ACUTE VIRAL RESPIRATORY INFECTIONS IN INTENSIVE CARE UNIT AND VENTILATOR SUPPORT PATIENTS IN MOI TEACHING AND REFERRAL HOSPITAL, UASIN-GISHU COUNTY, KENYA.

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DECLARATION

This is my original work. It has not been presented for any academic award or any other purpose in any institution.

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DEDICATION

This work is dedicated to all patients and individuals affected by respiratory diseases caused by viruses in Kenya.

I also dedicate this to my beloved parents Mr. and Mrs. David Kipsang Kwambai and my siblings for their financial and moral support that contributed to my success.

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ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
BAL	Bronchoalveolar lavage
Bp	Base pairs
CAP	Community acquired pneumonia
cDNA	Complementary DNA
CFT	Complement Fixation test
CMV	Cytomegalovirus
COPD	Chronic pulmonary obstructive disease
DFA	Direct fluorescent antibody
DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
FDA	Food and drug administration
HAI	Haemaglutination assay
HCOV	*Human Coronavirus*
HIV	Human immunodeficiency virus
hMPV	*Human metapneumovirus*

HRV	*Human Rhinovirus*
PIV	*Parainfluenza Virus*
IA	Immunoassays
IF	Immunofluorescense
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IREC	Institutional Research and Ethics committee
kbp	kilo base pairs
KEMRI	Kenya medical research institute
LRTI	Lower respiratory tract infection
LDTs	Laboratory developed tests
MTRH	Moi teaching and referral Hospital
NASBA	Nucleic acid sequence based amplification
PBS	Phosphate buffer saline
Qpcr	Quantitative polymerase chain reaction
RSV	*Respiratory syncytial virus*
RPM	Revolutions per minute
RNA	Ribonucleic acid
SARI	Severe acute respiratory infections

SPSS	Statistical package for social sciences
TAT	Turn-around time
URTI	Upper respiratory tract infection
WHO	World Health organization

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ABSTRACT

Acute respiratory tract infections (ARTIs) are among the five most common causes of morbidity and mortality globally, accounting for approximately 3.9 million deaths annually. Most of these deaths occur among young children in developing countries Mechanical ventilation supports the breathing system but does not change any pre-existing condition. Associated challenges include viral and bacterial infections. The prevalence of these infections is high, however these infections mimic bacterial infections. There is no documentation of these infections in many healthcare facilities In Africa. This study aimed at assessing the prevalence rates of *Influenza virus*, HRV, RSV, HPIV, hMPV, *Human Adenovirus* and HCoV in study subjects on ventilator support as well as those on critical care in the intensive care unit. The study was done at MTRH in Uasin Gishu County. Samples were collected from April 2017 to August 2017. 200 samples of bronchoalveolar lavage were collected. The samples were then transported to KEMRI Nairobi at 2-8°C for analysis. The RNA/DNA of the viruses was detected using real time PCR and multiplex PCR. Data analysis as well as coding and entry were done using statistical package for social studies (SPSS). The results were log-transformed to obtain equal distribution. The results were also expressed as mean±standard deviation. The results were then compared with respect to whether in ICU or on mechanical ventilation as well age using ANOVA with Bonferroni's post-test using GenStat Release 14.1 (PC/Windows). Presentation of the data was done using graphs, pie charts and tables/figures. The samples that tested positive for *Influenza A virus*, HPIV-1, HPIV-2, HPIV-3, RSV, *Adenovirus,* HRV *Human Metapneumovirus and* HCoV was 33 (16.5%) 12 (6%), 8 (4%), 11 (5.5%), 19 (9.5%), 5(2.5%), 42(21%), 22 (11%) and 9(4.5%) respectively. However significant difference in viral infection among study participants in the intensive care unit and those on ventilator support in the different age groups of the patients analyzed was noted. There was noted difference among the patients in different age categories based on whether in ICU or ventilator support by *Influenza A Virus*, HPIV-1, HRV and hMPV viral infections at P-Value ≤ 0.05. Highest infection means were indicated in age group >65 for *Influenza A Virus*, HPIV-1 and HRV and age group <5 for hMPV in both ICU and those on ventilator support. Lowest infection means were also observed only in age group 20-34 for *Influenza A Virus* HPIV-1, HRV and *Human metapneumovirus* in the intensive care unit and also those on ventilator support. There were 34 cases of multiple viral infections. 20 cases were in those on ventilator support while 14 cases were present in those in the intensive care unit. It is evident that these infections are common in patients in ICU and those under ventilator support at MTRH. It is also clear that these infections are common in the various age categories. Those below 5 years and those above 50 years have higher prevalence of majority of the infections in comparison to other age groups. Surveillance for viral respiratory infections should be improved in order to implement treatment and also understand seasonality of these viruses and other new respiratory viruses. Co-infections should be closely monitored especially in mechanical ventilation in order to understand the impact of ventilator support on infection rates by these viruses. More studies needs to be done focusing on nosocomial respiratory viral infections.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Acute respiratory tract infections (ARTIs) are among the five most common causes of morbidity and mortality globally, accounting for approximately 3.9 million deaths annually. Most of these deaths occur among young children in developing countries. Infections of the respiratory tract by viruses are common in all individuals regardless of their age. Adults and children are mostly affected. It also increases mortality in older adults and those with chronic diseases. For instance, 90% of seasonal influenza and 78% of RSV infections occur in adults aged above 65 years in the US, however many studies on *RSV, Human Metapneumovirus* virus and HRV focus on children below 5 years and elderly (Hall CB, 2001).There is close association between mechanical ventilation and pneumonia due to mechanical ventilation as well as other associated risks like airway injury. This can lead to alveolar damage and pneumothorax. It can also cause decreased cardiac output, oxygen toxicity diaphragm atrophy. Serious complications that may occur in patients on ventilator support include acute respiratory distress syndrome (ARDS) and acute lung injury (ALI)/ and (Ryland *et al.,* 2011). These contribute to increased levels of mortality as well as morbidity. Apart from these primary complications, secondary complications are also a challenge. Bacterial and viral respiratory infections are a major complication in this category of patients. Clinicians usually confuse acute Viral respiratory infections and bacterial infections which are more common (Balmes *et al.*, 2003).

Patients with chronic diseases and those that require critical are given specialized services in the intensive care units. A professional team highly trained in critical care monitor these patients and also provides special care and observation. These patients in the ICU are closely monitored and are usually kept comfortable by the medication that they are given. However consciousness in these patients may diminish, but sedation level may be different from patient to patient, and also condition of the patient. They become easily aroused and are also able to converse. Other patients may need higher levels of sedation to an extent of not being responsive to stimulation (Dominic *et al.,* 2000).

One of the most common reasons for people being admitted into intensive care is severe pneumonia. Some people have symptoms that are similar to common cold or flu. When their health deteriorates and they have difficulties breathing, they get admitted in hospital and later into intensive care. Some people who have not been admitted because of pneumonia have septicemia. This is a serious infection which affects the circulation as well as the lungs (Wendy and Palma., 2015).

These infections are common in humans regardless of their age or gender. They cause higher levels of morbidity at community level. The prevalence annually of theses illnesses increase in children at 2years and fall during the coming years. These infections will then increase at child bearing age then decreases as an individual grows older. However infections tend to increase in the elderly population (Fransen *et al.,* 1969).

Antiviral treatment of these viruses is available for *Influenza Virus*. Immediate diagnosis of these infections by viruses is important in management of patients admitted in the hospital, thus accurate and prompt diagnosis of these infections will help understand the cause of disease and reduce spread of the infection. These infections should be diagnosed early to help to stop administration of unnecessary antimicrobial agents and begin the use of antiviral drugs like for influenza. This will in turn reduce cost by shortening length of hospital stay (Adcock *et al*., 1997).

1.2 Statement of the problem

Viral respiratory tract infections have many effects on health. Acute viral infections by these viruses contribute a large proportion of respiratory diseases that are common in patients across all age groups worldwide. Individuals admitted in the ICU and those on ventilator support have a greater risk of being infected by respiratory pathogens especially if hygiene is not observed. This is due to pre-existing conditions like diabetes, hypertension and also immunosuppression due to chemotherapeutic agents they are using. Length of hospital stay is increased impacting on the individual and the hospital economically. Many of these patients have been infected when admitted but the infections are poorly understood to inform interventions. This study therefore will form a basis of understanding the prevalence of these infections so that necessary interventions can be put in place to reduce morbidity and mortality.

1.3 Justification

Viral etiology of acute respiratory infections affects families and society both emotionally and socially. They are not actually diagnosed but neglected in comparison to infections by bacteria. Documenting will help understand the prevalence of these viruses in the ICU and those who are mechanically ventilated and also prevalence of specific viruses in different age categories. Assessment of exposure risk and prompt diagnosis is important in management of these infections. Understanding Coinfections rates will help develop proper therapeutic measures that can treat infections with two or more viruses. In addition to increasing morbidity and mortality in affected patients, these infections also impact on available resources hence it is important for quick diagnosis and management to be implemented.

1.4 Research Questions

1. What is the prevalence of viral respiratory tract infections in patients on ventilator support and those in the intensive care unit?
2. What is the proportion of viral respiratory tract infections among patients on ventilator support and those in the intensive care unit in relation to age?
3. What is the prevalence of viral respiratory co-infections in patients on ventilator support and those in the intensive care unit?

1.5 Objectives

1.5.1 General Objective

1. To evaluate severe viral respiratory tract infections in patients on ventilator support and those in the intensive care unit.

1.5.2 Specific Objectives

1. To determine the prevalence of viral respiratory tract infections in patients on ventilator support and those in the intensive care unit?
2. To compare the prevalence of specific viral respiratory infections in patients on ventilator support and those in the intensive care unit.
3. To determine the prevalence of viral respiratory co-infections in patients on ventilator support and those in the intensive care unit.

1.6. Significance of the study

Documenting the prevalence of these infections will help in understanding the impact of all the respiratory viruses that affect these groups of patients in the ICU and the effect of ventilator support on infection rates by respiratory viruses. This will improve management of patients affected leading to reduction in morbidity and mortality.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of viral respiratory tract infections

Viral infections contribute to higher respiratory infections than previously thought; approximately 5% of these infections are due to viruses, but this has been underestimated (Martin *et al.,* 2018). The risk of infection by these viruses tends to increase when there is an epidemic. The impact of nosocomial viral respiratory infections has been neglected for a long time especially due to the challenges encountered in diagnosis and non-specific clinical manifestations of these infections (Nusrat *et al.*, 2009).

Viral infections are common in infants born prematurely and young children; however there is increased risk of infection by these viruses in preterm infants, the immunosuppressed and those with pulmonary diseases. In addition reinfection is more frequent in these groups of patients (Nicholson *et al.,* 1997). Neonatal infections are not well understood and there is little literature available describing these infections. Early diagnosis will help the clinicians to choose correct therapeutic measures and control the spread of viral pathogens within the healthcare facility (Effros RB, 2000).

In children below 5 years, mortality due to respiratory infections is estimated at 20% especially in underdeveloped countries, (Scott *et al.*, 2008). The cause of pneumonia in the community includes *Human Rhinovirus, Respiratory Syncytial virus* and Bocavirus. According to WHO report (WHO., 2008), respiratory viral infections account for greater than 90% of viral bronchiolitis cases in infants, an estimated 50% of pneumonia in the community in young children and over 90% of worsening asthma cases in

children. 30-50% of CAP and 20-60% in cases of chronic lung disease in the elderly people have been associated to these infections.

Respiratory viruses are transmitted in the hospital setting from direct contact with infected visitors and family, infected healthcare workers, other infected patients, indirectly through contact with contaminated fomites, or from patient-to-patient spread due to poor hand hygiene practices among healthcare providers. The frequency of specific respiratory viruses causing nosocomial infections reflects their activity in the community (Graman *et al.,* 1989).

Few studies have assessed the outcomes of nosocomial respiratory viral infections in noncritically ill, non-immunocompromised adult and pediatric patients. In one study, 1 in 5 children admitted to a pediatric intensive care unit (ICU) due to a respiratory viral infection had acquired the infection in the hospital. These children had an approximately 6-fold increased likelihood of mortality compared with those who had community-acquired respiratory viral infections (Spaeder *et al.,*2011). In another pediatric study, 49% of nosocomial respiratory viral infections occurred in premature infants (Simon *et al.,* 2006).

Respiratory viruses had been detected in respiratory specimens in 18.3% of critically ill adults requiring invasive mechanical ventilation (Van *et al.,* 2018). The detection rates are higher, ranging from 20.5 to 49.0%, in patients with community or hospital-acquired lower respiratory tract infections admitted to intensive care units (ICUs) (Loubet *et al.*, 2017). Human rhinovirus, influenza virus, and human parainfluenza viruses (HPIVs)

are the most frequently detected viruses in terms of disease severitity requiring timely actions, critically ill patients are considered appropriate candidates for respiratory viral panel testing (Shorr *et al.,* 2018).

2.2 Overview of mechanical ventilation

Mechanical ventilation is an important aspect of intensive care however technical issues make it complicated and difficult for majority of the healthcare providers. The recent trend in this area of respiratory medicine worsens the situation. Most of the available literature on complications of ventilator support applies to a few patients who are on intubation and ventilator support (Burns, 2007). This small proportion includes those with respiratory failure due to ALI or ARDS. This also applies to those with chronic pulmonary disease (COPD) or asthma. Less difficult issues occur in the remaining 80 to 90 percent of ventilated patients (Pierson, 2004).

Mechanical ventilation supports gas exchange and normal functioning of the lungs. Ventilator support may replace normal functioning of the lungs and the chest if it is done properly, however due to the adverse effects of ventilator support and positive pressure associated ventilation as well as intubation, it is advisable to reduce the time of ventilation as much as possible (MacIntyre et al., 2009).

2.3 Viruses that infect the respiratory tract

2.3.1 *Adenovirus*

Adenovirus is a non-enveloped DNA virus. The genome is double stranded. It causes mild illness in humans. It may also cause fever, diarrhea, conjunctivitis, bladder infection and a rash. Anyone can get infected especially infants and people with weakened immune response or an existing respiratory disease like tuberculosis**.** The incubation period is 2-6 days (File *et al.,* 2003; Smith *et al*., 2010).

2.3.2 *Human Rhinovirus*

Human Rhinoviruses are non-enveloped single stranded RNA viruses. It belongs to Picornaviridae family. They are associated with common cold but may also cause ear infection, sore throat, pneumonia and bronchitis. The incubation period is 8 hours to 2 days (Wald *et al*., 1995; Fransen *et al*., 1969).

2.3.3 *Influenza virus*

Influenza virus is a single stranded RNA virus with a segmented genome. It is an Orthomyxovirus. This virus has three genera, *Alphainfluenzavirus, Betainfluenzavirus, Gammainfluenzavirus and Deltainfluenzavirus. Influenza A* causes severe disease (Palese *et al*., 2007). The virus is spread through inhalation of aerosols produced by an infected person. It can also be transmitted through contact to the eye or hand to nose and also hand to mouth transmission. Symptoms appear after 1-2 days (Thompson *et al.,* 2004).

2.3.4 Human *Parainfluenza Virus*

Human Parainfluenza Viruses (HPIV) is a single stranded RNA virus. It is in the family of *Paramyxoviridae* family. In young children it causes pneumonia and bronchitis (Denny *et al.,* 1986). Endemic infections are mainly due to HPIV type 3, while infections caused by HPIV-1 and HPIV-2 rise as the months fall (Karron *et al.,* 2007). Reinfection occurs with advancing age. Acute respiratory illnesses due to this virus account for 1%–15% of respiratory infections in young adults. Symptoms appear after 2-8 days (Nicholson *et al*., 1997).

2.3.5 *Respiratory Syncytial Virus*

RSV is an RNA virus in the family of Paramyxoviridae (Mark *et al., 2004*). It causes a large proportion of LRTI in infancy and childhood. It is an agent of bronchiolitis and pneumonia in young children and infants (Giovanni *et al.,* 2014). Vaccine development for RSV is a major concern worldwide. Information is accessible on the epidemiological features and clinical presentation of this virus especially in young patients with infections of the respiratory tract in hospitals. (Yıldız *et al*., 2018). In the elderly population little is known about infections by RSV (Emelda *et al*., 2012).

2.3.6 *Human Coronavirus*

Human Coronaviruses are RNA viruses with crown-like appearance. It causes common cold just like rhinovirus (McIntosh *et al*., 1970). Growing this virus in cell culture has been difficult. This has led to limited studies over the past years *(*Geller *et al., 2012;* Bastien *et al., 2005).* 4% of pediatric pneumonia admissions were associated with three

endemic HCoVs, with a high proportion of cases co-occurring with another respiratory virus, no clear seasonal pattern, and with the age-distribution of cases following that of pneumonia admissions (Grieven *et al.,* 2020).

2.3.7 *Human Metapneumovirus*

Human Metapneumovirus is an RNA virus. It belongs to the family of *Paramyxoviridae* (Wilczyński *et al.,*2004). It causes acute viral respiratory tract infection in children and the elderly. Global prevalence of this virus is approximately 5-15%. In developing countries especially Africa, data is not available on genetic diversity and epidemiology of this virus (Mullins *et al.,* 2004)

2.4 Epidemiology of viral respiratory viruses.

Viruses are responsible for a large proportion of acute respiratory tract infections (ARTIs). *Human influenza, Human parainfluenza, Respiratory-syncytial-virus,* and *Adenoviruses* are among the leading cause of ARTIs. Epidemiological evidence of those respiratory viruses is limited in the East Africa Community (EAC) region (Therese *et al.,* 2021). These infections cause illnesses in children and also the elderly people worldwide. URTI are prevalent in young children and infants. These infections will continue to be more prevalent in adults and older children. Those attending daycare centers usually have more episodes per year (Monto *et al.,* 1974). Diagnosis of viruses such as RSV, *Influenza Viruses* and *Adenoviruses* was easily done using traditional methods but as time passed by other viruses like HCoV and HRV were discovered.

Detection methods have also improved. 50% and 75% of upper respiratory tract infections are attributed to HCoV and HRV (Sebastian and Stephen., 1996).

The patterns of epidemiology and evolution of circulating strains of hMPV are not well documented sub-Saharan Africa. From 2000 to 2011 4.8% of hospitalizations due to pneumonia in children were positive for hMPV in Kilifi (Betty *et al.,* 2016).

Human Coronavirus are endemic to many countries globally (Woo *et al*; Zaki *et al.,* 2012). There is little information about coronavirus especially in sub-Saharan Africa. The diversity, molecular features and circulation dynamics is not well documented (Owusu *et al.,* 2014).

Human Rhinovirus has recorded a higher prevalence in most regions of Kenya. A prevalence of (33%) has been recorded along the coastal region, the Western and eastern region has a prevalence of 32.7% and 11% respectively. However those aged between 2 months -7 years have highest infection rates. Those above 60 years had low infection rates (Kaburu *et al.,* 2014).

There is insufficient data on *Influenza Virus* in sub-Saharan Africa because of poor disease surveillance .This is a setback in detection of other strains of *Influenza Virus* that could have an effect on influenza pandemic (Gessner *et al.,* 2011). Kenya has a good framework for surveillance of *Influenza virus* countrywide. However it is a main cause of hospitalizations and deaths annually in Kenya (Ope *et al.,* 2011)In Kenya, *Influenza Virus* is common all through the year but with some peaks at some seasons of

the year. The incidence is high during the winter and also during the rainy months: March-April, October-November and cold month of July (Viboud et al., 2006).

The incidence of respiratory disease in infants in urban and rural settings associated with RSV is high in Kenya (Godfrey et al., 2013). 470 (12 %) were positive for RSV among 4714 children hospitalized with acute respiratory infection (Bryan *et al.,* 2016).

2.5 Risk factors associated with viral respiratory tract infections

Premature infants, those with congenital heart disease (CHD), the immune-compromised, patients with bronchopulmonary diseases are at risk of infection by these viruses (Jo Won Jung., 2011). Risk factors have not been evidently associated to increasing mortality in severe infections (Nair *et al.,* 2010).

Children below 5 years are at risk of LRTI if they are infected with RSV (Van Woensel *et al*., 2003). Approximately 3.4 million cases of severe LRTI that require hospitalization in children were attributed to RSV in 2005 worldwide. Out of these, there were almost 200,000 deaths in children below 5 years (Matías *et al*., 2015). Infections by RSV cause ICU admissions of up to 2% of all neoantes annually in Switzerland (Berger *et al.,* 2009).

2.6 Diagnosis of viral respiratory tract infection

2.6.1 Virus culture

Prompt diagnosis of respiratory viruses has significant impact on patient management due to the increased availability of antiviral drugs. Culture of these viruses will depend on examination of CPEs and hemadsorption of cell culture. A rapid culturing method is available for *Adenovirus, Influenza virus A and B,* RSV, hMPV HPIV 1, 2, and 3**,** *HRV* and *HCoV* (Diane *et al.,* 2006). However, Immunofluorescence technique is used for detection and identification of viral antigens. Due to the high sensitivity TAT less than 3 days, however negative results can be reported within 2 days (Leland and Ginoccio., 2007; Fong *et al*., 2000).

2.6.2 Rapid antigen detection

Viruses that infect the respiratory tract can be diagnosed by rapid immunoassays. Results are read manually but others involve the use of an automated reader (James., 2008). The sensitivity of these assays depends on titers of the respiratory viruses that have been shed; children shed higher titers of these viruses compared to adults (Leland *et al.,* 2007). Detection of *Influenza Virus* is poor when using immunoassays due to low sensitivity however positive diagnosis in hospitalized patients will help improve management of the patients (Keipp *et al.,* 2010) Diagnosis of H1N1 by rapid immunoassays has demonstrated poor sensitivity compared to real time PCR (Michael and Chonmaitree, 2010).

2.6.3 Direct Fluorescent Antibody Tests

This technique is more sensitive than rapid immunoassays. Sensitivity of 95% versus RT-PCR has been demonstrated for hMPV performed on aspirates from the nasopharynx of children (Terho *et al.*, 2008). The sensitivity of DFAT for *Human metapneumovirus* in comparison to real time PCR, showed a specificity of 100%, sensitivity of 95.2%, and accuracy of 98.9% (Ingram *et al.,* 2006).

2.6.4 Serology

Serology is integral part of diagnosis of viruses. Specific antibodies against these viruses usually appear 14 days after exposure. Antibodies against RSV, *Adenovirus, Influenza Virus* A and B, HPIV *1-3* can be detected by serological techniques. This technique can detect co-infections from hospitalized children with viral respiratory tract infection. However, in infants antibody response may be undetected (Hall *et al.,* 1991). Serological assays are less sensitive for the detection of HPIV and *Adenovirus* when compared to nucleic acid amplification techniques (Kuypers *et al.,* 2006).

2.6.5 Nucleic acid amplification techniques

It is a highly sensitive technique for diagnosing respiratory viruses. Correct primers and probes as well as correct optimization, amplification conditions and interpretation of results are required. False positives results may be obtained due to contamination (Michael and Chonmaitree, 2010).

PCR is the best molecular method for detecting sequence of viral RNA or DNA (Zumla *et al*., 2014). Primers are available for different viruses. These primers can be used in a multiplex PCR assay to detect several respiratory viruses. However it is important to optimize all conditions including annealing temperature and extension time that is required for all the primers that can be used in such PCR amplification (Osiowy, 1998). Detection of these viruses can be done in RT-PCR from clinical samples both in a one-step and two-step Reverse Transcriptase (RT) PCR. After extraction of viral RNA, complementary DNA is prepared. This is then used as a template for detecting the target sequences by PCR amplification (Poddar, 2002).

2.6.5.1 Real-Time PCR

In this technique detection occurs in real-time. Real-PCR is mainly used in quantitative analysis. It is a challenge to quantify the viral load in conventional methods (Wathuo *et al.,* 2016). The specificity of this assay is improved by use of fluorescent probes in the PCR amplification. This technique can perform multiplex detection and amplification. In addition; some platforms in real-time PCR have ability to amplify four different products that can be distinguished in one tube (Van Elden *et al.,* 2001). Most real-time PCR machines can detect less than four nucleic acid targets. PCR has demonstrated accuracy in diagnosis of respiratory viruses than other methods of detection (Templeton *et al.,* 2005).

Detection systems in Real-time PCR measures fluorescence that is indicative of DNA amplification within a closed tube in a 96 well plate format. This eliminates the need for

post-detection via agarose gel electrophoresis. This significantly reduces turn-around time. In every amplification cycle detection of the amplicon occurs which allow for the measurement of reaction kinetics. Detection by fluorescence is more sensitive than detection of DNA via agarose gel electrophoresis (Choudhary *et al.*, 2013).

2.6.5.2 Conventional PCR

The PCR product undergoes post-amplification procedures using specific DNA probes or electrophoresis. In this method of diagnosis, beads that are specific for target sequences can detect *Influenza A, B,* HPIV 1, 2, 3, RSV, *Adenoviruses, Human Metapneumovirus and hMPV* in one reaction (Bai *et al.*, 2006). A comparison between conventional PCR and RT-PCR showed that conventional PCR missed 60% of the positive RSV cases (Dagher *et al.*, 2004). False negatives can be associated with the ineffectiveness of gel electrophoresis detection system in conventional PCR as it may leave samples with lower viral load undetected (Ginocchio and Miadam, 2011).

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Study area

This study was done at Moi Teaching and referral Hospital. It is located in Eldoret Town; Uasin Gishu county.

3.1.1 Population

The population of Uasin Gishu was 1,163,186 according to housing and population census of 2019. The growth rate of the population was 3.8% while the density of the population was 267 persons per square kilometer **(**www.uasingishu.go.ke**).**

3.1.2 Catchment area

The bed capacity at MTRH is 800. The hospital serves a large population in the neighboring counties of West Pokot, Nandi, Turkana, Elgeyo Marakwet, Bungoma, and Tranzoia. It also receives patients from parts of Sudan and Uganda.

3.2 Study Population

It comprised of consented study participants in the ICU and those on ventilator support in MTRH.

3.3 Inclusion criteria

All patients in the intensive care unit and those under ventilator support in Moi teaching and referral hospital who consented in the study were sampled.

3.3 Study Design

The design of this study was cross-sectional study. Samples collected from consented participants were tested for *Influenza virus*, HPIV, HRV HAdV, RSV, HCoV and hMPV using Real time polymerase chain reaction.

3.4 Sampling Technique

In this study, all patients in the intensive care unit and those on ventilator support who consented were sampled hence non random sampling technique.

3.5 Sample size determination

The sample size calculation was determined by the formula of Fisher *et al*., 1998 based on 95% level of confidence and anticipated prevalence of 53.7%. The estimated prevalence was based on an earlier study in Thailand (Turner *et al.,* 2013).

$N=Z^{2} X P (1-P)/E^{2}$

Where:

N=Desired sample size

Z=Standard normal deviation=1.96(from the tailed normal table).

P=Incidence rate of viral respiratory infections

E=the desired degree of accuracy at 95% confidence level=0.5

$N=1.96^{2} X 0.537(0.463)/0.05=186$ samples.

A total of 186 study participants in the ICU and those on ventilator support at MTRH were involved.

3.6 Sample collection

Using bronchoscopy BAL (bronchoalveolar lavage) technique, normal saline was used to lavage the bronchoalveolar region. The specimen was then placed in a sterile container. Only one specimen was collected from each patient. After collection, VTM was added as soon as possible. The volume of VTM that was added and the sample collected was equal. Patient's number, age, gender and whether from ICU or MV was written on the specimen container. The sample was then stored at 4^0C for transportation to KEMRI Nairobi for analysis.

3.7 Laboratory analysis

3.7.1 RNA extraction. (QiaAmp RNA Mini Kit)

The primary samples were frozen at -80°C to -40°C. 350 µl of lysis buffer was added to the sample aliquots. 350 µl 70% ethanol was then added to the sample and mixed well by pipetting three times.700 µl of sample was placed on the spin column and centrifuged for 30 seconds at 13000 rpm, the supernatant was discarded. 700 µl of RW1 buffer was added to spin column and centrifuged for 30 seconds at 13000 rpm and the flow-through liquid discarded. This is followed by adding 500 µl of buffer (RPE) to spin column and centrifuged for 2 minutes at 13000 rpm. After discarding the supernatant, the spin column is placed in clean 2 ml tube and centrifuged for 1 minute at 13000 rpm. This is then placed in another clean 1.5 ml tube. 30-50 µl of RNase free water was finally added onto the membrane of the column and centrifuged for 1 minute at 13000 rpm. RNA will now be at the bottom of the tube.

3.7.2 DNA extraction ((QiaAmp DNA Mini Kit)

20µl of proteinase K was placed on the bottom of a 1.5 ml microcenrtifuge tube and 200µl of the specimen was added to the microcenrtifuge tube. 200µl AL buffer was then added and vortexing for 15seconds to ensure sufficient mixing and efficient lysis. This was followed by 10 minutes incubation at 56°C to ensure maximum DNA yield after lysis for 10 min at 56°C. 200µl of 96–100% ethanol was then added to the sample and mixed by vortexing for 15 seconds. 1.5 ml microcenrtifuge tube was then centrifuged to get rid of drops in the lid. The cap was closed and centrifuged at 6000 x *g* (8000 rpm) for 1 minute and placed in a clean 2 ml collection tube. The Mini spin column was then opened and 500 µl of AW1 buffer was added without wetting the rim and then closed and centrifuged at 6000 x *g* (8000 rpm) for 1 min. The QIAamp Mini spin column was carefully opened and 500 µl of AW2 buffer added without wetting the rim and then closed and centrifuged for three minutes at 20,000 x *g*; 14,000 rpm. The column was then placed in a new 2 ml collection tube and centrifuged for one minute at maximum speed. It was carefully opened and added 200 µl AE buffer or distilled water. This was Incubated at 15–25°C for 1 minute and then centrifuged at 6000 x *g* (8000 rpm) for 1 minute. Elution was done with 200 µl off Buffer AE. It is recommended that elution be done in Buffer AE and stored at –30 to –15°C.

3.7.3 Preparation of master mix

Preparation of the master mix was done by adding the real time PCR components as shown on table 3.2 below in an Eppendorf tube before dispensing on Applied Bio systems PCR 96-well plate for the real time PCR to be carried out. The real-time PCR

was carried out on AB 7500 Real Time Fast thermocycler, microcenrtifuge tubes and mixing gently by pipetting the master mix up and down ten times.

Table 3.1: Master Mix preparation components

Reagent	**Volume(µl) for 1X reaction**
Water (molecular grade)	6.125
Real Time PCR buffer	12.5
Forward primer (100µmol/l)	0.25
Reverse primer (100µmol/l)	0.25
Probe	0.25
QIAGEN one step RT-PCR enzyme mix (5U/µl)	0.625
Total volume	**20**

After preparing the master mix, 20µl of the master mix was dispensed to each well on the real time PCR 96 well-plate. 5µl of the sample RNA or DNA was then added to the master mix, 5µl of extracted nuclease free water was used as negative control and 5µl of the specific viral RNA or DNA was used as positive control.

The thermal cycler was programmed as per the following thermal cycling conditions. There was an initial reverse transcription reaction at 45^{O}C for 10 minutes. Thereafter the reverse transcriptase enzyme was inactivated and Taq polymerase enzyme activated at 95^{O}C for another 10 minutes. This was followed by 40 cycles where the template was unzipped for 15 seconds at 95°C. Annealing and extension was carried out 55°C for

one minute. With every cycle a detector would pass over the wells to detect any amplification and plot a sigmoid graph in case of positivity.

Table 3.2: Primer sequence of the selected respiratory viruses

NAME OF PRIMER/PROBE	SEQUENCE 5' > 3'
H5HA- For	TGG AAA GTG TAA RAA ACG GAA CGT
H5HA- Rev	TGA TTG CCA GYG CTA GGG AAC T
H5HA- Probe1	**FAM** -TGA CTA CCC GCA G"T"A TTC AGA AGA AGC AAG ACT AA-
H5HA-Probe2	**FAM**-CAA CTA TCC GCA G"T"A TTC AGA AGA AGC AAG
hMPV For	CAA GTG TGA CAT TGC TGA YCT RAA
hMPV Rev	ACT GCC GCA CAA CAT TT A GRA A
hMPV Probe#	TG GCY GTY AGC TTC AGT CAA TTC AAC AGA
RSV For	GGC AAA TAT GGA AAC ATA CGT GAA
RSV Rev	TCT TTT TCT AGG ACA TTG TAY TGA ACA G
RSV Probe#	CTG TGT ATG TGG AGC CTT CGT GAA GCT
HPIV-1 For	AGT TGT CAA TGT CTT AA T TCG TAT CAA T
HPIV-1 Rev	TCG GCA CCT AAG T AA TTT TGA GTT
HPIV-1 Probe**	AT A GGC CAA AGA "T"TG TTG TCG AGA CT A TTC CAA
HPIV-2 For	GCA TTT CCA A TC T AC AGG ACT A TG A
HPIV-2 Rev	ACC TCC TGG TAT AGC AGT GAC TGA AC
HPIV-2 Probe	CCA TTT ACC "T"AA GTG ATG GAA TCA ATC GCA AA
HPIV-3 For	TGG YTC AA T CTC AAC AAC AAG A TT T AA G
HPIV-3 Rev	TACCCGAGAAAT ATT ATTTTGCC
HPIV-3 Probe**	CCC A TC TG"T" TGG ACC AGG GAT AT A CT A CAA A

Adeno For	GCC CCA GTG GTC TT A CAT GCA CAT C	
Adeno Rev	GCC ACG GTG GGG TTT CT A AAC TT	
Adeno Probe#	TG CAC CAG ACC CGG GCT CAG GT A CTC CGA	
RNP For	AGA TTT GGA CCT GCG AGC G	
RNP Rev	GAG CGG CTG TCT CCA CAA GT	
RNP Probe#	TTC TGA CCT GAA GGC TCT GCG CG	
HRV Forward	CPXGCCZGCGTGGC	
HRV Reverse	GAAACACGGACACCCAAAGTA	
HRV Probe	TCCTCCGGCCCCTGAATGYGGC	
HCoV-OC43 Forward	CGATGAGGCTATTCCGACTAGGT	
HCoV-OC43 Reverse	CCTTCCTGAGCCTTCAATATAGTAACC	
HCoV-OC43 Probe	TCCGCCTGGCACGGTACTCCCT	
HCoV-299E Forward	CAGTCAAATGGGCTGATGCA	
HCoV-299E Reverse	AAGGGCTATAAAGAGAATAAGGTATTCT	
HCoV-299E Probe	CCCTGACGACCACGTTGTGGTTCA	
A(H1)pdm09HA gene	H1pdm-169-F	AAACTATGCAAACTAAGAGGGGT
	H1pdm-297-R	TGTTTCCACAATGTAGGACCA
	H1pdm-244-P	**FAM**–CCAGAGTGTGAATCACTCTCCACA–**BHQ**
A(H3)HA gene	H3-266-F	ACCCTCAGTGTGATGGCTTTCAAA
	H3-373-R	TAAGGGAGGCATAATCCGGCACAT
	H3-315-P	**FAM**-ACGAAGCAAAGCCTACAGCAACTGTT-**BHQ**

3.8 Data analysis

Descriptive statistics such as percentages, mean and standard deviation was used to analyze quantitative data. SPSS was used in entry, coding and also to analyze the data. The data was log-transformed to attain normal distribution and tabulated as mean±standard deviation. The results were compared based on whether in ICU or on ventilator support. This was also done based on gender of the study subjects using ANOVA with Bonferroni's post-test using GenStat Release 14.1 (PC/Windows). Data presentation was done using graphs, pie charts, tables and figures.

3.9 Ethical consideration

All patients that were recruited in this study gave informed consent that was written. Parental assent was sought for the study participants below 18 years. Approval for this study was from the Institutional research and ethics committee (IREC) of Moi University (School of medicine) and MTRH (Appendix 1 and 2 respectively). The data obtained from this study was confidential. Patients' details were not included, only codes were used.

CHAPTER FOUR: RESULTS

The data was analysed to address the objective of the study. 200 samples were collected, out of these 82 samples (41%) were from ICU and 118 (59%) were from MV.

4.1 Demographic information of the study population

Gender and age of the population studied was tabulated to aid in investigating differences in prevalence of these infections in the various age groups and gender.

4.1.1 Age

In this study, majority of the population studied were >65 years. This was 28.8% (58) of the population studied. However those between 50- 64, 35-49, 5-19 and <5 years were 24.2% (48), 20% (40), 13% (26) and 11% (22) respectively. 3% (6) of the population studied was between 20 to 34 years. The relevance of this age categories is because many studies have been done on children and the elderly hence it is important understand the distribution of these infections across all age groups.

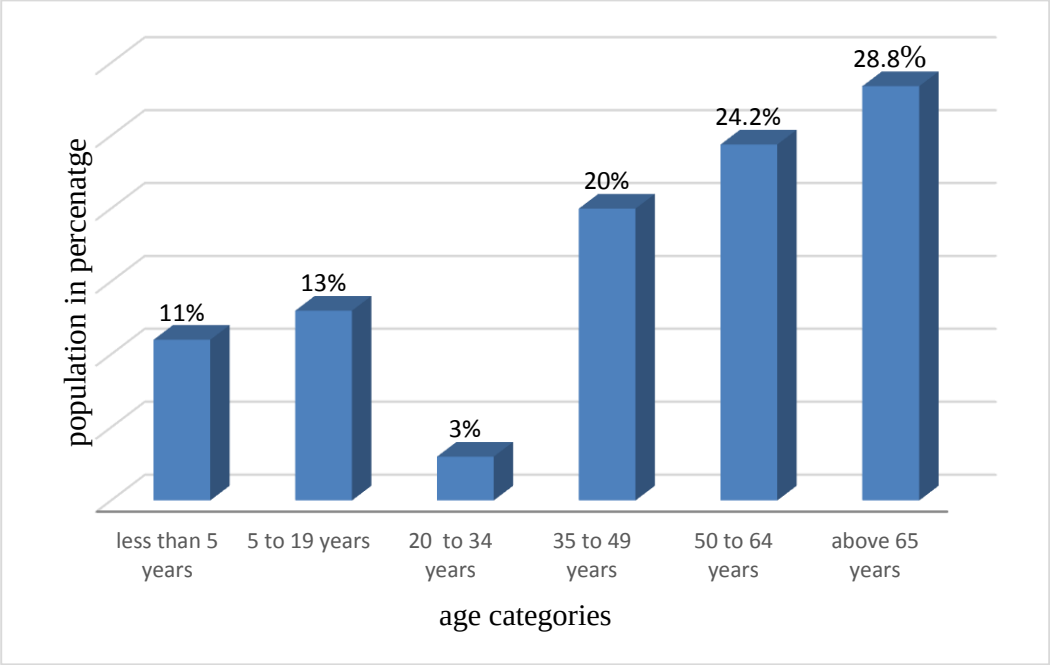

Figure 4.1: Age categories of the study population

4.1.2 Gender

Out of the 200 samples, 115 (57.5%) were male while the female were 85 samples (42.5%).

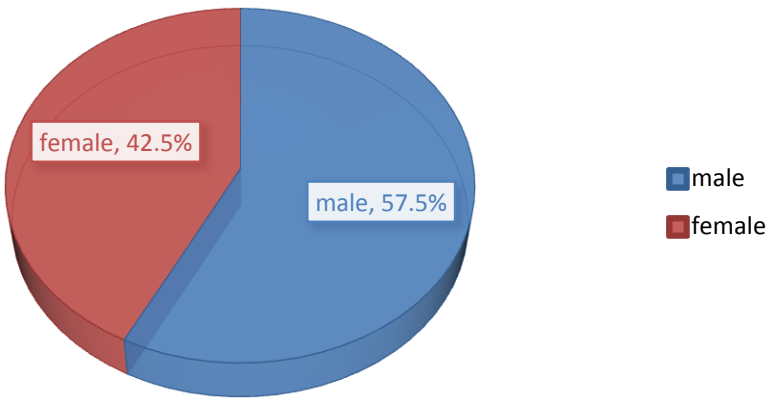

Figure 4.2: Gender of the study population

4.2 Prevalence of viral infections in ICU and MV

Table 4.1: Number of positive samples per virus

Virus type	ICU#	%	MV#	%	Total positives	Overall prevalence (%)
Influenza virus A	18	22.5%	15	18.51%	33	16.5%
HPIV-1	8	10.0%	4	4.93%	12	6.0%
HPIV-2	2	2.50%	6	7.40%	8	4.0%
HPIV-3	6	7.50%	5	6.17%	11	5.5%
RSV	14	17.5%	5	6.17%	19	9.5%
HAdv	3	3.75%	2	2.46%	5	2.5%
HRV	17	21.25%	25	30.86%	42	21.0%
hMPV	8	10.0%	14	17.28%	22	11.0%
HCoV	4	5.0%	5	6.17%	9	4.5%

The infections in the ICU and those on ventilator support are as shown in table 4.1. There were higher infection rates in patients in the intensive care unit by Influenza A virus 22.5% (18), HPIV-1 10% (8), HPIV-3 7.5% (6), RSV 17.5% (14) and Adenovirus 3.75% (3) as compared to those on mechanical ventilation where higher infection rates was noted in HPIV-2 7.40% (6), HRV 30.86% (42), *Human Metapneumovirus* 17.28% (14) and HCoV 6.17% (5).

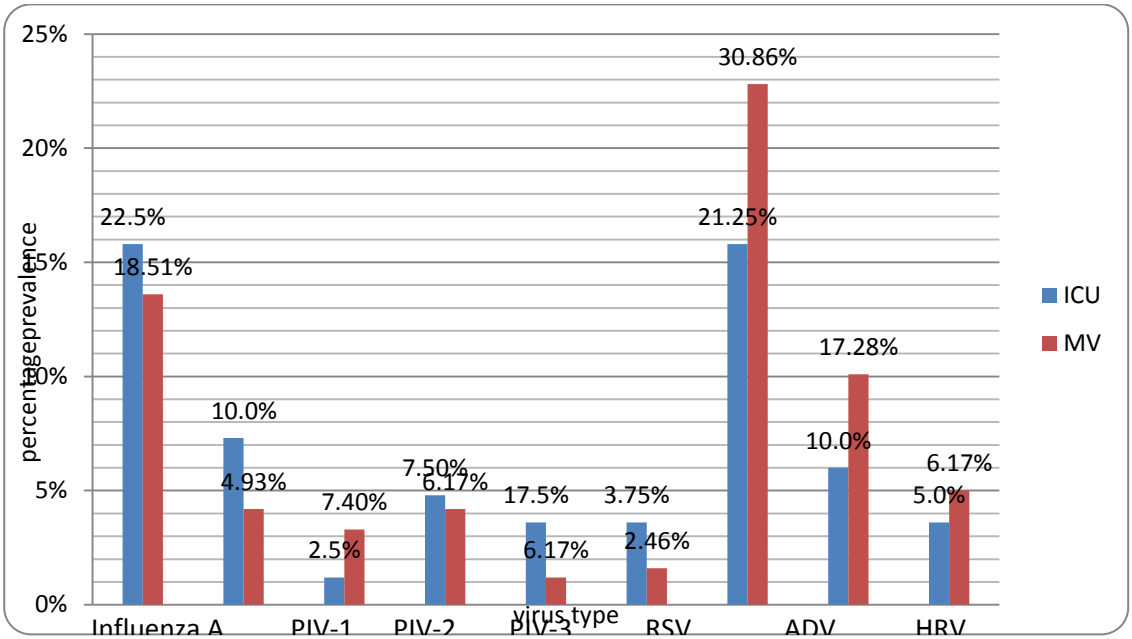

Figure 4.3: Viral infection rates in in the intensive care and those on ventilator support.

4.3 Subtypes of *Influenza A virus*

33 samples were positive for *Influenza A virus*, out of these 31 samples (15.5%) were positive for H3N2 while 2 samples (1%) were positive for H1N1. Both cases of H1N1 were on patients in the intensive care unit while 13 cases (16%) and 18 cases (22.5%) of H3N2 were detected in those on ventilator support and in the intensive care unit respectively.

4.4 Subtypes of Human coronavirus

There were 5 cases (6.17%) of HCoV identified in those under ventilator support, out of these 4.9% (4) were subtype OC43, only one case (1.2%) was subtype 229E. In the

intensive care unit a total of 4 cases was identified, 3.75% (3) were subtype OC43 while 1.25% (1) was 229E.

4.5 Prevalence of specific viral infections in the intensive care unit in relation to age

Table 4.2: Prevalence of specific viral infections in the intensive care unit in relation to age

	Influenza A virus		HPIV-1		HPIV-2		HPIV-3		RSV		HAdv		HRV		hMPV		HCoV	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
<5years	3	3.75	2	2.5	2	2.5	2	2.5	5	6.25	0	0	2	2.5	4	5.0	1	1.25
5-19 years	1	1.25	0	0	0	0	0	0	4	5.0	0	0	3	3.75	2	2.5	0	0
20-34 years	2	2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35-49 years	2	2.5	1	1.25	0	0	1	1.25	2	2.5	1	1.25	3	3.75	0	0	1	1.25
50-64 years	3	3.75	2	2.5	0	0	0	0	1	1.25	1	1.25	3	3.75	0	0	0	0
>65 years	7	8.75	3	3.75	0	0	3	3.75	2	2.5	1	1.25	6	7.50	2	2.5	2	2.5
TOTAL	18		8		2		6		14		3		17		8		4	

From table 4.2 above, those < 5years, infection by RSV was the highest at 6.25% (5), followed by *Human Metapneumovirus* 5.0%(4), *Influenza A virus* 3.75% (3), HPIV-1 2.5% (2), HPIV-2 2.5% (2), HPIV-3 2.5% (2), HRV 2.5%(2) and HCoV 1.25% (1). No case of Adenovirus was identified in those < 5 years in the intensive care unit. Among the study participants between 5-19 years, infection by RSV was the highest at 5.0% (4), followed by HRV 3.75% (3), *Human Metapneumovirus 2.5%* (2) and Influenza

virus 1.25% (1). No cases of HPIV-1, HPIV-2, HPIV-3, Adenovirus and HCoV were identified. There were only 2 cases (2.5%) of *Influenza A virus* in those between 20-34 years in the intensive care unit. No cases of the remaining viruses were identified in this age category. Among those between 35-49 years, infection by HRV 3.75% (3) was the highest. This was followed by *Influenza A virus* and RSV both having a prevalence of 2.5% (2). HPIV-1, HPIV-3, Adenovirus and HCoV recorded a prevalence of 1.25% in those between 35-49 years in the intensive care unit. No case of HPIV-2 was identified. *Influenza A virus* and HRV had the highest prevalence among those between 50-64 years at 3.75% (3) in the intensive care unit. This was followed by HPIV-1 2.5% (2), RSV 1.25% (1) and Adenovirus 1.25% (1). No cases of HCoV and *Human Metapneumovirus* was identified. *Influenza A virus and* HRV recorded a prevalence of 8.75% (7), 7.50% (6) respectively in those above 65 years. HPIV-1 and HPIV-3 had a prevalence of 3.75% (3). RSV, *Human Metapneumovirus* and HCoV recorded a prevalence of 2.5% (2). No case of HPIV-2 was identified in the intensive care unit among study participants above 65 years. Generally higher burden of the viral infections was noted in those <5 years and > 65 years in study participants in the intensive care unit.

4.6 Prevalence of specific viral infections in those on ventilator support in relation to age.

Table 4.3: Prevalence of specific viral infections in those on ventilator support in relation to age

	Influenza A virus		HPIV-1		HPIV-2		HPIV-3		RSV		HAdv		HRV		hMPV		HCoV	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
<5years	3	3.7	2	2.4	2	2.4	0	0	1	1.2	0	0	4	4.9	5	6.2	0	0
5-19 years	1	1.2	1	1.2	2	2.4	1	1.2	2	2.4	0	0	5	6.2	3	3.7	0	0
20-34 years	1	1.2	0	0	1	1.2	1	1.2	0	0	0	0	1	1.2	0	0	0	0
35-49 years	2	2.4	0	0	0	0	1	1.2	0	0	0	0	4	4.9	0	0	1	1.2
50-64 years	3	3.7	1	1.2	0	0	1	1.2	0	0	0	0	4	4.9	2	2.4	2	2.4
>65 years	5	6.2	1	1.2	1	1.2	1	1.2	2	2.4	2	2.4	7	8.6	4	4.9	2	2.4
TOTAL	15		4		6		5		5		2		25		14		5	

From table 4.3 above, those < 5 years, infection by *Human Metapneumovirus* had the highest prevalence at 6.2% (5), HRV, Influenza A virus, HPIV-1, HPIV-2, and RSV recorded a prevalence of 4.9% (4), 3.7% (3), 2.4 (2), 2.4% (2) and 1.2% (1) respectively. There were no cases of Adenovirus and HCoV that were detected in those below 5 years in those under ventilator support. High infection rates was noted in those between 5-19 years by HRV 6.2% (5), *Human Metapneumovirus* 3.7% (3) HPIV-2 2.4% (2) and RSV 2.4% (2). However, infection by *Influenza A virus*, HPIV-1 and HPIV-3 all had a prevalence of 1.2% (1). No case of HCoV was detected in this age category in those on ventilator support.

Lowest infection rates was noted in those between 20-34 years by *Influenza A virus*, HPIV-2, HPIV-3 and HRV all recording a prevalence of 1.2% (1). No cases of HPIV-1, RSV, Adenovirus, *Human Metapneumovirus* and HCoV were detected. In study participants between 35-49 years, HRV recorded the highest infection rates at 4.9% (4) followed by *Influenza A virus* 2.4% (2). Both HPIV-3 and HCoV had equal infection rate at 1.2% (1). No cases of HPIV-1, HPIV-2, RSV, *Adenovirus* and *Human Metapneumovirus* were detected in study participants on ventilator support between 35-49 years.

Among those between 50-64 years, HRV and *Influenza A virus* recorded higher prevalence at 4.9% (4) and 3.7% (3) respectively. The prevalence of *Human Metapneumovirus* and HCoV was at 2.4% (2) while HPIV-1 and HPIV-3 was 1.2% (1). There were no cases of HPIV-2, RSV and *Adenovirus* identified. However the age category > 65 years recorded the highest prevalence by HRV, *Influenza A virus* and *Human Metapneumovirus* at 8.8% (7), 6.2% (5) and 4.9% (4) respectively. RSV, *Adenovirus* and HCoV all recorded a prevalence of 2.4% (2) while HPV-1, HPIV-2 and HPIV-3 all had a prevalence of 1.2% (1) those >65 years on ventilator support. In general, the age categories < 5 years, 50-64 years and those >65 years had higher burden of the viral infections in those on ventilator support as compared to other age groups.

4.7 Comparison of viral type infections based on whether in ICU or on ventilator support and age

Table 4.4: ANOVA table showing comparison of viral type infections based on whether in ICU or on ventilator support and age. (95.0 percent LSD intervals, P-Value <0.05).

Virus type		AGE RANGE (yrs)						SED	P
		<5	5-19	20-34	35-49	50-64	>65		
Influenza	**ICU**	**0.66+0.03**	**-0.42**	**-0.69**	**0.34**	**0.58**	**1.29**	**0.431**	**0.003**
A	**MV**	**0.66+0.15**	**-0.42**	**-0.69**	**0.34**	**0.58**	**1.29**	**0.423**	**0.002**
PIV-1	**ICU**	**-0.42+0.09**	**-0.42**	**-0.69**	**-0.41**	**0.41**	**0.53**	**0.287**	**0.002**
	MV	**-0.42+0.04**	**-0.42**	**-0.69**	**-0.41**	**0.41**	**0.53**	**0.292**	**0.002**
PIV-2	ICU	0.11+0.02	-0.02	-0.69	-0.42	-0.69	-0.42	0.412	0.296
	MV	0.11+0.02	-0.02	-0.69	-0.42	-0.69	-0.42	0.422	0.319
PIV-3	ICU	-0.29+0.03	-0.42	-0.42	-0.14	-0.42	0.26	0.436	0.599
	MV	-0.29+0.01	-0.42	-0.42	-0.14	-0.42	0.26	0.462	0.653
RSV	ICU	0.07+0.02	0.34	-0.69	-0.42	-0.42	0.47	0.568	0.285
	MV	0.07+0.03	0.34	-0.69	-0.42	-0.42	0.47	0.522	0.208
ADV	ICU	-0.69+0.07	-0.69	-0.69	-0.41	-0.41	0.131	0.280	0.061
	MV	-0.69+0.07	-0.69	-0.69	-0.41	-0.41	0.131	0.280	0.061
HRV	**ICU**	**0.20+0.05**	**0.74**	**-0.42**	**0.89**	**0.87**	**1.11**	**0.457**	**0.035**
	MV	**0.20+0.03**	**0.74**	**-0.42**	**0.89**	**0.87**	**1.11**	**0.422**	**0.020**
Hmpv	**ICU**	**0.83+0.01**	**0.39**	**-0.69**	**-0.42**	**-0.29**	**0.66**	**0.404**	**0.006**
	MV	**0.83+0.16**	**0.39**	**-0.69**	**-0.42**	**-0.29**	**0.66**	**0.394**	**0.005**
HCOV	ICU	-0.69+0.03	-0.42	-0.69	-0.14	-0.14	0.26	0.371	0.133
	MV	-0.69+0.02	-0.41	-0.693	-0.14	-0.14	0.25	0.346	0.092

The positive values indicates significant levels of viral infection in that specific age group and whether in ICU or MV. Negative values indicate insignificant levels of infection. The bolded values shows infection levels at P-Value <0.05, these are

significant levels of infections of the specific viruses across the different age groups and gender.

Overall, there was noted difference among the patients in different age categories based on whether in ICU or ventilator support by *Influenza A Virus*, HPIV-1, HRV and hMPV viral infections at P-Value ≤0.05 as shown in Table 4.3. Among the age groups of the patients, highest infection means were indicated in age group >65 for *Influenza A Virus* (1.29±0.03), PIV-1 (0.533±0.09) and HRV (1.11±0.05) and age group >5 for hMPV (0.83±0.01), while lowest infection means were observed only in age group 20-34 for *Influenza A Virus* (-0.69±0.03), PIV-1 (-0.69±0.09), HRV (-0.69±0.05) and hMPV (-0.69±0.01).

Similarly, highest infection means were indicated in age group >65 for *Influenza A Virus* (1.29±0.15), PIV-1 (0.533±0.04) and HRV (1.11±0.03) and age group <5 for hMPV (0.83±0.16) in both ICU and those on ventilator support. Lowest infection means were also observed only in age group 20-34for *Influenza A Virus* (-0.69±0.15), PIV-1 (-0.69±0.04), HRV (-0.69±0.03) and hMPV (-0.69±0.16).

Comparatively, significantly (P-value <0.05) higher levels of viral infection was noted in those <5 and >65 while low infection levels was noted in those aged 5-19 and 20-34 years.

Similar pattern of infection was also seen in the subsets where there was no significant difference in infection levels.

4.8 Prevalence of Coinfections in the intensive care unit and those on ventilator support

The study also targeted at the prevalence of co-infections in the population studied. From the findings, it is clear that there were a significant prevalence of at least one virus in the samples analyzed. There were 34 cases of multiple viral infections. 20 cases were in those on ventilator support while 14 cases were present in those in the intensive care unit.

4.8.1 Co-infections in relation to age

Table 4.5: Co-infections in relation to Age Cross-tabulation

		Age categories						Total
		<5 years	5 to 19 years	20 to 34 years	35 to 49 years	50 to 64 years	above 65 years	
mixed infections	yes	7	3	2	3	8	11	34
	no	15	23	4	37	41	46	166
Total		22	26	6	40	49	57	200

From table 4.5 above, it is evident that those above 65 years had higher burden of co-infections, 11 cases identified. The age categories < 5 years, 5-19 years, 35-49 years and 50-64 years recorded 7, 3, 3, and 8 cases of multiple viral infections respectively. Those between 20-34 years had the least cases of co-infections, only 2 cases were identified.

4.8.2 Co-infections in relation to whether in the intensive care unit or on ventilator support and age

Age	Coinfections	MV/ICU
<5 years	RSV/HRV	MV
	RSV/hMPV/HRV	MV
	RSV/ Influenza A	ICU
	RSV/hMPV	ICU
	hMPV/Influenza A/HCoV	MV
	HCoV/ HRV	MV
	hMPV/RSV	ICU
5-19 years	HPIV-3/HRV	MV
	HPIV-1/Influenza A	ICU
	Influenza A/HRV	MV
20-34 years	HRV/HCoV	ICU
	Inluenza A A/HPIV-3	MV
35-49 years	RSV/hMPV	MV
	HPIV-3/hMPV	ICU
	Influenza A/HRV	MV
50-64 years	RSV/hMPV	ICU
	HCoV/HRV	MV
	Influenza A/HPIV-3/HRV	MV
	Influenza A/RSV	ICU
	RSV/HRV/hMPV	MV
	RSV/HRV	MV
	Influenza A/hMPV/HPIV-3	ICU
	hMPV/ Influenza A	ICU
>65 years	Influenza A/hMPV/PIV-3	MV
	HCoV/HRV/RSV	MV
	HRV/RSV/hMPV	ICU
	RSV/HRV	MV
	HPIV-1/HRV	MV
	HPIV-3/hMPV	ICU
	RSV/hMPV	MV
	HRV/HPIV-1/ADV	MV
	HRV/HCOV	ICU
	Influenza A/hMPV/PIV-3	MV
	RSV/HRV	ICU

The results in table 4.6 show that 3 viruses were identified in 10 samples while 2 viruses were detected in 24 samples. Co-infections were common across all age groups, however co-infections where more common in age groups 50-64 years and those above 65 years. There were also significant levels of co-infections in patients below 5 years. RSV was found to be the most co-infected with other viruses in age group below five years. HRV and *Human Metapneumovirus* were also found to be the most co-infected with other viruses in the age group above 65 years as indicated in figure 4.5 above. *Influenza A virus* and HRV were the most co-infected with other viruses in those between 50-64 years. Two cases of *Influenza A virus* were identified co-infected with HPIV-1 and HRV in those between 5-19 years. *Adenovirus* was the least co-infected with other viruses, only one case identified that had co-infection with HRV and PIV-1 in those above 65 years. RSV and HRV were the most co-infected with other viruses in study participants under ventilator support below < 5 years. There were 2 cases of co-infections combination by *Influenza A virus*/hMPV/PIV-3 in those > 65 years, also one case with similar co-infection combination was noted in those between 50-64 years. In study participants in the intensive care unit, co-infections by *Influenza A virus*, RSV and *Human Metapneumovirus* were the most co-infected with other viruses.

CHAPTER FIVE: DISCUSSION

Acute viral respiratory tract infections are a major cause of disease that has been underestimated for a very long time in our healthcare facilities. Improved diagnosis of these infections in the last 25 years has proved that these infections have been implicated in significant cases of morbidity and mortality that is actually on the same level as nosocomial pneumonia and community acquired bacterial infections (Christopher *et al.,* 2015).

In the current study 40.5% (81) and 40% (80) of the study subjects had at least one virus detected in those under ventilator support and those in the intensive care unit respectively. Two viruses were detected in 24 samples (12%), and three viruses were detected in 10 samples (6%). HRV was the most common at 21% (42), *Influenza A* virus was at 16.5% (33), *Human metapneumovirus* 11% (22), RSV 9.5% (19), HPIV-1 6% (12), HPIV-3 5.5% (11), HCoV 4.5% (9) and HPIV-2 4% (8). *Adenovirus* was the least at 2.5% (5). There is similarity with a study done in Kenya where out of 5,647 study subjects, 53.7% were <5 years. 2,380 (42.2%) of the samples had at least one virus detected. HRV was the most common at 8.6% followed by *Influenza virus,* HCoV, HPIV, RSV and *Adenovirus* at 6.9%, 6.8%, 6.6%, 3.9% and 2.7% respectively (Nyiro *et al.,* 2018).

H3N2 and H1N1had a prevalence of 15.5% and 1% respectively. In the current study no sample tested positive for *Influenza B Virus,* however the two subtypes (A and B) are present in Kenya. Other strains have also been reported in Kenya. In a study done

between 2006-2011 the prevalence for *Influenza B,* H1N1 2009 pandemic, H3N3 seasonal, H1N1 seasonal and unsubtyped was 31%, 28%, 24%, 10% and 7% respectively (Duncan *et al.,* 2013). Admissions in the intensive care unit are relatively common. There was an estimated record of 8% of intensive care unit admissions due to unspecified LRTI related to influenza infection in a 3-year observational study (Daubin *et al.,* 2005).

Human Rhinovirus causes infection both in the upper and lower respiratory tract in immunosuppressed individuals that require special care in the ICU. In the current study, *Human Rhinovirus* recorded a prevalence of 22%. However there was a higher prevalence in patience under mechanical ventilation compared to those in the ICU. Among adults studied in the intensive care unit with respiratory disease, 9% were positive for *Human Rhinovirus*. There was *Human Rhinovirus* infections associated case fatality of an estimated 30% in a study of immunosuppressed individuals requiring intensive care (Karhu *et al*., 2014).

HCoV usually cause a mild infection in the upper respiratory tract. It also causes severe LRTI in the elderly. In this study *Human coronavirus* recorded a prevalence of 6.17% (5) in those under ventilator support and 3.75% (4) in those in the intensive care unit. Subtype OC43 and 229E recorded a prevalence of 4.9% and 1.2% respectively in ventilator support respectively. In a study done in Kenya, 417 specimens were analyzed. Out of these 35 (8.4 %) tested positive for *Human coronavirus.* This comprised NL63,

OC43, HKU1, and 229E with a prevalence of 2.4 %, 2.9 %, 2.1 % and 1 % respectively (Lenata *et al.*, 2016).

RSV is a major cause of LRTI in children below 5 years. In this study RSV accounted for 6.25% of all the infections in the intensive care unit among those< 5 years while in those under ventilator support, the prevalence was at 1.2%. Among patients with respiratory failure in the intensive care unit, 2% to 6% have RSV infection. (Van *et al.*, 2002; Anderson *et al.*, 1990). However in other age categories in the current study, there were significant levels of infection both in the intensive care unit and those under ventilator support.

In the current study, the population aged <5 years had a prevalence of 6.2% for *Human metapneumovirus* in the ICU. The prevalence for RSV was 1.2% in the intensive care unit. *Human Rhinovirus*, *Influenza A*, HPIV-1 and HPIV-2 recorded a prevalence of 4.9%, 3.7%, 2.4% and 2.4% % respectively in the intensive care unit. In a study done in 78 children <5 years on ventilator support, there was nosocomial viral etiology in 29.5% of the cases. 14.1%, of the cases was due to RSV. *Influenza A Virus* was at 10.2%. There was co-infection of Respiratory syncytial virus with *Influenza A virus* at 2.6%, and RSV with PIV-1 at 1.3% (Edna *et al*., 2005).

Among the elderly, the impact of respiratory disease caused by viruses is increasing. Significant number of cases was noted in the elderly both in the intensive care and those on ventilator support in this study. *Influenza A virus* HRV, HPIV-1 and HPIV-3 recorded a prevalence of 8.75%, 7.5%, 3.75% and 3.75 % respectively in patients above

65 years. There was also significant levels of infection by RSV, *Human metapneumovirus* and HCoV. *Influenza Virus* and *Respiratory syncytial virus* are the main cause of LRTI. However; among the advanced in age, *Human Parainfluenzavirus,* HRV, HCoV and *Human metapneumovirus* have also been implicated to cause LRTI. They cause between 13-31% of LRTI in the elderly population (Talbot and Falsey, 2010). Several other studies have identified various viral etiologies as causes of ARTIs. In Kenya, a study conducted at the Kilifi district hospital reported a high prevalence (34%) of HRV infections in young children which was associated with severe pneumonia. Human parainfluenza, adenoviruses, and other respiratory viruses were also reported (Berkley *et al.,* 2010).

The study found that children <5 years and the elderly >65 years had higher burden of respiratory viruses compared to other age groups. This can be attributed to increased levels of immunosuppression especially in the elderly and the young.

Human parainfluenza viruses (HPIVs) account for a large percentage of pediatric respiratory infections, including upper respiratory tract infections (URTIs), bronchiolitis, and pneumonia. Findings in this study indicate a prevalence of HPIV-1 and HPIV-2 of 2.4% in those < 5 years on ventilator support and 2.5% for HPIV-1, HPIV-2 and HPIV-3 in the intensive care unit. Previous studies have reported generally similar prevalence estimates of HPIV. In Cameroon, HPIV prevalence of 7.5% was reported in 2012 among influenza-illness like patients (Njouom *et al.,* 2009). A slightly

lower HPIV prevalence estimate of 3.2% has been reported in Latin America (Villaran *et al.,* 2014).

In the current study co-infections were common across all age groups, the overall prevalence of co-infections was 17% (34), however co-infections where more common in age groups 50-64 years and those above 65 years. There were also significant levels of co-infections in patients below 5 years. RSV was found to be the most co-infected with other viruses in age group below five years. RSV was found to be the most co-infected with other viruses in age group below five years. Rhinovirus was also found to be the most co-infected with other viruses in study subjects above 65 years and 50-64. In a study performed in Netherlands where five ICUs were involved from 2013 to 2014, 1,499 patients were studied. There was infection with at least one virus in 18% of the samples analyzed. Two viruses were detected in 17 patients. Three viruses were present in two samples. PIV-3 was the common with a prevalence of 5.7%, 1.1% (17) had an infection with *Influenza Virus*

Comparing co-infections in those in the intensive care unit and those under ventilator support indicated that those on ventilator support had 20 cases while those in the intensive care unit were 14 cases. Co-infection combination of HRV with other viruses was the most common in those on ventilator support while co-infection combination of *Influenza A virus* with either RSV or *Human metapneumovirus* was the most common in the intensive care unit.

From this study *Human Metapneumovirus* was found to be mainly co-infected with RSV, *Human Rhinovirus* and *Influenza A Virus.* This is comparable to a study done in Kilifi, Kenya; where 16928 nasopharyngeal swabs were collected and analyzed. 25.2% tested positive for *Human Metapneumovirus.* There were co-infections in 13.8% of the samples analyzed. (Patrick *et al.*, 2018).

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In Moi Teaching and Referral Hospital, infections of the respiratory tract due to viruses are common in the ICU and those on ventilator support. The infections are common across all age categories. However, the young and the elderly have higher burden of these infections in comparison to other age categories. *Influenza A virus* and HRV are the most common viruses identified across all age groups. However other viruses studied had significant levels of infection. *Human Adenovirus* was the least detected in this study.

Generally patients in ICU had a higher prevalence of infection compared to those in ventilator support. Overall, there was noted difference among the patients in different age categories based on whether in ICU or ventilator support by *Influenza A Virus,* HPIV-1, HRV and hMPV viral infections at P-Value ≤ 0.05. Highest infection means were indicated in age group >65 for *Influenza A Virus,* HPIV-1 and HRV and age group <5 for hMPV in both ICU and those on ventilator support. Lowest infection means were also observed only in age group 20-34 for *Influenza A Virus* HPIV-1, HRV and *Human metapneumovirus* in the intensive care unit and also those on ventilator support.

There was a high prevalence of co-infections in both ICU and MV. There were 34 cases of multiple viral infections. 20 cases were in those on ventilator support while 14 cases were present in those in the intensive care unit. 3 viruses were identified in 10 samples while 2 viruses were detected in 24 samples. Co-infection combination of *Influenza A Virus*

+HPIV-3 + hMPV had the highest prevalence followed by RSV + hMPV + HRV. *Adenovirus* was the least co-infected with other viruses, only one case identified that had co-infection with HRV and PIV-1 in those above 65 years.

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6.2 Recommendation

- Surveillance for viral respiratory infections especially in the hospital set up should be intensified for specific treatment to be implemented in order to understand and document the prevalence of these infections.

- Due to high prevalence of these viruses in ICU patients, surveillance should be enhanced as well as clinical correlation in order to know their effect on mortality and morbidity. Those < 5 years and > 65 years should be closely monitored since they carry a huge burden of the respiratory infections.

- Co-infections should be closely monitored especially in mechanical ventilation in order to understand the impact of ventilator support on infection rates by these viruses. More studies needs to be done focusing on nosocomial respiratory viral infections.

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APPENDICES

APPENDIX 1: RESEARCH AND ETHICS COMMITTEE APPROVAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/108
Approval Number: 0001807

2nd February, 2017

Mr. Kipsang K. Amos,
Kenyatta University,
School of Medicine,
P.O. Box 43844-00100,
NAIROBI-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
02 FEB 2017
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Mr. Kipsang,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled: -

"Determination of the Prevalence of Acute Viral Respiratory Infectious In-Patients on Mechanical Ventilation in Moi Teaching and Referral Hospital Eldoret, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1807*** on 2nd February, 2017. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 1st February, 2018. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

APPENDIX 2: MOI TEACHING AND REFERRAL HOSPITAL APPROVAL

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R&P/10/2/V. II/2010

7th February, 2017

Mr. Kipsang K. Amos,
Kenyatta University,
School of Medicine,
P.O. Box 43844-00100,
NAIROBI-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Determination of the Prevalence of Acute Viral Respiratory Infectious In-Patients on Mechanical Ventilation in Moi Teaching and Referral Hospital Eldoret, Kenya".

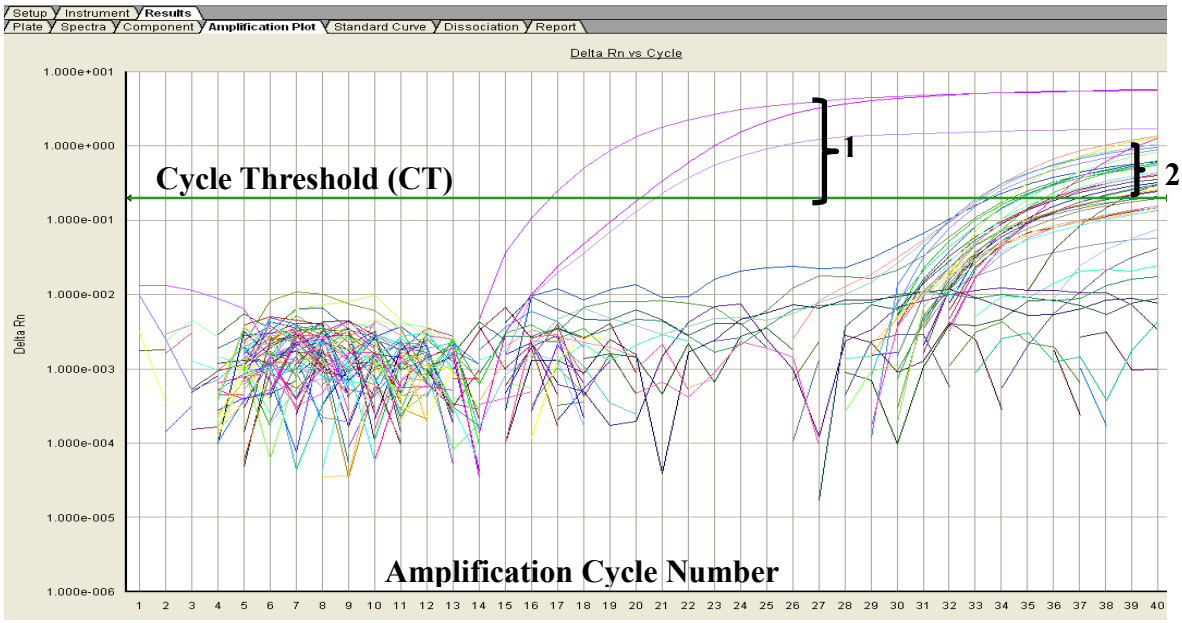
You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

07/02/2017

DR. WILSON K. ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

APPENDIX 3: REPRESENTATIVE REAL TIME PCR RESULTS



APPENDIX 4: REPRESENTATIVE MULTIPLEX PCR RESULTS