa | Cohen et al. 2014 | 2009-2012 | N | - | 6 | - | 16005 | - | 1239 (8) | May-Sept |

| Country | Study | Surveillance period | Full years | ILI sites | SARI sites | No. of specimens tested for influenza | | No. of influenza-positive specimens | | Peak season |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ILI (N) | SARI (N) | Flu+ ILI (N,%) | Flu+ SARI (N,%) | |
| ***South (continued)*** | | | | | | | | | | |
| South Africa | Budgell et al. 2015 | 2009-2012 | N | 205 | 6 | 9104 | 18293 | NS (41) | NS (8) | May-Sept |
| South Africa | Cohen et al., 2015c | 2009-2012 | N | - | 6 | - | 7067 | - | 621 (9) | May-Sept |
| Zambia | Theo et al, 2012◆ | 2008-2009 | N | 4 | 2 | 334 | 900 | 17 (5) | 40 (4) | Jun-Aug |
| ***Multiple countries*** | | | | | | | | | | |
| Cameroon, Côte d'Ivoire, Madagascar, Niger, Senegal | Heraud et al., 2012◆ | 2008-2009 | Y | NS | | Senegal 1458<br>Niger* 113<br>Côte d'Ivoire 2473<br>Cameroon 811<br>Madagascar 3457 | | Senegal 409 (28)<br>Niger 21 (19)<br>Côte d'Ivoire 547 (22)<br>Cameroon 212 (26)<br>Madagascar 1417 (41) | | 2008: year-round activity in Côte d'Ivoire, Cameroon, and Madagascar; Senegal - Aug-Oct. 2009: May-Nov for all countries except Madagascar = Jan-Mar & May-Sep |
| Cameroon, Cape Verde, Cote d'Ivoire, Ghana, Guinea, Mali, Mauritania, Niger, Nigeria, Senegal | (Nzussouo et al., 2012)◆ | 2009-2010 | N | 110 | | Cameroon 470<br>Cape Verde 498<br>Cote d'Ivoire 1272<br>Ghana 4032<br>Guinea 98<br>Mali 422<br>Mauritania 127<br>Niger 388<br>Nigeria 1322<br>Senegal 1546 | | Cameroon 165 (35)<br>Cape Verde 133 (27)<br>Cote d'Ivoire 492 (39)<br>Ghana 883 (22)<br>Guinea 12 (12)<br>Mali 53 (13)<br>Mauritania 31 (24)<br>Niger 90 (23)<br>Nigeria 123 (9)<br>Senegal 599 (39) | | |

ILI, influenza-like illness; SARI, severe acute respiratory illness; DRC, Democratic Republic of Congo; Y, Yes; N, No; NS, not stated; HH, household.

[a]Enrolled patients between January and March each year; [b]Patients with acute respiratory infection (undefined) were enrolled from a hospital.

[c]Includes both ILI & SARI patients; ◆Published in Journal of Infectious Diseases 'Influenza in Africa' supplement.

encompassed cough OR difficulty breathing OR chest pain AND documented axillary temperature ≥38.0°C OR oxygen saturation <90% OR hospitalization (Feikin et al., 2012b; Katz et al., 2012). In contrast to the studies in the earlier review (1980-2009), influenza was diagnosed using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) in all studies except one (McAnerney et al., 2012).

Most studies included all age groups, with the exception of Feikin *et al.* (>5 years) (Feikin et al., 2012b), Cohen *et al*. (>5 years) (Cohen et al., 2015c), and Dia *et al.* (>50 years) (Dia et al., 2014b). The surveillance populations were predominantly young children (the median age was <5 years in 22/30 studies). 14 studies included both ILI and SARI surveillance, 15 undertook ILI surveillance only, and 7 solely SARI surveillance. Just over half the studies had complete years of surveillance (table 1.3); among those, influenza-PCR positivity ranged from 10 to 41% (median 24%) for ILI cases; and 4 to 27% (median 9%) for SARI cases (Table 1.4). In studies that included both ILI and SARI surveillance, the detection rate of influenza was consistently higher in ILI compared to SARI cases.

Seasonality is difficult to discern since all but 5 studies (Njouom et al., 2010; Razanajatovo et al., 2011; Feikin et al., 2012c; McAnerney et al., 2012; Niang et al., 2012) included part of the influenza A(H1N1)pdm09 period, which disrupted seasonal patterns on the continent in 2009 and 2010. The available data suggest that countries in Southern Africa had clearly defined seasons – influenza activity was usually highest between May and September each year, in line with Southern Hemisphere winters. In contrast, seasonality is less clear-cut in the equatorial regions of the continent. Some have year-round activity, while in several countries in central and western Africa, influenza peaked in their corresponding rainy seasons (Niang et al., 2012; Kadjo et al., 2012; Nyatanyi et al., 2012).

Most the surveillance studies overlapped with the 2009 A(H1N1) pandemic period (June 2009 to August 2010), hence were able to shed some light on the epidemiological impact of the pandemic. Consistent with the findings from the serosurveys (Section 1.2.3), several studies found that children and young adults aged <25 years had a higher risk of infection with A(H1N1)pdm09 compared to other age groups (Dia et al., 2013; Orelle et al., 2012; Cohen et al., 2014). This finding is supported by a global pooled analysis of 70,000 laboratory-confirmed hospitalized A(H1N1)pdm09 patients from 19 countries (including Madagascar and South Africa), which found the highest risk of hospitalization in patients aged <5 years and 5-14 years (relative risk (RR) 3.3 and 3.2, respectively, compared to the general population). However, the highest risk of death per capita was found in the age groups 50–64 (RR 1.5) and >65 years (RR 1.6) (Van Kerkhove et al., 2011b). Increased exposure or outbreaks at schools could explain the increased risk seen in older children and adolescents

(Rajatonirina et al., 2011; Orelle et al., 2012).

In summary, substantial progress has been made to enhance influenza surveillance capacity in SSA, with an increasing number of countries, and sentinel sites conducting surveillance. These data show that, similar to other parts in the world, influenza is an important contributor to mild and severe acute respiratory disease in African individuals of all ages. Improved surveillance data on SARI have bettered our understanding of the role of influenza in hospitalised patients, a group that is especially pertinent from a clinical and public health perspective. The consistent detection of influenza in a higher percentage of ILI compared to SARI cases likely reflects the higher specificity of ILI case definition for influenza (Murray et al., 2013).

Nevertheless, data are absent from approximately half the countries in SSA. A large proportion of published studies did not include complete years of surveillance. Influenza seasonality outside of Southern Africa remains unclear. Additional years of surveillance post 2009A(H1N1) pandemic may help to elucidate influenza transmission patterns in the equatorial regions (see also Section 1.2.5 on the challenges of influenza surveillance in SSA).

#### *1.2.4.2 Incidence estimates of influenza disease in SSA*

Since 2009, a number of studies have estimated the burden of influenza-associated medical attendances or hospitalisations (Table 1.5). Although slightly different case definitions for acute respiratory infection were used, the incidence rates for influenza-related hospitalisations were similar in the three Kenyan (Feikin et al., 2012b; Emukule et al., 2014; Fuller et al., 2013) and one South African study (Cohen et al., 2013). Cohen *et al*. (Cohen et al., 2013) demonstrated higher incidence of influenza-related hospitalisations in individuals aged 25-44 years compared to the 5-24 and $\geq$45 age groups, which may be due to the underlying high HIV prevalence in this group. In contrast, Katz *et al.* (Katz et al., 2012) reported an incidence of influenza-associated medical attendances for acute lower respiratory tract infection (LRTI) of 1630 per 100,000 (95% CI 1500-1700), which is ~40 time higher than the other two Kenyan studies. Although genuine variations in disease burden may exist, the substantial disparity may be partly explained by the inclusion of children <5years, which constituted a large proportion of the study population (mean age 9.8 years), and had considerably higher rates of acute respiratory illness. Moreover, the incidence measure was of influenza-associated medical attendances, rather than hospitalisations. It is likely that only a very small proportion of medical attendances would have resulted in hospital admission.

**Table 1.5 Summary of studies that estimated influenza incidence in SSA**

| Country | Study | Surveillance period | Outcome | Age group (years) | Per 100,000 | | Adjustments |
|---|---|---|---|---|---|---|---|
| | | | | | Incidence | 95% CI | |
| Kenya | Feikin e al., 2012c | 2007-2009 | Influenza-associated respiratory illness[a] hospitalisation | ≥5 | 36.7 | 33.0-44.4 | - proportion of eligible patients who presented to hospital but not sampled<br>- proportion of patients from whom swabs were taken but not tested for influenza |
| Kenya | Katz et al., 2012 | 2007-2010 | Medically-attended influenza-associated acute LRTI[b] | All ages | 1630 | 1500-1700 | - proportion of eligible patients who presented to health facility but not sampled |
| Kenya | Fuller et al., 2013 | 2009-2011 | Influenza-associated SARI[c] hospitalisation | All ages | 21-24 | - | - Provincial proportion of influenza-positive SARI applied to provincial rates hospitalized SARI, and adjusted for prevalence of risk factors and health-seeking behaviour |
| Kenya | Emukule et al., 2014 | 2009-2012 | Influenza-associated SARI[c] hospitalisation | ≥5 | 30 | 20-40 | - proportion of eligible patients who presented to hospital but not sampled |
| South Africa | Cohen et al., 2013 | 2009-2011 | Influenza-associated acute LRTI[d] hospitalisation | 5-24<br>25-44<br>≥45 | 8-27<br>58-68<br>47-67 | -<br>-<br>- | - proportion of eligible patients not enrolled |

Abbreviations: CI = confidence interval; LRTI = lower respiratory tract infection; SARI = severe acute respiratory illness.

[a]Defined as cough, difficulty breathing or (if aged ≥ 5 years) pleuritic chest pain.

[b]Defined as cough, difficulty breathing, or chest pain, and either axillary temperature ≥38.0°C or oxygen saturation ≤90%.

[c]Defined as hospitalization with cough or difficulty breathing or pleural chest pain with an onset within the last 14 days.

[d]Defined as hospitalization with sudden onset fever (>38.0°C) or reported fever, cough or sore throat, and shortness of breath or difficulty breathing, with onset within 7 days.

#### *1.2.4.3 Risk factors for complications of seasonal influenza*

Beyond baseline demographics and clinical symptoms, few surveillance studies in SSA had recorded individual-level data to allow evaluation of risk factors for severe presentations of influenza. Only five studies have reported HIV status (Feikin et al., 2012b; Cohen et al., 2013; Emukule et al., 2014; Cohen et al., 2015b; Cohen et al., 2015a). Recent publications of surveillance data from South Africa found that HIV infection was strongly associated with severe influenza-related morbidity and mortality in SARI patients (Cohen et al., 2013; Cohen et al., 2015a; Tempia et al., 2015b). High prevalence of co-infections with *Mycobacterium tuberculosis* (10%) and *Streptococcus pneumoniae* (7%), as well as underlying medical co-morbidities such as chronic lung or heart disease and diabetes (7%) was also found among influenza-positive patients (Cohen et al., 2013). Another study from South Africa in the pre-ART era, found that HIV-infected adults aged 25 to 54 years had a 150 to 200-fold increased risk influenza-associated death compared with the general population (Cohen et al., 2012a).

A retrospective case control study in rural Kenya of 64 hospitalised patients >5 years of age with laboratory-confirmed influenza, and 190 age-, sex- and neighbourhood-matched healthy controls found that persons with HIV infection (adjusted odds ratio (aOR) 3.56, 95%CI 1.25-10.1) and chronic lung disease[iii] (aOR 6.83, 95%CI 1.37-34.0) were at greater risk of influenza-associated hospitalisation (Ope et al., 2011).

The limited data from SSA to date suggest that HIV may be an important risk factor for severe presentations of influenza. The existing evidence on the impact of HIV infection on influenza illness is further explored in Chapter 1.4.

### 1.2.5 Challenges of influenza surveillance in SSA

From Section 1.2.3, it is clear that major strides have been made towards improving influenza surveillance in SSA and elucidating the regional burden of influenza. Nevertheless, there are still enormous challenges to obtaining good quality and representative surveillance data in the region. Surveillance capacities remain limited in certain SSA countries (Steffen et al., 2012). Outcome data are rarely reported. A recently published review on mortality associated with SARI patients enrolled in influenza surveillance found that few African countries systematically collect data on respiratory hospitalization outcomes. Only 11 of 23 (48%) surveyed countries systematically collected mortality data (McMorrow et al., 2015). Of 37,714 SARI patients included from 8 countries between 2009 and 2012, 1073 deaths were reported of whom 57 tested positive for influenza (case fatality proportion (CFP) = 1.8%) (McMorrow et al., 2015).

[iii] Excluded active pulmonary tuberculosis or asthma.

In existing programmes, variability in surveillance methods, including case definitions, testing algorithms, age categorisations, methods of sample collection (nasopharyngeal (NP) aspirate, nasopharyngeal and/or oropharyngeal swabs) and consumables (e.g. type of viral transport medium) limits easy comparisons within and between countries. Secondly, many sentinel surveillance programmes have limited geographical representation, thus affecting generalizability of their findings. Thirdly, RT-PCR is now considered the gold standard for influenza diagnosis (WHO working group on polymerase chain reaction protocols for detecting subtype influenza A viruses, 2011). However, the requirements for reliable power source, access to reagents, consumables, trained technicians and skilled maintenance, in addition to robust sample transfer and storage systems, present significant logistical challenges to achieve high quality diagnostic standards in low-resource settings (Fields et al., 2013). Fourthly, timely dissemination of surveillance data is often hindered by suboptimal data analysis and reporting mechanisms (Steffen et al., 2011). Furthermore, the sustainability of the current costly surveillance programmes is unclear. At present, many programmes are funded by external institutions, such as the CDC and Institut Pasteur, but this will not remain indefinite (Radin et al., 2012). Active engagement and commitment from local governments are needed to sustain surveillance programmes in the long-term.

### 1.2.6 Interventions against influenza

In developed countries, annual vaccination (with IIV or LAIV, Chapter 1.1.5.1) is the cornerstone of influenza prevention (Monto, 2010; 2013). However, few countries in SSA have national influenza vaccine policies (Duque et al., 2014), and the requirement for annual vaccination is a major financial and logistical obstacle for many sub-Saharan African countries. A CDC survey in 2012 reported seasonal influenza vaccine availability in 14 of 31 (45%) surveyed African countries, in either the public or private sector (Duque et al., 2014). Only 2 SSA countries (Côte d'Ivoire and Mauritius) reported to have an active national public policy for influenza vaccination (Duque et al., 2014). Of note, South Africa has standing guidelines for the prevention and treatment of influenza but has yet to develop a national public policy regarding vaccination (Walaza and Cohen, 2015).

Only a handful of studies have evaluated efficacy or effectiveness of IIV in Africa; and all were conducted in South Africa. A randomised double blind placebo-controlled trial of IIV3 in HIV-infected adults demonstrated vaccine efficacy of 75.5% (95%CI 9.2%-95.6%) (Madhi et al., 2011), which is comparable to that in developed settings (Anema et al., 2008). However, patients with underlying co-morbidities and advanced immunosuppression (ART naïve with CD4 <100 cells/µL) were excluded. From an ILI surveillance programme between 2005 and 2009, age-adjusted vaccine effectiveness of IIV3 ranged from -14% (95%CI -100% to 35%) to 67% (95%CI 12%-90%) (Ntshoe et al., 2014). Low

vaccine coverage (approximately 4% among ILI cases) may have reduced the power to estimate vaccine effectiveness (Ntshoe et al., 2014). More recently, an RCT of IIV3 in HIV-infected and HIV-uninfected pregnant women demonstrated partial clinical efficacy in both groups (VE in HIV-infected pregnant women 57.7%, 95%CI 0.2-82.1, HIV-uninfected pregnant women 50.4%, 95%CI 14.5-71.2) as well as in infants unexposed to HIV (VE 48.8%, 95%CI 11.6-70.4) (Madhi et al., 2014). LAIV is not recommended in immunosuppressed adults or pregnant women, thus unsuitable for widespread use in sub-Saharan Africa where HIV prevalence is high. Even if annual seasonal influenza vaccination became national policy, its implementation would be logistically challenging. There is currently no indigenous vaccine production in the region (Schoub et al., 2013). Additionally, the vaccine donation initiative that provided 2009 pandemic H1N1 vaccines also highlighted problems in timely vaccine procurement, distribution and uptake in the region (Schoub et al., 2013).

Neuraminidase inhibitors (NI) is only available in limited quantities in some SSA countries (19/31 (65%) from a CDC survey (Duque et al., 2014)). NIs are most effective if administered within 48 hours of symptom onset (Aoki et al., 2003); though as discussed in Section 1.1.6, their widespread use outside at-risk groups in developed settings is being questioned (Jefferson et al., 2014b). At present, given the broad differential diagnosis of acute respiratory presentations in high HIV prevalence settings, lack of 'real-time' viral testing, in addition to the restricted availability and prohibitive cost, the widespread and effective use of antivirals in the region is improbable.

Estimation of vaccine and antiviral needs in SSA will depend on further evaluation of epidemiology and disease burden. In 2012, the WHO Strategic Advisory Group of Experts on Immunization (SAGE) designated pregnancy as the highest priority target group for vaccination (2012), and advised countries considering the introduction of seasonal influenza vaccination programme to evaluate influenza burden in at-risk populations to facilitate the development of evidence-based immunization policies based on country-specific priority target groups (2012). The relative priority of at-risk groups in SSA is likely to differ from that of developed settings. Furthermore, the development of a tailored response to influenza in Africa will require a better understanding of how influenza interacts with prevalent diseases such as HIV, TB and pneumonia; more data on circulating strains in the region to aid decision on the most appropriate vaccine formulation (Northern/Southern hemisphere formulations); and vaccine effectiveness studies in priority groups in Africa such as young children (<5 years), pregnant women and HIV-infected individuals with advanced immunosuppression and co-morbidities.

## 1.3 Contribution of Influenza and other respiratory viruses to the aetiology and outcome of adult pneumonia in sub-Saharan Africa

### 1.3.1 Introduction

Community-acquired pneumonia (CAP) is a leading cause of morbidity and mortality in all age groups worldwide (World Health Organization, 2008). The African continent bears a disproportionate burden, accounting for approximately 30% of the estimated 430 million episodes of lower respiratory tract infections (LRTI) each year (World Health Organization, 2008). Until recently, worldwide, pneumonia was largely attributed to bacterial pathogens, e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae* (Welte et al., 2012; Peto et al., 2014). However, the advent of sensitive molecular tests (Ruuskanen et al., 2011; Lanaspa et al., 2014), emergence of severe acute respiratory syndrome (SARS), avian influenza A(H5N1) and 2009 pandemic influenza A(H1N1) virus, which often manifested as severe pneumonia, in addition to the recent discovery of novel respiratory viruses (e.g. bocavirus, coronaviruses NL63, HKU1, Middle East respiratory syndrome [MERS] and human metapneumovirus [hMPV]), have led to the growing recognition of viruses as major contributors to severe respiratory infections.

Available data on the contribution of viruses to adult CAP are predominantly from studies in developed settings (Ruuskanen et al., 2011; Lanaspa et al., 2014), where up to a third of adult pneumonia were associated with viral infection (Jennings et al., 2008; Johansson et al., 2010; Lieberman et al., 2010; Jain et al., 2015). The epidemiology and viral aetiology of pneumonia in African adults are less well characterised (Ho, 2014), due to comparatively limited surveillance and diagnostic capacities, but are likely to substantially differ from developed settings for a variety of reasons. First, respiratory viruses tend to follow different seasonal patterns of activity. Epidemics of influenza occur during winter months in temperate regions, whereas emerging data from SSA indicate less distinct influenza peaks, particularly in equatorial regions (World Health Organization, 2014b) where a number of countries have reported year-round transmission (Dalhatu et al., 2012; Katz et al., 2012; Mmbaga et al., 2012). Secondly, high prevalence of HIV and other co-morbidities (e.g. tuberculosis and malaria) in SSA are likely to impact on the epidemiology and microbial aetiology of adult viral respiratory presentations (Joint United Nations Programme on HIV/AIDS, 2010). In contrast to the U-shaped age distribution seen in developed settings (Jokinen et al., 1993), several pneumonia aetiology studies in SSA have described a preponderance of younger adults (aged 15-45 years) (Scott et al., 2000; Tornheim et al., 2007). Thirdly, individuals with immunosuppression are at increased risk of severe disease. A study from South Africa described higher incidence of influenza-related hospitalization and mortality in HIV-infected compared with HIV-uninfected adults (also see Chapter 1.4) (Cohen et al.,

2013). Lastly, targeted (Monto, 2010) or universal (Grohskopf et al., 2014) seasonal influenza vaccination is recommended in many developed settings, but few African countries have implemented national influenza vaccination programmes (Duque et al., 2014).

### 1.3.2 Diagnosis of respiratory viral infections

There is currently no gold standard for respiratory viral aetiology detection. Conventional methods, such as viral culture and immunofluorescence microscopy of respiratory specimens, or serological testing of acute and convalescent serum samples, have poor sensitivity (Gunson et al., 2005). Nucleic acid amplification tests (NAATs), such as PCR-based methods, have far greater sensitivity (Mahony, 2010), are able to detect viruses that are difficult to culture (e.g. hMPV, coronaviruses NL63 and HKU1, and rhinovirus), can rapidly characterise new viral pathogens (Murdoch et al., 2010), and can yield results in a clinically relevant time frame. Studies in well-resourced settings have demonstrated higher microbial yield when conventional diagnostic techniques are augmented by NAATs (Templeton et al., 2005; Jennings et al., 2008; Johansson et al., 2010; Diederen et al., 2009), the latter now form the backbone of respiratory viral testing in both well resourced and surveillances sites in low-income settings.

A variety of upper and lower respiratory tract specimens have been used for aetiological studies of ARI, including nasopharyngeal aspirates (NPA) or washes; swabs from the nasopharynx or throat; expectorated or induced sputum; bronchoalveolar lavage (BAL) or transthoracic needle aspiration (Loens et al., 2009). Viral recovery varies according to specimen type. For the aetiological diagnosis of pneumonia, lower respiratory tract samples are preferred since they are taken from the site of infection (Murdoch et al., 2009; Karhu et al., 2014), but they are difficult to obtain without contamination from upper respiratory flora and are unavailable in many low resource settings. Viral diagnosis therefore mostly relies on upper respiratory specimens, but the high sensitivity of molecular tests complicates the interpretation of a positive result (Jansen et al., 2011). Aside from being the causative agent, a positive result may represent colonisation or post-infectious shedding. Thus, ascribing causality to every positive result may overestimate the contribution of respiratory viruses. Quantifying viral load (Bosis et al., 2008; Gerna et al., 2009) or ascertaining the background prevalence of asymptomatic nasopharyngeal viral infection in a control group (Feikin et al., 2012b; Levine et al., 2012) may aid characterisation the pathogenic roles of detected viruses, but neither approach has commonly been used in aetiological studies.

### 1.3.3 Viral aetiology of acute respiratory infection in adults in sub-Saharan Africa

Most aetiology studies undertaken in developed settings have focused on radiologically-confirmed pneumonia (Jennings et al., 2008; Templeton et al., 2005; Johansson et al., 2010; Diederen et al., 2009; Saito et al., 2006; Lieberman et al., 2010; de Roux et al., 2006; Charles et al., 2008; Johnstone et al., 2008; Angeles Marcos et al., 2006). Given the inconsistent availability of chest radiographs in SSA health settings (Saito et al., 2012), a broader definition of pneumonia was adopted to include clinical evidence of LRTI with or without confirmed chest radiograph changes. Studies that evaluated viral aetiology in patients with mild respiratory symptoms (not requiring hospitalisation) were also included to assess the relative contribution of respiratory viruses in mild and severe ARI.

To identify studies in SSA that had evaluated respiratory viruses in adults presenting with mild and severe ARI, a literature search using the PubMed database from inception till 15/03/2015 was conducted using the following search strategy: (“acute respiratory infection*”[All Fields] OR “acute respiratory illness*” [All Fields] OR “influenza-like illness*” OR “acute lower respiratory tract infection*” [All Fields] OR “pneumonia”[All Fields] OR “pneumonia”[MeSH Terms]) AND (“virus*”[All Fields] OR “viral”[All Fields]) AND (“Africa”[MeSH Terms] OR “Africa*”[All fields]). Only studies with patients aged five years and over were included. Those that exclusively examined for bacterial pathogens or a single virus (e.g. influenza surveillance) were excluded (Figure 1.5).

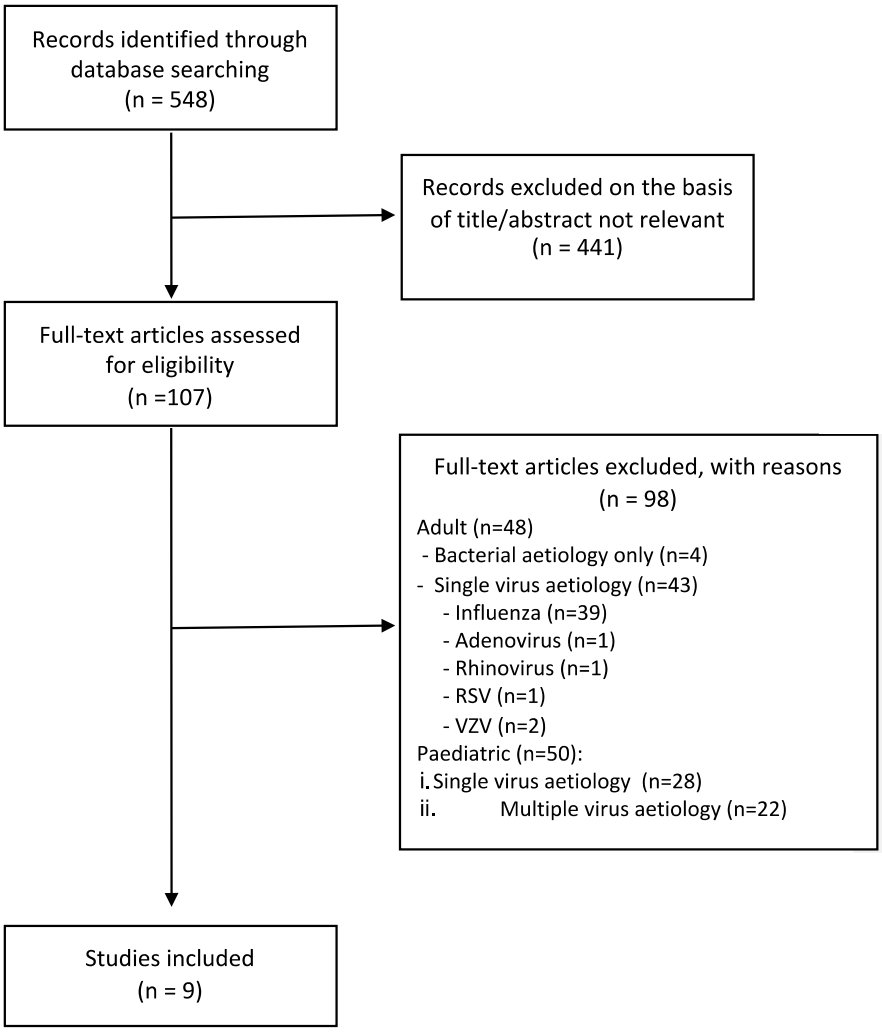



**Figure 1.5 Identification and selection of studies that have evaluated viral aetiology of acute respiratory infection in adults in SSA**

RSV, respiratory syncytial virus; VZV, varicella zoster virus.

Nine studies were identified; four in patients with ILI (Razanajatovo et al., 2011; Kadjo et al., 2013; Lekana-Douki et al., 2014; Dia et al., 2014a), four in hospitalised patients with pneumonia or clinical LRTI (Joosting et al., 1979; Scott et al., 2000; Hartung et al., 2011; Pretorius et al., 2012), and one that included both outpatient and inpatient ARI patients (Feikin et al., 2012b) (Figure 1.5, Tables 1.7 and 1.8). There were substantially more respiratory viral aetiology studies in young children (n=50) compared to adults, and a large proportion of excluded adult studies were reports from influenza surveillance following the 2009 A(H1N1) pandemic (Koegelenberg et al., 2010; Njouom et al., 2010; Barakat et al., 2011a; Barakat et al., 2011b; Byarugaba et al., 2011; Lahlou Amine et al., 2011; Van Kerkhove et al., 2011a; Archer et al., 2012; Barakat et al., 2012; Bulimo et al., 2012; Cohen et al.,

2012c; Dalhatu et al., 2012; Heraud et al., 2012; Katz et al., 2012; Lenzi et al., 2012; McAnerney et al., 2012; Muyembe Tamfum et al., 2012; Niang et al., 2012; Orelle et al., 2012; Radin et al., 2012; Steffen et al., 2012; Theo et al., 2012; Venter et al., 2012; Byarugaba et al., 2013; Cohen et al., 2013; Matheka et al., 2013; Cohen et al., 2014; Katz et al., 2014; Meseko et al., 2014; Ntshoe et al., 2014; Tarnagda et al., 2014; Thiam et al., 2014; Wolter et al., 2014a; Cohen et al., 2015a; Cohen et al., 2015c; McMorrow et al., 2015).

The nine included studies comprised 1753 non-hospitalised ILI patients, and 5746 hospitalised LRTI patients. There was substantial heterogeneity in the study populations, clinical case definitions of 'ILI' and 'pneumonia', and clinical specimens obtained for viral testing (Table 1.6). Four of the 9 studies did not include whole years of observation (Lekana-Douki et al., 2014; Scott et al., 2000; Hartung et al., 2011; Pretorius et al., 2012), which may have resulted in the over- or underestimation of the burden of viral infections depending on the study period included and the seasonal peaks. Two studies conducted prior to 2000 used viral culture and serology (Joosting et al., 1979; Scott et al., 2000); the others utilised PCR-based methods. All studies tested for influenza A and B, adenovirus and RSV, and most also included parainfluenza viruses (PIV) and enterovirus. The more recent studies have encompassed testing for rhinovirus, coronaviruses, hMPV and bocavirus. HIV prevalence was high (49-94%) among the 3 studies that reported serostatus (Scott et al., 2000; Hartung et al., 2011; Feikin et al., 2012b).

One or more viral pathogens were detected in 37.7 to 84.2% of ILI patients (Razanajatovo et al., 2011; Kadjo et al., 2013; Lekana-Douki et al., 2014; Dia et al., 2014a; Feikin et al., 2012b), and 5.7 to 58% of hospitalised pneumonia patients (Joosting et al., 1979; Scott et al., 2000; Hartung et al., 2011; Pretorius et al., 2012; Feikin et al., 2012b) (Table 1.7). This is similar to that reported in contemporary studies in developed settings (Jennings et al., 2008; Templeton et al., 2005; Hohenthal et al., 2008; Johansson et al., 2010; Diederen et al., 2009; Saito et al., 2006; Lieberman et al., 2010), though proportions tested positive for virus are not strictly comparable due to the variable number of respiratory viruses tested among studies.

With the exception of one Gabonese study (Lekana-Douki et al., 2014), influenza and rhinovirus were the most ubiquitous among the 5 studies that included non-hospitalised ILI patients (Razanajatovo et al., 2011; Kadjo et al., 2013; Lekana-Douki et al., 2014; Dia et al., 2014a; Feikin et al., 2012b). Adenovirus (2.2-12.1%), parainfluenza viruses (3.8-12.2%), RSV (1.9-7.5%), coronaviruses (2.3-11.3%), hMPV (0.9-6.1%) and enteroviruses (2.3-5.5%) were also frequently detected. Conversely, human bocavirus was rarely detected in patients with ILI.

**Table 1.6 Characteristics of studies on viral aetiology in adults with ARI in SSA**

| Study, year [Reference] | Country | Study period | Setting | Study population | Case definition | Specimen | Viral diagnostic test |
|---|---|---|---|---|---|---|---|
| ***Non-hospitalised*** | | | | | | | |
| Razanajatovo et al, 2011 | Madagascar | Jul 2008-Jun 2009 | Outpatient clinic-based surveillance | ≥5 years | ILI (Fever >38.0 °C and cough and ≥2 symptoms of sore throat, rhinorrhea, headache and muscular pain; symptoms < 3 days) | NP & OP swabs | rRT-PCR |
| Feikin et al, 2012b (Feikin et al., 2012b) | Kenya | Mar 2007-Feb 2010 | Population-based surveillance | ≥5 years | ARI (cough or difficulty breathing or chest pain and temperature >38.0 °C or oxygen saturation <90%) | NP & OP swabs | rRT-PCR |
| Njouom et al., 2012 | Cameroon | Jan - Dec 2009 | Outpatient clinic-based surveillance | ≥5 years | ILI (Fever >38.0 °C and cough or sore throat; symptoms < 5 days) | NP & OP swabs | rRT-PCR |
| Lekana-Douki et al, 2014 | Gabon | Mar 2010-Jun 2011 | Outpatient clinic-based surveillance | ≥5 years | ILI (Fever >38.0 °C and cough or sore throat) | NP swab | rRT-PCR |
| Dia et al, 2014b | Senegal | Jan 2009-Dec 2011 | Outpatient clinic-based surveillance | ≥50 years | ILI (Fever >38.0 °C and cough or sore throat; symptoms < 5 days) | NP & OP swabs | rRT-PCR |
| ***Hospitalised*** | | | | | | | |
| Joosting et al., 1979 | South Africa | May 1966-Apr 1972 | Hospital-based observational study | Black miners | Acute respiratory disease (definition not stated) | Throat swabs & serum | Viral culture & serology (HAI or complement fixation) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Scott et al., 2000 | Kenya | Mar 1994-May 1996 | Hospital-based observational study | ≥15 years | Pneumonia (≥2 symptoms of fever, cough, sputum, chest pain, SOB, or haemoptysis; symptoms <14 days; consolidation on CXR) | Serum | Complement fixation |
| Hartung et al., 2011 | Malawi | Feb-Sept 2006 | Hospital-based observational study | ≥18 years | Pneumonia (≥1 symptom of cough, sputum, chest pain, SOB, chest pain or haemoptysis; CXR changes) + admission to HDU | BAL fluid | rRT-PCR |
| Feikin et al., 2012 | Kenya | Mar 2007-Feb 2010 | Population-based surveillance | ≥5 years | ARI (cough or difficulty breathing or chest pain and temperature >38.0 °C or oxygen saturation <90% or hospitalisation) | NP & OP swabs | rRT-PCR |
| Pretorius et al., 2012 | South Africa | Feb 2009-Dec 2010 | Hospital-based surveillance (6 hospitals) | ≥5 years | SARI (fever; cough or sore throat; shortness or breath or difficulty breathing; symptoms ≤7 days) | NP & OP swabs | rRT-PCR |

HAI, haemaglutination inhibition assay; SOB, shortness of breath; CXR, chest radiograph (x-ray); HDU, high dependency unit; BAL, bronchoalveolar lavage; rRT-PCR, real-time reverse transcriptase polymerase chain reaction; ARI, acute respiratory infection; NP, nasopharyngeal; OP,oropharyngeal; SARI, severe acute respiratory infection.

**Table 1.7 Results of studies on viral aetiology in hospitalised adults with ARI in sub-Saharan Africa**

| Study, year | n | HIV prevalence (%) | Virology result | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ≥1 virus | Influenza | Adenovirus | Rhinovirus | PIV 1-4 | RSV | hMPV | Other |
| ***Non-hospitalized*** | | | | | | | | | | |
| Razanajatovo et al.,2011[a] | 159 | NS | 134 (84.2) | Any: 52 (32.7)<br>A: 45 (28.3)<br>B: 7 (4.4) | 6 (3.8) | 39 (24.5) | Any: 6 (3.8)<br>PIV1: 2 (1.3)<br>PIV2: 0 (0)<br>PIV3: 4 (2.5) | 12 (7.5) | 1 (0.6) | Bocavirus 0 (0)<br>HCoV OC43 12 (7.5); NL63 3 (1.9); 229E 2 (1.3); HKU1 1 (0.6) |
| Njouom et al, 2012[b] | 311 | NS | 176 (56.6) | Any: 110 (35.4)<br>A: 108 (34.7)<br>B: 1 (0.3)<br>C: 1 (0.3) | - | 36 (11.6) | Any: 12 (3.9)<br>PIV1: 4 (1.3)<br>PIV2: 0 (0)<br>PIV3: 6 (1.9)<br>PIV4: 2 (0.6) | 6 (1.9) | 11 (3.5) | Enterovirus: 17 (5.5) |
| Lekana-Douki et al., 2014[c] | 231 | NS | 87 (37.7) | Any: 23 (9.9)<br>A(H1N1)pdm09: 13 (5.6)<br>B: 10 4.3) | 28 (12.1) | 4 (1.7) | Any 19 (8.2)<br>PIV1: 4 (1.7)<br>PIV2: 4 (1.7)<br>PIV3: 9 (3.9)<br>PIV 4: 2 (0.9) | 15 (6.5) | 4 (1.7) | Enterovirus 9 (3.9)<br>HCoV OC43: 2 (0.9); NL63: 5 (2.2); 229E: 0 (0); HKU1: 1 (0.6) |
| Dia et al., 2014[d] | 232 | NS | 150 (64.6) | Any: 67 (28.9)<br>A: 52 (22.4)<br>B: 15 (6.5) | 5 (2.2) | 40 (17.2) | Any 13 (5.6)<br>PIV1: 3 (1.3)<br>PIV2: 0 (0)<br>PIV3: 4 (1.7)<br>PIV4: 6 (2.6) | 7 (3.0) | 5 (2.2) | Bocavirus 1 (0.4)<br>Enterovirus 6 (2.3)<br>HCoV Any: 6 (2.3) |
| Feikin et al., 2012b[e] | 820[j] | 49% | 237 (28.9) | Any: 84 (10.2)<br>22 (2.7)<br>62 (7.5) | 84 (10.2) | 108 (13.2)[k] | Any: 100 (12.2)<br>PIV1: 17 (2.1)<br>PIV2: 28 (3.4)<br>PIV3: 55 (6.7) | 59 (7.2) | 50 (6.1) | |

| Study, year | n | HIV prevalence (%) | Virology results | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ≥1 virus | Influenza | Adenovirus | Rhinovirus | PIV 1-4 | RSV | hMPV | Other |
| ***Hospitalised*** | | | | | | | | | | |
| Joosting et al., 1979[f] | 1012 | NS | 477 (47.1) | Any: 384 (37.9)<br>A: 287 (28.4)<br>B: 87 (8.6)<br>C: 10 (1) | 45 (4.4) | - | 17 (1.7) | 8 (0.8) | - | Enterovirus – 1 (0.1) |
| Scott et al., 2000b[g] | 281 | 52% | 16 (5.7) | 17 (5)<br>A: 12 (4.3)<br>B: 2 (0.7) | 2 (0.7) | - | - | 0 | - | |
| Hartung et al., 2011[h], | 51 | 94% | 11 (21.6) | A: 1 | 1 (9) | 4 (36) | PIV1: 1 (9) | 1 (9) | | Bocavirus: 1 (9)<br>HCoV OC43: 1 (9); NL63: 1 (9) |
| Feikin et al., 2012b[e] | 396[J] | 49% | 65 (16.4) | Any: 37 (9)<br>A: 29 (7)<br>B: 8 (2) | 29 (7) | 39 (35)[k] | 22 (6.5) | 33 (8) | 10 (3) | |
| Pretorius et al., 2012[i] | 4006[m] | NS | 5-24 years – 51%<br>25-44 years – 33.8%<br>45-64 years – 28.7%<br>≥65 years – 24.2% | Any – 343 (8.6)<br>A(H3N2): 102 (2.5)<br>A(H1N1): 104 (2.6)<br>B: 137 (3.4) | 251 (6.3%) | 653 (16) | Any: 145 (3.4)<br>PIV1: 12 (0.3)<br>PIV2: 27 (0.7)<br>PIV3: 96 (2.4) | 134 (3.3) | 62 (1.5) | Enterovirus: 63 (1.6) |

HIV, human immunodeficiency virus; NS, not stated; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus.

[a] Tested for Influenza A & B, adenovirus, rhinovirus, PIV1-3, RSV, hMPV, coronavirus OC43, NL63, 229E & HKU1, and bocavirus.

[b] Tested for Influenza A, B & C, rhinovirus, PIV1-3, RSV, hMPV, and enterovirus.

[c] Tested for Influenza A & B, adenovirus, rhinovirus, PIV1-3, RSV, hMPV, coronavirus OC43, NL63, 229E & HKU1 and enterovirus.

[d] Tested for Influenza A & B, adenovirus, rhinovirus, PIV1-4b, RSV, hMPV, coronaviruses, bocavirus, and enterovirus.

[e] Tested for Influenza A & B, adenovirus, PIV 1-3, RSV, and hMPV (also rhinovirus, enterovirus and parechovirus from January 2009).

[f] Tested for Influenza A, B & C, adenovirus, PIV1-3, RSV, herpesvirus, and enterovirus.

[g] Tested for Influenza A & B, adenovirus, and RSV.

[h] Tested for Influenza A & B, PIV 1-4, adenovirus, rhinovirus, RSV, hMPV, coronavirus OC43, NL63 & 229E, and bocavirus.

[i] Tested for Influenza A & B, adenovirus, PIV 1-3, RSV, hMPV, rhinovirus and enterovirus.

[j] Only included patients aged ≥5 years in whom naso/oropharngeal specimens taken.

[j]Include enterovirus due to cross-reactivity on real-time transcriptase polymerase chain reaction (RT-PCR).

[m] Only included patients aged ≥5 years

Five studies included hospitalised patients with clinical or radiologically-confirmed pneumonia (Joosting et al., 1979; Scott et al., 2000; Hartung et al., 2011; Pretorius et al., 2012; Feikin et al., 2012b). The earliest study, conducted in black South African miners hospitalised with acute respiratory disease (definition not stated), demonstrated respiratory viral infection in 47% of cases using viral culture and serology (Joosting et al., 1979). In particular, a high proportion of influenza A (28%) was found. The protracted influenza epidemic was attributed to the close working conditions that facilitated transmission, and high staff turnover, which maintained a pool of susceptible individuals. In contrast, a viral pathogen was only detected in 5.7% adults with hospitalised pneumonia in Kenya (Scott et al., 2000), though serology was the only viral diagnostic method used to test for a small number of viruses.

A prospective observational study in pneumonia patients admitted to a high dependency unit (HDU) at a tertiary referral hospital in Malawi found a viral pathogen in 11/51 patients (22%) (Hartung et al., 2011) – three were assigned a final diagnosis of viral pneumonitis, while the rest had an alternative diagnosis (pulmonary Kaposi's sarcoma) or pathogen (e.g. TB) that was considered the primary cause for their severe respiratory presentation. Selection bias towards those that were less severely ill was likely in this study, since it was limited to patients who could tolerate a bronchoscopy (with resultant exclusion of 39% of eligible patients, primarily with severe hypoxia). Additionally, two-thirds of patients had symptoms for greater than 3 weeks, which likely explains the high proportions of P*neumocystis jirovecii* pneumonia (PCP) (27%) and pulmonary TB (22%) seen.

The most recent studies were derived from epidemiological surveillance in Kenya (Feikin et al., 2012b) and South Africa (Pretorius et al., 2012). Both reported high detection rates of viral infection (16-51%); rhinovirus and influenza were again the most prevalent viruses, followed by adenovirus, RSV and parainfluenza viruses. Feikin *et al*. (Feikin et al., 2012b) also enrolled asymptomatic hospital controls. The authors identified significant association of influenza A and B, hMPV and RSV with hospitalised ARI. Conversely, rhinovirus, enterovirus, and adenovirus were equally common among cases and controls. Pretorius *et al*. (Pretorius et al., 2012), the largest study to date with greater than 4,000 patients, demonstrated the highest prevalence of viruses in hospitalised SARI patients aged 5-24 years (51%), compared to older age groups (25-44 years, 34%; 45-64 years, 29%; $\geq$65 years, 24%). There was little difference in the prevalence of individual respiratory viruses between ILI and pneumonia (Table 1.5).

Outcome data were sparse – mortality of 10% and 22% were reported by two of the hospitalised pneumonia studies by Scott *et al*. (Scott et al., 2000) and Hartung *et al.* (Hartung et al., 2011); Feikin *et al*. (Feikin et al., 2012b) found a case fatality ratio of 6% in individuals aged $\geq$5 years with hospitalised ARI. However, none of the studies reported clinical outcome according to underlying

viral or bacterial aetiology. Additionally, there were limited data on high-risk groups such as individuals with HIV infection or other co-morbidities; although Feikin *et al*. described higher incidence rates of all tested respiratory pathogens in HIV-infected compared to HIV-uninfected adults (Feikin et al., 2012b).

### 1.3.4 Detection of multiple viruses

Viral co-infections are increasingly identified as a result of the enhanced diagnostic sensitivity of PCR tests compared to conventional methods (Mahony, 2010) and the development of multiplex assays that enable simultaneous detection of multiple viruses from a single specimen (Mahony et al., 2007). The presence of several viruses could indicate: i) two or more viruses are contributing to ARI pathogenesis; ii) $\geq$1 detected virus is an innocent bystander; or iii) one virus is the predisposing factor (such as by damaging the respiratory epithelium) for pneumonia by the second viral pathogen. Some studies, mainly in children, report increased disease severity with mixed viral infection (Drews et al., 1997; Greensill et al., 2003; Semple et al., 2005; Templeton et al., 2005), but others have not (Subbarao et al., 1989; Peng et al., 2009). Hence the clinical significance of detecting multiple viruses remains unclear.

Among the included ILI studies, detection of >1 virus ranged from 4.7% (Njouom et al., 2012) to 31.4% (Razanajatovo et al., 2011) of enrolled patients. Only two of the hospitalised pneumonia studies from Kenya and South Africa reported viral co-infection (Feikin et al., 2012b; Pretorius et al., 2012). Pretorius *et al*. (Pretorius et al., 2012) found mixed viral infection in 17% of hospitalised pneumonia patients (including children <5 years), but were unable to correlate co-infection with clinical outcome due to the low number of specific co-infection combinations. The Kenyan surveillance study demonstrated no difference in co-infection between inpatients and outpatients (15 vs. 19%, p=0.51) (Feikin et al., 2012b). Similar prevalence of rhinovirus and adenovirus were also found in cases and healthy controls, implying that their identification in ARI cases may not be clinically significant (Feikin et al., 2012b). These findings highlight the difficulty in the clinical interpretation of positive PCR tests. In the majority of included ILI and LRTI studies, viruses were identified from upper respiratory tract specimens (table 1.4).

### 1.3.5 Limitations of existing studies

The existing viral aetiology studies in SSA suggest a high prevalence of viral pathogens in adults presenting with mild and severe ARI, in particular, influenza, rhinovirus, adenovirus, coronaviruses, parainfluenza viruses and RSV. Viral co-infections are not uncommon. However, there are wide variations in time period, study population, ARI definitions, clinical specimens, diagnostic methods,

and number of viruses tested. Key questions that need to be answered in SSA include the contribution of respiratory viruses to hospitalised pneumonia and mortality in high HIV prevalence settings, and the impact of HIV on the frequency and severity of viral respiratory infections. Moreover, concurrent recruitment of a control group to define the background prevalence of asymptomatic viral infections may aid interpretation of the significance of positive PCR results.

## 1.4 The impact of HIV infection on Influenza Illness

### 1.4.1 Overview

HIV infection increases susceptibility to a number of respiratory infections, including bacterial pneumonia (de Gaetano Donati et al., 2000; Sogaard et al., 2008) and pulmonary tuberculosis (Sonnenberg et al., 2005; Wood et al., 2000). Although influenza is commonly identified in HIV-infected adults with acute respiratory infection (Klein et al., 2007; Klein et al., 2010), little is known about the epidemiology of influenza in HIV-infected individuals. Published evidence on the impact of HIV infection on the susceptibility and severity of seasonal and pandemic influenza illness, as well as on influenza virus shedding and transmission in both low and high HIV prevalence settings will be reviewed in this chapter.

### 1.4.2 Susceptibility

#### *1.4.2.1 High-income countries*

**Seasonal influenza.** The only published data on the susceptibility of seasonal influenza in HIV-infected adults in low HIV prevalence settings include three outbreak investigations of influenza A in residential drug rehabilitation facilities (Table 1.9); two in the United States (Cohen and Macauley, 1989; Fine et al., 2001) and one in Italy (Boschini et al., 2006). The first two studies, conducted in the pre-ART era, found no association between HIV infection and ILI (Cohen and Macauley, 1989; Fine et al., 2001). Cohen and Macauley (Cohen and Macauley, 1989) described a non-significant difference in ILI incidence between 7 HIV-infected and 38 HIV-infected residents in Boston. Fine *et al.* (Fine et al., 2001) reported a higher incidence of ILI in HIV-infected, compared to HIV-uninfected individuals (52 vs. 25%), but the association was confounded by cigarette smoking. Both studies had a small sample size. In the third study, a 2004 influenza A(H3N2) outbreak in Italy, HIV-infected residents (n=171) were more likely to report ILI compared with HIV-uninfected residents (n=1140) (26 vs. 14%, RR 1.77, 95%CI 1.32–2.37) (Boschini et al., 2006). CD4 cell count and HIV viral load were not associated with the risk of ILI among HIV-infected persons. Although this study was larger than the other two, it did not describe or account for potential confounders, such as smoking. Of note, ILI,

**Table 1.8 Studies that evaluated influenza susceptibility between HIV-infected and HIV-uninfected adults**

| Author, year of publication | Country | N | Period | CD4 count (cells/µl) | Influenza virus strain | Outcome | Outcome frequency |
|---|---|---|---|---|---|---|---|
| ***Seasonal influenza*** | | | | | | | |
| Cohen & Macauley, 1989 | United States | 45 | Mar 1988 | NS | A(H1N1) | ILI – fever, chills, myalgia, sore throat & productive cough | HIV+ 0/7 (0%)<br>HIV- 14/38 (37%) |
| Fine et al., 2001 | United States | 133 | Nov-Dec 1996 | 92% CD4 <300<br>Median 141, range 6-437 | A(H3N2) | ILI - temp ≥37.8°c + cough or sore throat OR ≥1 respiratory symptom + laboratory-confirmed influenza | HIV+ 38/73 (52%)<br>HIV- 15/60 (25%) |
| Boschini et al, 2006 | Italy | 1311 | Feb-Mar 2004 | 87% CD4 >200 | A(H3N2) | ILI – Fever (>38°c) + ≥1 constitutional symptom + ≥1 respiratory symptom | HIV+ 44/171 (26%)<br>HIV- 165/1140 (14%)<br>**RR 1.77 (1.32-2.37)** |
| ***Pandemic infl.uenza A(H1N1)*** | | | | | | | |
| Campos-Loza et al., 2010 | Mexico | 832 | Apr-Jun 2009 | NS | A(H1N1)pdm09 | Laboratory-confirmed influenza (rRT-PCR) | HIV+ 11/20 (55%)<br>HIV- 120/812 (15%)<br>**OR 7.0 (2.8-17.3)** |
| Martinez et al., 2011 | Spain | 2106 | Apr-Dec 2009 | 5% CD4 <200 | A(H1N1)pdm09 | Laboratory-confirmed influenza (rRT-PCR) | HIV+ 56/623[a] (9%) |
| Althoff et al., 2011 | United States | 1790 | Mar-Sep 2009 | 39% CD4 <200 | A(H1N1)pdm09 | Seroincidence[b] | Incidence (per 100 PYs):<br>HIV+ 33.4 (26.4-42.3)<br>HIV- 29.7 (21.3-41.3)<br>**aIRR 1.20 (0.79-1.82)** |
| Hung et al., 2013 | Taiwan | 1497 | Sept-Nov 2009 | 11% CD4 <200<br>Median - 446 | A(H1N1)pdm09 | Seroprevalence[c]<br>Seroincidence[d] | Seroprevalence:<br>HIV+ 137/931 (14.7%)<br>HIV- 192/566 (33.9%)<br>Seroincidence:<br>HIV+ 85/907 (9.4%)<br>HIV- 44/429 (10.3%) |
| Garg et al., 2014 | Thailand | 368 | May 2008-May 2009<br>Apr-May 2010<br>Jan-Mar 2011 | NS | A(H1N1)pdm09 | Seroincidence | HIV+ 55/157 (35%)<br>HIV- 71/211 (34%) |

NS, not stated; ILI, influenza-like illness; RR, risk ratio; rRT-PCR, real-time reverse transcriptase polymerase chain reaction; OR, odds ratio; HAI, haemagglutination inhibition assay; aIRR, adjusted incidence rate ratio.

[a] HIV status of all patients tested (n=2106) not described in the paper

[b] ≥4-fold increase in HAI titres

[c] HAI titre > 1:40

[d] ≥4-fold increase in HAI titres or conversion from seronegative to HAI titres >1:40 in 2 sequential serum samples

rather than laboratory-confirmed influenza, was the outcome in all three studies. Overall, data on the impact of HIV infection on susceptibility to seasonal influenza are limited.

**2009 Pandemic influenza A(H1N1).** The 2009-2010 A(H1N1) influenza pandemic provided an opportunity to further investigate susceptibility to influenza infection by HIV status. Five studies compared rates of influenza A(H1N1)pdm09 infection between HIV-infected and HIV-uninfected adults; two based on PCR testing and three on serology (Table 1.9). At an emergency department in Mexico City, 11/20 (55%) HIV-infected and 120/812 (15%) HIV-uninfected patients attending with ILI were positive for A(H1N1)pdm09 ($p<0.001$) (Campos-Loza et al., 2010). Of note, the HIV-infected patients were older (median age, 47 vs. 36 years, $p=0.12$), and more likely to be a smoker (63 vs. 13%, $p=0.001$). A case series of adults presenting with acute respiratory illness in Barcelona, Spain found that 56/623 (9%) with confirmed influenza A(H1N1) infection were HIV-positive (Martinez et al., 2011). This suggested overrepresentation of HIV-infected patients as the proportion far exceeded the HIV prevalence in Catalonia (0.6%) (Centre d'Estudis Epidemiològics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya (CEEISCAT), 2008) . Similar to the Mexican study, HIV-infected patients were older (mean age, 44 vs. 39 years, $p=0.015$), more frequently male (79 vs. 44%, $p<0.001$), and more likely to smoke (54 vs. 13%, $p=0.001$) than the HIV-uninfected patients, but were less likely to be pregnant (0 vs. 11%, $p=0.008$) or have chronic lung disease (5 vs. 26%, $p<0.001$) (Martinez et al., 2011).

Three serology studies, conducted in the US, Taiwan and Thailand, found no association between HIV status and seroprevalence or seroincidence of pandemic influenza A(H1N1). Althoff et al. (Althoff et al., 2011) demonstrated no difference in seroincidence of A(H1N1)pdm09 infection (defined as a $\geq$4-fold increase in A(H1N1)pdm09 hemagglutination inhibition (HAI) antibody titre in serum samples collected between March and September 2009, compared with the same months in 2007 or 2008) between HIV-infected women with CD4 $\geq$350 cells/µl and HIV-uninfected women (incidence rates of 33.3 (95%CI 26.4-42.3) and 29.7 (95% CI, 21.3-41.3) per 100 person years (PYs), respectively. Comparatively fewer seroconversion events occurred in HIV-infected individuals with CD4 cell count <350 cells/µl in this study (16.9/100 PYs), which the authors suggested might have been due to an inability to mount an adequate antibody response in this group thus infections may have been missed (Althoff et al., 2011). A Taiwanese study found a significantly lower seroprevalence (HAI titre >1:40) in HIV-infected individuals, compared to HIV-uninfected patients (14.7 vs. 33.9%, $p<0.001$), though seroconversion rates were similar (85/907 (9.4%) and 44/429, 10.3%), in HIV-infected and HIV-uninfected patients respectively, $p=0.60$) (Hung et al., 2013). Of note, being heterosexual and ART treatment was associated with seropositivity. The authors suggested the former could be related to increased exposure to children with influenza, whilst the

latter may be explained by the recovery of memory B-cell subpopulation associated with ART (Hung et al., 2013).Lastly, Garg *et al.* (Garg et al., 2014) collected paired serum specimens from 368 Thai men who have sex with men (MSM) before and during the 2009 influenza A(H1N1) pandemic, and found similar seroincidences among HIV-infected (n=157) and HIV-uninfected (n=211) adults (35 vs. 34%, p=0.78).

In summary, the limited and heterogeneous available data do not suggest greater susceptibility to seasonal or pandemic influenza infection in HIV-infected adults than in HIV-uninfected persons. The role of CD4 cell count in influenza susceptibility remains unclear. Other risk factors for influenza infection, such as smoking, are often more prevalent in HIV-infected individuals (Arcavi and Benowitz, 2004), and need to be accounted for in studies assessing the relationship between HIV and influenza susceptibility.

#### *1.4.2.2 Low- or middle-income countries*

Largely due to a lack of diagnostic capability, few studies in high HIV prevalence settings, such as SSA, have estimated the incidence of influenza disease (Chapter 1.2.3.2), or evaluated the impact of HIV infection on influenza burden. Although influenza surveillance capacity on the African continent has expanded considerably over the past decade (Radin et al., 2012), few published studies have reported HIV status. Only two SSA studies have studied the impact of HIV infection on the burden of influenza disease, both from population-based surveillance. Feikin *et al.* (Feikin et al., 2012b) found that HIV-infected Kenyan adults with acute respiratory infection had a greater burden of both influenza A (incidence rates 4.49 [95%CI 2.80-6.18] vs. 1.42 [95%CI 1.04-1.80] per 100 person-years of observation (PYO) and influenza B (incidence rate 0.92 [95%CI 0.32-1.52] vs. 0.44 [95%CI 0.25-0.63] per 100 PYO) compared to HIV-uninfected adults. Cohen *et al.* (Cohen et al., 2013) also reported a 4-8 times higher incidence of influenza-associated acute lower respiratory tract infection (LRTI) in HIV-infected persons in South Africa compared with HIV-uninfected persons. Both studies were predominantly comprised of children ((Feikin et al., 2012b) 59% 5-17 years; (Cohen et al., 2013) 53% <5 years), and had considerable missing data on HIV status. Furthermore, only 36% of eligible patients in the Kenyan study had viral testing (Feikin et al., 2012b).

### 1.4.3 Severity

#### *1.4.3.1 Defining severity*

Accurate attainment of influenza-associated mortality is difficult; influenza infection is rarely confirmed by laboratory diagnosis, and influenza-related deaths are often attributed to co-morbid conditions or secondary infections. Consequently, severity of influenza disease is often inferred from

hospitalisation, duration of hospital stay, admission to intensive care unit, or requirement for mechanical ventilation.

#### *1.4.3.2 High-income countries*

**Seasonal influenza.** Early studies on the severity of influenza illness in HIV-infected adults were predominantly based on anecdotal reports or ecological studies. Several case series early in the HIV epidemic described severe clinical presentation and prolonged hospitalisation among HIV-infected individuals admitted with influenza, but did not include HIV-uninfected comparators (Thurn and Henry, 1989; Safrin et al., 1990; Radwan et al., 2000). An ecological study from the US noted a correlation between trends in pneumonia-attributable deaths among 25–44 year-old adults and influenza seasonality in cities with high incidence of acquired immunodeficiency syndrome (AIDS) (1988).

Several other US studies conducted before the advent of ART described excess influenza-associated morbidity and mortality in HIV-infected compared with HIV-uninfected persons (Table 1.9) (Neuzil et al., 1999; Lin and Nichol, 2001; Fine et al., 2001). Neuzil *et al.* (Neuzil et al., 1999) compared the incidence rates of acute cardiopulmonary hospitalisations and deaths among women enrolled in a Medicaid programme in the US between 1974 and 1993 during and around influenza seasons. Among those with high-risk conditions, HIV-infected women had the highest influenza-attributable risk of acute cardiopulmonary events (152 per 10,000 person-months, 95%CI 34-279). However, confidence intervals overlapped with estimates for other high-risk conditions, as HIV prevalence was only 0.02% (Neuzil et al., 1999). Lin *et al*. (Lin and Nichol, 2001) estimated excess deaths due to pneumonia or influenza in persons aged >13 years with AIDS from Cause-of-Death data from the National Center for Health Statistics. Patients with AIDS were found to have a 8.5 to 10.3-fold excess mortality attributable to pneumonia or influenza compared to the general US population, and 106-161 times higher when restricted to adults aged 25–54 years (Lin and Nichol, 2001). It is notable that neither study accounted for other respiratory infections, e.g. pneumococcal pneumonia, which likely contributed to excess hospitalization and death in this population. Moreover, CD4 cell count in HIV-infected populations was not available. The third study, the aforementioned outbreak investigation at a US drug rehabilitation centre (Chapter 1.4.2.1) (Fine et al., 2001), found more severe ILI presentation (as defined by illness duration >14 days, requiring an emergency department visit, or hospitalization) in HIV-infected, compared to HIV-uninfected residents (8/38 (21%) vs. 0/15). However, all 8 HIV-infected individuals with severe presentation were smokers, and 3 had additional co-morbidities, thus the independent effect of HIV on influenza severity was undeterminable.

**Table 1.9 Studies that have evaluated severity of seasonal influenza between HIV-infected and HIV-uninfected adults in high-income countries**

Adapted from Sheth et al. 2011a

| Author, year of publication | Country | Study population | Period | Outcome | Outcome frequency |
|---|---|---|---|---|---|
| Neuzil et al., 1999 (Neuzil et al., 1999) | United States | Women (15-64 years) enrolled in Tennessee Medicaid programme | 1974-1993 | Influenza-attributable rates of acute cardiopulmonary hospitalisation and death during influenza season per 10,000 person-months of observation | Women with high risk (including HIV):<br>15-45 years: 10.3<br>45-64 years- 26.4<br>Women with low risk:<br>15-45 years: 2.0<br>45-64 years- 2.9 |
| Lin & Nichol, 2001 | United States | Persons with AIDS vs. general population ≥13 years from Multiple cause-of-death data | 1991-1994 | Deaths due to pneumonia & influenza (P&I) during influenza seasons per 10,000 person-years of observation | Persons with AIDS: 9.4-14.7<br>General population: 1.1-1.2 |
| Fine et al., 2001 | United States | 133 residents & staff at drug rehabilitation facility during an influenza A outbreak | Nov-Dec 1996 | Severe influenza – prolonged illness (duration ≥14 days), required hospital evaluation or admission. | Prolonged illness:<br>HIV+ 6 (16%), HIV- 0<br>Emergency room evaluation:<br>HIV+ 3 (8%) HIV- 0<br>Hospitalisation:<br>HIV+ 2 (5%) HIV- 0 |
| Skiest et al, 2001 | United States | 43 cases of laboratory-confirmed influenza diagnosed at an HIV clinic | 1997-1999 | Influenza-associated pneumonia | Pneumonia: 7 (16%)<br>Mean CD4 340, viral load 3.34 $\log_{10}$ copies/ml<br>No pneumonia: 36 (84%)<br>Mean CD4 346, viral load 3.25 $\log_{10}$ copies/ml |
| Neuzil et al., 2003 | United States | HIV+ adults (15-50 years) enrolled in Tennessee Medicaid programme | 1995-1999 | Influenza-attributable hospitalisation rates during influenza seasons | 1995-96 (pre-ART): 48/1000 persons<br>1996-99 (ART): 5/1000 persons |
| Boschini et al., 2006 | Italy | 44 HIV+ and 154 HIV- residents at drug rehabilitation facility during an influenza A outbreak with ILI | Feb-Mar 2004 | ILI-related complications – e.g. pneumonia, bronchitis, sinusitis, meningitis, pharyngitis and tracheitis. | Risk of complication related to ILI<br>HIV+ OR 5.13 (95% CI 2.52-10.20) |

AIDS, acquired immunodeficiency syndrome; ART, highly active antiretroviral therapy; ILI, influenza-like illness.

Since the widespread introduction of ART, several more studies have assessed the relationship between HIV and influenza severity. In a case series of 43 HIV-infected adults with laboratory-confirmed influenza from an HIV clinic in Texas, 7 (16%) had pneumonia (Skiest et al., 2001). The majority of patients had a CD4 cell counts of >200 cells/µl (74%) and were on ART (42/43). No significant difference in CD4 count or viral load were noted in patients with, and without pneumonia (Skiest et al., 2001). A second study by Neuzil *et al* (Neuzil et al., 2003) using the Medicaid database described a dramatic decline in influenza-attributable hospitalizations following the introduction of ART (from 48 to 5 hospitalisations per 1000 persons, for 1995 and 1996-1999 respectively), but CD4 count was not reported. Lastly, in the above-mentioned outbreak investigation at the drug treatment centre in Italy (Chapter 1.2.3.2) (Boschini et al., 2006), HIV-infected residents with ILI had a higher risk of complications (such as pneumonia, bronchitis, otitis and meningitis) than HIV-uninfected residents (OR 5.13, 95% CI: 2.52-10.20), though CD4 cell count and viral load were not associated with the risk of complications (Boschini et al., 2006).

Although a number of studies, mostly from the pre-ART or early-ART era, suggest increased severity and mortality from seasonal influenza among HIV-infected individuals, these data should be cautiously interpreted. The case series were limited by small sample sizes, and failed to control for confounders, while results from ecological studies are prone to ecological fallacy.

**2009 Pandemic influenza A(H1N1).** Initial reports on the 2009 H1N1 pandemic from North America and Europe found that the proportion of patients hospitalized with A(H1N1)pdm09 that were HIV-infected (1-3.4%) (Kumar et al., 2009; Louie et al., 2009; Fuhrman et al., 2010; Peters et al., 2011) exceeded the prevalence of HIV infection in those regions (<0.5%) (UNAIDS, 2013). However, selection bias is possible since HIV-infected individuals were more likely to be tested and admitted if they presented with illness.

Two cases series of HIV-infected patients had contrasting findings. Perez *et al.* (Perez et al., 2010) described mild clinical presentation in 30 Chilean HIV-infected patients with confirmed or suspected influenza A(H1N1)pdm09 infection. Most were on ART (93%) and with a mean CD4 cell count of 423 cells/µl. Only 3 patients required hospital admission, and none died. The estimated incidence of 1.74% was similar to that of the general population of Chile (2.1%) (Perez et al., 2010). In contrast, a study from Mexico reported high rates of hospitalisation (n=16, 53%) and death (n=6, 20%) among 30 HIV-infected patients (median CD4 cell count 163 cells/µl) with confirmed influenza A(H1N1)pdm09 infection (Ormsby et al., 2011). Of note, 40% (n=12) had a concurrent opportunistic infection (OI); this sub-group had significantly lower median CD4 count (87 vs. 294 cells/µl, p=0.007), and a more complicated clinical course, as indicated by more prolonged admission (p=0.001), higher requirement for mechanical ventilation (p=0.009) and higher mortality (p=0.03) (Ormsby et al.,

2011). These findings suggest that influenza may be more severe in HIV-infected patients with advanced HIV disease. Conversely, the severity of presentation may have been attributable to the OI rather than influenza.

Studies that have compared the severity of clinical presentation of A(H1N1)pdm09 between HIV-infected and HIV-uninfected persons have demonstrated similar clinical outcomes (Table 1.10). Campos-Loza *et al.* (Campos-Loza et al., 2010) reported similar rates of hospitalization (27 vs. 25%, p=0.81), severe disease (9 vs. 7%, p=0.68), and death (9 vs. 5%; p=0.86) in HIV-infected and HIV-uninfected persons who presented to an emergency room in Mexico city with laboratory-confirmed pandemic influenza A(H1N1) infection. A study from Singapore that compared 11 HIV-infected persons (cases; median CD4 cell count 223 cells/ µl; 82% on ART) to 66 HIV-negative individuals (no comorbid conditions (n=33); with ≥1 comorbid condition (n=33)) with confirmed A(H1N1)pdm09 infection found no significant differences between cases and either control groups with regards to duration of illness or hospitalisation, and proportion of patients that required hospitalization or intensive care unit (ICU) support (Isais et al., 2010). A multi-centre Spanish study that included 26 HIV-infected (median CD4 count 604 cells/µl; 84% had undetectable viral load) and 559 HIV-uninfected patients who were hospitalised with influenza A(H1N1)pdm09 also demonstrated similar clinical outcomes in the two groups (Riera et al., 2010). Another study of Spanish adults hospitalised with pandemic A(H1N1) found that respiratory complications, such as pneumonia (9 vs. 25%, in HIV-infected and HIV-uninfected groups respectively) and respiratory failure (9 vs. 21%, respectively) were less common in the HIV-positive group (Martinez et al., 2011). Finally, comparable rates of ICU admission (29 vs. 34%) and death (13 vs. 13%) were found among HIV-infected and HIV-uninfected adults hospitalised with pandemic A(H1N1) in the US (Peters et al., 2011).

Two large multi-country reports described the prevalence of immunosuppression among hospitalised and fatal cases of confirmed A(H1N1)pdm09. The first, by the French Institute for public health surveillance (Institut de Veille Sanitaire), included 684 influenza-associated deaths in 28 countries. Underlying comorbidities were reported in 193 (28%) deaths, but HIV infection was not specifically listed (Vaillant et al., 2009). Sixteen patients had ‘‘immunodepression’’ and 19 had ‘‘infectious diseases other than flu’’. The majority of countries that contributed data to this report had low HIV infection prevalence (<1%). A second report that included 70,000 patients with laboratory-confirmed A(H1N1)pdm09 patients from 19 countries, found that “immunocompromised” patients had substantially increased risks of hospitalization (risk ratio (RR) 24.3, 95%CI 16.1-32.6) and death (RR 27.7, 95%CI 14.0-66.5), compared to patients with normal immune function (Van Kerkhove et al., 2011b). Unfortunately, specific evaluation of HIV as a risk factor was not possible as most of the countries that contributed data had low HIV prevalence (with

**Table 1.10 Reported rates of hospitalisation, ICU admission, mechanical ventilation and death by HIV status during the 2009 influenza A(H1N1) pandemic** (Sheth et al., 2011)

| Author, year of publication | Country | HIV prevalence | Study population | Total n(%) | | Hospitalisation n(%) | | ICU admission n(%) | | Mechanical ventilation n(%) | | Died n(%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | HIV+ | HIV- | HIV+ | HIV- | HIV+ | HIV- | HIV+ | HIV- | HIV+ | HIV- |
| Campos-Loza et al., 2010 | Mexico | 0.3% | HIV+ & HIV- adults with LCII | 11 | 94 | 3 (27) | 24 (25) | 1 (9) | 7 (7) | 1 (9) | 7 (7) | 1 (9) | 5 (5) |
| Isais et al., 2010 | Singapore | 0.2% | HIV+ adults & matched HIV- controls with LCII | 11 | 66 | 6 (55) | 36 (55) | 2 (0) | 2 (3) | NR | NR | 0 (0) | 0 (0) |
| Riera et al., 2010 | Spain | 0.5% | Hospitalised adults with LCII | 26 (4) | 599 | 26 (100) | 559 (100) | 3 (12) | 68 (12) | 2 (8) | 50 (9) | 0 (0) | 13 (2) |
| Martinez et al., 2011 | Spain | 0.5% | Adults presenting to ED with LCII | 56 (9) | 168 | 15 (27)* | 70 (42)* | NR | NR | 5 (9)* | 36 (21)* | 0 (0) | 3 (2) |
| Peters et al., 2011 | US | 0.45% | Hospitalised adults with LCII | 31 (3) | 877 | 31 (100) | 877 (100) | 9 (29) | 300 (34) | 6 (21) | 223 (28) | 4 (13) | 118 (13) |

NR, not reported; ICU, intensive care unit; LCII, laboratory-confirmed influenza infection

*p<0.05

the exception of South Africa) (Van Kerkhove et al., 2011b).

Lastly, a study from New York (HIV prevalence ~1.4% (New York City Department of Health and Mental Hygiene, 2014)) found that 7 (15%) of 47 confirmed deaths associated with 2009 H1N1 influenza had HIV infection (Lee et al., 2010). In a separate analysis comparing hospitalised patients with confirmed A(H1N1)pdm09 who died (n=28) and survived (n=95), deceased patients had a significantly higher proportion of immunosuppression (HIV infection not specified) than those who survived (29 vs, 3%; $p<0.05$); though it should be noted that other underlying conditions, such as diabetes and cardiovascular disease, were also more prevalent among those who died (Lee et al., 2010).

In brief, with the exception of Ormsby *et al.* (Ormsby et al., 2011), which included patients with advanced immunosuppression, most studies with well-controlled HIV populations did not find increased severity and mortality related to pandemic influenza A(H1N1) in HIV-infected individuals.

#### *1.4.3.3 Low- or middle-income countries*

Contrary to the studies described above, a number of studies derived from countries with high prevalence of HIV infection strongly suggest increased severity and risk of death among HIV-infected individuals (Table 1.11). A retrospective case control study conducted within a hospital-based influenza surveillance programme in rural Kenya observed a greater risk of influenza-associated hospitalisation in HIV-infected persons compared with HIV-uninfected adults (adjusted odds ratio 3.56, 95% CI 1.25-10.07) (Ope et al., 2011). A hospital-based influenza surveillance study in South Africa between 2009 and 2011 found that HIV-infected individuals had more prolonged hospital stay (>7 vs. <2 days, OR 4.5, 95% 2.1-9.5) and were more likely to die (OR 3.9, 95%CI 1.1-14.1) (Cohen et al., 2013). However, HIV status was only available for 78% of patients who had a positive influenza PCR, and only a third of HIV-infected individuals had available CD4 cell count data (60% had CD4 cell count <200 cells/µl) (Cohen et al., 2013).

A case series of 91 adult deaths due to influenza A(pdm09) in South Africa found that 17 (53%) of 32 patients with known serostatus were HIV-infected (Archer et al., 2009), which was substantially higher than the national HIV prevalence of 17.9% (UNAIDS, 2013). Data on CD4 cell counts and ART status were not available. Additionally, among the fatal cases, 10% had tuberculosis and over a quarter were pregnant. The relative contribution of HIV-associated immunosuppression to these deaths is therefore difficult to discern.

Another South African study modelled seasonal and pandemic influenza-associated deaths among persons aged ≥5 years between 1998 and 2009 using monthly surveillance and mortality data (Tempia et al., 2015b). HIV-infected persons aged 5-64 years had an excess risk of influenza-

**Table 1.11 Studies that have evaluated severity of seasonal influenza between HIV-infected and HIV-uninfected adults in low- or middle income countries**

| Author, year of publication | Country | Study population | Period | CD4 (cells/µl) & ART status | Severity outcome | Effect of HIV |
|---|---|---|---|---|---|---|
| Ope et al., 2011 | Kenya | 64 Cases: Influenza-related hospitalization in persons aged ≥5 years<br>190 Controls: Age-, sex-, and neighbourhood-matched controls | 2006-2008 | HIV+: cases 24.5%, controls 12.5%<br>Mean CD4: cases 399, controls 387 | Influenza-associated hospitalisation | aOR[a] 3.56 (95%CI 1.25-10.1) |
| Cohen et al., 2013 | South Africa | 11925 individuals (aged >2 days) enrolled with hospitalised acute LRTI | 2009-2011 | 60% CD4 <200[b] | Prolonged hospital stay (>7 vs. <2 days)<br>Death | aOR[c] 4.5 (95% 2.1-9.5)<br>aOR[c] 3.9 (95%CI 1.1-14.1) |
| Archer et al, 2009 | South Africa | 91 adults deaths with confirmed influenza A(H1N1) | 2009 | NR | Death | 17/32 (53%) with known HIV status |
| Tempia et al., 2015 | South Africa | Persons aged >5 years who died | 1998-2009 | NR | Influenza-associated mortality rate | Age-adjusted relative risk: 7.9 (7.1-8.9) |
| Cohen et al., 2015a | South Africa | 1376 individuals with influenza-associated SARI | 2009-2013 | 50% on ART[d] | Case fatality proportion<br>Mortality incidence | HIV+ 22/419 (5%)<br>HIV- 13/620 (2%)<br>HIV+ 24.8/100000 PYs<br>HIV- 1.7/100000 PYs<br>**IRR 20.4 (95%CI 15.0-27.8)** |

CI, confidence interval; LRTI, lower respiratory tract infection; aOR, adjusted odds ratio; NR, not reported; SARI, severe acute respiratory infection; PYs, person years; IRR, incidence rate ratio.

[a]Adjusted for chronic lung disease, HIV status, cattle and chicken ownership.

[b]Available for 33% HIV-infected individuals

[c]Adjusted for age, underlying medical condition excluding tuberculosis and HIV, pneumococcal co-infection, influenza type, duration of hospitalisation and death.

[d]Available for 50% HIV-infected individuals

associated death compared to HIV-uninfected persons (age-adjusted relative risk: RR 7.9, 95%CI 7.1-8.9). Case fatality proportion (CFP) was higher in HIV-infected, compared with HIV-negative individuals (22/419 (5%) vs. 13/620 (2%) respectively, p=0.006) enrolled with influenza-positive SARI in a surveillance programme in South Africa between 2009 and 2013 (Cohen et al., 2015a). CD4 cell count was available for <30% HIV-infected individuals; CFP was higher in those with severe immunosuppression (CD4 <200 cells/μl) compared to those without severe immune compromise (16 vs. 4% respectively, p=0.012). Among 251 (59%) HIV-infected individuals with data on ART status, 126 (50%) reported receiving ART, but no difference in CFP was observed among those receiving or not receiving ART (5 vs. 7%, p=0.39). Adjusting for age, mortality rate was 20 times (95%CI 15.0-27.8) higher in HIV-infected individuals than HIV-uninfected individuals (Cohen et al., 2015a).

In studies undertaken high HIV prevalence settings, a clear association between HIV infection and influenza-associated severe morbidity and mortality is emerging. Nevertheless, studies with comprehensive HIV data, and further understanding of the impact of CD4 and ART treatment are needed.

### 1.4.4 Shedding

The quantity of viral shedding is generally accepted as a proxy measure of infectiousness (Bell et al., 2006), thus comparison of influenza virus shedding between HIV-infected and HIV-uninfected individuals would help inform infection control measures and influenza prevention and treatment guidelines for this population. Data comparing influenza virus shedding in adults with and without HIV infection have yet to be published. In healthy individuals, the average duration of viral shedding is around 5 days, but can last up to 10 days (Carrat et al., 2008). Studies on patients with other forms of immunosuppression suggest that prolonged shedding of seasonal influenza virus (Klimov et al., 1995; Boivin et al., 2002; Weinstock et al., 2003; Gooskens et al., 2009) and influenza A(H1N1)pdm09 virus (2009b; Hill-Cawthorne et al., 2010) can occur for months, and has been shown to be associated with antiviral resistance.

A pilot study that compared influenza viral load by rRT-PCR serially up to 28 days patients with ILI was presented at the Options for the Control of Influenza VIII Conference (Hellferscee et al., 2013). No differences were observed in the duration (median 9 days, range 2-14 days) and magnitude of influenza virus shedding between enrolled HIV-infected (n=2) and HIV-uninfected (n=9) patients. However, the lack of association may be due to the small number of patients enrolled. The final results of this ongoing study are awaited.

### 1.4.5 Transmission

Few studies have evaluated the impact of HIV infection on influenza transmission dynamics. A study from a population-based influenza surveillance programme in an urban slum in Nairobi, Kenya, compared rates of ILI among household contacts of HIV-infected individuals, and household members of exclusively HIV-negative households (Wong et al., 2015). The presence of an HIV-infected person in the household was found to be a risk factor for ILI in non-HIV-infected household contacts (RR 1.44, 95%CI 1.06-1.94), and the risk increases with the number of HIV-infected persons in the household (Wong et al., 2015). However, this analysis did not account for the HIV status of the household contacts. Moreover, almost two-thirds of adults in households labelled as exclusively HIV-negative were not tested, which would have resulted in misclassification.

A retrospective cohort study of 176 influenza index cases and their 874 household contacts from the same surveillance programme in Kenya, examined the association between the HIV status of influenza index cases and the risk of developing secondary ILI among household contacts (Judd et al., 2015). Household contacts of HIV-infected influenza index cases were twice as likely to develop ILI, compared to household contacts of HIV-negative index cases (adjusted relative risk 2.36, 95%CI 1.19-4.66) (Judd et al., 2015). However, being an HIV-infected household contact of the index case was not significantly associated with the development of secondary ILI, compared with being an HIV-negative household contact. Influenza virus shedding data for the index cases was not available to substantiate the authors' hypothesis that HIV-infected index cases may shed virus for longer and at higher titres. Secondly, HIV status was not available for 62% index cases and 48% household contact, thus limiting the representativeness of the study population if those who were tested were systematically different from those who were not tested.

### 1.4.6 Summary

The impact of HIV infection on the susceptibility and severity of influenza illness appears to differ in low HIV-prevalence and HIV-prevalence settings. In regions with low prevalence of HIV infection, HIV infection does not appear to significantly increase susceptibility or severity to seasonal influenza infection, though many of the included studies were anecdotal reports or ecological studies that did not account for potential confounders such as smoking or comorbidities. Several studies suggest that a greater proportion of persons hospitalized with confirmed H1N1pdm virus infections are HIV-infected compared with the general population. However, increased propensity to test and hospitalise HIV-infected individuals who present with illness may result in overrepresentation of HIV-infected persons in the studies. Among patients hospitalized with pandemic influenza A(H1N1) in low HIV prevalence settings, severity of presentation and clinical outcomes appear to be equivalent among HIV-infected

and HIV-uninfected adults, especially in settings where the majority of patients are receiving ART and do not have severe immunosuppression.

In contrast, data from one Mexican study that included a high proportion of patients with advanced HIV disease (Ormsby et al., 2011), and studies emerging from countries with high HIV prevalence (Feikin et al., 2012b; Cohen et al., 2013; Ope et al., 2011; Archer et al., 2009; Tempia et al., 2015b) suggest that influenza susceptibility, severity, as well as risk of death may be increased among HIV-infected persons. This is likely due to more advanced immunosuppression, poorer virological control, in addition to a higher prevalence of other comorbidities, such as tuberculosis. To date, the majority of studies from high HIV prevalence settings have derived from South Africa, and information on CD4 cell count and ART status have been incomplete.

Further prospective studies in high HIV prevalence settings that comprehensively record CD4 cell counts, ART use, and co-morbid conditions are therefore needed, to evaluate the impact of these factors on the relationship between HIV infection and severity of influenza. Such data may allow policymakers to make informed decisions about targeted annual influenza immunization, which are currently unavailable in many high HIV prevalence countries (Chapter 1.5).

## 1.5 Influenza Vaccination in HIV-infected Adults

### 1.5.1 Current guidelines

From Chapter 1.4, the limited available evidence suggests that HIV-infected individuals experience greater susceptibility and severity of influenza illness compared to the general population, particularly in high HIV prevalence settings. Hence annual seasonal influenza vaccination is recommended for HIV-infected individuals by many national immunisation guidelines, including the UK (Geretti et al., 2008; National Advisory Committee on Immunization (NACI), 2014; Grohskopf et al., 2015). In South Africa, the only sub-Saharan African country with published immunisation guidelines, inactivated vaccines are recommended in HIV-infected individuals with CD4 cell count >100 cells/μL (Walaza, 2014).

As described in Section 1.1.5.1, two types of influenza vaccines are available: inactivated and live-attenuated vaccines (IIV & LAIV). As with other live virus vaccines, LAIV is not recommended in HIV-infected adults (Geretti et al., 2008; National Advisory Committee on Immunization (NACI), 2014; Grohskopf et al., 2015). HIV-infected persons are also advised to avoid close contact with anyone who has received LAIV for 7 (Grohskopf et al., 2015) to 21 (Geretti et al., 2008) days after receipt, due to the theoretical risk for transmission of the live-attenuated vaccine virus.

## 1.5.2 Evidence of efficacy in HIV-infected adults

### *1.5.2.1 Vaccine immunogenicity*

Vaccine-induced antibody responses are lower in HIV-infected persons compared to HIV-negative controls (Miotti et al., 1989; Kroon et al., 1994; Iorio et al., 1997; Fowke et al., 1997; Nelson et al., 1988; Amendola et al., 2001; Crum-Cianflone et al., 2011; Parmigiani et al., 2013; Yanagisawa et al., 2011; Nunes et al., 2015), and are correlated with CD4 count (Fowke et al., 1997; Fuller et al., 1999; Kroon et al., 2000; Tebas et al., 2010; Tiu et al., 2011; Yamanaka et al., 2005). Several studies examining the effect of ART on influenza vaccine immunogenicity have found higher seroprotection rates in patients on ART for 6 months or more compared to those not on treatment (Launay et al., 2011; Durier et al., 2013). Moreover, HIV-infected patients with CD4 counts >300 cells/μL while on ART appear to have humoral and cellular responses to influenza vaccination similar to those of healthy controls (Kroon et al., 1998).

The use of higher or booster doses of inactivated vaccines, in addition to adjuvanted preparations have been explored as strategies to enhance immunogenicity in HIV-infected persons. Additional or higher doses of inactivated vaccines (IIV3 and monovalent 2009 pandemic A/H1N1 (pAH1N1) vaccine) in HIV-infected adults have resulted in improved serconversion rates in some studies (Bickel et al., 2011; Nielsen et al., 2012; Santini-Oliveira et al., 2012; McKittrick et al., 2013; El Sahly et al., 2012; Cooper et al., 2012) but not in others (Cooper et al., 2011; Miotti et al., 1989; Kroon et al., 1994; Iorio et al., 1997; Hatakeyama et al., 2011; Lagler et al., 2012), though most of the studies that demonstrated no difference in immunogenicity were conducted in the pre-ART era (Miotti et al., 1989; Kroon et al., 1994; Iorio et al., 1997) or were non-randomised (Miotti et al., 1989; Kroon et al., 1994; Iorio et al., 1997; Hatakeyama et al., 2011; Lagler et al., 2012). A number of studies have also demonstrated improved humoral responses associated with adjuvanted seasonal IIV3 (Iorio et al., 2003) and pandemic A/H1N1 vaccine compared with non-adjuvanted preparations (Ho et al., 2011; Launay et al., 2011; Durier et al., 2013). Current guidelines recommend a single standard dose of IIV only (i.e. 15μg of haemagglutinin antigen per strain in each 0.5ml dose).

### *1.5.2.2 Clinical efficacy*

A recent Cochrane Review that included 116 studies on TIVs reported an overall efficacy of 60% [95% CI 53-66%] in preventing laboratory-confirmed influenza in healthy adults (see also Section 1.1.5.3) (Demicheli et al., 2014). In contrast, data on the clinical efficacy of influenza vaccination in HIV-infected adults are limited. A pooled clinical efficacy of 85% [95% CI 22-97%] was reported in a recent systematic review and meta-analysis of two randomised placebo-controlled trial (RCT) (Tasker et al., 1998; Madhi et al., 2011) and three observational studies (Fine et al., 2001; Ranieri et al., 2005;

Yamanaka et al., 2005) (Table 1.12 & 1.13). However, no significant effect on all-cause pneumonia, hospitalisation or mortality was found (Remschmidt et al., 2014). The majority of included studies were conducted in the pre-ART or early-ART era, with the exception of Madhi et al. (Madhi et al., 2011). Of note, this randomised trial of IIV3 in South African adults excluded patients with CD4 cell count <100 cells/μL and naïve to ART and those with co-morbidities (Madhi et al., 2011).

Only one RCT of IIV3 has been published in HIV-infected pregnant women. The study, which included 149 pregnant women from South Africa (mean age - 26 years), demonstrated a vaccine efficacy of 58% (95%CI 0.2-82%), but only 27% (p=0.60) among the infants of the participants (Madhi et al., 2014).

**Table 1.12 Characteristics of studies included in the systematic review on influenza vaccination in HIV-infected adults** (Remschmidt et al., 2014)

| Study, year of publication | Setting, country | Age (years) (mean or range) | N vaccinated CD4 count/μl | N unvaccinated CD4 count/μl | Vaccine type |
|---|---|---|---|---|---|
| ***Randomised placebo-controlled trial*** | | | | | |
| Tasker et al., 1999 | Outpatient military clinic, USA | 33 | 55<br>n=5 <200 | 47<br>n=8 <200 | Whole virion<br>A/Johannesburg/33/94<br>A/Texas/36/91<br>B/Harbin/07/94 |
| Madhi et al., 2011 | Outpatient clinic, South Africa | 34 | 255<br>Median 340[a] | 251<br>Median 356[a] | IIV3, Mutagrip® |
| ***Cohort study*** | | | | | |
| Fine et al., 2001[b] | Outbreak at residential facility, USA | 19-62 | 42<br>n=6 ≤100 | 29<br>n=8 ≤100 | A/Nanching/933/95<br>A/Texas/36/91<br>B/Harbin/07/94 |
| Ranieri et al., 2005 | Outpatient clinic, Italy | 20-69 | 90<br>n=12 <200 | 55<br>n=16 <200 | Virosomal adjuvanted, Inflexa V® |
| Yamanaka et al., 2005 | Outpatient clinic, Japan | 40-41 | 262<br>n=51 <200 | 66<br>n=9 <200 | Subunit<br>A/New Caledonia/20/99<br>A/Panama/2007/99<br>B/Shanton/7/87 |

[a]Patients naïve to antiretroviral therapy with CD4 cell count <100 per microlitre (μl) were excluded.

[b]Published as a case-control study, but only the cohort of HIV-infected persons were included.

**Table 1.13 Included studies in the systematic review on influenza vaccination in HIV-infected adults: Risk factors for influenza-related outcomes in vaccinated vs. non-vaccinated individuals** (Remschmidt et al., 2014)

| Study, year of publication | Risk ratio (95% confidence interval) | | | | | |
|---|---|---|---|---|---|---|
| | Laboratory-confirmed influenza | Influenza-like illness | Any respiratory illness | Pneumonia (all cause) | Hospitalisation (all cause) | Mortality (all-cause) |
| ***Randomised placebo-controlled trial*** | | | | | | |
| Tasker et al., 1999 | 0.04 (0.00-0.68) | - | 0.59 (0.36-0.99) | Not estimable[a] | Not estimable[a] | - |
| Madhi et al., 2011 | 0.24 (0.07-0.85) | 1.08 (0.47-2.50) | 0.84 (0.57-1.23) | 0.33 (0.01-8.02) | 0.98 (0.20-4.83) | 0.49 (0.04-5.39) |
| ***Cohort study*** | | | | | | |
| Fine et al., 2001 | - | 0.73 (0.47-1.13) | - | - | 0.23 (0.05-1.06) | - |
| Ranieri et al., 2005 | - | 0.22 (0.12-0.38) | - | Not estimable[a] | Not estimable[a] | - |
| Yamanaka et al., 2005 | 0.29 (0.15-0.56) | - | - | - | - | - |

[a]No cases

#### *1.5.2.3 Vaccine safety*

Inactivated influenza vaccine are safe and well tolerated in HIV-infected individuals (Zanetti et al., 2002), including pregnant women (Madhi et al., 2014; Abzug et al., 2013). As described in Section 1.1.5.2, injection site reactions are the most frequent side effects. Systemic side effects are uncommon, and include fever, malaise, myalgia, arthralgia and allergic reactions (most likely due to hypersensitivity to residual egg protein). Guillain–Barré syndrome has been reported very rarely; no association with seasonal or H1N1 pandemic vaccine has been found (Demicheli et al., 2014).

There have been concerns that vaccine-induced CD4 cell activation might promote HIV replication (Clouse et al., 1989). Minor CD4 count and viral load fluctuations have been described following vaccination with IIV3 (Ho, 1992; O'Brien et al., 1995; Rosok et al., 1996; Staprans et al., 1995; Fuller et al., 1999; Gunthard et al., 2000; Tasker et al., 1998) and adjuvanted pA/H1N1 vaccine (Calmy et al., 2012; Kelly et al., 2012), but not thought to be clinically relevant. No negative long-term sequelae have been demonstrated (Sullivan et al., 2000).

### 1.5.3 Summary

IIV is safe, and is recommended for HIV-infected individuals in many developed settings. Limited available evidence suggest that it is immunogenic and efficacious among HIV-infected individuals without significant immunosuppression. However, further studies are needed to evaluate the immunogenicity and clinical efficacy of IIV among HIV-infected individuals with advanced immunosuppression (CD4 cell count <100 cells/µl) and those with co-morbidities, which will be applicable to a substantial proportion of the HIV-infected population in SSA.

## 1.6 Interactions between respiratory viruses and *Streptococcus pneumoniae*

### 1.6.1 Overview

The first observation that viral infections predisposed to bacterial pneumonia was made by René Laënnec, the French physician who invented the stethoscope, in 1803 (Laënnec, 1819). He noted an increase in the prevalence of pneumonia following an influenza epidemic. It is now recognised that a substantial proportion of influenza-related morbidity and mortality is due to concurrent or secondary bacterial infection, particularly with *Streptococcus pneumoniae* and *Staphylococcus aureus* (Morens et al., 2008). Other respiratory viruses, including adenovirus, rhinovirus, respiratory syncytial virus (RSV), and parainfluenza viruses may also predispose to secondary infections, but

evidence of such interactions is lacking. In this chapter, current literature on the association between respiratory viruses, with a predominant focus on influenza, and S. *pneumoniae* infection will be reviewed.

### 1.6.2 The epidemiology of respiratory viruses and *Streptococcus pneumoniae*

#### *1.6.2.1 Pandemic influenza*

The devastating impact of influenza and pneumococcal co-infection was brought into focus by the 1918 influenza pandemic, which was associated with up to 50 million deaths globally (Chapter 1.1.4.3). Recent reviews of epidemiological, histological, and microbiological evidence from the pandemic suggest that the majority of severe illness and deaths were attributable to secondary bacterial pneumonia (Morens et al., 2008; Brundage and Shanks, 2007). Most deaths occurred 7 to 14 days after onset of influenza symptoms (Mills et al., 2004). Histopathological examination of lung tissue sections from 68 influenza victims demonstrated evidence of severe acute bacterial pneumonia in most cases. In the same study, over 90% lung tissue from 96 fatal cases were culture-positive for ≥1 bacterium; S. *pneumoniae* was the most frequently isolated organism, followed by *Streptococcus pyogenes* and S. *aureus* (Morens et al., 2008). Moreover, a review of lung-tissue and autopsy studies found that bacteria were more likely to be isolated from patients with influenza-associated pneumonia (mean 16%), particularly in those who died (40%), than those without pneumonia (<1%) (Chien et al., 2009). S. *pneumoniae* comprised 71% of positive cultures.

The subsequent pandemic in 1957-58 was associated with lower mortality. Nevertheless, pneumonia accounted for a higher proportion of deaths in 1957-58 (44%) than in the preceding inter-pandemic years (~20%, range 4–44%) (Trotter et al., 1959; Dauer, 1958; Collins and Lehmann, 1951). *S. aureus* was the predominant bacterium identified in pneumonia patients in this pandemic (Hers et al., 1958; Robertson et al., 1958; Martin et al., 1959). The shift in microbiological aetiology may have been due to temporal changes in bacterial circulation, but may also have been a consequence of widespread penicillin use, resulting in fewer penicillin-susceptible organisms, including pneumococci. However, the predominant pathogen reverted to S. *pneumoniae* during the 1968 pandemic (Bisno et al., 1971; Burk et al., 1971), suggesting that antibiotics were not the sole cause for the microbiological changes. It is therefore more likely that strain-related differences in the virus or the bacterial co-pathogens are responsible for the aetiological shifts between pandemics.

The emergence of the 2009 pandemic influenza A(H1N1) provided a further opportunity to study the role of bacterial co-infections in influenza morbidity/mortality in the context of widespread use of broad-spectrum antibiotics. In studies of severe or fatal cases of A(H1N1)pdm09, bacterial co-infection was identified in 25 to 50% cases (2009a; Dominguez-Cherit et al., 2009; Estenssoro et al.,

2010; Mauad et al., 2010; Shieh et al., 2010), with S. *pneumoniae* and S. *aureus* as the commonest pathogens identified. Those with bacterial co-infection were associated with increased severity and mortality (Rice et al., 2012; Palacios et al., 2009; Dhanoa et al., 2011; Cohen et al., 2012 ).

#### *1.6.2.2 Seasonal influenza*

A number of ecological studies have evaluated the association between influenza circulation and all invasive pneumococcal disease (IPD) during non-pandemic periods, with variable findings. Several US surveillance studies reported significant correlation between influenza activity and IPD occurrence (Kim et al., 1996; Talbot et al., 2005). A Swedish study between 1994 and 2004 estimated that 6-10% of annual IPD burden were influenza-related, with a lag time of 1 to 3 weeks for IPD following peaks in influenza incidence (Grabowska et al., 2006). In contrast, Zhou *et al.* (Zhou et al., 2012a) found substantial variation in the strength of association of invasive pneumococcal pneumonia incidence with influenza activity across seasons from 1994-2005; strength of association was highest when A(H3N2) was the predominant strain (Zhou et al., 2012a). Recent studies from the US (Walter et al., 2010) and Canada (Kuster et al., 2011) found that seasonality and time lag of pneumococcal disease was only partially related to influenza seasonality. These findings suggest that the impact of influenza on the epidemiology of pneumococcal pneumonia may differ during pandemic and non-pandemic periods. However, the studies did not distinguish pneumococcal pneumonia from other forms of invasive disease, such as bacteremia without pneumonia. Moreover, the majority of studies did not control for the underlying seasonality of either pathogen. In temperate regions, peaks in influenza and S. *pneumoniae* disease activity occur in winter, but similar seasonality is also observed in a wide range of infectious, environmental, and social factors (Kim et al., 1996; Watson et al., 2006). Unadjusted analyses of seasonally-correlated phenomena could result in overestimation of temporal associations (Bowie and Prothero, 1981).

#### *1.6.2.3 Other respiratory viruses*

Due to the availability of nucleic acid amplification techniques for identifying respiratory viral infections (Chapter 1.3.2), data on the frequency and outcomes of co-infections that involve other respiratory viruses are emerging. Contemporary pneumonia aetiology studies suggest that co-infections with bacteria and respiratory viruses other than influenza, including adenovirus, rhinovirus, RSV, coronaviruses, and parainfluenza viruses, are common (4-28%) (Angeles Marcos et al., 2006; Jennings et al., 2008; Johnstone et al., 2008; Templeton et al., 2005; Luchsinger et al., 2013; Damasio et al., 2015).

With regards to the impact of co-infection on illness severity, studies from Sweden (Johansson et al., 2011) and the Netherlands (Templeton et al., 2005) found that mixed viral-bacterial infection was

correlated with a more severe presentation (pneumonia severity index (PSI) (Fine et al., 1997) risk class IV/V) in adults with CAP. In the Dutch study, severe pneumonia was significantly associated with rhinovirus as well as coronaviruses OC43 and 229E (Templeton et al., 2005). Jennings et al. (Jennings et al., 2008) demonstrated increased disease severity (CURB65 score >2 (Lim et al., 2003)and PSI class IV/V) specifically in mixed rhinovirus-pneumococcal co-infection among adults with CAP in New Zealand. A retrospective case control study from Korea also found that preceding viral infection, particularly with rhinovirus, hMPV, RSV and parainfluenza viruses, was associated with increased severity (higher PSI score) of pneumococcal pneumonia (Yoon et al., 2014).

Conversely, viral-bacterial co-infections was not associated with increased illness severity in adult pneumonia cohorts from Chile (Luchsinger et al., 2013) and Brazil (Damasio et al., 2015). Choi *et al.* (Choi et al., 2012) demonstrated a high prevalence of respiratory viruses in Korean adults with community- and hospital-acquired pneumonia requiring ICU admission (rhinovirus (24%), parainfluenza virus (21%), human metapneumovirus (hMPV) (18%), influenza virus (17%), and RSV (14%)), but patients with viral-bacterial co-infections had similar mortality to those with single agent infection.

#### *1.6.2.4 Limitations of diagnostic tests for Streptococcus pneumoniae*

One of the key limitations of epidemiological studies evaluating the association between respiratory viruses and S. *pneumoniae* is the absence of a gold standard for pneumococcal detection. Current pneumococcal diagnostic tests lack sensitivity (Klugman et al., 2008); diagnostic yield have been reported to be <30% for blood culture and 57% for sputum culture (Roson et al., 2000). The recent advent of the BinaxNOW S. *pneumoniae* antigen (BinaxNOW-SP, Alere, USA) shows promise. It is an immunochromatographic membrane test (ICT) that tests for the presence of pneumococcal C-polysaccharide in urine. A meta-analysis of 27 studies estimated a pooled sensitivity of 74% and specificity of 97% for the diagnosis of adult pneumococcal pneumonia using ICT (Sinclair et al., 2013). Similar sensitivity and specificity have been reported in HIV-infected adults (Boulware et al., 2007) and critically ill patients (Lasocki et al., 2006) from well-resourced settings. However, limitations of this assay include cross-reaction with certain alpha-hemolytic streptococci (*Streptococcus mitis* and *Streptococcus oralis*) (Klugman et al., 2008), and poor specificity in young children due to high rates of asymptomatic nasopharyngeal (NP) carriage (Dowell et al., 2001; Hamer et al., 2002). In contrast, specificity of ICT was unaffected in a study of Thai women with a high prevalence of pneumococcal carriage (25%) (Turner et al., 2011).

There is also growing interest in the use of quantitative molecular techniques to diagnose pneumococcal pneumonia. Quantitative real-time PCR (qRT-PCR) with specific gene targets, such as

pneumococcal autolysin (*lyt*A) (Carvalho Mda et al., 2007), has been used to measure bacterial load of S. *pneumoniae* in blood (Kee et al., 2008; Peters et al., 2009; Rello et al., 2009), sputum (Yang et al., 2005), and NP specimens (Albrich et al., 2012). Pneumococcal detection rates are reportedly 2-5 times higher using blood qRT-PCR compared to blood culture (Resti et al., 2010; Wolter et al., 2014a; Rello et al., 2009), and bacterial load appears to correlate with clinical severity and mortality (Rello et al., 2009; Wolter et al., 2014b). However, only 10-25% of adults with pneumococcal pneumonia are bacteraemic (Feldman and Anderson, 2011; Said et al., 2013b). Respiratory specimens are more accessible, though good quality sputum samples are difficult to obtain, and discrimination between infection and colonisation is challenging. Several studies have evaluated the clinical utility of qRT-PCR on NP specimens of adults with CAP. Albrich *et al.* (Albrich et al., 2012) demonstrated good correlation between quantitative lytA RT-PCR on NP swabs and quantitative NP cultures, and suggested that an NP pneumococcal density cut-off of >8000 copies/mL could differentiate between clinical disease and asymptomatic carriage. Adeldaim *et al*. (Abdeldaim et al., 2008) evaluated a qRT-PCR based on gene fragment Spn9802 on NP specimens, and reported a clinically significant cut-off of $10^4$ DNA copies/mL. The findings from these two studies support the hypothesis of a critical colonisation density (around $10^3$-$10^4$/mL), which when exceeded, leads to an increased risk of micro-aspirations and subsequent lower respiratory tract infection (Greenberg et al., 2011; Albrich et al., 2012).

Lastly, sequential infections are difficult to capture; the preceding influenza infection has often resolved, or viral shedding has ceased, by the time the patient presents with secondary bacterial pneumonia (Weinberger et al., 2012).

### 1.6.2 Vaccine probe studies

The introduction of a 7-valent pneumococcal conjugate vaccine (PCV7) has resulted in a significant decline in invasive disease caused by vaccine serotypes in vaccinated children, as well as impressive herd protection of unvaccinated adults (Centers for Disease Control and Prevention, 2005; Millar et al., 2008). This efficacious vaccine has been used as a probe to explore the role of *Streptococcus pneumoniae* in the aetiology of influenza-associated pneumonia (Simonsen et al., 2011; Madhi and Klugman, 2004). Children that received a 9-valent PCV (PCV9) in a randomised, double-blind trial in South Africa had a 45% lower hospitalisation rate of seasonal influenza-related pneumonia, compared to children that were not vaccinated (Madhi and Klugman, 2004). PCV9 was also associated with reductions in hospitalisations for pneumonia associated with adenovirus, RSV and parainfluenza 1-3 by 31% (Madhi and Klugman, 2004), and hMPV by 58% (Madhi et al., 2006). A US study also found that states with higher PCV vaccination coverage had significantly fewer influenza-

associated pneumonia hospitalisations among children (Simonsen et al., 2011). The impact of PCV on reducing the burden of confirmed virus-associated pneumonia provides further evidence of the role of superimposed pneumococcal infection as a cause of severe pneumonia.

### 1.6.3 Are respiratory viral infections required for pneumococcal transmission?

Since nasopharyngeal pneumococcal colonisation, the first step in the development of invasive disease (Gray et al., 1980; Bogaert et al., 2004), is asymptomatic, coryza related to viral upper respiratory infections may be crucial for the transmission of pneumococcus from the posterior nasopharynx (Klugman et al., 2009). A prospective study of factors associated with the spread of respiratory diseases among British families found increased pneumococcal colonisation during clinical upper respiratory illness (Brimblecombe et al., 1958). It is likely that coryza facilitates mucous sampling of S. *pneumoniae*, thereby enhances detection. However, it also allows easier transmission through direct spread by coughing or sneezing or via fomite contamination (Brimblecombe et al., 1958). A recent study in rural Kenya has also demonstrated an association between NP pneumococcal colonisation and coryza (adjusted odds ratio 2.29, 95%CI 1.48-3.56) (Abdullahi et al., 2008).

### 1.6.4 Pathogenic mechanisms underlying the interaction between respiratory viruses and S. *pneumoniae*

#### 1.6.4.1 *Influenza and S. pnuemoniae*

The synergistic interaction between Influenza and *S. pneumoniae* have been shown in a number of animal models (Berendt et al., 1975; Peltola and McCullers, 2004; Seki et al., 2004). Pre-exposure of squirrel monkeys to influenza A(H3N2) virus significantly increased blood pneumococcal load (from 55 to 30,000 colonies/mL) (Berendt et al., 1975). Similarly, pneumococcal load in the lungs of influenza A pre-exposed monkeys were >100,000 compared to 90 colonies/mL in monkeys exposed to S. *pneumoniae* alone. Murine studies also established the importance of the order of exposure to the two pathogens – mice that were sequentially challenged by influenza virus, followed by pneumococcus 7 days later all died (McCullers and Rehg, 2002; Peltola and McCullers, 2004). Conversely, mice challenged with influenza virus 7 days after pneumococcal exposure did not result in fatal outcome, and mice that were simultaneously infected with both pathogens had a higher mortality (60%) than mice infected with either influenza or pneumococcus alone. In a different study, lungs of influenza-exposed mice had higher density of pneumococci following pneumococcal challenge (LeVine et al., 2001). Sequential influenza-pneumococcal infection was also associated

with increased pulmonary neutrophil infiltrates, as well as higher levels of IL-1β and TNF-α, compared to mice inoculated solely with S. *pneumoniae*.

A transmission model in ferrets also demonstrated that influenza infection increased susceptibility to pneumococcal acquisition in terms of the percentage of ferrets infected and the distance over which they could acquire infection (McCullers et al., 2010). Moreover, these influenza-mediated effects on colonisation, transmission, and disease were pneumococcal strain-specific.

Murine models have also helped to expand our understanding of the pathogenic processes by which influenza infection increases susceptibility to pneumococcal colonisation and subsequent disease (McCullers, 2006; Short et al., 2012). The hypothesised mechanisms, summarized in Table 1.14, include viral-mediated changes to the respiratory tract that facilitate pneumococcal binding, as well as alterations of the innate immune response that result in diminished ability to clear pneumococcus, and amplification of host immunopathology. The precise contribution of the various pathogenic mechanisms described remains uncertain, but it is clear that the processes are complex and multifactorial.

#### *1.6.4.2 Other respiratory viruses and S. pnuemoniae*

*In vitro* and animal models have also shown synergistic interactions between S. *pneumoniae* and viruses other than influenza. As these viruses share common virulence traits with influenza viruses, many of the mechanisms for synergy are expected to be similar (Table 1.14). Adenovirus, RSV and hMPV enhance *in vitro* adherence of S. *pneumoniae* to respiratory epithelial cells (Hakansson et al., 1994; Kukavica-Ibrulj et al., 2009; Hament et al., 2004).

Preceding hMPV infection has also been shown to increase pneumococcal load in mice, and is also associated with increased levels of inflammatory cytokines and chemokines, as well as greater expression of Toll-like receptor 2 (TLR2), TLR6, TLR7, and TLR13 in the lungs of co-infected mice (Kukavica-Ibrulj et al., 2009). Co-infection with RSV has been shown to up-regulate the expression of key pneumococcal virulence genes, including pneumolysin (Smith et al., 2014). Lastly, parainfluenza viruses encode a neuraminidase, which promotes pneumococcal binding in a similar manner to that of influenza (Alymova et al., 2005).

**Table 1.14 Pathogenic mechanisms underlying the interaction between influenza and S. *pneumoniae*, as demonstrated in animal models**

| Pathogenic mechanism | **Details on the effect of influenza infection on pneumococcal disease** | **Outcome** |
|---|---|---|
| Epithelial damage | - Viral destruction of respiratory epithelium (Harford and Hara, 1950) | - Increase exposure of S. *pneumoniae* to binding sites in the basement membrane |
| Reduced activity in airway protection mechanism | - Impaired ciliary function (Pittet et al., 2010)<br>- Obstruction of small airways due to disruption of surfactant, increased mucinous secretions and influx of inflammatory cells (Harford and Hara, 1950; Loosli et al., 1975) | - Decrease mechanical clearance of S. *pneumoniae*<br>- Improve conditions for bacterial growth<br>- Reduce oxygen and carbon dioxide diffusion capacity (Horner and Gray, 1973) |
| Increased receptor availability/ bacterial adherence | - Cleavage of sialic acid by viral or bacterial neuroaminidases (NAs) from cell surface glycoconjugates (McCullers and Bartmess, 2003) *Parainfluenza viruses (Alymova et al., 2005)<br>- Pro-inflammatory cytokines up-regulate expression of cellular receptors (Peltola and McCullers, 2004) *Adenovirus (Lai et al., 2014), RSV (Hament et al., 2004) & hMPV (Kukavica-Ibrulj et al., 2009)<br>- Deposition of fibrin and fibrinogen deposition during regenerative process following viral infection | - Expose cryptic cell receptors for pneumococcal binding in lower airways (McCullers, 2006)<br>- Promote adherence of S. *pneumoniae*<br>- Provide further attachment sites for bacteria |
| Down-regulation of innate immune response | - Interferon-γ production by T cells (Sun and Metzger, 2008)<br>- Desensitization of alveolar macrophages to bacterial Toll-like receptor (TLR) ligands such as flagellin (TLR5), lipopolysaccharide (TLR4) and lipotechoic acid (TLR2) (Didierlaurent et al., 2008) *hMPV (Kukavica-Ibrulj et al., 2009)<br>- Increased pulmonary levels of alternatively activated macrophages (AAMs) in murine lungs at days 7-14 post influenza virus infection (Didierlaurent et al., 2008)<br>- Induced Type 1 interferons impair CXCL1/2 production (Shahangian et al., 2009)<br>- IFN1 production also inhibits CCL2 production reduced recruitment of | - Down-regulate alveolar macrophage scavenger receptors (MARCO) on macrophages that are involved in phagocytosis of unopsonized pneumococci<br>- Reduce chemokine production, NF-κB activation and consequently impaired neutrophil recruitment<br>- AAMs have poorer bactericidal activity than pro-inflammatory macrophages<br>- Reduce neutrophil chemotaxis<br>- Reduce recruitment of monocytes/macrophages<br>- Suppress inflammatory response to secondary pneumococcal infection |

| | | |
|---|---|---|
| | monocytes/macrophages (Nakamura et al., 2011)<br>- Increased glucocorticoids levels | |
| Bacterial enhancement of inflammation | - Pneumococcal cell wall, pneumolysin and pyruvate oxidase SpxB (Cockeran et al., 2001; Spellerberg et al., 1996) | - Induce cell damage and inflammation |

*Denote viral pathogens that have demonstrated similar pathogenic mechanism to influenza virus in facilitating pneumococcal infection.

### 1.6.5 Rationale for evaluating the association between respiratory viruses and pneumococcal density

Existing evidence from epidemiological and experimental studies support the biological plausibility that preceding respiratory viral infections, particularly with influenza, increase susceptibility to bacterial pneumonia. Since pneumococcal disease is preceded by nasopharyngeal pneumococcal carriage (Bogaert et al., 2004), another potential mechanism by which respiratory viruses predispose to pneumococcal disease may be by increasing carriage density to reach the 'critical' level, with subsequent lower respiratory tract invasion (Albrich et al., 2012). Few epidemiological studies have evaluated the impact of respiratory viral infection on pneumococcal carriage density. A case control study of paediatric hospitalised LRTI in Vietnam found significantly higher NP pneumococcal load in children with viral co-infections than children without co-infection (Vu et al., 2011). Wolter *et al.* (Wolter et al., 2014b) also demonstrated higher pneumococcal colonization density, and in turn, increased invasive disease, in individuals with respiratory viral co-infection within a severe acute respiratory illness (SARI) surveillance programme in South Africa. However, both studies were cross-sectional, therefore unable to assess the dynamic relationship between onset of viral infection and NP pneumococcal carriage. If this hypothesis was true, it will have important implications for populations with high pneumococcal carriage, such as young children and HIV-infected individuals (Gill et al., 2008; Abdullahi et al., 2012).

We therefore plan to investigate the relationship between respiratory viral infections and NP pneumococcal carriage density in a region with a high prevalence of HIV infection and pneumococcal carriage. The aim is to determine whether adults with confirmed respiratory viral infection have increased density of NP pneumococcal carriage (Chapter 6).

## 1.7 Malawi

### 1.7.1 Demographic and health indicators

Malawi is located in Southeast Africa, with a population of around 15.9 million as of 2012 (UNdata, 2014), and is estimated to exceed 26 million by 2030 (Population Reference Bureau, 2012). The average life expectancy at birth is 54.9 years for males, and 55.2 years for females (UNdata, 2014). Malawi has a young population with a median age of 17; 46% are under 15 years of age (Population Reference Bureau, 2012). One of the poorest countries in the world, Malawi has a gross domestic product (GDP) of US$355 per capita and is ranked 174$^{th}$ of 187 countries according to the Human Development Index (United Nations Development Programme, 2014). The economy is predominantly agricultural, with around 90% of the population living in rural areas. A substantial proportion of Malawians rely on subsistence farming, and therefore are vulnerable to natural disasters, such as drought or heavy rain.

Blantyre, the commercial and finance centre, is situated in the South of Malawi (Figure 1.6). It has a population of 1.3 million, around two-thirds of which reside in urban dwellings. Healthcare is free at the point of delivery. The government health care system in Blantyre consists of the Queen Elizabeth Central Hospital (QECH), the largest referral hospital in the country that provides secondary and tertiary care to the population of greater Blantyre (see also Chapter 2.1.1), and 18 health centres that are under the jurisdiction of the District Health Office. Additionally, there are a number of private hospitals and clinics that are run by non-governmental organisations (NGO) but the number of inpatient beds available is very small.


**Figure 1.6 Map of Malawi and location of Blantyre** (red dot)

Malawi is characterized by a heavy burden of disease as evidenced by high mortality rates in both children (infant & under-five mortality, 66 & 112/1000 live births) and adults (maternal mortality 675/100,000 live births) (National Statistics Office (NSO) and ICF Macro, 2011), as well as high prevalence of diseases such as tuberculosis, malaria, HIV (Section 1.7.2), and other tropical diseases. Despite this, Malawi has only 1.9 physicians per 100,000 population, constituting one of the lowest physician densities in the world (World Health Organization, 2009b).

### 1.7.2 HIV epidemic in Malawi

Adult HIV prevalence in Malawi has been declining steadily since reaching a peak of 16.4% in 1999 (Government of Malawi, 2014). In 2010, the national adult HIV prevalence was reported as 10.6% (National Statistics Office (NSO) and ICF Macro, 2011), with significant gender and geographical variation (Figure 1.7). Women have higher prevalence than men, and prevalence is twice as high in urban compared to rural settings. In urban Blantyre, prevalence is reported to be as high as 18.5% in a household HIV self-testing study (Choko et al., 2011); Heterosexual contact is the principal mode of HIV transmission in Malawi (88%), while mother-to-child transmission of HIV accounts for around 10% of all new HIV infections (Government of Malawi, 2014).

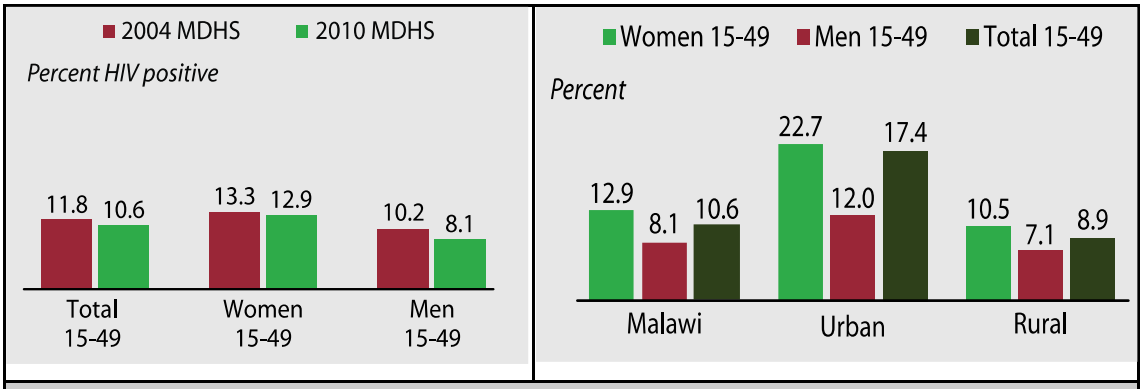



**Figure 1.7 Prevalence of HIV infection in Malawi, by A) sex; B) urban or rural distribution**(National Statistics Office (NSO) and ICF Macro, 2011).

MDHS, Malawi Demographic and Health Survey.

A free national programme to provide combined antiretroviral therapy (cART) for those living with HIV infection has been available in Malawi since 2004, following a grant from the Global Fund to Fight AIDS, TB and Malaria. As of December 2013, the total number of patients alive on ART was 472,865 (Government of Malawi, 2014). Using the previous ART eligibility criteria of CD4 $\leq$350 cells/mm$^3$ or WHO Clinical Stage 3 and 4, ART coverage in Malawi in 2013 was estimated at 83% (up from 65% in 2012) (Government of Malawi, 2014). However, the CD4 threshold for ART initiation was changed to $\leq$500 cells/mm$^3$ in 2014 (Malawi Ministry of Health, 2014) in line with the revised WHO guidelines (World Health Organization, 2013a). This has been fully implemented, thus increasing the pool of eligible patients. The initial first-line treatment had been a combination of stavudine, lamivudine and nevirapine, but was updated to tenofovir, lamivudine and efavirenz in July 2013 (Ministry of Health, 2008; Malawi Ministry of Health, 2014). Malawi was also one of the first countries to introduce lifelong ART for pregnant and breastfeeding women to prevent mother-to-child transmission (PMTCT) (Option B+) in 2011 (WHO Regional Office for Africa, 2014). Between 2011 and 2013, Option B+ increased ART coverage among HIV-infected pregnant women from 40 to 61% (Government of Malawi Ministry of Health, 2011; Government of Malawi Ministry of Health, 2013). However, ART uptake and retention remain a challenge. In a study of ~22,000 women who started Option B+ between October 2011 and March 2012, 17% women were lost to follow-up 6 months after ART initiation (Tenthani et al., 2014). More recently, the Malawi Ministry of Health reported that 77%, 72% and 71% of women who started ART as part of Option B+ were retained on treatment at six, 12, and 24 months after initiation, respectively (Government of Malawi Ministry of Health, 2014).

### 1.7.3 Acute respiratory infections in Malawi

There are scanty published data on acute respiratory infections in Malawian adults. Several reports in the pre-HIV era described ARI as the most common cause of adult admission in Malawi (Harries et al., 1988; Harries et al., 1990). More recently, a study at QECH from January 2010 to July 2011 identified both upper and lower respiratory infection as top ten diagnoses in the adult medical wards (SanJoaquin et al., 2013). Pneumonia was the commonest cause of admission (1149/7103, 16%). Of those patients, 85% had HIV infection with 10% inpatient mortality (SanJoaquin et al., 2013). However, the epidemiology and aetiology of ARI presentations in this setting have not yet been comprehensively described.

## 1.8 Aims and objectives

The aim of this thesis is to examine the relationship between HIV infection and influenza, in a high HIV prevalence setting. The main hypothesis is that HIV-infected adults are at an increased risk of influenza infection and experience greater severity of disease, compared to HIV-uninfected adults. Thus policy makers should consider HIV-infected individuals as a priority group when devising influenza control strategies in Malawi and other similar high HIV prevalence settings.

Key research questions that will be addressed include:

1. What is the epidemiology and burden of influenza in adults in an urban setting in Malawi?
2. Does HIV increase susceptibility to influenza infection?
3. What is the contribution of influenza to hospitalized lower respiratory tract infection (LRTI) in Malawian adults?
4. What are the clinical predictors of influenza in patients admitted with LRTI in Malawi?
5. Is HIV infection associated with increased severity of influenza-related respiratory presentation?
6. Does infection with influenza or other respiratory viruses predispose to secondary pneumococcal pneumonia by increasing risk of acquisition and nasopharyngeal density of *Streptococcus pneumoniae*?

This thesis sets out to address the above questions using the following studies:

1. Sentinel hospital-based surveillance of influenza and other respiratory viruses in adults presenting with mild and severe acute respiratory presentations (Q1)
2. Recruitment of a prospective cohort of HIV-infected and HIV-uninfected individuals to measure the incidence of influenza-confirmed illness episodes over a 2-year period (Q1&2)
3. A case control study of adults presenting with mild and severe influenza-related respiratory presentations in Blantyre, Malawi (Q3-5)
4. A cross-sectional and case control study nested within the prospective cohort (Q6)

# CHAPTER 2. GENERAL METHODS

This chapter discusses methodological aspects that are common to the studies described in Chapters 3 to 6, including study sites, laboratory procedures, data management and ethical considerations. Methods pertaining to each study – namely study design, population, clinical definitions, procedures, sample size, statistical analysis and timescale, will be discussed under the individual chapters.

## 2.1 Study sites

### 2.1.1 Queen Elizabeth Central Hospital

The Queen Elizabeth Central Hospital (QECH) is the largest government inpatient health facility (1250 beds) in Malawi, and is the referral centre for patients in the Southern Region. It provides secondary and tertiary care to the population of greater Blantyre (around 1.3 million), including internal medicine, general surgery, paediatrics, obstetrics and gynaecology, anaesthetics and radiology, in addition to specialised surgical specialties such as orthopaedics, Ear Nose and Throat, neurosurgery, and ophthalmology departments. A recently published paper on the electronic inpatient data collection system for adult inpatients (Surveillance Programme of Inpatients and Epidemiology (SPINE)) (SanJoaquin et al., 2013) reported over 7000 adult medical admissions at QECH between January 2010 and July 2011; median age was 37 years, 50% were male, 74% were HIV-positive (21% previously undiagnosed). The median stay in hospital was 5 days, and in-hospital mortality was 15% (SanJoaquin et al., 2013).

#### *2.1.1.1 Adult Emergency Trauma Centre (AETC)*

The current Adult Emergency Trauma Centre has been in operation since the beginning of 2012. Its facilities include a triage area, resuscitation area, and a short stay ward. On average, around 200 adults (range 100-250) attend daily, of which on average 20 to 30 result in medical admissions.

#### *2.1.1.2 Antiretroviral treatment (ART) and Voluntary Counselling and Testing (VCT) Clinics*

The ART clinic at QECH was founded initially as a fee-paying clinic in 2000, but has been providing ART free of charge to children and adults since 2004 as part of the Ministry of Health scale-up programme (van Oosterhout et al., 2005; van Oosterhout et al., 2007). Between 2004 and 2011, around 16,000 individuals were initiated on ART at the QECH clinic (Sloan et al., 2013). Currently,

~10,000 registered patients are alive and on treatment, of whom 9176 are on first-line ART treatment, and 560 are on second line therapy (July 2015).
The VCT service is located adjacent to the ART clinic. Trained counsellors provide HIV counselling and testing to approximately 40 walk-in patients daily.

### 2.1.2 Gateway Clinic

The Gateway clinic is a primary care clinic located close to QECH, run by the Ministry of Health Blantyre District Health Office (DHO). It was opened in October 2013 in response to growing pressures on the AETC at QECH, which had become overburdened with patients with primary care issues due to the lack of a district hospital and primary care services within Blantyre. The clinic has one consultation room (subdivided into two consultation areas using a screen), a short stay area, a laboratory where point-of-care tests, e.g. urinary pregnancy test and malaria rapid diagnostic test (RDT), are performed, and a pharmacy. The clinic also provides an HIV VCT service. The BASH-FLU study began recruitment at this clinic in January 2014. Recognition of the lack of space for patient consultation led to the donation of a container by MLW that has been refurbished into two clinic rooms, one of which is used for conducting research activities.

Since its opening, the number of patients attending the Gateway clinic has steadily increased. It currently sees up to 300 walk-in patients daily.

### 2.1.3 Malawi-Liverpool-Wellcome Trust (MLW) Clinical Research Programme

MLW is a clinical research programme that conducts laboratory, clinical, epidemiological and social science research on health problems affecting Malawi and sub-Saharan Africa. Established in 1995, the programme is part of the University of Malawi College of Medicine (COM) and has close links with the University of Liverpool and The Liverpool School of Tropical Medicine (LSTM) through the Liverpool-Glasgow Wellcome Trust Centre for Global Health Research (Wellcome Trust Liverpool Glasgow Centre for Global Health Research). It has also established collaborative links with numerous research institutions worldwide. Since 2003, it has been recognised as one the Wellcome Trust's Major Overseas Programme.

MLW is located within the grounds of QECH, with field research sites around urban Blantyre, Chikwawa and Thyolo. The programme hosts a large number of local and international PhD and Masters Students, as well as post-doctoral researchers. It also provide operational support, including administration, finance, information technology, human resources, data management, laboratory, in addition to ordering and storage of research consumables. Most MLW fellows with a clinical

background contribute to clinical duties, as well as undergraduate and postgraduate teaching programmes at QECH.

The research activities of MLW are focused under four main themes: Malaria, HIV and tuberculosis, Non-communicable diseases, and Microbes, immunity and vaccines.


**Figure 2.1 Study sites of influenza surveillance and BASH-FLU study within Blantyre, Malawi**

## 2.2 Laboratory Procedures

### 2.2.1 Sample registration and storage

With the exception of the haemagglutination inhibition (HAI) assay (see section 2.2.9), all clinical specimens collected from study participants were processed at the MLW or COM-MLW laboratories. Both laboratories participate in the UK National External Quality Assessment Scheme (NEQAS).

Each study specimen was labelled with a pre-printed laboratory specimen barcode and the participant's unique study barcode (section 2.3), with a corresponding laboratory specimen submission form that contained the same details, in addition to the participant's name and date of birth. This allowed treating clinicians to access to the results from the Laboratory Information Management System (LIMS) in a timely fashion. Laboratory test results were subsequently linked to the participants' demographic information by their unique study number.

Nasopharyngeal/oropharyngeal and serum samples were stored for batch processing at a later date. Stored specimens were anonymised (identifiable by laboratory specimen number and patient study

number only) and indexed in accordance with regulatory requirements for human specimen storage. They may be stored for up to five years after the end of the study, after which they will be destroyed in accordance to Category II waste procedure of the University of Liverpool protocol. Specific consent was sought for the storage and transfer of nasopharyngeal/oropharyngeal specimens to the United Kingdom (University of Liverpool and/or Liverpool School of Tropical Medicine laboratories), and serum specimens to the United States (CDC laboratory, Atlanta). A COMREC Material Transfer Agreement was completed and approved.

### 2.2.2 HIV test

HIV status of study participants were determined using rapid HIV tests as per WHO-recommended algorithm and national guidelines (figure 2.2 (World Health Organization, 2004)); Alere Determine™ HIV-1/2 assay (Alere Medical Co. Ltd., Chiba, Japan) was used as the initial screening test, and Uni-Gold™ Recombigen HIV test (Trinity BioTech, Wicklow, Ireland) as the confirmatory test.

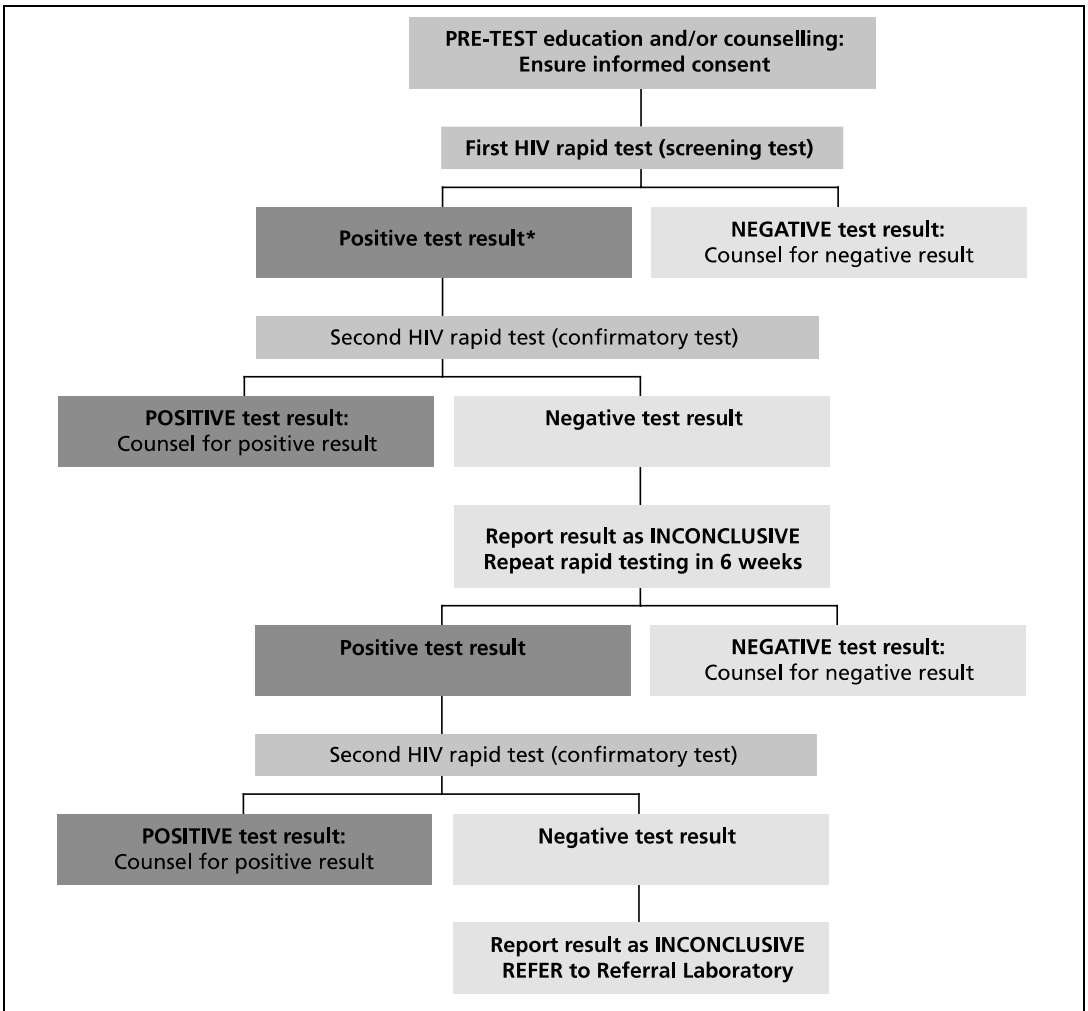



**Figure 2.2 Algorithm for use of rapid HIV tests (World Health Organization, 2004)**

### 2.2.3 CD4+ cell count

CD4+ T-cell counts were performed on a FACScount™ flow cytometer (Becton Dickinson, BD Biosciences, San Jose, USA).

### 2.2.4 Blood culture

5 to 10 millilitres of blood were collected in a single aerobic blood culture bottle containing the manufacturer's standard culture medium and incubated in a BACTEC 9050 blood culture system (Becton Dickenson, BD Biosciences, San Jose, USA) for up to 7 days at the MLW microbiology laboratory (Gordon et al., 2001; Everett et al., 2011).

### 2.2.5 Sputum tests for tuberculosis

#### *2.2.5.1 Sputum smear for acid-fast bacilli (AFB)*

Sputum specimens were processed at the COM-MLW TB laboratory. Samples were smeared onto a glass slide and allowed to air-dry then heat-fixed. Slides were then examined for acid-fast bacilli (AFB) after staining with Auramine. All Auramine-positive smears were confirmed with Ziehl-Neelsen staining. Each slide was examined by two independent readers.

**Table 2.1 Grading of sputum AFB smear**
(International Union Against Tuberculosis and Lung Disease, 2000)

| AFB count | Recording/Reporting |
|---|---|
| No AFB in >100 fields | Negative |
| 1 to 9 AFB per 100 fields | Specify the actual number of AFB per 100 fields |
| 10-99 AFB per 100 fields | + |
| 1 to 10 AFB per fields | ++ |
| >10 AFB per field | +++ |

AFB, acid-fast bacilli

#### *2.2.5.2 Sputum culture*

Sputum was incubated in liquid medium for up to 6 weeks using the BACTEC960 Mycobacterial Growth Index Tube (MGIT; Becton Dickinson, BD Biosciences, San Jose, USA) system, which exploits the fluorescence of an oxygen sensor to detect growth of mycobacteria in culture. A positive and a negative control were included with each batch.

#### *2.2.5.3 Gene Xpert*

Xpert MTB/RIF test was also performed on sputum in patients suspected to have pulmonary TB in the cohort and case control studies. It is an automated molecular test for *Mycobacterium tuberculosis*

*(*M*tb*) and resistance to rifampicin, using a nested rRT-PCR assay to amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with rifampicin resistance (Boehme et al., 2010). The MTB/RIF test platform (GeneXpert, Cepheid) integrates sample processing and PCR in a single-use disposable cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection, in addition to a sample processing control to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The only manual step is the addition of a bactericidal buffer to sputum before transferring a defined volume to the cartridge. The cartridge is then inserted into the GeneXpert device, which provides results within 2 hours.

### 2.2.6 BinaxNOW Urinary Antigen for *Streptococcus pneumoniae*

BinaxNOW® *Streptococcus pneumoniae* Antigen test (Alere North America, Freehold, US) was performed on unconcentrated urine specimens in patients enrolled with hospitalized LRTI, and a subset of participants in the cohort study according to manufacturer's instructions. It is a point-of-care immunochromatographic (ICT) assay for the detection of pneumococcal soluble antigen. Recent meta-analyses of adult studies reported sensitivity and specificity of 74-75% and 94-97% respectively (Said et al., 2013a; Sinclair et al., 2013; Horita et al., 2013; Boulware et al., 2007).

### 2.2.7 Nasopharyngeal and oropharyngeal samples for respiratory pathogen testing

In the cohort study (Chapter 4), paired nasopharyngeal and oropharyngeal specimens were taken using flocked swabs (FLOQswabs™, Copan Diagnostics, Brescia, Italy). For nasopharyngeal sampling, the swab is inserted along the floor of the nose to the posterior nasopharynx, and withdrawn with a rotating motion after a maximum of 5 seconds. The swab tips were placed in 3mls Universal Transport Medium (UTM™, Copan Diagnostics, Brescia, Italy) and 1ml aliquots are stored in cryovials. It is recognised that the type of swab and operator technique can affect microbial yield. Flocked swabs have been shown to be equivalent or superior to Dacron or Rayon swabs with regards to the sensitivity of microbial detection, and the quantity of organism recovered (Daley et al., 2006; Hernes et al., 2011). They have also demonstrated superiority in the recovery of pneumococci from the nasopharynx (Dube et al., 2013). A Kenyan study found no appreciable increase in sensitivity in taking two swabs at the same time-point, compared to one (Abdullahi et al., 2007), and current WHO guidelines recommend a single swab (Satzke et al., 2013). We therefore opted for a single nasopharyngeal sample only. This was also more acceptable to our participants, who had to undergo nasopharyngeal sampling up to 10 times over the study period.

In the influenza surveillance (Chapter 3) and case control study (Chapter 5), 1 ml aliquots of nasopharyngeal aspirates were placed in UTM and skim-milk triptone glucose glycerol (STGG) respectively. UTM aliquots were stored at -80°C, and batch-tested for influenza and other respiratory pathogens by rRT-PCR at the MLW molecular laboratory. Total nucleic acids were extracted from 300µl aliquots of each specimen with the Qiagen BioRobot® Universal System using the QIAamp One-For-All nucleic acid kit (Qiagen ltd., Manchester, UK) by the MLW molecular diagnostic staff.

**Detection of respiratory viruses and bacteria**

5 or 10 µl of nucleic acid extract was used in each qRT-PCR reaction. The CDC Human Influenza qRT-PCR diagnostic panel (CDC Influenza division) detecting influenza A and B, influenza A subtyping kit (CDC Influenza division) detecting seasonal A(H1), seasonal A(H3), and pandemic 2009A(H1) (World Health Organization, 2009a; Selvaraju and Selvarangan, 2010), and a real-time multiplex PCR assay, Fast-track Diagnostics® (FTD) Respiratory 33 kit (Fast-track Diagnostics Ltd., Luxembourg) (Table 2.2) were used (Bierbaum et al., 2014; Driscoll et al., 2014), in combination with the AgPath one-step qRT-PCR reagents (Applied Biosystems, California, USA). PCR conditions were according to the manufacturer's instructions. Appropriate negative and positive control specimens were run alongside each reaction. Samples with cycle threshold (Ct) value <33 were recorded as positive.

**Table 2.2 Pathogens included in the FTD Respiratory 33 multiplex PCR, and their determined clinical significance**

| | |
|---|---|
| **Viral (potentially pathogenic)** | Adenovirus<br>Bocavirus<br>Coronavirus OC43, NL63, 229E, HKU1<br>Enterovirus/parechovirus<br>Influenza A, B, C<br>Human metapneumovirus A & B<br>Parainfluenza viruses 1, 2, 3, 4<br>Respiratory syncytial virus (RSV) A & B<br>Rhinovirus |
| **Atypical bacteria (potentially pathogenic)** | *Bordetella pertussis*<br>*Chlamydia pneumoniae*<br>*Legionella* species<br>*Mycoplasma pneumoniae*<br>*Pneumocystis jirovecii* |
| **Commensal bacteria in the nasopharynx** | *Haemophilus influenzae species &* type B<br>*Moraxella catarrhalis* |

| | |
|---|---|
| | *Streptococcus pneumoniae*<br>*Staphylococcus aureus* |
| **Pathogens of unknown significance in the nasopharynx therefore excluded from analysis** | Cytomegalovirus<br>*Klebsiella pneumoniae*<br>*Salmonella* species |

### 2.2.8 Nasopharyngeal samples for quantitative *lyt*-A PCR for *Streptococcus pneumoniae*

The nasopharyngeal density of S. *pneumoniae* was measured in a sub-study of the BASH-FLU cohort study (Chapter 6), utilising real-time PCR targeting the autolysin-encoding *lytA* gene (Carvalho Mda et al., 2007; Albrich et al., 2012). *Lyt*A is a single-copy gene present in all encapsulated and non-encapsulated pneumococci. The assay does not cross-react with other *mitis* group streptococci (Greve and Moller, 2012). Total nucleic acids were extracted from 300ul universal transport medium (UTM) with combined nasopharyngeal and oropharyngeal specimens. The QIAamp One-For-All nucleic acid kit (Qiagen) was used with the Qiagen BioRobot Universal System following the standard protocol provided by the manufacturer. Five µl of the extracted nucleic acids were used in the quantitative *LytA* PCR assay. PCR primers and cycling conditions were used as described in Carvalho *et al* (Carvalho Mda et al., 2007). A standard curve for quantification was generated using paired tenfold serial dilutions of DNA extracted from a reference bacterial culture, spanning $7x10^2$-$7x10^9$ genomic copies per millilitre (copies/ml). DNA concentrations of these standards were measured using the Qubit DNA BR assay kit (Life Technologies). Sample density was reported as the qPCR concentration in copies/ml as calculated from the standard curve.

### 2.2.9 HAI assay

Serum specimen will be transferred to the laboratory at the Infiuenza Division of the National Center for Immunization and Respiratory Diseases, CDC, in Atlanta, Georgia, US, for processing of HAI assay. The results of this test will not be reported in this thesis.

## 2.3 Data management

Data for this study were collected in accordance with the ethical principles of Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation guidelines. MLW Standard Operating Procedures were adhered to in relation to data handling and consent.

Each participant was assigned a unique study number at enrolment. Data collection forms for a particular participant were linked by a scannable barcode containing this number. Data were

collected on paper-based case record forms (CRFs) (Appendix 8.3-8.5) and converted to electronic form using Intelligent Character Recognition (ICR) scanning software (Cardiff TeleForm® Version 10.7, Vista, CA). The software highlights data entries that are ambiguous or are outwith a pre-determined range, which were verified by the data officer or principal investigator. The verified data was subsequently exported to a Microsoft Access database on a secure server at MLW. No patient-identifiable information is kept on the electronic database, which is password-protected and access is restricted to the principal investigator. Data files were exported to Stata 12.1 (Statacorp, USA) for analysis. The paper CRFs were stored in locked filing cabinets in the MLW research office that were accessible only to the members of the study team.

The patient/guardian consent form and the participant tracking form contain the only sources of person-identifiable information. The latter contain the subjects' name, contact details and the unique study number, in order to provide a link with the anonymised clinical data. Both were kept in a secured locked cabinet held separately from the CRF hard copies.

The paper CRFs will be stored in the MLW archives for up to 5 years following study completion, after which time they will be destroyed.

## 2.4 Statistical analysis

Statistical analysis plans are described in the Methods section of the individual studies. All analyses were undertaken using STATA 12.0 (StataCorp, College Station, USA). Missing observations were included in the analysis of each study by creating missing value categories. For tables where denominators are not included, the numbers of missing values for individual variables are detailed in Appendix 8.4.

## 2.5 Ethical considerations

Ethical approval for the sentinel influenza surveillance was obtained from the College of Medicine Research Ethics Committee (COMREC), University of Malawi (P.07/10/958) and the Centers for Disease Control and Prevention (CDC) through an ethical reliance. The BASH-FLU cohort and case control studies were approved by the Research Ethics Committees of the Liverpool School of Tropical Medicine, UK (12.43) and COMREC (11/12/1310). Written informed consent was obtained from all participants or their guardians. Patient and guardian consent forms are in the appendix.

# CHAPTER 3. The epidemiology and risk factors for influenza in Malawian adults presenting with acute respiratory infection: a sentinel surveillance study

## 3.1 Introduction

Influenza is an important cause of acute respiratory disease worldwide; annual attack rate is estimated to be between 20 to 30% in children and 5 to 10% in adults, and an annual mortality of up to 500,000 (World Health Organization, 2014c). Although thought to be considerable, the epidemiology and burden of influenza in most SSA countries, particularly life-threatening lower respiratory disease, are poorly characterised (Chapters 1.2 & 1.3). The lack of diagnostic capacity, similarity of influenza presentation with other common febrile illnesses such as malaria and bacterial pneumonia, and prioritisation of other high-burden public health problems are likely contributory factors. A recent systematic review concluded that most SSA countries had insufficient epidemiological data to develop rational strategies for influenza prevention and control (Gessner et al., 2011). It is unsurprising therefore that while the World Health Organisation (WHO) recommends seasonal influenza vaccine for at-risk groups, such as young children, pregnant women, and HIV-infected individuals (2012), few countries in the region have implemented these recommendations or have a national public policies (Duque et al., 2014).

Since the 2009 influenza A(H1N1) virus (influenza A(H1N1)pdm09) pandemic, governments and international agencies, such as the CDC and WHO, have strengthened surveillance capacity for influenza and other respiratory viruses in SSA (Steffen et al., 2012). Emerging data suggest that influenza is prevalent in both mild and severe acute respiratory presentations (Chapter 1.2), and may be associated with a greater mortality compared to developed settings (Cohen et al., 2012c). Data from South Africa suggest increased incidence of hospitalisation and mortality in HIV-infected individuals, which has considerable implications for many high HIV prevalence SSA countries (Cohen et al., 2013; Cohen et al., 2015a). However, outside South Africa and Kenya, there are scant epidemiological data, and where available, surveillance activities have largely focused on children (Dalhatu et al., 2012; Lutwama et al., 2012; Niang et al., 2012; Muyembe Tamfum et al., 2012; Katz et al., 2012; Mmbaga et al., 2012; Theo et al., 2012; Cohen et al., 2013; Dia et al., 2013; Katz et al., 2014; Cohen et al., 2014; Emukule et al., 2014), are predominantly based on outpatients, and lack individual-level information to evaluate risk factors for influenza or severe influenza presentations.

With the support of the CDC, sentinel hospital-based surveillance for influenza and other respiratory viruses was established in January 2011, at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi. In a high HIV prevalence setting (SanJoaquin et al., 2013), we utilised clinical and molecular techniques to define the contribution of influenza and other respiratory pathogens to acute respiratory presentations in Malawian adults and characterise the risk factors associated with influenza-related respiratory presentations in the post-influenza A(H1N1)pdm09 period.

## 3.2 Methods

### 3.2.1 Study design

This was a sentinel surveillance study of adults presenting to QECH with mild and severe acute respiratory illness.

### 3.2.2 Study site

Active, prospective respiratory virus surveillance was introduced at QECH in January 2011. Patients were enrolled from the Adult Emergency Trauma Centre (AETC) (Chapter 2.1.1).

### 3.2.3 Study population

All patients aged 15 years and over who presented to the AETC during weekdays were eligible. Consecutive patients that fulfilled the case definitions were recruited. A maximum of 4 patients who met the case definition for ILI, and 4 patients with SARI were enrolled during weekdays. ILI recruitment was conducted in 2011 only.

The following adapted WHO case definitions (World Health Organization, 2013b) were used:

**Influenza-like illness (ILI)**

- Reported or recorded fever ($\geq$38°C)
- $\geq$ 2 of the following: cough, sore throat, myalgia, headache, OR diarrhoea/vomiting
- Symptom for 7 days or fewer

**Severe acute respiratory illness (SARI)**

- Reported **OR** recorded fever ($\geq$38°C)
- Cough **OR** sore throat
- Shortness of breath **OR** difficulty breathing
- Symptoms for 7 days or fewer

- Hospitalisation was not a requirement

### 3.2.4 Study procedures

#### *3.2.4.1 Staff*

The following staff were employed to maintain the study cohort:

- 2 study nurses: responsible for screening for eligible patients at the triage area in AETC; consent and enrolment of patients; obtaining clinical specimens, including nasopharyngeal aspirate, blood and sputum; instituting appropriate management, e.g. administering intravenous fluids or antibiotics; and completion of paper and electronic copies of screening and enrolment logs.
- 1 clinical officer: responsible for assessing eligibility, consent and enrolment of patients; clinical assessment and management of patients; conducting necessary clinical procedures, e.g. pleural aspiration; aiding study nurses in obtaining clinical specimens.
- 1 laboratory messenger: responsible for transporting clinical specimens from AETC to the MLW/COM laboratories.
- 1 molecular laboratory technician: responsible for aliquoting, storage and processing combined nasopharyngeal specimens, as part of a team of technicians in the core diagnostic laboratory.

In addition to screening and enrolment of study participants, study staff also contributed to routine clinical activities at the AETC.

#### *3.2.4.2 Enrolment*

ILI and SARI patients were recruited daily between 8am to 3pm in the AETC. Recruitment hours were limited by the necessity of clinical samples to reach the laboratory by 3pm for processing. Persons that met the eligibility criteria who were willing to consider participation in the study received a patient information document. Written informed consent was obtained.

Demographic and clinical information were obtained from study participants using a structured questionnaire (Appendix 8.3.1). Data collected included demographic details, clinical presentation, pregnancy status, history of pneumococcal conjugate or influenza vaccination, preceding antibiotic use, and smoking status. Physiological parameters at presentation were also recorded.

#### *3.2.4.3 Laboratory investigations*

The investigations that were performed on enrolment are listed in Table 3.1 (see Chapter 2.2 for details on individual tests).

| Table 3.1 Laboratory investigations undertaken in sentinel surveillance | | |
|---|---|---|
| **Investigations** | **ILI** | **SARI** |
| **Venous blood** | | |
| Malaria rapid diagnostic test | **X** | **X** |
| Rapid HIV test (if status unknown) | | **X** |
| Blood culture | | **X** |
| **Nasopharyngeal aspirate** | | |
| Influenza PCR (CDC H1N1 kit) | **X** | **X** |
| Multiplex PCR for 33 respiratory pathogens (Fast-track Diagnostics) | | **X** |

### 3.2.5 Statistical analysis

Between January 2011 and March 2013, demographic and clinical data on enrolled adults were entered through a touch screen electronic data collection system – the Surveillance Programme of Inpatients and Epidemiology (SPINE) (SanJoaquin et al., 2013). In April 2013, this was migrated to a scannable paper-based case report form using optical recognition software (Cardiff TeleForm® Version 10.7, Vista, CA, USA).

Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals to compare clinical variables between influenza-positive and influenza-negative patients. Monthly proportions of influenza positivity among SARI patients were plotted against meteorological parameters (mean monthly temperature, rainfall and relative humidity obtained from the Department of Climate Change and Meteorological Services in Blantyre) to assess potential associations.

The incidence of adult hospital-attended influenza-positive SARI per 100,000 persons was estimated using the number of enrolled SARI cases who tested positive for influenza, adjusting for the recruited proportion of all SARIs that attended QECH each month as the numerator (SanJoaquin et al., 2013), divided by census estimates of Blantyre population aged >15years (National Statistics Office (NSO), 2008) and multiplied by 100,000, with Poisson confidence intervals (CIs).

To identify factors associated with influenza positivity and clinical severity (defined as Modified Early Warning Score (MEWS) >4 (Subbe et al., 2001) (Table 3.2) among SARI patients, multivariable Poisson models with robust error estimates were constructed (log binomial convergence failure occurred (Williamson et al., 2013; Chen et al., 2014)). Covariates with a p-value of <0.2 on univariable analysis were assessed for significance in the multivariable analysis using backwards-stepwise selection. Risk ratios (RR) and 95% CIs were reported.

**Table 3.2 Modified Early Warning Score (MEWS)**

| | 3 | 2 | 1 | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|
| SBP(mmHg) | <70 | 71-80 | 81-100 | 101-199 | | ≥200 | |
| Heart rate (beats/min) | | <40 | 41-50 | 51-100 | 101-110 | 111-129 | ≥130 |
| Respiratory rate (breaths/min) | | <9 | | 9-14 | 15-20 | 21-29 | ≥30 |
| Temperature (°c) | | <35 | | 35-38.5 | | ≥38.5 | |
| AVPU score | | | | Alert | Reacting to voice | Reacting to pain | Unresponsive |

SBP, systolic blood pressure; AVPU, "Alert, voice, pain, unresponsive"

## 3.3 Results

### 3.3.1 Demographic and clinical characteristics of the surveillance population

From 1 January 2011 to 31 December 2013, 1705 patients (579 ILI (2011), and 1126 SARI patients (2011-2013)) aged 15 years and over were enrolled. Demographic characteristics and influenza results are described in Table 3.3. The median ages of SARI and ILI cases were 28.6 years (range 15–71 years) and 32.9 years (range 15-88 years) respectively. 301 (52%) ILI and 489 (43.4%) SARI patients were male. Twenty-eight pregnant women were enrolled (9 with ILI and 19 with SARI). HIV status was available for 1109 (98.5%) SARI patients, of whom 558 (50.3%) were HIV-infected. Very few patients (1.4% ILI and 1.2% SARI) reported receipt of influenza vaccination in the previous year.

Overall, 95 (16.4%) ILI cases and 163 (14.5%) SARI cases were positive for influenza viruses (18.3% (2011); 9.6% (2012); 16.1% (2013), Table 3.3). Annual cycles of influenza activity were observed (Figure 3.1), but timing of peak detection varied year to year. In 2011, influenza activity had a bimodal peak – in April and July. In 2012, influenza was detected between March and June only. In contrast, influenza was detected throughout 2013, but peaked in January and February. Increased influenza activity was predominantly seen between January and June (proportion of influenza-positive SARI patients: January to March 19.5%, April to June 18%, July to September 12.3%, and October to December 5%). Influenza activity was lowest in the hot, dry months, while the peaks were associated with high relative humidity, but not with rainfall or low temperature (Figures 3.1 to 3.3). Influenza A(H1N1)pdm09, A(H3N2) and influenza B circulated in all three years; influenza

**Table 3.3 Demographic characteristics and influenza PCR results for adults enrolled with ILI and SARI, 2011-2013 (N=1705)**

| Characteristic | 2011 | 2011-2013 |
|---|---|---|
| | ILI (n=579) n (%) | SARI (n=1126) n (%) |
| Gender | | |
| Male | 301 (52.0) | 489 (43.4) |
| Age (median, IQR) | 28.6 (23.5-35.2) | 32.9 (26.6-42) |
| HIV status [a.b] | | |
| Positive | N/A | 558 (50.3) |
| Pregnancy (Female only) | (**n=278**) | **(n=637)** |
| Yes | 9 (3.2) | 19 (2.7) |
| No | 269 (96.8) | 618 (97.3) |
| Current smoker | 37 (6.6) | 29 (2.8) |
| Antibiotics in the past two week | 63 (10.9) | 482 (46.5) |
| Reported Influenza vaccination in past year[c] | 8 (1.4) | 13 (1.2) |
| **Influenza-PCR positive** | 95 (16.4) | 163 (14.5) |
| Year | | |
| 2011 | 95 (16.4) | 46 (18.3) |
| 2012 | - | 35 (9.6) |
| 2013 | - | 82 (16.1) |
| Type/subtype | **(n=95)** | **(n=163)** |
| Influenza A | | |
| Pandemic A(H1N1) | 43 (45.3) | 61 (37.4) |
| Seasonal A(H3N2) | 33 (34.7) | 47 (28.8) |
| A(un-subtyped)[d] | 3 (3.2) | 1 (0.6) |
| Influenza B | 15 (15.8) | 50 (30.7) |
| Influenza A & B co-infection | 1 (1.0) | 3 (1.8) |
| **Other respiratory viruses identified[a]** | | |
| None | - | 717 (63.7) |
| 1 | - | 285 (25.3) |
| ≥2 | | 124 (11.0) |

ILI, influenza-like illness; SARI, severe acute respiratory illness; N/A, not applicable; IQR=inter-quartile range; PCR=polymerase chain reaction

The number of missing observations in each variable is detailed in Appendix 8.4.1; individuals with missing data were excluded from the denominator in percentage calculations.

[a]HIV test and multiplex-PCR for 33 respiratory pathogens were only performed on SARI patients

[b]HIV result – missing in 17 SARI patients.

[c]There is no routine influenza vaccination programme in Malawi.

[d]Influenza A sample with CT values ≤40 which could not be subtyped.

A(H1N1)pdm09 dominating in 2011 (39.1%) and 2013 (49.4%), while seasonal influenza A(H3N2) was the most prevalent in 2012 (48.6%).

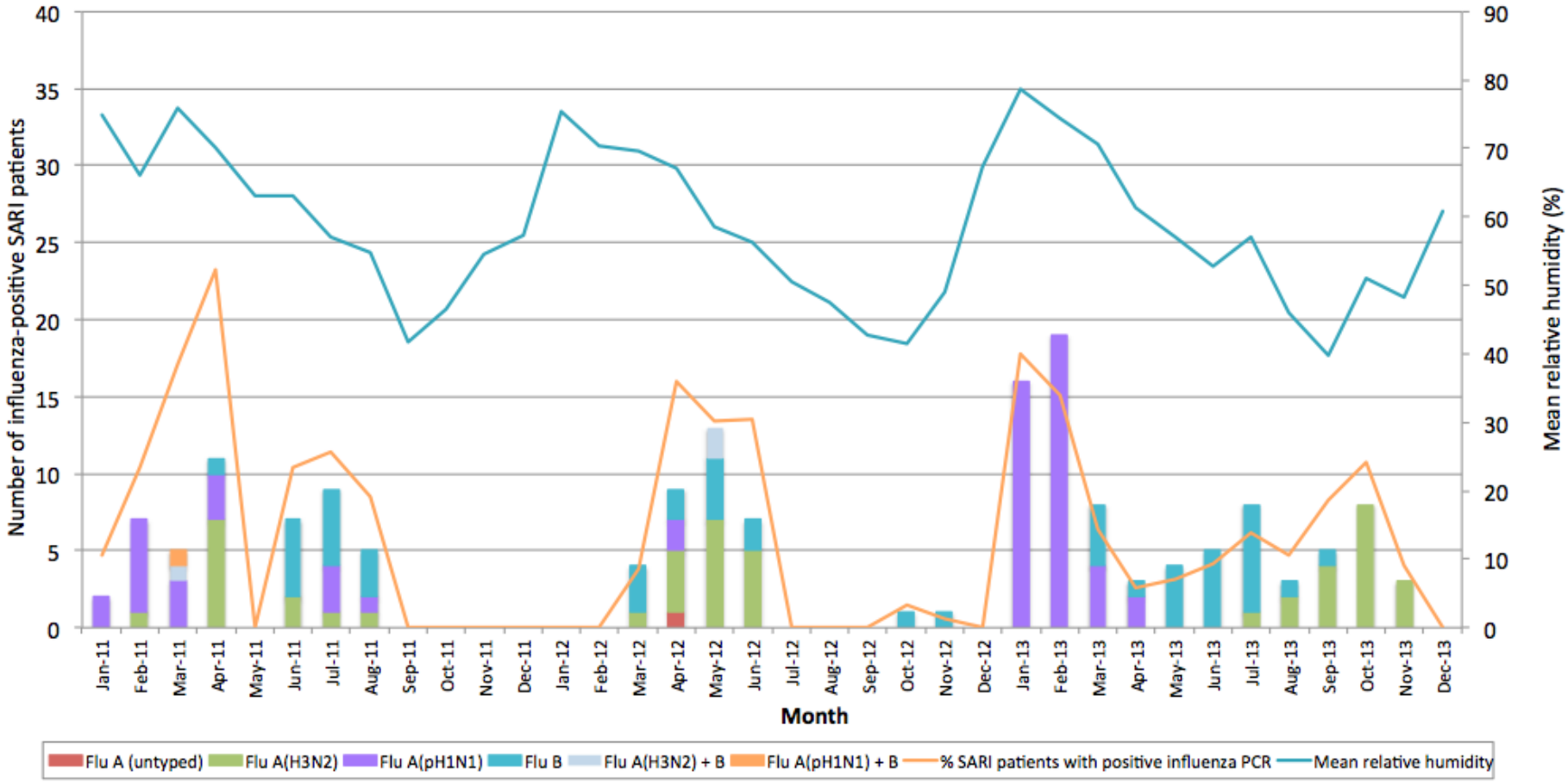



**Figure 3.1 Relationship between influenza positivity in enrolled adults with SARI, and monthly relative humidity, 2011-2013 (N=1126)**

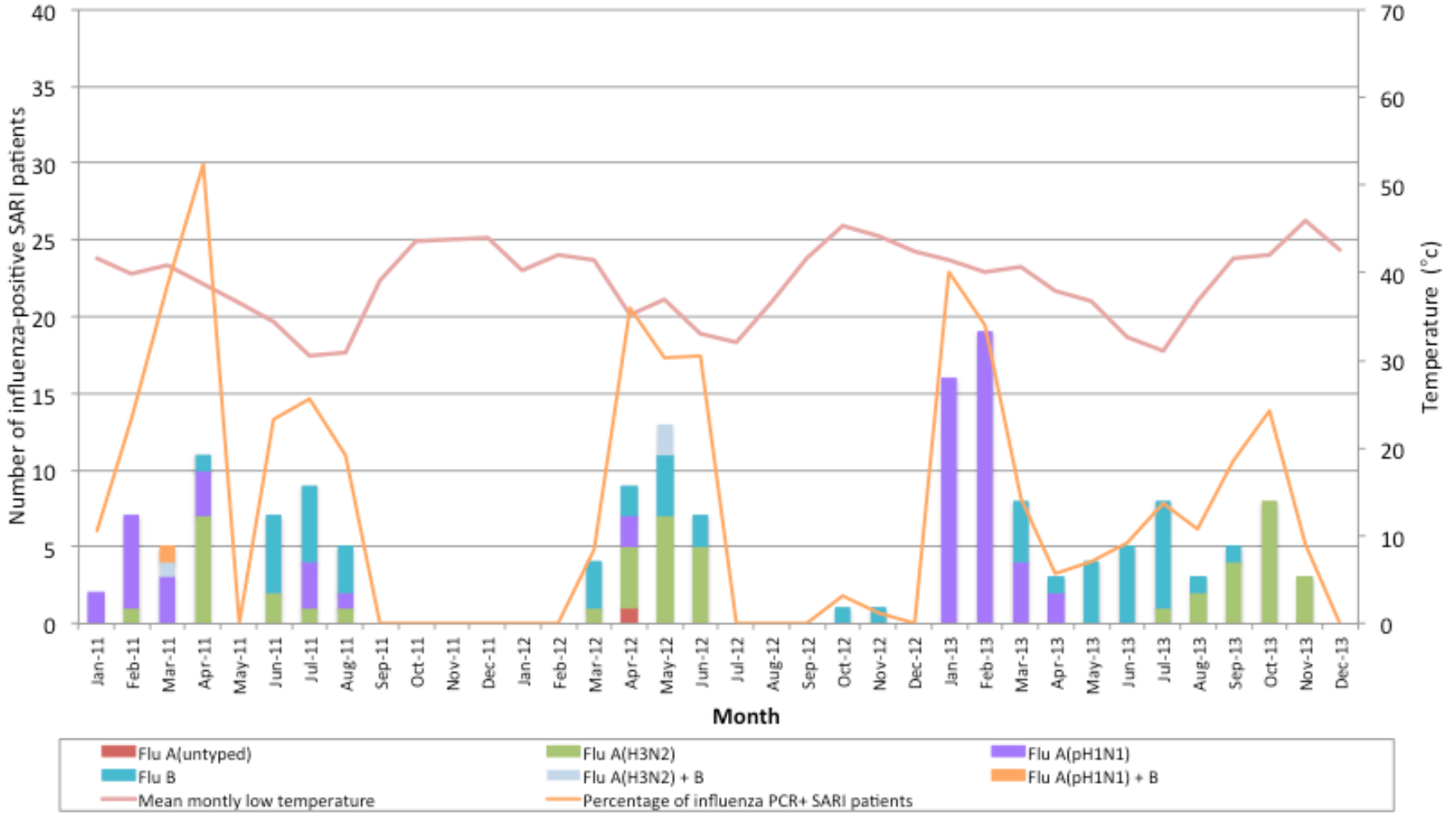



**Figure 3.2 Relationship between influenza positivity in enrolled adults with SARI, and monthly temperature, 2011-2013 (N=1126)**

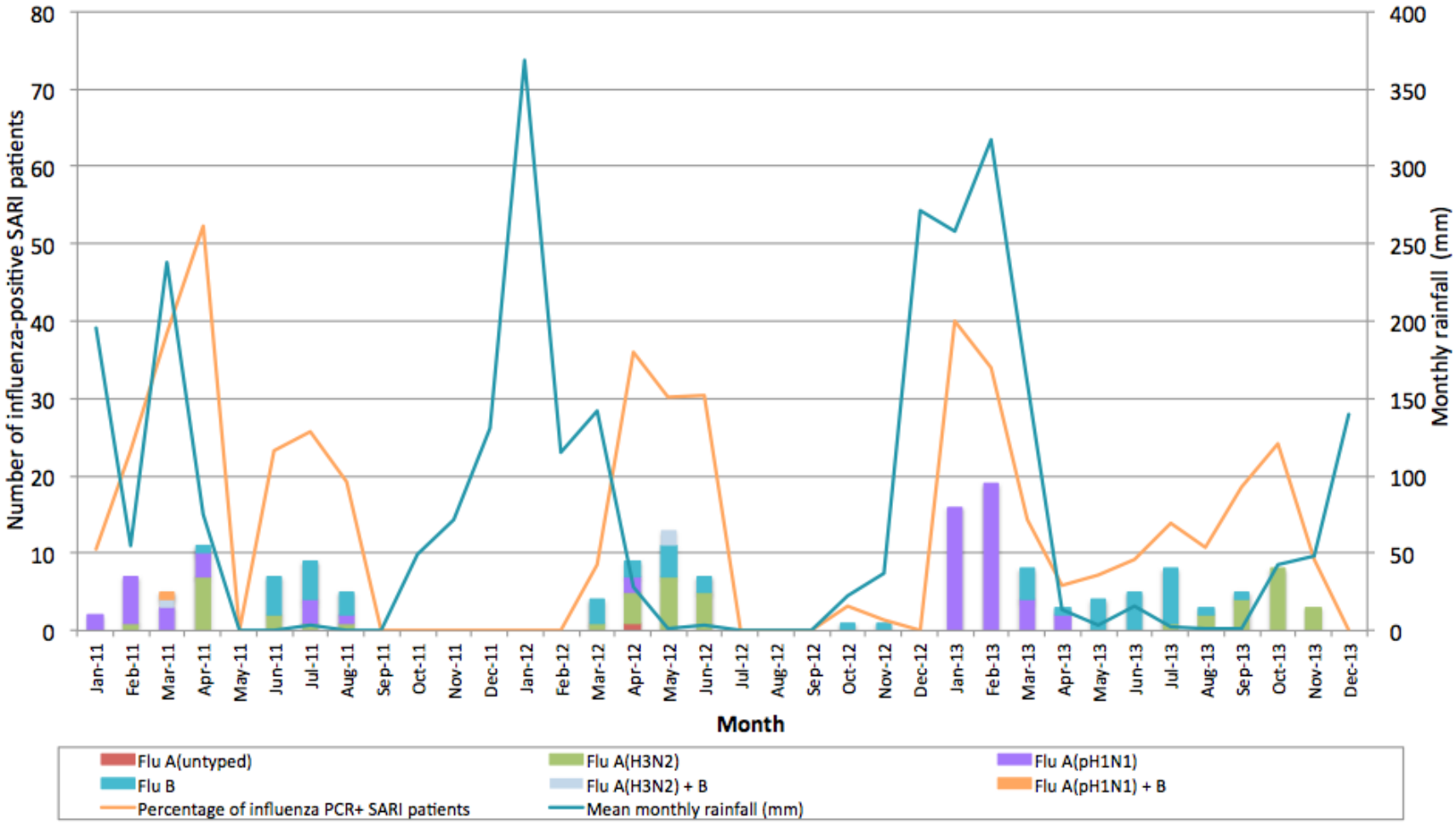



**Figure 3.3 Relationship between influenza positivity in enrolled adults with SARI, and monthly rainfall, 2011-2013 (N=1126)**

Malaria antigen test results were available for 562 (97.1%) ILI and 911 (80.9%) SARI patients; 3% of adults recruited with ILI or SARI had a positive malaria RDT; none were influenza positive.

Compared with influenza negative patients with SARI, headache was more (90.1 vs. 83.7%, OR 1.79 (95%CI 1.02-3.13), p=0.04) while rhinorrhoea was less (7.5 vs. 13.9%, OR 0.50 (95% CI 0.27-0.92, p=0.03) commonly reported among influenza positive patients. No other clinical differences by aetiology were found (Table 3.4).

**Table 3.4 Clinical characteristics of enrolled adults with SARI by influenza status (N=1126)**

| Characteristic | Number of cases (%) | | OR (95% CI) | P-value* |
|---|---|---|---|---|
| | Influenza PCR +ve (n=163) | Influenza PCR –ve (n=963) | | |
| **Symptoms** | | | | |
| Fever ≥38°c | 138/162 (85.2) | 770 (80.5) | 1.39 (0.88-2.23) | 0.16 |
| Cough | 149/152 (98.0) | 866/892 (97.1) | 1.49 (0.45-4.99) | 0.52 |
| Sore throat | 95/150 (63.3) | 592/890 (66.5) | 0.87 (0.61-1.25) | 0.45 |
| Headache | 137/152 (90.1) | 747/893 (83.7) | 1.79 (1.02-3.13) | 0.04 |
| Rhinorrhoea | 12/161 (7.5) | 132/950 (13.9) | 0.50 (0.27-0.92) | 0.03 |
| Myalgia | 129/152 (84.9) | 750/885 (84.7) | 1.02 (0.63-1.64) | 0.94 |
| Vomiting/diarrhoea | 19/151 (12.6) | 173/892 (16.5) | 0.73 (0.44-1.22) | 0.23 |
| **Signs** | | | | |
| Systolic BP <90mmHg | 27/152 (17.5) | 114/892 (12.8) | 1.44 (0.91-2.30) | 0.12 |
| HR >120/minute | 67/153 (44.0) | 340/892 (38.1) | 1.27 (0.90-1.80) | 0.18 |
| RR >30/minute | 76/151 (50.3) | 401/886 (45.3) | 1.23 (0.87-1.74) | 0.24 |
| Oxygen saturation ≤90% on air | 22/154 (14.0) | 104/894 (11.6) | 1.25 (0.76-2.08) | 0.37 |

PCR, polymerase chain reaction; OR, odds ratio; BP, blood pressure; HR, heart rate; RR, respiratory rate

### 3.3.2 Incidence of hospital-attended influenza-associated SARI

The incidence of hospital-attended influenza-associated SARI for Blantyre was estimated at 16.8 (95%CI 13.8-19.8) in 2011, 9.7 (95% CI 7.4-11.9) in 2012, and 16.9 (95%CI 14.0-19.8) in 2013, per 100,000 persons aged 15 years and over.

### 3.3.3 Prevalence of other respiratory viruses

Respiratory viruses other than influenza were identified in almost a third of enrolled SARI patients (32.2%, n=363) (Figure 3.4). The most ubiquitous were rhinovirus (n=149, 13.2%), RSV (n=48, 4.2%), adenovirus (n=47, 4.1%) and coronavirus OC43 (n=47, 4.1%). 253 (22.5%) had a single virus isolated, whereas co-detections were found in 150 (13.3%) adults.

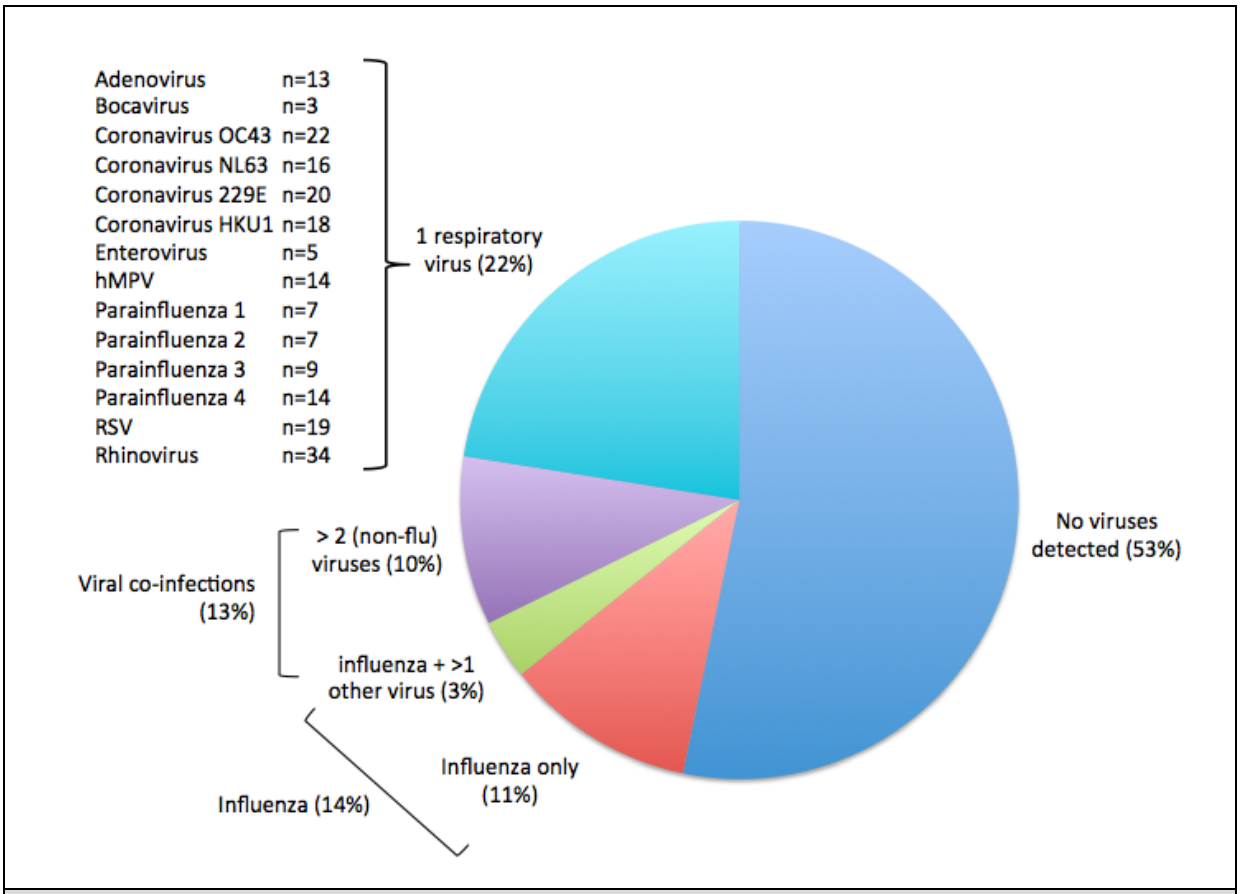



**Figure 3.4 Influenza and respiratory viruses detected in enrolled adults with SARI, 2011-2013 (N=1126)**

### 3.3.4 Factors associated with influenza positivity in SARI patients

Results from multivariable analysis (Table 3.5) indicate that, among SARI patients, non-pregnant women were more likely than men to be positive for influenza (adjusted risk ratio (aRR) 1.45, 95%CI 1.08-1.98). However, pregnant women had an almost three-fold increased risk of influenza infection, compared to men (aRR 2.82, 95%CI 1.41-5.83, p=0.007). A sub-analysis restricted to women of childbearing age (CBA) (15 to 45 years, n=505) with SARI was performed (Table 3.6). HIV prevalence was substantially lower in pregnant compared to non-pregnant women of CBA (21 vs. 54%, p=0.005). However, pregnant women were nearly twice as likely to be influenza positive than non-pregnant women, after adjustment for HIV status, recruitment year and viral co-infections (31 vs. 15%, aRR 1.84, 95%CI 0.99-3.43, p=0.05).

**Table 3.5 Factors associated with influenza PCR positivity in enrolled adults with SARI, 2011-2013 (N=1126)**

| Characteristic | Number of cases with +ve influenza-PCR (%) | Univariable[a] | | Multivariable[a,b] | |
|---|---|---|---|---|---|
| | | RR (95% CI) | P-value* | RR (95% CI) | P-value |
| **Sex** | | | | | |
| Male | 58/489 (11.9) | 1 | | | |
| Female | 105/636 (16.5) | 1.39 (1.03-1.88) | 0.03 | | |
| **Age group (years)** | | | | | |
| 15-24 | 40/230 (17.4) | 1.32 (0.92-1.91) | 0.33 | 1.35 (0.90-1.90) | 0.65 |
| 25-34 | 59/414 (14.3) | 1.08 (0.78-1.51) | | 1.06 (0.76-1.47) | |
| ≥ 35 | 62/472 (13.2) | 1 | | 1 | |
| **HIV status** | | | | | |
| Negative | 96/551 (17.5) | 1 | | 1 | |
| Positive | 60/558 (10.8) | 0.61 (0.46-0.83) | <0.001 | 0.59 (0.43-0.80) | <0.001 |
| **Medical history** | | | | | |
| Malaria RDT - negative | 138/882 (15.6) | | | | |
| - positive | 0/28 (0) | - | - | | |
| Recent antibiotics – no | 86/580 (14.8) | 1 | | | |
| – yes | 73/501 (14.6) | 0.99 (0.74-1.33) | 0.93 | | |
| Pregnancy – male (not applicable) | 58/494 (11.7) | 1 | | 1 | |
| – non-pregnant female | 99/612 (16.2) | 1.37 (1.02-1.86) | | 1.45 (1.08-1.98) | |
| – pregnant female | 6/19 (31.6) | 2.70 (1.33-5.44) | 0.02 | 2.82 (1.41-5.63) | 0.007 |
| Current smoking – no | 140/945 (14.8) | 1 | | | |
| – yes | 12/102 (11.8) | 0.79 (0.46-1.38) | 0.41 | | |
| **Year of surveillance** | | | | | |
| 2011 | 46/251 (18.6) | 1.91 (1.27-2.89) | | 2.38 (1.56-3.63) | |
| 2012 | 35/366 (9.6) | 1 | | 1 | |
| 2013 | 82/509 (16.1) | 1.69 (1.16-2.49) | 0.003 | 1.69 (1.14-2.49) | <0.001 |
| **Viral co-infections** | | | | | |
| None | 124 /717 (17.3) | 1 | | 1 | |
| >1 other respiratory virus | 39/408 (9.6) | 0.55 (0.39-0.78) | 0.001 | 0.51 (0.36-0.73) | <0.001 |

PCR, polymerase chain reaction; RR, risk ratio; CI, confidence interval; RDT, rapid diagnostic test

[a] Generalised linear model using Poisson distribution with robust error estimates.

[b]Backward stepwise approach, including *a priori* confounders (age, sex, HIV status and year of surveillance) and all variables with p<0.20 in univariate analysis.

**Table 3.6 Sub-analysis of factors associated influenza PCR positivity in females of childbearing age (15-45 years) with SARI, 2011-2013 (N=505)**

| Characteristic | Number of cases with +ve influenza-PCR (%) | Univariable[a] | | Multivariable[a,b] | |
|---|---|---|---|---|---|
| | | RR (95% CI) | P-value* | RR (95% CI) | P-value* |
| Pregnant | | | | | |
| No | 74/486 (15.2) | 1 | | 1 | |
| Yes | 6/19 (31.6) | 2.06 (1.03-4.14) | 0.04 | 1.84 (0.99-3.43) | 0.05 |
| HIV status | | | | | |
| Negative | 51/237 (21.5) | 1 | | 1 | |
| Positive | 27/262 (10.3) | 0.61 (0.46-0.83) | <0.001 | 0.45 (0.29-0.70) | <0.001 |
| Year of surveillance | | | | | |
| 2011 | 22/126 (17.5) | 2.36 (1.21-4.58) | | 2.82 (1.48-5.41) | |
| 2012 | 12/162 (7.4) | 1 | | 1 | |
| 2013 | 46/216 (21.3) | 2.88 (1.58-5.25) | <0.001 | 2.45 (1.35-4.45) | <0.001 |
| Viral co-infections | | | | | |
| None | 62/304 (20.4) | 1 | | 1 | |
| >1 other respiratory virus | 18/200 (9.0) | 0.44 (0.27-0.72) | 0.001 | 0.42 (0.26-0.69) | <0.001 |

RR, risk ratio; CI, confidence interval

[a]Generalised linear model using Poisson regression with robust error estimates

[b]Adjusted for all variables in the model

Influenza-positive adults with SARI were less likely to be HIV-infected (aRR 0.59; 95%CI 0.43-0.80, p<0.001) or have co-detection with an additional respiratory virus (aRR 0.51; 95%CI 0.36-0.73, p<0.001). Small numbers prohibited the evaluation of specific viral co-detection combinations with influenza.

### 3.3.5 Factors associated with clinical severity among SARI patients

422 (37.5%) SARI patients had clinically severe disease (MEWS score >4). No inpatient deaths were recorded, though patient follow-up after enrolment was incomplete. In the multivariable analysis (Table 3.7), HIV infection (aRR 1.58, 95%CI 1.23-2.04), and PCR-detected *P. jirovecii* (aRR 1.61, 95%CI 1.05-2.47) were associated with clinical severity. Influenza positivity was not associated with clinical severity. However, HIV infection predicted severity in both influenza-positive (RR 2.30, 95%CI 1.33-3.99) and influenza-negative (RR 1.46, 95%CI 1.13-1.89) SARI patients. Other viral aetiology (e.g. hMPV, parainfluenza 2) did not predict severity. Smoking was reported in 102 (9.7%) individuals, but was not associated with severity.

**Table 3.7 Factors associated with clinical severity in adults with SARI, 2011-2013 (N=1126)**

| Characteristic | Number of cases with clinical severity (n=422) (%) | Univariable[b] | | Multivariable[b,c] | |
|---|---|---|---|---|---|
| | | RR (95% CI) | P-value | RR (95% CI) | P-value |
| **Sex** | | | | | |
| Male | 105/489 (21.5) | 1 | | 1 | |
| Female | 133/636 (20.9) | 0.97 (0.77-1.22) | 0.81 | 0.88 (0.70-1.11) | 0.27 |
| **Age group** | | | | | |
| 15-24 | 46/231 (19.9) | 1 | | 1 | |
| 25-34 | 87/419 (20.8) | 1.04 (0.76-1.43) | | 0.96 (0.68-1.35) | |
| ≥ 35 | 105/476 (22.1) | 1.11 (0.81-1.51) | | 1.00 (0.73-1.39) | 0.95 |
| **HIV –** Negative | 91/551 (16.5) | 1 | | 1 | |
| **–** Positive | 143/558 (25.6) | 1.55 (1.23-1.96) | <0.001 | 1.58 (1.23-2.04) | <0.001 |
| **Medical history** | | | | | |
| Pregnancy - No | 236/1107 (21.3) | 1 | | | |
| - Yes | 2/19 (10.5) | 0.49 (0.13-1.84) | 0.29 | | |
| Recent antibiotics - No | 134/548 (24.5) | 1 | - | | |
| - Yes | 101/483 (20.9) | 0.85 (0.68-1.07) | 0.18 | | |
| Current smoker - No | 224/946 (23.7) | 1 | | | |
| - Yes | 14/102 (13.7) | 0.58 (0.35-0.96) | 0.03 | | |
| **Malaria RDT** - Negative | | | | | |
| - Positive | 213/883 (24.1) | 1 | | | |
| **Influenza PCR** - Negative | 6/28 (21.4) | 0.89 (0.43-1.82) | 0.75 | | |
| - Positive | 198/962 (20.6) | 1 | | | |
| | 40/163 (24.5) | 1.19 (0.89-1.60) | 0.24 | | |
| **Viral co-infections** | | | | | |
| No | 198/976 (20.3) | 1 | | | |
| Yes | 40/150 (26.7) | 1.31 (0.98-1.76) | 0.07 | | |
| **Respiratory pathogens** | | | | | |
| Adenovirus - Negative | 228/1076 (21.2) | 1 | | | |
| - Positive | 10/47 (21.3) | 1.00 (0.57-1.76) | 0.99 | | |
| Bocavirus - Negative | 232/1099 (21.1) | 1 | | | |
| - Positive | 6/24 (25.0) | 1.18 (0.59-2.39) | 0.64 | | |
| Coronavirus | | | | | |
| OC43 - Negative | 225/1074 (21.0) | 1 | | | |
| - Positive | 13/49 (26.5) | 1.27 (0.78-2.05) | 0.33 | | |
| NL63 - Negative | 227/1086 (20.9) | 1 | | | |
| - Positive | 11/37 (29.7) | 1.42 (0.86-2.37) | 0.18 | | |
| 229E - Negative | 227/1088 (20.9) | 1 | | | |
| - Positive | 11/35 (31.4) | 1.51 (0.91-2.49) | 0.11 | | |
| HKU1 - Negative | | 1 | | | |
| - Positive | | 0.85 (0.43-1.67) | 0.63 | | |
| Enterovirus - Negative | 236/1091 (21.6) | 1 | | | |
| - Positive | 2/32 (6.3) | 0.28 (0.08-1.11) | 0.07 | | |
| hMPV - Negative | 224/1089 (20.6) | 1 | | | |
| - Positive | 14/34 (41.2) | 2.00 (1.32-3.04) | 0.004 | | |
| Parainfluenza | | | | | |
| 1 - Negative | 230/1095 (21.0) | 1 | | | |
| - Positive | 8/28 (28.6) | 1.36 (0.75-2.47) | 0.31 | | |
| 2 - Negative | 231/1105 (20.9) | 1 | | | |
| - Positive | 7/18 (38.9) | 1.86 (1.03-3.36) | 0.04 | | |
| 3 - Negative | 234/1100 (21.3) | 1 | | | |
| - Positive | 4/23 (17.4) | 0.82 (0.33-2.01) | 0.66 | | |
| 4 - Negative | 233/1095 (21.3) | 1 | | | |

| | | | | | |
|---|---|---|---|---|---|
| - Positive | 5/28 (17.9) | 0.84 (0.38-1.87) | 0.67 | | |
| RSV - Negative | 226/1075 (21.0) | 1 | | | |
| - Positive | 12/47 (25.0) | 1.18 (0.72-1.97) | 0.50 | | |
| Rhinovirus - Negative | 201/974 (20.6) | 1 | | | |
| - Positive | 37/149 (24.8) | 1.20 (0.89-1.63) | 0.24 | | |
| *Chlamydia pneumoniae* | | | | | |
| - Negative | 234/1107 (21.1) | 1 | | | |
| - Positive | 4/16 (25.0) | 1.18 (0.50-2.78) | 0.70 | | |
| *Legionella species* | | | | | |
| - Negative | 189/944 (20.0) | 1 | | | |
| - Positive | 27/129 (20.9) | 1.05 (0.73-1.50) | 0.81 | | |
| *Mycoplasma pneumoniae* | | | | | |
| - Negative | 233/1099 (21.2) | 1 | | | |
| - Positive | 5/24 (20.8) | 0.98 (0.45-2.16) | 0.97 | | |
| *Pneumocystis jirovecii* | | | | | |
| - Negative | 205/1047 (19.6) | 1 | | 1 | |
| - Positive | 11/26 (42.3) | 2.16 (1.36-3.44) | 0.001 | 1.61 (1.05-2.47) | 0.03 |

RR, risk ratio; CI, confidence interval; RDT, rapid diagnostic test; PCR, polymerase chain reaction; hMPV, human metapneumovirus; RSV, respiratory syncytial virus.

[a] As defined by a MEWS score of >4

[b] Generalised linear model using Poisson distribution with robust error estimates.

## 3.4 Discussion

Our data substantiate the emerging evidence from the African region that the contribution of influenza to life-threatening respiratory disease, although previously under-recognised, is considerable (Radin et al., 2012). Comprehensive hospital-based sentinel surveillance in our high HIV seroprevalence, malaria-endemic African setting has illustrated influenza as an important contributor to mild (16.4%) and severe (14.6%) acute respiratory illnesses in adults. In the immediate post-pandemic period, influenza A(H1N1)pdm09 was the predominant strain in Malawi in 2011 and 2013, but was largely absent in 2012. Our data also highlight the strong association between pregnancy and influenza in SSA. Furthermore, around a quarter of influenza-positive adults with SARI presenting to hospital have severe disease, and that HIV infection is a predictor of severity in both influenza positive and influenza negative cases.

The identification of pregnancy as a potential risk factor for influenza has considerable public health importance. While epidemiological evidence from developed settings suggest that pregnant women are at increased risk for severe illness and death (Mosby et al., 2011; Van Kerkhove et al., 2011b), thus a priority group for influenza vaccination (2012), the association between pregnancy and influenza has not been described in SSA outside a pandemic. This finding has important implications in the region with the highest fertility rate (The World Bank, 2014). It is possible that pregnant women are a healthier group compared to non-pregnant women, thus the higher risk seen in this

group may be due to the greater contribution of influenza to SARI relative to other pathogens.

Over half of enrolled adults with SARI were HIV-positive, and HIV infection independently predicted clinical severity in both influenza positive and influenza negative cases. HIV is a risk factor for severe pneumonia (Mwachari et al., 2006; Feldman et al., 2009; Sogaard et al., 2008; Torres et al., 2013); indeed several SSA hospital pneumonia aetiology studies reported a high prevalence of HIV infection (26-94%) (Scott et al., 2000; Hartung et al., 2011; Koulla-Shiro et al., 1996). Furthermore, recent studies from Kenya (Ope et al., 2011) and South Africa (Cohen et al., 2013) have suggested an increased risk of influenza-related hospitalisation in HIV-infected persons.

Influenza was less commonly identified in HIV-infected compared to HIV-uninfected adults with SARI. This has also been described in HIV-infected children (Madhi et al., 2000) and adults (Cohen et al., 2015c) in South Africa, and likely reflects the different spectrum of organisms affecting HIV-infected adults (Park et al., 2001), with greater relative contribution of opportunistic pathogens such as *P. jirovecii*, *S. pneumoniae* and *M. tuberculosis* (Raju et al., 2012; Benito et al., 2012), rather than a lower absolute risk (i.e. the converse of pregnancy). Although we were able to ascertain HIV status for the majority of enrolled SARI patients (98.5%), our study was not powered to evaluate the impact of HIV infection on the burden and severity of influenza infection, particularly across CD4 cell strata. This association will be evaluated in the studies described in this thesis (Chapters 4 and 5).

The minimum incidence estimates of hospital-attended influenza-positive SARI (9.6 to 16.9 per 100,000 adult population) were lower than other published estimates from Kenya (30 (Emukule et al., 2014) and 36.7 (Feikin et al., 2012b) per 100,000) and South Africa (71-260 per 100,000 (in HIV-infected patients) and 5-44 per 100,000 (in HIV-uninfected patients)) (Cohen et al., 2013). These differences may be due to a genuine difference in disease burden, but may also in part be due to heterogeneous methodologies. All three previous African studies included children aged 5-14 years, a group that typically has higher annual rates of influenza infection (Feikin et al., 2012b; Cohen et al., 2013). Variations in health-seeking behavior and thresholds for hospital admission between the sites are also likely contributory factors. Moreover, ours are likely to represent minimum estimates, since our surveillance only detects individuals that access care at QECH. There are numerous reasons why access to QECH may not occur: patients may have presented to other healthcare facilities or a traditional healer; they may not consider their symptoms severe enough to warrant care; may be too ill or too poor to attend; or may have died prior to presentation (Desmond et al., 2013).

There is increasing recognition that, viruses such as rhinovirus, adenovirus, hMPV, parainfluenza viruses, and coronaviruses can cause clinically severe disease (Arden et al., 2005; Choi et al., 2012; Fry et al., 2011; Cao et al., 2014). Although other respiratory viruses were prevalent in patients with

SARI, and detection of multiple viruses was not uncommon, influenza tended to occur in isolation. Studies that have evaluated the association between viral co-infection and disease severity have yielded mixed results (Drews et al., 1997; Greensill et al., 2003; Semple et al., 2005; Templeton et al., 2005; Subbarao et al., 1989; Peng et al., 2009). We identified two or more viral pathogens in 13% adults with SARI, and found a non-significant trend towards increased severity in adults with viral co-infection (26.7% vs. 20.3% with MEWS >4). Further understanding of the interactions and contribution of these viruses to severe respiratory disease will help to narrow the focus on pertinent targets for vaccine and antiviral development.

A number of limitations need to be considered. First, we conducted hospital-based surveillance at a single site. While there are no other large inpatient facilities in Blantyre, we have not sampled from elsewhere in Malawi. Secondly, data on whether household contacts were unwell or also recruited in the study were not captured. We were therefore unable to account for potential household clustering. Thirdly, co-morbidities were poorly recorded, thus we were unable to evaluate chronic lung disease as a potential risk factor for influenza, or adjust for underlying co-morbidities in the multivariable analysis for clinical severity. Furthermore, data on hospitalisation and mortality were not systematically captured. Instead, we utilised the MEWS score as a surrogate marker for clinical severity. The score has been widely used in developed healthcare settings to identify patients at risk of deterioration, and a threshold of greater than four is predictive of inpatient mortality (Subbe et al., 2001). It has also been validated in other African settings (Burch et al., 2008; Rylance et al., 2009; Wheeler et al., 2013).

Vaccination with inactivated influenza vaccine (IIV) is safe, and effective in HIV-infected adults (Madhi et al., 2011), as well as pregnant women (both HIV-infected or uninfected) in Africa (Madhi et al., 2014). Moreover, vaccination of pregnant women also confers protection to their young infants less than 6 months of age (Madhi et al., 2014; Zaman et al., 2008), who respond poorly to vaccination (Englund et al., 2010). At present, IIV is unavailable through the public sector in Malawi and many similar African countries. Difficulties encountered by the vaccine donation initiative in the procurement, distribution and uptake of A(H1N1)pdm09 vaccine highlight the potential challenges of implementing annual vaccination (Schoub et al., 2013). Nevertheless, a targeted adult vaccination programme may utilise existing health infrastructure, namely HIV care and antenatal services.

In conclusion, there is a substantial burden of previously unstudied influenza-related severe respiratory disease in Malawian adults, a finding likely to be generalizable to other SSA urban areas. Surveillance for influenza and other respiratory viruses, with specific focus on severe disease in pregnant women and HIV-infected individuals, and greater effort to capture outcome data, is critical to further characterise disease burden in these high-risk groups. Annual vaccination is indicated for

both groups under current WHO recommendations, with pregnant women designated the highest priority (2012). Moreover, the GAVI Alliance are considering funding routine influenza immunization of pregnant women at first antenatal visit in 2018 (GAVI the Vaccine Alliance, 2013), many of whom in SSA, will be HIV-infected. How, when and who to administer vaccine remains a considerable challenge.

# CHAPTER 4. Incidence of Laboratory-confirmed Influenza Illness Among HIV-infected and HIV-uninfected Adults in Malawi: A Prospective Cohort Study

## 4.1 Introduction

Influenza infection and its complications are leading causes of morbidity and mortality worldwide (World Health Organization, 2014c). Immunocompromised persons, including those with HIV infection, are considered to be at greater risk of influenza-associated morbidity and mortality, thus are a priority group for annual seasonal influenza vaccination in many developed settings (Geretti et al., 2008; Grohskopf et al., 2014; National Advisory Committee on Immunization (NACI), 2014). However, influenza vaccine is not available in most sub-Saharan African countries (Duque et al., 2014). In 2009, the WHO highlighted the urgent need for more data on the burden of influenza in African countries and its effects on immunosuppressed persons due to the high prevalence of HIV infection in the region (World Health Organization, 2012a). Emerging data from surveillance programmes in SSA (Radin et al., 2012), including Malawi (Chapter 3), suggest that influenza is prevalent in adults with acute respiratory illness. However, few published studies have reported HIV status.

Data on the impact of HIV infection on susceptibility to influenza infection have been limited to several outbreak investigations (Cohen and Macauley, 1989; Fine et al., 2001; Boschini et al., 2006), in addition to several case series (Campos-Loza et al., 2010; Martinez et al., 2011) and serology studies (Althoff et al., 2010; Hung et al., 2013; Garg et al., 2014) from the recent 2009 A(H1N1) pandemic (Chapter 1.4). All were conducted in low HIV prevalence settings with conflicting results.

Two recently published surveillance studies from Kenya (Feikin et al., 2012b) and South Africa (Cohen et al., 2013) reported higher incidence of influenza infection in HIV-infected persons, compared to HIV-uninfected persons. However, they had incomplete data on HIV status (missing from 31% (Feikin et al., 2012b) and 22% (Cohen et al., 2013) enrolled participants, respectively), as well as CD4+ cell count and antiretroviral treatment. Additionally, both studies included children and adults, and did not include individual-level data to account for potential confounding factors.

We therefore conducted a prospective cohort study in an urban SSA setting with a high HIV prevalence, to examine the impact of HIV infection on the incidence of laboratory-confirmed

influenza illness, while controlling for possible confounders and defining additional risk factors that increase influenza burden in Malawian adults.

## 4.2 Methods

### 4.2.1 Study design

This was a prospective cohort study of HIV-infected, and HIV-uninfected adults, with a follow-up period of two years (Figure 4.1).

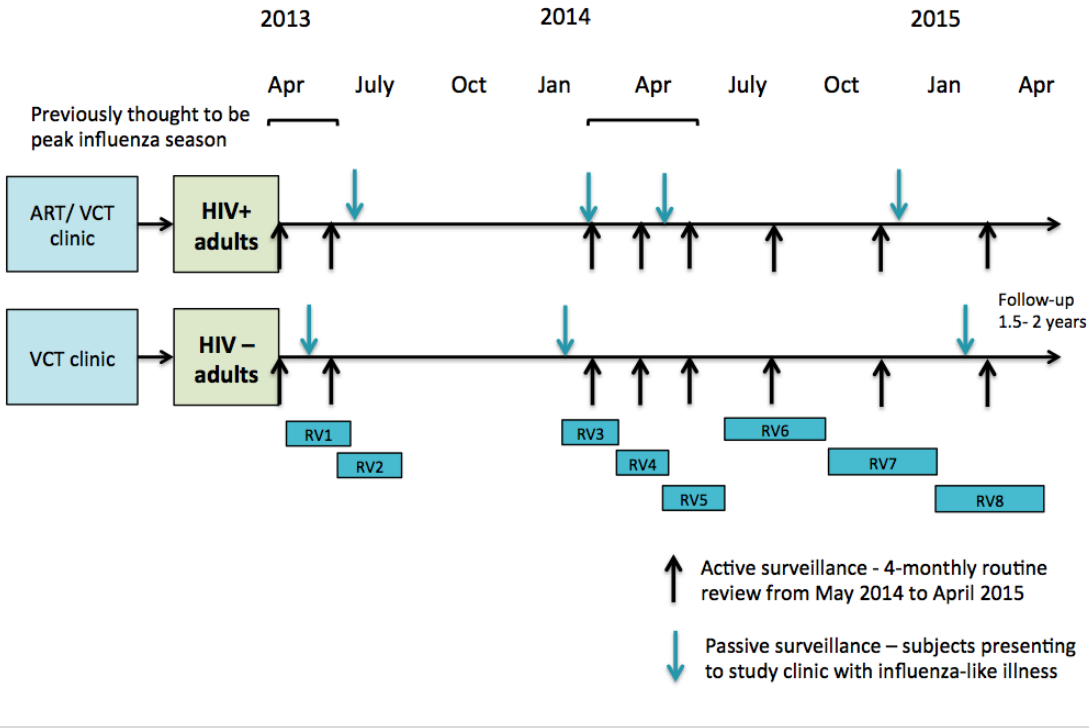



**Figure 4.1 Overview of study site, study population, and active and passive surveillance**
ART-antiretroviral treatment; VCT-voluntary counselling and testing; RV-routine review

### 4.2.2 Study site

Patients were enrolled from the ART and VCT clinics at QECH (Section 2.1.1.2). The study clinic was located within the VCT clinic (Chapter 2.1.2, figure 2.1). Study staff were present between 8am and 5pm on weekdays.

### 4.2.3 Study population

Adults aged ≥18 years were recruited from the ART and VCT clinics at QECH between April 2013 and January 2014. The following eligibility criteria were applied:

*Inclusion criteria*

- Aged 18 years or over
- Intending to stay in Blantyre in the next year
- Willing to attend QECH for scheduled and illness visits
- Willing to undergo an HIV test (if status unknown)
- Able to give informed consent (personally or by legal proxy)

*Exclusion criteria*

- Acute active lung disease (including acute pneumonia, suspected TB not on treatment, and intensive phase of tuberculosis treatment)
- Terminal illness (e.g. metastatic malignancy, terminal AIDS)
- Previous influenza vaccination
- Another household member already enrolled in study

Persons that met the eligibility criteria who were willing to consider participation in the study received a patient information document (in Chichewa or English). Written informed consent was obtained from the patient/guardian.

### 4.2.4 Study procedures

#### *4.2.4.1 Staff*

The following staff were employed to maintain the study cohort:

- 1 nurse coordinator: responsible for consent and enrolment of participants during recruitment period; taking the majority of clinical specimens, including naso-/oropharyngeal swabs, blood and urine; conducted routine reviews; and was in charge of coordinating participant follow-up and field worker visit schedule.
- 1 clinical officer: responsible for consent and enrolment of participants during recruitment period; aided nurse coordinator in obtaining clinical specimens; and conducted routine reviews and clinical assessment of participants attending with ILI episodes.

- 2 qualified HIV testing counsellors/ field workers: performed sensitisation talks at ART/VCT clinic and HTC counselling during recruitment period; trained in nasopharyngeal and oropharyngeal swab sampling; and conducted home visits of defaulted participants.
- 1 molecular laboratory technician: responsible for aliquoting, storage and processing combined nasopharyngeal & oropharyngeal specimens, as part of a team of technicians in the core diagnostic laboratory.

#### *4.2.4.2 Enrolment*

Demographic and clinical information, in addition to exposure to risk factors for influenza were obtained from study participants using a structured questionnaire (appendix 8.4). Data collected included: demographic details; clinical history (including co-morbidities, previous history of tuberculosis or pneumonia, pregnancy, history of pneumococcal conjugate vaccination, and HIV history); smoking and alcohol use; household characteristics (including household size, number of young children in household, number of rooms in dwelling); and socioeconomic status. The latter was defined by highest level of education attained (none, primary, secondary or tertiary), employment (yes or no), asset ownership (ownership of mobile phone, radio, refrigerator, bed and car/motorbike), food security (frequency of difficulty accessing food), access to water (type and distance water supply from dwelling) and type of sanitation facility.

Baseline clinical examination was performed to determine body mass index (BMI), oxygen saturation, evidence of chronic lung disease, in addition to the WHO clinical staging for HIV (if the participant was HIV-reactive).

A sticker was placed in the participant's health passport (figure 4.2) to identify them as a participant of the BASH-FLU cohort study; as a record of their study identification number; and to provide contact details in case the participant wished to contact the study team.

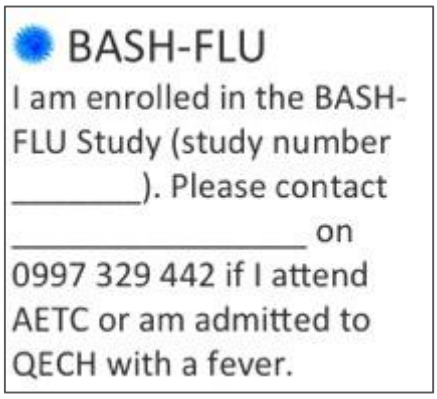



**Figure 4.2 Sticker that identifies the individual as a BASH-FLU cohort study participant**

#### *4.2.4.3 Active and passive surveillance*

The follow-up period was from the date of enrolment until 31 March 2015. Follow-up consisted of active and passive surveillance. Participants were reimbursed for their transport costs.

*Active surveillance*

Routine reviews (i.e. active surveillance) for the first year of the study took place monthly (x3) between April and July (thought to be peak influenza season from surveillance data 2011-2012). However, year-round influenza transmission was found in 2013 (Chapter 3.3), hence active surveillance was modified to 4-monthly visits in the second year of follow-up (figure 4.1). At each visit, participants were screened for influenza-like symptoms within the past 4 weeks, and nasopharyngeal and oropharyngeal swabs were obtained. The purpose of active surveillance was to ascertain the prevalence of asymptomatic carriage of influenza virus.

*Passive surveillance*

During the study period, participants were instructed to attend study clinic as soon as possible if they developed influenza-like symptoms. These were defined as reported or documented fever ($\geq$38°C) and $\geq$ 2 of the following symptoms: cough, rhinorrhoea, sore throat, myalgia, headache and vomiting/diarrhoea. A leaflet detailing the above symptoms was given to participants to take home at enrolment.

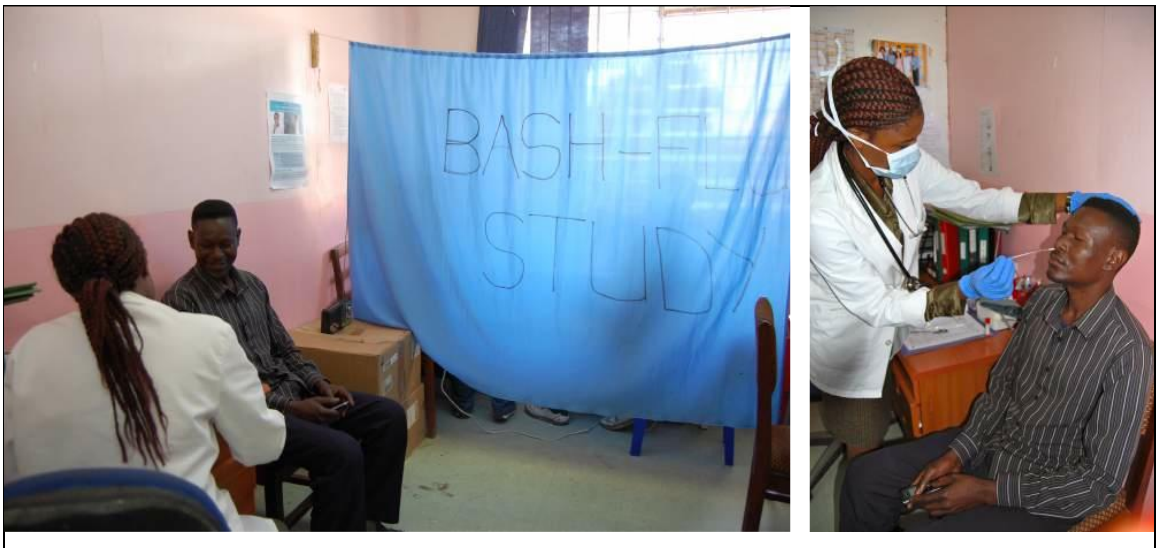


**Figure 4.3 A) The study clinical officer conducting a routine review of a participant at the study clinic; B) obtaining a nasopharyngeal specimen**

The study clinical officer assessed ill participants at the study clinic from 8am to 5pm during weekdays. Outwith these times, participants were advised to attend their local health centre or AETC if they were severely unwell, and inform the study team through the study phone. Any participant that was admitted to QECH was assessed by the study clinical officer at the earliest opportunity.

Data collected during the illness episodes included clinical presentation, physical examination, investigations, details of any hospital admission, outcome and final diagnosis.

#### 4.2.4.4 Laboratory investigations

The investigations that were performed on enrolment, routine visits and influenza-like illness episodes are listed below (see Section 2.2 for details of individual tests):

**Table 4.1 Laboratory investigations undertaken in the cohort study**

| Visit | Specimen type & test |
|---|---|
| **Enrolment** | **Venous blood**<br>- Rapid HIV test (if status unknown)<br>- CD4+ cell count |
| **Routine review (active surveillance)** | **Nasopharyngeal & oropharyngeal swabs**<br>- Influenza PCR (CDC H1N1 kit)<br>- Quantitative lytA PCR for *Streptococcus pneumoniae*<br>**Venous blood**<br>- Rapid HIV test (repeated annually in HIV-negative cohort)<br>**Urine** (in a subset of patients, December 2014 – March 2015)<br>**-** BinaxNOW *S. pneumoniae* urinary antigen |
| **Influenza-like illness episode (passive surveillance)** | **Venous blood**<br>- Malaria rapid diagnostic test<br>- Blood culture<br>- Haemagglutination inhibition (HAI) assay<br>(convalescent sample take 4-6 weeks after ILI episode)<br>**Nasopharyngeal & oropharyngeal swabs**<br>- Influenza PCR (CDC H1N1 kit)<br>- Quantitative lytA PCR for *Streptococcus pneumoniae*<br>**Sputum**<br>- Smear microscopy for AFB and culture<br>**Chest X-Ray** |

HIV testing was performed by trained counsellors in the study team. Participants that were newly diagnosed with HIV infection were referred for HIV care at the QECH ART clinic or their local health centre.

#### *4.2.4.5 Participant follow-up default procedures*

If a participant failed to attend a routine appointment, the study nurse attempted to contact him/her by telephone to arrange a further appointment. If the subject failed to attend within 2

weeks of the telephone contact, a field worker visited them at their home. Failure to locate the participant following 2 home visits led to one further attempt to locate them through local health surveillance assistants (HSA) or church. Thereafter, they were deemed lost to follow-up.

If a participant died, the study team attempted to ascertain whether the individual had influenza-like symptoms prior to death from his/her guardian or household contacts.

### 4.2.5 Sample size calculations

This study was powered for the primary outcome of incidence rate ratio (IRR) of laboratory-confirmed influenza illness by HIV status. No published data on influenza incidence in adults in SSA settings were available at the time of study inception. A cumulative incidence of 4 per 100 person years was estimated in the HIV-uninfected cohort. Assuming a 20% loss-to follow-up, a sample size of 608 individuals (with an HIV+: HIV- ratio of 60:40) was estimated to provide 80% power to detect an incidence rate ratio (IRR) of 3.0 or greater (i.e. to detect three times higher rate of influenza in HIV-infected vs. HIV-uninfected individuals).

### 4.2.6 Statistical analysis

For the descriptive comparison of baseline characteristics of the HIV-infected and HIV-uninfected cohorts, chi-square test was used to compare categorical variables; continuous variables were summarised using means and medians and compared using a Student independent-samples t-test or Wilcoxon rank-sum test as appropriate for the distribution properties of each variable.

The at-risk period commenced on day of enrolment and continued to the end of study (31 March 2015). All analyses were based on person-years observed to death, default or end of follow-up. In cases of default, follow-up was censored at the date of relocation from Blantyre, date of withdrawal of consent, or date of most recently attended clinic visit.

A Poisson regression model was used to estimate an incidence rate ratio (IRR) for the effect of HIV infection on influenza infection. For recurrent events (i.e. ILI events or recurrent influenza events), a population average Poisson regression model using generalised estimating equations (GEE) was constructed to account for clustering of ILI events in a participant. An exchangeable correlation matrix was specified. Additional Poisson regression models were performed with stratification of HIV-infected cohort by ART status (on or not on ART at enrolment) or CD4+ count (>350 or <350 cells/µl at enrolment), using the HIV-negative cohort as baseline.

The multivariable models adjusted for age and sex *a priori*, in addition to other exposures that potentially confounded the association between HIV and influenza illness. With few outcome events,

we adjusted for a maximum of three covariates to keep the number of events per model variable (EPV) at ~10, in order to avoid unreliable coefficients and biased estimates that have occurred in models with EPV <10 (Peduzzi et al., 1996). Thus three separate multivariable models were constructed, all of which contained HIV: model A included the age and sex, the *a priori* confounders, model B included the other risk factors, namely household crowding and food security, and model C included all variables from A and B for comparison.

In addition to consideration of confounding, it was necessary to investigate some relationships for interactions. We hypothesised that the relationship between HIV and influenza might differ by food security. Consequently, a Poisson regression model was constructed with an interaction term for food security divided into two groups (difficulty obtaining food: never or sometimes vs. often or always). A likelihood ratio test compared the model with an interaction term with one without.

### 4.2.7 Study timescale

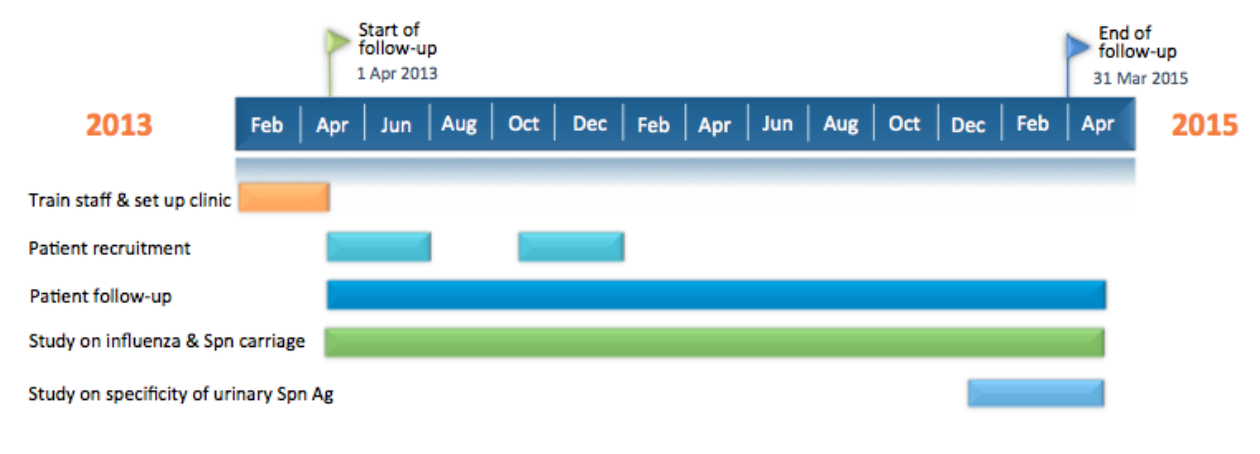



**Figure 4.4 Timescale of cohort study**

There were two periods of recruitment of cohort participants – i) April to June 2013, and ii) October to December 2013. The original aim had been to recruit the entire cohort (n=608) prior to the onset of peak influenza season in 2013. However, due to the combination of a three-month delay to the start of the study, under-recruitment of participants in the planned recruitment period (309 had been recruited up to end of June 2013), and subsequent finding of year-round influenza activity in Blantyre, a second period of recruitment was undertaken between October and December 2013.

The sub-study on the association between respiratory viral infection and nasopharyngeal carriage of S. *pneumoniae* is described in Chapter 6.

## 4.3 Results

### 4.3.1 Baseline characteristics of HIV-infected and HIV-uninfected cohort

Between April 2013 and January 2014, 655 individuals were screened for the cohort study. 608 participants were recruited, of whom 360 (59%) had HIV infection (Figure 4.5). The baseline characteristics of the HIV-infected and HIV-uninfected cohorts are summarised in Table 4.2.

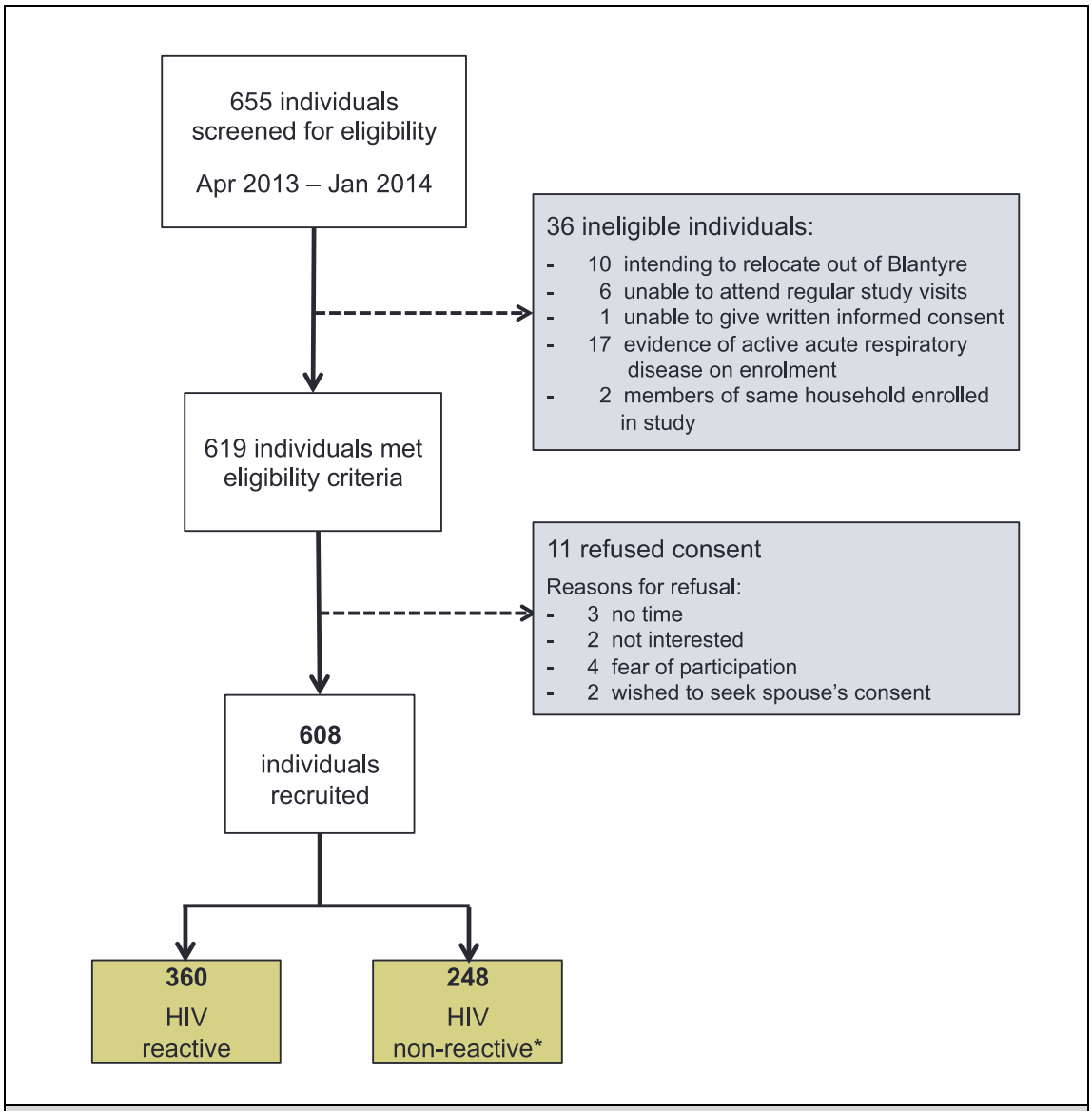



**Figure 4.5 Flow diagram of cohort study recruitment**

**Table 4.2 Demographic, clinical, household and socioeconomic characteristics of the cohort participants (N=608)**

| | HIV-infected cohort **n=360 (59%)** | HIV-uninfected cohort **n=248 (41%)** | p-value[a] |
|---|---|---|---|
| **Gender (male)** | 113 (31) | 111 (45) | 0.001 |
| **Age (years) – median (IQR)** | 37 (31-45) | 31 (25-39) | <0.001 |
| **Medical history** | | | |
| Asthma | 18 (5) | 8 (3) | 0.29 |
| Chronic lung disease | 1 (0.3) | 0 (0) | - |
| Heart failure | 2 (0.6) | 1 (0.4) | 0.80 |
| Chronic renal disease | 0 (0) | 0 (0) | - |
| Chronic liver disease | 2 (0.6) | 0 (0) | 0.24 |
| Diabetes | 2 (0.6) | 2(0.8) | 0.70 |
| Pregnant at enrolment | 4 (1) | 4 (2) | 0.02 |
| Previous pulmonary tuberculosis | 90 (25) | 6 (2) | <0.001 |
| Pneumonia in past 5 years | 59 (16) | 13 (5) | <0.001 |
| Smoking - current | 11 (3) | 4 (2) | 0.14 |
| - previous | 42 (12) | 19 (8) | |
| Drinks alcohol | 44 (12) | 35 (14) | 0.47 |
| BMI (kg/m$^2$)[c] – median (IQR) | 21.9 (19.7-24.7) | 22.0 (19.9-25.6) | 0.15 |
| <18.5 | 38 (10.6) | 17 (6.9) | |
| > 18.5 | 322 (89.4) | 231 (93.1) | 0.18 |
| **Housing characteristics** | | | |
| Children <5 years in household (n) | | | |
| 0 | 219 (61) | 132 (54) | |
| 1 | 115 (32) | 82 (33) | |
| ≥2 | 25 (7) | 32 (13) | 0.03 |
| Individuals aged ≥5years in household (n) | | | |
| 0-2 | 88 (25) | 56 (23) | |
| 3-4 | 162 (45) | 91 (37) | |
| ≥5 | 108 (30) | 100 (41) | 0.03 |
| Crowding index[b] | | | |
| <1.5 | 110 (30) | 66 (27) | |
| 1.5-2.9 | 144 (40) | 95 (38) | |
| ≥ 2.5 | 106 (30) | 87 (35) | 0.33 |
| Toilet | | | |
| None/non-VIP toilet | 327 (91) | 205 (83) | |
| VIP/flush toilet | 32 (9) | 43 (17) | 0.002 |
| Water supply | | | |
| River/stream/borehole | 75 (21) | 44 (18) | |
| Public tap/standpipe | 212 (60) | 131 (53) | |
| Piped to dwelling | 67 (19) | 71 (29) | 0.02 |
| Principal cooking fuel | | | |
| Firewood | 77 (22) | 49 (20) | |
| Charcoal | 244 (68) | 166 (68) | |
| Electricity | 36 (10) | 30 (12) | 0.66 |

| | | | |
|---|---|---|---|
| **Socioeconomic status** | | | |
| Highest level of education | | | |
| Never attended | 18 (5) | 10 (4) | |
| Primary | 152 (42) | 89 (36) | |
| Secondary – without MSCE | 138 (38) | 93 (38) | |
| Secondary – with MSCE/ Tertiary | 51 (15) | 56 (22) | 0.10 |
| Employment | | | |
| No formal employment (incl. students) | 87 (24) | 78 (31) | |
| Vocational/manual/farming | 35 (10) | 25 (10) | |
| Street seller | 119 (33) | 55 (23) | |
| Sales/shop/service worker | 90 (25) | 72 (29) | |
| Professional | 29 (8) | 16 (6) | 0.05 |
| Asset ownership[c] | | | |
| 1-2 | 141 (39) | 83 (33) | |
| 3 | 151 (42) | 113 (46) | |
| 4-5 | 68 (19) | 52 (21) | 0.36 |
| Food security[d] – difficulties obtaining food | | | |
| Never | 137 (38) | 133 (54) | |
| Sometimes | 149 (42) | 86 (35) | |
| Often/always | 70 (20) | 28 (11) | <0.001 |

IQR, interquartile range; VIP, ventilated improved pit; MSCE, Malawi School certificate of Education
[a]Chi-square or Fisher's exact test for categorical variables; Wilcoxon rank sum test for continuous variable.
The number of missing observations in each variable is detailed in Appendix 8.4.2; individuals with missing data were excluded from the denominator in percentage calculations.
[b]Total number of household members divided by the number of living rooms in household (kitchen or bathroom not included).
[c]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.
[d]Sometimes (1-2 times/month), often (up to once a week), always (every day)

In keeping with the demographic of the HIV epidemic in Malawi (Chapter 1.7.2), the HIV-infected cohort had a higher proportion of females and were older, compared to the HIV-uninfected cohort. The prevalence of chronic co-morbidities (reliant on self-reporting, in addition to checking the health passport) and smoking in both cohorts were low, though unsurprisingly a significantly higher proportion of HIV-infected participants reported a past history of tuberculosis (25% vs. 2%, $p<0.001$) and pneumonia in the past 5 years (16% vs. 5%, $p<0.001$).

The HIV-uninfected cohort had larger household sizes (both number of children <5, as well as individuals $\geq$5 years of age). However, the crowding index, derived from the total household size divided by the number of living rooms, did not differ between the two groups. There were also no significant differences in education level or asset ownership. The HIV-uninfected cohort had better sanitation facilities, water supply and food security.

Among the 360 enrolled HIV-infected individuals, the majority (85%) were diagnosed prior to the study (Table 4.3). Around two-thirds of the cohort were on ART at enrolment; most had been on ART for over 12 months. A further 47 (13%) were commenced on ART over the duration of the study. The relatively good immune reconstitution in this cohort is reflected in the median CD4+ cell count of 390 cells/µl (IQR 244-547).

**Table 4.3 Clinical characteristics of the HIV-infected cohort**

| | N (%) |
|---|---|
| **CD4+ at enrolment[a] – median, IQR** | 390 (244-547) |
| **WHO clinical stage** | |
| 1 | 138 (38) |
| 2 | 84 (23) |
| 3 | 130 (36) |
| 4 | 8 (2) |
| **Previously tested HIV-positive** | 307 (85) |
| **On ART** | |
| At enrolment | 234 (65) |
| Commenced during study | 47 (13) |
| **ART regimen at enrolment[b] (n=234)** | |
| Stavudine, lamivudine, nevirapine (1A) | 83 (36) |
| Zidovudine, lamivudine, nevirapine (2A) | 28 (12) |
| Stavudine, lamivudine, nevirapine (3A) | 2 (1) |
| Tenofovir, lamivudine, efavirenz (5A) | 110 (47) |
| Tenofovir, lamivudine, nevirapine (6A) | 3 (1) |
| Tenofovir, lamivudine, atazanavir/ritonavir (7A) | 7 (3) |
| **Duration on ART at enrolment (n=234)** | |
| <3months | 7 (3) |
| 3-12 months | 36 (15) |
| >12 months | 191 (82) |
| **On co-trimoxazole prophylaxis** | |
| At enrolment | 239 (67) |

IQR-interquartile range; ART-antiretroviral treatment

The number of missing observations in each variable is detailed in Appendix 8.4.2; individuals with missing data were excluded from the denominator in percentage calculations.

[a]Missing for 3 HIV-infected participants

[b]Regimen 1A had been the first line treatment in Malawi at the start of the recruitment period, but this was switched to 5A in July 2013 (Ministry of Health, 2011). Patients who were taking 1A were switched to 5A after July 2013.

### 4.3.2 Routine visits (active surveillance)

Between 1 April 2013 and 31 March 2015, 3094 routine reviews were conducted in 573 cohort participants (Figure 4.6). This included successful home visits by the field workers if participants had failed to attend 2 clinic appointments. Of note, intensive active surveillance during what was initially believed to be peak influenza season (January to June) was revised to 4-monthly reviews in the second year of follow up, as data that emerged from sentinel surveillance at QECH (Chapter 3) suggested year-round influenza transmission (see also Chapter 4.2.4.3). This explains the few visits that took place between September and November 2013, and high attendances from January to April 2014.

Symptoms of acute ILI (Chapter 4.2.4.3) were reported during 4 routine visits; these were recorded as ILI episodes. Furthermore, ILI symptoms within 4 week of review were reported in 40 visits (37 participants), but the participants did not contact study staff or attend the study clinic for review at the time of illness.

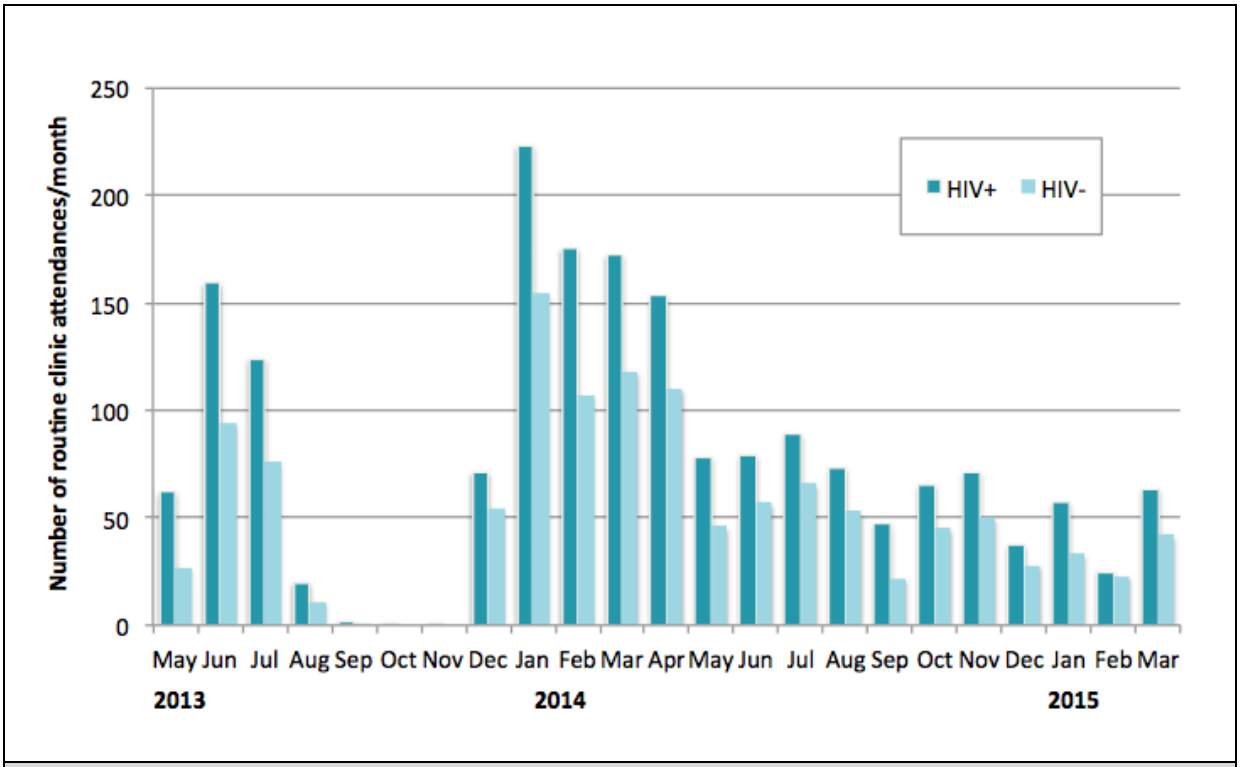



**Figure 4.6 Number of routine review attendances by cohort participants per month, April 2013–March 2015**

### 4.3.3 Cohort study follow-up

Among the 608 enrolled participants, 121 (19.9%, 71 HIV-infected, 50 HIV-uninfected) exited the study before the end date (Figure 4.7). Around half of the exited participants (n=61) had relocated out of Blantyre; 25 had withdrawn from the study, mainly due to reluctance to undergo repeated nasopharyngeal sampling; there were 11 deaths (10 HIV-infected (2.8%); 1 HIV-uninfected (0.4%)); and 25 participants were lost to follow-up. None of the deceased had reported respiratory symptoms or had attended the study clinic with an ILI episode in the 2 weeks prior to death.

There were no differences in the proportion of participants that exited the study or were loss-to-follow-up among the HIV-infected and HIV-uninfected cohorts. Total person-time follow-up was 520.1 and 347.8 person-years (PYs) in the HIV-infected and HIV-uninfected cohorts respectively.

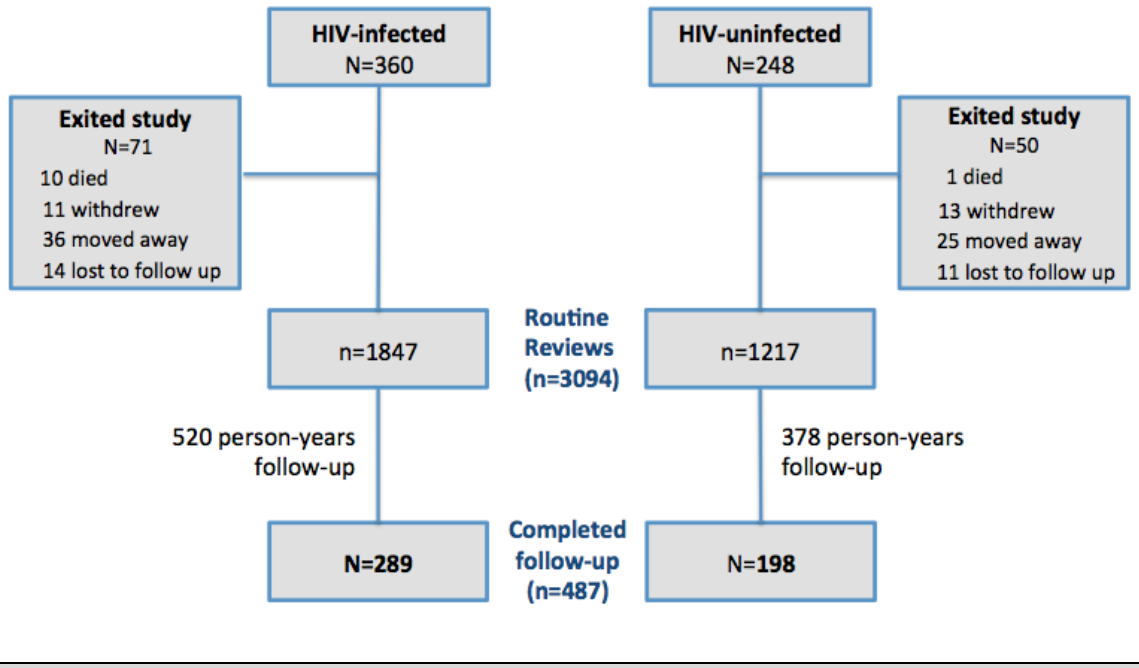



**Figure 4.7 Progress of cohort study participants, April 2013 – March 2015**

### 4.3.4 Influenza-like illness episodes (passive surveillance)

#### *4.3.4.1 ILI attendances*

348 illness attendances fulfilled the ILI criteria during the study period; 229 were in 130 (36.1%) HIV-infected participants, and 119 in 78 (31.5%) HIV-uninfected participants. HIV-infected participants were more likely to present with multiple ILI episodes (Figure 4.8). The incidence of ILI attendance

was 442 and 341 per 1000 PYs in HIV-infected and HIV-uninfected participants respectively, providing an incidence rate ratio (IRR) of 1.21 (95%CI 0.99-1.48, p=0.07).

#### *4.3.4.2 Pre-hospital treatment*

Antibiotic use within 2 weeks of ILI attendance was reported In 31 (8.9%) episodes (HIV-infected - 23); HIV-uninfected - 8)). Antibiotics included penicillins (n=18), cotrimoxazole (excluding prophylaxis) (n=7), tetracyclines (n=1), erythromycin (n=1) and ciprofloxacin (n=1). 14 participants (HIV-infected - 9; HIV-uninfected - 5) had also taken antimalarials prior to ILI visit (artemether/lumefantrine (LA) (n=9), quinine (n=2), and Fansidar® (n=2).

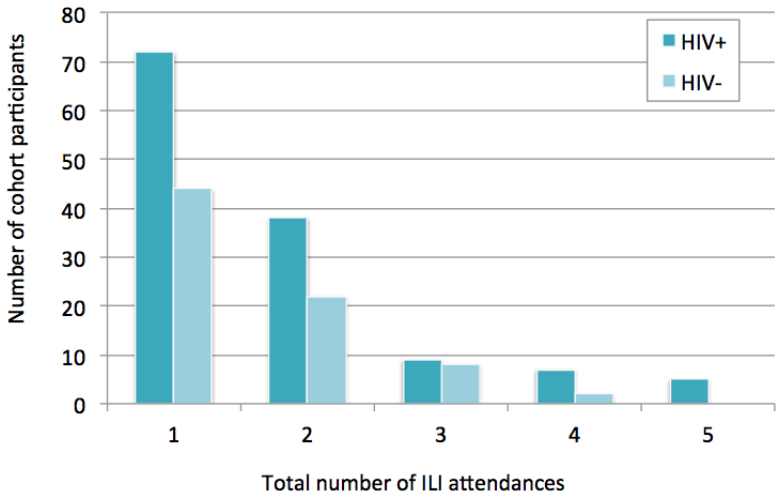



**Figure 4.8 Total number of ILI attendances by cohort participants, April 2013–March 2015**

#### *4.3.4.3 Clinical presentation*

The clinical characteristics of the ILI presentations, by HIV status, are summarised in Table 4.4. HIV-infected participants reported shortness of breath (11.3 vs. 4.3%, p=0.03) and diarrhoea (15.7 vs. 8.6%, p=0.07) with higher frequency compared to HIV-uninfected participants, and were more likely to have a documented fever (9.2 vs. 1.7%, p=0.009), tachycardia (11 vs 0%) and upper respiratory tract findings (12.6 vs. 5.1%, p=0.03). The prevalence of other symptoms and clinical signs were similar in the two groups. Most ILI presentations were mild; only five (1.4%) episodes resulted in hospital admission, 2 of which were associated with a positive influenza PCR (Table 4.5). Furthermore, no deaths resulted from the ILI episodes.

**Table 4.4 Clinical characteristics of participants presenting with ILI, April 2013–March 2015 (N=348)**

| Characteristic | HIV-infected participants (n=229) | HIV-uninfected participants (n=119) | p-value[a] |
|---|---|---|---|
| **Clinical presentation** | | | |
| Cough | 167 (72.6) | 85 (72.7) | 0.99 |
| Sore throat | 123 (53.5) | 59 (50.4) | 0.59 |
| Rhinorrhoea | 201 (87.4) | 101 (86.3) | 0.78 |
| Myalgia | 192 (83.5) | 99 (84.6) | 0.79 |
| Nausea/vomiting | 25 (10.9) | 10 (8.6) | 0.50 |
| Shortness of breath | 25 (11.3) | 5 (4.3) | 0.03 |
| Diarrhoea | 36 (15.7) | 10 (8.6) | 0.07 |
| Headache | 196 (85.2) | 102 (87.2) | 0.62 |
| **Clinical signs** | | | |
| Temperature ≥38°c | 21 (9.2) | 2 (1.7) | 0.009 |
| Heart rate >120 beats/min | 11 (4.8) | 0 (0) | 0.02 |
| Respiratory rate ≥30 breaths/min | 5 (2.2) | 0 (0) | 0.17 |
| Oxygen saturation <90% | 3 (1.3) | 0 (0) | 0.55 |
| Upper respiratory tract signs[b] | 29 (12.6) | 6 (5.1) | 0.03 |
| Lower respiratory tract signs[c] | 5 (2.2) | 1 (0.8) | 0.67 |
| **Hospital admission** | 5 (2.2) | 0 (0) | |

The number of missing observations in each variable is detailed in Appendix 8.4.2; individuals with missing data were excluded from the denominator in percentage calculations.

[a]Chi-squared or Fisher's exact test

[b]include redness or pus in throat, cervical lymphadenopathy or sinus tenderness.

[c]include crepitations, bronchial breathing, pleural rub, wheeze or signs consistent with pleural effusion.

#### *4.3.4.4 Investigation results*

Among 348 ILI episodes, 29 (8.3%) were found to be positive for influenza PCR (table 4.6); 24/229 (10.5%) in HIV-infected participants, and 5/119 (4.2%) in HIV-uninfected participants. Of the HIV-infected participants with a positive influenza PCR, two-thirds (n=16) had influenza B, the rest had influenza A(H3N2). In contrast, three HIV-uninfected participants had influenza A(H3N2), and two had influenza B. Figure 4.9 illustrates the frequency of ILI presentation and the type and subtype of influenza positive samples over the study period. There were no recurrent influenza events.

143/348 (41.1%) had at least 1 respiratory virus (including influenza) detected (Table 4.6). Rhinovirus was the most ubiquitous, followed by influenza and coronavirus NL63. There were no differences in the prevalence of respiratory viruses among HIV-infected and HIV-uninfected cohorts, though the numbers of individual viruses detected were small.

With regards to other investigations, RDT for malaria was positive in 10 (2.9%) ILI episodes; 2 had bacteraemia (both *Salmonella* species), and none were positive for acid-fast bacilli on sputum microscopy, or subsequent culture for *Mycobacterium tuberculosis*.

**Table 4.5 Investigation results and outcome of the hospitalised ILI episodes (n=5)**

| Sex (age, years) | HIV status | PCR result | Blood culture | CXR | AFB | Days of admission | Outcome |
|---|---|---|---|---|---|---|---|
| F, 25 | + | Influenza B hMPV | No growth | Lobar consolidation | – | 13 | Discharged |
| F, 32 | + | | No growth | Diffuse bilateral infiltrates | – | 4 | Discharged |
| F, 36 | + | Influenza A(H3) | No growth | Patchy bi-basal infiltrates | ND | 6 | Discharged |
| F, 33 | + | Legionella species | No growth | Normal | – | 3 | Discharged |
| F, 31 | + | *Mycoplasma pneumoniae* | *Salmonella typhimurium* | Diffuse bilateral infiltrates | ND | 12 | Discharged |

PCR, polymerase chain reaction, CXR, chest x-ray; AFB, sputum microscopy for acid-fast bacilli; hMPV, human metapneumovirus; ND, not done.

**Table 4.6 Investigation results of participants presenting with ILI, April 2013–March 2015 (N=348)**

| Investigation | HIV-infected cohort (n=229) | HIV-uninfected cohort (n=119) | p-value[a] |
|---|---|---|---|
| **Influenza-PCR positive** | 24 (10.5) | 5 (4.2) | 0.04 |
| *Influenza type/subtype* | | | |
| A(H3) | 8 (3.9) | 3 (3.4) | 0.89 |
| B | 16 (7.0) | 2 (1.7) | 0.049 |
| **Other respiratory viruses** | | | |
| Adenovirus | 3 1.3) | 5 (4.2) | 0.09 |
| Bocavirus | 2 (0.9) | 0 (0) | 0.55 |
| Coronavirus - 0C43 | 4 (1.8) | 3 (2.5) | 0.69 |
| - NL63 | 15 (6.6) | 9 (7.6) | 0.82 |
| - 229E | 7 (3.1) | 2 (1.7) | 0.72 |
| - HKU1 | 6 (2.6) | 2 (1.7) | 0.72 |
| Enterovirus | 3 (1.3) | 0 (0) | 0.55 |
| Human metapneumovirus | 6 (2.6) | 3 (2.5) | 1.00 |
| Parainfluenza virus 1 | 1 (0.4) | 1 (0.9) | 1.00 |
| Parainfluenza virus 2 | 0 (0) | 0 (0) | - |
| Parainfluenza virus 3 | 3 (1.3) | 3 (2.5) | 0.42 |
| Parainfluenza virus 4 | 2 (0.9) | 1 (0.9) | 1.00 |
| Rhinovirus | 32 (14.2) | 14 (11.9) | 0.55 |
| RSV | 8 (3.5) | 3 (2.5) | 0.76 |
| **At least 1 respiratory virus** | 97 (42.4) | 46 (38.7) | 0.51 |
| **Multiple viral co-detection** | 17 (7.4) | 5 (4.2) | 0.24 |
| **S. *pneumoniae* colonisation** | 104 (45.6) | 50 (42.4) | 0.57 |
| **Other aetiology identified** | | | |
| Malaria RDT positive | 5 (2.2) | 5 (4.2) | 0.27 |
| Blood culture positive[b] | 2/50 (4.0) | 0/22 (0) | 1.00 |
| Sputum positive for AFB | 0/6 (0) | 0/2 (0) | - |

PCR, polymerase chain reaction; RSV, respiratory syncytial virus; RDT, rapid diagnostic test; AFB, acid-fast bacilli

The number of missing observations in each variable is detailed in Appendix 8.4.2; individuals with missing data were excluded from the denominator in percentage calculations.

[a]Chi-squared or Fisher's exact test

b *Salmonella typhimurium* (n=1), *Salmonella typhi* (n=1)

#### *4.3.4.5 The impact of HIV infection on influenza incidence*

The HIV-infected cohort had 24 independent laboratory-confirmed influenza episodes over 520 PYs, and the HIV-uninfected cohort had 5 episodes over 348 PYs (Table 4.7). This equated to incidence rates of 46.0 (95%CI 30.8-68.6] vs. 14.5 (95%CI 6.0-34.7) per 1000PYs respectively, and a crude incidence rate ratio (IRR) for the effect of HIV infection of 3.21 (95% CI 1.22-8.41, p=0.02). To evaluate the impact of HIV infection, we sought to control for covariates that may potentially confound the relationship between HIV and influenza. In the univariable analysis, past history of pneumonia, high crowding index (denotes number of persons per sleeping room, used as proxy for household crowding), greater distance to water supply, lower education level, unemployment, and poor food security were associated with influenza infection. Results from a simulation study suggest that evaluation of less than 10 events per variable (EPV) in regression models may lead to biased and less precise estimates (Peduzzi et al., 1996). As there were only 29 outcome events, we decided to include a maximum of 3 covariates in a multivariable model. Three multivariable models were constructed (Table 4.8).

Multivariable model A included the *a priori* confounders, age and gender, in addition to HIV infection. The effect of HIV infection on influenza incidence did not change substantially after adjustment for age and sex (aIRR 3.01 (95%CI 1.11-8.21, p=0.02). For model B, HIV infection was included, along with up to two significant covariates using backwards stepwise regression. Household crowding and food insecurity were found to be independent risk factors for influenza infection, and the effect estimate for HIV infection was similar to model A (aIRR 2.94, 95%CI 1.11-7.80). In the final multivariable model (C), all five covariates from models A and B were included. The effect estimates from this model did not differ substantially from the first two; after adjusting for age, gender, household crowding and food security, HIV-infected adults still had an approximately three times increased rate of influenza infection compared to HIV-uninfected adults (aIRR 2.75 [1.02-7.44], p=0.03). Pearson's goodness-of-fit tests for all three models suggest reasonable fit with the data (Table 4.8).

Although none of the deceased participants reported respiratory symptoms (according to informal verbal autopsy) or had a documented ILI visit prior to death, we performed a sensitivity analysis including participant death as an influenza outcome event (10 in HIV+ & 1 in HIV- participants, Figure 4.7). Thus this analysis included 34 laboratory-confirmed influenza illness or death in the HIV-infected cohort, and 6 in the HIV-uninfected cohort, equating to incidence rates of 63.4 (95%CI 45.1-89.2] vs. 17.2 (95%CI 7.8-38.4) per 1000PYs respectively. Crude IRR for the effect of HIV infection was 3.68 (95% CI 1.54-8.78, p=0.002). In multivariable analysis, aIRR was 2.99 (95%CI 1.22-7.34), after adjusting for age, gender, household crowding and food insecurity (multivariable model C).

**Table 4.7 Risk factors for laboratory-confirmed influenza illness among cohort participants (N=608)**

| Characteristic | Number influenza +ve ILI episodes (n=29) | PYFU | IR (per 1000 PYFU) (95%CI) | Univariable[a] | | Multivariable C[a,e] | |
|---|---|---|---|---|---|---|---|
| | | | | IRR (95% CI) | P-value | IRR (95% CI) | P-value |
| **Gender** | | | | | | | |
| Male | 10 | 319 | 31.4 (16.9-58.3) | 1 | | 1 | |
| Female | 19 | 549 | 34.6 (22.1-54.2) | 1.21 (0.52-2.84) | 0.66 | 0.88 (0.40-1.93) | 0.74 |
| **Age group (years)** | | | | | | | |
| 18-29 | 5 | 239 | 20.9 (8.7-50.2) | 1 | | **1** | |
| 30-39 | 13 | 326 | 39.9 (23.2-68.7) | 1.73 (0.60-4.97) | | 1.55 (0.54-4.43) | |
| ≥ 40 | 11 | 303 | 36.3 (20.1-65.6) | 1.33 (0.43-4.05) | 0.30 | 1.42 (0.47-4.28) | 0.70 |
| **HIV status** | | | | | | | |
| Negative | 5 | 348 | 14.5 (6.0-34.7) | 1 | | **1** | |
| Positive | 24 | 520 | 46.0 (30.8-68.6) | 3.21 (1.22-8.41) | 0.02 | **2.75 (1.02-7.44)** | **0.03** |
| **Medical history** | | | | | | | |
| Previous pulmonary TB | 4 | 142 | 28.2 (23.3-51.1) | 0.82 (0.28-2.43) | 0.87 | | |
| Previous pneumonia | 6 | 110 | 54.3 (24.4-120.9) | 1.86 (0.75-4.57) | 0.14 | | |
| Low BMI (<18.5 kg/m$^2$) | 3 | 74 | 40.3 (13.0-125.0) | 0.81 (0.25-2.68) | 0.73 | | |
| **Housing characteristics** | | | | | | | |
| Children <5 yrs in household | 18 | 497 | 36.2 (22.8-57.5) | 1 | | | |
| 0 | 8 | 288 | 27.8 (13.9-55.6) | 0.77 (0.33-1.77) | | | |
| 1 | 3 | 81 | 37.3 (12.0-115.5) | 1.03 (0.30-3.50) | 0.80 | | |
| ≥2 | | | | | | | |
| Individual >5 yrs in household | | | | | | | |
| 0-3 | 5 | 196 | 25.5 (10.6-61.3) | 1 | | | |
| 3-4 | 10 | 356 | 28.1 (15.1-52.3) | 1.10 (0.38-3.22) | | | |
| ≥5 | 14 | 313 | 44.7 (26.5-75.4) | 1.76 (0.63-4.87) | 0.41 | | |
| Crowding index[b] | | | | | | 1 | |
| <1.5 | 4 | 245 | 16.3 (6.12-43.5) | 1 | | 3.41 (1.12-10.36) | |
| 1.5-2.4 | 16 | 340 | 47.1 (28.8-76.8) | 2.88 (0.96-8.62) | | 2.06 (0.62-6.83) | 0.056 |
| ≥2.5 | 9 | 282 | 32.0 (16.6-61.5) | 1.95 (0.60-6.35) | 0.11 | | |
| Water source | | | | | | | |
| River/stream/borehole | 8 | 184 | 43.5 (21.8-87.1) | 2.72 (0.72-10.26) | 0.25 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Public tap/standpipe | 18 | 486 | 37.0 (23.3-58.8) | 2.31 (0.68-7.86) | | | |
| Piped to dwelling | 3 | 188 | 16.0 (5.2-49.5) | 1 | | | |
| Distance from water supply | | | | | | | |
| <200 metres | 20 | 705 | 28.4 (18.3-44.0) | 1 | | | |
| ≥200 metres | 9 | 156 | 57.7 (30.0-110.9) | 2.02 (0.92-4.45) | 0.08 | | |
| Sanitation facility | | | | | | | |
| No/non-VIP toilet | 27 | 769 | 35.1 (24.1-51.2) | 1.71 (0.41-7.20) | 0.43 | | |
| VIP /flush toilet | 2 | 98 | 20.5 (5.1-81.9) | 1 | | | |
| Principal cooking fuel | | | | | | | |
| Electricity | 3 | 94 | 32.0 (10.3-99.3) | 1 | | | |
| Charcoal | 18 | 572 | 33.2 (21.2-52.0) | 1.04 (0.31-3.52) | | | |
| Firewood | 7 | 193 | 36.3 (17.3-76.1) | 1.14 (0.29-4.41) | 0.97 | | |
| **Socioeconomic status** | | | | | | | |
| Highest level of education | | | | | | | |
| Never attended/primary | 18 | 378 | 47.6 (30.0-75.6) | 1 | | | |
| Secondary/tertiary | 11 | 488 | 22.5 (12.5-40.7) | 0.47 (.22-1.00) | 0.05 | | |
| Employment | | | | | | | |
| No | 13 | 262 | 49.6 (28.8-85.4) | 1 | | | |
| Yes | 16 | 606 | 26.4 (16.2-43.1) | 0.53 (0.26-1.11) | 0.09 | | |
| Assets owned[c] | | | | | | | |
| 0-2 | 15 | 308 | 48.8 (29.4-80.9) | 1.42 (0.55-3.65) | 0.26 | | |
| 3 | 8 | 386 | 20.7 (10.4-41.5) | 0.60 (0.21-1.74) | | | |
| 4-5 | 6 | 175 | 34.4 (15.4-76.5) | 1 | | | |
| Difficulty obtaining food[d] | | | | | | | |
| Never | 10 | 386 | 25.9 (13.9-48.2) | 1 | | 1 | |
| Sometimes | 7 | 338 | 20.7 (9.9-43.5) | 0.80 (0.30-2.10) | | 0.71 (0.27-1.88) | |
| Often/always | 12 | 138 | 87.2 (49.5-153.6) | 3.35 (1.45-7.76) | 0.005 | 3.09 (1.30-7.36) | 0.006 |

ILI, influenza-like illness; PYFU, person-years follow-up; IR, incidence rate; IRR, incidence rate ratio; CI, confidence interval; TB, tuberculosis; BMI, body mass index; VIP, ventilated improved pit

[a]IRRs estimated for the incidence of laboratory-confirmed influenza using Poisson regression

[b]Total number of household members divided by the number of living rooms in household (kitchen or bathroom not included).

[c]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.

[d]Sometimes (1-2 times/month), often (up to once a week), always (every day)

[e]Adjusted for gender, age group, HIV status, crowding index and food security

**Table 4.8 Multivariable analyses of risk factors for laboratory-confirmed influenza infection among cohort participants (N=608)**

| Characteristic | IR of laboratory-confirmed influenza (per 1000 PYFU) (95%CI) | Multivariable A[a,b] | | Multivariable B[a,c] | | Multivariable C[a,d] | |
|---|---|---|---|---|---|---|---|
| | | IRR (95% CI) | P-value | IRR (95% CI) | P-value | IRR (95% CI) | P-value |
| **Gender** | | | | | | | |
| Male | 31.4 (16.9-58.3) | 1 | | | | 1 | |
| Female | 34.6 (22.1-54.2) | 0.97 (0.44-2.12) | 0.94 | | | 0.88 (0.40-1.93) | 0.74 |
| **Age group** | | | | | | | |
| 18-29 | 20.9 (8.7-50.2) | 1 | | | | **1** | |
| 30-39 | 39.9 (23.2-68.7) | 1.42 (0.44-2.12) | | | | 1.55 (0.54-4.43) | |
| ≥ 40 | 36.3 (20.1-65.6) | 1.23 (0.41-3.70) | 0.80 | | | 1.42 (0.47-4.28) | 0.70 |
| **HIV status** | | | | | | | |
| Negative | 14.5 (6.0-34.7) | **1** | | **1** | | **1** | |
| Positive | 46.0 (30.8-68.6) | **3.01 (1.11-8.21)** | **0.02** | **2.94 (1.11-7.80)** | **0.02** | **2.75 (1.02-7.44)** | **0.03** |
| **Crowding index**[e] | | | | | | | |
| <1.5 | 16.3 (6.12-43.5) | | | 1 | | 1 | |
| 1.5-2.4 | 47.1 (28.8-76.8) | | | 3.27 (1.09-9.80) | | 3.41 (1.12-10.36) | |
| ≥2.5 | 32.0 (16.6-61.5) | | | 1.97 (0.60-6.42) | 0.06 | 2.06 (0.62-6.83) | 0.056 |
| **Difficulty obtaining food**[f] | | | | | | | |
| Never | 25.9 (13.9-48.2) | | | 1 | | 1 | |
| Sometimes | 20.7 (9.9-43.5) | | | 0.71 (0.27-1.86) | | 0.71 (0.27-1.88) | |
| Often/always | 87.2 (49.5-153.6) | | | 2.99 (1.27-7.06) | 0.007 | 3.09 (1.30-7.36) | 0.006 |
| **Pearson's goodness-of-fit test** | | | 0.07 | | 0.42 | | 0.12 |

IR, incidence rate; PYFU, person-years follow-up; IRR, incidence rate ratio; CI, confidence interval
[a]IRRs estimated for the incidence of laboratory-confirmed influenza using Poisson regression
[b]Adjusted for gender, age group and HIV status.
[c]Adjusted for HIV status, crowding index and food security
[d]Adjusted for gender, age group, HIV status, crowding index and food security
[e]Total number of household members divided by the number of living rooms in household (kitchen or bathroom not included).
[f]Sometimes (1-2 times/month), often (up to once a week), always (every day)

#### *4.3.4.6 Other risk factors for influenza*

Those who lived in a household with 1.5-2.4 persons per sleeping room was associated with an increased risk of influenza, compared to those in households with <1.5 persons/sleeping room (aIRR 3.27, 95%CI 1.09-9.80). However, the increased risk was not observed in participants who lived in household with crowding index >2.5 (aIRR 1.97, 95%CI 0.60-6.42).

Participants who reported frequent (once a week or more) difficulties accessing food had a three-fold increased risk of influenza infection, compared to those who never had difficulties in food access (aIRR 2.99, 95%CI 1.27-7.06 – model B). Low BMI was not associated with influenza infection.

We also assessed covariates for potential effect modification of the impact of HIV on influenza illness. Among those that reported difficulty obtaining food once a week or more (often/always), the risk of influenza was almost 10-times greater in HIV-infected individuals (IRR 9.50, 95%CI 1.27-70.99) than HIV-uninfected individuals. Conversely, in those with no or infrequent difficulty obtaining food, the association between HIV and influenza disappears (IRR 1.18, 95%CI 0.32-4.39). This suggests an interaction between food insecurity and HIV (p-value for interaction, 0.01). Effect modification was not demonstrated with the other variables.

#### *4.3.4.7 Effects of CD4+ count and antiretroviral treatment status on influenza incidence*

To explore the effect of CD4+ T-lymphocyte cell count on influenza incidence, the HIV-infected cohort was stratified by their CD4+ count at enrolment (<200 or ≥200 cells/µl) (Table 4.8). We opted to adjust for *a priori* confounders only (age and gender) since the study was not powered for this stratified analysis. Using HIV-uninfected cohort as baseline, HIV-infected participants with CD4+ count <200 cells/µl at enrolment had nearly twice the incidence of influenza compared to HIV-infected participants with CD4+ ≥200 cells/µl (Incidence rate 40.5/ 1000 PYs, aIRR 2.59, 95%CI 0.92-7.27) vs. 79.1/1000 PYs, aIRR 5.50, 95%CI 1.68-18.02), compared to HIV-negative participants (incidence rate 14.5/1000 PYs). However, when restricted to the HIV-infected sub-group (Table 4.10), the association does not reach statistical significance (IRR 1.95, 95%CI 0.78-4.92). The effect of HIV on influenza did not differ by antiretroviral treatment status (Table 4.9 & 4.10).

**Table 4.9 The effect of CD4+ count at enrolment and antiretroviral treatment status on incidence rates of laboratory-confirmed influenza illness (N=608)**

| Characteristic | Number influenza +ve ILI episodes (n=29) | PYFU | IR (per 1000 PYFU) (95%CI) | Univariable | | Multivariable[a,b] | | Multivariable[a,c] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | IRR (95% CI) | P-value | IRR (95% CI) | P-value | IRR (95% CI) | P-value |
| **Gender** | | | | | | | | | |
| Male | 10 | 319 | 31.4 (16.9-58.3) | 1 | | 1 | | 1 | |
| Female | 19 | 549 | 34.6 (22.1-54.2) | 1.21 (0.52-2.84) | 0.66 | 1.06 (0.48-2.35) | 0.74 | 0.96 (0.44-9.20) | 0.92 |
| **Age group** | | | | | | | | | |
| 18-29 | 5 | 239 | 20.9 (8.7-50.2) | 1 | | **1** | | 1 | |
| 30-39 | 13 | 326 | 39.9 (23.2-68.7) | 1.73 (0.60-4.97) | | 1.42 (0.49-4.09) | | 1.39 (0.48-4.03) | |
| ≥ 40 | 11 | 8303 | 36.3 (20.1-65.6) | 1.33 (0.43-4.05) | 0.30 | 1.28 (0.43-3.86) | 0.70 | 1.17 (0.38-3.65) | 0.89 |
| **CD4+ category[d]** | | | | | | | | | |
| HIV-negative | 5 | 348 | 14.5 (6.0-34.7) | 1 | | 1 | | | |
| CD4+ ≥ 200 cells/µl | 14 | 316 | 40.5 (25.5-64.3) | 2.81 (1.04-7.58) | | 2.59 (0.92-7.27) | | | |
| CD4+ < 200 cells/µl | 10 | 202 | 79.1 (35.5-176) | 5.50 (1.68-18.02) | 0.02 | 5.11 (1.53-17.11) | 0.02 | | |
| **ART treatment status** | | | | | | | | | |
| HIV-negative | 5 | 348 | 14.5 (6.0-34.7) | 1 | | | | 1 | |
| HIV+ – on ART | 17 | 350 | 48.6 (30.2-78.1) | 3.37 (1.24-9.15) | | | | 3.20 (1.11-9.20) | |
| HIV+ – not on ART | 7 | 170 | 41.1 (19.6-86.2) | 2.86 (0.91-9.00) | 0.03 | | | 2.73 (0.85-8.72) | 0.055 |

ILI, influenza-like illness; PYFU, person-years follow-up; IR, incidence rate; IRR, incidence rate ratio; CI, confidence interval; ART, antiretroviral treatment

[a]IRRs estimated for the incidence of laboratory-confirmed influenza using Poisson regression

[b]Adjusted for gender, age group and CD4+ categories

[c]Adjusted for gender, age group and ART treatment status

[d]CD4+ result missing for 3 HIV-infected participants

**Table 4.10 The effect of CD4+ count at enrolment and antiretroviral treatment status on incidence rates of laboratory-confirmed influenza illness among HIV-infected participants (N=360)**

| Characteristic | Number influenza +ve ILI episodes (n=29) | PYFU | IR (per 1000 PYFU) (95%CI) | Univariable[a] | |
|---|---|---|---|---|---|
| | | | | IRR (95% CI) | P-value |
| **CD4+ category[b]** | | | | | |
| ≥ 200 cells/µl | 14 | 316 | 40.5 (25.5-64.3) | 1 | |
| < 200 cells/µl | 10 | 202 | 79.1 (35.5-176) | 1.95 (0.78-4.92) | 0.18 |
| **ART treatment status** | | | | | |
| Yes | 17 | 350 | 48.6 (30.2-78.2) | 1.18 (0.49-2.85) | 0.71 |
| No | 7 | 170 | 41.1 (19.6-86.2) | 1 | |

ILI, influenza-like illness; PYFU, person-years follow-up; IR, incidence rate; IRR, incidence rate ratio; CI, confidence interval; ART, antiretroviral treatment

[a]IRRs estimated for the incidence of laboratory-confirmed influenza using Poisson regression

[b]CD4+ result missing for 3 HIV-infected participants

#### *4.3.4.8 The impact of HIV infection on influenza incidence, accounting for the number of ILI attendances*

Lastly, we explored how a higher number of ILI attendances in the HIV-infected cohort than HIV-uninfected cohort affected the observed impact of HIV infection on laboratory-confirmed influenza illness (thus in this scenario the assumption is that HIV-infected persons were more likely to attend with ILI, rather than having greater propensity to develop ILI compared to HIV-uninfected persons). Logistic regression models using generalised estimating equations (GEE) were constructed, to account for the number of ILI attendances by cohort participants (table 4.10). Compared with the effect estimates derived from the Poisson regression models (Table 4.8), the population average odds ratios were marginally lower and confidence intervals broader, which were expected. Importantly, the elevated risk of influenza illness in HIV-infected participants compared to HIV-uninfected participants persisted (Model A: aOR 2.39, 95%CI 0.93-6.19; model B: aOR 2.54, 95%CI 1.00-6.47).

**Table 4.11 Risk factors for laboratory-confirmed influenza illness, accounting for the number of ILI attendances, April 2013-March 2015 (N=608)**

| Characteristic | Univariable | | Multivariable A[a,b] | | Multivariable B[a,c] | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | P-value | OR (95% CI) | P-value | OR (95% CI) | P-value |
| **Gender** | | | | | | |
| Male | 1 | | 1 | | | |
| Female | 1.21 (0.57-2.58) | 0.62 | 1.14 (0.54-2.39) | 0.73 | | |
| **Age group** | | | 1 | | | |
| 18-29 | 1 | | 1.58 (0.53-4.70) | | | |
| 30-39 | 2.05 (0.70-6.02) | | 1.56 (0.53-4.59) | | | |
| ≥ 40 | 2.09 (0.70-6.25) | 0.37 | | 0.70 | | |
| **HIV status** | | | | | | |
| Negative | 1 | | 1 | | 1 | |
| Positive | 2.75 (1.02-7.41) | 0.045 | 2.39 (0.93-6.19) | 0.07 | 2.54 (1.00-6.47) | 0.05 |
| **Crowding index**[d] | | | | | | |
| <1.5 | 1 | | | | 1 | |
| 1.5-2.4 | 2.71 (0.90-8.20) | | | | 3.60 (1.08-11.98) | |
| ≥2.5 | 1.47 (0.46-4.71) | 0.12 | | | 1.20 (0.36-4.03) | 0.02 |
| **Difficulty obtaining food**[e] | | | | | | |
| Never | 1 | | | | 1 | |
| Sometimes | 0.72 (0.27-1.91) | | | | 0.66 (0.25-1.71) | |
| Often/always | 2.13 (0.92-4.90) | 0.05 | | | 2.60 (1.06-6.41) | 0.03 |

OR, odds ratio; CI, confidence interval

[a]Odds ratios estimated by population average model using generalised estimating equation (GEE).

[b]Adjusted for gender, age group and HIV status.

[c]Adjusted for HIV status, crowding index and food security.

## 4.4 Discussion

This prospective cohort study has demonstrated that HIV-infected adults have an almost three times increased susceptibility to influenza infection, compared to HIV-uninfected adults. Among HIV-infected adults, there was a trend toward a higher risk of influenza among those with an enrolment CD4+ cell count of <200 cells/µl, but ART status had no impact. Household crowding and food insecurity were also independent risk factors for influenza infection. Furthermore, food insecurity may potentiate the impact of HIV on influenza. We did not find any significant associations between age, gender, previous history of pneumonia or tuberculosis, smoking, education, sanitation access, or exposure to young children in the household and risk of influenza.

Previous studies of HIV and risk of influenza, largely comprising anecdotal reports or retrospective studies in low HIV prevalence settings, have had contradictory findings (Chapter 1.4). However, our results support findings from Kenya and South Africa that clearly demonstrate an increased influenza incidence in HIV-infected individuals (Feikin et al., 2012b; Cohen et al., 2013). Our incidence rates of laboratory-confirmed influenza illness were similar to that reported by Feikin *et al.* (54.1 vs. 18.6 per 1000 PYs[iv], in HIV-infected and HIV-uninfected persons aged ≥18 years respectively, crude rate ratio ~2.91) (Feikin et al., 2012b). In contrast, Cohen *et al*. (Cohen et al., 2013) focused on influenza-associated LRTI hospitalisations rather than ILI, therefore had substantially lower incidence estimates despite including all ages (1.97-2.28 (HIV+) vs. 0.26-0.54 (HIV-), per 1000 PYs[i], relative risks 4.2-7.5). Both studies derived their incidence estimates from population-based surveillance, which lack precise denominators from which to calculate disease burden. Feikin *et al.* (Feikin et al., 2012b) estimated aetiology-specific incidence by applying the proportion of each aetiology to ARI incidence adjusted for health-seeking patterns; sampling bias could have affected the pathogens detected, since viral testing was only performed in 36% enrolled patients. Lastly, neither study accounted for potential confounders, nor had complete HIV data. Thus this Malawian cohort study provides strong evidence that HIV is an important risk factor for influenza infection.

Our HIV-infected cohort had a similar median age to that reported from a cross-sectional study undertaken at the QECH ART clinic (37 vs. 39 years) (van Oosterhout et al., 2005), but we had a higher proportion of females (69 vs. 55% (van Oosterhout et al., 2005)). This could have been due to recruitment bias, if women were more likely to present to the study clinic for eligibility screening. However, the implementation of new guidelines to initiate lifelong ART for all HIV-infected pregnant and breastfeeding women irrespective of WHO clinical stage or CD4+ cell count (Option B+) in 2011

[iv] Person-years adjusted to allow comparison with our incidence rates.

(Malawi Ministry of Health, 2011) likely contributed to the increased proportion of women attending HIV services. Since a large proportion of HIV-infected participants were recruited from the ART clinic, two-thirds were on ART at enrolment, and most had been taking ART for over 12 months. Viral load was not available in our study, but the median CD4+ cell count of 390 cells/μl implies that our HIV-infected cohort may have better-controlled HIV disease compared to the Malawian adult HIV population. The overall mortality rate of the HIV-infected cohort (2.8%) was also lower than that reported in other African settings (Geng et al., 2015; Boulle et al., 2014).

Acknowledging the limited number of influenza events, we found a significant excess of influenza B infection among the HIV-infected cohort (16/24 (67%) in HIV+ vs. 2/5 (40%) in HIV-, p=0.049, Table 4.6). This echoes the findings of a South African surveillance study that also observed an association with HIV infection in patients with hospitalised influenza B infection (Cohen et al., 2014). It is unclear why influenza B infection may be more common in HIV-infected individuals, but if confirmed, may imply that quadrivalent inactivated vaccines that contain both influenza B strains may yield better clinical efficacy in HIV-infected adults.

Household crowding, as measured by the number of persons per sleeping room in the household, was associated with increased risk of influenza infection. This may reflect transmission among household members at close proximity. A recent review suggested that influenza virus was predominantly transmitted at close range through large droplets generated by coughing, sneezing or talking (Chapter 1.1.2) (Brankston et al., 2007). Crowding may also be related to greater exposure to young children in the household, though the number of children under 5 years of age was not associated with influenza susceptibility. Household crowding has not been evaluated as a risk factor for influenza in adults, but has been implicated as a risk factor for ARI (Cardoso et al., 2004; Murray et al., 2012; Ram et al., 2014) and influenza (Doshi et al., 2015) in young children. Crowding has been suggested as a link between social behaviour and seasonality (Lofgren et al., 2007). Murray *et al*. (Murray et al., 2012) postulated that rainfall increases time spent indoors, intensifying close contact in crowded households, with subsequent increased transmission of respiratory pathogens. We did not evaluate meteorological data in this study, but no association was found between rainfall and influenza activity in our sentinel surveillance (Chapter 3.3).

It is intriguing that food insecurity emerged as a risk factor for influenza. Although linked with respiratory infections in young infants (Ohemeng et al., 2015), as well as increased incident opportunistic infections and hospitalisation in Ugandan adults (Weiser et al., 2012b), an association between food insecurity and influenza has not been previously described. Food insecurity can, but does not necessarily, lead to malnutrition (Kadiyala and Rawat, 2013) and low weight (Singh et al., 2014; Kac et al., 2012). In our cohort, 20% HIV-infected, and 11% HIV-uninfected participants

reported frequent difficulty obtaining food, but only 11% and 7% respectively, were underweight (BMI <18.5 kg/$m^2$); we did not find an association between low BMI and food insecurity or increased risk of influenza. Although poverty is one of the major causes, food insecurity is a complex phenomenon that is linked to lower standards of education, unemployment, lack of access to sanitation (Food and Agricultural Organization of the United Nations (FAO), 2000), in addition to socioeconomic factors that we have not measured (Jones et al., 2013). It is also inextricably linked with HIV infection (Gillespie and Kadiyala, 2005); among HIV-infected persons, it has been associated with non-adherence to ART (McKinney et al., 2014; Hong et al., 2014; Weiser et al., 2014), as well as poorer HIV control (Weiser et al., 2014; Feldman et al., 2015). Conversely, food insecurity significantly declines with increasing ART duration (Weiser et al., 2012a; Palar et al., 2012). Our analysis indicated effect modification between food insecurity and HIV; severe food insecurity amplified the effect of HIV on influenza risk. Given the small numbers involved (severe food insecurity was reported in 12 HIV+/influenza+ vs. 1 HIV-/influenza+ participants), these results require cautious interpretation, but merit further exploration, given the opportunities for intervening in nutritional support.

Other than influenza, we identified a host of other respiratory viruses among the ILI episodes, including rhinovirus, coronaviruses, hMPV, adenovirus, RSV, parainfluenza viruses, enterovirus, and bocavirus. Viral co-infections were found in approximately 5% of ILI episodes. With the exception of adenovirus, which was more frequently identified in HIV-uninfected individuals, there was no difference in the prevalence of other respiratory viruses detected between ILI samples from HIV-infected and HIV-uninfected individuals. As with influenza, incidence and disaggregated risk factors can be calculated. However, some of tested viruses, such as adenovirus, rhinovirus/enterovirus and bocavirus, are commonly identified in well individuals (Feikin et al., 2012b; Jansen et al., 2011; Garcia-Garcia et al., 2008), whereas asymptomatic carriage is uncommon for influenza A & B, RSV, hMPV, parainfluenza viruses and coronaviruses (Falsey et al., 2006; Feikin et al., 2012b; Kumar et al., 2008). Hence the clinical relevance of positive test results for these other viruses is difficult to discern. We plan to evaluate these results in context of asymptomatic nasopharyngeal samples that we have concurrently collected over the 2-year follow-up period (Chapter 7.5). The samples have yet to be processed, and will not be reported in this thesis.

This study has several strengths: to the best of our knowledge, it is the only longitudinal study to investigate the rates of influenza and its associations in a well-described cohort of HIV-infected and HIV-uninfected persons. Secondly, it was a prospective study with detailed characterisation of the clinical presentation and viral aetiology of ILI episodes, and the follow-up period included more than

one influenza season. Furthermore, we were able to investigate associations adjusted for key potential confounders.

This study did have several limitations. Cohort participants were recruited from the ART and VCT clinics at QECH, thus may have differed to the general population of Blantyre. Another observational study conducted at the same VCT clinic found significant differences in educational attainment and occupation between enrolled HIV-infected and uninfected patients (Kelly et al., 2014). In our cohort, the number of persons in the household (aged <5 and ≥5 years), sanitation facility, water supply and food insecurity differed significantly by HIV status (Table 4.2) We attempted to adjust for potential confounding by HIV status in the multivariable analysis. Although recruiting HIV-uninfected individuals in the community rather than at VCT clinic could have generated a more representative cohort, it seems unlikely that this would have better sampled individuals who didn't ordinarily present for HIV testing. Secondly, patient retention was our priority; it was felt that participants recruited close to the study clinic was more likely to return for review.

Under-ascertainment of ILI episodes, and therefore influenza cases, was likely. During routine reviews, 40 participants had admitted to symptoms in keeping with ILI but had not attended for review (Chapter 4.3.2). Many cited mild illness and financial constraints for non-attendance, which we attempted to mitigate by reimbursement of transport costs. Nonetheless, a healthcare utilisation study of QECH ART clinic attenders found that many do not seek medical care for perceived mild illness (Masangalawe et al., 2011). If non-presentation of ILI occurred equally in the HIV-infected and HIV-uninfected cohorts, then influenza burden would have been underestimated in both groups, and the observed effect would have been biased towards the null. We found that HIV-infected participants attended with ILI more frequently than HIV-uninfected participants (adjusted IRR 1.21), which implied either that HIV-infected individuals experienced more ILI events than HIV-uninfected individuals, or HIV-infected patients were more likely to present to the study clinic if they experienced an ILI. It is difficult to speculate which of the two scenarios was more likely. However, the latter would constitute a form of selection bias given the differential ascertainment of outcome data between exposure categories. We therefore performed an alternative analysis that accounted for the number of ILI attendances by cohort participants. As expected, the effect estimates derived from the population average model were slightly smaller with wider confidence intervals than the Poisson regression analysis, but the effect of HIV infection on influenza persisted.

A major source of bias in cohort studies is loss to follow-up. We employed a number of measures to encourage routine and illness attendances, and to minimise loss to follow-up. At enrolment, cohort participants were given comprehensive education on the purpose of the study and importance of attendance, reinforced by written information to take home. Since physical addresses are not

available in Blantyre (few street names and house numbers), we obtained detailed maps and directions to patients' dwellings, as well as contact numbers for the participant, guardian or relative/neighbour. A dedicated study contact number was given to all participants to allow ease of contact of study staff. Regular contact was maintained with participants through clinic visits and telephone. Furthermore, proactive field workers conducted home visits if participants failed to attend routine reviews; visits occasionally took place early morning or at weekends to increase likelihood of locating the participant. Around 20% participants exited the study prior to the end date, which is equivalent to the rates of loss to follow-up in other longitudinal studies in Blantyre (French et al., 2010). Importantly, attrition rates were similar between the HIV-infected and HIV-uninfected cohorts, and the reason for study exit was known for the majority of exited participants. Around half had relocated out of Blantyre, thus highlighting the difficulties of conducting a longitudinal study in an urban population. Only 14 (3.9%) HIV-infected and 11 (4.4%) HIV-uninfected participants were untraceable.

The risk of misclassification of HIV status was low as HIV testing were performed by experienced HTC counsellors and the tests used are associated with >99% sensitivity and specificity (World Health Organization, 2004). Additionally, HIV-uninfected participants were retested annually in case they seroconverted.

Although molecular assays are highly sensitive and specific, misclassification of influenza status could have occurred. False positive result is rare, but false negatives can occur from poor specimen collection or handling, or if the patient had stopped shedding virus (Centers for Disease Control and Prevention, 2015a). This was minimised by rigorous training of study team on specimen collection and handling, and advising participants to attend for review as soon as ILI symptoms develop. Furthermore, any misclassification was likely to be non-differential to HIV status and unlikely to skew the results.

The HIV-infected and HIV-uninfected cohorts were unmatched on any characteristics. This was primarily to allow us to assess the effect of age, gender and other factors on influenza risk, but also matching on age or sex would have been challenging. In a cohort study, the exposed and unexposed groups should be as similar as possible with respect to all factors that may be related to the outcome, except the exposure of interest (Hennekens and Buring, 1987). In our study, a number of important differences between the two cohorts could have potentially confounded the association between HIV and influenza. In particular, the HIV-infected participants had a higher prevalence of previous tuberculosis and pneumonia, smaller household size, poorer water supply and sanitation facility, and were more likely to report food insecurity. These variables were included after careful review of existing literature, and evaluated as potential confounders in the multivariable analysis.

With few outcome events, we adjusted for a maximum of three covariates to avoid unreliable coefficients and biased estimates that can occur in models with events per variable (EPV) <10 (Peduzzi et al., 1996). Thus three separate multivariable models were constructed, all containing HIV. The crude and adjusted effect estimates of HIV on influenza do not vary substantially, indicating little confounding by the included variables. However, we were limited in the number of variables we could include in a multivariable model, thus residual confounding is possible. We found a trend towards a higher risk of influenza among those with a low enrolment CD4+ cell count (<200 cells/µl), while ART status had no effect. However, the study was not powered for these stratified analyses.

Lastly, information on additional potential confounders would have been useful. For example, indoor air pollution may be an independent risk factor for influenza infection. Biomass fuel is extensively used in Blantyre (Piddock et al., 2014); the association between biomass fuel exposure and respiratory infection in young children is well established (Kirkwood et al., 1995), but has not been evaluated in adults. Data were collected on the principal type of fuel used in household, which did not differ by HIV status. However, we did not measure the magnitude of current air pollution exposure, which would have been a major additional undertaking. The association between biomass exposure and adult pneumonia is currently the subject of another research project in Blantyre.

This study therefore adds to the scant body of literature from high HIV prevalence settings that HIV-infected adults have an increased susceptibility to influenza infection, compared to HIV-uninfected adults. Although most of the influenza cases were mild, our findings have important public health implications. HIV-infected individuals, aside from having increased risk of influenza infection and therefore higher disease burden, may also have a substantial role in community and nosocomial influenza transmission. In particular, they may increase transmission to other HIV-infected individuals in overcrowded ART clinics and hospital wards, where up to 75% patients have HIV infection (SanJoaquin et al., 2013). Furthermore, given the synergistic interaction between influenza and *Streptococcus pneumoniae* (McCullers, 2006), this may be a potential mechanism for the higher risk of bacterial pneumonia (Sogaard et al., 2008) and IPD (Nuorti et al., 2000; Harboe et al., 2014) observed among HIV-infected persons. Consequently, preventative strategies against influenza in this risk group may be especially pertinent.

Influenza vaccines, the most effective preventative tool against influenza, are not widely available in most SSA countries, and require annual administration. Thus policymakers will likely require further detailed evidence of the impact of HIV infection on influenza, particularly severe disease (Chapter 5), as well as mortality, prior to consideration of HIV-infected adults as a target group for immunisation. As regards HIV and influenza susceptibility, several unanswered questions remain. The impact of the degree of immunosuppression, and duration of antiretroviral treatment on the risk of influenza

illness remain unclear. The contribution of environmental and socioeconomic factors to influenza risk, particularly the interaction between HIV and food insecurity, require further elucidation. A population-based household cohort study would be an appropriate study design to address these questions. A random sample generated from the population will increase representativeness of the cohort. Secondly, active surveillance (e.g. involving visit by field worker visit once or twice weekly) will allow more accurate ascertainment of influenza burden, as persons with ILI who would not seek healthcare or are prevented to do so due to financial or time constraints, or cultural preferences can be captured. Furthermore, it will provide the opportunity to assess the complex transmission dynamics of influenza. A large cohort study is costly and logistically difficult to perform, but cost efficiency can be optimised by concurrently evaluating a number of other respiratory pathogens, including bacteria, tuberculosis and atypical organisms, and their interactions with influenza.

In conclusion, HIV-infected Malawian adults have a greater burden of influenza illness than HIV-uninfected adults, particularly in those with advanced immunosuppression. Household crowding and food insecurity also emerged as previously unrecognised risk factors for adult influenza infection. Further evaluation of the effect of the degree of immunosuppression and duration of antiretroviral treatment will build a more complete picture of the effect of HIV on influenza susceptibility. The findings of this study, if accompanied by evidence of increased disease severity (Chapter 5), will present a strong case to policymakers to consider targeted influenza vaccination policy in HIV-infected adults in Malawi and other similar high HIV prevalence settings. The interaction between HIV and food insecurity also warrants further exploration.

# CHAPTER 5. Severe and Mild Influenza-Related Respiratory Presentations in Malawian Adults: a Case Control Study

## 5.1 Introduction

Pneumonia is the commonest reason for adult hospital admission in SSA, with an estimated 4 million episodes and 200,000 deaths annually (Scott et al., 2012). As a result of limited surveillance and diagnostic capacity until recently, the contribution of influenza to adult pneumonia in SSA has not been well characterised (Chapter 1.3). The few studies that have evaluated viral aetiology in adult pneumonia in SSA detected influenza in 2 to 38% cases (Joosting et al., 1979; Scott et al., 2000; Hartung et al., 2011; Feikin et al., 2012b; Pretorius et al., 2012). However, the studies comprised diverse study populations, pneumonia definitions, as well as viral diagnostic tests. Thus the precise contribution of influenza to pneumonia remains unclear.

Existing literature that have evaluated the impact of HIV infection on the severity of influenza presentation have demonstrated conflicting findings (Chapter 1.4). In low HIV prevalence settings, HIV infection did not appear to significantly increase severity of influenza. Conversely, recent studies from high HIV prevalence settings in SSA have shown increased risk of hospitalisation (Ope et al., 2011), prolonged duration of hospital stay (Cohen et al., 2013), and higher mortality (Cohen et al., 2013; Tempia et al., 2015b; Cohen et al., 2015a) in HIV-infected persons compared to HIV-uninfected persons, though HIV status, CD4+ cell count and ART treatment status were not comprehensively ascertained. The disparity in the observed impact of HIV on disease severity and mortality from influenza may be explained by more advanced immunosuppression in HIV-infected individuals, poorer access to ART, in addition to higher prevalence of co-morbid conditions, such as tuberculosis, pregnancy or malnutrition in HIV-infected adults in SSA compared to other regions, but requires further evaluation.

As described in Chapter 1.5, annual seasonal influenza vaccination is currently unavailable in much of SSA (Duque et al., 2014); most countries have neither the resources nor the infrastructure to implement this. Thus demonstrating an important contribution of influenza to hospitalised pneumonia, and defining risk factors for severe influenza presentation, are critical information to build a case for policymakers and international organisations to devise targeted influenza immunization policy, and identify appropriate priority groups to target in SSA.

We therefore conducted a case control study of adults presenting with severe (cases, hospitalized lower respiratory tract infection) and mild (controls, influenza-like illness) influenza illness to examine the role and impact of HIV as a risk factor for severe influenza-related respiratory presentation in Malawian adults, and to identify other risk factors prominent in this setting.

## 5.2 Methods

### 5.2.1 Study design

A case control study design was chosen to evaluate the association between HIV infection and severity of influenza presentation in adults since severe influenza-associated lower respiratory tract infection is a relatively rare outcome. It allowed efficient recruitment of 'cases' in the timeframe required to complete this study. Additionally, ILI patients were chosen over healthy community adult controls as the control group for two main reasons: first, to enrich for influenza-positivity in the control group; second, it was felt that ILI patients presenting to government primary and secondary care facilities were more likely to be from the same population from which cases were derived. We wished to examine age and gender as potential risk factors for severe influenza. Hence cases and controls were unmatched. An overview of cases and controls and the population from which they were derived are illustrated in figure 5.1.

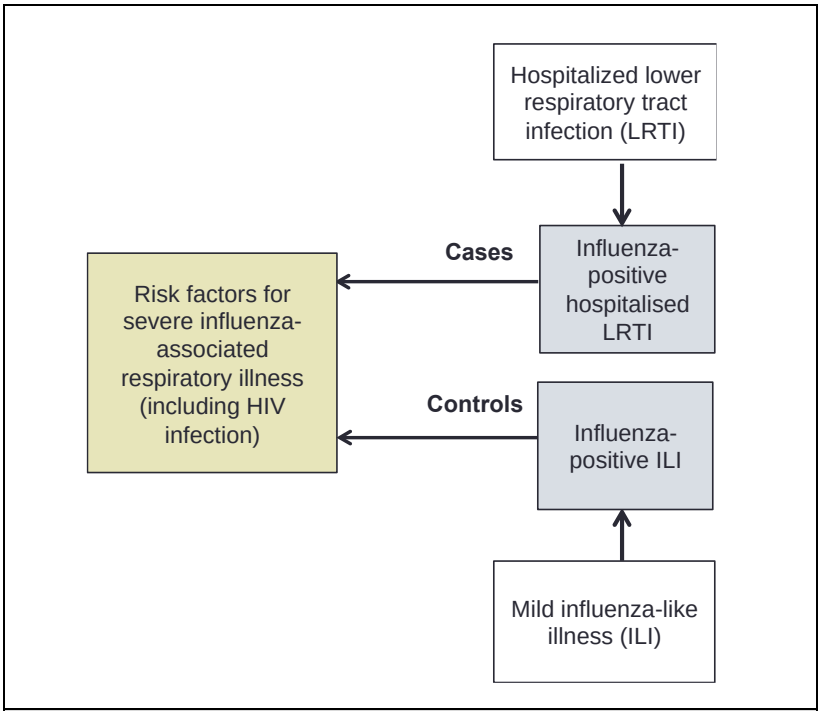



**Figure 5.1 Overview of case control study design**

### 5.2.2 Study site

Both potential cases and controls were enrolled from QECH at the start of the study. However, as a result of reorganisation of the health system in Blantyre, primary care patients were redirected to the Gateway Clinic. Following ethical approval, recruitment of patients with ILI took place at the Gateway clinic (Chapter 2.1.2) from January 2014.

### 5.2.3 Study population

#### *5.2.3.1 Definition of cases and controls*

**Cases**

Adults (aged ≥18 years) presenting to QECH with lower respiratory tract infection (LRTI) **AND** requiring hospitalisation **AND** influenza PCR-positive from a nasopharyngeal specimen.

**Controls**

Adults (aged ≥18 years) presenting to QECH or Gateway Clinic with influenza-like illness (ILI) **AND** not requiring hospitalisation **AND** influenza PCR-positive from a nasopharyngeal specimen.

#### *5.2.3.2 Eligibility criteria*

**Hospitalized LRTI**

***Inclusion criteria***

- Reported fever **OR** recorded fever (≥38°C oral[v])
- Reported cough **OR** chest pain **OR** breathlessness **OR** haemoptysis
- Crepitations **OR** pleural rub **OR** bronchial breathing **OR** signs suggestive of pleural effusion on clinical examination
- Require hospitalisation

***Exclusion criteria***

- Pre-admission diagnosis of terminal illness (e.g. metastatic malignancy)
- Current anti-tuberculous treatment
- Admission to hospital >24 hours prior to recruitment
- Symptoms for 14 days or greater
- Prior hospitalisation within last 4 weeks
- Suspected concurrent meningitis
- Prior participation in the study

[v] Obtained using SureTemp® Plus 690 electronic thermometer.

***Rationale for the inclusion/exclusion criteria***

Previous studies on pneumonia or LRTI have required the presence of infiltrates on chest radiograph (Templeton et al., 2005; de Roux et al., 2006; Jennings et al., 2008; Luchsinger et al., 2013). Since the availability of chest radiographs were variable at QECH, we opted for a clinical definition of LRTI – which comprised fever, ≥ one respiratory symptoms and ≥ one clinical sign suggestive of LRTI. In terms of exclusion criteria, the exclusion of patients who had been hospitalised for >24 hours, and prior hospitalisation within 4 weeks was to ensure that the detected influenza was community-acquired rather than due to nosocomial transmission. This study co-recruited patients with another study that aimed to define the aetiology of community-acquired pneumonia and factors associated with 30-mortality and treatment outcome (see section 5.2.5), hence patients that were deemed unlikely to live beyond 30 days, patients whose respiratory symptoms may have been due to another focus of infection (e.g. concurrent meningitis), as well as those on active TB treatment were excluded.

**ILI**

***Inclusion criteria***

- Reported fever **OR** recorded fever (≥38°C)
- ≥ 2 of the following: cough, sore throat, rhinorrhoea, myalgia, vomiting/diarrhoea **OR** headache
- Symptoms for 7 days or fewer
- Not requiring hospitalization for ILI presentation[vi]

***Exclusion criteria***

- Pre-admission diagnosis of terminal illness (e.g. metastatic malignancy)
- Current anti-tuberculous treatment
- Prior hospitalisation within last 4 weeks
- Suspected concurrent meningitis
- Prior participation in the study

***Rationale for the inclusion/exclusion criteria***

The inclusion criteria for ILI were identical to that used in the influenza surveillance at QECH (Chapter 3.2.3). Moreover, since the control group should be from the same study population from which cases are derived, the exclusion criteria that were applicable to hospitalised LRTI patients were also applied to enrolled patients with ILI.

[ii]May be hospitalized for an alternative indication e.g. fracture.

### 5.2.4 Related studies

The case control study recruited patients at QECH with acute respiratory symptoms alongside two other studies: the Malawian Adult Lower Respiratory Tract Infection Severity, Aetiology and Outcome (MARISO) study (COMREC reference P.11/12/1309) and the H1N1 sentinel influenza surveillance (P.07/10/958). The MARISO study, a prospective cohort study nested within this case control study, conducted more detailed clinical investigations and follow-up in a subset of patients with hospitalised LRTI. Eligibility criteria for adult LRTI were identical in the MARISO and BASH-FLU studies. There was also a significant overlap between hospitalized LRTI patients and patients with severe acute respiratory illness (SARI) recruited by the influenza surveillance. The overlap among the three studies is represented in Figure 5.2. Staff employed by the three studies were integrated into a single Acute Respiratory Infection Studies team. Recruitment procedures and laboratory investigations were streamlined to avoid obtaining duplicate clinical specimens and additional burden from participants. Depending on the fulfilment of eligibility criteria and daily recruitment target of each study, patients were recruited into one, two, or all three studies. Separate patient information leaflets were available for the three studies, but a joint informed consent procedure was completed (Appendix 8.3.3).

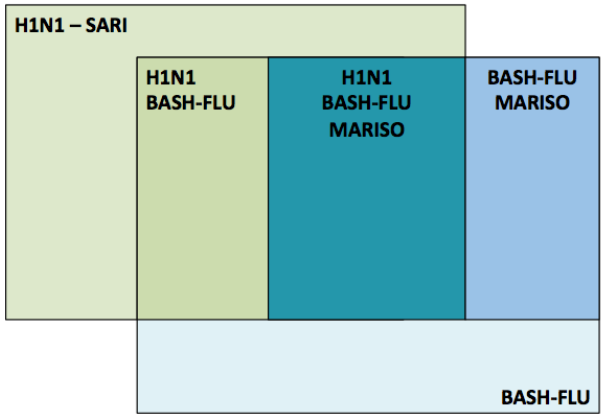



**Figure 5.2 Recruitment overlap among BASH-FLU, MARISO and H1N1 surveillance studies**

N.B. size of box in this diagram does not represent the relative proportion of patients recruited.

### 5.2.5 Study procedures

#### *5.2.5.1 Staff*

The following GCP-trained staff were employed to recruit for the BASH-FLU case control study, MARISO study and H1N1 influenza surveillance (i.e. the Acute Respiratory Infection studies):

- 3 study nurses: responsible for screening for eligible patients at the triage area in AETC, medical wards and the Gateway clinic; consent and enrolment of patients; obtaining clinical specimens, including nasopharyngeal aspirate, blood, sputum and urine; instituting appropriate management, e.g. administering intravenous fluids or antibiotics; and completion of paper and electronic copies of screening and enrolment logs.
- 3 clinical officers: responsible for assessing eligibility, consent and enrolment of patients; clinical assessment and management of patients; conducting necessary clinical procedures, e.g. pleural aspiration; aiding study nurses in obtaining clinical specimens.
- 1 laboratory messenger: responsible for transporting clinical specimens from the area of recruitment (AETC, medical ward or Gateway clinic) to the MLW/COM laboratories.
- 1 molecular laboratory technician: responsible for aliquoting, storage and processing nasopharyngeal specimens, as part of a team of technicians in the core diagnostic laboratory.

In addition to screening and enrolment of study participants, study staff also contributed to routine clinical activities at the AETC and Gateway Clinic during their working hours.

**Figure 5.3 Recruitment activities by study nurses in the Adult Emergency Trauma Centre**
**A)** One of the study nurses at the triage desk in AETC, **B)** Study nurses performing baseline observations and screening for eligible patients at the triage area.

#### *5.2.5.2 Enrolment*

Recruitment of ILI patients took place at the Gateway clinic between 8 and 11 am on weekdays. Hospitalised LRTI patients were recruited daily between 8am to 3pm in AETC; the study team also screened for eligible patients in the medical wards every morning that had been admitted in the preceding 24 hours. Recruitment hours were limited by the necessity of clinical samples to reach the laboratory by 3pm for processing.

Persons that met the eligibility criteria who were willing to consider participation in the study received a patient information document, and written informed consent was sought. A proportion of hospitalised LRTI patients were unable to provide informed consent at initial assessment due to confusion or clinical instability. In order to recruit a representative study population with hospitalized LRTI, these patients were recruited on the basis of proxy consent provided by an accompanying guardian (using a separate guardian consent form – Appendix 8.3.3). In the absence of a guardian, patients unable to give informed consent were not recruited. Formal consent from the patient was requested when they had recovered to the extent that they were able to have reasonable comprehension and retention of the information related to the study. If consent was denied at this stage, the participant's record was deleted from the study database.

Data were obtained from study participants and/or their guardian using a structured questionnaire (Appendix 8.3.3). Information collected included: demographic details; clinical presentation; medical history (including co-morbidities, previous history of tuberculosis or pneumonia, pregnancy, HIV and vaccination history); smoking and alcohol use; household characteristics (including household size, number of young children in household, number of rooms in dwelling and cooking fuel used); and socioeconomic status. The latter was defined by education, occupation, asset ownership, food security, and access to water and sanitation facilities.

Detailed clinical examination was performed to ascertain physiological parameters at presentation, nutritional status (body mass index (BMI) and mean upper arm circumference (MUAC)), mental status (AVPU score), upper and lower respiratory tract signs, other relevant systemic findings, as well as WHO clinical staging for HIV (if the participant was HIV-reactive). Of note, the same CRFs are completed for ILI and hospitalised LRTI patients, but the groups are differentiated by their designated study barcodes (section 2.3): hospitalised LRTI patients have barcodes beginning with 2200XXX (XXX is their study number), whereas ILI patients have barcodes beginning with 3300XXX.

In addition to clinical assessment of patients and completing study documents, the study clinical officers were also responsible for completion of hospital admission documents, entering patients'

clinical details in their health passport, and formulation of onward management plans. Empirical treatment was administered by the study nurses.

#### *5.2.5.3 Laboratory investigations*

The investigations that were performed on patients with ILI and hospitalised LRTI at recruitment are listed below (see Section 2.2 for details of individual tests):

**Table 5.1 Laboratory investigations undertaken in the case control study**

| Investigations | ILI | Hospitalised LRTI |
|---|---|---|
| **Venous blood** | | |
| Malaria rapid diagnostic test | **X** | **X** |
| Rapid HIV test (if status unknown) | **X** | **X** |
| CD4+ cell count | X | **X** |
| Blood culture | | **X** |
| **Nasopharyngeal & oropharyngeal swabs** | | |
| Influenza PCR (CDC H1N1 kit) | **X** | **X** |
| Multiplex PCR for 33 respiratory pathogens (Fast-track Diagnostics) | | **X** |
| Quantitative *lyt*A PCR for *S. pneumoniae* | | **X** |
| **Sputum** | | |
| Smear microscopy for AFB & culture | | **X** |
| GeneXpert MTB/RIF | | **X** |
| **Urine** | | |
| Rapid urinary antigen test for S. *pneumoniae* (BinaxNOW®) | | **X** |
| **Chest X-Ray** | | **X** |

All laboratory tests were done at the MLW or University of Malawi College of Medicine laboratories (Chapter 2.2). With the exception of PCR results on nasopharyngeal specimens, results of the above investigations were relayed back to patients and the clinical team looking after them as soon as they were available. HIV testing was performed by the MLW laboratory, and results were fed back to the participants by HTC-trained study staff. Patients that were newly diagnosed with HIV infection were referred for HIV care at the QECH ART clinic or their local health centre.

#### *5.2.5.4 Follow-up*

No formal follow-up was conducted beyond recruitment. However, the study team conducted daily morning ward rounds of enrolled patients at QECH to feedback clinically-relevant laboratory test results to the clinical team, obtain digital images of chest radiographs, and record duration of admission and patient outcome. Timing of hospital discharge was at the discretion of the clinical team.

### 5.2.6 Sample size calculations

In a Kenyan case control study, the OR of having HIV infection was 3.6 (95%CI 1.3-10.1) in hospitalised patients with influenza (cases) compared to healthy adults (controls) (Ope et al., 2011). A lower effect estimate was thought likely in this study since our controls were patients with ILI. With an estimated HIV prevalence of 37.5% in adults with ILI from preliminary influenza surveillance data (Garg et al., 2012), and a case: control ratio of 1:2, a sample size of 57 cases and 114 controls provided 80% power to detect risk factors with an odds ratio of 2.5 or greater. Based on estimated influenza prevalence of 10% and 16.4% in adults presenting to QECH with hospitalized LRTI and ILI respectively, this would involve recruitment of approximately 570 adults with hospitalized LRTI and 695 adults with ILI. Patient recruitment was discontinued once the target number of cases and controls are recruited.

### 5.2.7 Statistical analysis

In the descriptive comparison of baseline characteristics between enrolled hospitalised LRTI and mild ILI patients, as well as influenza-positive cases and controls, chi-square tests was used to compare categorical variables. Student independent-samples t-tests or Wilcoxon rank-sum tests were used to compare continuous variables, depending on the distribution properties of the variable.

Multivariable analyses were implemented to identify i) clinical predictors of influenza among hospitalised LRTI patients; and ii) risk factors for severe influenza presentation (i.le. being a case). A log binomial regression model was used to compute risk ratios (RR) and 95% confidence intervals for factors predicting influenza-associated LRTI.

The analysis of risk factors for severe influenza presentation was restricted to cases and controls. HIV infection and month of recruitment were included *a priori* in the multivariable model; the latter due to the discordant timing of recruitment of hospitalized LRTI and ILI patients (Figure 5.6). Since cases and controls were unmatched, unconditional logistic regression models were used to estimate odds ratios for the effect of HIV (primary exposure of interest) and other risk factors for severe influenza presentation. In both multivariable models, covariates with a p-value <0.10 on univariable analysis were assessed in the multivariable model using stepwise backwards elimination. Factors with two-sided p-values of <0.05 were considered significant.

Population attributable fractions (PAF) of modifiable risk factors for severe influenza were also estimated. PAF provides an estimate of the proportion of hospitalised influenza cases that would theoretically be prevented if the exposure was removed, assuming a causal relationship between

the exposure and outcome (Rockhill et al., 1998). The PAFs and confidence intervals for the risk factors for severe influenza were derived from the prevalence of exposure in cases and adjusted OR from the multivariable logistic regression model using the *punafcc* command in Stata 12.0 (Newson, 2013), which is based on the formulae by Greenland and Drescher (Greenland, 1993). As individual risk factors may interact in their impact on overall risk of disease, PAFs for multiple risk factors can add up to greater than 100%.

### 5.2.8 Study timescale

Study staff were recruited and trained in March and April 2013. Recruitment of hospitalised LRTI and ILI patients commenced in AETC on 15 May 2013, and at the Gateway Clinic (ILI patients only) from 27 January 2014. Patient recruitment was completed on 28 February 2015.

## 5.3 Results

Between May 2013 and February 2015, 1645 patients were screened for eligibility for hospitalised LRTI and 846 for ILI, with subsequent recruitment of 518 and 642 patients respectively (Figure 5.4). Recruitment was discontinued prior to reaching target sample size due to time constraints. Due to the strict eligibility criteria for hospitalized LRTI, a large proportion of screened patients were deemed ineligible for study inclusion (n=1109, 68.5%); the predominant reasons for exclusion were illness duration greater than 14 days, lack of focal chest signs, and the absence of fever. Eligible and non-eligible individuals had similar sex distribution (59 vs. 64% male, p=0.08) (Figure 5.5). However, ineligible individuals were slightly older with a median age 35 years and a higher proportion of individuals aged > 40years (40% vs. 31%. P=0.01).

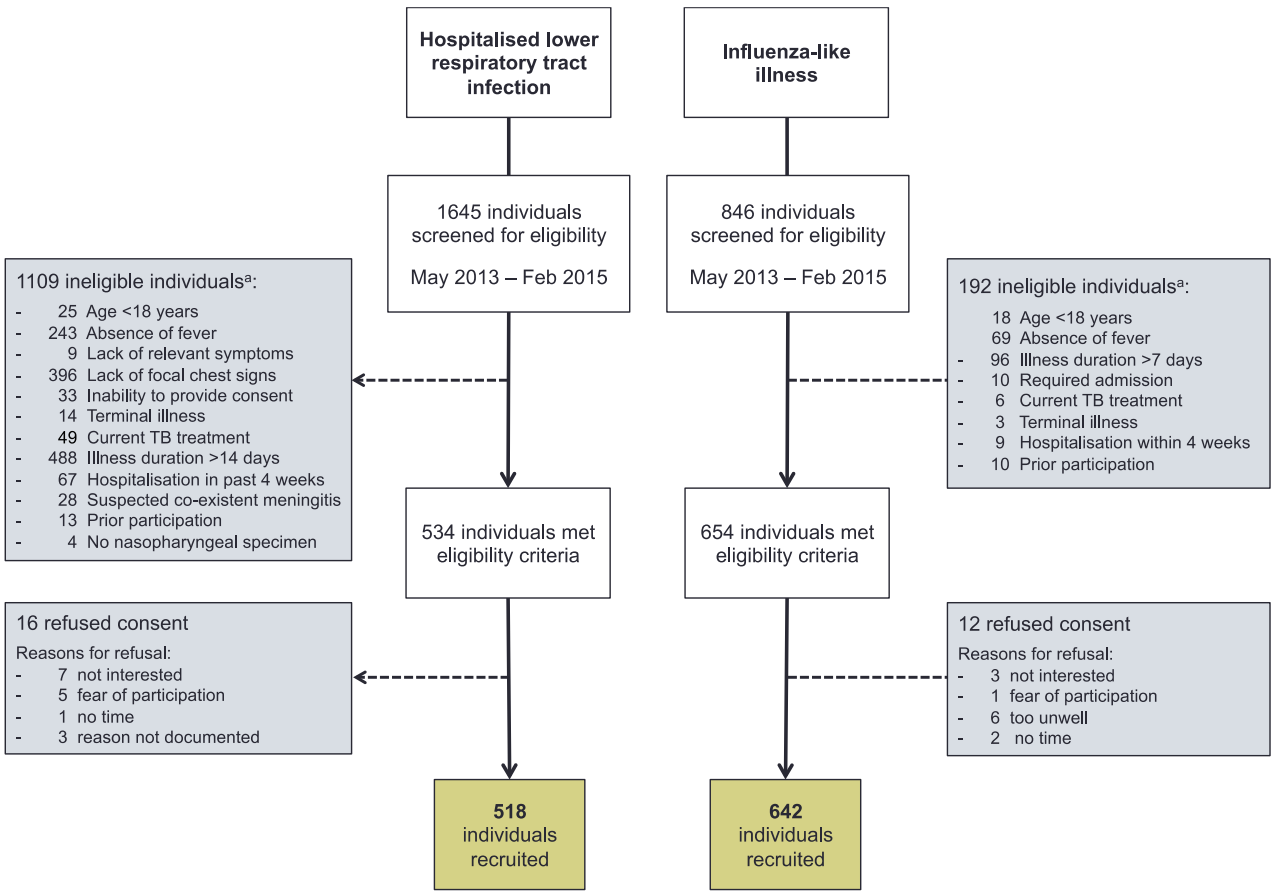



**Figure 5.4 Flow diagram of case control study recruitment**

[a]Some excluded for multiple reasons

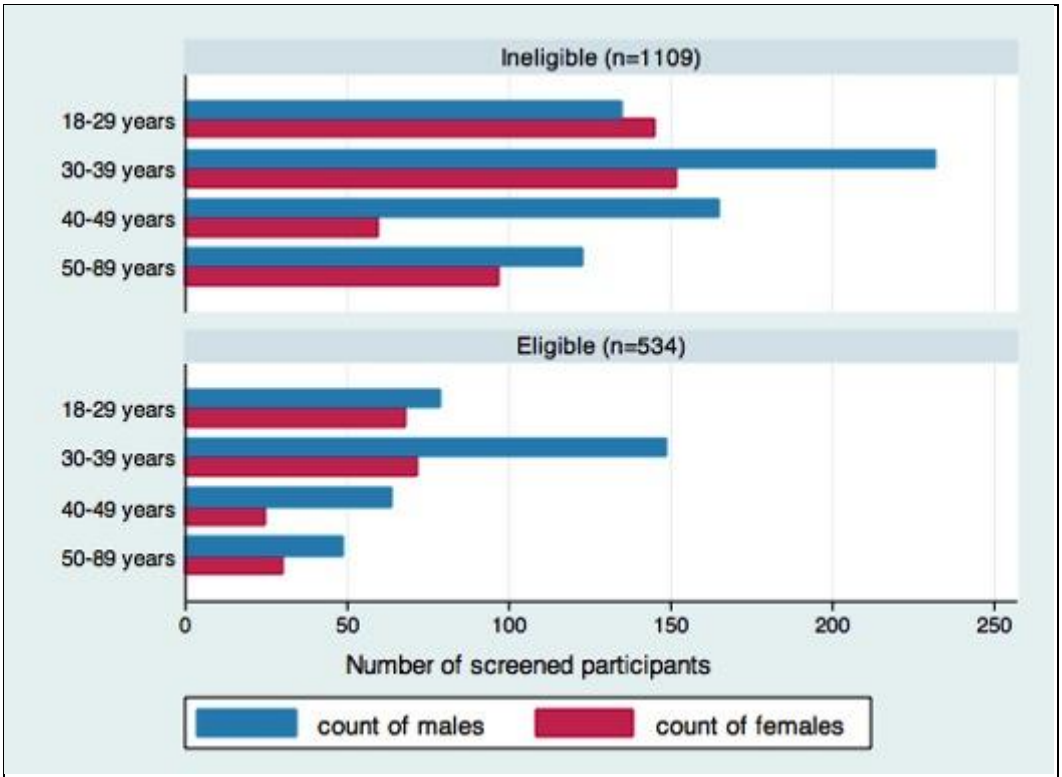



**Figure 5.5 Age and gender distribution among eligible and ineligible individuals screened for hospitalised LRTI**

Recruitment progress over the 22-month period is illustrated in Figure 5.6. The number of participants recruited each month varied from 11 to 37 for hospitalised LRTI, and 1 to 68 for ILI. Few ILI patients were recruited between September and December 2013 due to health system reorganisation in Blantyre that resulted in the diversion of patients with primary care issues away from AETC (Chapter 5.2.3). Enrolment numbers improved from January 2014 after the recruitment site of ILI patients was changed to the Gateway Clinic.

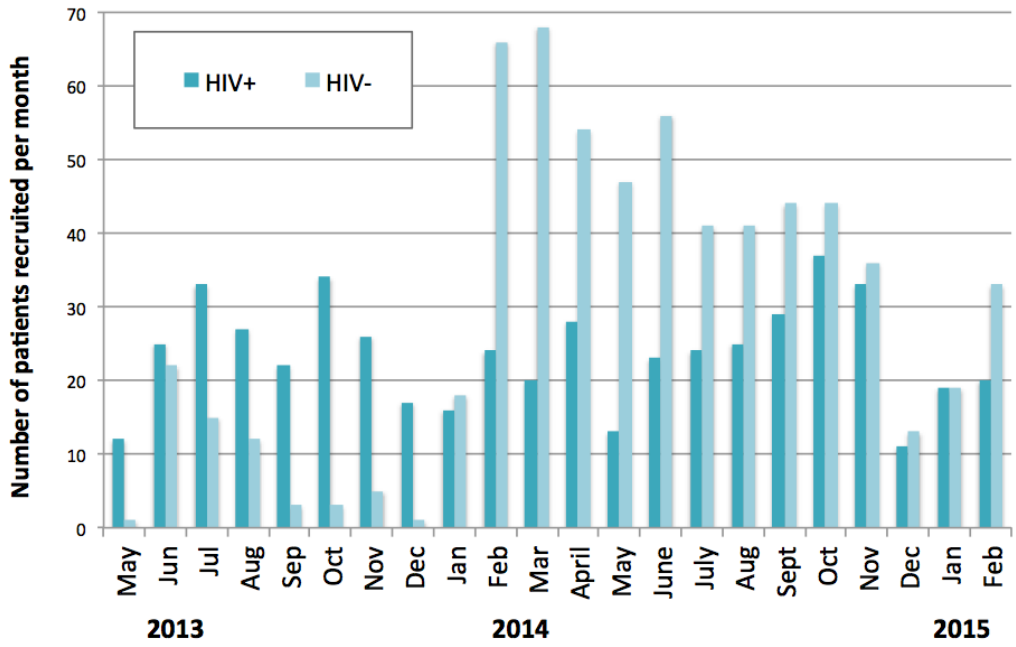



**Figure 5.6 Monthly patient recruitment in the BASH-FLU case control study**

### 5.3.1 Baseline characteristics of hospitalised LRTI and ILI patients

The baseline characteristics of the patients enrolled with hospitalised LRTI and mild ILI are summarized in Table 5.2. Compared to patients with ILI, those recruited with hospitalised LRTI were more likely to be male (62 vs. 42%) and were older (median age 35 vs. 32 years). Since QECH is a tertiary referral hospital, it is unsurprising that 61% of LRTI patients had attended another health facility prior to admission, with a similar proportion reporting pre-hospital antibiotic consumption. The LRTI patients also had a substantially higher HIV prevalence (77 vs. 30%), and had more advanced immunosuppression among those with HIV-infection (median CD4+ count 101 vs. 313 cells/µl). The prevalence of chronic co-morbidities in both groups were low, though a higher proportion of hospitalised LRTI patients had reported a previous history of tuberculosis and pneumonia within the past 5 years.

In terms of household characteristics, there were no differences in household crowding or exposure to young children (<5 years). However, patients with ILI had better sanitation (encompassing sanitation facility, water source, as well as distance to water source), level of education, asset ownership, and reported less food insecurity.

The presenting symptoms and clinical examination findings of the two groups are summarized in Table 5.3. The majority of symptoms, with the exception of headache, rhinorrhoea and sore throat, were more commonly reported among hospitalised LRTI patients. Additionally, a higher proportion

**Table 5.2 Demographic, clinical, household and socioeconomic characteristics of adults enrolled with hospitalised LRTI and mild ILI, Queen Elizabeth Central Hospital and Gateway Clinic, Blantyre, May 2013-February 2015 (N=1160)**

| Characteristic | Hospitalised LRTI (n=518) | ILI (n=642) | p-value[a] |
|---|---|---|---|
| **Demographic** | | | |
| Gender | | | |
| Male | 323 (62.4) | 273 (42.5) | <0.001 |
| Age (median, IQR) | 34.8 (29.6-42.2) | 31.9 (25.4-43.0) | <0.001 |
| Age group | | | |
| 18-29 | 138 (26.6) | 278 (43.3) | |
| 30-39 | 227 (43.8) | 165 (25.7) | |
| ≥ 40 | 153 (29.5) | 199 (31.0) | <0.001 |
| **Pre-hospital/clinic attendance & treatment** | | | |
| Attended another health facility[b] | 312 (60.8) | 62 (9.8) | <0.001 |
| Antibiotics within 2 weeks | 309 (60.7) | 70 (11.1) | <0.001 |
| Antimalarials within 2 weeks | 83 (16.3) | 13 (2.1) | <0.001 |
| Traditional medicines within 2 weeks | 39 (7.7) | 2 (0.3) | <0.001 |
| **HIV prevalence** | 396 (76.6) | 193 (30.2) | <0.001 |
| Known HIV+ at enrolment | 246/396 (62.1) | 92/189 (48.7) | <0.001 |
| On ART at enrolment | 188/233 (80.7) | 68/87 (78.2) | 0.62 |
| **CD4+ cell count (cells/µl)[c]** | | | |
| median, IQR | 101 (46-196) | 313 (167-450) | <0.001 |
| **Medical history** | | | |
| Chronic lung disease | 18 (3.6) | 15 (2.4) | 0.23 |
| Chronic cardiac disease | 2 (0.4) | 1 (0.2) | 0.59 |
| Hypertension | 9 (1.8) | 19 (3.0) | 0.19 |
| Cancer | 5 (1.0) | 1 (0.2) | 0.09 |
| Chronic kidney disease | 1 (0.2) | 0 (0) | 0.44 |
| Chronic liver disease | 1 (0.2) | 0 (0) | 0.44 |
| Stroke | 5 (1.0) | 1 (0.2) | 0.99 |
| Previous history of tuberculosis | 90 (17.5) | 45 (7.1) | <0.001 |
| Pneumonia in past 5 years | 113 (22.1) | 46 (7.2) | <0.001 |
| Pregnant | 2/191 (1.1) | 10/363 (2.8) | 0.19 |
| Smoking – current | 58 (11.3) | 39 (6.1) | <0.001 |
| Alcohol | 135 (26.4) | 78 (12.2) | <0.001 |
| **Household and socioeconomic** | | | |
| Children <5years | | | |
| 0 | 296 (57.7) | 381 (59.7) | |
| 1 | 156 (30.4) | 195 (30.6) | |
| ≥2 | 61 (11.9) | 62 (9.7) | 0.48 |
| Crowding index[d] | | | |
| <1.5 | 174 (35.0) | 219 (35.8) | |
| 1.5-2.4 | 203 (40.9) | 258 (42.2) | |
| ≥2.5 | 120 (24.1) | 135 (22.1) | 0.71 |
| Water supply | | | |
| River/borehole/well | 150 (29.2) | 134 (21.0) | |
| | 272 (52.9) | 308 (48.3) | |

| | | | |
|---|---|---|---|
| Public tap/standpipe | 92 (17.9) | 196 (30.7) | <0.001 |
| Piped to dwelling/plot | | | |
| Distance from water supply | 182 (35.4) | 272 (42.6) | |
| <200m | 332 (64.5) | 366 (57.4) | 0.01 |
| ≥200m | | | |
| Sanitation | 284 (55.3) | 260 (40.8) | |
| None/non-VIP toilet | 188 (36.6) | 252 (39.6) | |
| VIP toilet | 42 (8.2) | 125 (19.6) | <0.001 |
| Flush toilet | | | |
| Principal cooking fuel | 140 (27.2) | 129 (20.2) | |
| Firewood | 325 (63.2) | 421 (66.1) | |
| Charcoal | 49 (9.5) | 87 (13.7) | 0.006 |
| Electricity | | | |
| Highest level of education | 49 (9.7) | 52 (8.2) | |
| Never attended school | 274 (53.9) | 266 (41.7) | |
| Primary | 185 (36.4) | 320 (50.2) | <0.001 |
| Secondary/Tertiary | | | |
| Employment status | | | |
| Unemployed | 68 (13.2) | 91 (14.3) | 0.61 |
| Asset ownership[e] | | | |
| 0 | 84 (16.3) | 75 (11.8) | |
| 1 | 106 (20.6) | 112 (17.6) | |
| 2 | 122 (23.7) | 165 (25.9) | |
| 3 | 158 (30.7) | 206 (32.3) | |
| 4-5 | 35 (8.7) | 80 (12.5) | 0.04 |
| Food security – difficulty obtaining food | | | |
| Never | 247 (48.0) | 367 (57.5) | |
| Sometimes | 226 (44.0) | 250 (39.2) | |
| Often/always | 41 (8.0) | 31 (3.3) | <0.001 |
| **Season of recruitment** | | | |
| Jan-Mar | 99 (19.1) | 204 (31.8) | |
| Apr-Jun | 100 (19.3) | 180 (28.0) | |
| Jul-Sep | 161 (31.1) | 156 (24.3) | |
| Oct-Dec | 158 (30.5) | 102 (15.9) | <0.001 |

LRTI-lower respiratory tract infection; ILI-influenza-like illness; IQR-interquartile range; PCV, pneumococcal conjugate vaccine; VIP, ventilated improved pit latrine

The number of missing observations in each variable is detailed in Appendix 8.4.3; individuals with missing data were excluded from the denominator in percentage calculations.

[a]chi-squared test for categorical variables /Wilcoxon rank sum test for continuous variables

[b]Includes attendance to other hospital, health centre, private clinic, traditional healer or pharmacy

[c]CD4+ count not available for 45 hospitalised LRTI and 13 ILI patients

[d]Total number of household members divided by number of rooms in house (excluding kitchen and bathroom)

[e]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.

**Table 5.3 Clinical characteristics of adults enrolled with hospitalised LRTI and mild ILI, May 2013-February 2015 (N=1160)**

| Characteristic | Hospitalised LRTI (n=518) | Mild ILI (n=624) | p-value[a] |
|---|---|---|---|
| **Symptoms** | | | |
| Cough | 506 (98.6) | 612 (95.9) | 0.03 |
| Sputum | 406 (79.1) | 111 (17.4) | <0.001 |
| Shortness of breath | 492 (95.9) | 93 (14.6) | <0.001 |
| Chest pain | 501 (97.9) | 259 (40.6) | <0.001 |
| Myalgia | 405 (79.0) | 516 (80.9) | 0.42 |
| Headache | 233 (45.4) | 504 (79.0) | <0.001 |
| Rhinorrhoea | 83 (16.2) | 529 (82.9) | <0.001 |
| Sore throat | 122 (23.8) | 395 (61.9) | <0.001 |
| Nausea/vomiting | 83 (16.2) | 22 (3.5) | <0.001 |
| DIarrhoea | 81 (15.9) | 25 (3.9) | <0.001 |
| Weight loss | 310 (60.4) | 18 (2.8) | <0.001 |
| Night sweats | 331 (64.7) | 17 (2.7) | <0.001 |
| Skin rash | 38 (7.4) | 17 (2.7) | <0.001 |
| **Physiological parameters at enrolment** | | | |
| Disorientation[b] | 7 (1.4) | 3 (0.5) | 0.11 |
| Temperature >38°c or <35°c | 256 (49.4) | 48 (7.5) | <0.001 |
| Systolic blood pressure <90mmHg | 96 (18.7) | 10 (1.6) | <0.001 |
| Diastolic blood pressure <60mmHg | 132 (25.7) | 20 (3.1) | <0.001 |
| Heart rate >120 or <40 beats/min | 222 (43.2) | 23 (3.6) | <0.001 |
| Respiratory rate $\geq$30/min | 231 (46.1) | 4 (0.6) | <0.001 |
| Oxygen saturation $\leq$90% | 113 (22.0) | 7 (1.1) | <0.001 |
| Unable to stand | 97 (18.9) | 1 (0.2) | <0.001 |
| BMI (kg/m$^2$) – median (IQR) | 19.9 (18.2-21.7) | 21.7 (19.7-24.3) | <0.001 |
| <18.5 | 147(29.7) | 67 (10.5) | |
| 18.5-24.9 | 329 (66.5) | 437 (68.5) | |
| $\geq$25 | 19 (3.8) | 134 (21.0) | <0.001 |
| MUAC (mm) – median (IQR) | 250 (220-260) | 255 (240-270) | <0.001 |
| **Clinical signs** | | | |
| Red throat | 11 (2.1) | 38 (6.0) | 0.001 |
| Pus in throat | 1 (0.2) | 1 (0.2) | 1.00 |
| Cervical lymphadenopathy | 40 (7.8) | 5 (0.8) | <0.001 |
| Sinus tenderness | 1 (0.2) | 3 (0.5) | 0.63 |
| Crepitations | 436 (84.8) | 18 (2.8) | <0.001 |
| Bronchial breathing | 188 (36.6) | 7 (1.1) | <0.001 |
| Effusion | 85 (16.5) | 0 (0) | <0.001 |
| Pleural rub | 141 (27.4) | 0 (0) | <0.001 |
| Wheeze | 20 (3.9) | 4 (0.6) | <0.001 |

| | | | |
|---|---|---|---|
| Finger clubbing | 27 (5.3) | 0 (0) | <0.001 |
| Pale conjunctivae | 29 (5.6) | 1 (0.2) | <0.001 |
| Oral candidiasis | 34 (6.6) | 2 (0.3) | <0.001 |
| Kaposi's sarcoma lesions | 16 (3.1) | 0 (0) | <0.001 |
| Jaundice | 7 (1.4) | 1 (0.2) | 0.01 |
| Hepatomegaly | 25 (4.9) | 2 (0.3) | <0.001 |
| Splenomegaly | 17 (3.3) | 2 (0.3) | <0.001 |
| Pedal oedema | 14 (2.8) | 3 (0.5) | 0.002 |

LRTI, lower respiratory tract infection; ILI-influenza-like illness; BMI, body mass index, MUAC, mid upper arm circumference; mm-millimetres; IQR-interquartile range.
The number of missing observations in each variable is detailed in Appendix 8.4.3; individuals with missing data were excluded from the denominator in percentage calculations.

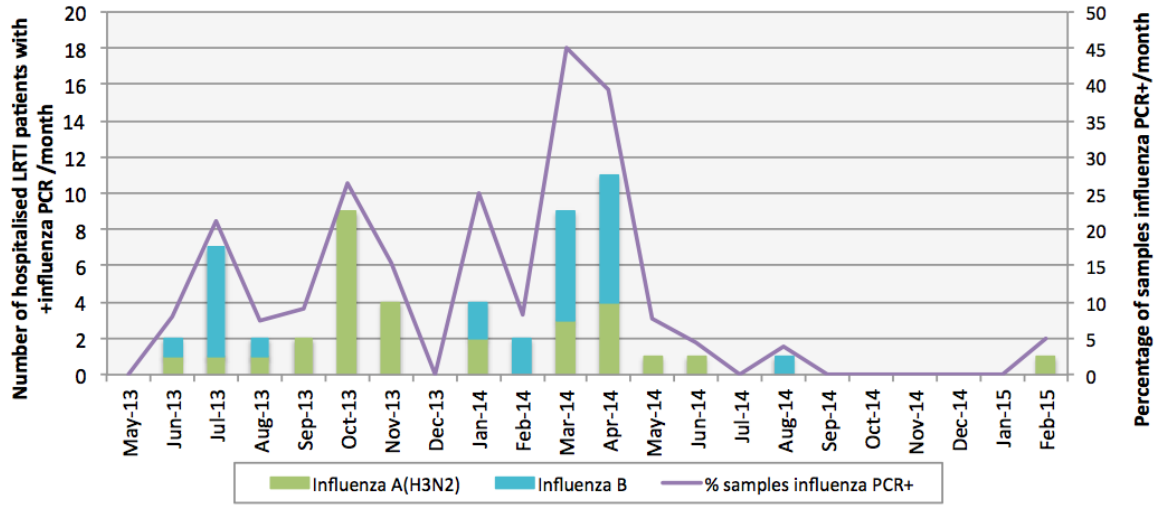
[a]Chi-square or Fisher's exact test

[b]Disorientated in time, place or person

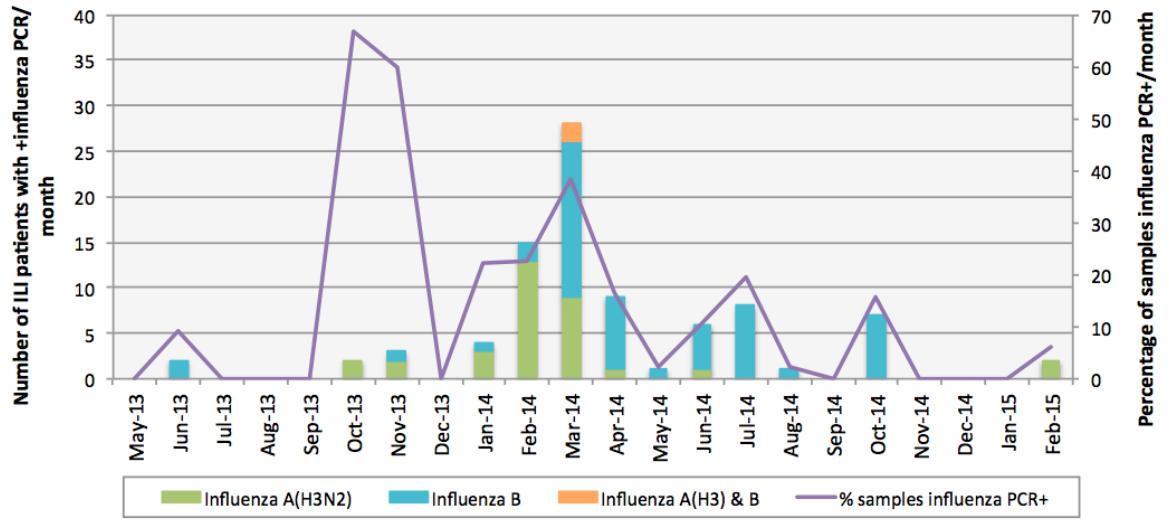
of LRTI patients had physiological parameters consistent with clinical instability (e.g. systolic blood pressure <90mmHg or oxygen saturation <90%), thus supporting greater severity of clinical presentation in this group.

### 5.3.2 The contribution of influenza to hospitalised LRTI and ILI

Influenza was identified in 56 (10.8%) patients with hospitalised LRTI and 88 (13.7%) patients with ILI. Figure 5.6 illustrates the number and proportion of enrolled hospitalised LRTI and ILI patients with a positive influenza PCR, including the type and subtype of influenza. Seasonal influenza A(H3N2) and influenza B, but not influenza A(H1N1)pdm09, were identified during the study period.




**A. Hospitalised lower respiratory tract infection**




**B. Influenza-like illness**

**Figure 5.7 Number and percentage of A) hospitalised LRTI (n=56), and B) ILI (n=88) patients with a positive influenza PCR per month, including influenza type and subtype, May 2013-February 2015**

### 5.3.3 Aetiology and outcome of hospitalised LRTI at QECH

#### *5.3.3.1 Aetiology*

From the diagnostic tests that were performed (Chapter 5.2.5.3), one or more aetiological agents were identified in 381 (67%) of 518 patients admitted with LRTI (Table 5.4). Respiratory viruses were detected by rRT-PCR from NPA from approximately a third of patients (n=177, 34.2%); the most commonly identified were influenza A and B (n=56, 10.8%), adenovirus (n=39, 7.7%), coronavirus 229E (n=22, 4.4%), and rhinovirus (n=22, 4.4%). Among those with $\geq$ 1 respiratory virus detected on NPA, 39 (22.3%) had viral co-detection. Figure 5.8 illustrates the seasonal distribution of respiratory viruses other than influenza that have 10 or more positive samples over the study period, detected from nasopharyngeal specimen by rRT-PCR.

A bacterial aetiology, including atypical organisms, was found in 203 (39%) hospitalised LRTI patients (Table 5.4). *Legionella* species (n=96, 18.6%) and *Streptococcus pneumoniae* (n=95, 18.3%) were the most frequently detected bacteria. S. *pneumoniae* was grown on blood culture in 4 patients (3 of whom also had a positive urinary antigen test), and was diagnosed by urinary antigen test alone in 91 patients. S. *pneumoniae* demonstrated no obvious seasonal patterns (Figure 5.9), whereas detection of *Legionella* species peaked in December 2013, followed by sustained activity from June 2014 to February 2015. Bacteraemia with Gram-negative organisms was also common (including *Salmonella* species (n=14; *Salmonella typhi* (n=8), non-typhoidal *Salmonella* (n=6)), *Escherichia coli* (n=2)*, Burkholderia cepacia* (n=1)*, and Enterobacter cloacae* (n=1)). Atypical pathogens were detected in a small proportion of patients (*Bordetella pertussis* (n=7), *Chlamydia pneumoniae* (n=2), *Mycoplasma pneumoniae* (n=7)).

*S. pneumoniae* was identified from nasopharyngeal aspirate by RT-PCR in 284 (55.1%) patients, which likely represents colonisation. Of note, urinary antigen for S. *pneumoniae* was positive for 73/233 (31.3%) of patients with a positive PCR on NPA, versus 21/189 (11.1%) who had a negative PCR.

Among the 389 patients that submitted sputum, pulmonary tuberculosis was diagnosed[vii] in 93 (23.9%) patients (by smear microscopy (n=43), liquid culture (n=59) or GeneXpert (n=58)). Malaria rapid diagnostic test was positive in 7 (1.4%) patients; 5 of which had an additional microbial agent detected (virus (n=3), bacteria (n=1), TB (n=1)). The overlap in aetiological diagnoses is illustrated in Figure 5.10. Mixed pathogen infections were common (n=113, 21.8%). Specific pathogen combinations are shown in Tables 5.5 to 5.8.

[vii] Patients could have been diagnosed with >1 method.

| Table 5.4 Aetiology of adults with hospitalised LRTI at QECH (n=518) | |
|---|---|
| **Investigation** | **N (%)** |
| **Aetiology undetermined** | 167 (32.2) |
| **NPA – respiratory viruses (n=517)** | |
| ≥ 1 respiratory virus | 177 (34.2) |
| Influenza A & B | 56 (10.8) |
| Adenovirus | 40 (7.7) |
| Bocavirus | 15 (2.9) |
| Coronavirus OC43 | 10 (1.9) |
| NL63 | 7 (1.4) |
| 229E | 22 (4.3) |
| HKU1 | 5 (1.0) |
| Enterovirus | 5 (1.0) |
| Human metapneumovirus | 11 (2.2) |
| Influenza C | 6 (1.2) |
| Parainfluenza virus 1 | 2 (0.4) |
| Parainfluenza virus 2 | 7 (1.4) |
| Parainfluenza virus 3 | 7 (1.4) |
| Parainfluenza virus 4 | 3 (0.6) |
| Rhinovirus | 22 (4.3) |
| Respiratory syncytial virus | 9 (1.7) |
| ≥ 2 respiratory viruses | 39 (7.5) |
| **NPA - atypical pathogens (n=517)** | |
| *Bordetella pertussis* | 6 (1.1) |
| *Chlamydia pneumoniae* | 2 (0 |
| L*egionella* species | 96 (18.6) |
| *Mycoplasma pneumoniae* | 7 (1.3) |
| *Pneumocystis jirovecii* | 7 (1.3) |
| **NPA - *Streptococcus pneumoniae* colonization (n=517)** | 284 (55.1) |
| **Blood culture** | 25/509 (4.9)[b] |
| *Streptococcus pneumoniae* | 4 |
| *Staphylococcus aureus* | 2 |
| *Escherichia coli* | 3 |
| S*almonella* species | 14 |
| *Burkholderia cepacia* | 1 |
| *Enterobacter cloacae* | 1 |
| Contaminants (e.g. Bacillus sp., Micrococcus sp., coagulase negative Staphylococcus) | 15 |
| **Tuberculosis (diagnosed by smear microscopy /culture/Gene Xpert)** | 93/389 (23.9) |
| **Urinary S. *pneumoniae* antigen** - positive | 94/421 (22.3) |
| **Malaria RDT** - positive | 7/488 (1.4) |
| **Outcome** | |
| Duration of admission[b] (days) – median (IQR) | 6 (3-8) |
| Required admission to HDU[c] | 72/477 (15.1) |
| Inpatient mortality | 52 (10.0) |

LRTI, lower respiratory tract infection; NPA, nasopharyngeal aspirate; RDT, rapid diagnostic test; IQR, interquartile range; HDU, high dependency unit

[a]Excludes contaminants

[b]Discharge date missing in 16 participants; [c]Missing in 41 participants

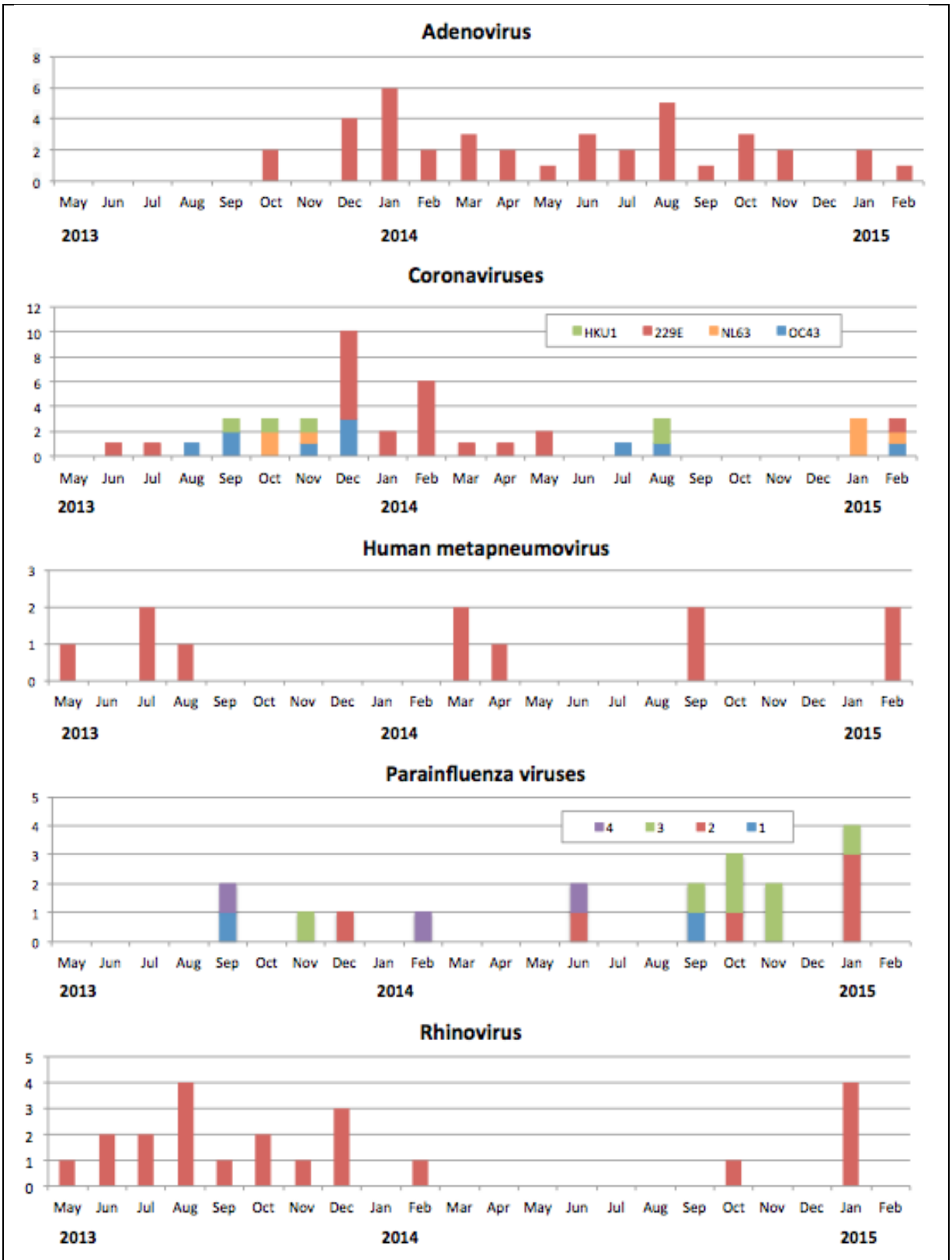



**Figure 5.8 Monthly distribution of respiratory viruses other than influenza A and B (with n>10) detected by rRT-PCR, among patients with hospitalized LRTI, May 2013-February 2015**

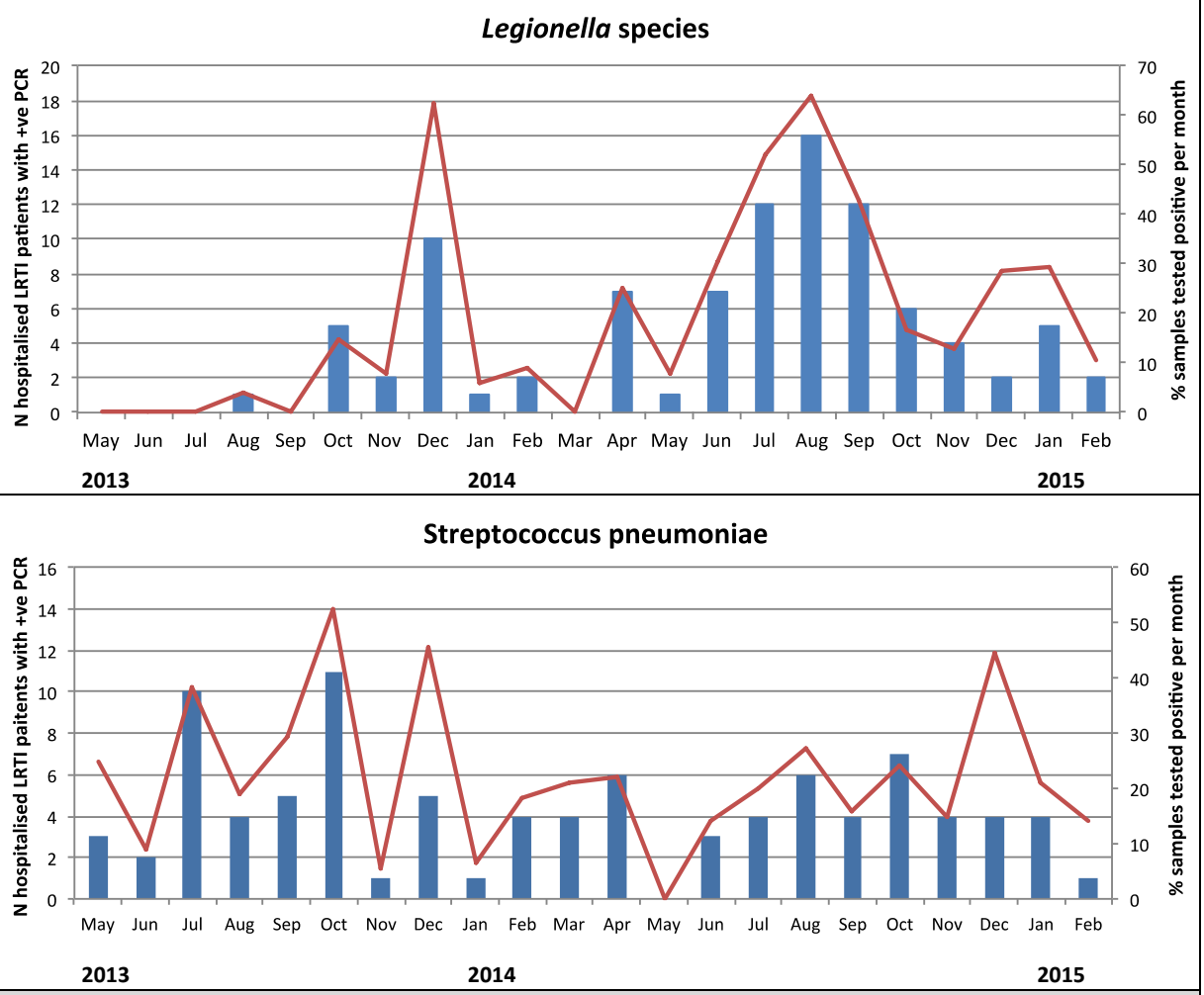



**Figure 5.9 Monthly distribution of *Legionella* species detected by rRT-PCR (n=518) and Streptococcus pneumoniae, detected by urinary antigen test (n=421), among patients with hosptialised LRTI, May 2013-February 2015**

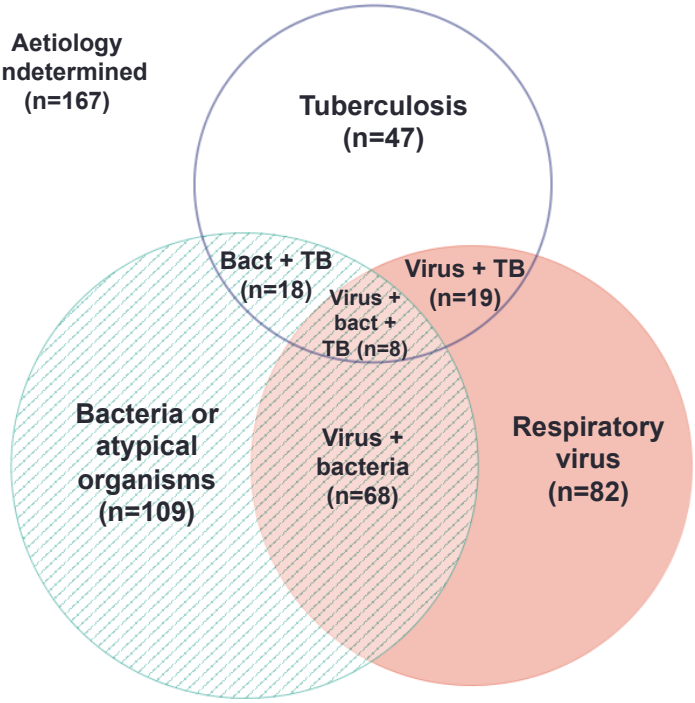



**Figure 5.10 Overlap of aetiological diagnoses among adults enrolled with hospitalised LRTI (N=518)**

One or more aetiological agents were detected in 351 patients.

Patients with positive malaria rapid diagnostic test (n=7) not represented in this Venn diagram; 3 had co-detection with virus, 1 with bacteria and 1 with TB.
Respiratory viruses were identified by CDC influenza PCR and multiplex PCR for 33 respiratory pathogens (FastTrack Diagnostics).
Bacterial aetiology were identified by positive blood culture or positive urinary antigen for *Streptococcus pneumoniae* (Alere BinaxNOW®). Atypical pathogens were identified by multiplex PCR for 33 respiratory pathogens (FastTrack Diagnostics).
Tuberculosis was diagnosed by smear microscopy, culture, or GeneXpert.
Venn diagram was generated using eulerAPE version 3.

| Table 5.5 Specific bacteria & virus combinations in adults with hospitalised LRTI (N=68) | | |
|---|---|---|
| **Bacterial organism** | **Virus(es)** | **N** |
| E. *cloacae* | Coronavirus 229E | 1 |
| *Salmonella* species | Coronavirus NL63 | 1 |
| S. *pneumoniae* (n=26) | Adenovirus | 3 |
| | Coronavirus 43 | 1 |
| | Coronavirus 229 | 1 |
| | hMPV | 3 |
| | Influenza | 6 |
| | Influenza + adenovirus | 1 |
| | Influenza + hMPV | 1 |
| | Influenza + bocavirus + coronavirus 229 | 1 |
| | Parainfluenza virus 1 | 1 |
| | Parainfluenza virus 2 | 1 |
| | Parainfluenza virus 2 + rhinovirus | 1 |
| | Parainfluenza virus 3 | 2 |
| | Parainfluenza virus 4 | 1 |
| | Rhinovirus | 2 |
| | RSV | 1 |
| S. *aureus* | Influenza | 2 |
| B. *pertussis* | Enterovirus | 1 |
| | Rhinovirus | 1 |
| *Legionella* species (n=22) | Adenovirus | 4 |
| | Adenovirus + coronavirus OC43 | 1 |
| | Adenovirus + hMPV | 1 |
| | Bocavirus | 1 |
| | Coronavirus OC43 | 1 |
| | Coronavirus OC43 + rhinovirus | 1 |
| | Coronavirus OC43 + parainfluenza virus2 + RSV | 1 |
| | Enterovirus | 1 |
| | Enterovirus + rhinovirus | 1 |
| | Influenza | 5 |
| | Influenza + RSV | 1 |
| | Parainfluenza virus 1 | 1 |
| | Parainfluenza virus 2 | 1 |
| | Parainfluenza virus 3 | 1 |
| | Parainfluenza virus 4 | 1 |
| M. *pneumoniae* | Parainfluenza virus 2 | 1 |
| *Salmonella typhi* | Rhinovirus | 1 |
| | RSV | 1 |
| S. *pneumoniae* + B. *pertussis* | Rhinovirus | 1 |
| S. *pneumoniae* + *Legionella* species | Adenovirus | 1 |
| | Coronavirus OC43 | 1 |
| | Coronavirus 229E | 1 |
| | Coronavirus HKU1 | 1 |
| | Rhinovirus | 1 |
| | RSV | 1 |
| *Legionella* species + M. *pneumoniae* | Bocavirus | 1 |
| hMPV, human metapneumovirus; RSV, respiratory syncytial virus | | |

| **Table 5.6 Specific TB and virus combinations in adults with hospitalised LRTI (N=19)** | |
|---|---|
| **Virus(es)** | **N** |
| Adenovirus | 6 |
| Adenovirus + coronavirus 229E | 1 |
| Bocavirus + RSV | 1 |
| Coronavirus OC43 | 1 |
| Coronavirus NL63 + rhinovirus | 1 |
| Coronavirus 229E | 2 |
| hMPV | 1 |
| hMPV + parainfluenza virus 3 | 1 |
| Influenza | 3 |
| Influenza + parainfluenza virus 3 + rhinovirus | 1 |
| Rhinovirus | 1 |
| hMPV, human metapneumovirus; RSV, respiratory syncytial virus | |

| **Table 5.7 Specific TB and bacteria combinations in adults with hospitalised LRTI (N=18)** | |
|---|---|
| **Bacterial organism** | **N** |
| Legionella species | 8 |
| M. *pneumoniae* | 1 |
| S. *pneumoniae* | 6 |
| *Legionella* species + S. *pneumoniae* | 3 |

| **Table 5 8 Specific TB, bacteria and virus combinations in adults with hospitalised LRTI (N=7)** | | |
|---|---|---|
| **Bacterial organism** | **Virus(es)** | **N** |
| *Burkholderia cepacia* | Coronavirus 229E | 1 |
| Chlamydia pneumoniae | Adenovirus | 1 |
| Legionella species | Parainfluenza virus 3 | 1 |
| Salmonella species | Adenovirus + parainfluenza virus 2 + parainfluenza virus 4 | 1 |
| S. *pneumoniae* | Adenovirus<br>Rhinovirus | 1<br>1 |
| *Legionella* species + *Mycoplasma pneumoniae* | Adenovirus | 1 |

#### *5.3.3.2 Outcome*

The median duration of hospital stay was 6 days (range 1-75 days). In terms of clinical outcome[viii]: 72 (13.9%) patients required admission to the high dependency unit, WHO-recommended anti-tuberculous chemotherapy was started in 104 (20.1%) patients; and 18 (3.5%) were treated for *Pneumocystis jirovecii* (PCP) with intravenous co-trimoxazole during their admission. 52 (10%) patients died, 46 (88.5%) of whom were HIV-reactive.

### 5.3.3 Clinical predictors of influenza among hospitalized LRTI patients

Factors that may predict a diagnosis of influenza among patients admitted with hospitalised LRTI were explored (Table 5.9). Univariable analysis revealed that being female (risk ratio (RR) 1.66, 95%CI 1.01-2.71, p=0.045), and symptom duration less than 7 days (RR 1.81, 95%CI 1.11-2.95) was associated with an influenza diagnosis. Conversely, those that had a laboratory-confirmed diagnosis of tuberculosis were less likely to have influenza. No particular symptoms or signs of clinical instability were associated with influenza. Additionally, influenza was not associated with inpatient mortality. On multivariable analysis, the strong negative association with tuberculosis persisted (RR 0.33, 95%CI 0.12-0.89, p=0.01), as did a weak association with shorter symptom duration (<7 days vs. 7-14 days, RR 1.62, 95%CI 0.99-2.65, p=0.06).

[viii]Number of patients with missing values: HDU status (n=41), inpatient TB treatment (n=18), inpatient PCP treatment (n=21), and inpatient mortality (n=12).

**Table 5.9 Predictors of laboratory-confirmed influenza in adults with hospitalised LRTI, May 2013-February 2015 (N=518)**

| Characteristic | | N (%) Influenza PCR+ (N=56) | Univariable[a] | | Multivariable[a,c] | |
|---|---|---|---|---|---|---|
| | | | RR (95% CI) | p-value | RR (95% CI) | p-value |
| **Demographic characteristics** | | | | | | |
| Gender | Male | 28/323 (8.7) | 1 | | | |
| | Female | 28/195 (14.4) | 1.66 (1.01-2.71) | 0.045 | | |
| Age group (years) | 18-29 | 17/138 (12.3) | 1.11 (0.59-2.09) | 0.73 | | |
| | 30-39 | 22/227 (9.7) | 0.87 (0.48-1.59) | | | |
| | ≥40 | 17/153 (11.1) | 1 | | | |
| **Underlying medical conditions** | | | | | | |
| HIV status | Negative | 17/121 (14.1) | 1 | | | |
| | Positive | 39/396 (9.9) | 0.70 (0.41-1.19) | 0.21 | | |
| Previous history of tuberculosis | No | 46/424 (10.9) | 1 | | | |
| | Yes | 10/90 (11.1) | 1.02 (0.54-1.95) | 0.94 | | |
| Pneumonia past 5 years | No | 39/399 (9.5) | 1 | | | |
| | Yes | 17/11 (15.0) | 1.58 (0.92-2.69) | 0.11 | | |
| Malnutrition (BMI <18.5) | No | 41/348 (11.8) | 1 | | | |
| | Yes | 12/147 (8.1) | 0.69 (0.38-1.28) | 0.22 | | |
| **Season** | Jan-Mar | 16/99 (16.2) | 1.96 (0.99-3.91) | | | |
| | Apr-Jun | 15/100 (15.0) | 1.82 (0.91-3.67) | | | |
| | Jul-Sep | 12/161 (7.5) | 0.91 (0.43-1.92) | | | |
| | Oct-Dec | 13/158 (8.2) | 1 | 0.06 | | |
| **Clinical presentation**[b] | | | | | | |
| Symptom duration (days) | <7 | 26/166 (15.7) | 1.81 (1.11-2.95) | 0.02 | 1.62 (0.99-2.65) | |
| | 7-14 | 30/346 (8.7) | 1 | | 1 | 0.06 |
| Symptoms | | | | | | |
| Cough | No | 0/7 (0) | | | | |
| | Yes | 56/506 (11.1) | - | - | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sputum | No | 7/107 (6.5) | 1 | | | |
| | Yes | 49/406 (12.1) | 1.84 (0.86-3.96) | 0.12 | | |
| Shortness of breath | No | 4/21 (19.1) | 1 | | | |
| | Yes | 52/482 (10.6) | 0.55 (0.22-1.39) | 0.21 | | |
| Chest pain | No | 1/11 (9.1) | 1 | | | |
| | Yes | 55/501 (11.0) | 1.21 (0.18-7.96) | 0.85 | | |
| Myalgia | No | 8/108 (7.4) | 1 | | | |
| | Yes | 48/405 (11.9) | 1.6 (0.78-3.28) | 0.20 | | |
| Headache | No | 28/280 (10.0) | 1 | | | |
| | Yes | 28/233 (12.0) | 1.20 (0.73-1.97) | 0.47 | | |
| Rhinorrhoea | No | 42/430 (9.8) | 1 | | | |
| | Yes | 14/83 (16.9) | 1.73 (0.99-3.02) | 0.07 | | |
| Sore throat | No | 38/391 (9.7) | 1 | | | |
| | Yes | 18/122 (14.8) | 1.52 (0.90.-2.56) | 0.12 | | |
| **Co-infections** | | | | | | |
| ≥ 1 other respiratory virus | No | 41/384 (10.7) | 1 | | | |
| | Yes | 15/134 (11.1) | 1.05 (0.60-1.83) | 0.87 | | |
| Bacteria | No | 35/308 (11.4) | 1 | | | |
| | Yes | 21/210 (10.0) | 0.88 (0.53-1.47) | 0.62 | | |
| Tuberculosis | No | 43/297 (14.5) | 1 | | 1 | |
| | Yes | 4/92 (4.4) | 0.30 (0.11-0.81) | | 0.33 (0.12-0.89) | |
| | Don't know[c] | 9/129 (7.0) | 0.48 (0.24-0.96) | 0.004 | 0.51 (0.25-1.01) | 0.01 |
| S. *pneumoniae* carriage | No | 20/225 (8.9) | 1 | | | |
| | Yes | 36/379 (12.9) | 1.45 (0.87-2.44) | 0.15 | | |
| **Outcome** | | | | | | |
| Died during inpatient stay | No | 53/454 (11.7) | 1 | | | |
| | Yes | 3/52 (5.8) | 0.49 (0.16-1.53) | 0.22 | | |

RR, risk ratio; CI, confidence interval; BMI, body mass index

[a]Log binomial regression

[b]No associations were demonstrated between physiological parameters (Temperature <35 or >38°c, systolic BP<90mmHg, diastolic BP<0mmHg, heart rate >120 or <40/minute, respiratory rate ≥30/min, oxygen saturation <90%, and disorientation) and influenza positivity.

[c] No sputum submitted.; [d]Adjusted for symptom duration at presentation and current tuberculosis.

### 5.3.4 Risk factors for severe influenza presentation

A total of 56 cases and 88 controls were enrolled (Section 5.3.2). The demographic characteristics of cases and controls are shown in Table 5.10. Influenza A virus was identified in 30 (53.6%) cases and 35 (39.8%) controls; all were subtype A(H3N2) except one control (unsubtyped). Of note, both influenza A(H3) and B were identified in two controls. No difference was observed in age and sex. Thirty-nine (69.6%) cases and 26 (29.6%) controls were HIV-reactive (p<0.001). HIV-infected cases had more advanced immunosuppression (median CD4+ count 140 vs. 265 cells/µl, p=0.03) and were more likely to be on ART treatment at enrolment (35.7 vs. 9.1%, p<0.001) compared to HIV-infected controls. Cases were more likely to be underweight and a higher proportion of cases reported previous tuberculosis and pneumonia within the past 5 years.

Cases and controls did not differ in terms of household exposure to children <5 years or crowding. However, cases were more likely to be exposed to children who had received pneumococcal conjugate vaccine. Controls had better sanitation, level of education, and food security.

In the univariable analysis (Table 5.11), HIV infection, reported history of tuberculosis, pneumonia within past 5 years, being underweight, month of recruitment, type and distance from water supply, sanitation facility, lower education level, and food insecurity were all associated with being a case. Two multivariable models were constructed; model A included influenza regardless of type/subtype, whereas model B incorporated the type of influenza as a covariate. Month of recruitment was deemed an *a priori* confounder, due to the seasonal discrepancy in the recruitment of hospitalised LRTI and ILI patients (Figure 5.5). In multivariable model A, HIV infection was strongly associated with severe influenza presentation (adjusted odds ratio (aOR) 4.98, 95%CI 2.09-11.88, p<0.001). Additionally, those that reported pneumonia within the past 5 years (aOR 6.49, 95%CI 2.00-21.07, p<0.001), those with poor sanitation facility, i.e. having no toilet or a non-ventilated improved pit (VIP) latrine toilet (aOR 3.14, 95%CI 1.25-7.84, p=0.01), and frequent difficulty accessing food (aOR 20.85, 95%CI 1.97-221.16, p=0.01) were also at increased risk of severe influenza.

Significant risk factors remained the same in multivariable model B as Model A (Table 5.11). However, in this model, cases were over 5 times more likely to have influenza type A compared to B (aOR 5.33, 95%CI 1.88-15.10, p<0.001).

Possible interaction between HIV infection and food security was explored. The effect of HIV on the risk of severe influenza was similar in those who report no food insecurity (OR 6.03, 95%CI 2.23-16.32), and those who report food insecurity “sometimes, often or always” (OR 4.93, 95%CI 1.62-14.99). No effect modification was demonstrated (p=0.58).

**Table 5.10 Description of influenza-positive cases and controls**

| Characteristic | Cases n=56 | Controls n=88 | p-value[a] |
|---|---|---|---|
| **Influenza type** | | | |
| Influenza A PCR positive | 30 (53.6) | 35 (38.9) | |
| Influenza B PCR positive | 26 (46.4) | 55 (61.1) | |
| **Month of recruitment** | | | |
| Jan-Mar | 16 (28.6) | 49 (55.7) | |
| Apr-Jun | 15 (26.8) | 18 (20.5) | |
| Jul-Sep | 12 (21.4) | 9 (10.2) | |
| Oct-Dec | 13 (23.2) | 12 (13.6) | 0.01 |
| **Gender** | | | |
| Male | 28 (50.0) | 43 (48.9) | 0.89 |
| **Age, years (median, IQR)** | 35.9 (28.1-43.4) | 31.9 (26.4-42.2) | 0.13 |
| **Age group, years** | | | |
| 18-29 | 17 (30.4) | 36 (40.9) | |
| 30-39 | 22 (39.3) | 25 (28.4) | |
| ≥ 40 | 17 (30.4) | 27 (30.7) | 0.32 |
| **HIV status** | | | |
| HIV-reactive | 39 (69.6) | 26 (29.6) | <0.001 |
| CD4+ count (cells/µl) -median, IQR | 140 (87-268) | 265 (161-341) | 0.03 |
| On ART at enrolment | 20/39 (51.3) | 8/26 (30.8) | 0.10 |
| **Medical history** | | | |
| Previous history of tuberculosis | 10 (17.9) | 5 (5.7) | 0.01 |
| Pneumonia in past 5 years | 17 (30.9) | 7 (8.0) | <0.001 |
| Pregnant | 0/28 (0) | 1/45 (2.2) | 1.00 |
| BMI <18.5 (kg/m$^2$) | 12 (22.6) | 9 (10.2) | 0.05 |
| Smoking (current) | 5 (8.9) | 7 (8.0) | 0.59 |
| Alcohol | 8 (14.3) | 8 (9.1) | 0.33 |
| **Household and socioeconomic factors** | | | |
| Children <5 years in household | | | |
| 0 | 31 (55.4) | 55 (62.5) | |
| 1 | 19 (33.9) | 22 (25.0) | |
| ≥2 | 6 (10.7) | 11 (12.5) | 0.51 |
| Crowding index[b] | | | |
| <1.5 | 16 (36.4) | 18 (26.5) | |
| 1.5-2.4 | 20 (45.5) | 31 (45.6) | |
| ≥2.5 | 8 (18.2) | 19 (27.9) | 0.83 |
| Water supply | | | |
| River/borehole/well | 15 (26.8) | 19 (21.6) | |
| Public tap/standpipe | 32 (57.1) | 40 (45.5) | |
| Piped to dwelling/plot | 9 (16.1) | 29 (33.0) | 0.08 |
| Distance to water supply | | | |
| <200 metres | 15 (26.8) | 37 (42.0) | |
| ≥200 metres | 41 (73.2) | 41 (58.0) | 0.06 |
| Sanitation facility | | | |
| None/non-VIP toilet | 34 (60.7) | 37 (42.1) | |
| VIP/flush toilet | 22 (39.3) | 51 (57.9) | 0.03 |
| Principal cooking fuel | | | |
| Firewood | 14 (25.0) | 14 (15.9) | |

| | | | |
|---|---|---|---|
| Charcoal | 35 (66.1) | 55 (62.5) | |
| Other | 5 (8.9) | 19 (21.6) | 0.62 |
| Highest level of education | | | |
| Never attended school | 5 (9.1) | 15 (17.1) | |
| Primary | 34 (61.8) | 28 (31.8) | |
| Secondary/Tertiary | 16 (29.1) | 45 (51.1) | 0.002 |
| Employment | | | |
| Unemployed | 5 (8.9) | 10 (11.4) | 0.64 |
| Asset ownership[c] | | | |
| 0-1 | 22 (39.3) | 29 (33.0) | |
| 2-3 | 29 (51.8) | 47 (53.4) | |
| 4-5 | 5 (8.9) | 12 (13.6) | 0.59 |
| Difficulty accessing food | | | |
| Never | 28 (50.0) | 58 (65.9) | |
| Sometimes | 21 (37.5) | 29 (33.0) | |
| Often/always | 7 (12.5) | 1 (1.1) | 0.008 |

IQR, interquartile range; BMI, body mass index, PCV, pneumococcal conjugate vaccine; VIP, ventilated improved pit latrine

[a]Chi-square or Fisher's exact test

[b]Total number of household members divided by number of rooms in house (excluding kitchen and bathroom)

[c]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.

**Table 5.11 Risk factors for severe influenza presentation in influenza PCR positive cases and controls, May 2013-February 2015**

| Characteristic | Cases[a] n=56 | Controls[b] n=88 | Univariable[c] OR (95% CI) | p-value | Multivariable A [c,d] OR (95% CI) | p-value | Multivariable B[c,e] OR (95% CI) | p-value |
|---|---|---|---|---|---|---|---|---|
| **Gender** | | | | | | | | |
| Male | 28 (50.0) | 43 (48.9) | 0.96 (0.49=1.88) | 0.89 | | | | |
| **Age group (years)** | | | | | | | | |
| 18-29 | 17 (30.4) | 36 (40.9) | 0.75 (0.32-1.73) | | | | | |
| 30-39 | 22 (39.3) | 25 (28.4) | 1.40 (0.61- 3.22) | | | | | |
| ≥40 | 17 (30.4) | 27 (30.7) | 1 | 0.32 | | | | |
| **Month of recruitment** | | | | | | | | |
| Jan-Mar | 16 (28.6) | 49 (55.7) | 1 | | 1 | | 1 | |
| Apr-Jun | 15 (26.8) | 18 (20.5) | 2.55 (1.05-5.20) | | 3.36 (1.13-9.95) | | 5.15 (1.58-16.81) | |
| Jul-Sep | 12 (21.4) | 9 (10.2) | 4.08 (1.45-11.46) | | 6.34 (1.69-23.80) | | 11.69 (2.54-53.8) | |
| Oct-Dec | 13 (23.2) | 12 (13.6) | 3.32 (1.25-8.72) | 0.01 | 3.60 (1.07-12.10) | 0.01 | 2.89 (0.82-10.12) | 0.003 |
| **HIV status** | | | | | | | | |
| HIV-reactive | 39 (69.6) | 26 (29.6) | 5.47 (2.63-11.36) | <0.001 | 4.98 (2.09-11.88) | <0.001 | 6.72 (2.56-17.66) | <0.001 |
| **Previous history of tuberculosis** | 10 (17.9) | 5 (5.7) | 3.61 (1.16-11.20) | 0.03 | | | | |
| **Pneumonia past 5 years** | 17 (30.9) | 7 (8.0) | 5.18 (1.98-13.53) | 0.001 | 6.49 (2.00-21.07) | 0.001 | 6.43 (1.95-21.25) | 0.001 |
| **Malnutrition (BMI <18.5)** | 12 (22.6) | 9 (10.2) | 2.57 (1.00-6.60) | 0.05 | | | | |
| **Water supply** | | | | | | | | |
| River/borehole/well | 15 (26.8) | 19 (21.6) | 2.54 (0.93-6.98) | 0.07 | | | | |
| Public tap/standpipe | 32 (57.1) | 40 (45.5) | 2.58 (1.07-6.22) | | | | | |
| Piped to dwelling/plot | 9 (16.1) | 29 (33.0) | 1 | | | | | |
| **Distance from water supply** | | | | | | | | |
| <200 metres | 15 (26.8) | 37 (42.1) | 1 | | | | | |
| ≥200 metres | 41 (73.1) | 51 (57.9) | 1.98 (0.96-4.10) | 0.07 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sanitation facility** | | | | | | | | |
| None/non-VIP toilet | 34 (60.7) | 37 (42.1) | 2.13 (1.08-4.22) | 0.03 | 3.14 (1.25-7.84) | 0.01 | 3.64 (1.38-9.55) | 0.006 |
| VIP/ flush toilet | 22 (39.3) | 51 (57.9) | 1 | | 1 | | 1 | |
| **Highest level of education** | | | | | | | | |
| None | 5 (9.1) | 15 (17.1) | 1 | | | | | |
| Primary | 34 (61.8) | 28 (31.8) | 3.64 (1.18-11.27) | | | | | |
| Secondary & above | 16 (29.1) | 45 (51.1) | 1.07 (0.33-3.41) | 0.002 | | | | |
| **Difficulties obtaining food** | | | | | | | | |
| Never | 28 (50.0) | 58 (65.9) | 1 | | 1 | | 1 | |
| Sometimes | 21 (37.5) | 29 (33.0) | 1.50 (0.73-3.08) | | 1.15 (0.47-2.84) | | 1.26 (0.48-3.26) | |
| Often/always | 7 (12.5) | 1 (1.1) | 14.4 (1.70-123.65) | 0.007 | 20.85 (1.97-221.16) | 0.01 | 48.43 (3.72-631.34) | 0.002 |
| **Influenza type** | | | | | | | | |
| Influenza A virus | 30 (53.6) | 33 (38.4) | 1.85 (0.94-3.66) | 0.08 | | | 5.33 (1.88-15.10) | <0.001 |
| Influenza B virus | 26 (46.4) | 53 (61.6) | 1 | | | | 1 | |
| **Hosmer-Lemeshow test** | | | | | | 0.30 | | 0.92 |

OR, odds ratio; CI, confidence interval; BMI, body mass index; pneumococcal conjugate vaccine; VIP, ventilated improved pit latrine

[a]Influenza-PCR positive hospitalised LRTI

[b]Influenza-PCR positive ILI

[c]Unconditional logistic regression

[d]Adjusted for HIV status, history of pneumonia within 5 years, month of recruitment, sanitation facility, and food security

[e]Adjusted for HIV status, history of pneumonia within 5 years, month of recruitment, sanitation facility, food security, and influenza type

#### *5.3.4.1 Effect of CD4+ and HIV treatment status*

To explore the roles of CD4+ T-lymphocyte cell count and ART treatment status, a sub-analysis restricting to HIV-infected cases and controls was performed (Table 5.12). There was a suggestion that patients with a CD4+ cell count <200 cells/µl had increased severity of influenza, compared to those with CD4+ ≥200 cells/µl (OR 2.72, 95%CI 0.97-7.60, p=0.06), but the association was not statistically significant. No association was found between ART treatment status and influenza severity. Stratified analysis by duration of ART treatment was not possible due to the small numbers included.

**Table 5.12 The effect of CD4+ cell count and HIV treatment status on the risk of severe influenza presentation, restricted to HIV-infected cases and controls (N=65)**

| **Characteristic** | Cases[a] **n=39** | Controls[b] **n=26** | Univariable[c] **OR (95% CI)** | **p-value** |
|---|---|---|---|---|
| **CD4+ cell count (cells/µl)** | | | | |
| ≥200 | 16 (41.0) | 17 (58.9) | 1 | |
| <200 | 23 (59.0) | 9 (34.6) | 2.72 (0.97-7.60) | 0.06 |
| **Antiretroviral therapy** | | | | |
| No | 20 (51.3) | 18 (69.3) | 1 | |
| Yes | 19 (48.7) | 8 (30.8) | 2.14 (0.75-6.07) | 0.15 |

#### *5.3.4.2 Population attributable fraction*

The adjusted PAFs (aPAF) (Table 5.13) were computed using multivariable model A (Table 5.11). In our study population, the highest proportion of cases of hospitalized influenza can be attributed to HIV infection (aPAF 56.7%, 95%CI 42.3-67.4) and poor sanitation (aPAF 40.9%, 95%CI 20.5-56.0). In contrast, despite the observed strong effect, frequent difficulty accessing food only accounted for 12% (95%CI 10.6-13.6) of cases, due to the low prevalence in our population.

**Table 5.13 Population attributable fraction of modifiable risk factors for severe influenza**

| Characteristic | % exposed among cases | Adjusted OR (95% CI) | p-value | Adjusted PAF (95%CI) |
|---|---|---|---|---|
| **Being HIV-infected** | 69.6 | 4.98 (2.09-11.88) | <0.001 | 56.7% (42.3-67.4) |
| **Sanitation facility** | | | | |
| None/non-VIP toilet | 60.7 | 3.14 (1.25-7.84) | 0.01 | 40.9% (20.5-56.0) |
| VIP/ flush toilet | 39.3 | 1 | | - |
| **Difficulties obtaining food** | | | | |
| Never | 50.0 | 1 | | - |
| Sometimes | 37.5 | 1.15 (0.47-2.84) | | 6% (-40.9-38.0) |
| Often/always | 12.5 | 20.85 (1.97-221.16) | 0.01 | 12.0% (10.3-13.7) |

OR, odds ratio; CI, confidence interval; PAF, population attributable fraction

## 5.4 Discussion

Lower respiratory tract infections are the leading cause of adult hospital admission in sub-Saharan Africa (SSA) (Scott et al., 2012). Bacteria such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* (M*tb*) are key causative organisms in the region (Scott et al., 2000; Nyamande et al., 2007). However, the contribution of respiratory viruses, such as influenza, have not been well characterised (Ho, 2014). This prospective case control study confirms influenza as an important contributor to hospitalised lower respiratory tract infection in Malawian adults. Importantly, in the context of a high HIV prevalence setting, HIV-infected adults were five times as likely to have severe influenza than HIV-uninfected adults. Patients with advanced immunosuppression (CD4+ <200 cells/μl) are at particular risk. In fact, over 50% of hospitalised influenza cases were attributable to HIV infection. We also found that those who reported a history of pneumonia within five years, poor sanitation, frequent difficulty accessing food and who had infection with influenza A virus were at increased risk of severe influenza disease.

Whilst studies in low HIV prevalence settings have produced conflicting data regarding HIV as a risk factor for severe influenza disease, our findings corroborate with the handful of studies from SSA that have demonstrated increased severity of influenza presentation in HIV-infected persons (Chapter 1.4.3.3). A retrospective case control study in rural Kenya found a 3.5 times increased risk of influenza-associated hospitalisation in HIV-infected persons aged ≥5 years than HIV-uninfected individuals (Ope et al., 2011). The study district had a similar HIV prevalence to Blantyre, but the proportion of cases and controls that were HIV-infected were comparatively lower than ours (24.5 vs. 12.5%, respectively). This is likely due to their inclusion of older children (aged 5-17 years), which

comprised two-thirds of their study population, as well as their use of health individuals, rather than patients with ILI, as controls. When restricted to those aged ≥18 years (22/64 cases and 64/190 controls), the HIV prevalence among their cases and controls were more comparable to our study population (59 vs. 24%, corresponding to a crude odds ratio of 4.53). The study may have been particularly prone to recall bias, since cases were interviewed weeks to months after the illness event (Ope et al., 2011).

Cohen *et al.* (Cohen et al., 2013) also reported a 4 to 8-fold greater incidence of influenza-related hospitalisation among HIV-infected individuals enrolled in sentinel surveillance in South Africa. HIV-infected individuals also had more prolonged hospitalisation and higher mortality. However, no difference in case fatality ratio (CFR) was demonstrated between those with CD4 cell count <200 or >200 cells/μl (Cohen et al., 2013). Both these previous studies were limited by substantial missing data with regards to HIV status, degree of immunosuppression and ART treatment status. Hence this case control study, conducted exclusively in adults, with well-defined cases and controls, near complete ascertainment of HIV status, CD4 cell count and ART use, and estimates of PAF, provides the most robust data to date that HIV is a key risk factor for severe influenza presentation in a high HIV prevalence setting. Moreover, patients with CD4 count <200 cells/μl had an almost 3-fold greater risk of severe presentation compared to those with CD4+ >200 cells/μl, which has not been previously described.

We did not find any association between ART treatment status and influenza severity, even though most of the HIV-infected patients reportedly on ART had been taking it for over 12 months. In industrialised settings, the introduction of ART coincided with a dramatic decline in influenza-attributable hospitalisations (Neuzil et al., 2003), and was associated with improved survival following pandemic influenza A(H1N1) infection (Ormsby et al., 2011). However, similar to our finding, this beneficial effect has not been reported in high HIV prevalence settings. Cohen *et al.* (Cohen et al., 2015c) observed no difference in CFR among HIV-infected individuals with influenza-positive SARI who were or were not receiving ART. Jambo *et al*. (Jambo et al., 2012) also found poor reconstitution of influenza-specific CD4+ T-cell response in HIV-infected Malawian adults with Stage III/IV disease following 12 months of ART, despite a marked reduction in viral load and rise in CD4+ count (Jambo et al., 2012). These results suggest that ART may not protect HIV-infected participants from influenza-related complications. Alternatively, several years of ART may be required before sufficient pathogen-specific immune reconstitution is achieved. Thus the impact of ART on the relationship between HIV and influenza severity requires further evaluation, particularly in light of the recent revision of CD4+ threshold for initiating ART from 350 to 500 cells/μl in 2013 (World Health Organization, 2013a).

CD4+ T-cells are an essential component of the defence against influenza infection (Thomas et al., 2006), thus cell-mediated immune defects in the lungs in HIV-infected individuals may be a potential mechanism for increased severity of influenza virus. Jambo *et al*. (Jambo et al., 2011) found impaired pulmonary antigen-specific CD4+ T-cell response to influenza in asymptomatic HIV-infected Malawian adults. The authors also demonstrated a loss of influenza-specific proliferative CD4+ T-cell responses in patients with early, as well as advanced HIV disease (Jambo et al., 2012), implying that the loss of influenza-specific immunity occurs early in HIV infection prior to total CD4 T-cell depletion, rendering HIV-infected individuals vulnerable to influenza infection, but also to secondary bacterial infection such as pneumococcal pneumonia, particularly in an area of intense pneumococcal exposure such as Malawi (Everett et al., 2011).

Bacterial co-infections could have contributed to the severity of influenza presentation in the HIV-infected group. Epidemiological evidence and murine models suggest that influenza predisposes to invasive bacterial infections, particularly *Streptococcus pneumoniae* (McCullers and Rehg, 2002; McCullers, 2006; McNamee and Harmsen, 2006; Brundage, 2006). Among individuals with hospitalised LRTI in South Africa, both influenza and HIV infection were associated with increased blood pneumococcal load (Wolter et al., 2014a), as well as invasive pneumococcal pneumonia (Wolter et al., 2014b). Cohen *et al*. (Cohen et al., 2013) observed an increased risk of pneumococcal co-infection among HIV-infected individuals with influenza-associated LRTI. Although none of our influenza cases had documented pneumococcal bacteraemia, 11/46 (24%) had a positive pneumococcal urinary antigen test.

Aside from HIV infection, several other risk factors for severe influenza were identified (Figure 5.11). Firstly, patients who reported a pneumonia episode within the past 5 years had a greater risk of severe influenza. We believe previous history of severe respiratory infection partly mediates the effect of HIV on influenza severity (red arrow in Figure 5.9), since HIV is associated with increased risk of bacterial pneumonia (Sogaard et al., 2008). However, a past episode of severe respiratory infection could also act as a proxy for chronic lung disease, thus in itself an independent risk factor for severe influenza. Chronic lung disease is a known risk factor of severe outcomes due to influenza infection (Ope et al., 2011; Pebody et al., 2010; Van Kerkhove et al., 2011b), and has been identified as a priority group for vaccination in industrialised countries (England, 2013; Grohskopf et al., 2014). Previous pulmonary TB was associated with influenza severity in the univariable, but not the multivariable analysis. This was thought be to due to confounding by its association with HIV infection. Of note, we were unable to assess whether current TB infection was associated with influenza severity, as ILI patients were not investigated for TB. However, this was unlikely, as TB/influenza co-infection was only identified in 4 of 518 hospitalised LRTI patients (Table 5.9).

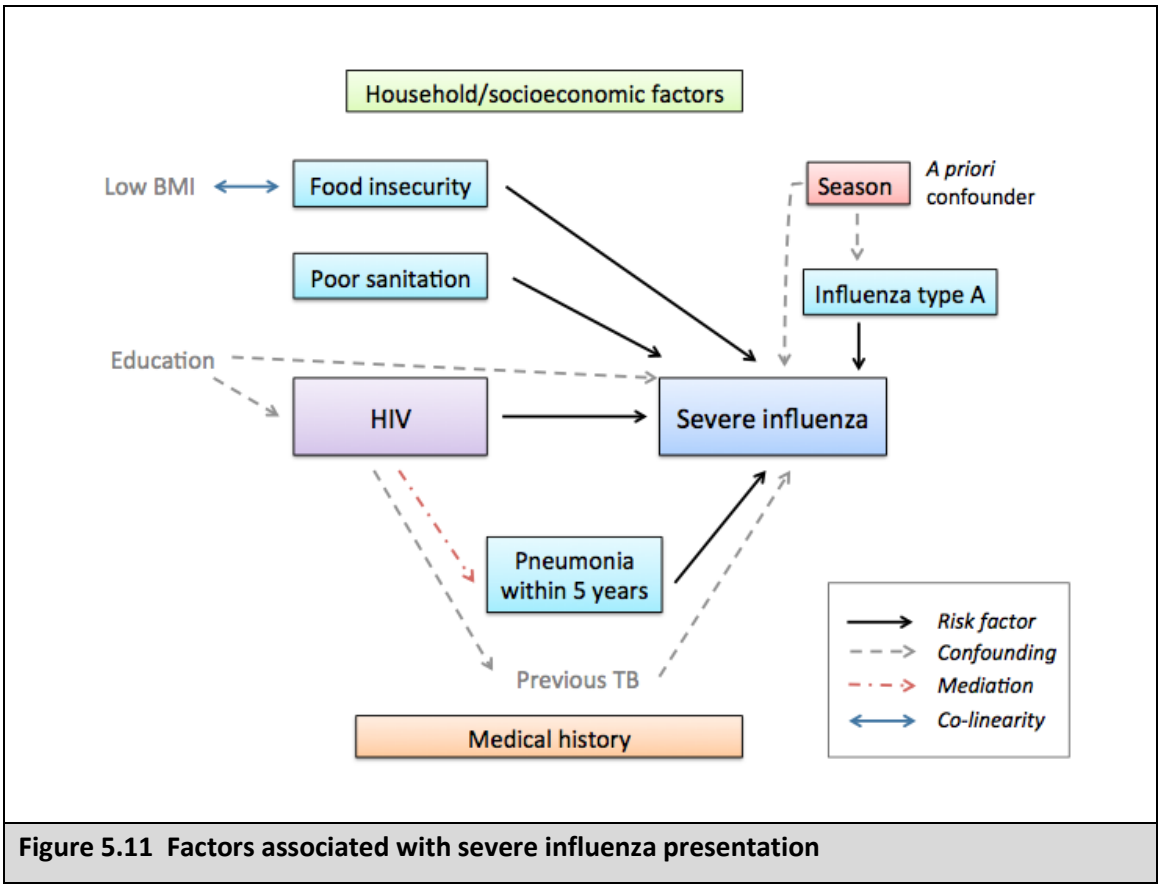



**Figure 5.11 Factors associated with severe influenza presentation**

Cases were also found to be associated with poor (or unimproved (World Health Organization and UNICEF, 2015)) sanitation and food insecurity. The link between poor sanitation and infection is well known (Mara et al., 2010). Although the effect estimate for poor sanitation was lower than that for the other risk factors (aOR 3.14), it emerged as the second most important risk factor for severe influenza in our analysis (PAF 40.9%), due to the high prevalence of cases with unimproved sanitation (61%). Food insecurity can lead to low weight; an association between malnutrition and increased severity of infections has been described in children (Jones and Berkley, 2014), though not in adults. In our study, those that reported frequent difficulty accessing food, but not those with low BMI, were at significantly greater risk of severe influenza presentation. Although the two variables are closely related, low weight is not only due to inadequate dietary intake, but also malabsorption and an increased metabolic rate in HIV-infected adults (Hommes et al., 1991; Melchior et al., 1991). Food insecurity remains strongly associated with severe influenza following adjustment for HIV status, suggesting it is an independent risk factor, and may be associated with underlying unmeasured socioeconomic factors. In contrast to the cohort study, we did not demonstrate any interaction between HIV and food security on the effect of influenza severity. However, as few patients reported 'often' or 'always' having difficulties obtaining food, interaction was evaluated

with food security as a binary variable. This would have limited our ability to assess for effect modification on the more severe end of the food insecurity spectrum.

Influenza A (all subtype H3N2) was associated with increased disease severity compared to influenza B. This may be partly due to the discrepant recruitment of hospitalised LRTI and ILI cases; influenza A(H3N2) was prevalent in the months where more hospitalised LRTI cases were recruited (see Figures 5.6 and 5.7). We attempted to minimise selection bias by controlling for month of recruitment. Influenza B virus is commonly perceived to cause milder infection than influenza A, though available literature have inconsistent findings. A large US viral surveillance study from 1976 to 1999 found that the highest influenza-related mortality rates occurred during A(H3N2)-predominant seasons (Thompson et al., 2003). Esposito *et al.* (Esposito et al., 2011) also reported more LRTI presentations, as well as higher rate and duration of hospitalisation in Italian children aged <15 years with A(H3N2) compared to A(H1N1) and B infections between 2007 and 2009. During the pandemic period, A(H1N1) was found to cause more severe disease than A(H3N2) and B in a population-based influenza hospitalization surveillance study in the US (Chaves et al., 2013). A multi-centre study from Korea reported increased rate of hospitalisation and duration of hospital stay in patients infected with influenza A(H3N2) compared to B in the post-pandemic period, but this was confounded by the higher proportion of elderly and patients in co-morbidities among those with A(H3N2) infection (Wie et al., 2013). Conversely, several studies found similar clinical characteristics and outcome among outpatients (Irving et al., 2012) and hospitalised patients (Su et al., 2014; Gutierrez-Pizarraya et al., 2012) with confirmed influenza A and B infections, though two of the three studies did not distinguish between A subtypes (Irving et al., 2012; Su et al., 2014). It is likely that the comparative severity of influenza A and B viruses in any given season will depend on the virulence of the circulating strain, as well as the degree of antigenic drift from the previous season, which in turn will affect the impact of population immunity.

One or more aetiological agents were identified in two-thirds of patients with hospitalised LRTI. Influenza was detected in 11% of adults enrolled with hospitalised LRTI at QECH, which is comparable to the proportions reported in pneumonia studies from developed settings (mean influenza positivity 7.5%, range 3.0-18.7%) (Angeles Marcos et al., 2006; Charles et al., 2008; Johnstone et al., 2008; Saito et al., 2012; Jennings et al., 2008; Diederen et al., 2009; Johansson et al., 2010; Templeton et al., 2005; Hohenthal et al., 2008; Lieberman et al., 2010; Lim et al., 2001; Lauderdale et al., 2005; Almirall et al., 2007; Ruiz et al., 1999). Other respiratory viruses were also prevalent. Overall, around one third of patients were positive for at least 1 respiratory virus (including influenza) and viral co-infection was found in 7.5% cases. The isolation of L*egionella* species in almost 20% of LRTI is surprising. The incidence of *Legionella* reported from several

pneumonia cohorts from South Africa has ranged from 1.7 to 8.7% (Potgieter and Hammond, 1992; Maartens et al., 1994; Mpe et al., 2001; Nyamande et al., 2007). Odera and Anzara (Odera and Anzala, 2009) found *Legionella pneumophila* in 9% of adult pneumonia patients in Nairobi, though a large proportion was associated with air conditioner exposure. Other pneumonia series in SSA have not identified any *Legionella* infection (Feldman et al., 1989; Scott et al., 2000; Hartung et al., 2011). In these studies, the presence of *Legionella* was evaluated using direct fluorescent antibody or urinary antigen. Although PCR on a throat swab has been shown to correlate with serology and urinary antigen results (Ramirez et al., 1996), the significance of identifying *Legionella* from the nasopharynx is unclear. It may represent the causative organism, but may also reflect asymptomatic carriage or environmental contamination. We plan to evaluate this further by performing urinary *Legionella* antigen test on stored specimens.

In contrast, the proportion of patients with proven pneumococcal pneumonia in our study (18%) was lower than that reported in other SSA pneumonia cohorts (25-46% (Koulla-Shiro et al., 1996; Scott et al., 2000; Yoshimine et al., 2001; Charalambous et al., 2003; Nyamande et al., 2007; Albrich et al., 2012)), most of whom had similarly high prevalence of HIV infection. This may be partly due to the fact that around 20% patients were unable to provide a urine specimen for the BinaxNOW antigen test. Additionally, only four patients had *S. pneumoniae* bacteraemia. The low blood culture yield may be attributable to the high prevalence of pre-hospital antibiotic utilisation (61%). This has prompted a 4$^{th}$ year medical student project to evaluate pre-hospital antibiotic utilisation in Blantyre (analysis pending). Lastly, M. *tuberculosis* was identified in a quarter of patients that submitted sputum. This reflects the high incidence of TB in Malawi (Bank, 2015), and is in keeping with studies from other high TB prevalence settings that have confirmed M*tb* as a key contributor to acute community-acquired pneumonia (Scott et al., 2000; Nyamande et al., 2007). Thus patients with respiratory symptoms should be screened for TB regardless of symptom duration.

The clinical manifestation of influenza-associated LRTI was indistinguishable from other causes of LRTI, rendering the rationalisation of antimicrobial therapy by clinical assessment difficult. Influenza-positive patients did have shorter symptom duration (<7 days), and were less likely to have TB co-infection. There have been concerns that influenza virus infection may play a role in the development of active TB disease either directly after exposure to M*tb* or through reactivation of latent M*tb* infection, particularly in TB-endemic countries (de Paus et al., 2013). However, consistent with our findings, most of the studies that have evaluated both influenza and tuberculosis have not found an association (Bradshaw et al., 2008; de Paus et al., 2013; Oei and Nishiura, 2012; Roth et al., 2013). The exception is Walaza *et al.* (Walaza et al., 2015), who reported an increased mortality among patients with influenza and TB co-infection in South Africa, compared to those with TB

infection alone. However, this only applied in patients with symptoms >7 days, and their sample size was small.

This study has a number of limitations. Firstly, recall bias could not be entirely discounted but was unlikely, since both cases and controls were ill at enrolment. Secondly, the main exposure of interest (HIV status) was objectively measured, and differential recall of other risk factors of interest (e.g. household exposure to young children, household and socioeconomic characteristics) between cases and controls was also unlikely.

Few eligible participants refused enrolment (2.8% hospitalized LRTI and 1.8% ILI patients), thus unlikely to have resulted in selection bias. However, Berkson's bias (Berkson, 1946) could have occurred if HIV-infected participants had an increased propensity to present to hospital with severe respiratory symptoms than HIV-negative individuals, and secondly, if the study clinician was more likely to admit an individual with acute respiratory illness that was HIV-infected. We tried to minimise the latter firstly by rigorous training of study staff to follow standard operating procedures and manage patients according to standardised hospital protocols (Sloan and Zijlstra, 2012). Secondly, the eligibility criteria for hospitalized LRTI were strict. Furthermore, the HIV status was unknown to the study clinician at the time of assessment for the majority of patients (300 (58%) hospitalized LRTI; 549 (86%) ILI).

RT-PCR tests for influenza are very sensitive and specific (Mahony, 2010). However, as the average duration of influenza virus shedding is 5 days (Carrat et al., 2008), LRTI patients who presented with longer symptom duration may have ceased shedding virus at the time of enrolment, thus erroneously categorised as influenza negative. If this misclassification occurred equally in HIV-infected and HIV-uninfected patients, the effect of HIV on severity would have been underestimated. However, if the likelihood of influenza detection given infection differed according to HIV status, for example, if influenza virus shedding occurred for longer duration in HIV-infected persons, this differential misclassification could have biased the effect estimate away from null. At present, the impact of HIV infection on the duration and magnitude of influenza virus shedding is unclear, but is under evaluation in ongoing studies in South Africa (Hellferscee et al., 2013) and Malawi (Chapter 1.4.4).

Although HIV was the primary exposure of interest, we assessed a number of additional risk factors for severe influenza. When evaluating multiple variables, it is possible that a significant association was discovered by chance, when a true association does not exist. We tried to mitigate this in two ways: firstly, all variables included in the analysis were carefully considered after consulting existing literature, as possible confounders of the association between HIV and influenza severity, or

potential risk factors for severe influenza. Secondly, since there were only 56 cases, we included a maximum of five variables in the multivariable analysis to avoid biased estimates and over-adjustment (Peduzzi et al., 1996). On the other hand, by limiting the number of variables we included in our model, residual confounding was possible. Moreover, the small number of cases and controls certain continuous variables, such as age group, required broad categorisation. This may have limited the ability to adequately adjust for age, therefore a small, linear association between age and influenza severity could have been missed.

Lastly, due to time constraints, recruitment was discontinued after 22 months in February 2015, and therefore did not take place over 2 full calendar years. Taking into account the seasonal patterns in influenza activity in Blantyre, this likely resulted in an underestimation of the contribution of influenza to both ILI and hospitalised LRTI, as March and April months typically have high influenza activity.

This study confirms a prominent aetiological role for influenza in mild and severe acute respiratory illness in Malawian adults. Since pneumonia is the commonest cause of adult medical admissions at QECH (SanJoaquin et al., 2013), constituting 1 in 6 primary diagnosis, effective preventative strategies against influenza could substantially reduce the burden of ARI in Malawi and other similar settings with overstretched health systems. Moreover, HIV infection increases the risk of severe influenza presentation; greater than 50% severe influenza cases is attributable to HIV infection. This finding is especially important in SSA, a region with 25 million people living with HIV (UNAIDS, 2013).

Several interventions may reduce influenza burden in HIV-infected persons. Firstly, ART has been shown to reduce influenza-related hospitalisations in the US (Neuzil et al., 2003), as well as influenza-related deaths in South Africa (Cohen et al., 2012b). Although we have not demonstrated an impact in our study, continued expansion of HIV testing and ART scale-up means an increasing number of HIV-infected individuals will have access to ART (Malawi Ministry of Health, 2014). Whether ART will have an impact on influenza hospitalisation in the long term needs assessment. Secondly, inactivated influenza vaccines (IIV) has been shown to be safe and effective in HIV-infected persons without severe immunosuppression, though no significant effects on other clinical outcomes, such as all-cause pneumonia, hospitalisation and mortality, have been observed (Remschmidt et al., 2014). The only randomized controlled trial that have evaluated efficacy of IIV in HIV-infected persons in SSA demonstrated a 76% reduction in laboratory-confirmed influenza, but the study excluded patients with co-morbidities and ART-naïve patients with a CD4 cell count <100 cells/μl (Madhi et al., 2011). However, IIV is not currently available in the public sector in Malawi, and most SSA countries (Palache et al., 2014; Duque et al., 2014).

The identification of poor sanitation and food insecurity as risk factors of severe influenza in adults highlight the importance of ongoing public health interventions to combat these poverty-related issues; both are targets of Millennium Development Goals (MDGs): Goal 1C – to halve the proportion of people who suffer from hunger, and Goal 7C – to halve the proportion of people without sustainable access to safe drinking water and basic sanitation, by 2015 (United Nations, 2015a). Whilst community hand-hygiene interventions have been shown to reduce respiratory illness in developed settings (Aiello et al., 2008), this is irrelevant while 60% of our study population have to travel over 200m from their dwelling to a water source, and almost half have unimproved sanitation facility.

Although we have identified HIV-infected adults as an important risk group, and effective vaccines exists, a number of unanswered questions remain. Firstly, the impact of the degree of immunosuppression and duration of antiretroviral treatment on influenza severity among HIV-infected individuals needs further evaluation as more effective ART regimens are available, and a growing number of HIV-infected individuals gain access to ART (World Health Organization, 2013a). Secondly, HIV-infected individuals with advanced immunosuppression were found to have the greatest risk of influenza severity. However, data on the safety, immunogenicity and efficacy of IIV in this group is lacking. Additional factors that require elucidation include the feasibility of administering influenza vaccine at ART clinics, acceptability of an annual vaccine to patients in SSA, genomic data to inform policymakers as to whether which vaccine recommendation (northern or southern hemisphere) to follow, and optimal timing of vaccine administration given the absence of clear seasonality.

In conclusion, we have demonstrated that influenza has an important aetiological role in severe respiratory presentations in Malawian adults. Additionally, in a high HIV prevalence setting, HIV infection is a major risk factor for severe influenza disease in Malawian adults, especially in those with a CD4+ cell count of <200 cells/μl. Further evaluation of the effect of degree of immunosuppression and ART treatment status on influenza severity are needed. Additionally, feasibility and effectiveness studies of IIV in HIV-infected adults with advanced immunosuppression or co-morbidities may better guide targeted immunisation polices in similar low resource and high HIV prevalence settings.

# CHAPTER 6. Association Between Respiratory Viral Infection and the Nasopharyngeal Carriage of *Streptococcus pneumoniae*: a Cross-sectional and Nested Case Control Analysis

## 6.1 Introduction

There is convincing epidemiological evidence, particularly from previous influenza pandemics, that a substantial proportion of influenza-related morbidity and mortality is due to secondary bacterial pneumonia, particularly with *Streptococcus pneumoniae* (Morens et al., 2008). Emerging data suggest that respiratory viruses other than influenza, such as rhinovirus, RSV, adenovirus and parainfluenza viruses, also predispose to pneumococcal pneumonia (Angeles Marcos et al., 2006; Jennings et al., 2008; Johnstone et al., 2008; Templeton et al., 2005; Luchsinger et al., 2013; Damasio et al., 2015). As detailed in Chapter 1.6, the pathogenic synergism between respiratory viruses and *S. pneumoniae* are supported by several lines of evidence, including epidemiological data from previous influenza pandemics (Morens et al., 2008; Brundage and Shanks, 2008; Fleming-Dutra et al., 2013); *in vitro* and animal models that have demonstrated a variety of pathogenic mechanisms through which viral infections may predispose to pneumococcal pneumonia (Peltola et al., 2005; Pittet et al., 2010; Nakamura et al., 2011); a high proportion of confirmed pneumococcal pneumonia with viral co-infection found in pneumonia aetiology studies (Lieberman et al., 1996; Ruiz et al., 1999; de Roux et al., 2006; Camps Serra et al., 2008; Jennings et al., 2008); in addition to vaccine probes studies that have shown reduced incidence of influenza A, PIV and hMPV in children who received PCV (Madhi and Klugman, 2004; Madhi et al., 2006). Pneumonia, therefore, commonly manifests as a polymicrobial disease.

Acquisition of nasopharyngeal carriage of a new pneumococcal serotype is the first step in the process that leads to invasive disease (Bogaert et al., 2004). Animal models and human studies suggest that respiratory viral infection may predispose to incident carriage of new pneumococcal serotypes, and enhance carriage density, with subsequent increased susceptibility to invasive infection (Albrich et al., 2012; Wolter et al., 2014b).

Several murine models have demonstrated that influenza A infection significantly increased the density of colonising pneumococci (Nakamura et al., 2011; Mina et al., 2013), as well as duration of carriage in the nasopharynx (Mina et al., 2013). A recent cohort study in Andean children

demonstrated increased acquisition new pneumococcal serotypes in ARI related to influenza and parainfluenza (Grijalva et al., 2014). Studies from Vietnam (Vu et al., 2011) and South Africa (Wolter et al., 2014b) have also found higher pneumococcal colonization density in individuals with respiratory viral co-infection. However, the latter studies were cross-sectional in design, thus were unable to assess the dynamic relationship between the onset of viral infection and nasopharyngeal pneumococcal carriage.

We therefore conducted two studies nested within the BASH-FLU prospective cohort (Chapter 4): i) a cross-sectional study to examine the relationship between influenza or other respiratory viral infections and nasopharyngeal carriage of S. *pneumoniae* at ILI presentation, and ii) a case control study to determine whether respiratory viral infections are associated with an increase in pneumococcal carriage density as well an increased risk of pneumococcal acquisition in Malawian adults.

## 6.2 Methods

### 6.2.1 Study design and population

The first part consists of a cross-sectional analysis of the association between laboratory-confirmed influenza infection and pneumococcal carriage among all influenza-like illness (ILI) episodes (n=348) of the BASH-FLU cohort participants that took place between April 2013 and March 2015 (Chapter 4.3.4). The second is a nested case control study within the prospective cohort. Figure 6.1 illustrates the method by which cases and controls were identified. Cases were cohort participants with an ILI episode that was PCR-positive for one or more respiratory viruses, and had an asymptomatic swab taken less than six weeks before the ILI episode. Controls were a random sample of participants with virus-negative ILI episodes, who also had an asymptomatic swab taken within six weeks of the ILI episode. Controls were frequency-matched by calendar month of ILI episode to cases. A cohort participant who serves as a control at one point may subsequently become a case; and a cohort member may be a case or control more than once. This study design was chosen as a time- and cost-efficient method of addressing the research question: viral aetiology of the ILI episodes had already been determined; performance of quantitative *lyt*A PCR was confined to cohort participants with a confirmed virus-positive ILI event, and a sample of those with virus-negative ILI; and data on exposures and risk factors were collected prior to the ILI episodes, therefore eliminating recall bias. Moreover, regular nasopharyngeal sampling throughout the study enabled a longitudinal assessment of the impact of viral infection on pneumococcal density. However, the compromise is

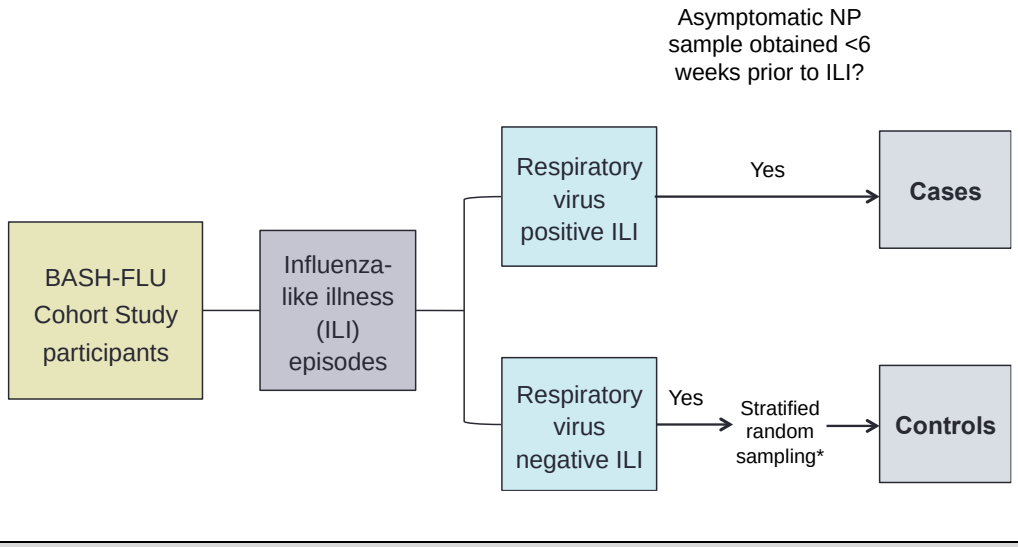



**Figure 6.1 Identification of cases and controls**

NP, nasopharyngeal; ILI, influenza-like illness

*Frequency-matched for calendar month of cases

the reduced precision in effect estimates that results from the clustering by individuals (see also Chapter 6.4).

#### *6.2.1.1 Definition of cases and controls*

**Cases**

Cohort study participant with a positive PCR for influenza A & B, adenovirus, bocavirus, coronaviruses HKU1, 229E, OC43 & NL63, enterovirus, human metapneumovirus (hMPV), parainfluenza virus 1-4, rhinovirus or respiratory syncytial virus (RSV) from an ILI episode **AND** asymptomatic when a swab taken <6 weeks prior to ILI episode.

**Controls**

Cohort study participants with a virus-negative ILI episode, frequency-matched to calendar month of cases **AND** asymptomatic when a swab taken <6 weeks prior to ILI episode.

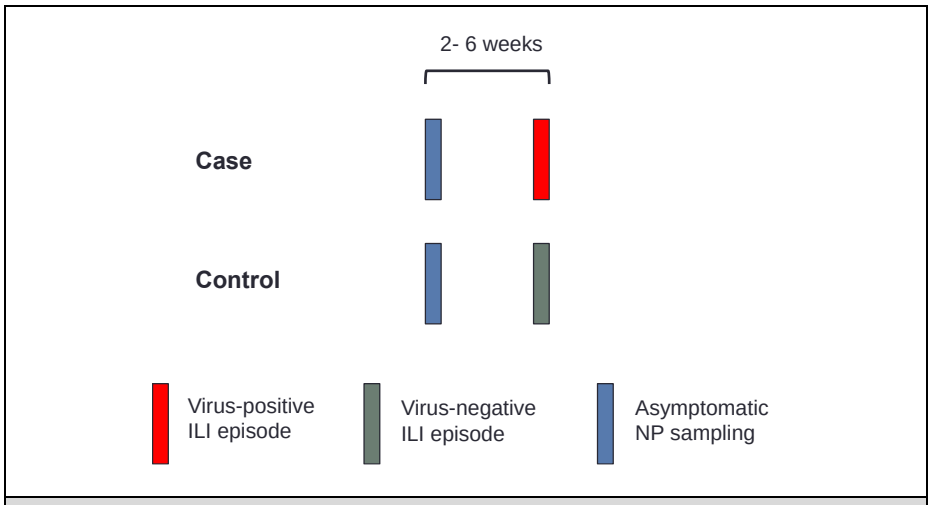



**Figure 6.2 Timing of asymptomatic and ILI NP samples in case control study**
ILI, influenza-like illness; NP, nasopharyngeal

## 6.2.2 Study procedures

At every routine visit and ILI presentation, combined nasopharyngeal and oropharyngeal swabs (FLOQswabs™, Copan Diagnostics, Brescia, Italy) were obtained and placed in Universal Transport Medium (UTM™, Copan Diagnostics, Brescia, Italy).

### *6.2.2.1 Detection of respiratory viruses*

For all ILI episodes, the CDC Human Influenza RT-PCR diagnostic panel (CDC Influenza Division), and the Fast-track Diagnostics® (FTD) Respiratory 33 kit (Fast-track Diagnostics Ltd., Luxembourg) were performed on combined nasopharyngeal and oropharyngeal specimens (Chapter 2.2.7). Respiratory viruses tested included influenza A & B, adenovirus, bocavirus, coronaviruses HKU1, 229E, OC43 & NL63, enterovirus, hMPV, parainfluenza virus 1-4, rhinovirus and RSV.

### *6.2.2.2 Detection and quantification of S. pneumoniae*

For all ILI episodes, nasopharyngeal pneumococcal colonisation was identified using the FTD Respiratory 33 kit. In the case control study, a *lyt*A quantitative PCR assay (Chapter 2.2.8) was also performed on the ILI episodes and preceding asymptomatic samples of cases and controls. Bacterial load (genomic copies/mL) was calculated from a standard curve using serially diluted DNA extracts with a known quantity of *S. pneumoniae*. *lyt*A PCR results was considered positive only if the *lyt*A gene was amplified (cycle threshold [$C_T$] <40).

### 6.2.3 Sample size

The cross-sectional study included all 348 ILI presentations in 208 cohort participants (130 HIV-infected). The case control study was exploratory, hence the number of cases comprised all virus-positive ILI episodes with a corresponding pre-ILI asymptomatic swab. Controls were frequency-matched to cases by calendar month of ILI episode, in a 1:1 ratio.

### 6.2.4 Statistical analysis

In the cross-sectional study, pneumococcal carriage was assessed as a binary outcome (S. *pneumoniae* PCR positive or negative from combined naso/oropharyngeal swabs). To account for the cohort participants' multiple ILI attendances in the longitudinal follow-up, we used a Generalised Estimating Equations (GEE) (Hanley et al., 2003) model with an exchangeable correlation structure to examine the association between influenza and pneumococcal carriage. GEE uses robust estimation of standard errors to account for the correlated observations.

In the case control study, a base 10 logarithmic transformation of pneumococcal bacterial load of S. *pneumoniae* was used to standardise and normalise the distribution. *Change in bacterial density* was defined as nasopharyngeal pneumococcal load at ILI episode minus pneumococcal load at the preceding asymptomatic visit. For the purposes of calculating changes in pneumococcal load, a sample with a negative lytA qPCR was assigned a value of 1 copy/ml. A *new colonisation episode* was defined as a positive S. *pneumoniae* lytA PCR at ILI episode after having none detected in the previous sample.

Virus-positive ILI was the main study exposure. Chi-square or Fisher's exact test was used to compare categorical variables, and median log-transformed bacterial loads were compared using Wilcoxon rank sum test. Multivariable GEE logistic regression models were implemented to identify factors associated with two primary end points: i) a 1-$\log_{10}$ (i.e. 10-fold) increase in nasopharyngeal pneumococcal density; and ii) a new nasopharyngeal pneumococcal colonization episode among virus-positive and virus-negative ILI cases. There was insufficient power to assess the effect of individual respiratory viruses on pneumococcal acquisition or increase in pneumococcal density.

Since no published study has evaluated the temporal change in pneumococcal carriage density in ILI, the decision to utilise a 1-1$\log_{10}$ increase in pneumococcal density as the model outcome was based on the effect estimates from the above-mentioned cross-sectional studies (Vu et al., 2011; Wolter et al., 2014b). As a sensitivity analysis, a lower magnitude of density increase (log2, i.e. doubling) was also investigated.

## 6.3 Results

### 6.3.1 The association between influenza and pneumococcal carriage at ILI visit

Of the 348 ILI episodes, 154 (44.5%) were FTD PCR positive for S.*pneumoniae* (see also Table 4.6). The univariable and multivariable analyses of factors associated with pneumococcal carriage during ILI are shown in Table 6.1. In the univariable analysis, influenza-positive ILI episodes were associated with twice the odds of concurrent pneumococcal carriage, compared to influenza-negative episodes (62.1 vs 42.9%, OR 2.06 [95%CI 0.95-4.47], p=0.07). Participants that have two or more young children (< 5 years) in the household (67.7 vs. 39.7%, OR 3.21 [95%CI 1.25-8.21]), compared to no children], unimproved water supply (OR 2.51 [95%CI 1.20-5.26]), and those with fewer assets (OR 2.72, 95%CI 1.24-5.95, 0-2 vs. 4-5 household assets owned) also had higher pneumococcal carriage. In contrast, age, sex, HIV status, past history of tuberculosis and pneumonia, recent antibiotic use, season of ILI attendance, household crowding, sanitation facility, principal cooking fuel, education, employment status, and presence of respiratory viruses other than influenza were not associated with pneumococcal colonisation**.**

In the multivariable analysis, the association between influenza infection and pneumococcal carriage remained borderline significant after adjusting for number of young children in household and water supply (aOR 2.03, 95%CI 0.93-4.46, p=0.08).

**Table 6.1 Factors associated with nasopharyngeal carriage of S. *pneumoniae* during ILI episodes (N=346)**

| Characteristic | N (%) S.*pneumoniae* PCR positive | Univariable[a] | | Multivariable[a,b] | |
|---|---|---|---|---|---|
| | | OR (95% CI) | p-value | OR (95%CI) | p-value |
| **Demographics** | | | | | |
| Sex | | | | | |
| Female | 96/213 (45.1) | 1 | | | |
| Male | 58/133 (43.6) | 1.02 (0.63-1.67) | 0.92 | | |
| Age group (years) | | | | | |
| 18-29 | 40/95 (42.1) | 1 | | | |
| 30-39 | 57/128 (44.5) | 1.10 (0.63-1.92) | | | |
| ≥ 40 | 57/123 (46.3) | 1.18 (0.67-2.08) | 0.85 | | |
| **Medical history** | | | | | |
| HIV status | | | | | |
| Negative | 50/118 (42.4) | 1 | | | |
| Positive | 104/228 (45.6) | 1.21 (0.74-1.96) | 0.45 | | |
| Previous pulmonary TB | | | | | |
| No | 129/298 (43.3) | 1 | | | |
| Yes | 25/48 (52.1) | 1.32 (0.69-2.55) | 0.40 | | |
| Pneumonia within 5 years | | | | | |
| No | 123/290 (42.4) | 1 | | | |
| Yes | 31/56 (56.4) | 1.66 (0.93-2.94) | 0.08 | | |
| Recent antibiotics (<2 weeks) | | | | | |
| No | 141/315 (44.8) | 1 | | | |
| Yes | 13/31 (41.9) | 0.94 (0.45-1.94) | 0.86 | | |
| **Season** | | | | | |
| Hot & rainy (Dec-Apr) | 58/126 (46.0) | 1.14 (0.62-2.08) | 0.91 | | |
| Hot & dry (Sept-Nov) | 62/140 (44.3) | 1.08 (0.62-1.88) | | | |
| Cool & dry (May-Aug) | 34/80 (42.5) | 1 | | | |
| **Household characteristics** | | | | | |
| Children <5 yrs in household | | | | | |
| 0 | 83/209 (39.7) | 1 | | 1 | |
| 1 | 50/106 (47.2) | 1.43 (0.86-2.36) | | 1.33 (0.80-2.22) | |
| ≥2 | 21/31 (67.7) | 3.21 (1.25-8.20) | 0.03 | 3.28 (1.27-8.47) | 0.04 |
| Crowding index[c] | | | | | |
| <1.5 | 33/84 (39.3) | 1 | | | |
| 1.5-2.4 | 61/130 (46.9) | 1.42 (0.80-2.52) | | | |
| 2.5 | 60/132 (45.5) | 1.37 (0.73-2.54) | 0.47 | | |
| Water supply | | | | | |
| River/stream/borehole | 41/70 (58.6) | 2.51 (1.20-5.26) | 0.03 | 2.27 (1.06-4.87) | 0.04 |
| Public tap/standpipe | 90/211 (42.7) | 1.31 (0.70-2.46) | | 1.16 (0.61-2.22) | |
| Piped to dwelling | 21/60 (35.0) | 1 | | 1 | |
| Distance from water supply | | | | | |
| <200 metres | 124/281 (44.1) | 1 | | | |
| ≥200 metres | 27/61 (44.3) | 0.99 (0.57-1.72) | 0.96 | | |
| Sanitation facility | | | | | |
| None/non-VIP toilet | 143/317 (45.1) | 1 | | | |
| VIP/flush toilet | 11/29 (37.9) | 0.75 (0.32-1.80) | 0.53 | | |
| Principal cooking fuel | | | | | |
| Electricity | 22/43 (34.4) | 1 | | | |
| Charcoal | 99/227 (43.6) | 1.51 (0.63-4.14) | | | |
| Firewood | 42/82 (51.2) | 2.18 (0.78-6.08) | 0.29 | | |
| **Socioeconomic status** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Highest level of education | | | | | |
| Never attended/primary | 61/132 (46.2) | 1 | | | |
| Secondary/tertiary | 93/214 (43.5) | 0.92 (0.58-1.46) | | | |
| Employment | | | | | |
| No | 24/47 (51.1) | 1 | | | |
| Yes | 130/299 (43.5) | 0.72 (0.40-1.28) | | | |
| Assets owned[d] | | | | | |
| 0-2 | 76/158 (48.1) | 2.72 (1.24-5.95) | 0.04 | | |
| 3 | 65/140 (46.4) | 2.43 (1.10-5.36) | | | |
| 4-5 | 13/48 (27.1) | 1 | | | |
| Difficulty obtaining food[e] | | | | | |
| Never | 61/135 (45.2) | 1 | | | |
| Sometimes | 57/129 (44.2) | 0.97 (0.58-1.63) | | | |
| Often/always | 36/82 (43.9) | 1.00 (0.54-1.85) | 0.99 | | |
| **Viral PCR results** | | | | | |
| Influenza infection | | | | | |
| No | 136/317 (42.9) | 1 | | 1 | |
| Yes | 18/29 (62.1) | 2.06 (0.95-4.47) | 0.07 | 2.03 (0.93-4.46) | 0.08 |
| Other respiratory viral infection[f] | | | | | |
| No | 102/232 (44.0) | 1 | | | |
| Yes | 52/114 (45.6) | 1.08 (0.68-1.70) | 0.74 | | |

PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval; TB, tuberculosis; VIP, ventilated improved pit latrine.

[a]Odds ratios estimated by population average model using generalised estimating equation (GEE).

[b]Adjusted for influenza PCR status, number of children < 5 years in household, and water supply.

[c]Total number of household members divided by the number of living rooms in household (kitchen or bathroom not included).

[d]Number of the following assets owned in household: working refrigerator, radio, mobile phone, bed and car/motorbike.

[e]Sometimes (1-2 times/month), often (up to once a week), always (every day)

fIncludes adenovirus, bocavirus, coronavirus OC43/NL63/229E/HKU1, enterovirus, human metapneumovirus, parainfluenza viruses 1-4, rhinovirus and respiratory syncytial virus.

### 6.3.2 The impact of respiratory viral infections on pneumococcal carriage density and acquisition

#### *6.3.2.1 Demographic and clinical characteristics of cases and controls*

143 of 348 (41.1%) ILI attendances by cohort participants had one or more respiratory viruses detected (Figure 6.3). Of those, 80 (55.9%) episodes had a corresponding pre-ILI NP specimen (taken <6 weeks before ILI episode). 80 controls were selected by stratified random sampling from the 210 virus-negative ILI episodes, frequency-matched for calendar month to the cases. Overall, this nested case control study included data from 131 participants, or 21.5% of the BASH-FLU prospective cohort (Chapter 4).

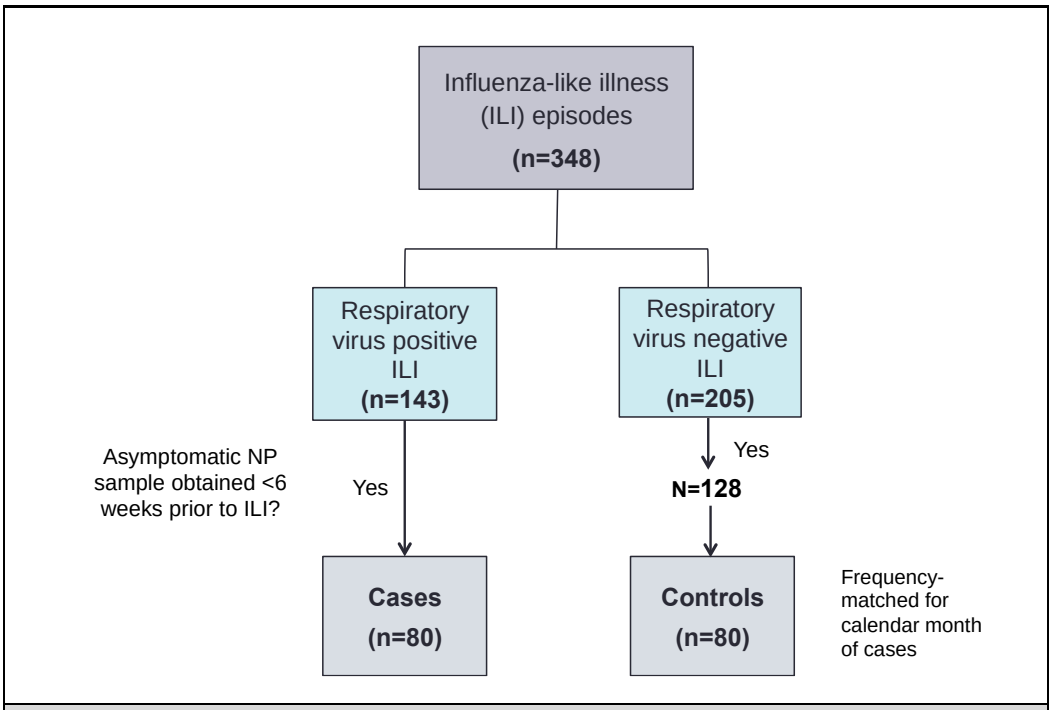



**Figure 6.3 Flow diagram of cases ad controls**

ILI, influenza-like illness; NP, nasopharyngeal

The demographic characteristics of the cases and controls are compared in Table 6.2. The 80 cases and 80 controls comprised 131 cohort participants. Twenty-two participants contributed more than 1 ILI episode as either a case or control; sixteen contributed two episodes (1 x case and 1 x control (n=10); 2 x case (n=3); 2 x control (n=3)), and six contributed three episodes (2 x case & 1 x control (n=5); 1 x case & 2 x control (n=1)). The minimum time interval between two included ILI episodes within the same participant was 1 month. Additionally, no ILI episode was included more than once. Cases and controls were evenly matched in terms of age, gender, HIV status, past medical history, household characteristics and antibiotic exposure prior to ILI event. The only significant difference

identified was in food security – cases were more likely to report difficulty in accessing food compared to controls.

**Table 6.2 Demographic and clinical characteristics of cases and controls**

| Characteristic[a] | Case (n=80) N (%) | Control (n=80) N (%) | p-value[b] |
|---|---|---|---|
| **Number of cohort participants[c]** | 71 | 76 | - |
| **Gender - male** | 28 (35.0) | 35 (43.8) | 0.26 |
| **Age (years) – median (IQR)** | 37.4 (31.3-48.0) | 36.7 (29.7-45.9) | 0.69 |
| **HIV status** | 56 (70.0) | 49 (62.0) | 0.29 |
| CD4 count (cells/μl) – median (IQR) | 411 (293-630) | 380 (255-564) | 0.49 |
| On ART | 38 (79.2) | 30 (65.0) | 0.13 |
| **Children <5 years in household (n)** | | | |
| 0 | 53 (66.3) | 48 (60.0) | |
| 1 | 20 (25.0) | 20 (25.0) | |
| ≥2 | 7 (8.8) | 12 (15.0) | 0.46 |
| **Crowding index** | | | |
| <1.5 | 21 (26.2) | 16 (20.0) | |
| 1.5-2.4 | 31 (38.8) | 31 (38.8) | |
| ≥2.5 | 28 (35.0) | 33 (41.2) | 0.58 |
| **Difficulty accessing food** | | | |
| Never | 22 (27.5) | 30 (37.5) | |
| Sometimes | 28 (35.0) | 35 (43.8) | |
| Often/always | 30 (37.5) | 15 (18.7) | 0.03 |
| **Antibiotics within 2 weeks of ILI episode** | 9 (11.2) | 11 (13.8) | 0.63 |
| **Interval between asymptomatic & ILI samples (days)** – median (IQR) | 20 (12-28) | 22.5 (12-28) | 0.43 |

IQR, interquartile range; ART, antiretroviral treatment; ILI, influenza-like illness

[a]No difference was observed between cases and controls in past medical conditions, including asthma, chronic lung disease, heart failure, chronic kidney disease, chronic liver disease, diabetes, pregnancy, previous history of tuberculosis or pneumonia, smoking, alcohol use, level of education, employment status, sanitation or water access and principal fuel use.

[b]Chi-square or Wilcoxon rank sum test

[c]16 participants contributed to both case and control ILI episodes.

To assess for possible bias, the demographic characteristics and viral aetiology of the 80 cases were compared to the 63 virus-positive ILI attendances that were excluded as cases due to the absence of a preceding asymptomatic specimen. Age, gender and the prevalence of HIV infection were similar between the two groups (Table 6.3). However, as a result of low number of routine reviews (and therefore fewer asymptomatic samples collected) between September and November 2013 (Chapter 4.3.2), proportionally fewer virus-positive ILI episodes were included as cases during that period (figure 6.4A). Consequently, viruses that were prevalent around that period were under-represented, including influenza A(H3N2) and RSV (figure 6.4B). Conversely, influenza B, coronavirus 229E, parainfluenza virus 3, and rhinovirus were over-represented.

**Table 6.3 A comparison of the demographic characteristics of virus-positive ILI that were included and excluded as cases in the analysis (N=143)**

| Characteristic | Case (n=80) N (%) | Excluded (n=63) N (%) | p-value[a] |
|---|---|---|---|
| **Gender - male** | 28 (35.0) | 22 (34.5) | 0.95 |
| **Age (years) – median (IQR)** | 37.4 (31.3-48.0) | 38.9 (28.3-47.9) | 0.43 |
| **HIV-positive** | 56 (70.0) | 44 (69.8) | 0.93 |
| CD4 count (cells/μl) – median (IQR) | 411 (293-630) | 416 (291-552) | 0.72 |

IQR, interquartile range; ILI, influenza-like illness [a]Chi-square or Wilcoxon rank sum test

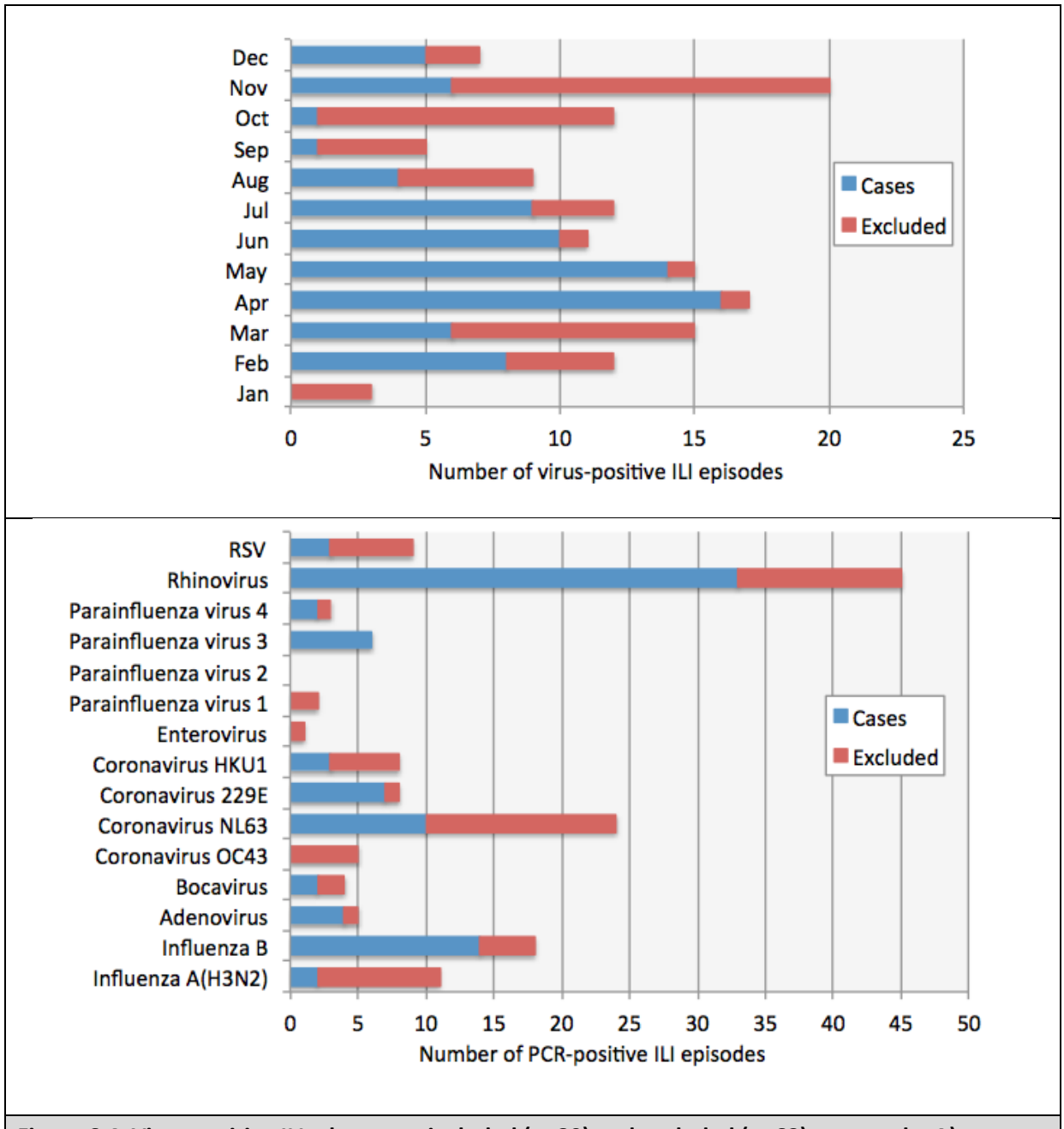



**Figure 6.4 Virus-positive ILIs that were included (n=80) and excluded (n=63) as cases by A) calendar month; and B) individual respiratory viruses.**

RSV, respiratory syncytial virus; PCR, polymerase chain reaction; ILI, influenza-like illness

#### *6.3.2.2 Comparison of nasopharyngeal density of Streptococcus pneumoniae between ILI episode and preceding asymptomatic visit*

The viral aetiology identified in the ILI episodes among cases is illustrated in Figure 6.5. Rhinovirus (n=33), influenza B (n=14) and coronavirus NL63 (n=10) were the most frequently detected viruses. 13/80 (16.2%) cases had viral co-infection.

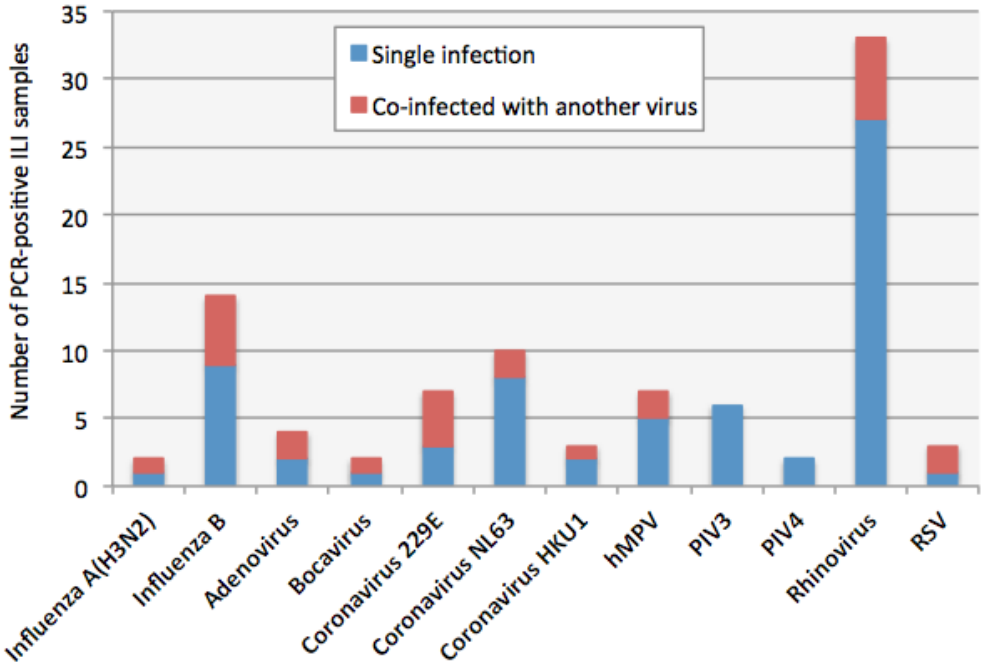



**Figure 6.5 Viral aetiology from ILI episodes of cases (N=80)**

hMPV, human metapneumovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Pneumococcal carriage at ILI time-point was determined by both the Fast-track Diagnostics® (FTD) multiplex kit and quantitative *lyt*A PCR; *lyt*A qPCR detected a much higher prevalence of nasopharyngeal carriage than the FTD PCR (74.4 vs. 46.3%, p <0.001), though FTD PCR was positive in 7 cases that were *lyt*A PCR negative (Inter-observer agreement between the two PCR tests, $\kappa$= 0.2885). Only *lyt*A PCR was performed on the pre-ILI samples. Therefore, for the purposes of the analysis of pneumococcal density change and acquisition, only *lyt*A PCR results were used.

Pneumococcal carriage prevalence by quantitative *lyt*A PCR did not differ between cases and controls, at both pre-ILI (60 vs. 60%, p=1.00) and ILI (73 v. 76%, p=0.59) time-points (Table 6.4). The median bacterial load among those that were *lyt*A PCR positive were also similar between cases and

controls at asymptomatic visit (4.10 vs. 4.21 $log_{10}$ copies/ml, respectively) and ILI visit (4.07 vs. 4.11 $log_{10}$ copies/ml).

**Table 6.4 Nasopharyngeal carriage of S. *pneumoniae* during ILI episodes and asymptomatic visits in cases and controls**

| Characteristic | Case (n=80) N (%) | Control (n=80) N (%) | p-value[1] |
|---|---|---|---|
| ***ILI episode*** | | | |
| FTD PCR positive | 39 (49) | 35 (44) | 0.53 |
| *Lyt*A qPCR positive | 59 (73) | 61 (76) | 0.59 |
| Median (IQR), $log_{10}$ copies/ml | 4.10 (3.66-5.05) | 4.21 (3.72-6.55) | 0.51 |
| ***Asymptomatic visit*** | | | |
| *Lyt*A qPCR positive | 48 (60) | 48 (60) | 1.00 |
| Median (IQR), $log_{10}$ copies/ml | 4.07 (1-4.86) | 4.11 (1-4.78) | 0.84 |
| ***Difference between ILI episode & asymptomatic visit*** | | | |
| Median Δ in *lyt*A qPCR (IQR), $log_{10}$ copies/ml | 0 (-0.72-1.81) | 0.05 (-0.20-2.40) | 0.12 |
| 1-$log_{10}$ increase in *lyt*A qPCR between ILI & asymptomatic episode | 23 (29) | 29 (36) | 0.31 |
| 1-$log_2$ increase in *lyt*A qPCR between ILI & asymptomatic episode | 31 (39) | 36 (45) | 0.42 |
| New colonisation episode[2] | 12 (15.0) | 15 (18.8) | 0.53 |

qPCR, quantitative polymerase chain reaction; IQR, interquartile range; ILI, influenza-like illness

[1] Chi-square or Wilcoxon rank sum test

[2] Spn negative at asymptomatic visit, but positive at ILI episode

The magnitude of change in NP pneumococcal density between the NP specimens taken at ILI episode and the preceding asymptomatic visit varied widely in both cases (-6.3 to +5.2 $log_{10}$ copies/ml) and controls (-7.4 to +5.3 $log_{10}$ copies/ml). However, the median difference in NP pneumococcal load was similar between cases and controls (0 vs. +0.05 $log_{10}$ copies/ml (p=0.25) respectively; Table 6.4 & figure 6.6A). There was also no difference when cases were stratified into influenza (median change 0 $log_{10}$ copies/ml, p=0.89) and other respiratory viruses (median change +0.12 $log_{10}$ copies/ml, p=0.17).

Additionally, we investigated whether the time interval between asymptomatic and illness sampling impacted on the magnitude of change in NP colonisation density (Figure 6.7). No correlation was demonstrated.

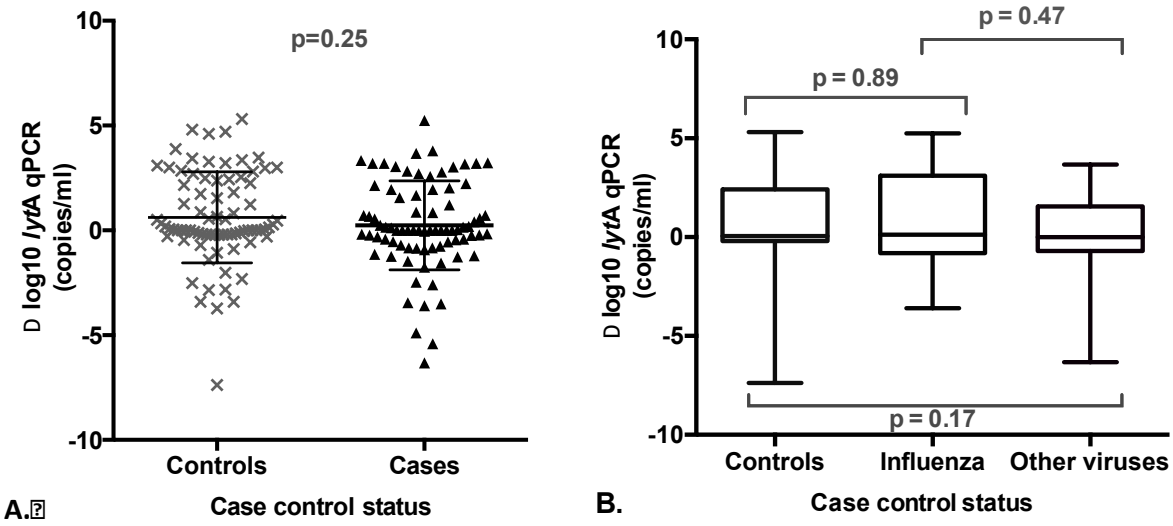



**Figure 6.6 Changes in quantitative pneumococcal colonisation densities between asymptomatic visit and ILI episode among A) controls (n=80) and cases (n=80); and B) controls and cases stratified by episodes in which influenza A & B (n=16) and other respiratory viruses (n=64) were identified.**

NP colonisation densities were obtained by *lyt*A real-time PCR from nasopharyngeal swab samples. Data are presented as medians, interquartile ranges (box) and minimum and maximum values (whiskers). P-values are calculated by Wilcoxon rank sum test.

qPCR, quantitative polymerase chain reaction.

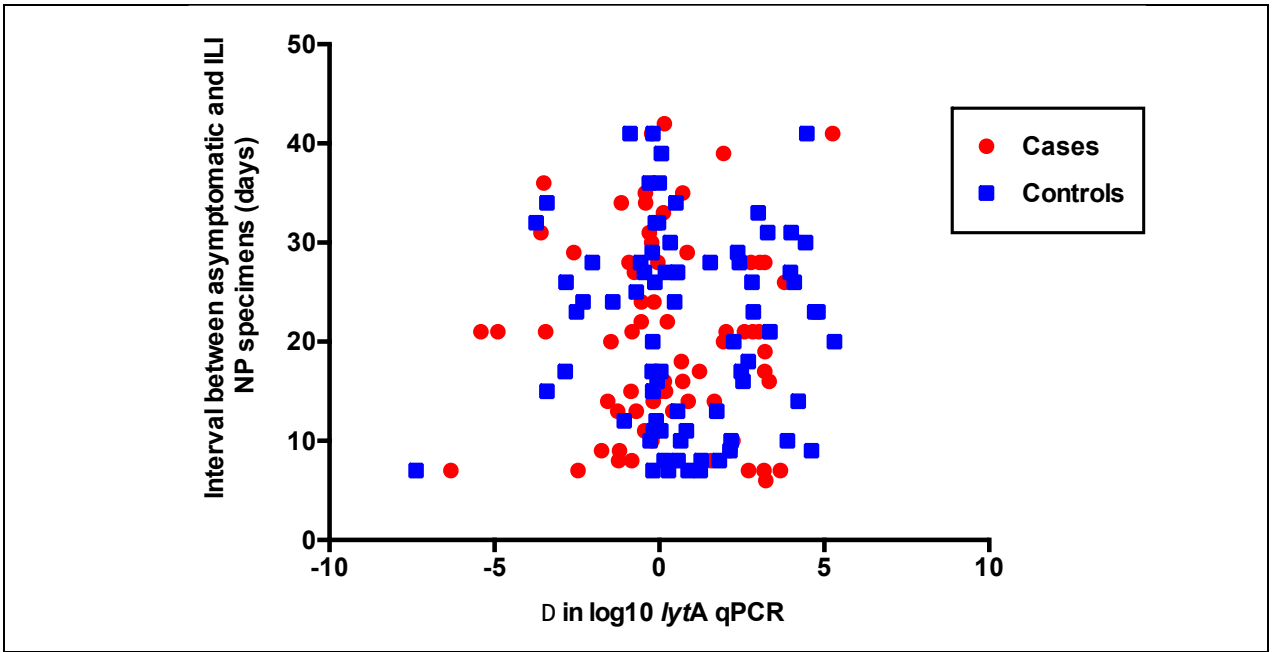



**Figure 6.7 Correlation between NP sampling interval and change in quantitative lytA qPCR between asymptomatic visit and ILI episode among cases and controls**

ILI, influenza-like illness; NP, nasopharyngeal; qPCR, quantitative polymerase chain reaction

#### *6.3.2.3 Factors associated with an increase in pneumococcal colonisation density during influenza-like illness*

Factors associated with a 1-$\log_{10}$ increase in pneumococcal colonisation density between at ILI visit were explored using a GEE logistic regression model (Table 6.5). A 10-fold rise in NP pneumococcal load between asymptomatic visit and ILI episode was observed in 23 (29%) of cases vs. 29 (36%) controls. In the univariable analysis, cases (virus-positive ILI) were not associated with an increase in pneumococcal load compared to controls (virus-negative ILI) (28.8 vs. 36.3%, OR 0.72, 95% CI 0.38-1.34, p=0.30). Individuals that did not take antibiotics in the 2 weeks preceding an ILI episode were more likely to have an increase in pneumoocccal load than those that did not (35 vs. 15%, OR 3.21, 95%CI 0.87-11.87). Furthermore, an increase in pneumococcal load was found in those who used firewood as their principal cooking fuel versus those that did not (OR 2.66, 95%CI 1.15-6.16). Other factors, such as age, gender, HIV infection, viral co-infection, living with children <5 years, household crowding, sampling interval, and season in which ILI occurred were not associated with increased colonisation density.

Case control status was entered Into the multivariable model as the main exposure of interest. However, only firewood use was independently associated with an increase in NP pneumococcal load at ILI visit (aOR 2.62, 95%C 1.14-5.98).

**Table 6.5 Factors associated with a $log_{10}$ increase in pneumococcal colonisation density at ILI episode**

| Characteristic | 1-$log_{10}$ increase in Spn density n=52 | Univariable[a] | | Multivariable [a,b] | |
|---|---|---|---|---|---|
| | | OR (95% CI) | p-value | OR (95% CI) | p-value |
| **Case control status** | | | | | |
| Control | 29/80 (36.3) | 1 | | 1 | |
| Case | 23/80 (28.8) | 0.72 (0.38-1.34) | 0.30 | 0.74 (0.39-1.41) | 0.35 |
| **Gender** | | | | | |
| Male | 22/63 (34.9) | 1 | | | |
| Female | 30/97 (30.9) | 0.80 (0.41-1.58) | 0.53 | | |
| **Age group (years)** | | | | | |
| 18-29 | 11/37 (29.7) | 1 | | | |
| 30-39 | 24/64 (37.5) | 1.45 (0.63-3.32) | | | |
| ≥40 | 17/59 (28.8) | 0.94 (0.32-2.05) | 0.50 | | |
| **HIV status** | | | | | |
| Negative | 16/54 (29.6) | 1 | | | |
| Positive | 35/105 (33.3) | 1.17 (0.60-2.30) | 0.64 | | |
| **Antibiotics in past 2 weeks** | | | | | |
| No | 49/140 (35.0) | 3.21(0.87-11.87) | 0.08 | | |
| Yes | 3/20 (15.0) | 1 | | | |
| **Season** | | | | | |
| Hot and rainy | 19/71 (26.7) | 1 | | | |
| Cold and dry | 28/76 (36.8) | 1.58 (0.77-3.26) | | | |
| Hot and dry | 5/13 (38.5) | 1.68 (0.47-5.98) | 0.43 | | |
| **Interval between AV & ILI (days)** | | | | | |
| 7-14 | 17 (32.1) | 1 | | | |
| 15-28 | 27 (37.0) | 1.23 (0.55-2.74) | | | |
| 29-42 | 8 (23.5) | 0.66 (0.27-1.62) | 0.42 | | |
| **Viral co-infection** | | | | | |
| No | 46/147 (31.3) | 1 | | | |
| Yes | 6/13 (46.1) | 1.84 (0.58-5.85) | 0.30 | | |
| **Children <5 years in household (n)** | | | | | |
| 0 | 37/101 (36.6) | 1 | | | |
| 1 | 7/40 (17.5) | 0.39 (0.17-0.89) | | | |
| ≥2 | 8/19 (42.1) | 1.47 (0.43-5.06) | 0.055 | | |
| **Crowding index** | | | | | |
| <1.5 | 11/37 (29.7) | 1 | | | |
| 1.5-2.4 | 17/62 (27.4) | 0.87 (0.40-1.90) | | | |
| ≥2.5 | 24/61 (39.3) | 1.63 (0.74-3.62) | 0.25 | | |
| **Principal cooking fuel** | | | | | |
| Electricity | 5/7 (41.7) | 2.12 (0.65-6.94) | | 2.06 (0.60-7.13) | |
| Charcoal | 29/107 (27.1) | 1 | | 1 | |
| Firewood | 18/38 (47.4) | 2.66 (1.15-6.16) | 0.047 | 2.62 (1.14-5.98) | 0.05 |

OR, odds ratio; CI, confidence interval; AV, asymptomatic visit; ILI, influenza-like illness

[a]Logistic regression with generalised estimating equation

[a,b]Adjusted for case control status (main exposure of interest) and principal cooking fuel

As a sensitivity analysis, we also examined factors associated with a 1-log base 2 increase (i.e. doubling) in colonisation density. However, no significant associations, including case control status, were found (data not shown).

#### *6.3.2.4 Factors associated with a new episode of pneumococcal colonisation during influenza-like illness*

Among the 32 cases and 32 controls that were not colonised with S. *pneumoniae* at the pre-ILI visit, a new pneumococcal colonisation episode was found at 27 ILI visits (Table 6.6). Virus-positive ILI (i.e. being a case) was not associated with pneumococcal acquisition (12 (37.5%) vs. 15 (46.9%), in cases and controls respectively). HIV-infected adults had a significantly higher risk of pneumococcal acquisition, compared to HIV-uninfected adults (56.5 vs. 20.0%, p=0.004). In contrast, age, sex, preceding antibiotic use, season of ILI episode, sampling interval, in addition to household and socioeconomic variables were not associated with pneumococcal acquisition. There were insufficient numbers to construct a multivariable model using logistic regression with GEE.

**Table 6.6 Factors associated with pneumococcal acquisition at ILI episode among cases (n=32) and controls (n=32) that had no pneumococcal colonisation at pre-ILI visit**

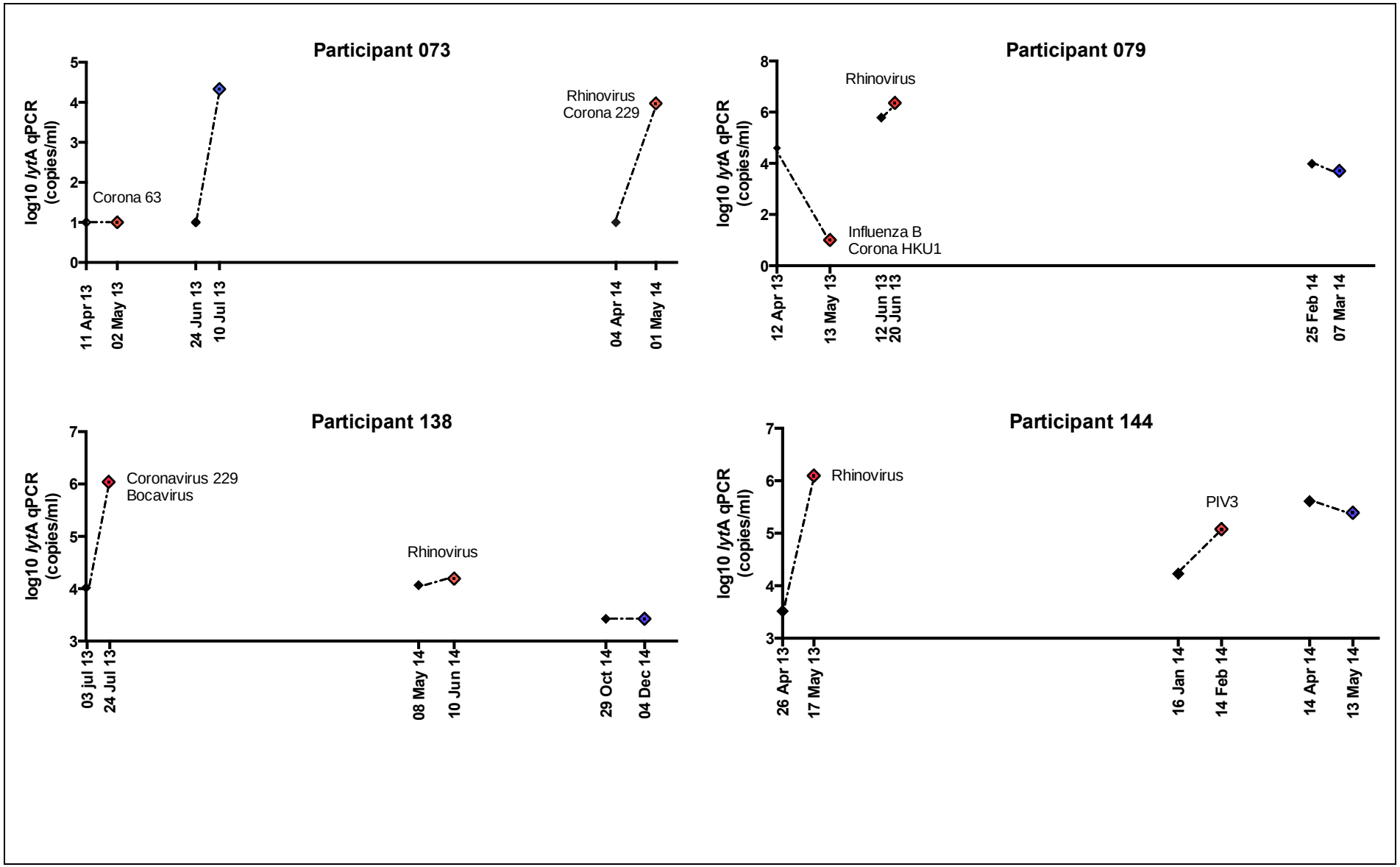
| Characteristic | Pneumococcal acquisition (n=27) | P-value[a] |
|---|---|---|
| **Case control status** | | |
| Control | 15/32 (46.9) | |
| Case | 12/32 (37.5) | 0.45 |
| **Gender** | | |
| Male | 9/27 (33.3) | |
| Female | 18/37 (48.7) | 0.22 |
| **Age group (years)** | | |
| 18-29 | 7/17 (41.2) | |
| 30-39 | 10/25 (40.0) | |
| ≥40 | 10/22 (45.5) | 0.93 |
| **HIV status** | | |
| Negative | 5/25 (20.0) | |
| Positive | 22/39 (56.4) | **0.004** |
| **Season** | | |
| Hot and rainy | 12/223 (52.2) | |
| Cold and dry | 12/37 (33.3) | |
| Hot and dry | 3/5 (60.0) | 0.25 |
| **Interval between AV & ILI (days)** | | |
| 7-14 | 6/21 (28.6) | |
| 15-28 | 17/33 (51.5) | |
| 29-42 | 4/10 (40.0) | 0.25 |
| **Children <5 years in household (n)** | | |
| 0 | 21/57 (45.7) | |
| 1 | 4/14 (28.6) | |
| ≥2 | 2/4 (50.0) | 0.50 |
| **Crowding index** | | |
| <1.5 | 8/15 (53.3) | |
| 1.5-2.4 | 7/22 (31.8) | |
| ≥2.5 | 12/27 (44.4) | 0.41 |
| **Principal cooking fuel** | | |
| Electricity | 3/5 (60.0) | |
| Charcoal | 16/40 (40.0) | |
| Firewood | 8/17 (47.1) | 0.66 |

OR, odds ratio; CI, confidence interval; AV, asymptomatic visit; ILI, influenza-like illness

[a]Chi-square or Fisher's exact test

#### *6.3.2.5 Exploring fluctuations in nasopharyngeal pneumococcal density in cohort participants that have contributed multiple ILI episodes*

The inclusion of multiple ILI episodes in a number of participants provided an opportunity to study the fluctuations in NP pneumococcal carriage density within an individual over time. In participants that had contributed more than two ILI episodes, quantitative *lyt*A qPCR results from asymptomatic and ILI episodes were plotted on a timeline (Figure 6.8). Marked variations in pneumococcal colonisation densities were observed in all six participants. Significant increases in pneumococcal load between pre-ILI and ILI specimens were observed in several virus-positive ILI episodes (participant 73 (rhinovirus & coronavirus 229E), 138 (bocavirus & coronavirus 229E), 144 (rhinovirus), 228 (coronavirus NL63) and 514 (coronavirus NL63)), but not in others (participant 073 (coronavirus NL63), 079 (influenza B & coronavirus HKU1; rhinovirus), participant 138 (rhinovirus). Conversely, large rises in pneumococcal densities were also detected in a number of virus-negative ILI episodes (participants 073 & 514). Overall, no individual- or virus-specific patterns in relation to changes in colonisation density were identified. However, substantial rises in pneumococcal load were noted in a number of instances where an asymptomatic sample was taken one month after an ILI episode. In participant 079, a 5-log increase in lytA qPCR was detected 30 days after an influenza B & coronavirus HKU1 ILI episode. Similarly, a 6-log increase in pneumococcal density was seen 29 days after a rhinovirus ILI episode in participant 228.


Participant 073
log10 lytA qPCR (copies/ml)
Corona 63
Rhinovirus
Corona 229
11 Apr 13
02 May 13
24 Jun 13
10 Jul 13
04 Apr 14
01 May 14
Participant 079
log10 lytA qPCR (copies/ml)
Rhinovirus
Influenza B
Corona HKU1
12 Apr 13
13 May 13
12 Jun 13
20 Jun 13
25 Feb 14
07 Mar 14
Participant 138
log10 lytA qPCR (copies/ml)
Coronavirus 229
Bocavirus
Rhinovirus
03 jul 13
24 Jul 13
08 May 14
10 Jun 14
29 Oct 14
04 Dec 14
Participant 144
log10 lytA qPCR (copies/ml)
Rhinovirus
PIV3
26 Apr 13
17 May 13
16 Jan 14
14 Feb 14
14 Apr 14
13 May 14

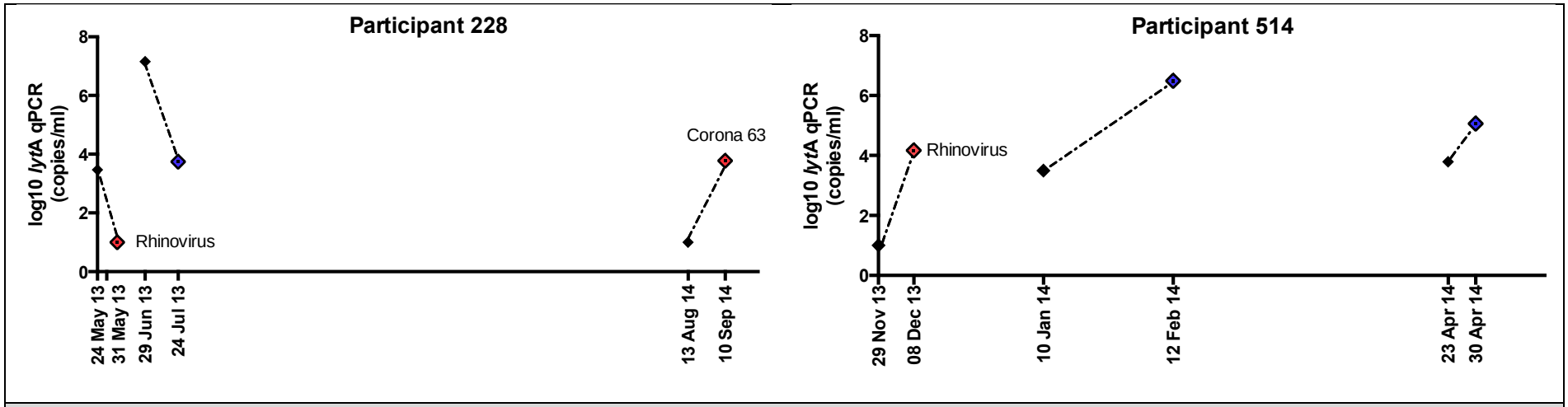



**Figure 6.8 Sequential nasopharyngeal pneumococcal load in asymptomatic and ILI visits in participants that contributed 3 episodes to the case control study (N=6)**

virus-positive ILI episode (case) virus-negative ILI episode (control) ♦ pre-ILI asymptomatic sample

## 6.4 Discussion

As highlighted in Chapter 1.6, the synergistic interaction between influenza and S. *pneumoniae* has been well described in the context of influenza pandemics and animal models. However, few epidemiological studies have evaluated the relationship between influenza and other respiratory viruses and pneumococcal carriage, the necessary precursor to invasive disease. We explored this relationship through two studies nested within the BASH-FLU prospective cohort: a cross-sectional and a case control study. We identified a high prevalence of pneumococcal carriage in Malawian adults (*lyt*A-positive in 60% asymptomatic, and 74% ILI samples). At ILI presentation, infection with influenza, but not other respiratory viruses, was associated with a higher prevalence of pneumococcal carriage (62 vs. 43%, aOR 2.03), compared to those that were influenza-negative. Having two or more children <5 years in the household and inadequate water supply also predisposed to pneumococcal carriage during ILI. We also examined the dynamic impact of respiratory viral infections on nasopharyngeal pneumococcal density, before and during ILI, and the risk of pneumococcal acquisition using a quantitative *lyt*A PCR. Contrary to our hypothesis, respiratory viral infection did not predispose to a greater increase in pneumococcal colonisation density, or a higher risk of pneumococcal acquisition. Firewood use was associated with an increase in pneumococcal bacterial load, while HIV infection predisposed to pneumococcal acquisition during ILI. Additionally, dynamic variations in nasopharyngeal pneumococcal load (up to 6 $\log_{10}$ copies/ml) were observed among participants that had quantitative *lyt*A PCRs performed at multiple time-points.

Only one other study has evaluated the association between influenza and pneumococcal carriage in a high HIV prevalence setting; Wolter *et al.* (Wolter et al., 2014a) found that NP pneumococcal carriage was significantly associated with influenza virus (aOR 2.2), as well as adenovirus (aOR 1.7) and rhinovirus (aOR 1.6). Due to limited numbers, we were unable to examine the effect of individual respiratory viruses except for influenza. Collectively, viruses other than influenza had no impact on colonisation.

Exposure to young children in the household is a known determinant of adult pneumococcal carriage (Hussain et al., 2005; Regev-Yochay et al., 2004), since children <5 years have the highest carriage incidence (Leiberman et al., 1999; Ghaffar et al., 1999), and are thought to be the main source of transmission to adults in the household (Hussain et al., 2005; Hendley et al., 1975; Mosser et al., 2014). Pneumococcal colonisation has not been directly linked to poor sanitation. However, inadequate water supply has been identified as a risk factor for IPD in Alaskan children (Wenger et al., 2010). Poor access to water likely compromises hand hygiene, leading to increased transmission

of respiratory pathogens. Handwashing has been shown to reduce pneumococcal carriage among children attending day care centres (Gudnason et al., 2014), as well as the risk of respiratory illness in a military population (Ryan et al., 2001).

With regards to the case control study, our findings contrast with existing studies that have evaluated the impact of viral infection on NP pneumococcal colonisation density (Vu et al., 2011; Wolter et al., 2014b) and acquisition (Grijalva et al., 2014). Among Vietnamese children with radiologically confirmed pneumonia, those with viral co-infection had a 15-fold higher NP pneumococcal load, compared to children without viral co-infection (Vu et al., 2011). Wolter *et al.* (Wolter et al., 2014b) also demonstrated higher NP pneumococcal colonisation density in hospitalised LRTI adults and children with respiratory viral co-infection in South Africa (adjusted OR 1.7 [95%CI 1.1-2.6], per $\log_{10}$ increase in colonization density). A case control study nested within a cohort of Peruvian children with acute respiratory illness (ARI) found that influenza and parainfluenza-related ARI facilitated the acquisition of new pneumococcal serotypes (Grijalva et al., 2014). This, however, only applied to children who were already colonised with pneumococcus. Infections with other respiratory viruses (adenovirus, hMPV, rhinovirus and RSV) were not associated with pneumococcal acquisition.

Our case control study differs from those described above in many respects. They were focused on young children (48% were <5 years of age in Wolter et al. (Wolter et al., 2014b)), while our study was undertaken exclusively in adults. The South African study demonstrated significantly lower carriage density in adults compared to young children (>15 years vs. <2 years, aOR 0.4) (Wolter et al., 2014b). Two-thirds of our study population were HIV-infected, whereas only the South African study included HIV-infected individuals. Furthermore, the studies that examined pneumococcal colonisation density were cross-sectional. In contrast, our case control study is the first to evaluate temporal changes in nasopharyngeal pneumococcal density in relation to respiratory viral infections in adults. Cases and controls were derived from the same study population, thus reducing selection bias. Recall bias is also minimised since exposures and potential risk factors were ascertained before ILI episodes. The fact that an individual can be enrolled as a case or a control on more than one occasion is both a strength and a limitation. Inclusion of several ILI episodes in the same individual permitted the longitudinal assessment of pneumococcal density in relation to illness episodes. However, the need to account for within-individual correlation of observations in statistical analysis inevitably resulted in a decrease in precision in effect estimates.

Longitudinal assessment of pneumococcal colonisation density has only occurred in the context of the Experimental Human Pneumococcal Challenge (EHPC) model (Ferreira et al., 2011; Gritzfeld et al., 2014), where healthy adults are inoculated intranasally with a controlled dose of a

pneumococcal isolate; carriage status and bacterial density are then evaluated at regular time-points. A 4-5 $\log_{10}$ increases in carriage densities have been observed among carriage-positive volunteers following pneumococcal challenge, with an average carriage duration of 28-35 days (Gritzfeld et al., 2014). Nonetheless, this model has not assessed pneumococcal carriage density in relation to viral infections.

Several factors may explain why we did not observe a greater increase in pneumococcal density among the cases, compared to controls. Firstly, our sample size was small. The total number of cases was restricted to ILI attendances that were PCR positive for at least one virus, and had an NP specimen taken within 6 weeks of ILI visit. Our sample of 80 cases and 80 controls provided 80% power to detect factors associated with an increase in pneumococcal density with an odds ratio of 2.5 or greater, therefore compromising our ability to detect smaller effect sizes. Similarly, we were unable to assess the impact of influenza virus on pneumococcal density due to the limited number of influenza infections among cases (n=16). The inclusion of respiratory viruses that are often detected in asymptomatic individuals, such as rhinovirus and adenovirus, and more recently discovered viruses that have uncertain clinical significance, e.g. bocavirus (Jartti et al., 2008), could have diluted any real effect of influenza infection on pneumococcal colonisation density if one had been present.

Secondly, both studies that observed higher NP pneumococcal density with viral co-infection comprised individuals with severe LRTI (Vu et al., 2011; Wolter et al., 2014b). In particular, Vu *et al*. (Vu et al., 2011) observed significantly higher pneumococcal load only in virally co-infected children with radiologically confirmed pneumonia, but not other types of LRTI. It is therefore possible that substantial increases in carriage density only occur in patients with severe respiratory presentations, while mild viral illness may be associated with subtler density changes. The majority of ILI presentations among the cohort participants were mild (Chapter 4.3.4.3).

Thirdly, pneumococcal carriage status and densities were compared at ILI presentation and the preceding well visit, with a sampling interval ranging between 7 to 42 days. The magnitude of density change was not related to the varying time interval between samples. However, acquisition episodes could have been missed, as limited evidence suggest that adults have a shorter duration of carriage compared to children (14-17 [23-31 in mothers] vs. 13-71 days respectively) (Ekdahl et al., 1997; Melegaro et al., 2004; Darboe et al., 2010; Turner et al., 2012). This potential misclassification error is likely to be non-differential to case control status, therefore biasing towards the null. Furthermore, the absence of serotype information is an important limitation, as we could only ascertain new colonisation in individuals that were not previously colonised, but not the acquisition of a new pneumococcal serotype, in those that were already colonised.

Lastly, pneumococcal density increases may have been missed if they occurred after, rather than at the time of respiratory viral infection. In several participants, we noted a 5-6 $\log_{10}$ increase in pneumococcal load around 4 weeks after a virus-positive ILI episode (Chapter 6.3.5), though it is unclear whether this rise is related to the preceding viral infection. In murine models, rapid increases in pneumococcal carriage density occurred within 7 days of inoculation of influenza virus (Nakamura et al., 2011; Mina et al., 2013). However, observations in mice are not necessarily translatable to humans, given the differences in their NP microbial ecology and immunological responses (Ferreira et al., 2011). Carriage duration also appear to be shorter in mice than human subjects (McCool et al., 2002; McCool et al., 2003; McCool and Weiser, 2004). Thus the timing and magnitude of density increase in relation to viral infections in humans remain unclear. We are investigate this further by performing *lyt*A PCR on asymptomatic NP samples that were taken 4-8 weeks after an ILI episode.

This study highlights the challenges of studying the temporal effects of respiratory viral infection on pneumococcal colonisation density in human subjects. Although an inadequate sample size and the above-mentioned methodological issues may have hampered our ability to detect an association if one was present, our observations that respiratory viral infections have no effect on pneumococcal colonisation density or acquisition risk could be real. Instead of enhancing bacterial load in the nasopharynx, respiratory viruses may facilitate the direct extension of S. *pneumoniae* into the lower respiratory tract through other mechanisms as shown in animal models (Chapter 1.6.4), including virus-mediated damage to the respiratory epithelium (Harford and Hara, 1950; Loosli et al., 1975; Pittet et al., 2010), increased receptor availability to aid pneumococcal binding (Peltola and McCullers, 2004; McCullers and Bartmess, 2003), promotion of bacterial emergence from biofilms (Marks et al., 2013), as well as down-regulation of host innate immune responses (Sun and Metzger, 2008; Didierlaurent et al., 2008; Shahangian et al., 2009; Nakamura et al., 2011). Alternatively, the association observed between influenza and pneumococcal carriage in cross-sectional studies to date may be due to the converse of our hypothesis, i.e. NP carriage of S. *pneumoniae* increases the probability of clinically manifesting influenza infection. This is unlikely, but worth further exploration.

Other potential factors associated with an increase in NP pneumococcal load at ILI were also explored. The use of firewood as the principal cooking fuel was associated with a 2.5 times greater risk of an increase in pneumococcal density during ILI, compared to charcoal use. Exposure to indoor air pollution (IAP) is a well-known risk factor of severe pneumonia, particularly in young children (Dherani et al., 2008). However, the impact of IAP, or more specifically, firewood on pneumococcal carriage has not been studied. Pneumococcal carriage surveys are currently being conducted within clinical trials on the use of biomass briquettes in the Gambia (Medical Research Council Unit The

Gambia, 2002-), and advanced cookstoves in Malawi (Jary et al., 2014), to assess whether reduced exposure to IAP will affect pneumococcal carriage in babies and mothers.

Among the cases and controls that did not have pneumococcal colonisation at the pre-ILI visit, HIV-infection was associated with a significantly increased risk of pneumococcal acquisition (56 vs. 20%, p=0.004, in HIV-infected and HIV-uninfected participants respectively). This is unsurprising, since the host immune response has an important regulatory role in the acquisition and clearance of pathogens in the upper respiratory tract (Garcia-Rodriguez and Fresnadillo Martinez, 2002). Poor mucosal immunity associated with HIV infection may result in increased risk of acquisition and persistence of colonisation. Our finding is consistent with the other studies have longitudinally assessed pneumococcal carriage in adults. Rodriguez-Barradas et *al*. (Rodriguez-Barradas et al., 1997) observed higher rate of pneumococcal acquisition and persistent carriage in HIV-infected individuals. In a cohort study of Zambia, HIV-infected mothers were more likely to have repeated colonisation, earlier new colonisation, as well as more prolonged duration of colonisation, than HIV-negative mothers (Gill et al., 2008). These observations likely explain why HIV-infected individuals have higher rates of pneumococcal carriage than HIV-uninfected individuals (Gill et al., 2008; Paul, 1997; Sepako et al., 2014), which in turn, may partly contribute to the increased frequency of pneumococcal pneumonia and bacteraemia and also severity of influenza disease seen in HIV-infected individuals (as illustrated in Chapter 5). Nevertheless, this result should be cautiously interpreted as we were unable to control for confounders, such as smoking, exposure to young children and crowding in household, or socioeconomic status due to small numbers.

The rate of NP pneumococcal colonisation in our study (60% in asymptomatic, and 74% in ILI samples) is substantially higher than that reported in other African settings. A recent review of pneumococcal carriage studies in SSA reported a pooled prevalence of 28% (95%CI 19-37%) in adults >15 years (Usuf et al., 2014). The disparity in carriage rates may in part be due to the different detection methods. All studies included in the review used culture to determine pneumococcal carriage. Although culture and *lyt*A qPCR have shown good correlation in terms of bacterial quantitation (Albrich et al., 2012; Gritzfeld et al., 2014), *lyt*A qPCRs are unaffected by the effects of previous antibiotics (Albrich et al., 2012) and have superior sensitivity at low pneumococcal densities (Gritzfeld et al., 2014). On the other hand, qPCR can detect both viable and non-viable bacterial cells, thus can overestimate pneumococcal colonisation prevalence (Chien et al., 2013). Interestingly, the *lyt*A qPCR detected a significantly higher pneumococcal carriage prevalence than the FTD multiplex PCR, even though both PCRs target the *lyt*A gene. Since there is currently there is no gold standard for NP colonisation (Satzke et al., 2013), it is unclear whether this represents under-detection by FTD PCR, or over-detection by *lyt*A qPCR. There are no published data comparing

the performance of the two PCR tests, but we are investigating this further in other carriage studies in Blantyre.

A number of other limitations in this study should be considered. Since the selection of cases and controls was contingent on the presence of an asymptomatic sample within 6 weeks before an ILI attendance, ILI episodes were more likely to be included if they occurred at certain times of year. In the first year of follow-up, routine visits only occurred between May and August. Thus certain viruses that were prevalent outside that period, such as influenza A(H3N2) and RSV, were under-represented (Figure 6.4B). The demographic characteristics did not differ between virus-positive ILIs that were or were not included as cases, but the distribution of respiratory viruses among the 80 cases was not representative of all virus-positive ILI events. This is likely affected cases and controls equally, since they were frequency-matched for the calendar month of ILI episode.

Lastly, given the dynamic variations in pneumococcal colonisation densities observed, it is likely that there are a number of confounding factors at play that have not been accounted for in our analysis. These may include climatic factors, detailed biomass exposure, and antibiotic usage in the community. An important unmeasured confounder is the PCV vaccination status of the children in the participant's household. PCVs have been found to reduce vaccine-type pneumococcal carriage in adults through herd protection (Davis et al., 2013). In this study, 34% case and 40% control households had children under the age of five; many of whom would have received $\geq$1 dose of the 13-valent pneumococcal conjugate vaccine (PCV13), which has been part of the national immunisation programme in Malawi since November 2011.

PCV has been shown to reduce the overall incidence of IPD (Conklin et al., 2014), as well as influenza-related pneumococcal pneumonia (Madhi and Klugman, 2004). A recent murine study has shown that a live-attenuated influenza vaccine (LAIV) significantly reduced influenza-mediated excess pneumococcal carriage density and duration of carriage, while no benefit was observed with PCV (Mina et al., 2013). The latter observation reinforces existing knowledge that PCV reduces pneumococcal serotype-specific carriage by reducing acquisition rather than density or duration of existing carriage (Klugman, 2001). These findings imply that influenza vaccination may have an important role in reducing excess pneumococcal co-infections during influenza epidemics, by interrupting influenza-associated pneumococcal transmission. A study utilising the Experimental Human Pneumococcal Carriage model is under way, to compare the impact of LAIV and inactivated influenza vaccine (IIV) on pneumococcal carriage in human volunteers.

In summary, we found that infection with influenza, but not other respiratory viruses, was associated with pneumococcal colonisation during ILI, but respiratory viral infection did not impact

impact on pneumococcal carriage density or the risk of pneumococcal acquisition. The role of respiratory viruses in the transition from asymptomatic pneumococcal carriage to invasive disease remains unclear. Further assessment of the dynamic interaction between respiratory viruses and pneumococcal colonisation in human subjects is warranted to better understand the pathogenic mechanisms underlying the strong link between respiratory viral infections and secondary pneumococcal pneumonia.

# CHAPTER 7. OVERALL DISCUSSION

## 7.1 Overview

The epidemiology of influenza infection in adults has been rigorously studied in developed settings with temperate climates. In contrast, as highlighted in Chapter 1, relatively little is known about the clinical manifestation, epidemiology and risk factors of influenza in sub-Saharan Africa. In a region with high prevalence of HIV infection and co-morbid conditions, scarce health resources, as well as poverty-related factors, influenza is likely to have considerable impact on morbidity and mortality. Annual vaccination of vulnerable high-risk groups is the cornerstone of influenza prevention in many developed settings, but influenza vaccine is not available for anyone in the public sector in most SSA countries (Palache et al., 2014). Therefore, gathering robust evidence on influenza disease burden and groups that are most at-risk of severe influenza complications in SSA are critical for the development of influenza prevention and control strategies.

Key areas of research focus required for decision making include influenza disease burden, its impact on health systems, risk factors for severe influenza presentations, in addition to its interaction with other prevalent co-morbidities such as tuberculosis, malaria and malnutrition (Gessner et al., 2011; World Health Organization, 2012a). Furthermore, understanding the impact of immunosuppression on influenza presentation is particularly pertinent in a continent that accounts for 70% of all people living with HIV globally (UNAIDS, 2014a). HIV-infected individuals are considered to be at greater risk of influenza-related complications, and thus designated a priority group in many national immunisation guidelines (Geretti et al., 2008; Grohskopf et al., 2014; National Advisory Committee on Immunization (NACI), 2014). However, available data suggest that the impact of HIV may differ in low and high prevalence settings (Chapter 1.4).

This thesis sets out to address some of these important unanswered questions in a high HIV prevalence urban setting in Malawi. First of all, the epidemiology, seasonality and risk factors for influenza infection were characterised in Malawian adults enrolled in a sentinel surveillance study, in the three years following the influenza A(H1N1)pdm09 pandemic. Secondly, the impact of HIV infection on the frequency and severity of influenza illness, and potential risk factors for severe influenza presentation (i.e. hospitalised LRTI) were assessed through two prospective observational studies. Lastly, as a potential mechanism through which influenza and other respiratory viral infections predispose to secondary bacterial pneumonia, the association between respiratory viral infections and nasopharyngeal *Streptococcus pneumoniae* density was investigated in a case control

study nested within the existing cohort. This final chapter will review and summarise the key findings of this thesis, discuss the clinical, research and policy implications of the findings, and suggest future studies.

## 7.2 Principal findings of the research

### 7.2.1 The epidemiology of HIV and influenza in sub-Saharan Africa

As the first description of adult influenza epidemiology in Southern Africa outside of South Africa, the comprehensive hospital-based sentinel surveillance study identified influenza as a common cause of mild (16%) and severe (15%) acute respiratory infections in Malawian adults. Although cyclical, no consistent, distinct seasonality to influenza activity was observed. A minimum incidence of influenza-positive hospital-attended SARI of 9.6 to 16.8 per 100,000 adult population was estimated; a finding that is likely generalisable to other urban settings in SSA. Importantly, pregnancy was identified as a risk factor for influenza; pregnant women had nearly double the risk of hospitalised influenza infection compared to non-gravid women of childbearing age (aRR 1.84 (95%CI 0.99-3.43), which has not been described in SSA outside a pandemic. Furthermore, HIV infection, which was detected in >50% adults presenting with SARI, predicted clinical severity (as defined by Modified Early Warning Score >4 (Subbe et al., 2001)) in both influenza-positive and influenza-negative adults with SARI.

Through the 2-year follow-up of a prospective cohort, a high burden of influenza illness was found in both HIV-infected and HIV-uninfected adults (46 vs. 14.5 per 1000 person-years). After accounting for potential confounders, HIV-infected adults had an approximately three times greater susceptibility to influenza infection compared to HIV-uninfected adults. To the best of our knowledge, this is the first longitudinal study to investigate the incidence rates of influenza and its associations in a well-described cohort of HIV-infected and HIV-uninfected persons. In the case control study of patients with influenza-confirmed hospitalised LRTI and ILI, HIV was the single most important risk factor for severe influenza-related presentation; HIV-infected adults experienced a five-fold greater risk of severe influenza disease compared to HIV-uninfected adults, and over 50% of influenza-related hospitalised LRTI were attributable to HIV. Neither study was designed to specifically investigate the effect of HIV at different levels of immunosuppression. However, the trend towards a higher incidence of influenza, as well as greater disease severity in those with an CD4+ cell count of <200 cells/µl would support the hypothesis of greater susceptibility and risk of severe complications among those with advanced immunosuppression.

Data from the surveillance, cohort and case control studies, taken together, present a convincing argument for a causal relationship between HIV and influenza susceptibility and severity.

Surveillance data highlighted the high prevalence of HIV infection and its role in severe respiratory hospital presentations in Malawian adults. The cohort and case control studies confirmed strong associations between HIV and influenza susceptibility as well as severity, even after controlling for potential confounders. The trend towards greater risk in those with more profound immunosuppression suggests a dose-response relationship. Moreover, the finding that almost 60% of severe/hospitalised influenza presentations in Malawian adults were attributable to HIV infection further emphasises the critical role of HIV infection in severe influenza in this population. These findings are consistent with that of the few studies that have examined this association in SSA (Feikin et al., 2012b; Cohen et al., 2013; Ope et al., 2011). What sets this study apart from the surveillance studies in South Africa and Kenya are the comprehensive ascertainment of HIV status, CD4+ cell count and information on antiretroviral treatment, and the prospective collection of exposures and risk factors, including household or socio-economic characteristics that may confound the association between HIV and influenza. Furthermore, HIV infection is known to increase risk of infection and disease severity in other respiratory pathogens, such as S. *pneumoniae*, thus the relationship is biologically plausible.

Our results, together with other data from SSA, contrast with those from developed setting. As expected, the effect of HIV infection on Influenza differs in settings where the majority of HIV-infected patients are receiving ART and have good virological control, in stark contrast to high HIV prevalence populations in SSA accompanied by a high proportion of advanced disease and co-existent morbidities, such as tuberculosis, malnutrition, as well as external poverty-related factors. Some of the risk factors for influenza identified from this study potentially explain why disease is may be more severe in HIV-infected adults in SSA, compared to other settings (Section 7.2.2). The marked difference in impact also highlights the danger of extrapolating findings from developed setting, to inform policy regarding targeted influenza control strategies in SSA.

The incidence estimates of influenza-associated SARI and ILI presentations from the sentinel surveillance and cohort studies respectively, were substantially different. This underscores the fact that health facility-based surveillance greatly underestimates the number of mild community cases. Studies on healthcare utilisation in Malawi and other African settings have shown that most individuals do not seek medical care for mild illness, particularly adults and those from poor socioeconomic backgrounds (Desmond et al., 2013; Burton et al., 2011). As the cohort participants were actively encouraged to attend for mild ILI and reimbursed their transport costs, the incidence rates derived from the cohort study are more likely to reflect community burden of disease.

Furthermore, most influenza surveillance studies from SSA have included individuals of all ages; few have examined influenza epidemiology exclusively in adults. Adults and children have different co-

morbidity profiles, social contact patterns (Johnstone-Robertson et al., 2011; Ferraro et al., 2014), viral aetiology of acute respiratory illnesses (Feikin et al., 2012b; Zhang et al., 2014; Self et al., 2015), as well as varying influenza attack rates (World Health Organization, 2014c) and transmission dynamics (White et al., 2014). The epidemiology and risk factors for influenza in the two groups are therefore likely to be distinct, and should be evaluated separately.

### 7.2.2 Other risk factors for increased burden and severity of influenza in SSA

Aside from HIV, a number of risk factors for influenza infection and severity previously unrecognised in adults were identified; some may be unique to SSA settings. Household crowding was an independent risk factor for acquiring influenza infection, whereas a past history of pneumonia and poor sanitation facility were associated with severe influenza presentation. Those that reported difficulties accessing food at least once a week had a significantly higher rate of influenza infection, as well as increased risk of severe disease, compared to those that reported no food insecurity. Furthermore, severe food insecurity potentiated the effect of HIV on influenza risk. A correlation between food insecurity and low BMI (<18.5 $kg/m^2$) was found in both studies, but low BMI was not associated with increased influenza incidence or severity.

### 7.2.3 Other viral and bacterial aetiology of acute respiratory presentation in adults in SSA

Respiratory viruses were prevalent in Malawian adults presenting with ILI (40%), SARI (47%) and hospitalised LRTI (34%). Viral co-infections were detected in 14-27% of those with a viral aetiology. In the case control study, we identified one or more aetiological agents in two-thirds of patients with hospitalised LRTI – bacteria in 39%, virus 34%, and M. *tuberculosis* in 18% [24% among those that submitted sputum]). 22% had mixed pathogen infections. These findings concur with contemporary pneumonia studies (de Roux et al., 2006; Jennings et al., 2008; Johansson et al., 2010; Luchsinger et al., 2013) that are increasingly recognising pneumonia as a polymicrobial disease. However, the epidemiology of pneumonia in our study differs in many respects to that of developed settings. Key distinctions include a younger age group, lower prevalence of chronic medical conditions (except HIV), a greater incidence of pulmonary tuberculosis, and higher mortality. Most of these factors are likely driven by HIV infection.

### 7.2.4 The association between respiratory viruses and pneumococcal nasopharyngeal density

In the cross-sectional analysis of ILI presentations by the cohort participants, infection with influenza, but not other respiratory viruses, were associated with pneumococcal colonisation.

However, proven respiratory viral infection was not associated with an increase in nasopharyngeal pneumococcal density or increased risk of pneumococcal acquisition. HIV-infected participants were more likely to acquire pneumococcal carriage during an ILI.

## 7.3 Limitations

Before considering the wider implications of the results, a number of aspects of the design and conduct of the study, that have not been previously discussed, should be considered in interpreting the findings.

Firstly, this study was conducted in a single centre in an urban setting, which may limit the generalisability of the findings. However, patients were recruited from three different sites, ranging from well individuals from the outpatient department to patients with severe LRTI requiring hospitalisation. It is likely that the findings are applicable to other urban SSA populations with a high prevalence of HIV infection.

Secondly, the sample sizes of the cohort and case control studies were calculated according to the estimated impact of HIV infection on influenza incidence and severity, but were not powered to evaluate different CD4+ stratifications or ART status. Achieving adequate statistical power for the stratified analyses would have required a substantially larger sample size, which was unlikely to be achievable within the time frame and budget of this project.

Thirdly, several household and socioeconomic indicators emerged as risk factors for influenza. As they were not the main exposures of interest, some were crudely measured. Furthermore, a number of household and socioeconomic variables with multiple categories were collapsed, and several continuous variables, namely crowding index and asset ownership, were also converted to categorical form, due to small numbers. This loss of information and precision could have resulted in residual confounding or missed associations if a small linear effect was present. We recommend further detailed evaluation of sanitation, household crowding and, particularly food insecurity, as risk factors for influenza and other respiratory pathogens.

Lastly, ascertainment of cases in all three studies relied on passive surveillance, i.e. patients presenting to healthcare facility when they developed symptoms. This could have led to a partial or biased picture. Potential under-ascertainment of ILI cases in the cohort study was discussed in Chapter 4.4. In a recent household cohort study in England, around 20% of the study population showed serological evidence of influenza infection, but most (75%) were asymptomatic (Hayward et al., 2014). It is possible that HIV-infected individuals are more likely to develop symptoms given infection compared to HIV-infected individuals, instead of an absolute higher risk of influenza

acquisition. We aim to examine this further by analysing a proportion of the control samples, in order to establish the background prevalence of asymptomatic influenza carriage over the study period (Section 7.5).

## 7.4 Implications

### 7.4.1 Clinical implications of the work in this thesis

#### *7.4.1.1 Recognition of influenza*

The results of this thesis show that influenza is commonly associated with both upper and lower respiratory tract illness in Malawian adults. However, the majority of healthcare workers as well as the general public do not perceive influenza as a important problem. Consequently, it is rarely considered in the differential diagnosis of patients presenting with an acute febrile illness. In the absence of laboratory confirmation, non-specific febrile illnesses are often empirically treated with antimalarials and/or antimicrobials. Therefore, improving clinicians' awareness of influenza and other respiratory viruses could facilitate a reduction of polypharmacy, as well as the propagation of antimalarial and antimicrobial resistance. Differentiation of influenza from other acute respiratory infections and other febrile disease, such as malaria, can be challenging. In the case control study, influenza-related LRTI was clinically indistinguishable from other causes of LRTI, thus making the rationalisation of antibiotic use at clinical presentation difficult.

#### *7.4.1.2 Infection control and antivirals*

In developed settings, the management of individuals with severe hospitalised influenza comprise isolation, infection control, antiviral ± antimicrobial treatment to prevent/treat secondary bacterial infection. The identification of influenza in one in nine patients admitted with severe respiratory presentations indicates a substantial risk of nosocomial transmission in the overcrowded wards at QECH. Few studies have examined health care-associated infections in SSA, and none have studied nosocomial spread of respiratory viruses (Bagheri Nejad et al., 2011). It also emphasises the need for infection control measures in hospitals, particularly during the months of peak influenza transmission. While patient isolation is impractical in most resource-poor health facilities, physical interventions such as handwashing, and the use of face masks, gloves and aprons could be feasibly implemented in some settings to curtail nosocomial spread (Jefferson et al., 2011; Murni et al., 2013). Furthermore, any new health facilities in SSA should be designed to minimise crowding and maximise airflow.

Antiviral treatment, namely neuraminidase inhibitors (NI), is prohibitively expensive and is largely unavailable in SSA (Duque et al., 2014). They are most effective if administered within 48 hours of

symptom onset (Aoki et al., 2003), but very few present to health care within this timeframe (3.1% hospitalised LRTI and 7.2% ILI patients in the case control study). The lack of 'real-time' viral testing, in addition to the broad differential diagnosis of acute respiratory presentations, high cost and lack of availability, suggest there is currently no place for antiviral treatment in patients suspected to have severe influenza-related presentation in most SSA healthcare settings.

#### *7.4.1.3 The impact of ART*

Trends toward lower risk of influenza infection and severe disease were observed among HIV-infected individuals with higher CD4+ cell counts (>200 cells/μl). This implies that ART-associated immune reconstitution may protect against influenza illness, as is the case for other mucosal infections, notably pneumococcal disease (Feikin et al., 2004). No association was found between ART treatment status and influenza burden or severity, but the study was not designed to specifically assess the effect of HIV by CD4+ cell count or antiretroviral treatment status. Evaluating the impact of ART duration on influenza risk is likely to be more informative than whether a patient is receiving ART. As highlighted in Chapter 5.4, there are conflicting data regarding the effect of ART on influenza. In developed settings, the introduction of ART was associated with a reduction in influenza-attributable hospitalisations (Neuzil et al., 2003), and improved survival during the recent influenza A(H1N1) pandemic (Ormsby et al., 2011). Conversely, antiretroviral treatment status had no effect on CFR among HIV-infected individuals with influenza-positive SARI (Cohen et al., 2015c). A study in Malawi also found poor reconstitution of influenza-specific CD4+ T-cell response in HIV-infected adults with WHO clinical stage III/IV disease despite completing 12 months of ART (see Section 7.6.2) (Jambo et al., 2012). A possible explanation is that pathogen-specific immune reconstitution may vary according to nadir CD4+ count or the duration of ART treatment. In view of ongoing efforts to scale up antiretroviral treatment in Malawi and other high HIV prevalence settings (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2012), as well as the recent revision of CD4+ threshold for initiating ART from 350 to 500 cells/μl (World Health Organization, 2013a), further studies are needed to fully characterise the interplaying effects of CD4+ count and ART duration on the relationship between HIV and influenza burden and severity. In particular, to clarify whether long-term effective ART may attenuate the elevated burden and severity of influenza seen in HIV-infected adults.

### 7.4.2 Epidemiological/research implications of the work in this thesis

#### *7.4.2.1 Increased susceptibility = increased transmission?*

In the cohort study, HIV-infected individuals were approximately three times more likely to develop laboratory-confirmed influenza illness compared to HIV-uninfected individuals. Although the

majority of ILI presentations were mild, this could have important public health implications for two reasons. First, in the case control study, a five-fold greater risk of severe influenza presentation was demonstrated in HIV-infected individuals given infection. Secondly, increased susceptibility to influenza infection may also represent a greater risk of transmission. HIV-infected individuals with mild infection are less likely to be confined (at home or in hospital), therefore more likely to mix widely in the community. This risk may be further amplified if HIV-infected persons have more prolonged viral replication and shedding than HIV-uninfected individuals. Prolonged viral shedding has been demonstrated in non-HIV-related immunosuppression (Klimov et al., 1995; Boivin et al., 2002; Weinstock et al., 2003; Gooskens et al., 2009), but few studies have evaluated the effect of HIV infection on influenza virus shedding and transmission (Chapter 1.4.4). If the above hypotheses are true, HIV-infected individuals may play an important role in the community transmission of influenza, thus strengthening the argument to implement influenza preventative measures in this group. They may also play a key role in nosocomial transmission, particularly to other susceptible HIV-infected individuals in overcrowded ART clinics and hospital wards, where up to 75% adult patients have HIV infection (SanJoaquin et al., 2013).

#### *7.4.2.2 The immunology of HIV and influenza in SSA*

There is a paucity of information regarding the effect of HIV, and the likely impact of long-term ART, on innate and acquired immune responses to influenza infection. Several studies undertaken in Malawi have demonstrated lower proportions of total CD4+ T cells and impaired influenza-specific CD4+ T cell responses within the lungs of asymptomatic HIV-infected individuals, compared to HIV-uninfected adults (Jambo et al., 2011). These defects in pulmonary immunity were found in individuals with relatively high CD4+ cell counts (>350 cells/μl), and did not recover after 12 months on ART, despite immune recovery as indicated by an increase in CD4+ cell count and marked reduction in viral load (Jambo et al., 2012). This impairment in influenza-specific CD4+ T cell immunity may be partly responsible for the increased risk of influenza infection and associated complications in HIV-infected persons. HIV-infected patients on ART may be at risk to influenza-related complications. The evidence of poor influenza-specific immune reconstitution suggests that ART alone may not circumvent the elevated risk in HIV-infected persons. Although not the main focus of this thesis, further studies on the effect of HIV and ART on intra-pulmonary immune response to influenza infection, particularly CD8+ T cell and anti-influenza haemagglutinin (HA) antibodies, are warranted.

#### *7.4.2.3 Synergism between influenza and pneumococcus*

Given the synergistic interaction between influenza and *Streptococcus pneumoniae* (McCullers, 2006), increased risk of influenza acquisition may act as a potential mechanism for the higher rates

of bacterial pneumonia (Sogaard et al., 2008) and IPD (Nuorti et al., 2000; Harboe et al., 2014) observed among HIV-infected individuals, compared to HIV-uninfected persons, i.e. influenza may partly mediate the effect of HIV on pneumococcal infection (Figure 7.1). As highlighted in Chapter 1.6, few epidemiological studies have examined the relationship between influenza and S. *pneumoniae* outside of pandemics. Even fewer have done so in high HIV prevalence settings (Cohen et al., 2013; Wolter et al., 2014a; Wolter et al., 2014b). Further epidemiological studies of the inter-relationships among HIV, influenza and S. *pneumoniae* are warranted. Effective prevention of influenza-related complications in HIV-infected persons may necessitate targeting of both influenza and pneumococcus.

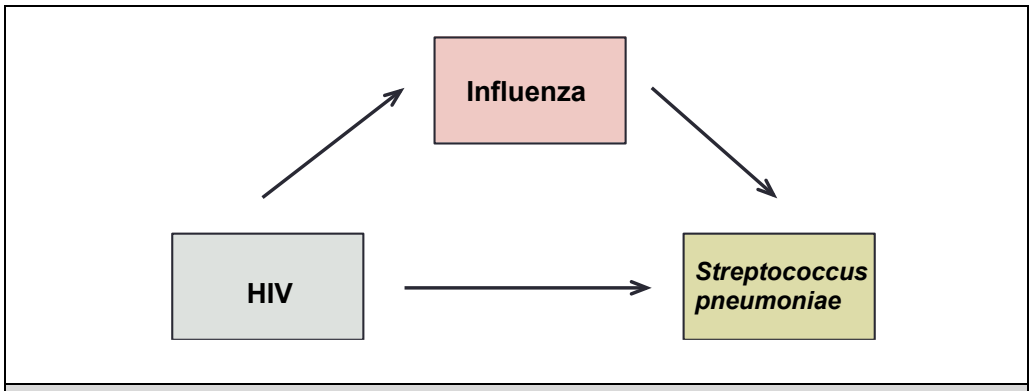



**Figure 7.1 Relationship between HIV, influenza and S. *pneumoniae***

#### *7.4.2.3 Modification of ongoing virological surveillance*

In response to the findings from this study, adult sentinel surveillance at QECH has adapted to focus on HIV-infected individuals and pregnant women. Furthermore, enhanced efforts have been made to capture pneumococcal diagnosis and outcome data, including hospitalisation, duration of hospital stay, and mortality.

### 7.4.3 Policy implications of the work in this thesis

Most countries in SSA do not have established seasonal influenza vaccination policies (World Health Organization, 2010c). The World Health Organization designated pregnant women as the highest priority target group in 2012, but individual nations were advised to tailor their influenza vaccination policies according to country-specific disease burden, risk groups, and cost effectiveness, that is achievable within their local capacity and resources (World Health Organization, 2012b). Up till now, due to a lack of awareness of influenza across healthcare workers and policy makers, poor understanding of the disease burden and groups at risk of severe influenza, as well as scarce resources in a region beset with a great number of other health priorities, the demand for influenza

vaccine in SSA has been low. Of 489 million influenza vaccine doses distributed in 2011, the WHO African (AFRO) region accounted for only 0.7% distributed doses (Palache et al., 2014).

The results of this study, together with published studies from South Africa and Kenya, provide substantive evidence of the high burden of influenza disease in adults in SSA, and identify HIV-infected persons at particular risk of infection and severe disease. Nevertheless, identification of priority groups is only the first step in the process towards developing influenza control policies that are specific to the continent's needs. Decision makers will also require evidence of vaccine efficacy and effectiveness, as well as cost benefits, in the target groups. Additionally, numerous regulatory, logistical, and financial obstacles will need to be overcome if targeted influenza vaccination policies were to be successfully and sustainably implemented in SSA.

#### *7.4.3.1 Clinical efficacy of IIV in HIV-infected population*

The findings from this thesis suggest that among HIV-infected adults, those with advanced immunosuppression (CD4+ count <200 cells/μl) are more vulnerable to influenza infection and severe complications than those with higher CD4+ counts. However, they have poorer immune response to IIV (Kroon et al., 1994; Fuller et al., 1999; Yamanaka et al., 2005; Yanagisawa et al., 2011), and evidence of clinical efficacy in this group is lacking. The only study that has evaluated IIV in an African adult HIV population demonstrated a 75.5% reduction in laboratory-confirmed influenza illness in IIV3-vaccinated adults (Madhi et al., 2011). However, the study excluded individuals with CD4+ cell count <100 cells/μl that were naïve to ART, and those with co-morbidities. Aside from an ongoing study that is exploring the effect of HIV and/or active tuberculosis on IIV3 immunogenicity in South African adults (Groome, 2013), IIV has not been evaluated in HIV-infected individuals with co-morbidities. Additionally, the study was not powered to observe differences in the rates of hospitalisation or deaths between IIV and placebo recipients. Thus the effectiveness of IIV in adults with advanced immunosuppression and/or co-morbid conditions, and the impact of IIV on severe respiratory outcomes and death need to be assessed.

Quadrivalent inactivated vaccines (IIV4) have not been evaluated in SSA. Encompassing both circulating influenza B strains, IIV4 may yield better clinical efficacy in HIV-infected individuals than IIV3, as influenza B viruses were more frequently detected in HIV-infected adults with hospitalised SARI in South Africa (Cohen et al., 2014), as well as in our cohort participants that presented with ILI (Chapter 4.3). However, the potential benefits of enhanced efficacy will need to be weighed against the additional costs.

#### *7.4.3.2 Cost effectiveness of IIV in HIV-infected population*

Seasonal influenza vaccination has been shown to provide substantial public health benefits as well

as cost savings in industrialised settings (Clements et al., 2011; Preaud et al., 2014), but there are no data from low income countries (Peasah et al., 2013). Economic evaluation of targeted vaccine introduction in SSA will need to be undertaken. The high burden of influenza and the high PAF of HIV in severe influenza presentations suggest that vaccine interventions targeted at HIV-infected individuals are likely to be cost-effective.

#### *7.4.3.3 IIV source and delivery to HIV-infected populations in SSA*

With the exception of tetanus toxoid immunization in pregnant women, there are currently no adult vaccination programmes in Malawi. The optimal vaccine delivery mechanism will need to be evaluated. IIV may be deliverable through antiretroviral treatments clinics, since the majority of HIV-infected adults receiving both pre-ART and ART care attend for regular clinical assessments (Malawi Ministry of Health, 2014).

The absence of clear seasonal patterns of influenza activity from the sentinel surveillance data (Figure 3.1) also presents a challenge in determining the ideal timing for vaccination. From the available data, the predicted best time would be between October and December (before the onset of influenza activity). This will favour the use of the vaccine composition recommended for the Northern Hemisphere, rather than the Southern Hemisphere. Additional surveillance data from 2014 and 2015 should be included.

In terms of vaccine supply, sustainable local manufacturing capacity will be required for feasible deployment of influenza vaccination policies. Although there is currently no indigenous influenza vaccine production in SSA, South Africa is one of 14 developing countries that have been awarded a grant to develop in-country manufacturing capacity for influenza vaccine, as part of the Global Action Plan for Influenza Vaccines (GAP) (World Health Organization, 2015c). The Biovac Institute (www.biovac.co.za) in Cape Town, South Africa, has developed the capacity for multi-product formulation and filling, including that for imported influenza vaccine. A licence to manufacture IIV is anticipated this year (Schoub et al., 2013). Additional challenges in vaccine cold chain storage, distribution and handling delays in importation will need to be considered. Lastly, the requirement for annual vaccination renders it financially and logistically unfeasible in most African settings.

Given the challenges highlighted above, what are the realistic prospects of influenza vaccine becoming available in SSA? In developing countries, access to new and underused vaccines has been hugely improved over the past 15 years by the launch of the Global Alliance for Vaccines and

Immunisation (GAVI), a unique public-private global health partnership[ix] whose mission is to save children's lives and protect people's health by increasing access to immunisation in poor countries. At present, GAVI supports 11 vaccines (including PCV, rotavirus, and polio) in 72 countries. Although its main focus has been on childhood vaccination, GAVI explored the possibility of funding influenza vaccination in pregnant women as part of their Vaccine Investment Strategy in 2013 (GAVI the Vaccine Alliance, 2013), but are awaiting further data on the impact of maternal immunisation in developing settings (Section 7.6).

#### *7.4.3.4 Alternative strategies to prevent influenza in HIV-infected populations*

Strategies to boost sub-optimal immune responses in HIV-infected individuals have been explored, particularly during the 2009 influenza A(H1N1) pandemic. Administration of higher or more frequent doses of standard non-adjuvanted vaccine preparations in HIV-positive adults was not consistently associated with improved immunogenicity (Bickel et al., 2011; Cooper et al., 2011; El Sahly et al., 2012; Hatakeyama et al., 2011; McKittrick et al., 2013; Nielsen et al., 2012), though enhanced responses (Ho et al., 2011; Launay et al., 2011; Iorio et al., 2003) have been observed with novel adjuvanted preparations.

As stated early, the requirement for annual vaccination represent a major hurdle for the introduction of current influenza vaccines in SSA. Therefore new vaccines with better efficacy, and more prolonged and heterosubtypic protection may be valuable in African settings. Researchers have recently engineered several novel vaccine candidates targeting the stem of the HA molecule, which is similar across different strains and mutates much less readily than the head. These vaccine candidates have demonstrated broad protection against multiple influenza strains in mice and non-human primates (Impagliazzo et al., 2015; Yassine et al., 2015). This exciting development suggests we may be one step closer to a universal influenza vaccine that protects against all seasonal and pandemic strains, and eliminate the need for annual vaccination.

PCV vaccination in HIV-infected adults could be an alternative approach to prevent influenza-related complications, since secondary pneumococcal pneumonia is a common severe complication. PCV was efficacious against recurrent pneumococcal infection in HIV-infected Malawian adults (French et al., 2010). As discussed earlier, a probe study of PCV9 in South Africa children demonstrated a 45% efficacy against influenza-associated pneumonia hospitalization. A similar study could be undertaken in HIV-infected adults. However, the herd protection arising from the widespread introduction of PCV13 in SSA (Hammitt et al., 2014) is likely to limit the impact of PCV13 in lessening pneumococcal

[ix] Includes developing country and donor governments, the WHO, UNICEF, the World Bank, research and technical agents, the vaccine industry, the Bill & Melinda Gates Foundation and other philanthropic organisations.

burden in adults.

#### *7.4.3.5 Strategies against poverty*

The identification of household crowding, poor sanitation and food insecurity as risk factors for influenza highlight the importance of ongoing public health interventions to alleviate hunger and poverty, and to improve access to clean water and sanitation (United Nations, 2015b). Many of these factors are likely to coexist in persons living in SSA. Nearly half of the case control study participants had unimproved[x] sanitation, which is comparable to the country estimates by the l WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation. Water supply and sanitation facilities have seen substantial improvements in rural parts of Malawi over the past 25 years, but they have remained unchanged in the urban areas (WHO/UNICEF, 2015).

Food insecurity emerged as a previously unrecognised risk factor for influenza. Since this exposure was derived from a single question – "How often have you had difficulty accessing food?", more in-depth evaluation of food insecurity (Jones et al., 2013) and its association with influenza and other respiratory infections are warranted.

Great strides have been made to alleviate hunger on the continent, through national and regional initiatives to improve food availability, access, as well as food stability and utilisation. The proportion of undernourished persons in Malawi has fallen from 44.7 to 20.7% in the past 25 years, making it one of 18 SSA countries to have successfully achieved the MDG target of halving the number of persons suffering from hunger. Nevertheless, one in five in the Malawian population are still undernourished. This finding, if confirmed, could have important policy implications. Current WHO guidelines for seasonal influenza vaccination do not include undernourished persons in the list of high-risk groups, but the new findings would perhaps support its inclusion.

### 7.5 Outstanding investigations

Several planned aspects of work are not covered in this thesis. Firstly, paired acute and convalescent sera were collected from cohort participants during, and 4 to 6 weeks after ILI presentation for haemagglutination inhibition (HAI) antibody titres. We aim to correlate PCR with serology results, in particular, to establish influenza diagnosis in patients presenting with ILI who may have stopped shedding virus at the time of nasopharyngeal sampling, thus could have been missed by PCR testing. The HAI assays are due to be processed at the National Center for Immunization and Respiratory Diseases Laboratory, CDC, Atlanta, US, at the end of 2015.

[x]Sanitation facilities that do not ensure a hygienic separation of human excreta from human contact, including pit latrines without slabs or open pit, hanging or bucket latrines, and open defecation.

Secondly, we aim to ascertain the background prevalence of respiratory viruses in asymptomatic Malawian adults during the study period. From the 3094 combined NP/OP specimens taken at routine visits of cohort participants (when they were asymptomatic), 15 samples from HIV-infected participants, and 15 from HIV-uninfected participants per calendar month will be randomly selected, and tested for adenovirus, bocavirus, coronaviruses OC43/NL63/229E/HKU1, enterovirus, hMPV, influenza A & B, parainfluenza viruses 1-4, rhinovirus, and RSV, using the CDC Human Influenza qRT-PCR diagnostic panel (CDC Influenza division) and Fast-track Diagnostics® (FTD) Respiratory 33 kit (Fast-track Diagnostics Ltd., Luxembourg) (Chapter 2.2.7). These 'controls' will be compared to the prevalence of viruses identified from i) the ILI presentations by cohort participants, and ii) the patients hospitalised with LRTI in the case control study. The comparison will allow a better understanding of the causative role of these respiratory viruses in mild and severe acute respiratory presentations.

## 7.6 Future directions

From the findings presented in this thesis, important research questions that have arisen include:

1. Where do HIV-infected individuals contract influenza from?
2. What is the most effective strategy to reduce the burden of influenza in resource-limited settings in SSA?

This thesis has established a high burden of infection and severe disease in HIV-infected Malawian adults. However, young children (<5 years) and pregnant women are the other key target groups in SSA. In light of limited resources and competing health needs, decisions are required as to which at-risk groups should be prioritised. Factors that require consideration include the contribution of the risk group to the overall influenza disease burden in the population, disease severity within individual risk groups, vaccine effectiveness, feasibility of delivery, likely acceptability of vaccine, indirect effects of vaccination, as well as cost-effectiveness (Table 7.1). The goal is to develop influenza vaccine policies that maximise health benefit through efficient use of limited resources.

Children under 5 years of age experience the highest burden of influenza infection and illness, compared to other age groups (Nair et al., 2011; Cohen et al., 2013). They also believed to be the principal transmitters of influenza in the community (Viboud et al., 2004; Cauchemez et al., 2008). Furthermore, there is evidence of more intense and prolonged viral shedding in children (Hall et al., 1979; Frank et al., 1981), and for frequent contacts among children as well as between children and adults (Mossong et al., 2008). Therefore vaccinating children may efficiently disrupt the transmission and spread of influenza, conferring indirect protection of close household contacts as well as the

wider community (Vynnycky et al., 2008). Indeed, dynamic transmission models have suggested that the extension of influenza vaccination to include children of all ages, instead of restricting to those with clinical risk, is cost-effective due to the anticipated herd protection (Pitman et al., 2012; Baguelin et al., 2012). As a result, several countries, including the US (Fiore et al., 2010) and UK (Joint Committee on Vaccination and Immunisation, 2012), have since adopted universal childhood influenza vaccination.

With regards to SSA, a cluster-randomised trial (CRT) of IIV3 vs. control vaccine in children aged 6 months to 10 years was undertaken in Senegal between 2009 and 2011 (Diallo, 2014a). Preliminary results suggest moderate overall vaccine effectiveness (44%, 95%CI 16-63%); VE was higher among older (6-10 years) compared to younger (<5 years) children (VE 60-64% vs. 25%). Although vaccination programmes are in place, immunising children against influenza will present logistical challenges. Firstly, they are the largest of the three risk groups. Secondly, LAIV is the preferred vaccine for children in developed settings due to superior efficacy compared to IIV3 (Ambrose et al., 2012), as well as observed indirect protection to older children when delivered through schools (Glezen et al., 2010). However, only one study in Senegal has evaluated LAIV in children (aged 2-6 years). Initial report suggested that LAIV was well-tolerated, but efficacy data are awaited (Diallo, 2014b). In the UK, LAIV is not recommended in HIV-infected children and adolescents who are not established on ART or those with advanced immunosuppression (England, 2013). In terms of IIV, children <9 years are recommended two doses due to inadequate immune response (Grohskopf et al., 2015). Furthermore, an RCT of IIV3 in HIV-infected children aged 6 months to 5 years in South Africa demonstrated poor immunogenicity and efficacy (Madhi et al., 2013). Thus further studies on the role of LAIV and adjuvanted preparations of IIV in protecting children in SSA against influenza are needed.

**Table 7.1 Identification of current knowledge and data gaps among the key clinical risk groups in SSA**

| | Target population | | |
|---|---|---|---|
| | **Children (<5 years)** | **Pregnant women** | **HIV-infected adults** |
| **Estimated population size in Malawi** | ~2,860,000 (UNICEF, 2012) | ~113,120[a] | 930,000 (UNICEF, 2012) |
| **Disease burden** | High | High | High |
| **Vaccine efficacy/ effectiveness** | 44% (16-63%) (Diallo, 2014a) | HIV- 50.4% [14.5-71.1%] (Madhi et al., 2014)<br>HIV+ 57.7% [0.2-82.1%]<br>Await Mali trial results | 75.5% [9.2-95.6%][b] (Madhi et al., 2011) |
| **Public health impact** | NA | To be addressed by RCTs in Mali and South Africa | NA |
| **Cost-effectiveness** | NA | NA | NA |
| **Pros** | - Existing vaccination programme<br>- Evidence of indirect protection | - Existing vaccination programme<br>- Increase birth weight<br>- Protect infants < 6 months | - May reduce community and nosocomial transmission (i.e. indirect protection) |
| **Cons** | - Poor immunogenicity in younger children (require a 2nd dose) | - Require year-round IIV supply | - No existing vaccination programme<br>- Poor immunogenicity in those with advanced immunosuppression |

NA, not assessed

[a]Estimated from number of women of childbearing age (15-49 years) that are HIV-infected (n=560,000) (UNAIDS, 2014b) multiplied by general fertility rate, i.e. the number of total live births per 1000 women of reproductive age, in Malawi (202 per 1,000 women(National Statistics Office (NSO) and ICF Macro, 2011)). This is likely to represent an underestimate as the survey may not have captured all pregnancies (e.g. some women may not have known they were pregnant at the time of survey), and a proportion of pregnancies do not result in live births.

[b]Excluded individuals with co-morbidities or CD4+ cell count < 100 cells/μl naïve to ART.

With regards to pregnant women, there is compelling evidence of substantially greater risk of severe disease and mortality in this group, both in developed settings (Neuzil et al., 1998; Callaghan et al., 2010; Louie et al., 2010; Jamieson et al., 2009) and in SSA (Archer et al., 2009; Tempia et al., 2015a), hence they are deemed the highest priority for vaccination (World Health Organization, 2012b). Vaccination of pregnant women have the additional benefits of protecting against adverse birth outcomes such as prematurity and small for gestational age (SGA) (Steinhoff et al., 2012), as well as protecting infants less than 6 months, who have high disease burden but are ineligible for influenza vaccine (Englund et al., 1993; Zaman et al., 2008). On one hand, antenatal clinics in Malawi and other SSA settings have substantial experience in administering tetanus toxoid vaccine in pregnancy. On the other hand, careful consideration of supply chain logistics will be needed as maternal immunisation will require a year-round supply of IIV, which the vaccine industry is not currently set up to provide. It may also necessitate a mid-year switch in formulation. Furthermore, the acceptability of the vaccine among pregnant women in SSA should be explored.

Three RCTs evaluated the safety and efficacy of maternal IIV3 immunisation in preventing maternal and infant (<6 months) influenza disease in Nepal, Mali and South Africa between 2011 and 2013 (Omer et al., 2015). The South African study was recently published, while results from the other two trials are anticipated at the end of 2015. Madhi *et al*. (Madhi et al., 2014) demonstrated partial protection of IIV3 against confirmed influenza in HIV-uninfected pregnant women (VE 50.4%, 95%CI 14.5-71.1%), as well as HIV-infected women (VE 57.7%, 95%CI 0.2-82.1%). Pooled analyses of data from the three trials are planned to assess outcomes for which the individual trials may not have been powered, including the impact of maternal IIV3 vaccination on neonatal outcomes, such as pre-term and SGA births, infant growth, infant pneumonia, maternal and infant mortality, as well as indirect effects on household contacts (Omer et al., 2015). These results will inform GAVI's decision regarding the adoption of maternal influenza immunization in developing countries. Pregnant women could potentially be the first clinical risk group to receive targeted influenza immunisation in SSA.

From the above, it appears that the evidence base for immunising young children and HIV-infected adults against influenza require strengthening before comparison of potential vaccine impact can be made between the risk groups. Since this thesis has confirmed elevated disease burden and severity in HIV-infected adults, key data gaps include the potential public health impact of targeted influenza vaccination in HIV-infected adults, as well as the feasibility of vaccine delivery at ART clinic, clinical effectiveness of IIV among individuals with advanced immunosuppression ± co-morbidities, and cost-effectiveness. A community cluster-randomised vaccine probe study of IIV in HIV-infected adults could efficiently address most of these gaps. Vaccine probe studies utilise a randomised

clinical trial design to evaluate the impact of a vaccine against a wide range of clinical syndromes associated with a particular infection for which aetiological confirmation may be difficult (Feikin et al., 2014) (Figure 7.2). This is particularly applicable to influenza, as it can cause asymptomatic infection, respiratory presentations of varying severity, in addition to increased susceptibility to secondary bacterial pneumonia. The impact of IIV on severe disease (e.g. hospitalisation) and mortality end-points could be studied, provided there is an adequate sample size. Probe studies therefore allows estimation of the absolute burden of disease that is preventable by vaccine (Feikin et al., 2014), i.e. the vaccine preventable disease incidence (VPDI), by deriving the difference in disease incidence between vaccinated and unvaccinated persons.

Furthermore, a household transmission study could also be nested within the vaccine trial, in order to gain a better understanding of the effect of HIV on the viral shedding and transmission dynamics of influenza, as well as estimating the potential indirect benefit from reduced community transmission of influenza by HIV-infected adults. Other factors that may affect influenza transmission, such as the effects of season, household size, age of household members, as well as magnitude and duration of viral shedding, can be evaluated concurrently. These data will inform transmission models in SSA settings of high HIV prevalence.

Madhi *et al.* (Madhi and Klugman, 2004) have probed the role of pneumococcal super-infections in virus-associated pneumonia. South African children vaccinated with a 9-valent PCV were associated with a 45% reduction in influenza-associated pneumonia hospitalization, thus confirming that a significant fraction of severe influenza presentation is attributable to pneumococcal co-infection. This interaction can alternatively be explored by using IIV to probe the contribution of influenza in the pathogenesis of culture-confirmed pneumococcal pneumonia.

Vaccine probe studies have been instrumental in driving public health decisions regarding *Haemophilus influenzae* type b (Hib), pneumococcal (Cutts et al., 2005), as well as rotavirus (Feikin et al., 2012a) vaccines. Our proposed cluster-randomised vaccine probe study of IIV in HIV-infected adults could provide a clear picture of the overall preventable disease burden and hence the public health value of the vaccine. In a setting with a high background incidence of influenza and severe respiratory disease, such as Malawi, even a vaccine with a relatively low VE can lead to a large vaccine-preventable disease burden and thus targeted vaccine introduction could be justified (Feikin et al., 2014).

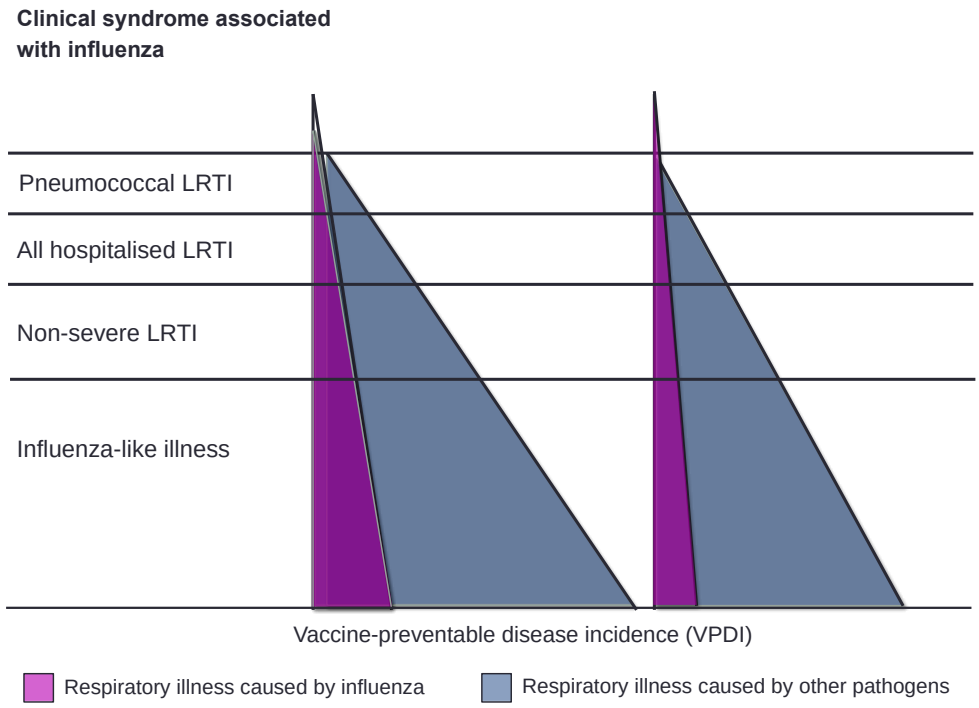



**Figure 7.2 Proportions of different clinical syndromes attributable to influenza (left) and the effect of inactivated influenza vaccine (right).** Adapted from Feikin *et al*. (Feikin et al., 2014)
LRTI, lower respiratory tract infection

## 7.7 Conclusion

This study has demonstrated that the high prevalence of HIV infection and poverty-related factors, characteristics specific to sub-Saharan Africa, render the epidemiology of influenza in the region distinct from other parts of the world. In an urban setting with high HIV prevalence, we have clearly shown that HIV infection increases the susceptibility to influenza infection, as well as the risk of severe influenza disease. Individuals with advanced immunosuppression may be particularly vulnerable. Reasons for the elevated risk in HIV-infected individuals are likely to be multifactorial, and may include impaired immune response to influenza, higher prevalence of co-morbid conditions, greater susceptibility to co-existent or secondary bacterial infection, in addition to higher prevalence of other identified risk factors, such as household crowding, sanitation and food insecurity. All these factors are likely to be exacerbated by the continuing population expansion on the continent (Population Reference Bureau, 2014).

The findings from this thesis suggest that influenza preventative strategies should be an important aspect of the management of HIV-infected adults in SSA. Inactivated vaccine would be the obvious

choice to prevent influenza infection and its complications in this risk group, but the high cost, logistical, programmatic and production difficulties, and inadequate data on clinical efficacy in persons with advanced immunosuppression, as well as public health impact of targeted influenza vaccination in HIV-infected adults represent important barriers to its introduction. Further studies to improve this knowledge are needed in Malawi and other high HIV prevalence settings.

# CHAPTER 8. APPENDICES

## 8.1 Publications arising from PhD

**Viral pneumonia in adults in sub-Saharan Africa – epidemiology, aetiology, diagnosis and management**
**Ho A**. *Pneumonia* 2014; 5:18-29

**British HIV Association Guidelines on the use of vaccines in HIV-positive adults 2015**
Geretti AM, Brook G, Cameron C, Chadwick D, French N, Heyderman R, **Ho A**, Hunter M, Ladhani S, Lawton M, MacMahon E, McSorley J, Pozniak A, Rodger A. www.bhiva.org/Guidelines. HIV Medicine (in press)

## 8.2 Manuscripts submitted and in preparation

**The Epidemiology and Risk Factors of Influenza in Malawian Adults with High HIV Prevalence following the 2009 AH1N1 Pandemic, 2011-2013**
**Ho A**, Mallewa J, Peterson I, SanJoaquin M, Garg S, Bar-Zeev N, Menyere M, Alaerts M, Mapurisa G, Chilombe M, Nyirenda M, Lalloo DG, Rothe C, Widdowson MA, McMorrow M, French N, Everett D, Heyderman RS.

**Predictors of mortality among Malawian infants hospitalized with pneumonia**
Newberry N, Pollock L, **Ho A**, Heyderman R, Peterson I, Everett D, McMorrow M, Bar-Zeev N.

**Coronavirus Surveillance in Malawi: No MERS-CoV but temperature and age associated hCoV infections in SARI patients**
Alaerts M, Nagelkerke N, Peterson I, Bar-Zeev N, **Ho A**, Aston S, Peno C, Sisya T, Chammudzi P, Brown C, Chinyama E, Mkandawire C, French N, McMorrow M, Heyderman RS, Everett DB

## 8.3 Patient consent and case report forms (see attached CD)

### 8.3.1 Influenza surveillance study

i. Consent form (English & Chichewa)
ii. Case report forms

### 8.3.2 Cohort study

i. Consent form (English & Chichewa)

ii. Case report forms

### 8.3.3 Case control study

i. Consent form – Hospitalised LRTI patients (English & Chichewa)

ii. Consent form – ILI patients (English & Chichewa)

iii. Case report forms – Hospitalised LRTI & ILI patients[xi]

## 8.4 Missing values

### 8.4.1 Influenza surveillance study

| Variable | N missing | % missing |
|---|---|---|
| **Table 3.3** | | |
| HIV status (SARI patients only) | 17 | 1.5 |
| Pregnancy | 54 | 3.2 |
| Current smoker | 49 | 2.9 |
| Antibiotics in past 2 weeks | 53 | 3.1 |
| Reported influenza vaccination in past year | 47 | 2.8 |

### 8.4.2 Cohort study

| Variable | N missing | % missing |
|---|---|---|
| **Table 4.2** | | |
| Drinks alcohol | 2 | 0.3 |
| Highest level of education | 1 | 0.2 |
| Children <5 years in household | 3 | 0.5 |
| Individuals > years in household | 1 | 0.2 |
| Crowding index | 2 | 0.3 |
| Toilet | 1 | 0.2 |
| Water supply | 8 | 1.3 |
| Principal cooking fuel | 6 | 1.0 |
| Highest level of education | 1 | 0.2 |
| Employment | 4 | 0.7 |
| Food security | 5 | 0.8 |
| **Table 4.3** | | |
| CD4 at enrolment (HIV+ only) | 3 | 0.8 |

[xi] Same CRFs were used for hospitalised LRTI and ILI patients, except for eligibility criteria form.

| | | |
|---|---|---|
| On co-trimoxazole prophylaxis at enrolment | 2 | 0.6 |
| **Table 4.4** | | |
| Cough | 3 | 0.9 |
| Sore throat | 3 | 0.9 |
| Rhinorrhoea | 3 | 0.9 |
| Myalgia | 3 | 0.9 |
| Nausea/vomiting | 3 | 0.9 |
| Shortness of breath | 3 | 0.9 |
| Diarrhoea | 3 | 0.9 |
| Headache | 3 | 0.9 |
| Temperature (°c) | 4 | 1.2 |
| Heart rate | 4 | 1.2 |
| Respiratory rate | 4 | 1.2 |
| Oxygen saturation | 3 | 0.9 |
| Upper respiratory tract signs | 3 | 0.9 |
| Lower respiratory tract signs | 3 | 0.9 |
| **Table 4.6** | | |
| Other respiratory viruses[xii] | 3 | 0.9 |
| Malaria RDT | 1 | 0.3 |

### 8.4.3 Case control study

| Variable | N missing | % missing |
|---|---|---|
| **Table 5.2** | | |
| Attended another health facility | 11 | 1.0 |
| Antibiotics within 2 weeks | 21 | 1.8 |
| Antimalarials within 2 weeks | 19 | 1.6 |
| Traditional medicine within 2 weeks | 20 | 1.7 |
| Known HIV+ at enrolment (in HIV+) | 4 | 0.7 |
| On ART at enrolment (in HIV+) | 18 | 3.1 |
| CD4 count (in HIV+) | 58 | 9.9 |
| Medical history[xii] | 22 | 1.9 |
| Past history of tuberculosis | 8 | 0.7 |
| Pneumonia within last 5 years | 10 | 0.9 |
| Pregnant | 10 | 0.9 |
| Smoking - current | 9 | 0.8 |
| Alcohol | 10 | 0.9 |
| Children <5 years | 9 | 0.8 |
| Crowding index | 51 | 4.4 |
| Highest level of education | 1 | 0.2 |
| Water supply | 8 | 0.7 |
| Distance to water supply | 8 | 0.7 |
| Toilet | 9 | 0.8 |
| Principal cooking fuel | 8 | 0.7 |
| Highest level of education | 14 | 1.2 |
| Employment status | 21 | 1.8 |

[xii] Including chronic lung/cardiac/kidney/liver disease, hypertension, cancer, and stroke.

| | | |
|---|---|---|
| Asset ownership | 7 | 0.6 |
| Food security | 8 | 0.7 |
| **Table 5.3** | | |
| Cough | 9 | 0.8 |
| Sputum | 9 | 0.8 |
| Shortness of breath | 9 | 0.8 |
| Chest pain | 10 | 0.9 |
| Myalgia | 9 | 0.8 |
| Headache | 9 | 0.8 |
| Rhinorrhoea | 9 | 0.8 |
| Sore throat | 9 | 0.8 |
| Nausea/vomiting | 11 | 1.0 |
| Diarrhoea | 12 | 1.0 |
| Weight loss | 9 | 0.8 |
| Night sweats | 11 | 1.0 |
| Skin rash | 9 | 0.8 |
| Disorientation | 19 | 1.6 |
| Temperature | 8 | 0.7 |
| Systolic/diastolic blood pressure | 8 | 0.7 |
| Heart rate | 9 | 0.8 |
| Respiratory rate | 26 | 2.2 |
| Oxygen saturation | 18 | 1.6 |
| Unable to stand | 13 | 1.1 |
| BMI | 27 | 2.3 |
| MUAC | 19 | 1.6 |
| Red throat | 12 | 1.0 |
| Pus in throat | 12 | 1.0 |
| Cervical lymphadenopathy | 17 | 1.5 |
| Sinus tenderness | 19 | 1.6 |
| Crepitations | 12 | 1.0 |
| Bronchial breathing | 11 | 1.0 |
| Effusion | 10 | 0.9 |
| Pleural rub | 12 | 1.0 |
| Wheeze | 11 | 1.0 |
| Finger clubbing | 14 | 1.2 |
| Pale conjunctivae | 13 | 1.1 |
| Oral candidiasis | 15 | 1.3 |
| Kaposi's sarcoma lesions | 14 | 1.2 |
| Jaundice | 16 | 1.4 |
| Hepatomegaly | 16 | 1.4 |
| Splenomegaly | 16 | 1.4 |
| Pedal oedema | 18 | 1.6 |
| **Table 5.10** | | |
| CD4 count (in HIV+) | 4 | 6.2 |
| On ART at enrolment (in HIV+) | 2 | 3.1 |
| Pregnant | 2 | 1.4 |
| BMI | 3 | 2.1 |
| Crowding index | 10 | 6.9 |
| Highest level of education | 1 | 0.7 |
| Employment status | 3 | 2.1 |

### 8.4.4 Association between respiratory viral infection and pneumococcal carriage

| **Table 6.2** | | |
|---|---|---|
| CD4 count (in HIV+) | 1 | 1.0 |

# REFERENCES

(1920) *Report on the pandemic of influenza 1918-19. Report No. 4,* London: Minstry of Health.

(1980) A revision of the system of nomenclature for influenza viruses: a WHO memorandum. *Bull World Health Organ* 58: 585-591.

(1988) Increase in pneumonia mortality among young adults and the HIV epidemic--New York City, United States. *MMWR Morb Mortal Wkly Rep* 37: 593-596.

(2002) Influenza outbreak--Madagascar, July-August 2002. *MMWR Morb Mortal Wkly Rep* 51: 1016-1018.

(2003) Influenza outbreak in the district of Bosobolo, Democratic Republic of the Congo, November-December 2002. *Wkly Epidemiol Rec* 78: 94-96.

(2009a) Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - United States, May-August 2009. *MMWR Morb Mortal Wkly Rep* 58: 1071-1074.

(2009b) Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients - Seattle, Washington, 2009. *MMWR Morb Mortal Wkly Rep* 58: 893-896.

(2010) Update: influenza activity - United States, 2009-10 season. *MMWR Morb Mortal Wkly Rep* 59: 901-908.

(2012) Vaccines against influenza WHO position paper - November 2012. *Wkly Epidemiol Rec* 87: 461-476.

(2013) Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices--United States, 2013-2014. *MMWR Recomm Rep* 62: 1-43.

Abdeldaim GM, Stralin K, Olcen P, et al. (2008) Toward a quantitative DNA-based definition of pneumococcal pneumonia: a comparison of Streptococcus pneumoniae target genes, with special reference to the Spn9802 fragment. *Diagn Microbiol Infect Dis* 60: 143-150.

Abdullahi O, Karani A, Tigoi CC, et al. (2012) The prevalence and risk factors for pneumococcal colonization of the nasopharynx among children in Kilifi District, Kenya. *PLoS One* 7: e30787.

Abdullahi O, Nyiro J, Lewa P, et al. (2008) The descriptive epidemiology of Streptococcus pneumoniae and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi district, Kenya. *Pediatr Infect Dis J* 27: 59-64.

Abdullahi O, Wanjiru E, Musyimi R, et al. (2007) Validation of nasopharyngeal sampling and culture techniques for detection of Streptococcus pneumoniae in children in Kenya. *J Clin Microbiol* 45: 3408-3410.

Abzug MJ, Nachman SA, Muresan P, et al. (2013) Safety and immunogenicity of 2009 pH1N1 vaccination in HIV-infected pregnant women. *Clin Infect Dis* 56: 1488-1497.

Aderaye G. (1994) The etiology of community acquired pneumonia in adults in Addis Ababa. *West Afr J Med* 13: 142-145.

Ahmed JA, Katz MA, Auko E, et al. (2012) Epidemiology of respiratory viral infections in two long-term refugee camps in Kenya, 2007-2010. *BMC Infect Dis* 12: 7.

Aiello AE, Coulborn RM, Perez V, et al. (2008) Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. *Am J Public Health* 98: 1372-1381.

Albrich WC, Madhi SA, Adrian PV, et al. (2012) Use of a rapid test of pneumococcal colonization density to diagnose pneumococcal pneumonia. *Clin Infect Dis* 54: 601-609.

Almirall J, Boixeda R, Bolibar I, et al. (2007) Differences in the etiology of community-acquired pneumonia according to site of care: a population-based study. *Respir Med* 101: 2168-2175.

Althoff K, Gange S and Sharp G. (2010) Elevated 2009 H1N1 antibody titers in HIV-infected and unifnected women in the US; a sero-study, March 1 to September 2009 (#807). *Conference on Retroviruses and Opportunistic Infections.* San Francisco, USA.

Althoff KN, Eichelberger M, Gange SJ, et al. (2011) Seroincidence of 2009 H1N1 infection in HIV-infected and HIV-uninfected women prior to vaccine availability. *AIDS* 25: 1229-1232.

Alymova IV, Portner A, Takimoto T, et al. (2005) The novel parainfluenza virus hemagglutinin-neuraminidase inhibitor BCX 2798 prevents lethal synergism between a paramyxovirus and Streptococcus pneumoniae. *Antimicrob Agents Chemother* 49: 398-405.

Ambrose CS, Luke C and Coelingh K. (2008) Current status of live attenuated influenza vaccine in the United States for seasonal and pandemic influenza. *Influenza Other Respir Viruses* 2: 193-202.

Ambrose CS, Wu X, Knuf M, et al. (2012) The efficacy of intranasal live attenuated influenza vaccine in children 2 through 17 years of age: a meta-analysis of 8 randomized controlled studies. *Vaccine* 30: 886-892.

Amendola A, Boschini A, Colzani D, et al. (2001) Influenza vaccination of HIV-1-positive and HIV-1-negative former intravenous drug users. *J Med Virol* 65: 644-648.

Ampofo K, Gesteland PH, Bender J, et al. (2006) Epidemiology, complications, and cost of hospitalization in children with laboratory-confirmed influenza infection. *Pediatrics* 118: 2409-2417.

Andayi F, Crepey P, Kieffer A, et al. (2014) Determinants of individuals' risks to 2009 pandemic influenza virus infection at household level amongst Djibouti city residents--a CoPanFlu cross-sectional study. *Virol J* 11: 13.

Anema A, Mills E, Montaner J, et al. (2008) Efficacy of influenza vaccination in HIV-positive patients: a systematic review and meta-analysis. *HIV Med* 9: 57-61.

Angeles Marcos M, Camps M, Pumarola T, et al. (2006) The role of viruses in the aetiology of community-acquired pneumonia in adults. *Antivir Ther* 11: 351-359.

Antes G and Meerpohl JJ. (2014) Statistical and methodological concerns about the beneficial effect of neuraminidase inhibitors on mortality. *Lancet Respir Med* 2: e10.

Aoki FY, Macleod MD, Paggiaro P, et al. (2003) Early administration of oral oseltamivir increases the benefits of influenza treatment. *J Antimicrob Chemother* 51: 123-129.

Arcavi L and Benowitz NL. (2004) Cigarette smoking and infection. *Arch Intern Med* 164: 2206-2216.

Archer B, Cohen C, Naidoo D, et al. (2009) Interim report on pandemic H1N1 influenza virus infections in South Africa, April to October 2009: epidemiology and factors associated with fatal cases. *Euro Surveill* 14: 1-5.

Archer BN, Timothy GA, Cohen C, et al. (2012) Introduction of 2009 pandemic influenza A virus subtype H1N1 into South Africa: clinical presentation, epidemiology, and transmissibility of the first 100 cases. *J Infect Dis* 206 Suppl 1: S148-153.

Arden KE, Nissen MD, Sloots TP, et al. (2005) New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. *J Med Virol* 75: 455-462.

Ayala E, Kagawa FT, Wehner JH, et al. (2009) Rhabdomyolysis associated with 2009 influenza A(H1N1). *JAMA* 302: 1863-1864.

Azziz Baumgartner E, Dao CN, Nasreen S, et al. (2012) Seasonality, timing, and climate drivers of influenza activity worldwide. *J Infect Dis* 206: 838-846.

Bagheri Nejad S, Allegranzi B, Syed SB, et al. (2011) Health-care-associated infection in Africa: a systematic review. *Bull World Health Organ* 89: 757-765.

Baguelin M, Jit M, Miller E, et al. (2012) Health and economic impact of the seasonal influenza vaccination programme in England. *Vaccine* 30: 3459-3462.

Balkovic ES, Goodman RA, Rose FB, et al. (1980) Nosocomial influenza A (H1N1) infection. *Am J Med Technol* 46: 318-320.

Bank W. (2015) *Incidence of tuberculosis (per 100,000 people)*. Available at: http://data.worldbank.org/country/malawi.

Barakat A, Benjouad A, Manuguerra JC, et al. (2011a) Virological surveillance in Africa can contribute to early detection of new genetic and antigenic lineages of influenza viruses. *J Infect Dev Ctries* 5: 270-277.

Barakat A, Ihazmad H, Benkaroum S, et al. (2011b) Influenza surveillance among outpatients and inpatients in Morocco, 1996-2009. *PLoS One* 6: e24579.

Barakat A, Ihazmad H, El Falaki F, et al. (2012) 2009 Pandemic influenza A virus subtype H1N1 in Morocco, 2009-2010: epidemiology, transmissibility, and factors associated with fatal cases. *J Infect Dis* 206 Suppl 1: S94-100.

Baxter R, Ray GT and Fireman BH. (2010) Effect of influenza vaccination on hospitalizations in persons aged 50 years and older. *Vaccine* 28: 7267-7272.

Bell D, Nicoll A, Fukuda K, et al. (2006) Non-pharmaceutical interventions for pandemic influenza, international measures. *Emerg Infect Dis* 12: 81-87.

Belshe RB, Edwards KM, Vesikari T, et al. (2007) Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med* 356: 685-696.

Benito N, Moreno A, Miro JM, et al. (2012) Pulmonary infections in HIV-infected patients: an update in the 21st century. *Eur Respir J* 39: 730-745.

Beran J, Peeters M, Dewe W, et al. (2013) Immunogenicity and safety of quadrivalent versus trivalent inactivated influenza vaccine: a randomized, controlled trial in adults. *BMC Infect Dis* 13: 224.

Berendt RF, Long GG and Walker JS. (1975) Influenza alone and in sequence with pneumonia due to Streptococcus pneumoniae in the squirrel monkey. *J Infect Dis* 132: 689-693.

Berkson J. (1946) Limitations of the application of fourfold table analysis to hospital data. *Biometrics* 2: 47-53.

Besselaar TG, Botha L, McAnerney JM, et al. (2004) Antigenic and molecular analysis of influenza A (H3N2) virus strains isolated from a localised influenza outbreak in South Africa in 2003. *J Med Virol* 73: 71-78.

Bickel M, von Hentig N, Wieters I, et al. (2011) Immune response after two doses of the novel split virion, adjuvanted pandemic H1N1 influenza A vaccine in HIV-1-infected patients. *Clin Infect Dis* 52: 122-127.

Bierbaum S, Forster J, Berner R, et al. (2014) Detection of respiratory viruses using a multiplex real-time PCR assay in Germany, 2009/10. *Arch Virol* 159: 669-676.

Biggerstaff M, Cauchemez S, Reed C, et al. (2014) Estimates of the reproduction number for seasonal, pandemic, and zoonotic influenza: a systematic review of the literature. *BMC Infect Dis* 14: 480.

Bisno AL, Griffin JP, Van Epps KA, et al. (1971) Pneumonia and Hong Kong influenza: a prospective study of the 1968-1969 epidemic. *Am J Med Sci* 261: 251-263.

Boehme CC, Nabeta P, Hillemann D, et al. (2010) Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 363: 1005-1015.

Bogaert D, De Groot R and Hermans PW. (2004) Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *Lancet Infect Dis* 4: 144-154.

Boivin G, Goyette N and Bernatchez H. (2002) Prolonged excretion of amantadine-resistant influenza a virus quasi species after cessation of antiviral therapy in an immunocompromised patient. *Clin Infect Dis* 34: E23-25.

Boschini A, Longo B, Caselli F, et al. (2006) An outbreak of influenza in a residential drug-rehabilitation community. *J Med Virol* 78: 1218-1222.

Bosis S, Esposito S, Osterhaus AD, et al. (2008) Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. *J Clin Virol* 42: 286-290.

Boulle A, Schomaker M, May MT, et al. (2014) Mortality in patients with HIV-1 infection starting antiretroviral therapy in South Africa, Europe, or North America: a collaborative analysis of prospective studies. *PLoS Med* 11: e1001718.

Boulware DR, Daley CL, Merrifield C, et al. (2007) Rapid diagnosis of pneumococcal pneumonia among HIV-infected adults with urine antigen detection. *J Infect* 55: 300-309.

Bowie C and Prothero D. (1981) Finding causes of seasonal diseases using time series analysis. *Int J Epidemiol* 10: 87-92.

Bradshaw BS, Smith DW and Blanchard S. (2008) A cohort study of tuberculosis and influenza mortality in the twentieth century. *Biodemography Soc Biol* 54: 74-94.

Brankston G, Gitterman L, Hirji Z, et al. (2007) Transmission of influenza A in human beings. *Lancet Infect Dis* 7: 257-265.

Bridges CB, Thompson WW, Meltzer MI, et al. (2000) Effectiveness and cost-benefit of influenza vaccination of healthy working adults: A randomized controlled trial. *JAMA* 284: 1655-1663.

Brimblecombe FS, Cruickshank R, Masters PL, et al. (1958) Family studies of respiratory infections. *Br Med J* 1: 119-128.

Brundage JF. (2006) Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infect Dis* 6: 303-312.

Brundage JF and Shanks GD. (2007) What really happened during the 1918 influenza pandemic? The importance of bacterial secondary infections. *J Infect Dis* 196: 1717-1718; author reply 1718-1719.

Brundage JF and Shanks GD. (2008) Deaths from bacterial pneumonia during 1918-19 influenza pandemic. *Emerg Infect Dis* 14: 1193-1199.

Bulimo WD, Achilla RA, Majanja J, et al. (2012) Molecular characterization and phylogenetic analysis of the hemagglutinin 1 protein of human influenza A virus subtype H1N1 circulating in Kenya during 2007-2008. *J Infect Dis* 206 Suppl 1: S46-52.

Burch VC, Tarr G and Morroni C. (2008) Modified early warning score predicts the need for hospital admission and inhospital mortality. *Emerg Med J* 25: 674-678.

Burk RF, Schaffner W and Koenig MG. (1971) Severe influenza virus pneumonia in the pandemic of 1968-1969. *Arch Intern Med* 127: 1122-1128.

Burton DC, Flannery B, Onyango B, et al. (2011) Healthcare-seeking behaviour for common infectious disease-related illnesses in rural Kenya: a community-based house-to-house survey. *J Health Popul Nutr* 29: 61-70.

Byarugaba DK, Ducatez MF, Erima B, et al. (2011) Molecular epidemiology of influenza A/H3N2 viruses circulating in Uganda. *PLoS One* 6: e27803.

Byarugaba DK, Erima B, Millard M, et al. (2013) Genetic analysis of influenza B viruses isolated in Uganda during the 2009-2010 seasons. *Virol J* 10: 11.

Callaghan WM, Chu SY and Jamieson DJ. (2010) Deaths from seasonal influenza among pregnant women in the United States, 1998-2005. *Obstet Gynecol* 115: 919-923.

Calmy A, Bel M, Nguyen A, et al. (2012) Strong serological responses and HIV RNA increase following AS03-adjuvanted pandemic immunization in HIV-infected patients. *HIV Med* 13: 207-218.

Campos-Loza A, Soto-Ramirez L and Sierra-Madero J. (2010) New swine origin influneza A in HIV-inected patients during the 2009 outbreak in Mexico City (#801). *Conference on Retroviruses and Opportunistic Infections.* San Francisco, USA, (#801).

Camps Serra M, Cervera C, Pumarola T, et al. (2008) Virological diagnosis in community-acquired pneumonia in immunocompromised patients. *Eur Respir J* 31: 618-624.

Cao B, Huang GH, Pu ZH, et al. (2014) Emergence of community-acquired adenovirus type 55 as a cause of community-onset pneumonia. *Chest* 145: 79-86.

Cardoso MR, Cousens SN, de Goes Siqueira LF, et al. (2004) Crowding: risk factor or protective factor for lower respiratory disease in young children? *BMC Public Health* 4: 19.

Carrat F, Vergu E, Ferguson NM, et al. (2008) Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *Am J Epidemiol* 167: 775-785.

Carvalho Mda G, Tondella ML, McCaustland K, et al. (2007) Evaluation and improvement of real-time PCR assays targeting lytA, ply, and psaA genes for detection of pneumococcal DNA. *J Clin Microbiol* 45: 2460-2466.

Casado I, Martinez-Baz I, Burgui R, et al. (2014) Household transmission of influenza A(H1N1)pdm09 in the pandemic and post-pandemic seasons. *PLoS One* 9: e108485.

Cassel-Beraud AM, Rakotonirina G and Coulanges P. (1988) [Study of an influenza epidemic in Tananarive (February 1987)]. *Arch Inst Pasteur Madagascar* 54: 125-130.

Cauchemez S, Ferguson NM, Fox A, et al. (2014) Determinants of influenza transmission in South East Asia: insights from a household cohort study in Vietnam. *PLoS Pathog* 10: e1004310.

Cauchemez S, Valleron AJ, Boelle PY, et al. (2008) Estimating the impact of school closure on influenza transmission from Sentinel data. *Nature* 452: 750-754.

Centers for Disease Control and Prevention. (2005) Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease--United States, 1998-2003. *MMWR Morb Mortal Wkly Rep* 54: 893-897.

Centers for Disease Control and Prevention. (2014) *Influenza (Flu) - Types of influenza viruses*. Available at: http://www.cdc.gov/flu/about/viruses/types.htm

Centers for Disease Control and Prevention. (2015a) *Guidance for Clinicians on the Use of RT-PCR and Other Molecular Assays for Diagnosis of Influenza Virus Infection*. Available at: http://www.cdc.gov/flu/professionals/diagnosis/molecular-assays.htm.

Centers for Disease Control and Prevention. (2015b) *Transmission of Influenza Viruses from Animals to People*. Available at: http://www.cdc.gov/flu/about/viruses/transmission.htm.

Centre d'Estudis Epidemiològics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya (CEEISCAT). (2008) SIVES 2008: Sistema Integrat de Vigilancia Epidemiolo´gica del SIDA/VIH/ITS en Catalunya (SIVES). In: Generalitat de Catalunya DdS (ed). Barcelona, Espana.

Charalambous S, Day JH, Fielding K, et al. (2003) HIV infection and chronic chest disease as risk factors for bacterial pneumonia: a case-control study. *AIDS* 17: 1531-1537.

Charles PG, Whitby M, Fuller AJ, et al. (2008) The etiology of community-acquired pneumonia in Australia: why penicillin plus doxycycline or a macrolide is the most appropriate therapy. *Clin Infect Dis* 46: 1513-1521.

Chaves SS, Aragon D, Bennett N, et al. (2013) Patients hospitalized with laboratory-confirmed influenza during the 2010-2011 influenza season: exploring disease severity by virus type and subtype. *J Infect Dis* 208: 1305-1314.

Chaves SS, Perez A, Farley MM, et al. (2014) The Burden of Influenza Hospitalizations in Infants from 2003- 2012, United States. *Pediatr Infect Dis J*.

Chen SC and Liao CM. (2008) Modelling control measures to reduce the impact of pandemic influenza among schoolchildren. *Epidemiol Infect* 136: 1035-1045.

Chen W, Shi J, Qian L, et al. (2014) Comparison of robustness to outliers between robust poisson models and log-binomial models when estimating relative risks for common binary outcomes: a simulation study. *BMC Med Res Methodol* 14: 82.

Chertow DS and Memoli MJ. (2013) Bacterial coinfection in influenza: a grand rounds review. *JAMA* 309: 275-282.

Chien YW, Klugman KP and Morens DM. (2009) Bacterial pathogens and death during the 1918 influenza pandemic. *N Engl J Med* 361: 2582-2583.

Chien YW, Vidal JE, Grijalva CG, et al. (2013) Density interactions among Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus in the nasopharynx of young Peruvian children. *Pediatr Infect Dis J* 32: 72-77.

Children's HIV Association. (2015) CHIVA Vaccination of HIV-infected children (2015). 1-2.

Choi SH, Hong SB, Ko GB, et al. (2012) Viral infection in patients with severe pneumonia requiring intensive care unit admission. *Am J Respir Crit Care Med* 186: 325-332.

Choko AT, Desmond N, Webb EL, et al. (2011) The uptake and accuracy of oral kits for HIV self-testing in high HIV prevalence setting: a cross-sectional feasibility study in Blantyre, Malawi. *PLoS Med* 8: e1001102.

Clements KM, Chancellor J, Nichol K, et al. (2011) Cost-effectiveness of a recommendation of universal mass vaccination for seasonal influenza in the United States. *Value Health* 14: 800-811.

Clerc Y, Coulanges P, Ratsirahonana S, et al. (1980) [Epidemic of influenza in Antananarivo (with isolation of virus A/Texas/77 (H3N2))]. *Arch Inst Pasteur Madagascar* 47: 101-120.

Clouse KA, Robbins PB, Fernie B, et al. (1989) Viral antigen stimulation of the production of human monokines capable of regulating HIV1 expression. *J Immunol* 143: 470-475.

Cockeran R, Theron AJ, Steel HC, et al. (2001) Proinflammatory interactions of pneumolysin with human neutrophils. *J Infect Dis* 183: 604-611.

Coffin SE, Zaoutis TE, Rosenquist AB, et al. (2007) Incidence, complications, and risk factors for prolonged stay in children hospitalized with community-acquired influenza. *Pediatrics* 119: 740-748.

Cohen AL, Hellferscee O, Pretorius M, et al. (2014) Epidemiology of influenza virus types and subtypes in South Africa, 2009-2012. *Emerg Infect Dis* 20: 1162-1169.

Cohen C, Moyes J, Tempia S, et al. (2013) Severe influenza-associated respiratory infection in high HIV prevalence setting, South Africa, 2009-2011. *Emerg Infect Dis* 19: 1766-1774.

Cohen C, Moyes J, Tempia S, et al. (2015a) Mortality amongst Patients with Influenza-Associated Severe Acute Respiratory Illness, South Africa, 2009-2013. *PLoS One* 10: e0118884.

Cohen C, Moyes J, Walaza S, et al. (2012a) Increased risk of death amongst HIV-infected persons hospitalized with influenza-associated acute lower respiratory illness in South Africa 2009-2011. *African African Network for Influenza Surveillance and Epidemiology (ANISE) meeting.* Nairobi.

Cohen C, Simonsen L, Sample J, et al. (2012b) Influenza-Related Mortality Among Adults Aged 25-54 Years With AIDS in South Africa and the United States of America. *Clin Infect Dis*.

Cohen C, Simonsen L, Sample J, et al. (2012c) Influenza-related mortality among adults aged 25-54 years with AIDS in South Africa and the United States of America. *Clin Infect Dis* 55: 996-1003.

Cohen C, Walaza S, Moyes J, et al. (2015b) Epidemiology of viral-associated acute lower respiratory tract infection among children <5 years of age in a high HIV prevalence setting, South Africa, 2009-2012. *Pediatr Infect Dis J* 34: 66-72.

Cohen C, Walaza S, Moyes J, et al. (2015c) Epidemiology of Severe Acute Respiratory Illness (SARI) among Adults and Children Aged >/=5 Years in a High HIV-Prevalence Setting, 2009-2012. *PLoS One* 10: e0117716.

Cohen C, Wolter N, Moyes J, et al. (2012 ) Pneumococcal co-infection in patients with seasonal and pandemic influenza in South Africa, 2009 (abstract 331). *Annual African Network for Influenza Surveillance and Epidemiology (ANISE) Meeting.* Nairobi, 55.

Cohen JP and Macauley C. (1989) Susceptibility to influenza A in HIV-positive patients. *JAMA* 261: 245.

Collins SD and Lehmann J. (1951) Trends and epidemics of influenza and pneumonia: 1918-1951. *Public Health Rep* 66: 1487-1516.

Conklin L, Loo JD, Kirk J, et al. (2014) Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type invasive pneumococcal disease among young children. *Pediatr Infect Dis J* 33 Suppl 2: S109-118.

Cooper C, Klein M, Walmsley S, et al. (2012) High-level immunogenicity is achieved vaccine with adjuvanted pandemic H1N1(2009) and improved with booster dosing in a randomized trial of HIV-infected adults. *HIV Clin Trials* 13: 23-32.

Cooper C, Thorne A, Klein M, et al. (2011) Immunogenicity is not improved by increased antigen dose or booster dosing of seasonal influenza vaccine in a randomized trial of HIV infected adults. *PLoS One* 6: e17758.

Cordell R, Pickering L, Henderson FW, et al. (2004) Infectious diseases in childcare settings. *International Conference on Women and Infectious Disease.* Atlanta, US., e9.

Cowling BJ, Ip DK, Fang VJ, et al. (2013) Aerosol transmission is an important mode of influenza A virus spread. *Nat Commun* 4: 1935.

Cox NJ and Subbarao K. (1999) Influenza. *Lancet* 354: 1277-1282.

Crum-Cianflone NF, Eberly LE, Duplessis C, et al. (2011) Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine in an immunocompromised population: a prospective study comparing HIV-infected adults with HIV-uninfected adults. *Clin Infect Dis* 52: 138-146.

Cunha BA, Corbett M and Mickail N. (2012) Influenza A presenting as viral encephalitis in an adult. *Heart Lung* 41: 308-309.

Cutts FT, Zaman SM, Enwere G, et al. (2005) Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 365: 1139-1146.

Daley P, Castriciano S, Chernesky M, et al. (2006) Comparison of flocked and rayon swabs for collection of respiratory epithelial cells from uninfected volunteers and symptomatic patients. *J Clin Microbiol* 44: 2265-2267.

Dalhatu IT, Medina-Marino A, Olsen SJ, et al. (2012) Influenza viruses in Nigeria, 2009-2010: results from the first 17 months of a national influenza sentinel surveillance system. *J Infect Dis* 206 Suppl 1: S121-128.

Damasio GA, Pereira LA, Moreira SD, et al. (2015) Does virus-bacteria coinfection increase the clinical severity of acute respiratory infection? *J Med Virol* 87: 1456-1461.

Darboe MK, Fulford AJ, Secka O, et al. (2010) The dynamics of nasopharyngeal streptococcus pneumoniae carriage among rural Gambian mother-infant pairs. *BMC Infect Dis* 10: 195.

Dauer CC. (1958) Mortality in the 1957-58 influenza epidemic. *Public Health Rep* 73: 803-810.

Davis SM, Deloria-Knoll M, Kassa HT, et al. (2013) Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects. *Vaccine* 32: 133-145.

Dawood FS, Iuliano AD, Reed C, et al. (2012) Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infect Dis* 12: 687-695.

de Gaetano Donati K, Bertagnolio S, Tumbarello M, et al. (2000) Effect of highly active antiretroviral therapy on the incidence of bacterial pneumonia in HIV-infected subjects. *Int J Antimicrob Agents* 16: 357-360.

de Paus RA, van Crevel R, van Beek R, et al. (2013) The influence of influenza virus infections on the development of tuberculosis. *Tuberculosis (Edinb)* 93: 338-342.

de Roux A, Ewig S, Garcia E, et al. (2006) Mixed community-acquired pneumonia in hospitalised patients. *Eur Respir J* 27: 795-800.

De Serres G, Lampron N, La Forge J, et al. (2009) Importance of viral and bacterial infections in chronic obstructive pulmonary disease exacerbations. *J Clin Virol* 46: 129-133.

Dellagi K, Rollot O, Temmam S, et al. (2011) Pandemic influenza due to pH1N1/2009 virus: estimation of infection burden in Reunion Island through a prospective serosurvey, austral winter 2009. *PLoS One* 6: e25738.

Demicheli V, Jefferson T, Al-Ansary LA, et al. (2014) Vaccines for preventing influenza in healthy adults (Review): CD001269. *Cochrane Data- base Syst Rev.*

Desmond NA, Nyirenda D, Dube Q, et al. (2013) Recognising and treatment seeking for acute bacterial meningitis in adults and children in resource-poor settings: a qualitative study. *PLoS One* 8: e68163.

Dhanoa A, Fang NC, Hassan SS, et al. (2011) Epidemiology and clinical characteristics of hospitalized patients with pandemic influenza A (H1N1) 2009 infections: the effects of bacterial coinfection. *Virol J* 8: 501.

Dherani M, Pope D, Mascarenhas M, et al. (2008) Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis. *Bull World Health Organ* 86: 390-398C.

Dia N, Diene Sarr F, Thiam D, et al. (2014a) Influenza-like illnesses in Senegal: not only focus on influenza viruses. *PLoS One* 9: e93227.

Dia N, Ndiaye MN, Monteiro Mde L, et al. (2013) A subregional analysis of epidemiologic and genetic characteristics of influenza A(H1N1)pdm09 in Africa: Senegal, Cape Verde, Mauritania, and Guinea, 2009-2010. *Am J Trop Med Hyg* 88: 946-953.

Dia N, Richard V, Kiori D, et al. (2014b) Respiratory viruses associated with patients older than 50 years presenting with ILI in Senegal, 2009 to 2011. *BMC Infect Dis* 14: 189.

Diallo A. (2014a) Effectiveness of seasonal influenza vaccinatin of children in tropical developing Africa: a cluster-randomised trial. *4th African Network for Influenza Surveillance and Epidemiology Meeting.* Cape Town, South Africa.

Diallo A. (2014b) LAIV clinical efficacy and safety trial in Senegal. *4th African Network for Influenza Surveillance and Epidemiology Meeting.* Cape Town, South Africa.

Didierlaurent A, Goulding J, Patel S, et al. (2008) Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. *J Exp Med* 205: 323-329.

Diederen BM, Van Der Eerden MM, Vlaspolder F, et al. (2009) Detection of respiratory viruses and Legionella spp. by real-time polymerase chain reaction in patients with community acquired pneumonia. *Scand J Infect Dis* 41: 45-50.

Dietz K. (1993) The estimation of the basic reproduction number for infectious diseases. *Stat Methods Med Res* 2: 23-41.

Dobson J, Whitley RJ, Pocock S, et al. (2015) Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. *Lancet Respir Med* 385: 1729-1737.

Dodds L, McNeil SA, Fell DB, et al. (2007) Impact of influenza exposure on rates of hospital admissions and physician visits because of respiratory illness among pregnant women. *CMAJ* 176: 463-468.

Dominguez-Cherit G, Lapinsky SE, Macias AE, et al. (2009) Critically Ill patients with 2009 influenza A(H1N1) in Mexico. *JAMA* 302: 1880-1887.

Doshi P. (2011) The elusive definition of pandemic influenza. *Bull World Health Organ* 89: 532-538.

Doshi S, Silk BJ, Dutt D, et al. (2015) Household-level risk factors for influenza among young children in Dhaka, Bangladesh: a case-control study. *Trop Med Int Health* 20: 719-729.

Dosseh A, Ndiaye K, Spiegel A, et al. (2000) Epidemiological and virological influenza survey in Dakar, Senegal: 1996-1998. *Am J Trop Med Hyg* 62: 639-643.

Dosseh A and Rogier C. (1996) Influenza survey in Senegal. *Trans R Soc Trop Med Hyg* 90: 377.

Dowell SF, Garman RL, Liu G, et al. (2001) Evaluation of Binax NOW, an assay for the detection of pneumococcal antigen in urine samples, performed among pediatric patients. *Clin Infect Dis* 32: 824-825.

Drews AL, Atmar RL, Glezen WP, et al. (1997) Dual respiratory virus infections. *Clin Infect Dis* 25: 1421-1429.

Driscoll AJ, Karron RA, Bhat N, et al. (2014) Evaluation of fast-track diagnostics and TaqMan array card real-time PCR assays for the detection of respiratory pathogens. *J Microbiol Methods* 107C: 222-226.

Dube FS, Kaba M, Whittaker E, et al. (2013) Detection of from Different Types of Nasopharyngeal Swabs in Children. *PLoS One* 8: e68097.

Duque J, McMorrow ML and Cohen AL. (2014) Influenza vaccines and influenza antiviral drugs in Africa: are they available and do guidelines for their use exist? *BMC Public Health* 14: 41.

Durier C, Desaint C, Lucht F, et al. (2013) Long-term immunogenicity of two doses of 2009 A/H1N1v vaccine with and without AS03(A) adjuvant in HIV-1-infected adults. *AIDS* 27: 87-93.

Earhart KC, Beadle C, Miller LK, et al. (2001) Outbreak of influenza in highly vaccinated crew of U.S. Navy ship. *Emerg Infect Dis* 7: 463-465.

Edwards KM, Dupont WD, Westrich MK, et al. (1994) A randomized controlled trial of cold-adapted and inactivated vaccines for the prevention of influenza A disease. *J Infect Dis* 169: 68-76.

Ekdahl K, Ahlinder I, Hansson HB, et al. (1997) Duration of nasopharyngeal carriage of penicillin-resistant Streptococcus pneumoniae: experiences from the South Swedish Pneumococcal Intervention Project. *Clin Infect Dis* 25: 1113-1117.

El Sahly HM, Davis C, Kotloff K, et al. (2012) Higher antigen content improves the immune response to 2009 H1N1 influenza vaccine in HIV-infected adults: a randomized clinical trial. *J Infect Dis* 205: 703-712.

Emukule GO, Khagayi S, McMorrow ML, et al. (2014) The burden of influenza and RSV among inpatients and outpatients in rural western Kenya, 2009-2012. *PLoS One* 9: e105543.

England PH. (2013) Immunisation against infectious disease (Green Book). In: D. S and Ramsay M (eds). London: Crown, 185-218.

Englund JA, Mbawuike IN, Hammill H, et al. (1993) Maternal immunization with influenza or tetanus toxoid vaccine for passive antibody protection in young infants. *J Infect Dis* 168: 647-656.

Englund JA, Walter E, Black S, et al. (2010) Safety and immunogenicity of trivalent inactivated influenza vaccine in infants: a randomized double-blind placebo-controlled study. *Pediatr Infect Dis J* 29: 105-110.

Esposito S, Cantarutti L, Molteni CG, et al. (2011) Clinical manifestations and socio-economic impact of influenza among healthy children in the community. *J Infect* 62: 379-387.

Estabragh ZR and Mamas MA. (2013) The cardiovascular manifestations of influenza: a systematic review. *Int J Cardiol* 167: 2397-2403.

Estenssoro E, Rios FG, Apezteguia C, et al. (2010) Pandemic 2009 influenza A in Argentina: a study of 337 patients on mechanical ventilation. *Am J Respir Crit Care Med* 182: 41-48.

Everett DB, Mukaka M, Denis B, et al. (2011) Ten years of surveillance for invasive Streptococcus pneumoniae during the era of antiretroviral scale-up and cotrimoxazole prophylaxis in Malawi. *PLoS One* 6: e17765.

Falsey AR, Criddle MC and Walsh EE. (2006) Detection of respiratory syncytial virus and human metapneumovirus by reverse transcription polymerase chain reaction in adults with and without respiratory illness. *J Clin Virol* 35: 46-50.

Feikin DR, Feldman C, Schuchat A, et al. (2004) Global strategies to prevent bacterial pneumonia in adults with HIV disease. *Lancet Infect Dis* 4: 445-455.

Feikin DR, Laserson KF, Ojwando J, et al. (2012a) Efficacy of pentavalent rotavirus vaccine in a high HIV prevalence population in Kenya. *Vaccine* 30 Suppl 1: A52-60.

Feikin DR, Njenga MK, Bigogo G, et al. (2012b) Etiology and Incidence of viral and bacterial acute respiratory illness among older children and adults in rural western Kenya, 2007-2010. *PLoS One* 7: e43656.

Feikin DR, Ope MO, Aura B, et al. (2012c) The population-based burden of influenza-associated hospitalization in rural western Kenya, 2007-2009. *Bull World Health Organ* 90: 256-263A.

Feikin DR, Scott JA and Gessner BD. (2014) Use of vaccines as probes to define disease burden. *Lancet* 383: 1762-1770.

Feldman C, Alanee S, Yu VL, et al. (2009) Severity of illness scoring systems in patients with bacteraemic pneumococcal pneumonia: implications for the intensive care unit care. *Clin Microbiol Infect* 15: 850-857.

Feldman C and Anderson R. (2011) Bacteraemic pneumococcal pneumonia: current therapeutic options. *Drugs* 71: 131-153.

Feldman C, Kallenbach JM, Levy H, et al. (1989) Community-acquired pneumonia of diverse aetiology: prognostic features in patients admitted to an intensive care unit and a "severity of illness" core. *Intensive Care Med* 15: 302-307.

Feldman MB, Alexy ER, Thomas JA, et al. (2015) The association between food insufficiency and HIV treatment outcomes in a longitudinal analysis of HIV-infected individuals in New York City. *J Acquir Immune Defic Syndr*.

Fernal J. (2005) *On the pestilent diseases: that their cause is hidden,* Leiden, Netherlands: Brill.

Ferraro CF, Trotter CL, Nascimento MC, et al. (2014) Household crowding, social mixing patterns and respiratory symptoms in seven countries of the African meningitis belt. *PLoS One* 9: e101129.

Ferreira DM, Jambo KC and Gordon SB. (2011) Experimental human pneumococcal carriage models for vaccine research. *Trends Microbiol* 19: 464-470.

Fields BS, House BL, Klena J, et al. (2013) Role of global disease detection laboratories in investigations of acute respiratory illness. *J Infect Dis* 208 Suppl 3: S173-176.

Fine AD, Bridges CB, De Guzman AM, et al. (2001) Influenza A among patients with human immunodeficiency virus: an outbreak of infection at a residential facility in New York City. *Clin Infect Dis* 32: 1784-1791.

Fine MJ, Auble TE, Yealy DM, et al. (1997) A prediction rule to identify low-risk patients with community-acquired pneumonia. *N Engl J Med* 336: 243-250.

Fiore AE, Shay DK, Broder K, et al. (2008) Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. *MMWR Recomm Rep* 57: 1-60.

Fiore AE, Uyeki TM, Broder K, et al. (2010) Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Recomm Rep* 59: 1-62.

Flannery B, Clippard J, Zimmerman RK, et al. (2015) Early estimates of seasonal influenza vaccine effectiveness - United States, January 2015. *MMWR Morb Mortal Wkly Rep* 64: 10-15.

Fleming DM, Crovari P, Wahn U, et al. (2006) Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. *Pediatr Infect Dis J* 25: 860-869.

Fleming-Dutra KE, Taylor T, Link-Gelles R, et al. (2013) Effect of the 2009 influenza A(H1N1) pandemic on invasive pneumococcal pneumonia. *J Infect Dis* 207: 1135-1143.

Food and Agricultural Organization of the United Nations (FAO). (2000) *The underlying cause of food insecurity*. Available at: http://www.fao.org/docrep/003/x8406e/x8406e00.htm.

Forgie IM, Campbell H, Lloyd-Evans N, et al. (1992) Etiology of acute lower respiratory tract infections in children in a rural community in The Gambia. *Pediatr Infect Dis J* 11: 466-473.

Forrest BD, Steele AD, Hiemstra L, et al. (2011) A prospective, randomized, open-label trial comparing the safety and efficacy of trivalent live attenuated and inactivated influenza vaccines in adults 60 years of age and older. *Vaccine* 29: 3633-3639.

Fowke KR, D'Amico R, Chernoff DN, et al. (1997) Immunologic and virologic evaluation after influenza vaccination of HIV-1-infected patients. *AIDS* 11: 1013-1021.

Frank AL, Taber LH, Wells CR, et al. (1981) Patterns of shedding of myxoviruses and paramyxoviruses in children. *J Infect Dis* 144: 433-441.

French N, Gordon SB, Mwalukomo T, et al. (2010) A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. *N Engl J Med* 362: 812-822.

Fry AM, Lu X, Olsen SJ, et al. (2011) Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. *PLoS One* 6: e17780.

Fuhrman C, Bonmarin I, Paty AC, et al. (2010) Severe hospitalised 2009 pandemic influenza A(H1N1) cases in France, 1 July-15 November 2009. *Euro Surveill* 15.

Fuller JA, Summers A, Katz MA, et al. (2013) Estimation of the national disease burden of influenza-associated severe acute respiratory illness in Kenya and Guatemala: a novel methodology. *PLoS One* 8: e56882.

Fuller JD, Craven DE, Steger KA, et al. (1999) Influenza vaccination of human immunodeficiency virus (HIV)-infected adults: impact on plasma levels of HIV type 1 RNA and determinants of antibody response. *Clin Infect Dis* 28: 541-547.

Gachara G, Ngeranwa J, Magana JM, et al. (2006) Influenza virus strains in Nairobi, Kenya. *J Clin Virol* 35: 117-118.

Garcia-Garcia ML, Calvo C, Pozo F, et al. (2008) Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. *Pediatr Infect Dis J* 27: 358-360.

Garcia-Rodriguez JA and Fresnadillo Martinez MJ. (2002) Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother* 50 Suppl S2: 59-73.

Garg S, Everett D, SanJoaquin M, et al. (2012) Seasonality and Burden of Influenza among Children and Adults Presenting to Queen Elizabeth Central Hosital with Influenza-like Illness or Severe Acute Respiratory Illness - Blantyre, Malawi, January-September 2011. *Annual African Network for Influenza Surveillance and Epidemiology (ANISE) Meeting.* Nairobi, 55.

Garg S, Olsen SJ, Fernandez S, et al. (2014) Seroincidence of Influenza Among HIV-infected and HIV-uninfected Men During the 2009 H1N1 Influenza Pandemic, Bangkok, Thailand. *Open Forum Infect Dis* 1: ofu082.

Garten RJ, Davis CT, Russell CA, et al. (2009) Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 325: 197-201.

Gauzere BA, Bussienne F, Bouchet B, et al. (2011) [Severe cases of A(H1N1)v2009 infection in Reunion Island in 2009 and 2010]. *Bull Soc Pathol Exot* 104: 97-104.

GAVI the Vaccine Alliance. (2013) *Vaccine investment strategy*. Available at: http://www.gavi.org/about/strategy/vaccine-investment-strategy/

Geng EH, Odeny TA, Lyamuya RE, et al. (2015) Estimation of Mortality among HIV-infected people on antiretroviral therapy treatment in east Africa: a sampling based approach in an observational, multisite, cohort study. *Lancet HIV* 2: e107-e116.

Geretti AM, Brook G, Cameron C, et al. (2008) British HIV Association guidelines for immunization of HIV-infected adults 2008. *HIV Med* 9: 795-848.

Gerna G, Piralla A, Rovida F, et al. (2009) Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. *J Med Virol* 81: 1498-1507.

Gessner BD, Shindo N and Briand S. (2011) Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. *Lancet Infect Dis* 11: 223-235.

Ghaffar F, Friedland IR and McCracken GH, Jr. (1999) Dynamics of nasopharyngeal colonization by Streptococcus pneumoniae. *Pediatr Infect Dis J* 18: 638-646.

Gill CJ, Mwanakasale V, Fox MP, et al. (2008) Impact of human immunodeficiency virus infection on Streptococcus pneumoniae colonization and seroepidemiology among Zambian women. *J Infect Dis* 197: 1000-1005.

Gillespie S and Kadiyala S. (2005) *HIV/AIDs and food and nutrition security,* Washington, D.C.: International Food Policy Research Institute (IFPRI).

Glezen WP, Gaglani MJ, Kozinetz CA, et al. (2010) Direct and indirect effectiveness of influenza vaccination delivered to children at school preceding an epidemic caused by 3 new influenza virus variants. *J Infect Dis* 202: 1626-1633.

Gooskens J, Jonges M, Claas EC, et al. (2009) Prolonged influenza virus infection during lymphocytopenia and frequent detection of drug-resistant viruses. *J Infect Dis* 199: 1435-1441.

Gordon MA, Walsh AL, Chaponda M, et al. (2001) Bacteraemia and mortality among adult medical admissions in Malawi--predominance of non-typhi salmonellae and Streptococcus pneumoniae. *J Infect* 42: 44-49.

Government of Malawi. (2014) Global AIDS Response Progress Report (GARPR): Malawi Progress Report for 2013. 45.

Government of Malawi Ministry of Health. (2011) Integrated HIV Program Report April-June 2011

Government of Malawi Ministry of Health. (2013) Integrated HIV Program Report October-December 2013.

Government of Malawi Ministry of Health. (2014) Integrated HIV Program Report January-March 2014.

Grabowska K, Hogberg L, Penttinen P, et al. (2006) Occurrence of invasive pneumococcal disease and number of excess cases due to influenza. *BMC Infect Dis* 6: 58.

Gray BM, Converse GM, 3rd and Dillon HC, Jr. (1980) Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. *J Infect Dis* 142: 923-933.

Greenberg D, Givon-Lavi N, Newman N, et al. (2011) Nasopharyngeal carriage of individual Streptococcus pneumoniae serotypes during pediatric pneumonia as a means to estimate serotype disease potential. *Pediatr Infect Dis J* 30: 227-233.

Greenberg DP, Robertson CA, Noss MJ, et al. (2013) Safety and immunogenicity of a quadrivalent inactivated influenza vaccine compared to licensed trivalent inactivated influenza vaccines in adults. *Vaccine* 31: 770-776.

Greenland S. (1993) Methods for epidemiologic analyses of multiple exposures: a review and comparative study of maximum-likelihood, preliminary-testing, and empirical-Bayes regression. *Stat Med* 12: 717-736.

Greensill J, McNamara PS, Dove W, et al. (2003) Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. *Emerg Infect Dis* 9: 372-375.

Greve T and Moller JK. (2012) Accuracy of using the lytA gene to distinguish Streptococcus pneumoniae from related species. *J Med Microbiol* 61: 478-482.

Grijalva CG, Griffin MR, Edwards KM, et al. (2014) The role of influenza and parainfluenza infections in nasopharyngeal pneumococcal acquisition among young children. *Clin Infect Dis* 58: 1369-1376.

Gritzfeld JF, Cremers AJ, Ferwerda G, et al. (2014) Density and duration of experimental human pneumococcal carriage. *Clin Microbiol Infect* 20: O1145-1151.

Grohskopf LA, Olsen SJ, Sokolow LZ, et al. (2014) Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP) -- United States, 2014-15 influenza season. *MMWR Morb Mortal Wkly Rep* 63: 691-697.

Grohskopf LA, Olsen SJ, Sokolow LZ, et al. (2015) Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP) -- United States, 2015-16 influenza season. *MMWR Morb Mortal Wkly Rep* 64: 818-825.

Groome M. (2013) Effect of HIV and/or Active Tuberculosis on the Immune Responses to Trivalent Influenza Vaccine (TIV) in Adults (TIV_HIV_TB). ClinicalTrials.gov identifier: NCT01811823.

Gudnason T, Hrafnkelsson B, Laxdal B, et al. (2014) Risk factors for nasopharyngeal carriage of Streptococcus pneumoniae and effects of a hygiene intervention: repeated cross-sectional cohort study at day care centres. *Scand J Infect Dis* 46: 493-501.

Gunson RN, Collins TC and Carman WF. (2005) Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. *J Clin Virol* 33: 341-344.

Gunthard HF, Wong JK, Spina CA, et al. (2000) Effect of influenza vaccination on viral replication and immune response in persons infected with human immunodeficiency virus receiving potent antiretroviral therapy. *J Infect Dis* 181: 522-531.

Gutierrez-Pizarraya A, Perez-Romero P, Alvarez R, et al. (2012) Unexpected severity of cases of influenza B infection in patients that required hospitalization during the first postpandemic wave. *J Infect* 65: 423-430.

Haffizulla J, Hartman A, Hoppers M, et al. (2014) Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomised, placebo-controlled, phase 2b/3 trial. *Lancet Infect Dis* 14: 609-618.

Hakansson A, Kidd A, Wadell G, et al. (1994) Adenovirus infection enhances in vitro adherence of Streptococcus pneumoniae. *Infect Immun* 62: 2707-2714.

Hall CB, Douglas RG, Jr., Geiman JM, et al. (1979) Viral shedding patterns of children with influenza B infection. *J Infect Dis* 140: 610-613.

Hall CE, Cooney MK and Fox JP. (1973) The Seattle virus watch. IV. Comparative epidemiologic observations of infections with influenza A and B viruses, 1965-1969, in families with young children. *Am J Epidemiol* 98: 365-380.

Halloran ME. (2005) *Encyclopaedia of Biostatistics (2nd Edition): Secondary Attack Rate*: John Wiley & Sons, Ltd.

Hament JM, Aerts PC, Fleer A, et al. (2004) Enhanced adherence of Streptococcus pneumoniae to human epithelial cells infected with respiratory syncytial virus. *Pediatr Res* 55: 972-978.

Hamer DH, Egas J, Estrella B, et al. (2002) Assessment of the Binax NOW Streptococcus pneumoniae urinary antigen test in children with nasopharyngeal pneumococcal carriage. *Clin Infect Dis* 34: 1025-1028.

Hammitt LL, Akech DO, Morpeth SC, et al. (2014) Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae and non-typeable Haemophilus influenzae in Kilifi, Kenya: findings from cross-sectional carriage studies. *Lancet Glob Health* 2: e397-405.

Hanley JA, Negassa A, Edwardes MD, et al. (2003) Statistical analysis of correlated data using generalized estimating equations: an orientation. *Am J Epidemiol* 157: 364-375.

Harboe ZB, Larsen MV, Ladelund S, et al. (2014) Incidence and risk factors for invasive pneumococcal disease in HIV-infected and non-HIV-infected individuals before and after the introduction of combination antiretroviral therapy: persistent high risk among HIV-infected injecting drug users. *Clin Infect Dis* 59: 1168-1176.

Harford CG and Hara M. (1950) Pulmonary Edema in Influenzal Pneumonia of the Mouse and the Relation of Fluid in the Lung to the Inception of Pneumococcal Pneumonia. *J Exp Med* 91: 245-260.

Harries AD, Speare R and Wirima JJ. (1988) A profile of respiratory disease in an African medical ward. *J R Coll Physicians Lond* 22: 109-113.

Harries AD, Speare R and Wirima JJ. (1990) Medical admissions to Kamuzu Central Hospital, Lilongwe, Malawi in 1986: comparison with admissions to Queen Elizabeth Central Hospital, Blantyre in 1973. *Trop Geogr Med* 42: 274-279.

Hartung TK, Chimbayo D, van Oosterhout JJ, et al. (2011) Etiology of suspected pneumonia in adults admitted to a high-dependency unit in Blantyre, Malawi. *Am J Trop Med Hyg* 85: 105-112.

Hatakeyama S, Iwatsuki-Horimoto K, Okamoto K, et al. (2011) Unadjuvanted pandemic H1N1 influenza vaccine in HIV-1-infected adults. *Vaccine* 29: 9224-9228.

Hayward AC, Fragaszy EB, Bermingham A, et al. (2014) Comparative community burden and severity of seasonal and pandemic influenza: results of the Flu Watch cohort study. *Lancet Respir Med* 2: 445-454.

Hazlett DT, Bell TM, Tukei PM, et al. (1988) Viral etiology and epidemiology of acute respiratory infections in children in Nairobi, Kenya. *Am J Trop Med Hyg* 39: 632-640.

Hellferscee O, von Mollendorf C, Treurnicht F, et al. (2013) Influenza viral shedding in HIV-infected and HIV-uninfected individuals, South Africa, 2012. #P2-559. *Options for the Control of Influenza VIII.* Cape Town, South Africa.

Hendley JO, Sande MA, Stewart PM, et al. (1975) Spread of Streptococcus pneumoniae in families. I. Carriage rates and distribution of types. *J Infect Dis* 132: 55-61.

Hennekens CH and Buring JE. (1987) *Epidemiology in medicine,* Philadelphia, USA.: Lippincott Willliams & Wilkins.

Heraud JM, Njouom R, Rousset D, et al. (2012) Spatiotemporal circulation of influenza viruses in 5 African countries during 2008-2009: a collaborative study of the Institut Pasteur International Network. *J Infect Dis* 206 Suppl 1: S5-13.

Hernes SS, Quarsten H, Hagen E, et al. (2011) Swabbing for respiratory viral infections in older patients: a comparison of rayon and nylon flocked swabs. *Eur J Clin Microbiol Infect Dis* 30: 159-165.

Hers JF, Masurel N and Mulder J. (1958) Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. *Lancet* 2: 1141-1143.

Hill-Cawthorne GA, Schelenz S, Lawes M, et al. (2010) Oseltamivir-resistant pandemic (H1N1) 2009 in patient with impaired immune system. *Emerg Infect Dis* 16: 1185-1186.

Ho A. (2014) Viral pneumonia in adults and older children in sub-Saharan Africa - epidemiology, aetiology, diagnosis and management. *Pneumonia* 5: 18-29.

Ho DD. (1992) HIV-1 viraemia and influenza. *Lancet* 339: 1549.

Ho J, Moir S, Wang W, et al. (2011) Enhancing effects of adjuvanted 2009 pandemic H1N1 influenza A vaccine on memory B-cell responses in HIV-infected individuals. *AIDS* 25: 295-302.

Hohenthal U, Vainionpaa R, Meurman O, et al. (2008) Aetiological diagnosis of community acquired pneumonia: utility of rapid microbiological methods with respect to disease severity. *Scand J Infect Dis* 40: 131-138.

Hommes MJ, Romijn JA, Endert E, et al. (1991) Resting energy expenditure and substrate oxidation in human immunodeficiency virus (HIV)-infected asymptomatic men: HIV affects host metabolism in the early asymptomatic stage. *Am J Clin Nutr* 54: 311-315.

Hong SY, Fanelli TJ, Jonas A, et al. (2014) Household food insecurity associated with antiretroviral therapy adherence among HIV-infected patients in Windhoek, Namibia. *J Acquir Immune Defic Syndr* 67: e115-122.

Horcajada JP, Pumarola T, Martinez JA, et al. (2003) A nosocomial outbreak of influenza during a period without influenza epidemic activity. *Eur Respir J* 21: 303-307.

Horimoto T and Kawaoka Y. (2005) Influenza: lessons from past pandemics, warnings from current incidents. *Nat Rev Microbiol* 3: 591-600.

Horita N, Miyazawa N, Kojima R, et al. (2013) Sensitivity and specificity of the Streptococcus pneumoniae urinary antigen test for unconcentrated urine from adult patients with pneumonia: a meta-analysis. *Respirology* 18: 1177-1183.

Horner GJ and Gray FD, Jr. (1973) Effect of uncomplicated, presumptive influenza on the diffusing capacity of the lung. *Am Rev Respir Dis* 108: 866-869.

Hung CC, Zhao TL, Sun HY, et al. (2013) Seroepidemiology of novel influenza A (H1N1) infection among HIV-infected patients in the era of highly active antiretroviral therapy. *J Microbiol Immunol Infect* 46: 151-157.

Hussain M, Melegaro A, Pebody RG, et al. (2005) A longitudinal household study of Streptococcus pneumoniae nasopharyngeal carriage in a UK setting. *Epidemiol Infect* 133: 891-898.

Impagliazzo A, Milder F, Kuipers H, et al. (2015) A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science* 349: 1301-1306.

International Union Against Tuberculosis and Lung Disease. (2000) Technical Guide: Sputum Examination for Tuberculosis by Direct Microscopy in Low-Income Countries. 5th ed. Paris, France, 25.

Iorio AM, Alatri A, Francisci D, et al. (1997) Immunogenicity of influenza vaccine (1993-94 winter season) in HIV-seropositive and -seronegative ex-intravenous drug users. *Vaccine* 15: 97-102.

Iorio AM, Francisci D, Camilloni B, et al. (2003) Antibody responses and HIV-1 viral load in HIV-1-seropositive subjects immunised with either the MF59-adjuvanted influenza vaccine or a conventional non-adjuvanted subunit vaccine during highly active antiretroviral therapy. *Vaccine* 21: 3629-3637.

Irving SA, Patel DC, Kieke BA, et al. (2012) Comparison of clinical features and outcomes of medically attended influenza A and influenza B in a defined population over four seasons: 2004-2005 through 2007-2008. *Influenza Other Respir Viruses* 6: 37-43.

Isais F, Lye D, Llorin R, et al. (2010) Pandemic (H1N1) 2009 influenza in HIV-infected adults: clinical features, severity, and outcome. *J Infect* 61: 437-440.

Jackson C, Vynnycky E and Mangtani P. (2010) Estimates of the transmissibility of the 1968 (Hong Kong) influenza pandemic: evidence of increased transmissibility between successive waves. *Am J Epidemiol* 171: 465-478.

Jain S, Self WH, Wunderink RG, et al. (2015) Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med* 373: 415-427.

Jambo KC, Sepako E, Fullerton DG, et al. (2011) Bronchoalveolar CD4+ T cell responses to respiratory antigens are impaired in HIV-infected adults. *Thorax* 66: 375-382.

Jambo KC, Sepako E, Glennie SJ, et al. (2012) Naturally-acquired influenza-specific CD4+ T-cell proliferative responses are impaired in HIV-infected African adults. *PLoS One* 7: e38628.

Jamieson DJ, Honein MA, Rasmussen SA, et al. (2009) H1N1 2009 influenza virus infection during pregnancy in the USA. *Lancet* 374: 451-458.

Jansen RR, Wieringa J, Koekkoek SM, et al. (2011) Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol* 49: 2631-2636.

Jartti T, Jartti L, Peltola V, et al. (2008) Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatr Infect Dis J* 27: 1103-1107.

Jary HR, Kachidiku J, Banda H, et al. (2014) Feasibility of conducting a randomised controlled trial of a cookstove intervention in rural Malawi. *Int J Tuberc Lung Dis* 18: 240-247.

Jefferson T, Del Mar CB, Dooley L, et al. (2011) Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane Database Syst Rev*: CD006207.

Jefferson T, Di Pietrantonj C, Rivetti A, et al. (2014a) Vaccines for preventing influenza in healthy adults. *Cochrane Database Syst Rev* 3: CD001269.

Jefferson T, Jones MA, Doshi P, et al. (2014b) Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. *Cochrane Database Syst Rev* 4: CD008965.

Jennings LC, Anderson TP, Beynon KA, et al. (2008) Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax* 63: 42-48.

Johansson N, Kalin M and Hedlund J. (2011) Clinical impact of combined viral and bacterial infection in patients with community-acquired pneumonia. *Scand J Infect Dis* 43: 609-615.

Johansson N, Kalin M, Tiveljung-Lindell A, et al. (2010) Etiology of community-acquired pneumonia: increased microbiological yield with new diagnostic methods. *Clin Infect Dis* 50: 202-209.

Johnson NP and Mueller J. (2002) Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. *Bull Hist Med* 76: 105-115.

Johnstone J, Majumdar SR, Fox JD, et al. (2008) Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. *Chest* 134: 1141-1148.

Johnstone-Robertson SP, Mark D, Morrow C, et al. (2011) Social mixing patterns within a South African township community: implications for respiratory disease transmission and control. *Am J Epidemiol* 174: 1246-1255.

Joint Committee on Vaccination and Immunisation. (2012) JCVI statement on the annual influenza vaccination programme – extension of the programme to children. JCVI, 1-5.

Joint United Nations Programme on HIV/AIDS. (2010) UNAIDS Report on the Global AIDS Epidemic 2010. 1-364.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2012) Treatment 2015. Geneva, Switzerland: UNAIDS, 1-42.

Jokinen C, Heiskanen L, Juvonen H, et al. (1993) Incidence of community-acquired pneumonia in the population of four municipalities in eastern Finland. *Am J Epidemiol* 137: 977-988.

Jones AD, Ngure FM, Pelto G, et al. (2013) What are we assessing when we measure food security? A compendium and review of current metrics. *Adv Nutr* 4: 481-505.

Jones KD and Berkley JA. (2014) Severe acute malnutrition and infection. *Paediatr Int Child Health* 34 Suppl 1: S1-S29.

Jones M, Del Mar C and Hama R. (2014) Statistical and methodological concerns about the beneficial effect of neuraminidase inhibitors on mortality. *Lancet Respir Med* 2: e9-e10.

Joosting AC, Harwin RM, Orchard M, et al. (1979) Respiratory viruses in hospital patients on the Witwatersrand. A 7-year study. *S Afr Med J* 55: 403-408.

Jordan E. (1923) *Epidemic influenza: a survey,* Chicago: American Medical Association.

Judd MC, Emukule GO, Njuguna H, et al. (2015) The Role of HIV in the Household Introduction and Transmission of Influenza in an Urban Slum, Nairobi, Kenya, 2008-2011. *J Infect Dis*.

Juurlink DN, Stukel TA, Kwong J, et al. (2006) Guillain-Barre syndrome after influenza vaccination in adults: a population-based study. *Arch Intern Med* 166: 2217-2221.

Kac G, Schlussel MM, Perez-Escamilla R, et al. (2012) Household food insecurity is not associated with BMI for age or weight for height among Brazilian children aged 0-60 months. *PLoS One* 7: e45747.

Kadiyala S and Rawat R. (2013) Food access and diet quality independently predict nutritional status among people living with HIV in Uganda. *Public Health Nutr* 16: 164-170.

Kadjo HA, Ekaza E, Coulibaly D, et al. (2012) Sentinel surveillance for influenza and other respiratory viruses in Cote d'Ivoire, 2003-2010. *Influenza Other Respi Viruses*.

Kadjo HA, Ekaza E, Coulibaly D, et al. (2013) Sentinel surveillance for influenza and other respiratory viruses in Cote d'Ivoire, 2003-2010. *Influenza Other Respir Viruses* 7: 296-303.

Karhu J, Ala-Kokko TI, Vuorinen T, et al. (2014) Lower Respiratory Tract Virus Findings in Mechanically Ventilated Patients With Severe Community-Acquired Pneumonia. *Clin Infect Dis*.

Karstaedt AS, Hopley M, Wong M, et al. (2009) Influenza- and respiratory syncytial virus-associated adult mortality in Soweto. *S Afr Med J* 99: 750-754.

Katz MA, Lebo E, Emukule G, et al. (2012) Epidemiology, seasonality, and burden of influenza and influenza-like illness in urban and rural Kenya, 2007-2010. *J Infect Dis* 206 Suppl 1: S53-60.

Katz MA, Muthoka P, Emukule GO, et al. (2014) Results from the first six years of national sentinel surveillance for influenza in Kenya, July 2007-June 2013. *PLoS One* 9: e98615.

Kee C, Palladino S, Kay I, et al. (2008) Feasibility of real-time polymerase chain reaction in whole blood to identify Streptococcus pneumoniae in patients with community-acquired pneumonia. *Diagn Microbiol Infect Dis* 61: 72-75.

Kelly CM, van Oosterhout JJ, Ngwalo C, et al. (2014) HIV associated neurocognitive disorders (HAND) in Malawian adults and effect on adherence to combination anti-retroviral therapy: a cross sectional study. *PLoS One* 9: e98962.

Kelly D, Burt K, Missaghi B, et al. (2012) Responses to pandemic ASO3-adjuvanted A/California/07/09 H1N1 influenza vaccine in human immunodeficiency virus-infected individuals. *BMC Immunol* 13: 49.

Kieninger D, Sheldon E, Lin WY, et al. (2013) Immunogenicity, reactogenicity and safety of an inactivated quadrivalent influenza vaccine candidate versus inactivated trivalent influenza vaccine: a phase III, randomized trial in adults aged >=18 years. *BMC Infect Dis* 13: 343.

Kim PE, Musher DM, Glezen WP, et al. (1996) Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. *Clin Infect Dis* 22: 100-106.

Kirkwood BR, Gove S, Rogers S, et al. (1995) Potential interventions for the prevention of childhood pneumonia in developing countries: a systematic review. *Bull World Health Organ* 73: 793-798.

Klein MB, Lu Y, DelBalso L, et al. (2007) Influenzavirus infection is a primary cause of febrile respiratory illness in HIV-infected adults, despite vaccination. *Clin Infect Dis* 45: 234-240.

Klein MB, Yang H, DelBalso L, et al. (2010) Viral pathogens including human metapneumovirus are the primary cause of febrile respiratory illness in HIV-infected adults receiving antiretroviral therapy. *J Infect Dis* 201: 297-301.

Klimov AI, Rocha E, Hayden FG, et al. (1995) Prolonged shedding of amantadine-resistant influenzae A viruses by immunodeficient patients: detection by polymerase chain reaction-restriction analysis. *J Infect Dis* 172: 1352-1355.

Klugman KP. (2001) Efficacy of pneumococcal conjugate vaccines and their effect on carriage and antimicrobial resistance. *Lancet Infect Dis* 1: 85-91.

Klugman KP, Chien YW and Madhi SA. (2009) Pneumococcal pneumonia and influenza: a deadly combination. *Vaccine* 27 Suppl 3: C9-C14.

Klugman KP, Madhi SA and Albrich WC. (2008) Novel approaches to the identification of Streptococcus pneumoniae as the cause of community-acquired pneumonia. *Clin Infect Dis* 47 Suppl 3: S202-206.

Kmietowicz Z. (2014) Study claiming Tamiflu saved lives was based on "flawed" analysis. *BMJ* 348: g2228.

Koegelenberg CF, Irusen EM, Cooper R, et al. (2010) High mortality from respiratory failure secondary to swine-origin influenza A (H1N1) in South Africa. *QJM* 103: 319-325.

Koita OA, Sangare L, Poudiougou B, et al. (2012) A seroepidemiological study of pandemic A/H1N1(2009) influenza in a rural population of Mali. *Clin Microbiol Infect* 18: 976-981.

Koulla-Shiro S, Kuaban C and Belec L. (1996) Acute community-acquired bacterial pneumonia in Human Immunodeficiency Virus (HIV) infected and non-HIV-infected adult patients in Cameroon: aetiology and outcome. *Tuber Lung Dis* 77: 47-51.

Kroon FP, Rimmelzwaan GF, Roos MT, et al. (1998) Restored humoral immune response to influenza vaccination in HIV-infected adults treated with highly active antiretroviral therapy. *AIDS* 12: F217-223.

Kroon FP, van Dissel JT, de Jong JC, et al. (1994) Antibody response to influenza, tetanus and pneumococcal vaccines in HIV-seropositive individuals in relation to the number of CD4+ lymphocytes. *AIDS* 8: 469-476.

Kroon FP, van Dissel JT, de Jong JC, et al. (2000) Antibody response after influenza vaccination in HIV-infected individuals: a consecutive 3-year study. *Vaccine* 18: 3040-3049.

Kukavica-Ibrulj I, Hamelin ME, Prince GA, et al. (2009) Infection with human metapneumovirus predisposes mice to severe pneumococcal pneumonia. *J Virol* 83: 1341-1349.

Kumar A, Zarychanski R, Pinto R, et al. (2009) Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA* 302: 1872-1879.

Kumar S, Wang L, Fan J, et al. (2008) Detection of 11 common viral and bacterial pathogens causing community-acquired pneumonia or sepsis in asymptomatic patients by using a multiplex reverse transcription-PCR assay with manual (enzyme hybridization) or automated (electronic microarray) detection. *J Clin Microbiol* 46: 3063-3072.

Kunisaki KM and Janoff EN. (2009) Influenza in immunosuppressed populations: a review of infection frequency, morbidity, mortality, and vaccine responses. *Lancet Infect Dis* 9: 493-504.

Kuster SP, Tuite AR, Kwong JC, et al. (2011) Evaluation of coseasonality of influenza and invasive pneumococcal disease: results from prospective surveillance. *PLoS Med* 8: e1001042.

Kwong JC, Campitelli MA, Gubbay JB, et al. (2013) Vaccine effectiveness against laboratory-confirmed influenza hospitalizations among elderly adults during the 2010-2011 season. *Clin Infect Dis* 57: 820-827.

Laënnec RTH. (1819) *De l'Auscultation Médiate ou Traité du Diagnostic des Maladies des Poumons et du Cœur,* Paris.

Lagler H, Grabmeier-Pfistershammer K, Touzeau-Romer V, et al. (2012) Immunogenicity and tolerability after two doses of non-adjuvanted, whole-virion pandemic influenza A (H1N1) vaccine in HIV-infected individuals. *PLoS One* 7: e36773.

Lahlou Amine I, Bajjou T, El Rhaffouli H, et al. (2011) Pandemic influenza A(H1N1)2009 in Morocco: experience of the Mohammed V Military Teaching Hospital, Rabat, 12 June to 24 December 2009. *Euro Surveill* 16.

Lai SH, Liao SL, Wong KS, et al. (2014) Preceding human metapneumovirus infection increases adherence of Streptococcus pneumoniae and severity of murine pneumococcal pneumonia. *J Microbiol Immunol Infect* 49: 216-224.

Lamb R and Krug RM. (2001) *Fields - Virology (Two Volumes). Chapter 46. Orthomyxoviridae: The Viruses and Their Replication,* Philadelphia: Lippincott Williams & Wilkins.

Lanaspa M, Annamalay AA, Lesouef P, et al. (2014) Epidemiology, etiology, x-ray features, importance of co-infections and clinical features of viral pneumonia in developing countries. *Expert Rev Anti Infect Ther* 12: 31-47.

Lasocki S, Scanvic A, Le Turdu F, et al. (2006) Evaluation of the Binax NOW Streptococcus pneumoniae urinary antigen assay in intensive care patients hospitalized for pneumonia. *Intensive Care Med* 32: 1766-1772.

Lassalle C, Grizeau P, Isautier H, et al. (1998) [Epidemiological surveillance of grippe and dengue. Reunion, 1996]. *Bull Soc Pathol Exot* 91: 61-63.

Lau LL, Nishiura H, Kelly H, et al. (2012) Household transmission of 2009 pandemic influenza A (H1N1): a systematic review and meta-analysis. *Epidemiology* 23: 531-542.

Lauderdale TL, Chang FY, Ben RJ, et al. (2005) Etiology of community acquired pneumonia among adult patients requiring hospitalization in Taiwan. *Respir Med* 99: 1079-1086.

Launay O, Desaint C, Durier C, et al. (2011) Safety and immunogenicity of a monovalent 2009 influenza A/H1N1v vaccine adjuvanted with AS03A or unadjuvanted in HIV-infected adults: a randomized, controlled trial. *J Infect Dis* 204: 124-134.

Lee EH, Wu C, Lee EU, et al. (2010) Fatalities associated with the 2009 H1N1 influenza A virus in New York city. *Clin Infect Dis* 50: 1498-1504.

Leiberman A, Dagan R, Leibovitz E, et al. (1999) The bacteriology of the nasopharynx in childhood. *Int J Pediatr Otorhinolaryngol* 49 Suppl 1: S151-153.

Lekana-Douki SE, Mouinga-Ondeme A, Nkoghe D, et al. (2013) Early introduction and delayed dissemination of pandemic influenza, Gabon. *Emerg Infect Dis* 19: 644-647.

Lekana-Douki SE, Nkoghe D, Drosten C, et al. (2014) Viral etiology and seasonality of influenza-like illness in Gabon, March 2010 to June 2011. *BMC Infect Dis* 14: 373.

Lenzi L, Mello AM, Silva LR, et al. (2012) Pandemic influenza A (H1N1) 2009: risk factors for hospitalization. *J Bras Pneumol* 38: 57-65.

LeVine AM, Koeningsknecht V and Stark JM. (2001) Decreased pulmonary clearance of S. pneumoniae following influenza A infection in mice. *J Virol Methods* 94: 173-186.

Levine OS, O'Brien KL, Deloria-Knoll M, et al. (2012) The Pneumonia Etiology Research for Child Health Project: a 21st century childhood pneumonia etiology study. *Clin Infect Dis* 54 Suppl 2: S93-101.

Lichenstein R, Magder LS, King RE, et al. (2012) The relationship between influenza outbreaks and acute ischemic heart disease in Maryland residents over a 7-year period. *J Infect Dis* 206: 821-827.

Lieberman D, Schlaeffer F, Boldur I, et al. (1996) Multiple pathogens in adult patients admitted with community-acquired pneumonia: a one year prospective study of 346 consecutive patients. *Thorax* 51: 179-184.

Lieberman D, Shimoni A, Shemer-Avni Y, et al. (2010) Respiratory viruses in adults with community-acquired pneumonia. *Chest* 138: 811-816.

Lim WS, Macfarlane JT, Boswell TC, et al. (2001) Study of community acquired pneumonia aetiology (SCAPA) in adults admitted to hospital: implications for management guidelines. *Thorax* 56: 296-301.

Lim WS, van der Eerden MM, Laing R, et al. (2003) Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. *Thorax* 58: 377-382.

Lin JC and Nichol KL. (2001) Excess mortality due to pneumonia or influenza during influenza seasons among persons with acquired immunodeficiency syndrome. *Arch Intern Med* 161: 441-446.

Loens K, Van Heirstraeten L, Malhotra-Kumar S, et al. (2009) Optimal sampling sites and methods for detection of pathogens possibly causing community-acquired lower respiratory tract infections. *J Clin Microbiol* 47: 21-31.

Lofgren E, Fefferman NH, Naumov YN, et al. (2007) Influenza seasonality: underlying causes and modeling theories. *J Virol* 81: 5429-5436.

Longini IM, Jr., Koopman JS, Monto AS, et al. (1982) Estimating household and community transmission parameters for influenza. *Am J Epidemiol* 115: 736-751.

Loosli CG, Stinson SF, Ryan DP, et al. (1975) The destruction of type 2 pneumocytes by airborne influenza PR8-A virus; its effect on surfactant and lecithin content of the pneumonic lesions of mice. *Chest* 67: 7S-14S.

Louie JK, Acosta M, Jamieson DJ, et al. (2010) Severe 2009 H1N1 influenza in pregnant and postpartum women in California. *N Engl J Med* 362: 27-35.

Louie JK, Acosta M, Winter K, et al. (2009) Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. *JAMA* 302: 1896-1902.

Luchsinger V, Ruiz M, Zunino E, et al. (2013) Community-acquired pneumonia in Chile: the clinical relevance in the detection of viruses and atypical bacteria. *Thorax* 68: 1000-1006.

Lutwama JJ, Bakamutumaho B, Kayiwa JT, et al. (2012) Clinic- and hospital-based sentinel influenza surveillance, Uganda 2007-2010. *J Infect Dis* 206 Suppl 1: S87-93.

Lyman GH and Kuderer NM. (2005) The strengths and limitations of meta-analyses based on aggregate data. *BMC Med Res Methodol* 5: 14.

Maartens G, Lewis SJ, de Goveia C, et al. (1994) 'Atypical' bacteria are a common cause of community-acquired pneumonia in hospitalised adults. *S Afr Med J* 84: 678-682.

Madhi SA, Cutland CL, Kuwanda L, et al. (2014) Influenza vaccination of pregnant women and protection of their infants. *N Engl J Med* 371: 918-931.

Madhi SA, Dittmer S, Kuwanda L, et al. (2013) Efficacy and immunogenicity of influenza vaccine in HIV-infected children: a randomized, double-blind, placebo controlled trial. *AIDS* 27: 369-379.

Madhi SA and Klugman KP. (2004) A role for Streptococcus pneumoniae in virus-associated pneumonia. *Nat Med* 10: 811-813.

Madhi SA, Ludewick H, Kuwanda L, et al. (2006) Pneumococcal coinfection with human metapneumovirus. *J Infect Dis* 193: 1236-1243.

Madhi SA, Maskew M, Koen A, et al. (2011) Trivalent inactivated influenza vaccine in African adults infected with human immunodeficient virus: double blind, randomized clinical trial of efficacy, immunogenicity, and safety. *Clin Infect Dis* 52: 128-137.

Madhi SA, Schoub B, Simmank K, et al. (2000) Increased burden of respiratory viral associated severe lower respiratory tract infections in children infected with human immunodeficiency virus type-1. *J Pediatr* 137: 78-84.

Mahony J, Chong S, Merante F, et al. (2007) Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay. *J Clin Microbiol* 45: 2965-2970.

Mahony JB. (2010) Nucleic acid amplification-based diagnosis of respiratory virus infections. *Expert Rev Anti Infect Ther* 8: 1273-1292.

Majanja J, Njoroge RN, Achilla R, et al. (2013) Impact of influenza A(H1N1)pdm09 virus on circulation dynamics of seasonal influenza strains in Kenya. *Am J Trop Med Hyg* 88: 940-945.

Malawi Ministry of Health. (2011) Clinical Management of HIV in Children and Adults - Malawi Integrated Guidelines for Providing HIV Services in: Antenatal Care, Maternal Care, Under 5 Clinics, Family Planning Clinics, Exposed Infant/Pre-ART Clinics and ART Clinics. In: Health Mo (ed). Lilongwe, Malawi, 1-79.

Malawi Ministry of Health. (2014) Clinical Management of HIV in Children and Adults - Malawi Integrated Guidelines for Providing HIV Services in: Antenatal Care, Maternal Care, Under 5 Clinics, Family Planning Clinics, Exposed Infant/Pre-ART Clinics and ART Clinics. In: Health Mo (ed). Lilongwe, Malawi, 1-95.

Maman I, Badziklou K, Landoh ED, et al. (2014) Implementation of influenza-like illness sentinel surveillance in Togo. *BMC Public Health* 14: 981.

Mangtani P, Cumberland P, Hodgson CR, et al. (2004) A cohort study of the effectiveness of influenza vaccine in older people, performed using the United Kingdom general practice research database. *J Infect Dis* 190: 1-10.

Mara D, Lane J, Scott B, et al. (2010) Sanitation and health. *PLoS Med* 7: e1000363.

Marks G and Beatty WK. (1976) *Epidemics,* New York, USA.: Charles Scribner's Sons.

Marks LR, Davidson BA, Knight PR, et al. (2013) Interkingdom signaling induces Streptococcus pneumoniae biofilm dispersion and transition from asymptomatic colonization to disease. *MBio* 4.

Martin CM, Kunin CM, Gottlieb LS, et al. (1959) Asian influenza A in Boston, 1957-1958. II. Severe staphylococcal pneumonia complicating influenza. *AMA Arch Intern Med* 103: 532-542.

Martin-Loeches I, Sanchez-Corral A, Diaz E, et al. (2011) Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza A(H1N1) virus. *Chest* 139: 555-562.

Martinez E, Marcos MA, Hoyo-Ulloa I, et al. (2011) Influenza A H1N1 in HIV-infected adults. *HIV Med* 12: 236-245.

Masangalawe C, Kandulu A and van Oosterhout JJ. (2011) Which health care facilities do adult Malawian antiretroviral therapy patients utilize during intercurrent illness?A cross sectional study. *BMC Health Serv Res* 11: 345.

Matheka DM, Mokaya J and Maritim M. (2013) Overview of influenza virus infections in Kenya: past, present and future. *Pan Afr Med J* 14: 138.

Matias G, Taylor R, Haguinet F, et al. (2014) Estimates of mortality attributable to influenza and RSV in the United States during 1997-2009 by influenza type or subtype, age, cause of death, and risk status. *Influenza Other Respir Viruses*.

Mauad T, Hajjar LA, Callegari GD, et al. (2010) Lung pathology in fatal novel human influenza A (H1N1) infection. *Am J Respir Crit Care Med* 181: 72-79.

McAnerney JM, Cohen C, Moyes J, et al. (2012) Twenty-five years of outpatient influenza surveillance in South Africa, 1984-2008. *J Infect Dis* 206 Suppl 1: S153-158.

McAnerney JM, Johnson S and Schoub BD. (1994) Surveillance of respiratory viruses. A 10-year laboratory-based study. *S Afr Med J* 84: 473-477.

McCool TL, Cate TR, Moy G, et al. (2002) The immune response to pneumococcal proteins during experimental human carriage. *J Exp Med* 195: 359-365.

McCool TL, Cate TR, Tuomanen EI, et al. (2003) Serum immunoglobulin G response to candidate vaccine antigens during experimental human pneumococcal colonization. *Infect Immun* 71: 5724-5732.

McCool TL and Weiser JN. (2004) Limited role of antibody in clearance of Streptococcus pneumoniae in a murine model of colonization. *Infect Immun* 72: 5807-5813.

McCullers JA. (2006) Insights into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev* 19: 571-582.

McCullers JA and Bartmess KC. (2003) Role of neuraminidase in lethal synergism between influenza virus and Streptococcus pneumoniae. *J Infect Dis* 187: 1000-1009.

McCullers JA, McAuley JL, Browall S, et al. (2010) Influenza enhances susceptibility to natural acquisition of and disease due to Streptococcus pneumoniae in ferrets. *J Infect Dis* 202: 1287-1295.

McCullers JA and Rehg JE. (2002) Lethal synergism between influenza virus and Streptococcus pneumoniae: characterization of a mouse model and the role of platelet-activating factor receptor. *J Infect Dis* 186: 341-350.

McKinney O, Modeste NN, Lee JW, et al. (2014) Determinants of Antiretroviral Therapy Adherence among Women in Southern Malawi: Healthcare Providers' Perspectives. *AIDS Res Treat* 2014: 489370.

McKittrick N, Frank I, Jacobson JM, et al. (2013) Improved immunogenicity with high-dose seasonal influenza vaccine in HIV-infected persons: a single-center, parallel, randomized trial. *Ann Intern Med* 158: 19-26.

McMorrow ML, Wemakoy EO, Tshilobo JK, et al. (2015) Severe acute respiratory illness deaths in Sub-Saharan Africa and the role of influenza: a case-series from 8 countries. *J Infect Dis*.

McNamee LA and Harmsen AG. (2006) Both influenza-induced neutrophil dysfunction and neutrophil-independent mechanisms contribute to increased susceptibility to a secondary Streptococcus pneumoniae infection. *Infect Immun* 74: 6707-6721.

Medical Research Council Unit The Gambia. (2002-) *Use of Biomass Briquettes: Its Effect on Indoor Air Pollution and on Pneumococcal Nasopharyngeal Carriage. A Randomized Clinical Trial. In ClinicalTrials.gov.* . Available at: https://clinicaltrials.gov/show/NCT01660659.

Melchior JC, Salmon D, Rigaud D, et al. (1991) Resting energy expenditure is increased in stable, malnourished HIV-infected patients. *Am J Clin Nutr* 53: 437-441.

Melegaro A, Gay NJ and Medley GF. (2004) Estimating the transmission parameters of pneumococcal carriage in households. *Epidemiol Infect* 132: 433-441.

Memoli MJ, Athota R, Reed S, et al. (2014) The natural history of influenza infection in the severely immunocompromised vs nonimmunocompromised hosts. *Clin Infect Dis* 58: 214-224.

Meseko CA, Odaibo GN and Olaleye DO. (2014) Detection and isolation of 2009 pandemic influenza A/H1N1 virus in commercial piggery, Lagos Nigeria. *Vet Microbiol* 168: 197-201.

Metersky ML, Masterton RG, Lode H, et al. (2012) Epidemiology, microbiology, and treatment considerations for bacterial pneumonia complicating influenza. *Int J Infect Dis* 16: e321-331.

Millar EV, Watt JP, Bronsdon MA, et al. (2008) Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal colonization among unvaccinated household members. *Clin Infect Dis* 47: 989-996.

Mills CE, Robins JM and Lipsitch M. (2004) Transmissibility of 1918 pandemic influenza. *Nature* 432: 904-906.

Mina MJ, Klugman KP and McCullers JA. (2013) Live attenuated influenza vaccine, but not pneumococcal conjugate vaccine, protects against increased density and duration of pneumococcal carriage after influenza infection in pneumococcal colonized mice. *J Infect Dis* 208: 1281-1285.

Ministry of Health M. (2008) Guidelines for the use of antiretroviral therapy in Malawi. In: Health Mo (ed) 3rd ed.

Ministry of Health M. (2011) Clinical Management of HIV in Children and Adults - Malawi Integrated Guidelines for Providing HIV Services in: Antenatal Care, Maternal Care, Under 5 Clinics, Family Planning Clinics, Exposed Infant/Pre-ART Clinics and ART Clinics. In: Health Mo (ed) 1st ed. Malawi, 1-95.

Miotti PG, Nelson KE, Dallabetta GA, et al. (1989) The influence of HIV infection on antibody responses to a two-dose regimen of influenza vaccine. *JAMA* 262: 779-783.

Mizuta K, Oshitani H, Mpabalwani EM, et al. (1995) An outbreak of influenza A/H3N2 in a Zambian school dormitory. *East Afr Med J* 72: 189-190.

Mizuta K, Oshitani H, Saijo M, et al. (1997) Epidemiology of influenza virus infections in children with acute respiratory infections in Zambia. *Ann Trop Paediatr* 17: 115-119.

Mmbaga VM, Mwasekaga MJ, Mmbuji P, et al. (2012) Results from the first 30 months of national sentinel surveillance for influenza in Tanzania, 2008-2010. *J Infect Dis* 206 Suppl 1: S80-86.

Mohan A, Chandra S, Agarwal D, et al. (2010) Prevalence of viral infection detected by PCR and RT-PCR in patients with acute exacerbation of COPD: a systematic review. *Respirology* 15: 536-542.

Monto AS. (2010) Seasonal influenza and vaccination coverage. *Vaccine* 28 Suppl 4: D33-44.

Monto AS, Ohmit SE, Petrie JG, et al. (2009) Comparative efficacy of inactivated and live attenuated influenza vaccines. *N Engl J Med* 361: 1260-1267.

Morens DM and Rash VM. (1995) Lessons from a nursing home outbreak of influenza A. *Infect Control Hosp Epidemiol* 16: 275-280.

Morens DM and Taubenberger JK. (2012) 1918 influenza, a puzzle with missing pieces. *Emerg Infect Dis* 18: 332-335.

Morens DM, Taubenberger JK and Fauci AS. (2008) Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 198: 962-970.

Morens DM, Taubenberger JK, Folkers GK, et al. (2010) Pandemic influenza's 500th anniversary. *Clin Infect Dis* 51: 1442-1444.

Morvan J, Rakotonirina G and Coulanges P. (1990) [Surveillance of influenza in Tananarive. 1989]. *Arch Inst Pasteur Madagascar* 57: 205-219.

Mosby LG, Rasmussen SA and Jamieson DJ. (2011) 2009 pandemic influenza A (H1N1) in pregnancy: a systematic review of the literature. *Am J Obstet Gynecol* 205: 10-18.

Mosser JF, Grant LR, Millar EV, et al. (2014) Nasopharyngeal carriage and transmission of Streptococcus pneumoniae in American Indian households after a decade of pneumococcal conjugate vaccine use. *PLoS One* 9: e79578.

Mossong J, Hens N, Jit M, et al. (2008) Social contacts and mixing patterns relevant to the spread of infectious diseases. *PLoS Med* 5: e74.

Mpe MJ, Jobo RD and Modise JP. (2001) The incidence of community-acquired Legionella pneumonia as determined by the urinary antigen test. A one-year prospective study in an academic hospital. *South African Journal of Epidemiology and Infection* 16: 54-56.

Mulholland EK, Ogunlesi OO, Adegbola RA, et al. (1999) Etiology of serious infections in young Gambian infants. *Pediatr Infect Dis J* 18: S35-41.

Murdoch DR, Jennings LC, Bhat N, et al. (2010) Emerging advances in rapid diagnostics of respiratory infections. *Infect Dis Clin North Am* 24: 791-807.

Murdoch DR, O'Brien KL, Scott JA, et al. (2009) Breathing new life into pneumonia diagnostics. *J Clin Microbiol* 47: 3405-3408.

Murni I, Duke T, Triasih R, et al. (2013) Prevention of nosocomial infections in developing countries, a systematic review. *Paediatr Int Child Health* 33: 61-78.

Murray EL, Khagayi S, Ope M, et al. (2013) What are the most sensitive and specific sign and symptom combinations for influenza in patients hospitalized with acute respiratory illness? Results from western Kenya, January 2007-July 2010. *Epidemiol Infect* 141: 212-222.

Murray EL, Klein M, Brondi L, et al. (2012) Rainfall, household crowding, and acute respiratory infections in the tropics. *Epidemiol Infect* 140: 78-86.

Muthuri SG, Venkatesan S, Myles PR, et al. (2014) Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. *Lancet Respir Med* 2: 395-404.

Muyembe Tamfum JJ, Nkwembe E, Bi Shamamba SK, et al. (2012) Sentinel surveillance for influenza-like illness, severe acute respiratory illness, and laboratory-confirmed influenza in Kinshasa, Democratic Republic of Congo, 2009-2011. *J Infect Dis* 206 Suppl 1: S36-40.

Mwachari CW, Nduba VN, Park DR, et al. (2006) Clinical presentation among HIV-infected and non-infected adults with community acquired pneumonia in Nairobi. *Int J Tuberc Lung Dis* 10: 516-522.

Nair H, Brooks WA, Katz M, et al. (2011) Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet* 378: 1917-1930.

Nakamura S, Davis KM and Weiser JN. (2011) Synergistic stimulation of type I interferons during influenza virus coinfection promotes Streptococcus pneumoniae colonization in mice. *J Clin Invest* 121: 3657-3665.

Naleway AL, Irving SA, Henninger ML, et al. (2014) Safety of influenza vaccination during pregnancy: a review of subsequent maternal obstetric events and findings from two recent cohort studies. *Vaccine* 32: 3122-3127.

National Advisory Committee on Immunization (NACI). (2014) Statement on Seasonal Influenza Vaccine for 2014-2015. Public Health Agency of Canada, 1-68.

National Institute for Health and Care Excellence. (2009) Amantadine, oseltamivir and zanamivir for the treatment of influenza: NICE technology appraisal guidance 168. National Institute for Health and Care Excellence, 1-42.

National Statistics Office (NSO). (2008) Malawi 2008 Population and Housing Census Results. Zomba, Malawi, 1-23.

National Statistics Office (NSO) and ICF Macro. (2011) Malawi Demographic and Health Survey 2010. Zomba, Malawi and Calverton, Maryland: NSO & ICF Macro 437.

Nelson KE, Clements ML, Miotti P, et al. (1988) The influence of human immunodeficiency virus (HIV) infection on antibody responses to influenza vaccines. *Ann Intern Med* 109: 383-388.

Neuzil KM, Coffey CS, Mitchel EF, Jr., et al. (2003) Cardiopulmonary hospitalizations during influenza season in adults and adolescents with advanced HIV infection. *J Acquir Immune Defic Syndr* 34: 304-307.

Neuzil KM, Reed GW, Mitchel EF, Jr., et al. (1999) Influenza-associated morbidity and mortality in young and middle-aged women. *JAMA* 281: 901-907.

Neuzil KM, Reed GW, Mitchel EF, et al. (1998) Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol* 148: 1094-1102.

New York City Department of Health and Mental Hygiene. (2014) HIV surveillance annual report, 2013. Queens, NY, 1-13.

Newson RB. (2013) Attributable and unattributable risks and fractions and other scenario comparisons. *Stata Journal* 13: 672-698.

Nguyen TH, Farrar J and Horby P. (2008) Person-to-person transmission of influenza A (H5N1). *Lancet* 371: 1392-1394.

Niang MN, Dosseh A, Ndiaye K, et al. (2012) Sentinel surveillance for influenza in Senegal, 1996-2009. *J Infect Dis* 206 Suppl 1: S129-135.

Nichol KL, Lind A, Margolis KL, et al. (1995) The effectiveness of vaccination against influenza in healthy, working adults. *N Engl J Med* 333: 889-893.

Nicoll A, Ciancio B, Tsolova S, et al. (2008) The scientific basis for offering seasonal influenza immunisation to risk groups in Europe. *Euro Surveill* 13.

Nielsen AB, Nielsen HS, Nielsen L, et al. (2012) Immune response after one or two doses of pandemic influenza A (H1N1) monovalent, AS03-adjuvanted vaccine in HIV infected adults. *Vaccine* 30: 7067-7071.

Njouom R, Mba SA, Noah DN, et al. (2010) Circulation of human influenza viruses and emergence of Oseltamivir-resistant A(H1N1) viruses in Cameroon, Central Africa. *BMC Infect Dis* 10: 56.

Njouom R, Yekwa EL, Cappy P, et al. (2012) Viral etiology of influenza-like illnesses in Cameroon, January-December 2009. *J Infect Dis* 206 Suppl 1: S29-35.

Nokleby H and Nicoll A. (2010) Risk groups and other target groups - preliminary ECDC guidance for developing influenza vaccination recommendations for the season 2010-11. *Euro Surveill* 15.

Ntshoe GM, McAnerney JM, Tempia S, et al. (2014) Influenza Epidemiology and Vaccine Effectiveness among Patients with Influenza-Like Illness, Viral Watch Sentinel Sites, South Africa, 2005-2009. *PLoS One* 9: e94681.

Nunes MC, Cutland CL, Dighero B, et al. (2015) Kinetics of Hemagglutination-Inhibiting Antibodies Following Maternal Influenza Vaccination Among Mothers With and Those Without HIV Infection and Their Infants. *J Infect Dis*.

Nuorti JP, Butler JC, Gelling L, et al. (2000) Epidemiologic relation between HIV and invasive pneumococcal disease in San Francisco County, California. *Ann Intern Med* 132: 182-190.

Nyamande K, Lalloo UG and John M. (2007) TB presenting as community-acquired pneumonia in a setting of high TB incidence and high HIV prevalence. *Int J Tuberc Lung Dis* 11: 1308-1313.

Nyatanyi T, Nkunda R, Rukelibuga J, et al. (2012) Influenza sentinel surveillance in Rwanda, 2008-2010. *J Infect Dis* 206 Suppl 1: S74-79.

Nzussouo NT, Michalove J, Diop OM, et al. (2012) Delayed 2009 pandemic influenza A virus subtype H1N1 circulation in West Africa, May 2009-April 2010. *J Infect Dis* 206 Suppl 1: S101-107.

O'Brien WA, Grovit-Ferbas K, Namazi A, et al. (1995) Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood* 86: 1082-1089.

Oba K, Nishihara A, Okamura K, et al. (2000) Two cases of acute myositis associated with influenza A virus infection in the elderly. *J Nippon Med Sch* 67: 126-129.

Odera AS and Anzala O. (2009) A survey of Legionella pneumophila among pneumonia patients at Kenyatta National Hospital. *East Afr Med J* 86: 565-571.

Oei W and Nishiura H. (2012) The relationship between tuberculosis and influenza death during the influenza (H1N1) pandemic from 1918-19. *Comput Math Methods Med* 2012: 124861.

Ohemeng A, Marquis GS and Lartey A. (2015) Household Food Insecurity is Associated with Respiratory Infections Among 6- to 11-Month Old Infants in Rural Ghana. *Pediatr Infect Dis J* 34: 821-825.

Ohmit SE, Victor JC, Rotthoff JR, et al. (2006) Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. *N Engl J Med* 355: 2513-2522.

Ohmit SE, Victor JC, Teich ER, et al. (2008) Prevention of symptomatic seasonal influenza in 2005-2006 by inactivated and live attenuated vaccines. *J Infect Dis* 198: 312-317.

Olaleye OD, Omilabu SA, Olabode AO, et al. (1989) Serological evidence for influenza virus activity in Nigeria (1985-1987). *Virologie* 40: 11-17.

Omer SB, Richards JL, Madhi SA, et al. (2015) Three randomized trials of maternal influenza immunization in Mali, Nepal, and South Africa: Methods and expectations. *Vaccine* 33: 3801-3812.

Ope MO, Katz MA, Aura B, et al. (2011) Risk factors for hospitalized seasonal influenza in rural western Kenya. *PLoS One* 6: e20111.

Orelle A, Razanajatovo NH, Rajatonirina S, et al. (2012) Epidemiological and virological characterization of 2009 pandemic influenza A virus subtype H1N1 in Madagascar. *J Infect Dis* 206 Suppl 1: S140-147.

Ormsby CE, de la Rosa-Zamboni D, Vazquez-Perez J, et al. (2011) Severe 2009 pandemic influenza A (H1N1) infection and increased mortality in patients with late and advanced HIV disease. *AIDS* 25: 435-439.

Ortiz JR, Lafond KE, Wong TA, et al. (2012) Pandemic influenza in Africa, lessons learned from 1968: a systematic review of the literature. *Influenza Other Respir Viruses* 6: 11-24.

Palache A, Oriol-Mathieu V, Abelin A, et al. (2014) Seasonal influenza vaccine dose distribution in 157 countries (2004-2011). *Vaccine* 32: 6369-6376.

Palacios G, Hornig M, Cisterna D, et al. (2009) Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. *PLoS One* 4: e8540.

Palar K, Wagner G, Ghosh-Dastidar B, et al. (2012) Role of antiretroviral therapy in improving food security among patients initiating HIV treatment and care. *AIDS* 26: 2375-2381.

Pan American Health Organization & Centers for Disease Control and Prevention (PAHO-CDC). (2006) PAHO-CDC Generic Protocol for Influenza Surveillance. Washington DC, 8-9.

Park DR, Sherbin VL, Goodman MS, et al. (2001) The etiology of community-acquired pneumonia at an urban public hospital: influence of human immunodeficiency virus infection and initial severity of illness. *J Infect Dis* 184: 268-277.

Parmigiani A, Alcaide ML, Freguja R, et al. (2013) Impaired antibody response to influenza vaccine in HIV-infected and uninfected aging women is associated with immune activation and inflammation. *PLoS One* 8: e79816.

Patriarca PA, Arden NH, Koplan JP, et al. (1987) Prevention and control of type A influenza infections in nursing homes. Benefits and costs of four approaches using vaccination and amantadine. *Ann Intern Med* 107: 732-740.

Patterson KD. (1981) The demographic impact of the 1918-1919 influenza pandemic in sub-Saharan Africa: a preliminary assessment. . In: Fyfe C and McMaster D (eds) *African History Demography II.* Centre of African Studies: University of Edinburgh, 401-433.

Patterson KD and Pyle GF. (1983) The diffusion of influenza in sub-Saharan Africa during the 1918-1919 pandemic. *Soc Sci Med* 17: 1299-1307.

Patterson KD and Pyle GF. (1991) The geography and mortality of the 1918 influenza pandemic. *Bull Hist Med* 65: 4-21.

Paul J. (1997) Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 12 December 1996. HIV and pneumococcal infection in Africa. Microbiological aspects. *Trans R Soc Trop Med Hyg* 91: 632-637.

Peasah SK, Azziz-Baumgartner E, Breese J, et al. (2013) Influenza cost and cost-effectiveness studies globally--a review. *Vaccine* 31: 5339-5348.

Pebody RG, McLean E, Zhao H, et al. (2010) Pandemic Influenza A (H1N1) 2009 and mortality in the United Kingdom: risk factors for death, April 2009 to March 2010. *Euro Surveill* 15.

Peduzzi P, Concato J, Kemper E, et al. (1996) A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* 49: 1373-1379.

Peltola VT and McCullers JA. (2004) Respiratory viruses predisposing to bacterial infections: role of neuraminidase. *Pediatr Infect Dis J* 23: S87-97.

Peltola VT, Murti KG and McCullers JA. (2005) Influenza virus neuraminidase contributes to secondary bacterial pneumonia. *J Infect Dis* 192: 249-257.

Peng D, Zhao D, Liu J, et al. (2009) Multipathogen infections in hospitalized children with acute respiratory infections. *Virol J* 6: 155.

Pepin S, Donazzolo Y, Jambrecina A, et al. (2013) Safety and immunogenicity of a quadrivalent inactivated influenza vaccine in adults. *Vaccine* 31: 5572-5578.

Perez CM, Dominguez MI, Ceballos ME, et al. (2010) Pandemic influenza A (H1N1) in HIV-1-infected patients. *AIDS* 24: 2867-2869.

Peters PJ, Skarbinski J, Louie JK, et al. (2011) HIV-infected hospitalized patients with 2009 pandemic influenza A (pH1N1)--United States, spring and summer 2009. *Clin Infect Dis* 52 Suppl 1: S183-188.

Peters RP, de Boer RF, Schuurman T, et al. (2009) Streptococcus pneumoniae DNA load in blood as a marker of infection in patients with community-acquired pneumonia. *J Clin Microbiol* 47: 3308-3312.

Peto L, Nadjm B, Horby P, et al. (2014) The bacterial aetiology of adult community-acquired pneumonia in Asia: a systematic review. *Trans R Soc Trop Med Hyg* 108: 326-337.

Petrie JG, Ohmit SE, Cowling BJ, et al. (2013) Influenza transmission in a cohort of households with children: 2010-2011. *PLoS One* 8: e75339.

Philip RN, Bell JA, Davis DJ, et al. (1961) Epidemiologic studies on influenza in familial and general population groups, 1951-1956. II. Characteristics of occurrence. *Am J Hyg* 73: 123-137.

Phillips CJ, Woolpert T, Sevick C, et al. (2013) Comparison of the effectiveness of trivalent inactivated influenza vaccine and live, attenuated influenza vaccine in preventing influenza-like illness among US military service members, 2006-2009. *Clin Infect Dis* 56: 11-19.

Piddock KC, Gordon SB, Ngwira A, et al. (2014) A cross-sectional study of household biomass fuel use among a periurban population in Malawi. *Ann Am Thorac Soc* 11: 915-924.

Pitman RJ, White LJ and Sculpher M. (2012) Estimating the clinical impact of introducing paediatric influenza vaccination in England and Wales. *Vaccine* 30: 1208-1224.

Pittet LA, Hall-Stoodley L, Rutkowski MR, et al. (2010) Influenza virus infection decreases tracheal mucociliary velocity and clearance of Streptococcus pneumoniae. *Am J Respir Cell Mol Biol* 42: 450-460.

Poehling KA, Edwards KM, Weinberg GA, et al. (2006) The underrecognized burden of influenza in young children. *N Engl J Med* 355: 31-40.

Population Reference Bureau. (2012) Malawi Population Data Sheet 2012. 8.

Population Reference Bureau. (2014) 2014 World Population Data Sheet In: USAID (ed). 1-20.

Potgieter PD and Hammond JM. (1992) Etiology and diagnosis of pneumonia requiring ICU admission. *Chest* 101: 199-203.

Preaud E, Durand L, Macabeo B, et al. (2014) Annual public health and economic benefits of seasonal influenza vaccination: a European estimate. *BMC Public Health* 14: 813.

Pretorius MA, Madhi SA, Cohen C, et al. (2012) Respiratory viral coinfections identified by a 10-plex real-time reverse-transcription polymerase chain reaction assay in patients hospitalized with severe acute respiratory illness--South Africa, 2009-2010. *J Infect Dis* 206 Suppl 1: S159-165.

Public Health England. (2015) Immunisation against infectious disease (Green Book). In: D. S and Ramsay M (eds). London: Crown, 1-31.

Puig-Barbera J, Diez-Domingo J, Arnedo-Pena A, et al. (2012) Effectiveness of the 2010-2011 seasonal influenza vaccine in preventing confirmed influenza hospitalizations in adults: a case-case comparison, case-control study. *Vaccine* 30: 5714-5720.

Qin Y, Horby PW, Tsang TK, et al. (2015) Differences in the Epidemiology of Human Cases of Avian Influenza A(H7N9) and A(H5N1) Viruses Infection. *Clin Infect Dis*.

Quandelacy TM, Viboud C, Charu V, et al. (2014) Age- and sex-related risk factors for influenza-associated mortality in the United States between 1997-2007. *Am J Epidemiol* 179: 156-167.

Radin JM, Katz MA, Tempia S, et al. (2012) Influenza surveillance in 15 countries in Africa, 2006-2010. *J Infect Dis* 206 Suppl 1: S14-21.

Radwan HM, Cheeseman SH, Lai KK, et al. (2000) Influenza in human immunodeficiency virus-infected patients during the 1997-1998 influenza season. *Clin Infect Dis* 31: 604-606.

Rajatonirina S, Heraud JM, Orelle A, et al. (2012a) The spread of influenza A(H1N1)pdm09 virus in Madagascar described by a sentinel surveillance network. *PLoS One* 7: e37067.

Rajatonirina S, Heraud JM, Randrianasolo L, et al. (2011) Pandemic influenza A(H1N1) 2009 virus outbreak among boarding school pupils in Madagascar: compliance and adverse effects of prophylactic oseltamivir treatment. *J Infect Dev Ctries* 5: 156-162.

Rajatonirina S, Rakotosolofo B, Rakotomanana F, et al. (2012b) Excess mortality associated with the 2009 A(H1N1)v influenza pandemic in Antananarivo, Madagascar. *Epidemiol Infect*: 1-6.

Raju R, Peters BS and Breen RA. (2012) Lung infections in the HIV-infected adult. *Curr Opin Pulm Med* 18: 253-258.

Rakoto-Andrianarivelo M, Morvan JM, Raobijaona H, et al. (1993) [Influenza surveillance in Tananarive during the year 1992]. *Arch Inst Pasteur Madagascar* 60: 9-13.

Ram PK, Dutt D, Silk BJ, et al. (2014) Household air quality risk factors associated with childhood pneumonia in urban Dhaka, Bangladesh. *Am J Trop Med Hyg* 90: 968-975.

Ramirez JA, Ahkee S, Tolentino A, et al. (1996) Diagnosis of Legionella pneumophila, Mycoplasma pneumoniae, or Chlamydia pneumoniae lower respiratory infection using the polymerase chain reaction on a single throat swab specimen. *Diagn Microbiol Infect Dis* 24: 7-14.

Ranieri R, Veronelli A, Santambrogio C, et al. (2005) Impact of influenza vaccine on response to vaccination with pneumococcal vaccine in HIV patients. *AIDS Res Hum Retroviruses* 21: 407-409.

Ravaoarinoro M, Clerc Y, Ratsirahonana S, et al. (1982) [Surveillance of influenza in Tananarive in the year 1981]. *Arch Inst Pasteur Madagascar* 49: 85-95.

Razanajatovo NH, Richard V, Hoffmann J, et al. (2011) Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. *PLoS One* 6: e17579.

Regev-Yochay G, Raz M, Dagan R, et al. (2004) Nasopharyngeal carriage of Streptococcus pneumoniae by adults and children in community and family settings. *Clin Infect Dis* 38: 632-639.

Reid AH, Taubenberger JK and Fanning TG. (2004) Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus. *Nat Rev Microbiol* 2: 909-914.

Rello J, Lisboa T, Lujan M, et al. (2009) Severity of pneumococcal pneumonia associated with genomic bacterial load. *Chest* 136: 832-840.

Remschmidt C, Wichmann O and Harder T. (2014) Influenza vaccination in HIV-infected individuals: systematic review and assessment of quality of evidence related to vaccine efficacy, effectiveness and safety. *Vaccine* 32: 5585-5592.

Resti M, Moriondo M, Cortimiglia M, et al. (2010) Community-acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real-time polymerase chain reaction using blood samples. *Clin Infect Dis* 51: 1042-1049.

Rice TW, Rubinson L, Uyeki TM, et al. (2012) Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. *Crit Care Med* 40: 1487-1498.

Ridenhour BJ, Campitelli MA, Kwong JC, et al. (2013) Effectiveness of inactivated influenza vaccines in preventing influenza-associated deaths and hospitalizations among Ontario residents aged >/= 65 years: estimates with generalized linear models accounting for healthy vaccinee effects. *PLoS One* 8: e76318.

Riera M, Payeras A, Marcos MA, et al. (2010) Clinical presentation and prognosis of the 2009 H1N1 influenza A infection in HIV-1-infected patients: a Spanish multicenter study. *AIDS* 24: 2461-2467.

Robertson L, Caley JP and Moore J. (1958) Importance of Staphylococcus aureus in pneumonia in the 1957 epidemic of influenza A. *Lancet* 2: 233-236.

Rockhill B, Newman B and Weinberg C. (1998) Use and misuse of population attributable fractions. *Am J Public Health* 88: 15-19.

Rodriguez-Barradas MC, Tharapel RA, Groover JE, et al. (1997) Colonization by Streptococcus pneumoniae among human immunodeficiency virus-infected adults: prevalence of antibiotic resistance, impact of immunization, and characterization by polymerase chain reaction with BOX primers of isolates from persistent S. pneumoniae carriers. *J Infect Dis* 175: 590-597.

Rosok B, Voltersvik P, Bjerknes R, et al. (1996) Dynamics of HIV-1 replication following influenza vaccination of HIV+ individuals. *Clin Exp Immunol* 104: 203-207.

Roson B, Carratala J, Verdaguer R, et al. (2000) Prospective study of the usefulness of sputum Gram stain in the initial approach to community-acquired pneumonia requiring hospitalization. *Clin Infect Dis* 31: 869-874.

Rossignol JF, La Frazia S, Chiappa L, et al. (2009) Thiazolides, a new class of anti-influenza molecules targeting viral hemagglutinin at the post-translational level. *J Biol Chem* 284: 29798-29808.

Roth S, Whitehead S, Thamthitiwat S, et al. (2013) Concurrent influenza virus infection and tuberculosis in patients hospitalized with respiratory illness in Thailand. *Influenza Other Respi Viruses* 7: 244-248.

Rudenko LG, Lonskaya NI, Klimov AI, et al. (1996) Clinical and epidemiological evaluation of a live, cold-adapted influenza vaccine for 3-14-year-olds. *Bull World Health Organ* 74: 77-84.

Ruiz M, Ewig S, Marcos MA, et al. (1999) Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. *Am J Respir Crit Care Med* 160: 397-405.

Ruuskanen O, Lahti E, Jennings LC, et al. (2011) Viral pneumonia. *Lancet* 377: 1264-1275.

Ryan MA, Christian RS and Wohlrabe J. (2001) Handwashing and respiratory illness among young adults in military training. *Am J Prev Med* 21: 79-83.

Rylance J, Baker T, Mushi E, et al. (2009) Use of an early warning score and ability to walk predicts mortality in medical patients admitted to hospitals in Tanzania. *Trans R Soc Trop Med Hyg* 103: 790-794.

Safrin S, Rush JD and Mills J. (1990) Influenza in patients with human immunodeficiency virus infection. *Chest* 98: 33-37.

Said MA, Johnson HL, Nonyane BA, et al. (2013a) Estimating the burden of pneumococcal pneumonia among adults: a systematic review and meta-analysis of diagnostic techniques. *PLoS One* 8: e60273.

Said MA, Johnson HL, Nonyane BA, et al. (2013b) Estimating the burden of pneumococcal pneumonia among adults: a systematic review and meta-analysis of diagnostic techniques. *PLoS One* 8: e60273.

Saito A, Kohno S, Matsushima T, et al. (2006) Prospective multicenter study of the causative organisms of community-acquired pneumonia in adults in Japan. *J Infect Chemother* 12: 63-69.

Saito S, Howard AA, Reid MJ, et al. (2012) TB diagnostic capacity in sub-Saharan African HIV care settings. *J Acquir Immune Defic Syndr* 61: 216-220.

Salmon DA, Proschan M, Forshee R, et al. (2013) Association between Guillain-Barre syndrome and influenza A (H1N1) 2009 monovalent inactivated vaccines in the USA: a meta-analysis. *Lancet* 381: 1461-1468.

Salonen O, Koshkiniemi M, Saari A, et al. (1997) Myelitis associated with influenza A virus infection. *J Neurovirol* 3: 83-85.

SanJoaquin MA, Allain TJ, Molyneux ME, et al. (2013) Surveillance Programme of IN-patients and Epidemiology (SPINE): implementation of an electronic data collection tool within a large hospital in Malawi. *PLoS Med* 10: e1001400.

Santini M, Kutlesa M, Zarkovic K, et al. (2012) Influenza A 2009 H1N1 encephalitis in adults with viral RNA in cerebrospinal fluid. *Scand J Infect Dis* 44: 992-996.

Santini-Oliveira M, Camacho LA, Souza TM, et al. (2012) H1N1pdm09 adjuvanted vaccination in HIV-infected adults: a randomized trial of two single versus two double doses. *PLoS One* 7: e39310.

Satzke C, Turner P, Virolainen-Julkunen A, et al. (2013) Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine* 32: 165-179.

Schoub BD. (2010) Surveillance and management of influenza on the African continent. *Expert Rev Respir Med* 4: 167-169.

Schoub BD, Gessner BD, Ampofo W, et al. (2013) Afriflu2--second international workshop on influenza vaccination in the African continent--8 November 2012, Cape Town (South Africa). *Vaccine* 31: 3461-3466.

Schoub BD, Johnson S, McAnerney JM, et al. (1986) Laboratory studies of the 1984 influenza epidemic on the Witwatersrand. *S Afr Med J* 70: 815-818.

Scott JA, Hall AJ, Muyodi C, et al. (2000) Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. *Lancet* 355: 1225-1230.

Scott JAG, Adegbola R and Gordon SB. (2012) *Pneumonia in adults.,* Cambridge: Cambridge University Press.

Seki M, Yanagihara K, Higashiyama Y, et al. (2004) Immunokinetics in severe pneumonia due to influenza virus and bacteria coinfection in mice. *Eur Respir J* 24: 143-149.

Self WH, Williams DJ, Zhu Y, et al. (2015) Respiratory Viral Detection in Children and Adults: Comparing Asymptomatic Controls and Patients With Community-Acquired Pneumonia. *J Infect Dis*.

Selvaraju SB and Selvarangan R. (2010) Evaluation of three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses. *J Clin Microbiol* 48: 3870-3875.

Semple MG, Cowell A, Dove W, et al. (2005) Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis* 191: 382-386.

Sepako E, Glennie SJ, Jambo KC, et al. (2014) Incomplete recovery of pneumococcal CD4 T cell immunity after initiation of antiretroviral therapy in HIV-infected malawian adults. *PLoS One* 9: e100640.

Shahangian A, Chow EK, Tian X, et al. (2009) Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. *J Clin Invest* 119: 1910-1920.

Sheth AN, Patel P and Peters PJ. (2011) Influenza and HIV: lessons from the 2009 H1N1 influenza pandemic. *Curr HIV/AIDS Rep* 8: 181-191.

Shieh WJ, Blau DM, Denison AM, et al. (2010) 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. *Am J Pathol* 177: 166-175.

Short KR, Habets MN, Hermans PW, et al. (2012) Interactions between Streptococcus pneumoniae and influenza virus: a mutually beneficial relationship? *Future Microbiol* 7: 609-624.

Simonsen L, Taylor RJ, Young-Xu Y, et al. (2011) Impact of pneumococcal conjugate vaccination of infants on pneumonia and influenza hospitalization and mortality in all age groups in the United States. *MBio* 2: e00309-00310.

Sinclair A, Xie X, Teltscher M, et al. (2013) Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by Streptococcus pneumoniae. *J Clin Microbiol* 51: 2303-2310.

Singh A, Singh A and Ram F. (2014) Household food insecurity and nutritional status of children and women in Nepal. *Food Nutr Bull* 35: 3-11.

Skiest DJ, Kaplan P, Machala T, et al. (2001) Clinical manifestations of influenza in HIV-infected individuals. *Int J STD AIDS* 12: 646-650.

Sloan DJ, van Oosterhout JJ, Malisita K, et al. (2013) Evidence of improving antiretroviral therapy treatment delays: an analysis of eight years of programmatic outcomes in Blantyre, Malawi. *BMC Public Health* 13: 490.

Sloan DJ and Zijlstra EE. (2012) *The Clinical Book, 2nd edition. Guidelines of the Department of Medicine, College of Medicine, Malawi.*

Smith A, Coles S, Johnson S, et al. (2009) An outbreak of influenza A(H1N1)v in a boarding school in South East England, May-June 2009. *Euro Surveill* 14.

Smith CM, Sandrini S, Datta S, et al. (2014) Respiratory syncytial virus increases the virulence of Streptococcus pneumoniae by binding to penicillin binding protein 1a. A new paradigm in respiratory infection. *Am J Respir Crit Care Med* 190: 196-207.

Smith W, Andrewes CH and Laidlaw PP. (1933) A virus obtained from influenza patients. *Lancet* 222: 66-68.

Soares JL, Ratsitorahina M, Rakoto Andrianarivelo M, et al. (2003) [Epidemics of acute respiratory infections in Madagascar in 2002: from alert to confirmation]. *Arch Inst Pasteur Madagascar* 69: 12-19.

Sogaard OS, Lohse N, Gerstoft J, et al. (2008) Hospitalization for pneumonia among individuals with and without HIV infection, 1995-2007: a Danish population-based, nationwide cohort study. *Clin Infect Dis* 47: 1345-1353.

Sonnenberg P, Glynn JR, Fielding K, et al. (2005) How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *J Infect Dis* 191: 150-158.

Spellerberg B, Cundell DR, Sandros J, et al. (1996) Pyruvate oxidase, as a determinant of virulence in Streptococcus pneumoniae. *Mol Microbiol* 19: 803-813.

Staprans SI, Hamilton BL, Follansbee SE, et al. (1995) Activation of virus replication after vaccination of HIV-1-infected individuals. *J Exp Med* 182: 1727-1737.

Steffen C, Debellut F, Gessner BD, et al. (2012) Improving influenza surveillance in sub-Saharan Africa. *Bull World Health Organ* 90: 301-305.

Steffen C, Diop OM, Gessner BD, et al. (2011) Afriflu--international conference on influenza disease burden in Africa, 1-2 June 2010, Marrakech, Morocco. *Vaccine* 29: 363-369.

Steinhoff MC, Omer SB, Roy E, et al. (2012) Neonatal outcomes after influenza immunization during pregnancy: a randomized controlled trial. *CMAJ* 184: 645-653.

Su S, Chaves SS, Perez A, et al. (2014) Comparing clinical characteristics between hospitalized adults with laboratory-confirmed influenza a and B virus infection. *Clin Infect Dis* 59: 252-255.

Subbarao EK, Griffis J and Waner JL. (1989) Detection of multiple viral agents in nasopharyngeal specimens yielding respiratory syncytial virus (RSV). An assessment of diagnostic strategy and clinical significance. *Diagn Microbiol Infect Dis* 12: 327-332.

Subbe CP, Kruger M, Rutherford P, et al. (2001) Validation of a modified Early Warning Score in medical admissions. *QJM* 94: 521-526.

Subbe CP, Slater A, Menon D, et al. (2006) Validation of physiological scoring systems in the accident and emergency department. *Emerg Med J* 23: 841-845.

Sullivan PS, Hanson DL, Dworkin MS, et al. (2000) Effect of influenza vaccination on disease progression among HIV-infected persons. *AIDS* 14: 2781-2785.

Sun K and Metzger DW. (2008) Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. *Nat Med* 14: 558-564.

Talbot TR, Poehling KA, Hartert TV, et al. (2005) Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. *Am J Med* 118: 285-291.

Tamma PD, Ault KA, del Rio C, et al. (2009) Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol* 201: 547-552.

Tarnagda Z, Yougbare I, Ilboudo AK, et al. (2014) Sentinel surveillance of influenza in Burkina Faso: identification of circulating strains during 2010-2012. *Influenza Other Respir Viruses* 8: 524-529.

Tasker SA, O'Brien WA, Treanor JJ, et al. (1998) Effects of influenza vaccination in HIV-infected adults: a double-blind, placebo-controlled trial. *Vaccine* 16: 1039-1042.

Taubenberger JK and Morens DM. (2006) 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis* 12: 15-22.

Taubenberger JK, Reid AH, Lourens RM, et al. (2005) Characterization of the 1918 influenza virus polymerase genes. *Nature* 437: 889-893.

Taylor G, Mitchell R, McGeer A, et al. (2014) Healthcare-associated influenza in Canadian hospitals from 2006 to 2012. *Infect Control Hosp Epidemiol* 35: 169-175.

Tebas P, Frank I, Lewis M, et al. (2010) Poor immunogenicity of the H1N1 2009 vaccine in well controlled HIV-infected individuals. *AIDS* 24: 2187-2192.

Tellier R. (2009) Aerosol transmission of influenza A virus: a review of new studies. *J R Soc Interface* 6 Suppl 6: S783-790.

Tempia S, Walaza S, Cohen AL, et al. (2015a) Mortality Associated With Seasonal and Pandemic Influenza Among Pregnant and Nonpregnant Women of Childbearing Age in a High-HIV-Prevalence Setting-South Africa, 1999-2009. *Clin Infect Dis* 61: 1063-1070.

Tempia S, Walaza S, Viboud C, et al. (2015b) Deaths Associated with Respiratory Syncytial and Influenza Viruses among Persons >/=5 Years of Age in HIV-Prevalent Area, South Africa, 1998-2009(1). *Emerg Infect Dis* 21: 600-608.

Templeton KE, Scheltinga SA, van den Eeden WC, et al. (2005) Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. *Clin Infect Dis* 41: 345-351.

Tenthani L, Haas AD, Tweya H, et al. (2014) Retention in care under universal antiretroviral therapy for HIV-infected pregnant and breastfeeding women ('Option B+') in Malawi. *AIDS* 28: 589-598.

The World Bank. (2014) World Development Indicators: Reproductive health.

Theo A, Liwewe M, Ndumba I, et al. (2012) Influenza surveillance in Zambia, 2008-2009. *J Infect Dis* 206 Suppl 1: S173-177.

Thiam D, Niang M, Dia N, et al. (2014) [Influenza sentinel surveillance network improvement in Senegal and results.]. *Bull Soc Pathol Exot*.

Thomas PG, Keating R, Hulse-Post DJ, et al. (2006) Cell-mediated protection in influenza infection. *Emerg Infect Dis* 12: 48-54.

Thompson WW, Shay DK, Weintraub E, et al. (2003) Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 289: 179-186.

Thurn JR and Henry K. (1989) Influenza A pneumonitis in a patient infected with the human immunodeficiency virus (HIV). *Chest* 95: 807-810.

Tinoco JC, Pavia-Ruz N, Cruz-Valdez A, et al. (2014) Immunogenicity, reactogenicity, and safety of inactivated quadrivalent influenza vaccine candidate versus inactivated trivalent influenza vaccine in healthy adults aged >/=18 years: a phase III, randomized trial. *Vaccine* 32: 1480-1487.

Tiu CT, Lin YS, Pagala M, et al. (2011) Antibody response to inactivated influenza A (H1N1) 2009 monovalent vaccine in patients with and without HIV. *J Acquir Immune Defic Syndr* 58: e99-102.

Toovey S, Jick SS and Meier CR. (2011) Parkinson's disease or Parkinson symptoms following seasonal influenza. *Influenza Other Respir Viruses* 5: 328-333.

Tornheim JA, Manya AS, Oyando N, et al. (2007) The epidemiology of hospitalized pneumonia in rural Kenya: the potential of surveillance data in setting public health priorities. *Int J Infect Dis* 11: 536-543.

Torres A, Peetermans WE, Viegi G, et al. (2013) Risk factors for community-acquired pneumonia in adults in Europe: a literature review. *Thorax* 68: 1057-1065.

Treanor JJ, Kotloff K, Betts RF, et al. (1999) Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. *Vaccine* 18: 899-906.

Tricco AC, Chit A, Soobiah C, et al. (2013) Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. *BMC Med* 11: 153.

Trilla A, Trilla G and Daer C. (2008) The 1918 "Spanish flu" in Spain. *Clin Infect Dis* 47: 668-673.

Trotter Y, Jr., Dunn FL, Drachman RH, et al. (1959) Asian influenza in the United States, 1957-1958. *Am J Hyg* 70: 34-50.

Truscott J, Fraser C, Cauchemez S, et al. (2012) Essential epidemiological mechanisms underpinning the transmission dynamics of seasonal influenza. *J R Soc Interface* 9: 304-312.

Turner P, Turner C, Jankhot A, et al. (2012) A longitudinal study of Streptococcus pneumoniae carriage in a cohort of infants and their mothers on the Thailand-Myanmar border. *PLoS One* 7: e38271.

Turner P, Turner C, Kaewcharernnet N, et al. (2011) A prospective study of urinary pneumococcal antigen detection in healthy Karen mothers with high rates of pneumococcal nasopharyngeal carriage. *BMC Infect Dis* 11: 108.

UNAIDS. (2013) Report on the global AIDS epidemic. In: UNAIDS (ed).

UNAIDS. (2014a) 2014 Global Statistics - fact sheet. *How AIDS changed everything. MDG 6: 15 years, 15 lessons of hope from the AIDS response.*: UNAIDS, 1-8.

UNAIDS. (2014b) *AIDSinfo country fact sheets*. Available at: http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/.

UNdata. (2014) United Nations Statistics Division; Country Profile, Malawi.

UNICEF. (2012) *Malawi statistics*. Available at: http://www.unicef.org/infobycountry/malawi_statistics.html.

United Nations. (2015a) *Millenium Development Goals* Available at: http://www.un.org/millenniumgoals/.

United Nations. (2015b) *Sustainable Development Goals*. Available at: http://www.un.org/sustainabledevelopment/sustainable-development-goals/.

United Nations Development Programme. (2014) Human Development Report - Sustaining Human Progress: Reducing Vulnerabilities and Building Resilience.

Usuf E, Bottomley C, Adegbola RA, et al. (2014) Pneumococcal carriage in sub-Saharan Africa--a systematic review. *PLoS One* 9: e85001.

Vaillant L, La Ruche G, Tarantola A, et al. (2009) Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009. *Euro Surveill* 14.

Van Kerkhove MD, Mounts AW, Mall S, et al. (2011a) Epidemiologic and virologic assessment of the 2009 influenza A (H1N1) pandemic on selected temperate countries in the Southern Hemisphere: Argentina, Australia, Chile, New Zealand and South Africa. *Influenza Other Respir Viruses* 5: e487-498.

Van Kerkhove MD, Vandemaele KA, Shinde V, et al. (2011b) Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. *PLoS Med* 8: e1001053.

van Oosterhout JJ, Bodasing N, Kumwenda JJ, et al. (2005) Evaluation of antiretroviral therapy results in a resource-poor setting in Blantyre, Malawi. *Trop Med Int Health* 10: 464-470.

van Oosterhout JJ, Kumwenda JK, Hartung T, et al. (2007) Can the initial success of the Malawi ART scale-up programme be sustained? The example of Queen Elizabeth Central Hospital, Blantyre. *AIDS Care* 19: 1241-1246.

Vellozzi C, Burwen DR, Dobardzic A, et al. (2009) Safety of trivalent inactivated influenza vaccines in adults: background for pandemic influenza vaccine safety monitoring. *Vaccine* 27: 2114-2120.

Vellozzi C, Iqbal S and Broder K. (2014) Guillain-Barre syndrome, influenza, and influenza vaccination: the epidemiologic evidence. *Clin Infect Dis* 58: 1149-1155.

Venter M, Naidoo D, Pretorius M, et al. (2012) Evolutionary dynamics of 2009 pandemic influenza A virus subtype H1N1 in South Africa during 2009-2010. *J Infect Dis* 206 Suppl 1: S166-172.

Viboud C, Boelle PY, Cauchemez S, et al. (2004) Risk factors of influenza transmission in households. *Br J Gen Pract* 54: 684-689.

Vu HT, Yoshida LM, Suzuki M, et al. (2011) Association between nasopharyngeal load of Streptococcus pneumoniae, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. *Pediatr Infect Dis J* 30: 11-18.

Vynnycky E, Pitman R, Siddiqui R, et al. (2008) Estimating the impact of childhood influenza vaccination programmes in England and Wales. *Vaccine* 26: 5321-5330.

Walaza S. (2014) Recommendations pertaining to the use of viral vaccines: influenza 2014. *S Afr Med J* 104: 166-167.

Walaza S and Cohen C. (2015) Recommendations pertaining to the use of influenza vaccines and influenza antiviral drugs: Influenza 2015. *South African Medical Journal* 105: 90-91.

Walaza S, Tempia S, Dawood H, et al. (2015) Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010-2011. *BMC Infect Dis* 15: 26.

Walter ND, Taylor TH, Shay DK, et al. (2010) Influenza circulation and the burden of invasive pneumococcal pneumonia during a non-pandemic period in the United States. *Clin Infect Dis* 50: 175-183.

Wang Z, Tobler S, Roayaei J, et al. (2009) Live attenuated or inactivated influenza vaccines and medical encounters for respiratory illnesses among US military personnel. *JAMA* 301: 945-953.

Warren-Gash C, Bhaskaran K, Hayward A, et al. (2011) Circulating influenza virus, climatic factors, and acute myocardial infarction: a time series study in England and Wales and Hong Kong. *J Infect Dis* 203: 1710-1718.

Watson M, Gilmour R, Menzies R, et al. (2006) The association of respiratory viruses, temperature, and other climatic parameters with the incidence of invasive pneumococcal disease in Sydney, Australia. *Clin Infect Dis* 42: 211-215.

Weber DJ, Rutala WA and Schaffner W. (2010) Lessons learned: protection of healthcare workers from infectious disease risks. *Crit Care Med* 38: S306-314.

Webster RG, Bean WJ, Gorman OT, et al. (1992) Evolution and ecology of influenza A viruses. *Microbiol Rev* 56: 152-179.

Weinberger DM, Simonsen L, Jordan R, et al. (2012) Impact of the 2009 influenza pandemic on pneumococcal pneumonia hospitalizations in the United States. *J Infect Dis* 205: 458-465.

Weinstock DM, Gubareva LV and Zuccotti G. (2003) Prolonged shedding of multidrug-resistant influenza A virus in an immunocompromised patient. *N Engl J Med* 348: 867-868.

Weiser SD, Gupta R, Tsai AC, et al. (2012a) Changes in food insecurity, nutritional status, and physical health status after antiretroviral therapy initiation in rural Uganda. *J Acquir Immune Defic Syndr* 61: 179-186.

Weiser SD, Palar K, Frongillo EA, et al. (2014) Longitudinal assessment of associations between food insecurity, antiretroviral adherence and HIV treatment outcomes in rural Uganda. *AIDS* 28: 115-120.

Weiser SD, Tsai AC, Gupta R, et al. (2012b) Food insecurity is associated with morbidity and patterns of healthcare utilization among HIV-infected individuals in a resource-poor setting. *AIDS* 26: 67-75.

Wellcome Trust Liverpool Glasgow Centre for Global Health Research. http://www.liverpoolwttc.org.uk/ Available at: http://www.liverpoolwttc.org.uk/

Welte T, Torres A and Nathwani D. (2012) Clinical and economic burden of community-acquired pneumonia among adults in Europe. *Thorax* 67: 71-79.

Wenger JD, Zulz T, Bruden D, et al. (2010) Invasive pneumococcal disease in Alaskan children: impact of the seven-valent pneumococcal conjugate vaccine and the role of water supply. *Pediatr Infect Dis J* 29: 251-256.

Wheeler I, Price C, Sitch A, et al. (2013) Early warning scores generated in developed healthcare settings are not sufficient at predicting early mortality in Blantyre, Malawi: a prospective cohort study. *PLoS One* 8: e59830.

White LF, Archer B and Pagano M. (2014) Determining the dynamics of influenza transmission by age. *Emerg Themes Epidemiol* 11: 4.

WHO Regional Office for Africa. (2014) Implementation of Option B+ for Prevention of Mother-to-Child Transmission of HIV: The Malawi Experience. Republic of Congo.

WHO working group on polymerase chain reaction protocols for detecting subtype influenza A viruses. (2011) The use of PCR in the surveillance and diagnosis of influenza. . Geneva, Switzerland, 1-10.

WHO/UNICEF. (2015) Estimates on the use of water sources and sanitation facilities - Malawi. Updated June 2015. *Joint Monitoring Programme for Water Supply and Santitation.* WHO/UNICEF.

Wie SH, So BH, Song JY, et al. (2013) A comparison of the clinical and epidemiological characteristics of adult patients with laboratory-confirmed influenza A or B during the 2011-2012 influenza season in Korea: a multi-center study. *PLoS One* 8: e62685.

Williamson T, Eliasziw M and Fick GH. (2013) Log-binomial models: exploring failed convergence. *Emerg Themes Epidemiol* 10: 14.

Wolter N, Cohen C, Tempia S, et al. (2014a) HIV and influenza virus infections are associated with increased blood pneumococcal load: a prospective, hospital-based observational study in South Africa, 2009-2011. *J Infect Dis* 209: 56-65.

Wolter N, Tempia S, Cohen C, et al. (2014b) High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia. *J Infect Dis* 210: 1649-1657.

Wong JM, Cosmas L, Nyachieo D, et al. (2015) Increased Rates of Respiratory and Diarrheal Illnesses in HIV-Negative Persons Living With HIV-Infected Individuals in a Densely Populated Urban Slum in Kenya. *J Infect Dis* 212: 745-753.

Wood R, Maartens G and Lombard CJ. (2000) Risk factors for developing tuberculosis in HIV-1-infected adults from communities with a low or very high incidence of tuberculosis. *J Acquir Immune Defic Syndr* 23: 75-80.

World Health Organization. *Integrated Management of Childhood Illness (IMCI)*. Available at: http://www.who.int/maternal_child_adolescent/topics/child/imci/en/.

World Health Organization. (2004) Rapid HIV Tests: Guidelines for Use in HIV Testing and Counselling Services in Resource-constrained Settings. Geneva: World Health Organization, 48.

World Health Organization. (2008) The global burden of disease: 2004 update. Switzerland: World Health Organization.

World Health Organization. (2009a) CDC protocol of realtime RTPCR for influenza A(H1N1). The WHO Collaborating Centre for influenza, CDC Atlanta, US: World Health Organization

World Health Organization. (2009b) *Density of physicians (total number per 1000 population, latest available year)*. Available at: http://www.who.int/gho/health_workforce/physicians_density/en/.

World Health Organization. (2009c) Strategic Advisory Group of Experts on Immunization - report of the extraordinary meeting on the influenza A (H1N1) 2009 pandemic, 7 July 2009. *Wkly Epidemiol Rec* 84: 301-304.

World Health Organization. (2010a) Limiting Spread: limiting the spread of pandemic, zoonotic and seasonal epidemic influenza. *Public Health Research Agenda for Influenza.* Geneva.

World Health Organization. (2010b) *Pandemic (H1N1) 2009 - update 112*. Available at: http://www.who.int/csr/don/2010_08_06/en/.

World Health Organization. (2010c) Seasonal influenza vaccination: plan to update the WHO position paper. In: meeting S (ed).

World Health Organization. (2010d) WHO Guidelines for Pharmacological Management of Pandemic Influenza A(H1N1) 2009 and other Influenza Viruses. Geneva, Switzerland.: World Health Organization, 1-32.

World Health Organization. (2012a) Public Health Research Needs for Influenza in Africa. *Public Health Research Agenda for Influenza.* Geneva, Switzerland: WHO, 1-10.

World Health Organization. (2012b) Vaccines against influenza WHO position paper - November 2012. *Wkly Epidemiol Rec* 87: 461-476.

World Health Organization. (2013a) Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. Geneva, Swtizerland: World Health Organization, 272.

World Health Organization. (2013b) Global Epidemiological Surveillance Standards for Influenza. Geneva, Switzerland: WHO, 1-73.

World Health Organization. (2014a) *Avian Influenza fact sheet*. Available at: http://www.who.int/mediacentre/factsheets/avian_influenza/en/.

World Health Organization. (2014b) *Global Influenza Surveillance and Response System (GISRS)*. Available at: http://www.who.int/influenza/gisrs_laboratory/en/.

World Health Organization. (2014c) *Influenza (Seasonal) - Fact sheet N°211*. Available at: http://www.who.int/mediacentre/factsheets/fs211/en/.

World Health Organization. (2014 ) *Global Influenza Surveillance and Response System (GISRS)*. Available at: http://www.who.int/influenza/gisrs_laboratory/en/.

World Health Organization. (2015a) *Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO* Available at: http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/.

World Health Organization. (2015b) *FluNet*. Available at: http://www.who.int/influenza/gisrs_laboratory/flunet/en/.

World Health Organization. (2015c) Global action plan for influenza vaccines (GAP).

World Health Organization and UNICEF. (2015) *WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation*. Available at: http://www.wssinfo.org/.

Yamanaka H, Teruya K, Tanaka M, et al. (2005) Efficacy and immunologic responses to influenza vaccine in HIV-1-infected patients. *J Acquir Immune Defic Syndr* 39: 167-173.

Yanagisawa N, Maeda K, Ajisawa A, et al. (2011) Reduced immune response to influenza A (H1N1) 2009 monovalent vaccine in HIV-infected Japanese subjects. *Vaccine* 29: 5694-5698.

Yang S, Lin S, Khalil A, et al. (2005) Quantitative PCR assay using sputum samples for rapid diagnosis of pneumococcal pneumonia in adult emergency department patients. *J Clin Microbiol* 43: 3221-3226.

Yassine HM, Boyington JC, McTamney PM, et al. (2015) Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat Med* 21: 1065-1070.

Yazdanbakhsh M and Kremsner PG. (2009) Influenza in Africa. *PLoS Med* 6: e1000182.

Yoon YK, Yang KS, Sohn JW, et al. (2014) Impact of preceding respiratory viral infections on the clinical severity of patients with pneumococcal pneumonia. *Influenza Other Respir Viruses* 8: 549-556.

Yoshimine H, Oishi K, Mubiru F, et al. (2001) Community-acquired pneumonia in Ugandan adults: short-term parenteral ampicillin therapy for bacterial pneumonia. *Am J Trop Med Hyg* 64: 172-177.

Zaman K, Roy E, Arifeen SE, et al. (2008) Effectiveness of maternal influenza immunization in mothers and infants. *N Engl J Med* 359: 1555-1564.

Zanetti AR, Amendola A, Besana S, et al. (2002) Safety and immunogenicity of influenza vaccination in individuals infected with HIV. *Vaccine* 20 Suppl 5: B29-32.

Zhang D, He Z, Xu L, et al. (2014) Epidemiology characteristics of respiratory viruses found in children and adults with respiratory tract infections in southern China. *Int J Infect Dis* 25: 159-164.

Zhou H, Haber M, Ray S, et al. (2012a) Invasive pneumococcal pneumonia and respiratory virus co-infections. *Emerg Infect Dis* 18: 294-297.

Zhou H, Thompson WW, Viboud CG, et al. (2012b) Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008. *Clin Infect Dis* 54: 1427-1436.