Risk factors and cost of illness for acute respiratory infections in children under five years of age attending selected health facilities in Nakuru County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis to Esther, Reginald and Sharleen for being my source of inspiration, love, encouragement and their support in conducting this study.

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LIST OF ABBREVIATIONS

ALRTIs	-	Acute Lower Respiratory Tract Infections
ARI	-	Acute Respiratory Infections
AURTIs	-	Acute Upper Respiratory Tract Infections
BPS	-	Board of Post graduate Studies
CDC	-	Centers for Disease Control (CDC)
cDNA	-	Complimentary DNA
CI	-	Confidence Interval
DALYs	-	Disability Adjusted Life Years
DCC	-	Day Care Centres
dNTPs	-	Deoxynucleotide Triphosphates
ERC	-	Ethical Review Committee
FITC	-	Forest Industrial Training Centre
GAPDH	-	Glyceraldehyde-3-phosphate Dehydrogenase
GISRS	-	Global Influenza Surveillance and Response System
HIV/AIDS	-	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
HMPV	-	Human metapneumovirus
HRSV	-	Human Respiratory Syncytial Virus
ICD	-	Informed Consent Document
IMCI	-	Integrated Management of Childhood Illness
IVs	-	Influenza viruses
JKUAT	-	Jomo Kenyatta University of Agriculture and Technology
KAP	-	Knowledge, Attitudes and Practices
KEMRI	-	Kenya Medical Research Institute
KEPI	-	Kenya Expanded Programme on Immunization
LRTIs	-	Lower Respiratory Tract Infections
MDG	-	Millennium Development Goal
MEME	-	Multiple Exposures Multiple Effects
MUAC	-	Mid Upper Arm Circumference
NK	-	Natural Killer
OR	-	Odds Ratio

PCR	-	Polymerase Chain Reaction
PHC	-	Primary Health Care
PIVs	-	Parainfluenza viruses
RNA	-	Ribonucleic Acid
RT-PCR	-	Reverse Transcription Polymerase Chain Reaction
SARS	-	Severe Acute Respiratory Syndrome
SD	-	Standard Deviation
SSC	-	Scientific Steering Committee
UNICEF	-	United Nations International Children's Fund
URTIs	-	Upper Respiratory Tract Infections
USA	-	United States of America
WHO	-	World Health Organization

DEFINITION OF TERMS

Acute Respiratory Infections (ARI) in children: ARI in children case were defined as a child with sudden onset of fever with cough and /or sore throat in the absence of other diagnoses.

Appropriate care: This is where health care was sought from qualified medical professionals in government health facilities and private hospitals/clinics

Inappropriate care: Other types of care such as purchasing medicines from pharmacy, home remedies, religious/faith healing and traditional healers which was defined as inappropriate care

Lower respiratory tract infections: The lower respiratory tract is the part of the respiratory tract below the vocal cords. Lower respiratory tract infection is often used as a synonym for pneumonia, the rubric of lower respiratory tract infection can also be applied to other types of infection including lung abscess and acute bronchitis.

Upper respiratory tract infections (URI or URTI): These are the illnesses caused by an acute infection which involves the upper respiratory tract: nose, sinuses, pharynx or larynx. This commonly includes: tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, and the common cold. Acute upper respiratory tract infections include rhinitis, pharyngitis/tonsillitis and laryngitis often referred to as a common cold, and their complications: sinusitis, ear infection and sometimes bronchitis (though bronchi are generally classified as part of the lower respiratory tract infection)

Health seeking behavior: was defined as mother's response for signs and symptoms of illnesses to reduce severity, complication or even death after she recognized her child's illness and if she reported visiting any health institutions; health center, health post, privet clinic or at least community health worker. Mothers who did not report visiting any health institution for the perceived common childhood were considered as healthcare non-seeker.

Rural: Inhabitants enrolled at a facility that is over 20 kilometers radius from the nearest urban centre (Nakuru County city centre).

Urban: Inhabitants enrolled at a facility that within a radius of less than 20 kilometers from nearest urban centre (Nakuru county city centre).

ABSTRACT

Acute Respiratory Infections (ARI) are among of the most common causes of both illness and mortality in children under five years of age. A prevalence of between 11% and 29% has been reported in studies conducted in the East African countries. In Kenya, ARI has been associated with 20-30% admissions and 19% deaths of children under five years of age. Evaluation of risk factors for ARI, assessment of health seeking behaviour that may influence care and management of ARI, knowledge and perceptions regarding ARI transmission and preventive measures as well as cost estimation of ARI episodes that can be used in budgeting and resource allocation is crucial in reducing childhood illnesses attributed to ARI. These factors have not been well investigated in Kenyan populations. This study aimed at identifying the aetiology, risk factors, and cost of illness for acute respiratory illnesses in subjects from selected health facilities in Nakuru County in Kenya. The study also assessed the caretakers' health care seeking behaviour, knowledge and perception on ARI transmission and prevention. This was a case control study which recruited caretakers (parents or guardians) with children less than 5 years of age who visited the selected health facilities with children suspected to have ARI. A corresponding control was enrolled for each case matching for age and sex. Data was collected using semi-structured questionnaires. A subgroup was sampled to collect specimen for determination of aetiology by laboratory analysis. Demographic data was analysed by descriptive statistics while chi-square and Fisher's Exact Test were used to analyze categorical data and student's t-test for continuous data. Logistic regression analysis was used to identify the factors influencing health care seeking behavior and potential risk factors for ARI. The study enrolled a total of 261 participants but 5 of them did not find matching controls and hence data was analysed for 256 participants. Specimens were taken from eighty two (33%) of the children for bacterial culture and detection of viral agents but four were spoilt during transportation and therefore seventy eight were tested for viral and bacteria detection. Bacteria were isolated from 24.4% of sampled patients with *Streptococcus pyogenes* and *Streptococcus viridans,* being the most predominant. At least one respiratory virus was detected in 44.9% of the specimen collected from the children. Of the viral agents detected, 20.5% were influenza A, 16.7% were respiratory syncytial virus (RSV) while 10.3% were influenza B viruses. Mixed infections were present in 29.5% of the children. The major risk factors identified were: malnutrition, crowding and smoking. Factors that showed a trend

towards protection were immunization, breastfeeding for more than 4 months, parental education above primary school, family income above $176 (kshs 15,000). Knowledge on preventive measures for ARI was generally low. Majority, 231 (92.8%) sought appropriate health care (health facility) but a few sought inappropriate care from traditional doctors, direct purchase of drugs from pharmacies, and others used home remedies. Family size, delivery in a hospital, income and education influenced the health seeking behavior among the respondents. The total mean cost of managing ARI was $17.70 with consultation and cost of prescribed medicine constituting the major cost drivers for management of ARI. The study strongly recommends basic health promotional measures like proper breastfeeding practices, proper nutrition of the child in prevention and control of ARI. Health education can change health care seeking behavior and attitude of caretakers and other family members to take appropriate care of the children with ARI and other childhood illnesses.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background information

The disease burden for Acute Respiratory Infections (ARI) is estimated at 94,037 000 disability adjusted life years (DALY) and 3.9 million deaths globally. Acute respiratory infections are among the most common causes of both illness and mortality in children aged below five years, who on average get three to six episodes of ARIs annually regardless of where they live or what their economic situation is (Williams *et al.,* 2002). Acute respiratory infections contributes 2 to 4% of deaths in children <5 years of age in the low mortality member states. These causes contribute 19 to 21% of child deaths in the Eastern Mediterranean, Africa, and South East Asia regions (Emmelin and Stig, 2007). Although the frequency of ARI is similar in both developed and developing countries, mortality due to ARI is 10–50 times higher in developing countries (Broor *et al.,* 2007). The proportion of mild to severe disease varies between high and low income countries because of differences in specific aetiologies and risk factors. The severity of ARIs in children under five is worse in developing countries, resulting in a higher case fatality rate. Estimates indicate that in 2000, 1.9 million of them died because of ARIs, 70% of them in Africa and Southeast Asia (Williams *et al.,* 2002). The World Health Organization (WHO) estimates that 2 million children under five die of pneumonia each year (Bryce *et al.,* 2005). Two separate studies conducted in Tanzania reported ARI prevalence of 11% and 29%, respectively, in children less than five years of age (Kilabuko and Nakai, 2007; Chandler *et al.,* 2008).

Although medical care can to some extent mitigate both severity and fatality, many severe ARIs do not respond to therapy, particularly those of viral aetiology, largely because of the lack of highly effective antiviral drugs. Preventive measures are therefore critical in controlling the ARI (Bryce *et al.,* 2005). Studies on aetiology of ARI indicate that about 50% of the infections are of viral aetiology (Maitreyi *et al.,* 2000) while among the rest, majority are attributed to bacterial causes. A study conducted in Dadaab and Kakuma refugee camps in Kenya to characterize the role of respiratory viruses in the epidemiology of ARI among refugees reported that 49.8% of the collected samples from the patients had at least one respiratory virus (Ahmed *et al.,* 2012). Another study carried out in North

Eastern and Rift Valley provinces of Kenya reported respiratory viruses detection rate of 60.1% in specimen collected from children and adults (Kim *et al.,* 2011).

Few studies conducted assessing potential risk factors associated with ARI have demonstrated among others roles various factors including indoor and outdoor pollution, child care, breastfeeding, parent age, crowding and child immunization. Bautista *et al.* (2009) carried out a study to evaluate relationship between exposure to indoor charcoal smoke and risks of acute upper respiratory infection (AURTIs) and acute lower respiratory infection (ALRTIs) in a cohort of children from the Dominican Republic. Findings of this study demonstrated that incidences of ALRTIs in children from charcoal-using households were 1.58 higher than those in children from households using gas. Another case control study with the aim of identifying environmental risk factors in the Dutch general population found smoking as a risk factor for increased general practitioner consultation due to ARI compared with non-smokers and was also contact with persons who had recent complaints of ARI (van Gageldonk-Laafeber *et al.,* 2005). Other studies conducted in other countries have shown exposure to various biomass increases the risk of ARI incidence (Smith *et al.,* 2000; Romieu *et al.,* 2002; Mishra, 2003; Dherani *et al.,* 2008).

Proper management of childhood illness including ARI depends on the caretaker's decision to utilise health services from the medical facilities. The World Health Organization estimates that seeking prompt and appropriate care could reduce child deaths due to acute respiratory infections by 20% (WHO, 1997). In more than 40 of the 82 countries Worldwide with available data, fewer than 50% of the children with ARI are taken to a health care provider (http://www.unicef.org). Limited studies have been conducted to assess the health care seeking behaviour among parents with young children in various countries. A study conducted in Ethiopia to assess the mother's care seeking behaviour for childhood illness between rural and urban populations indicated that care was sought from health facilities only for less than half of sick rural children 48 (43.2%) as compared to urban 41 (87.2%). Mothers' responses and actions were frequently influenced by their perception of severity or worsening of illness. Lack of money, distance, and perception of the illness not being serious were the major reasons for not seeking health care (Tsion *et al.,* 2008). Residence and knowledge were identified as the

major predictors of health care seeking practices from health facilities (Tsion *et al.,* 2008).

Effective outbreak and preventive measures to ARI infections and potential outbreaks requires support from the population at risk for measures undertaken to mitigate the diseases transmission and spread. Higher perceived effectiveness of measures undertaken and higher perceived threat of the disease can lead to higher rates of positive behavioural change, and better knowledge increases the uptake of preventive measures. The knowledge and perception of the community could influence both individual and community protective behaviour (Yap *et al.,* 2010; Iyun and Tomson, 1996). Hadi (2002) in a study in Bangladesh assessing mother's knowledge in transmission, clinical signs recognition and preventive measures; mothers reported presence of cough and common cold most frequently.

Success of public health preventive interventions to reduce the transmission and reduce incidence of ARI as well as improve care seeking behaviour and management of ARI depends on various factors. These include: evaluation of risk factors for ARI and aetiology, assessment of health seeking behaviour that may influence care and management of ARI, knowledge and perceptions regarding ARI transmission and preventive measures as well as cost estimation of ARI episodes that can be used in budgeting and resource allocation to reduce childhood illnesses attributed to ARI. These factors have not been well investigated in Kenya populations.

1.2. Statement of the problem and justification

Acute respiratory infections (ARIs) continue to be the leading cause of acute illnesses worldwide and remain the most important cause of infant and young children mortality, accounting for about two million deaths each year. Lower respiratory infections (LRIs) are responsible for more severe illnesses such as influenza, pneumonia and viral infections that are the leading contributors to ARIs' mortality. The incidence of ARIs in children aged less than 5 years is estimated to be 0.29 and 0.05 episodes per child-year in developing and industrialized countries, respectively, which translates into 151 million and 5 million new episodes each year. The situation is worse in developing countries and the risk factors in community studies remain largely under investigated.

Various studies conducted in Kenya have showed a high burden of ARI among children. Mohamed (2013) in a study among refugees in Dadaab Camp in Kenya showed that the overall mortality attributed to lower respiratory infection was 21.3% with atleast 43.8% of the patient population samples containing at least one respiratory virus. Ahmed *et al.* (2012) reported an annual Severe ARI hospitalisation rate of 57 per 1000 children per year for 2007-2010 among participants from Dadaab and Kakuma refugee camps. A study by Sikolia *et al.* (2004) in an urban set up in Kibera, Nairobi, revealed a high prevalence of ARI in the area with (69.7%).

Several studies conducted in Kilifi, Kenya have reported the role of RSV in ARI causing hospitalization of 3% and 23% of children with RSV developing severe ARI (Nokes *et al.,* 2004). A separate study reported RSV to be associated with LRTI (13%), severe-LRTI (19%) and hospitalizations (5%) among the children in the cohort (Nokes *et al.,* 2008). Nokes *et al.* (2009) in another study reported that 29% of all children admissions were attributed to severe or very severe pneumonia. Okiro *et al.* (2012) reported a burden of childhood of 6% URTI and LRTI 8% among the children enrolled in a study conducted in birth cohort in Kilifi District Hospital. Other studies conducted in Kenya have detected respiratory viruses in large proportions of patients with ARI; 60.1% (Kim *et al.,* 2011**),** 66.6% (Munywoki *et al.,* 2011) and 68% (Feikin *et al.,* 2012).

Community responses during ARI epidemics is important in preventing further spread, the responses depends on the community knowledge, perception and beliefs. Success in the prevention and management of ARI also depends on the knowledge about transmission, illness identification and prevention measures by the communities. Little is known about the community psychological and behavioural responses to influenza pandemics in Kenya. Identification of communities' practices and knowledge level on modes of transmission and prevention of spread of common ARI may present opportunities on the need for public education and health promotion to reduce the incidence and re-currencies of the conditions.

Respiratory infections are common in children under 5 years presenting as acute cough and have been indicated as the major cause of need to seek medical care. Though it poses

a major economic and health burden to the communities, cost of management and potential risk factors are not clearly documented. An estimate of the cost of managing the ARI in children under five will aid in budgetary decision-making, and provide an insight on economic justification for the need of interventions to reduce the burden of ARI. Knowledge of the potential risk factors would aid in supporting preventive public health interventions to reduce the incidence of ARI. Few reports are available for studies in Kenya that have investigated risk factors for ARI (Okiro, 2008) and some were conducted more than 10 years (Wafula *et al.,* 2000) and therefore current data is required to aid in making informed decisions on the appropriate interventions. Studies on economic burden of ARI have been conducted in other continents (Hollinghurst *et al.,* 2008; Lambert *et al.,* 2008) but scanty information is available regarding cost of ARI episode in Kenya. This information would be used in public health education and health promotion to educate the public on preventive measures for ARI.

1.3. Hypothesis

i. The risk factors for ARI are different between cases and control populations of the study population in Nakuru County.
ii. The aetiological agents isolated in ARI cases are different from those isolated from control populations of the study population in Nakuru County.
iii. Health seeking behaviours among parents of children with or without ARI are different.

1.4. Objectives

1.4.1. General objective

To determine the aetiology, risk factors, health seeking behaviour and cost of illness for acute respiratory illnesses among children less than 5 years of age in selected facilities in Nakuru County.

1.4.2. Specific objectives

i. To identify the aetiology for ARI among children less than 5 years of age visiting selected health facilities in Nakuru County in Kenya.
ii. To determine the risk factors for childhood ARI among under 5 years old cases and controls in the selected health facilities Nakuru County in Kenya.

iii. To determine the caretakers' healthcare seeking behaviour, knowledge and practices with regard ARI transmission and prevention

iv. To estimate the cost of illness for acute respiratory infection in the selected sites

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Definition of acute respiratory infections

The upper respiratory tract includes the sinuses, nasal passages, pharynx, and larynx. These structures direct the air breath from the outside to the trachea and eventually to the lungs for respiration to take place. An upper respiratory tract infection, or upper respiratory infection, is an infectious process of any of the components of the upper airway (Lambert *et al.,* 2008). Infection of the specific areas of the upper respiratory tract can be named specifically. Examples of these may include rhinitis (inflammation of the nasal cavity), sinus infection (sinusitis or rhino sinusitis) - inflammation of the sinuses located around the nose, common cold (nasopharyngitis) - inflammation of the nares, pharynx, hypopharynx, uvula, and tonsils, pharyngitis (inflammation of the pharynx, uvula, and tonsils), epiglottitis (inflammation of the upper portion of the larynx or the epiglottis), laryngitis(inflammation of the larynx), laryngotracheitis (inflammation of the larynx and the trachea), and tracheitis (inflammation of the trachea). Upper respiratory infections are one of the most frequent causes of medical visits with varying symptoms ranging from runny nose, sore throat, cough, to breathing difficulty, and lethargy (Oyejide and Osinusi, 1990; Dharmage *et al.,* 1996; Williams *et al.,* 2002; Lambert *et al.,* 2008; http://doktermudatrader.blogspot.com/ 2010/05).

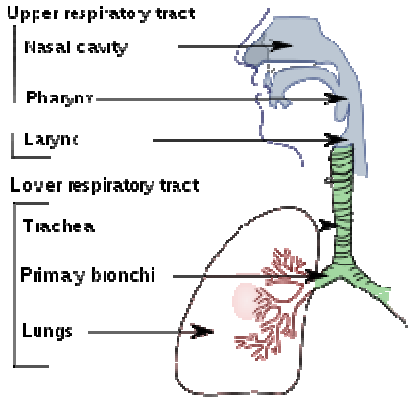

Figure 2.1: Major parts of respiratory tract system
Source: http://en.wikipedia.org/wiki/File:Illu_conducting_passages.svg

2.2. Classification of acute respiratory infections

Acute respiratory infections (ARIs) are classified as upper respiratory tract infections (URTIs) or lower respiratory tract infections (LRTIs). The upper respiratory tract consists

of the airways from the nostrils to the vocal cords in the larynx, including the paranasal sinuses and the middle ear. The lower respiratory tract covers the continuation of the airways from the trachea and bronchi to the bronchioles and the alveoli (Simoes *et al.,* 2006).

2.2.1. Lower respiratory tract infections

The lower respiratory tract is the part of the respiratory tract below the vocal cords. While often used as a synonym for pneumonia, the rubric of lower respiratory tract infection can also be applied to other types of infection including lung abscess and acute bronchitis. Symptoms include shortness of breath, weakness, high fever, coughing and fatigue (http://doktermudatrader.blogspot.com/ 2010/05). Lower respiratory tract infections place a considerable strain on the health budget and are generally more serious than upper respiratory infections. Since 1993 there has been a slight reduction in the total number of deaths from lower respiratory tract infection. However in 2002 they were still the leading cause of deaths among all infectious diseases, and they accounted for 3.9 million deaths worldwide and 6.9% of all deaths that year (Beaglehole *et al.,* 2004). There are a number of acute and chronic infections that can affect the lower respiratory tract. The two most common infections are bronchitis and pneumonia. Influenza affects both the upper and lower respiratory tracts (Antibiotic Expert Group, 2006).

2.2.2. Upper respiratory tract infections

Upper respiratory tract infections (URI or URTI) are the illnesses caused by an acute infection which involves the upper respiratory tract: nose, sinuses, pharynx or larynx. This commonly includes: tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, and the common cold (Eccles *et al.,* 2007). Acute upper respiratory tract infections include rhinitis, pharyngitis/tonsillitis and laryngitis often referred to as a common cold, and their complications: sinusitis, ear infection and sometimes bronchitis (though bronchi are generally classified as part of the lower respiratory tract.) Symptoms of URTI's commonly include cough, sore throat, runny nose, nasal congestion, headache, low grade fever, facial pressure and sneezing. Onset of the symptoms usually begins 1–3 days after the exposure to a microbial pathogen. The illness usually lasts 7–10 days. Group A beta haemolytic streptococcal pharyngitis or tonsillitis typically presents with a sudden onset of sore throat, pain with swallowing and fever. Tonsillitis does not usually cause runny

nose, voice changes or cough. Pain and pressure of the ear caused by a middle ear infection (Otitis media) and the reddening of the eye (Conjunctivitis) caused by are often associated with upper respiratory infections (Mika *et al.,* 1998; Eccles *et al.,* 2007).

2.2.3. Systemic effects of acute respiratory infections

ARIs are not confined to the respiratory tract and have systemic effects because of possible extension of infection or microbial toxins, inflammation, and reduced lung function. Diphtheria, pertussis (whooping cough), and measles are vaccine-preventable diseases that may have a respiratory tract component but also affect other systems (Simoes *et al.,* 2006).

2.3. Aetiology of acute respiratory infections

Acute respiratory infections are caused mainly by viruses but bacterial aetiologies have been confirmed. A Dutch case-controlled study of general practice patients found that viruses accounted for 58% of acute respiratory tract infections and bacteria such as Group A streptococcus was responsible for 11%, and 3% of patients had mixed bacterial and viral infections (van Gageldonk-Laafeber *et al.,* 2005). Various viral and bacterial strains have been associated with ARI as described below.

2.3.1. Viral aetiology

There are a number of viruses that are considered primarily as pathogens of the respiratory tract. Over 200 different viruses have been isolated in patients with URTIs. Until recently when molecular techniques were developed for detection and confirmation of various pathogens; viral pathogens have historically been detected using cell culture, antigen detection, or serological diagnostic panels. This group includes the influenza viruses (IVs), respiratory syncytial virus (HRSV), and the parainfluenza viruses (PIVs) types I, II and III. Adenoviruses cause respiratory disease, but specific serotypes can affect other organ systems and produce nonspecific illness, such as fever with no clear focus. There is a second line of agents, including rhinoviruses, other picornaviruses and coronaviruses that are relatively common, but have previously been thought of as typically associated with milder upper respiratory tract illness (Falsey *et al.,* 2002). Another group of viruses originally identified in individuals with respiratory disease, are rarely found in conjunction with respiratory illness: these include agents such as

mimivirus and Mossman virus (Arden *et al.,* 2006). Episodic respiratory infections caused by the transmission from animals to humans of viruses that are not well adapted for human-to-human spread include avian influenza strains (Peiris *et al.,* 2004) and apparent occasional episodes, such as that caused by a recently discovered reovirus, Melaka virus (Chua *et al.,* 2007; Schildgen *et al.,* 2008).

A number of viruses from the herpesviruses family, including human herpes virus type 6, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, and the herpes simplex viruses, have all been associated with respiratory tract disease, particularly in the immunocompromised (Mackie*,* 2003). A number of viruses identified for the first time in specimens from the human respiratory tract; are human metapneumovirus (HMPV) (Lambert *et al.,* 2008), a number of new coronavirus: SARS-associated coronavirus (Ksiazek *et al.,* 2003) human coronaviruses, human bocavirus (Allander *et al.,* 2005) and two human polyomaviruses: KI (KIPyV)24 and WU virus (WUPyV). Another new human polyomavirus, initially found in association with Merkel cell carcinoma tissue, has also been found in respiratory specimens (Feng *et al.,* 2008). These viruses comprise the core group of common childhood respiratory pathogens, as well as those that may only occasionally cause illness.

2.3.2. Bacterial aetiology

Up to 15% of acute pharyngitis cases are associated with by bacterial aetiology, commonly Group A streptococcus in streptococcal pharyngitis (Bisno, 2001). Major LRTIs are associated with various bacterial aetiologies such as *Streptococcus pneumoniae, Mycoplasma pneumoniae*, *Chlamydia* spp, *Pseudomonas* spp., *Escherichia coli*, and *Haemophilus influenzae* causing pneumonia. There are few data on the causes of neonatal pneumonia in developing countries, but studies of neonatal sepsis suggest that these include Gram-negative enteric organisms, particularly *Klebsiella* spp, and Gram-positive organisms, mainly Streptococcus pneumoniae, groupB *Streptococcus* and *S. aureus* (WHO, 1999). In a study conducted in Hongkong to investigate the bacterial etiology of ARI, *Mycoplasma* spp was found to contribute to 3% of the positive cases (Sung *et al.,* 2009). Vong *et al.* (2011) in a recent study on viral and bacterial aetiologies of community acquired acute lower respiratory infections found that bacteria commonly associated LRTIs in under 5 year-old children were *Burkholderia pseudomallei,*

*Streptococcus pneumoniae, Haemophilus influenza*e, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis*.

2.4. Transmission and significance of acute respiratory infections

Transmission is via respiratory droplets or by virus-contaminated hands. Upper respiratory tract (nose, throat, sinuses) mucosa inflammation causes increased secretions, rhinorrhea and results in sneezing, and coughing facilitating the spread. In many developing and developed countries, ARIs are the most common infectious illness in the general population and are major to contributors missing school and office particularly the URTIs (Cherry *et al.,* 2008). Acute respiratory tract infections are leading causes of morbidity and mortality in developed and developing countries. The importance of acute respiratory tract infections in working adults and the elderly has long been recognised, and the bulk of research efforts and prevention programs have been targeted to these groups. More recently, there has been renewed attention given to the impact respiratory tract infections have in infants and children and the prospects for prevention in this group (Lambert *et al.,* 2008).

2.5. Clinical manifestations of acute respiratory infections

In general, a respiratory disease begins with complaints and symptoms are mild. In the course of the disease symptoms may be more severe and if the weight can fall in a state of respiratory failure and possibly death. Danger signs can be seen based on clinical signs including; tachypnea, irregular breathing (apnea), thorax wall retraction, nostril breathing, cyanosis, weak or missing breath sounds, grunting and wheezing, restless, headache, convulsions, coughing, excessive sweating, sore throat, otitis and coma. In children less than 5 years of age, danger includes; inability to drink, convulsions, decreased consciousness, stridor, reduced appetite, seizures, decreased consciousness, wheezing, fever and cold (Oyejide and Osinusi, 1990; Dharmage *et al.,* 1996; Williams *et al.,* 2002; Lambert *et al.,* 2008).

2.6. Acute respiratory infections in children

2.6.1. Global burden of acute respiratory infections in children

Acute respiratory infections (ARI) constitute the major cause of mortality and morbidity among children in developing countries. Every year, approximately 15,000,000 children

under the age of 5 years die and 25-30% of this mortality is attributable to acute respiratory infections as an underlying or contributing to ARI in children (Forgie *et al.,* 1992). Pneumonia accounts for most of these deaths. Incidence of ARI is highest in the first two years of life, with up to eight episodes per year, and during the cold seasons averaging close to one infection per child-month (Monto, 2002). Whilst illnesses can often be managed in the community with supportive care from parents or other caring adults, complications requiring a medical visit and antibiotic therapy, such as otitis media (30%) and sinusitis (8%), are common (Fisher *et al.,* 1997; Revai *et al.,* 2007). In pre-school aged children, nearly 50% of general practitioner visits are for ARI. Further, up to 5% of all infants are hospitalised for viral lower respiratory tract infections (LRTI) such as croup, bronchiolitis, and pneumonia, or from secondary bacterial pneumonia. Rapid identification of respiratory viruses in children can reduce the duration of hospital admission, antibiotic use, and number of investigations performed, and has been shown to be cost effective in busy diagnostic settings (Woo *et al.,* 1997; van Woensel *et al.,* 2003).

2.6.2. Burden of acute respiratory infections in Kenya

Studies conducted in Kenya have shown a high burden of ARI among pre-school going children. Mohamed (2013) in a study on the burden of Lower Respiratory Tract Infections (LRTI) and the etiology of viral ARIs among refugees in Dadaab Camp in Kenya showed that the overall mortality attributed to LRTI was 21.3% with atleast 43.8% of the patient samples containing at least one respiratory virus. Ahmed *et al.* (2012) in a study in Dadaab and Kakuma refugee camps on epidemiology of ARI among refugees found an annual Severe ARI hospitalisation rate of 57 per 1000 children per year for 2007-2010. Viruses were attributed to more hospitalization particularly RSV and Adeno virus. A study by Sikolia *et al.* (2004) in an urban set up in Kibera, Nairobi, revealed a high prevalence of ARI in the area with 69.7% of the participants developing ARI. Nokes *et al.* (2004) conducted a study in Kilifi District Hospital on a cohort of 338 children to determine the role of RSV in causing acute respiratory infections. In this study, 10.5% of the patients specimen were antigen positive for RSV and 36% of the children with RSV developed ARI (23%) and 3% were hospitalized. Nokes *et al.* (2008) in another study of RSV associated ARI, reported RSV to be associated with LRTI (13%), severe-LRTI (19%) and hospitalizations (5%) among the children in the cohort. In a separate study of prospective surveillance of severe and very severe pneumonia in children aged less than 5

years admitted between 2002 and 2007 in Kilifi district hospital, Kenya, Nokes *et al.* (2009) showed that 29% of all children admissions were attributed to severe or very severe pneumonia. Respiratory syncytial virus prevalence was 15% (20% in infants) and 27% during epidemics (32% in infants) and admission rates due to ARI were double in the areas close to the hospital (Nokes *et al.,* 2009).

Okiro *et al.* (2012) reported a burden of childhood of 6% URTI and LRTI 8% among the children enrolled in a study conducted in birth cohort in Kilifi District Hospital. Another study by Munywoki *et al.* (2011) reported an overall detection rate of at least one respiratory virus in 66.6% of children enrolled in the study, with Rhinovirus being the most frequently detected (26.4%), followed by RSV (24.4%) and parainfluenza virus PIV (12.0%). Kim *et al.,* (2011) reported a high respiratory virus detection rate with 60.1% of children specimen testing positive for at least one virus, and 16.9% for more than one virus. Feikin *et al.* (2012) in another study conducted from 2007 to 2010 in rural western Kenya reported that at least one respiratory virus was detected in 68% of the tested samples, with detection being higher among outpatients (71%) than inpatients (58%). Influenza A virus was the most common virus (22%) while *Streptococcus pneumoniae* was the most common bacteria (16%) detected. Additionally, Influenza A virus, influenza B virus, respiratory syncytial virus (RSV) and human metapneumovirus were more prevalent among cases (22%, 6%, 8% and 5%, respectively) than controls. In this study, 2% of the ARI patients died (0.2% among outpatients, 6% among inpatients) within 30 days of their clinic visit (Feikin *et al.,* 2012).

2.7. Potential risk factors for acute respiratory infections

A lot of efforts to reduce mortality from ARI have concentrated on the prompt diagnosis and treatment of cases of ARI through primary health care (PHC) systems (De Francisco *et al.,* 1993). Although acute respiratory illnesses (ARI) are major causes of morbidity and mortality in early childhood worldwide, little progress has been made in their control and prophylaxis (Kusel *et al.,* 2006). Knowledge of the risk factors would eventually help in developing public health interventions and targeted health education to reduce ARI incidence and recurrence within the communities. Few studies conducted assessing potential risk factors associated with ARI have demonstrated among others roles various

factors including indoor and outdoor pollution, child care, breastfeeding, parent age, crowding and child immunisation. A study conducted to assess the magnitude of the risk of high morbidity (>7 episodes/year) for acute respiratory infections (ARI) in infants attending day care centres (DCC) showed an increased incidence of ARI with 14 episodes per child/year among DCC infants with a median of 74 sick days, while among children at home, the ARI incidence was 6 episodes, and the median was 40 days (Hernandez *et al.,* 1999). De Francisco *et al.* (1993) in a study to assess risk factors involved with death due to ARI found an increased risk of death from ARI for children in who lived in house where one of the residents was smoking and the child was carried while cooking. A reduced risk of death due to ARI was found in children who were exclusively breastfed while overcrowding in the house where the subject case lived was found to have increased risk. This study not find a significant increased risk of death from ARI and location of heating sources, location of the kitchen, maternal age, education and socioeconomic status. Bautista *et al.* (2009) carried out a study to evaluate relationship between exposure to indoor charcoal smoke and risks of acute upper respiratory infection (AURTIs) and acute lower respiratory infection (ALRTIs) in a cohort of children from the Dominican Republic. Findings of this study demonstrated that incidences of ALRTIs in children from charcoal-using households were 1.58 higher than those in children from households using gas. Another study case controlled study with the aim of identifying environmental risk factors in the Dutch general population found smoking as a risk factor for increased general practitioner consultation due to ARI compared with non-smokers and so was contact with persons outside the households with complaints of ARI (van Gageldonk-Laafeber *et al.,* 2005). Other studies conducted in other countries have shown exposure to various biomass increases the risk of ARI incidence (Smith *et al.,* 2000; Romieu *et al.*, 2002; Mishra, 2003; Dherani *et al.*, 2008). These studies have demonstrated potential risk factors in which varies from community to community, however, there is lack of information on risk factors associated with ARI in Kenya that could be used for public health interventions.

2.8. Treatment and management of acute respiratory infections

Treatment of ARI depends on the type of ARI, whether is it URTI or LRTI as well as the aetiological agent. The approaches that have been used and suggested to manage various ARIs are discussed below.

2.8.1. Management of lower respiratory tract infections

Mothers often take their children with an acute upper respiratory infection to a clinic because of concerns about the child's cough, fever, sore throat, or blocked nose, or because of problems with feeding. Cough is a symptom of most acute respiratory infections (ARI) including both upper respiratory infections (URTIs), such as coughs and colds (also known as the common cold, coryza, acute nasopharyngitis or acute pharyngorhinitis), and the more serious lower respiratory infections (LRI) such as pneumonia, bronchitis and bronchiolitis. Medicines for the symptoms of upper respiratory infections are sought both for the relief of discomfort and as a response to the fear that the illness is potentially serious. Parents usually do not understand the mechanisms of these infections, and do not appreciate that a "cure" does not exist (Lambert *et al.,* 2008).

Many upper respiratory tract infections are caused by viruses, and many of the bacterial infections in this area resolves without antibiotic therapy (Mandell, 2005). No drug therapy has been shown to cure or shorten the duration of viral upper respiratory tract infections (WHO, 2001). Unfortunately, antibiotics are over prescribed for upper respiratory tract infections; in many developed and developing countries, antibiotics are prescribed for 50% to 75% of ambulatory patients seen for viral upper respiratory tract infections (van Gageldonk-Laafeber *et al.*, 2005). The case management of ARI focuses on case detection and treatment of pneumonia but must also assure the adequate management of children with a cough or cold who do not have pneumonia. Among experimental therapies, alpha-2-interferon has shown some efficacy in preventing experimentally induced rhinovirus infections and their spread within families, but remains experimental due to expense and toxicity. Antiviral agents are available to treat specific infections for example, ribavirin for respiratory syncytial virus, and amantadine if given early in the course of influenza. There is no evidence that antibiotic or other medical therapy for the common cold virus (Fashner *et al.,* 2012; WHO, 2001). Educational programs can reduce inappropriate prescriptions. Because patient demand fuels antibiotic prescriptions for upper respiratory symptoms, another useful strategy is the delayed prescription, whereby the physician offers a prescription but suggests that it be filled only if symptoms fail to resolve without antibiotics (van Gageldonk-Laafeber *et al.,* 2005).

2.8.2. Treatment and management of lower respiratory tract infections with empirical treatment

Pneumonia is the cause of most serious lower respiratory tract infection. The most common cause of pneumonia, *Streptococcus pneumoniae* accounts for about a third of bacteremic pneumonia. This is a dangerous type of lung infection with a mortality rate of around 25% (Dear *et al.,* 2003). For optimal management of a pneumonia patient the following must be assessed; pneumonia severity, identification of causative organism, analgesia of chest pain, the need for supplemental oxygen, physiotherapy, hydration, bronchodilators and possible complications of emphysema or lung abscess. For community acquired respiratory infections the appropriate use of antibiotics such as fluoroquinolones is a therapeutic option. However it is clinical response that is the best indicator of efficacy (Wilson *et al.,* 1999). With increased development of drug resistance, traditional empirical treatments are becoming less effective, hence it is important to base antibiotic choice on isolated bacteria and sensitivity tests.

2.8.3. Non-pharmacological treatment

In 2003 a very high quality, published research was done about the risk of hospitalization due to respiratory illness and type of infant feeding in developed countries. It involved 3,201 breastfed babies and 1,324 non –breastfed babies. It showed an overall 72 % reduction in the risk of hospitalization in infants who exclusively breastfed for 4 or more months compared to those who were formula-fed. Therefore, exclusive breastfeeding for 4 or more months is associated with a reduction in the risk of hospitalization secondary to lower respiratory tract diseases (Ip *et al.,* 2007). The mainstay of non-pharmacological treatment for many years has been rest and increased fluid intake, however, some studies have reported harmful effects such as dilution of blood sodium concentration leading to headache, confusion or possibly seizures. Rest will allow the body to conserve energy to fight off the infection. Physiotherapy is indicated in some types of pneumonia and should be encouraged where appropriate (Guppy and Del Mar, 2007).

2.9. Preventive measures for acute respiratory infections

Risk factors for fatal pneumonia include poor socioeconomic status, incomplete immunization schemes, malnutrition, late care seeking and inadequate treatment (Black *et al.,* 2003). Yet cheap and effective tools exist for pneumonia prevention and care.

Generally, the recommendations focus on improvement in vaccine coverage for measles, *Haemophilus influenzae* type B and pertussis, community education, improved nutrition, training of health providers in diagnostic and treatment algorithms, use of effective antibiotics, and timely referral of severely ill cases (Jones *et al.,* 2003). The strategies for prevention of RTIs in children include: 1) parent education (normal prevalence, familial predisposition, risk factors modification, and general hygiene, natural course of ARI without and with antibiotics); 2) specific immunization; 3) chemoprophylaxis; and 4) specific and nonspecific immunostimulation.

Vaccines ultimately represent the best opportunity to reduce the morbidity and mortality associated with paediatric RTIs. A nested study of American Indian children 1–7 years of age who had participated in a group-randomized efficacy trial reported that the children from communities randomized to receive PCV-7 (10.3%) had a lower prevalence of vaccine-serotype colonization 27 months after vaccination than did the children from control communities (17.1%) (Millar *et al.,* 2006). In a separate study, a seven-valent pneumococcal conjugate vaccine (PCV7) introduced in the United States in 2000 has been shown to reduce invasive pneumococcal disease (IPD) in both vaccinated children and adults through induction of herd immunity. A model developed to calculate the total burden of pneumococcal pneumonia prevented by infant PCV7 vaccination in the United States from 2000 to 2006 estimated a reduction of 788,838 (CI, 695,406 to 875,476) hospitalizations for pneumococcal pneumonia (Simonsen *et al.,* 2010). Bruce *et al.* (2011) found that influenza vaccines shots reduced all-cause mortality by 4.6% during 9 laboratory-defined flu seasons in Northern California. Various pneumococcal vaccines are under development. The data on antiviral chemoprophylaxis during influenza in children are preliminary and conflicting interms of their safety and efficacy (Schaad, 2005).

Currently, antibacterial chemoprophylaxis is only exceptionally appropriate. The benefit is modest and the risk of selective pressure of the antibacterial drug for emergence of resistant pathogens is real (Klein, 2000; Schaad, 2005). Use of immunostimulant containing the lyophilized extract of the following bacteria: *Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans*, and *Moraxella catarrhalis* have

been cited to prevent ARI and reduce the course for antibiotic treatment (Jara-Pkrez and Berber, 2000; Schaad, 2005).

2.10. Factors that may influence effective management and preventive measures

2.10.1. Parental predictors of care seeking behaviours for childhood respiratory infections

Proper management of childhood illness including ARI depends on the caretaker's decision to utilize health services from the medical facilities. The World Health Organization estimates that seeking prompt and appropriate care could reduce child deaths due to acute respiratory infections by 20% (WHO; 1997). The integrated management of childhood illness (IMCI) strategy, besides improving providers' skills in managing childhood illness also aims to improve families' care seeking behaviour. The health workers are trained to teach the mothers about danger signs and counsel them about the need to seek care promptly if these signs occur (WHO, 1997). In more than 40 of the 82 countries Worldwide with available data, fewer than 50% of the children with ARI are taken to a health care provider (http://www.unicef.org). Limited studies have been conducted to assess the care seeking behaviour among children caretaker in various developing countries. A study conducted in Ethiopia to assess the Mother's care seeking behaviour for childhood illness between rural and urban populations indicated that care was sought from health facilities only for less than half of sick rural children 48 (43.2%) as compared to urban 41(87.2%). Mothers' responses and actions were frequently influenced by their perception of severity or worsening of illness. Lack of money, distance, and perception of the illness not being serious were the major reasons for not seeking care. Residence and knowledge were identified as the major predictors of health care seeking practices from health facilities (Tsion *et al.,* 2008). A study to investigate the contribution of poor case management and care-seeking behaviour to childhood deaths from acute respiratory infections (ARI) and diarrhoeal diseases in rural Mexico reported that late care seeking and/or poor case management contributed to more than half (68%) of deaths. The estimated contribution of care seeking alone was 32%, of case management alone 17% and of both care seeking and case management 18% of deaths. Doctors implicated as having contributed to a child's death had significantly lower clinical competence scores than those who were not. Private doctors accounted for 1.4 times more consultations prior to death than public doctors, but were implicated in 1.8

times the number of deaths (Bojalil *et al.,* 2007). In a study conducted in Grenada, mothers expressed lack of seeking health care due to ARI mainly because they perceived the costs were high and distance to the facilities was long. Almost half mothers lacked confidence in the health care offered to their child, the poorest mothers (living on less than ≤2 USD/day) showed greater interest in health education than the wealthiest mothers. Factors that mostly influenced their choice of care seeking were the child having symptoms of respiratory disease and experiencing a child death in the past (Sakisaka and Hanada, 2010). In a cross-sectional survey conducted in the immunization clinics by Sreeramareddy *et al.* (2006) in Nepal observed that a large proportion of mothers failed to seek appropriate and prompt care for childhood illness and most often the health care was sought from pharmacies instead of from qualified medical practitioners unless they perceived the illness that the illness was as serious. Another study in Equado reported low care seeking behaviour, primary obstacles reported for timely health care-seeking among the study participants were lack of money for medicines, transportation fares, and restrictive hours of the health centres (Luque *et al.,* 2008). Factors related to care seeking behaviour needs to be assessed to understand the potential influence in care seeking and targeted for communities' health promotion and education in the country. Information on health seeking behaviour for childhood illness in Kenya, is scare, this study assessed the care seeking behaviour of the study participants that would influence their decisions to seek appropriate medical services as well as provide information on the appropriate package for danger signs recognition and action in ARI education within IMCI.

2.10.2. Parental knowledge and perception on transmission and preventive measures for acute respiratory infections

Effective outbreak and preventive measures to ARI infections and potential outbreaks requires support from the population at risk for measures undertaken to mitigate the diseases transmission and spread. Higher perceived effectiveness of measures undertaken and higher perceived threat of the disease can lead to higher rates of positive behavioral change, and better knowledge also increased the uptake of preventive measures. The knowledge and perception of the community could influence, both individual and community protective behaviour (Yap *et al.,* 2010).

A study carried in Nigeria to assess the mothers' knowledge and perception to ARI recorded low knowledge level. The study reported that most respondents reported exposure to cold and perceive coldness of the body as a causal agent for ARI, whereas none of them mention viral or bacterial agents. The reported dominating practice of mothers was either the use of irritants to get rid of the cause of the disease ('coldness') through vomiting, by forcing the child to swallow bitter remedies such as cow urine, or to use a remedy with warming and soothing properties such as a commercial product with methyl salicylate being the most popular ointment as drug of choice to "warm the chest, both from outside and inside", either applied topically or dissolved in hot water to drink (Iyun and Tomson, 1996). Hadi (2002) in a study in Bangladesh assessing mother's knowledge in transmission, clinical signs recognition and preventive measures indicated reported that the mothers reported presence of cough and common cold was the most frequently cited. Other signs such as fast breathing, chest in-drawing, noisy breathing, and fever were also known to many rural women. On the other hand, convulsion, inability to drink and shrunken eyes were rarely known to most women. The knowledge of preventive measures of ARI was low with personal hygiene as a preventive measure was cited by about one thirds of the sample women. Very few women recognised safe environment could prevent ARI as well as child immunisation, adequate diet, breast-feeding during infancy and giving vitamin A. A study conducted in Kenya in Baringo district indicated that mothers had good knowledge of mild forms of ARI but not the severe forms. Only 18% of mothers described pneumonia satisfactorily and 60.2% knew that measles is preventable by immunisation. Their attitude to ARI was appropriate but subsequent practices were not; majority of the mothers (87.1%) of the mothers said they would seek health centre services for severe ARI (Simiyu *et al.,* 2003). Luque *et al.* (2008) is a separate study in Equado, reported that there was an overall lack of recognition of the signs and symptoms of serious lower respiratory infections by mothers leading to limited utilisation of health services for management of childhood illnesses including ARI. Studies conducted to assess parental or care takers knowledge and perceptions regarding ARI are limited and no recent reports on knowledge and perceptions regarding ARI cause, signs, transmission and preventive measures in Kenya. Health education programmes can only be effective when designed to take into account the prevailing knowledge, attitudes and practices (KAP) of the community towards ARI

in their children. This study assessed the parental or care takers knowledge regarding ARI aetiology, transmission, treatment and preventive measures.

2.10.3. Economic burden of the respiratory illnesses

Acute respiratory tract infections are the most common illness experienced by all age groups and, globally, it is estimated they are responsible for approximately four million of the 14 million deaths every year in children under the age of five years (Suara *et al.,* 1995). These deaths occur disproportionately in children in developing countries. Rates of lower respiratory tract infection as high as 240 per 1,000 children per year have been found in population based studies. In 1999 lower respiratory tract infections were identified as the leading cause of global disability-adjusted life-years (DALYs), and will remain in the top ten causes of DALYs if approaches to address reduction of incidence are not put in place (Murray and Lopez, 1997). This prominent role for ARIs in mortality and morbidity persists in updated burden of disease calculations (Mathers and Loncar, 2006). Despite this, global expenditure on health research into acute respiratory diseases is low in relative terms compared to other diseases where the annual research investment per DALY is much higher (Michaud *et al.,* 2001). Reasons for this may be that such infections disproportionately affect children, and that the serious consequences of such infections are more common in developing country settings; both relatively disenfranchised in relation to the selection of priority areas for spending on global medical care and medical research (Lambert *et al.,* 2008)

Economic effects of ARI may influence the care seeking by parents. An estimate of the cost to healthcare providers and parents would aid budgetary decision-making, and provide an insight into the need for interventions to reduce the burden (Hollinghurst *et al.,* 2008). Acute respiratory infection is a common cause of morbidity and mortality in Kenya, and worldwide among children under 5 years, however, with declining global economic status and increased costs of living without significant improvement in economic status among communities in developing countries, there is needs for proper budgetary allocation for medical costs and hence this brings the need to evaluate the annual cost of management of ARI and estimate the episodes per child annually. Development of novel therapeutic possibilities and the possible use of non-pharmaceutical interventions to contain ARI transmission all underline the need to more

critically weigh the costs and benefits of prevention and treatment for common respiratory tract infections. Such non pharmaceutical interventions include; use of face masks, improved hand hygiene, cough etiquette, disinfection, social distancing measures, health promotion and travel restrictions (Cowling *et al.,* 2008; Larson *et al.,* 2010). As well as non-pharmaceutical options, there are vaccine candidates and therapeutics against respiratory pathogens that may be suitable for such widespread prevention either already available, undergoing clinical trial, or in pre-clinical development. Limited studies have been carried out in other countries to evaluate the cost of managing ARI. Hollinghurst *et al.* (2008) conducted a cost of illness study in preschool going children in United Kingdom (UK) for acute cough and found that the cost burden on the healthcare provider of acute cough in pre-school children is substantial. The average cost per episode was £27.43 (US$52) with costs related to consultations with general practitioners accounting for accounted for 93% of this cost (£22.91; US$43), cost per episode to caretakers and caregivers was £14.77 (US$28) and mean expenditure per child on over-the-counter preparations was £1.32 (US$2.5). Caretaker's experienced other personal cost through travel and expenditure and suffered significantly due to days off work while attending to sick children. Lambert *et al.* (2008) in a separate study in Australia, evaluating the costs of managing ARI due to viral pathogens found an average cost of AU$309 (US$293) to manage all ARI's with a range of AU$180 to AU$553 (US$171-525) per episode. To prepare for the possibility of large scale preventative programs against childhood acute respiratory infections, rates of ARI episodes recurrence and health economics studies are required. A review conducted by McPake *et al.* (2011) reports that costs of drugs alone for treating ARI without including consultations, travel and time are about USD0.05 in Malawi. Total health spending in Kenya stands at about US$6.2 per capita, far short of the World Health Organisation's (WHO) recommended level of US$34 per capita. More than 16% of the total expenditure on healthcare originates from donors (http://www.allianzworldwidecare.com/healthcare-in-kenya). The ARI episode costs reported show discrepant ranges and many have been done in developed countries, such evaluations have not been conducted in Kenya. This study estimated the cost per episode among the study subjects presenting with ARI.

2.11. Conceptual framework

Acute respiratory infection and symptoms is dependent variable and indoor air pollution and poor nutritional status are independent variables which include determinants such as; socio-demographic factors including education, family size, marital status, occupation and age of the parent; child physiological and immunological factors which may comprise of modifiable factors such as nutritional status, immunization and breastfeeding; and environmental factors like smoking, cooking fuel, crowding and house make that expose the child to un-favourable environmental conditions. This conceptual framework is based on Multiple Exposures Multiple Effects (MEME) model from WHO (Briggs, 2003; Figure 2.2).

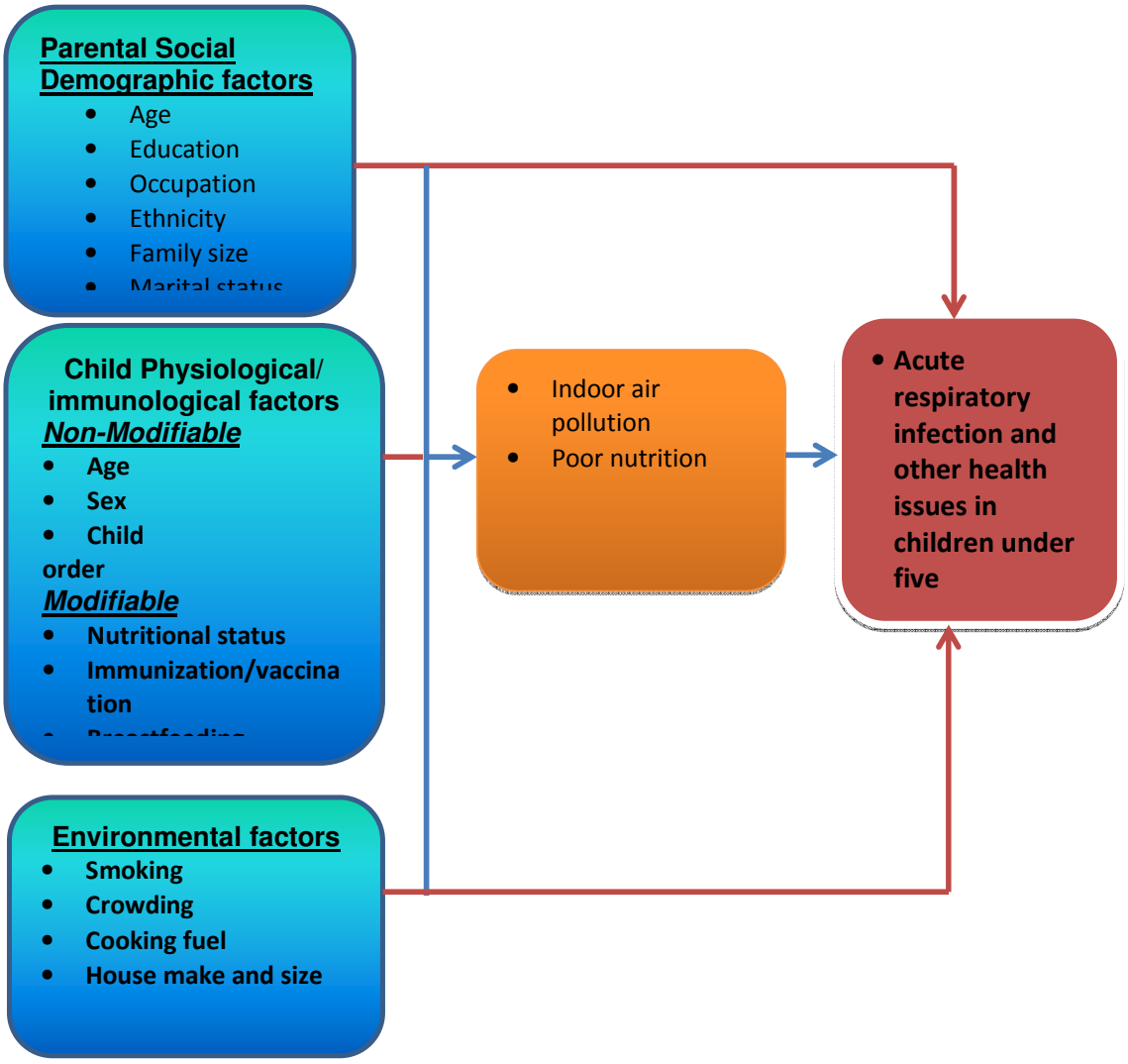

Figure 2.2: Conceptual framework of care seeking behavior for childhood illness

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study design and site

This was case control study conducted in Nakuru County in two selected facilities; a rural health facility (Mangu Health centre) and an urban health facility (FITC Health Centre) randomly selected from the list of health facilities in the County. The Nakuru district was selected based on heavy burden of infectious diseases using regular epidemiological data submitted by various districts to the Ministry of Health (MOH) through the Division of Disease Surveillance and Response (DDSR unpublished epidemiological reports). The study obtained a list of rural and urban health facilities from the district medical officer of health. The study obtained a list of rural and urban health facilities from Nakuru County from the Ministry of Health facilities master list available at the MoH website (http://www.ehealth.or.ke/facilities/). From the list rural and urban health facilities were entered separately into the Statistical Package for Social Scientist (SPSS) data base and a random selection of one facility was done from each category using the SPSS computer program (http://commfaculty.fullerton.edu/jreinard/bookweb/random.htm). The study selected a rural and an urban facilities to compare the risk factors and health seeking behavior among the two populations as some previous reports have indicated that critical inequities exist and children living in rural areas are more likely to suffer pneumonia and other infectious diseases than those in the urban areas (Igor *et al.,* 2008). The study recruited participants (parents or guardians) with children aged less than 5 years who visited the selected health facilities with suspected ARI for interview. Corresponding controls were identified for each case, matching for age and sex. The study was conducted between the months of April to November 2013.

3.2. Study subjects

The study enrolled parents or guardians of children less than 5 years diagnosed with ARI and their children. A case was defined as a child with airway complaints, who received a diagnosis of acute respiratory illness including influenza like illness (ILI) and who had not used antibiotics or antiviral medications in the previous 2 weeks. Control subjects were defined as children who had complaints other than respiratory complaints, who had no complaints of an ARI in the prior 2 weeks, who did not belong to the same household as the case patient, and who had not used antibiotics or antivirals in the previous 2 weeks.

Controls were recruited within the same facilities and matched for age and sex with each case recruited at each health facility. The study recruited the participants in both an urban and a rural health facility for comparison.

3.3. Eligibility criteria

3.3.1. Inclusion criteria

Care takers

i. Parents or guardians with children under 5 years of age who have suspected ARI at enrolment as well as those with children without ARI selected as controls
ii. Over 18 years of age
iii. Accept to give consent to participate and consent for their children to be sampled for ARI aetiology confirmation

Children

i. Children under 5 years of age with suspected ARI at enrolment (cases)
ii. Children under 5 years of age without signs of ARI matched with a case (controls)

3.3.2. Exclusion criteria

Care takers

i. Caretakers of both cases and controls who fail to give consent
ii. Caretakers of both cases and controls with children above 5 years of age

Children

i. Children with complaints of an ARI in the last two weeks were not selected as either cases or controls
ii. Children belonging to the same household a case as either cases or controls
iii. Children who had antibiotics or anti-viral medication in the last two weeks (controls and cases)

3.4. Sample size

Sample size formula for comparing two proportions by Casagrande *et al.* (1978) was applied to obtain the minimum sample size. This considered use of charcoal (biomass) for cooking as a key risk factor for ARI among the selected study populations. Since the level of usage of this cooking method is unknown, a proportion of 0.5 (50%) was used.

$$n = \frac{\{Z_{1-\alpha/2}\sqrt{[2P(1-P)]} + Z_{1-\beta}\sqrt{[P_1(1-P_1)+P_2(1-P_2)]}\}^2}{(P_1-P_2)^2}$$

Where;

α = Type I error (0.05)

β = Type II error (0.10)

At 95% confidence, $Z_{1-\alpha/2} = 1.96$

At 90% power, $Z_{1-\beta} = 1.28$

P_1= Assumed proportion of usage of charcoal (risk factor) as fuel for cooking among people with ARI (50%)

P_2= Estimated Proportion of people using charcoal without ARI (control group) (28%)

$$P = \frac{P_1 + P_2}{2}$$

The minimum sample size per group was calculated as 102. Allowing for 10% non-completeness, the sample size was adjusted upwards to 113. The study targeted to recruit a minimum of 113 parents/guardians with children with suspected ARI as cases and 113 controls matched for age and sex, making a total of 226. A total of 261 participants gave informed consent and were recruited to the study. However, 5 cases did not find matching controls and therefore their data was not included in the analysis, hence data was analysed for 256 participants i.e. 128 cases and 128 controls. Thirty percent of the enrolled children were selected to obtain a nasopharyngeal specimen for isolation and identification of aetiological agents.

3.5. Sampling procedure and participants recruitment

The Nakuru district was selected based on heavy burden of infectious diseases using regular epidemiological data submitted by various districts to the ministry of health through the division of disease surveillance and response. The study obtained a list of rural and urban health facilities from the district medical officer of health. The PI randomly selected one health facility from each category i.e rural and urban facility. From each facility the study recruited equal number of cases as controls i.e. children who met the case definition of ARI and the eligibility criteria stipulated earlier until the sample size was reached or surpassed. Cases and control subjects were recruited within the same

health facility within 1 week of each other and were matched by age and sex with the cases i.e. for each case recruited a corresponding control was recruited within a week. The study clinician at the health facility helped to identify a matching control for each case and recruited them to the study. The study principal investigator was assisted by research assistants, who explained the details of the study including the potential benefits and risks of participating in the study and seek their consent to participate (Appendix 1 and Appendix 2). They gave written consent in an entirely voluntary manner after all relevant information was provided in a language that they could understand. Participants who could not read or write were asked to make a thumb print on the informed consent form. A witness was present during the process of informed consent for any illiterate participants and the witness was also asked to sign the consent form. This witness was however not present during the interview process to ensure un-biased responses. The research assistants, who were clinicians (nurses and clinical officers) interviewed the parents or guardian after obtaining written informed consent and assigned each participant with 'participant identification numbers' (PIN). In addition to interviewing the caretakers, the study team conducted an assessment of malnutrition using the mid upper arm circumference (MUAC) method described later in section 3.6. No data identifying the participant were used on the forms; the study team used PIN numbers given to each participant.

The study randomly selected one third of the recruited cases and controls to collect specimen for laboratory testing of ARI aetiology. This selection employed simple random sampling. The PI developed stickers marked 'Yes' for one third of the facility sample size and the rest were written 'NO'. These stickers were folded and the parents/guardians of the recruited participants were requested to randomly pick one sticker (Figure 3.1). Those who select 'Yes' were requested to allow specimen to be collected from their children for laboratory. Detailed patient recruitment and selection procedure is attached as Appendix 3. To ensure consistency and professionalism, interviews were conducted by trained and qualified research assistants. This was to ensure quality control of the data collection procedure and recruitment of participants. The clinicians regularly examining patients in the selected facilities examined the patients and referred those with suspected ARI based on the case definition, for enrolment into the study.

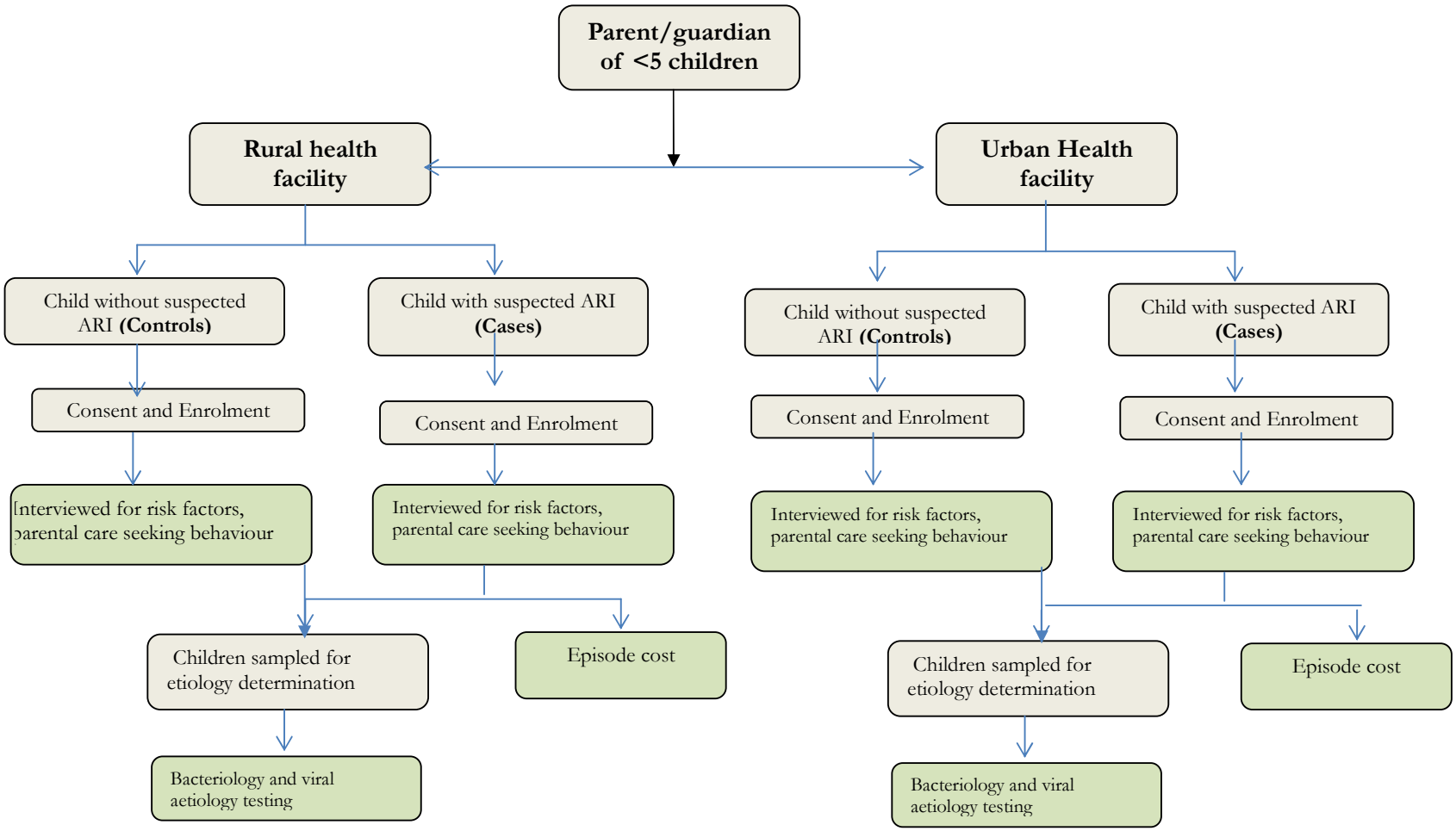

Figure 3.1: Participants enrollment flow chart

3.6. Questionnaire data collection

To assess the knowledge on preventive measures and risks associated with ARI and identify parental predictors of care seeking behaviours for childhood respiratory infections, data was collected using semi-structured questionnaires from the enrolled participants. The semi-structured questionnaire were used to collect information such as demographic information, employment status, potential risks of ARI and probability of ARI outbreaks, efficacy of preventive measures, knowledge on modes of transmission, perception on government preparedness to ARI outbreaks, control outbreaks and availability of medicines to treat ARI.

To identify relationship of socio-economic status and risk factors for ARI, data on household characteristics, including household construction, type of fuel used for cooking (gas/kerosene, electricity, wood, or a combination), whether they carried children while cooking, location of the cooking place, size of the houses, family size among others collected. The questionnaire also collected information on potential barriers to seeking health care for childhood ARI including distance to health facility, child delivery place, perceived severity of the disease and demographic determinants. Immunization status was indicted by the caretakers and verified from the child immunization card and record books at the health facilities. Detailed questionnaires used to collect information are attached as Appendix 4 and Appendix 5 (Swahili translation) for the cases while Appendix 6 and Appendix 7 (Swahili translation) were used for the controls respondents. Both cases and controls were assessed for clinical signs of visible severe wasting and used mid upper arm circumference (MUAC) to evaluate the nutritional status using a non-stretch cloth measuring MUAC tape. The mid-point between the elbow and the shoulder of the left arm was estimated and the MUAC tape placed around arm to measure mid-upper arm circumference

. The measurements were taken and recorded the nearest 0.1 cm or 1mm in the interview form. A record of MUAC below 11cm indicated that the child was severely malnourished, between 11-12.5cm the child was moderately malnourished, between 12.5-13.5cm the child was not malnourished but was at risk of malnourishment while measurement above 13.5cm indicated that the child was properly nourished. Detailed standard operating procedure (SOP) for measuring MUAC is attached as Appendix 8. Weight was also taken using an electronic scale and length using a measuring board of

standard design (for children less than 2 years old) or height using a wall-mounted scale (for those aged 2 years or older).

The study also estimated the costs related to management of ARI in the children. To estimate the financial/economic burden of the respiratory illnesses and risk factors episodes relationship evaluation, parents or guardians of children under five with ARI were requested to provide information on costs related to managing the current episode of ARI. The information sought included; costs for medical consultation, medicine, laboratory, transportation and also an estimation of days off work while taking care of the sick child due to ARI. The mean cost was calculated by taking the average of costs of all the cost items (Lambert *et al.,* (2008). The research assistants assisted in administration of questionnaires participants who could not read and write and those who were not confortable to write due to their held their babies or other reasons; while those who could read and write and were comfortable to independently complete the questionnaire were given to fill. The questionnaires were translated to Swahili for ease of understanding by participants who opted for self-administration of the questionnaire.

3.7. Ethical consideration and approval

The proposal was reviewed and approved by KEMRI Scientific Steering Committee (SSC) (Appendix 9) and KEMRI Ethical Review Committee (ERC) (Appendix 10) prior to commencement of the study (protocol number –SSC 2282). Participants were asked to give written informed consent for them to participate in the study and participation was voluntary. Failure to participate in the study did not undermine the standard care received from the health facilities. All data obtained from the study was handled in confidence by the principal investigator and the study questionnaires did not contain information linking the data to individuals. The questionnaires contained unique numbers and no names were put on the questionnaires. No social risk events and invasive procedures were carried out and no adverse events were experienced during the study. Participants were free to withdraw from the study at any time, and were guaranteed that their withdrawal would not interfere with the service they received from the health facility. Additionally, the participants were interviewed in a private room within the health facilities to ensure privacy and confidentiality. After consenting, the witnesses of persons who could read and write were requested to step aside during the interview to ensure un-biased responses.

3.8. Quality assurance of study results

To ensure data quality, questionnaires equivalent to 10% of the total sample size (25 questionnaires) were pre-tested at Kibera health centre that were purposively selected in Kibera slums in Nairobi where similar studies have been conducted previously. This ensured that the questions were well understood and corrected any ambiguous questions before using them in the main study. Data were collected by qualified and trained research assistants to ensure uniformity in the administration of the questionnaires. Collected data were double entered into an electronic database by two different clerks to ensure proper transcription of the study questionnaires details.

3.9. Specimen collection and transportation

Specimen collection for ARI etiological agents testing was done by trained clinicians to ensure the participants safety. Throat swabs were collected from tonsillar areas using sterile cotton swabs on wooden applicator sticks for bacterial isolation. The throat swabs were placed immediately in Amies transport media and sent to the KEMRI Centre for Microbiology Centre laboratory immediately at room temperature for bacterial culture. For viral agents, the nasopharyngeal swabs were collected by inserting the swab into one nostril straight back along the floor of the nasal passage for several centimetres until reaching the nasopharynx. The swabs were rotated gently for 5-10 seconds to loosen the epithelial cells and collect the sample. After swabbing, the swab applicator was cut off, and each absorbent swab was placed into a vial containing 1 mililitre of viral transport medium (VTM) and transported at 4–10^{o}C to KEMRI Center for Virus Research in cool boxes for further processing. The nasopharyngeal samples were stored at -80°C if not processed immediately until further analysis (Kim *et al.,* 2011). These samples were subjected to respiratory virus detection using multiplex PCR. Detailed standard operating procedures for specimen collection and transportation are attached as Appendix 11.

3.10. Laboratory analysis and identification of aetiological agents

3.10.1. Bacteria identification

3.10.1.1. Inoculation and incubation

The method of Wormser and Hanna (2004) was used in processing and identification of bacterial agents. Specimen collected were primarily inoculated on; (i) blood agar base

supplemented with 5% sheep blood and incubated in CO_2 at set temperature of 35-37^{o}C to isolate the bacteria such as *Streptococcus pneumonia*, *Staphylococcus aureus,* beta haemolytic streptococcus and other gram positive bacilli; (ii) Gonococci (GC) agar base with 2% haemoglobin and 1% isovitalex and incubated in CO_2 at 35-37^{o}C to allow isolation *Haemophilus influenza*; (iii) MacConkey with 0.01% crystal violet incubated at 35-37^{o}C in ambient air incubator for isolation of gram-negative bacteria such as *Klebsiella* species that may be associated with ARI. Detailed procedures for preparation of the culture media used in the study are attached as Appendix 12.

Swabs from the transport media for each child were rolled to make an inoculum covering between a quarter and a third of each of the plates to maximize transfer of organisms. The inoculation was done beginning with non-selective media (GC agar and blood agar) and to selective media (MacConkey). These were then spread to the whole plate using sterile loops to obtain separated colonies. The plates were incubated under the conditions indicated above for 24-48 hours (upto 36 hours for the GC agar plate to allow fastidious bacteria to grow). The swab was then used to make a smear for Gram staining and examination.

3.10.1.2. Identification of bacteria

After the incubation, the organisms were all presumptively identified based on morphological appearance of the colonies and the hemolysis (on blood agar). Additionally, Gram staining and other biochemical tests were conducted to identify the bacteria. Morphological appearance of each colony type in culture was described, transferred to fresh agar media (blood agar) to purify the bacteria isolates and Gram smear was made as an initial step for identification. Pure cultures were incubated for 24-48 hours at 35-37^{o}C in CO_2.

Various biochemical tests were conducted to identify the bacteria; for organisms appearing as gram positive cocci, catalase was conducted differentiate between *Staphylococcus* spp (catalase positive) and *Streptococcus* spp (catalase negative). *Staphylococcus* spp were differentiated on basis of coagulase test; *Staphylococcus aureus* (coagulase positive) and other coagulase negative *Staphylococcus* spp. A battery of tests were conducted to characterize *Streptococcus* spp; (i) for those that were β-hemolytic on

blood agar, bacitracin sensitivity test was conducted to distinguish Group A Streptococcus (Bacitracin sensitive) from Streptococcus Group C (bacitracin resistant); (ii) Bile esculin test was used to classify the α-hemolytic *Streptococcus* spp; *Streptococcus faecalis* (positive) and *Streptococcus pneumoniae* and the viridans group (both negative); (iii) Optochin disc sensitivity was used to differentiate between *Streptococcus* pneumonia *(sensitive)* and the viridans group (resistant). Detailed standard operating procedures for the identification tests indicated above and identification flow charts used in the study are attached as Appendix 13 (Ruoff *et al.,* 1999). All the test results and final identification of the bacteria were recorded in the laboratory worksheet (Appendix 14) before transferring the information to the study data base.

3.10.2. Detection of viruses from the clinical specimen

3.10.3. Nucleic acid extraction

Viral Ribonucleic acid (RNA) from the specimen was extracted by a commercial kit (Qiagen, 2010) according to the manufacturer's protocol (QIAamp MinElute Virus Spin Kit, Qiagen, Valencia, CA). The QIAamp MinElute Virus Vacuum Kit uses well-established technology for simultaneous purification of viral DNA and RNA. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 150 µl. The procedure comprises 4 steps (lyse, bind, wash, elute) and designed to ensure that there is no sample-to-sample cross-contamination and allows safe handling of potentially infectious samples (Qiagen, 2010). Briefly 140µl of the sample (from the viral transport medium) was added to 560µl of viral lysis buffer, incubated at room temperature (15-25^{0}C) for 10 minutes, then 560µl of molecular grade 100% ethanol was added and mixed by vortexing for 15 seconds. This was then centrifuged briefly to remove drops from inside the Eppendorf tube lid. From the lysed viral material, 630µl of RNA was then placed on to a spin column, spun at 6000 revolutions per minutes (rpm), twice binding the RNA to the spin column. The RNA was washed twice, first with 500µl of wash buffer 1 (AW1) at 8000rpm for 1 minute, then with 500µl of wash buffer 2 (AW2) at 20,000rpm for 3 minutes. The RNA was eluted from the spin column by adding 60µl elution buffer (AVE) and spinning at 6000 rpm for 1 minute in a 1.5 ml Eppendorf tube. The eluted RNA was then stored at −80^{0}C until the sample numbers were adequate to run PCR. Detailed step by step procedure for RNA extraction to detect respiratory viruses is attached as Appendix 15 (Qiagen, 2010).

3.10.4. Reverse Transcriptase Real-time polymerase chain reaction

3.10.4.1. Method principle

In order to detect and subtype influenza viruses from the samples, a real time RT-PCR protocol designed by World Health Organization (WHO) and the Centers for Disease Control (CDC) was used (CDC, 2009). This protocol was used to detect influenza A viruses and sub-type seasonal influenza A (H3N2) and pandemic influenza (H1N1) as well as detect influenza B from the samples. Real-time of quantitative PCR is a method of nucleic acid (DNA, RNA) quantification where the polymerase chain reaction (PCR) is monitored in real time (as the testing procedure is still continuing). In contrast to the conventional PCR in that it is quantitative i.e. it enables the determination of the exact concentration (relative or absolute) of the amplified DNA in the sample. Conversely, in conventional PCR the results of amplification are only seen after the PCR is completed. In case the template is RNA, reverse transcription is done to convert the RNA into DNA (complementary DNA or cDNA) before it is amplified with real-time PCR.

In real-time PCR, first the amplified DNA is fluorescently labelled and second, the amount of the fluorescence released during amplification is directly proportional to the amount of amplified DNA. Fluorescence is monitored during the whole PCR process (along all the 40 cycles). The higher the initial number of DNA molecules in the sample, the faster the fluorescence will increase during the PCR cycles. If a sample contains more targets the fluorescence will be detected in earlier cycles.

3.10.4.2. PCR amplification and results interpretation

In this study, real-time RT-PCR amplification and detection was performed on an ABI 7500 (Applied Biosystems) using the AgPath-ID one-step RT-PCR kit (Applied Biosystems, Foster City, CA, USA). Briefly, 25µl reaction volume for each sample contained 5 µl of extracted RNA, 6 µl of nuclease free water, 0.25µl of forward primer, 0.25µl of reverse primer, 0.25µl of the probe, 0.75µl of AgPath enzyme mix and 12.5µl of AgPath kit 2X buffer. The cycling conditions for the real time RT-PCR protocol were: an initial cycle at 40^0C for 30 minutes for the reverse transcription of the RNA to develop the complimentary DNA (cDNA). This was followed by incubation at 94^0C for 10 minutes to inactivate the reverse transcriptase and activate the Taq polymerase. This was

then followed by 40 cycles involving denaturation at 94^0C for 15 seconds, and an annealing and strand extension stage at 55^0C for 1 minute.

Cycle threshold ≤35 was interpreted as positive i.e. samples that produced amplification curves that crossed the threshold line at 35 cycles or earlier. The real time PCR was initially conducted to detect influenza A and influenza B in a singleplex PCR. All the samples that were positive with influenza A were tested for influenza A subtypes using specific primers for seasonal influenza (H3N2) and pandemic influenza (H1N1) applying the same preparation and amplification procedure as described above. Primers used for real-time PCR and a detailed procedure for preparation, specimen set up, amplification and interpretation of the real time RT-PCR results is attached as appendix 16 (CDC, 2009).

3.10.5. One-step Reverse Transcriptase polymerase chain reaction

3.10.5.1. Method principle

The multiplex reverse transcriptase PCR (RT-PCR) was done to identify non-influenza viruses; first PCR reaction for simultaneous detection of hMPV and RSV and second PCR (parainfluenza 1, 2 and 3) using the same PCR conditions. Multiplex PCR technique enables amplification of two or more products in parallel in a single reaction tube.

3.10.5.2. PCR amplification and results interpretation

The procedure of Bellau-Pujol *et al.* (2005) was applied which consisted of a single-step combining reverse transcription and PCR amplification, performed using the one-step RT-PCR kit from QIAGEN (Hilden, Germany) carried out on AB 9700 PCR thermocycler (GeneAmp® Applied Biosystem). The reaction mixture contained 13µl nuclease free water, 1µl of enzyme mix, 5µl of 5X RT-PCR buffer, 1µl of 0.4mM Deoxynucleotide Triphosphates (dNTPs), 1.25µl of each of the primers (forward and reverse primers in the multiplex PCR for RSV/hMPV) in one reaction, and a second reaction for parainfluenza viruses (1-3). A 2.5µl aliquot of the extracted RNA sample was added to give a final volume of 25µl (Bellau-Pujol *et al.,* 2005). Table 3.1 shows the primer sequences used for the viral multiplex PCR the different respiratory viruses. The cycling conditions for the multiplex RT-PCRs were: an initial cycle at 50^0C for 30 minutes for the reverse transcription followed by incubation at 94^0C for 15 minutes to

inactivate the reverse transcriptase and activate the Taq polymerase. This was followed by 40 cycles involving denaturation at 94^0C for 30 seconds, annealing at 55^0C for 30 seconds and strand extension at 72^0C for 1 minute. Finally, a final extension at 72^0C for 10 minutes was done to completely fill in the amplification products. An internal control was included to check the extraction step and the presence of inhibitors of the RT-PCR assay. This control consisted of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, which is normally transcribed in nasal mucosis cells. This gene was amplified with specific primers.

3.10.5.3. Agarose gel electrophoresis of the amplification products and interpretation of the results

The PCR products were separated using gel electrophoresis on 2% agarose gels and stained with ethidium bromide using an HP AlphaImager® (Alpha Immutech, South Africa) and visualized under UV light. Agarose (2%) was prepared in 1x TBE buffer and the solution was mixed by swirling gently and then heating in a microwave until all the agarose was completely dissolved. The gel was then left to cool for a few minutes and ethidium bromide was added to a final concentration of 0.5µg/ml. The gel was then poured onto an electrophoretic tank containing combs and left to set for 30 minutes. The combs were then carefully removed. Three microliters of the PCR products were mixed with the 2µl of the loading dye (blue orange dye) and then loaded onto the wells. A DNA marker was loaded on the first lane of each of the wells, negative control was loaded to the second well while third well was loaded with positive control containing (hMPV and RSV) for the first multiplex PCR and parainfluenza 1, 2, and 3 for the second multiplex PCR. The tank was then connected onto an electric current and run at 150 volts for about 30-45 minutes. The gel was then visualized and the gel photo printed using the AlphaImager gel documentation (Alpha Innotech, California, USA). Detailed procedure for preparation, running and interpretation of real time RT-PCR results is attached as appendix 17.

Table 3.1: Primer sequences and target genes used multiplex polymerase chain reaction in the detection of viral agents of acute respiratory infections

Virus	Primers	Sequence (5' – 3')	Gene	Amplicon size (bp)	Melting temp (°C)
hRSV	vrs 1	GGA ACA AGT TGT TGA GGT TTA TGA ATA TGC	Nucleocapsid	279	60
	vrs P2	TTC TGC TGT CAA GTC TAG TAC ACT GTA GT ACT TG			55
hMPV	hmpv 1	CCC TTT GTT TCA GGC CAA	Matrix protein	416	54
	hmpv 2	GCA GCT TCA ACA GTA GCT G			58
Parainfluenza virus 1	PIS1+	CCG GTA ATT TCT CAT ACC TAT G	Hemagglutinin-	317	48
	PIS1−	CCT TGG AGC GGA GTT GTT AAG	Neuraminidase		51
Parainfluenza virus 2	PIP2+	AAC AAT CTG CTG CAGCAT TT	Hemagglutinin-	507	56
	PIP2−	ATG TCA GAC AAT GGG CAA AT	Neuraminidase		56
Parainfluenza virus 3	Para3.1	CTC GAG GTT GTC AGG ATA TAG	Hemagglutinin-	189	46
	Para3.2	CTT TGG GAG TTG AAC ACA GTT	Neuraminidase		48
Internal control	GAPDH1	TCA TCC ATG ACA ACT TTG GTA TCG TG	GAPDH	564	59
	GAPDH2	CTC TTC CTC TTG TGC TCT TG			60

GAPDH-Glyceraldehyde-3-phosphate dehydrogenase gene; hRSV- respiratory syncytial virus; virus; hMPV - human metapneumovirus; bp-base pairs; vrs 1- hRSV forward primer; vrs 2- hRSV reverse primer; hmpv 1 – hMPV forward primer; hmpv 2- hMPV reverse primer; PIS1+ - parainfluenza 1 forward primer; PIS1- - parainfluenza 1 reverse primer; PIP2+ - parainfluenza 2 forward primer; PIP2- - parainfluenza 2 reverse primer; Para3.1 - parainfluenza 3 forward primer; Para3.2 - parainfluenza 2 reverse primer (Bellau-Pujol *et al.*, 2005).

3.11. Data management and analysis

Data from the questionnaires was entered into Microsoft Excel database and cleaned before analysis. The data was analysed using Statistical Package for Social Scientists (SPSS/PC^{TM}) software. Demographic data was analysed by calculating frequencies, proportions for categorical variables, and means, standard deviations and medians accordingly for continuous variables. Statistical tests of comparison using chi square test or Fisher's Exact Test was used for categorical variables while Student's *t* Test was used for continuous variables. The data on aetiology was analysed using descriptive statistics and results expressed proportions. To evaluate the potential risk factors for childhood ARI bivariate analysis was performed to describe the study population and identify risk factors. Logistic regression analysis was used to determine the independent risk factors associated with ARI for factors that were significant at bivariate analysis. The predictors of care seeking behaviour were estimated by the calculation of odds ratios (OR) and 95%

confidence intervals (CIs). To estimate the financial burden of ARI, descriptive statistics was used estimate the mean cost per ARI episode per child. Data was presented in tables and figures and p-value of less than 0.05 was considered significant.

3.12. Dissemination of research findings

The study findings will be disseminated through seminars with the communities under the study and presentations in national and international conferences. The investigator has also published manuscripts in peer reviewed journals for wider information and knowledge sharing. The titles of the manuscripts are; Matu M, Kikuvi G, Wanzala P, Karama M, Symekher S (2014). Risk factors for acute respiratory infections in children under five years in Nakuru, Kenya. *African Journal of Health Sciences* **27(4)**:376-387; and Matu M, Kikuvi G, Wanzala P, Karama M, Symekher S (2014). Aetiology of Acute Respiratory Infections in Children under Five Years in Nakuru, Kenya. *Journal of Microbiology and Experimentation* 1(4): 00021.

3.13. Study limitations

The study employed case control study which is known to be prone recall and selection bias. Participants were questioned mainly about the symptoms, ARI management expenses and events that have happened in not more than two weeks ago related to the child illness to minimize recall bias. In addition, matching controls were selected within a week of recruitment of a case to minimize variations in the findings due to change of environment as a result of time lapse.

CHAPTER FOUR

4.0. RESULTS

4.1. Participants characteristics

4.1.1. Distribution of study participants by age, body weight and marital status

Parents or guardians who provided informed consent to participate in the study were aged between 18 to 46 years with a median age of 27 years (Mean±SD; 27.6±5.6). The children were aged between 4 and 60 months with median age of 24 months (Mean±SD; 24.8±15.8). The children had a median birth weight of 3 kgs (Mean±SD; 3±0.6). Most (59%) of the children enrolled in the study were males. No significant difference was found between cases and control's age and mean birth weights (p=0.183). Distribution of the study participants by age and body weight is shown in Table 4.1.

Table 4.1: Distribution of participants by age and weight

	Total			Cases			Controls			
Characteristics	**Mean±SD**	**Median**	**Range**	**Mean±SD**	**Median**	**Range**	**Mean±SD**	**Median**	**Range**	**p**
Child Age (Months)	24.8±15.8	24	4 - 60	24.5±16.1	23.5	4 - 60	25±15.4	24	4 - 60	0.8
Child birth weight (kgs)	3±0.6	3	2 - 7.8	3.1±0.6	3	2 - 7.8	3±0.6	3	2 - 7.5	0.183
Parent Age	27.6±5.6	27	18 - 46	27.6±5.9	27	18 - 46	27.6±5.4	27	18 - 45	0.99

SD = Standard deviation; p = Level of significance using student's t-test, p=0.05 was considered significant

4.1.2. Relationship between parental demographic factors and acute respiratory infections

Majority of the respondents were young parents aged between 20 and 35 years with 45 (35.2%) caretakers of the cases having an age of between 26 and thirty years while those of the controls were between 20 and 43 years (43; 33.6%). As shown in Table 4.2, the odds of ARI were higher in caretakers in the age category of 36-40 years was (Odds Ratio, OR=1.7, 95% Confidence Interval, CI 0.4-7.29). Odds of ARI decreased odds non-significantly among caretakers who were over 40 years (OR=0.32, 95% CI: 0.02-3.56). Ninety four 94 (74.6%) and 99 (77.3%) caretakers of the cases and controls, respectively, were married or in a cohabiting relationship. About 20% were single parents, 30 (23.8%) cases and 12 (18%) controls. As shown in Table 2, few respondents, 4 (3.1%) were either

widowed or divorced/separated. The differences in the marital status between the groups was not significant (p=0.169).

Most of the respondents were literate with more than 60% having at least secondary school education. Forty one (32.5%), 42 (33.3%) and 39 (31%) among the cases and 38 (29.9%), 39 (30.7%) and 41 (32.3%) among the controls had primary, secondary and college education respectively (Table 4.2). There was a trend of reduced odds of ARI with increased parental education; caretakers with at least primary education (OR= 0.37, 95% CI: 0.068- 2.03), secondary school education (OR=0.37, 95% CI: 0.068- 2.03), college level (OR=0.42, 95% CI: 0.08-2.3), and university level (OR=0.8, 95% CI: 0.08-8.47). There was, however, no significant difference in level of education between the study groups (p=0.695). Majority of the respondents were Christians, 124 (99.2%) and 123 (96.9%) cases and control caretakers, respectively (Table 4.2). Religion was not found to be a significant risk factor for ARI (p=0.37).

Eighty two (65.6%) cases and 81 (64.3%) control caretakers indicated that they had a monthly income of less than sixty dollars (five thousand Kenya shillings). Although only less than 5% of the participants earned more than US$176 (kshs 15, 000) a month, the higher income had reduced odds (OR=0.6, 95% CI: 0.14-2.63; Table 4.2). As shown in table 4.2 over 60% of the homes had more than 3 persons living in the house. Children living in homes with more than eight occupants had slightly increased odds of ARI (OR=1.38, 95% CI: 0.23-7.99) although this was not statistically significant.

Table 4.2: Association of socio-demographic and economic characteristics of the study respondents with acute respiratory infections

	Participant type			
Characteristics	**Cases**	**Controls**	**OR (95%CI)**	**p**
Parent age (years)	n(%)	n(%)		
<20	9 (7)	7 (5.5)	1	
20-25	38 (29.7)	43 (33.6)	1.45 (0.49-4.28)	
26-30	45 (35.2)	40 (31.3)	1.14 (0.39-3.35)	0.684
31-35	26 (20.3)	29 (22.7)	1.43 (0.47-4.4)	
36-40	6 (4.7)	8 (6.3)	1.7 (0.4-7.29)	
>40	4 (3.1)	1 (0.8)	0.32 (0.02- 3.56)	
Marital status*				
Single	30 (23.8)	23 (18)	1	
Married/cohabited	94 (74.6)	99 (77.3)	1.37 (0.74-2.53)	0.169
Divorced/separated	0	4 (3.1)	-	
Widowed	2 (1.6)	2 (1.6)	1.3 (0.17-9.97)	
Level of education*				
No formal education	2 (1.6)	5 (3.9)	1	
Primary school education	41 (32.5)	38 (29.9)	0.37 (0.068- 2.03)	
Secondary school education	42 (33.3)	39 (30.7)	0.37 (0.068- 2.03)	0.695
College level	39 (31)	41 (32.3)	0.42 (0.08-2.3)	
University Level	2 (1.6)	4 (3.1)	0.8 (0.08-8.47)	
Parent religion*				
Christian	124 (99.2)	123 (96.9)	1	
Muslim	1 (0.8)	3 (2.4)	3.02 (0.31- 29.48)	0.37
Hindu	0	1 (0.8)	-	
Family income (kshs)*				
<5000	82 (65.6)	81 (64.3)	1	
5000-10000	34 (27.2)	34 (27)	1.0 (0.57-1.78)	0.607
10001-15000	4 (3.2)	8 (6.3)	2.0 (0.59-6.99)	
<15000	5 (4)	3 (2.4)	0.6 (0.14-2.63)	
Number of people living in a house				
1-3	31 (24.2)	45 (35.1)	1	
3-5	61 (47.7)	55 (43)	0.62 (0.35-1.11)	0.182
5-8	34 (26.6)	24 (18.8)	0.48 (0.24-0.97)	
More than 8	2 (2)	4 (3.1)	1.38 (0.23-7.99)	

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, $p<0.05$ was considered significant; Kshs – Kenya shillings; *a few of the participants declined to respond to some of the questions such as disclose their marital status, income, religion and level of education.

4.1.3. Association of nutritional, vaccination status and other child characteristics with acute respiratory infections

Eighty one (31.6%) of the enrolled children were less than 1 year, followed by children aged 2-3 years. The study did not find age to be a significant factor for increased risk of ARI (p=0.957). However, slightly increased odds were found in children between 2-4

years while age of over 4 years had reduced odds (OR=0.91, 95% CI: 0.37-2.27). As shown in Table 4.3, more children enrolled in the study were males, 151 (59%) with about equivalent number of cases, 76 (59.4) as controls, 75 (58.6%) among the male children and a similar distribution among females with 52 (40.6%) cases and 53 (41.1%) female controls (Table 4.3). Sex was not associated with altered odds of ARI among the study subjects (OR=1.03, 95% CI: 0.61-1.77; p=0.957).

Overall, malnutrition (severe and moderate malnutrition; MUAC<12.5mm) was found in 68 (27.8%) of the children. Many cases than controls were malnourished, with 45 (35.2%) being malnourished compared to 23 (18.3%) among the control group (Table 4.3). Malnutrition was significantly associated with increased risk of ARI (crude OR=2.4, 95% CI: 1.36-4.33; p=0.003), this association remained significant even after adjusting for relevant factors (Adjusted OR=2.8, 95% CI: 1.25-6.15; p=0.01)

Nine percent of the children had not completed childhood immunization under the Kenya Expanded Programme on Immunization (KEPI) compared to 143 (56%) who had completed and 88 (35%) who were still continuing with the program. There was reduced odds of ARI among the children who had completed immunization (OR=0.88, 95% CI: 0.37-2.09). However, the difference did not reach statistical significance (p=0.428). The study observed that more cases than controls had received vaccination against seasonal influenza in the past one year, 40 (32.8%) and 28 (25.2%), respectively (Table 4.3). There was no significant difference between the acquisition of ARI and vaccination against seasonal influenza (p=0.205).

Few respondents, 5 (10%) indicated that their children had not breastfed at all, with 2 (11.6%) and 3 (2.4%) caretakers of cases and controls, respectively. Fifty (40%) cases and 57 (45%) control children were breastfed for more than 6 months. Breastfeeding associated with slightly reduced odds in children breastfed for 4-6 months (OR=0.61, 95% CI: 0.09- 4.0), more than 6 months (OR=0.76, 95% CI: 0.12-4.73) or currently breastfeeding (OR=0.59, 95% CI: 0.095-3.7). The differences in the length of breastfeeding among the cases and controls was however not statistically significant (p=0.817). The higest proportion of children had between 1 and 3 siblings, among them were 35 (30.4%) cases and 38 (33.9%) control children. There were non-significantly

increased odds of ARI among children with more than 5 siblings living in the same house (OR=1.6, 95% CI: 0.25-9.81). This study found no significant association of birth order with risk of ARI, although it was observed that being last born was associated with reduced odds of ARI (OR=0.47, 95% CI: 0.17-1.27; Table 4.3).

Table 4.3: Association of nutritional, vaccination status and other child characteristics with acute respiratory infections

	Participant type			
Characteristics	**Cases**	**Controls**	**OR (95%CI)**	**p**
Child age (years)				
0 - <1	42 (32.8)	39 (30.5)	1	
>1 - 2	25 (19.5)	24 (18.8)	1.03 (0.51-2.1)	
>2 - 3	29 (22.7)	33 (25.8)	1.23 (0.63- 2.38)	0.957
>3 - 4	19 (14.8)	21 (16.4)	1.19 (0.56- 2.54)	
>4 - 5	13 (10.2)	11 (8.5)	0.91(0.37- 2.27)	
Sex of the child				
Male	76 (59.4)	75 (58.6)	1	0.899
Female	52 (40.6)	53 (41.4)	1.03 (0.61-1.77)	
Nutritional status				
Malnourished (≤12.5cm)	45 (35.2)	23 (18.3)	1	0.003
Well nourished(>12.5cm)	83 (64.8)	103 (81.7)	2.43(1.36-4.33)	
Completion of childhood immunization				
No	12 (9.4)	12 (9.4)	1	
Yes	76 (59.8)	67 (52.3)	0.88 (0.37-2.09)	0.428
Continuing	39 (30.8)	49 (38.3)	1.26 (0.51-3.1)	
Vaccination for seasonal influenza				
No	82 (67.2)	83 (74.8)	1	0.205
Yes	40 (32.8)	28 (25.2)	0.69 (0.39-1.22)	
Period of Breastfeeding				
Not breastfed	2 (1.6)	3 (2.4)	1	
Less than 4 months	2 (1.6)	3 (2.4)	1(0.08-12.56)	
4-6 months	22 (17.8)	20 (15.9)	0.61 (0.09- 4.0)	0.817
6 months and above	50 (40.3)	57 (45.2)	0.76 (0.12-4.73)	
Continuing	48 (38.7)	43 (34.1)	0.59 (0.095- 3.7)	
Number of siblings				
0	59 (51.3)	56 (50)	1	
1-3	35 (30.4)	38 (33.9)	1.14(0.64-2.06)	0.842
4-5	19 (16.6)	15 (13.4)	0.83 (0.385- 1.79)	
>More than 5	2 (1.7)	3 (2.7)	1.6 (0.25- 9.81)	
Child birth order				
First born	44 (34.9)	47 (37)	1	
Second born	45(35.7)	45 (35.5)	0.94 (0.523-1.68)	
Between second and last born	23 (18.3)	28 (22)	1.14 (0.57-2.27)	0.404
Last born	14 (11.1)	7 (5.5)	0.47 (0.17-1.27)	

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, $p<0.05$ was considered significant; *p = 0.01 (logistic regression analysis); a few of the participants did not respond to some of the questions such as disclose their child birth order, number of siblings, period of breastfeeding, vaccination against seasonal influenza, while three participants decline to allow their children MUAC taken.

4.1.4. Clinical symptoms observed among the acute respiratory infections cases

Comparing the clinical characteristics among cases in the rural and urban participants, fever and cough were the most frequently reported symptoms in 74.2% and 71.9%, respectively. Significantly more cases from the urban facility had fever, 60 (100%) compared with 35 (51.5%) from the rural facility (p=0.0001). Other characteristics that were common were wheezing 24 (18.7%) and high pulse rate 19 (14.8%). Dysponea was reported in 13 (19.1%) cases from the rural site and none from the urban health facility (Table 4.4).

Table 4.4: Distribution of clinical features of acute respiratory infections among the cases in rural and urban settings

Observed clinical features		n (%)	Facility location		p	OR (95%CI)
			Urban n (%)	Rural (n%)		
Fever	No	33 (25.8)	33 (48.5)	0		1
	Yes	95 (74.2)	35 (51.5)	60 (100)	.0001	2.714 (2.086-3.532)
High respiratory pulse rate	No	109 (85.2)	61 (89.7)	48 (80)		1
	Yes	19 (14.8)	7 (10.3)	12 (20)	.123	2.179 (.0797-5.957)
Wheezing	No	104 (81.3)	59 (86.8)	45 (75)		1
	Yes	24 (18.7)	9 (13.2)	15 (25)	.089	2.185 (0.877-5.445)
Tachypnoea	No	122 (95.3)	64 (94.1)	58 (96.7)		1
	Yes	6 (4.7)	4 (5.9)	2 (3.3)	.496	0.552 (0.097-3.125)
Rhinorrhoea	No	126 (98.4)	67 (98.5)	59 (98.3)		1
	Yes	2 (1.6)	1 (1.5)	1 (1.7)	.929	1.136 (0.069-18.559)
Cough	No	36 (28.1)	18 (26.5)	18 (30)		1
	Yes	92 (71.9)	50 (73.5)	42 (70)	.658	0.84 (0.388-1.817)
Sore throat	No	123 (96.1)	64 (94.1)	59 (98.3)		1
	Yes	5 (3.9)	4 (5.9)	1 (1.7)	.219	0.271 (0.029-2.496)
Earache	No	125 (97.7)	67 (98.5)	58 (96.7)		1
	Yes	3 (2.3)	1 (1.5)	2 (3.3)	.487	2.310 (0.204-26.14)
Stridor	No	125 (97.7)	67 (98.5)	58 (96.7)		1
	Yes	3 (2.3)	1 (1.5)	2 (3.3)	.487	2.310 (0.204-26.14)
Dysponea	No	115 (89.8)	55 (80.9)	60 (100)		1
	Yes	13 (10.2)	13 (19.1)	0	.0001	0.478 (0.395-0.579)

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using Fisher's exact test, $p<0.05$ was considered significant.

4.2. Aetiology of acute respiratory infections

Thirty three percent of the children recruited in the study were sampled to collect throat and nasopharyngeal specimen for bacterial isolation and detection of viral agents respectively. Four specimen were unsuitable upon arrival due to transportation conditions and therefore 78 specimen were tested for isolation of bacteria and detection of viruses

using PCR. Bacteria were isolated from 19 of 78 (24.4%) throat swabs while at least one type of virus was detected in 35 out of 78 (44.9%) nasopharyngeal specimen (Figure 4.1).

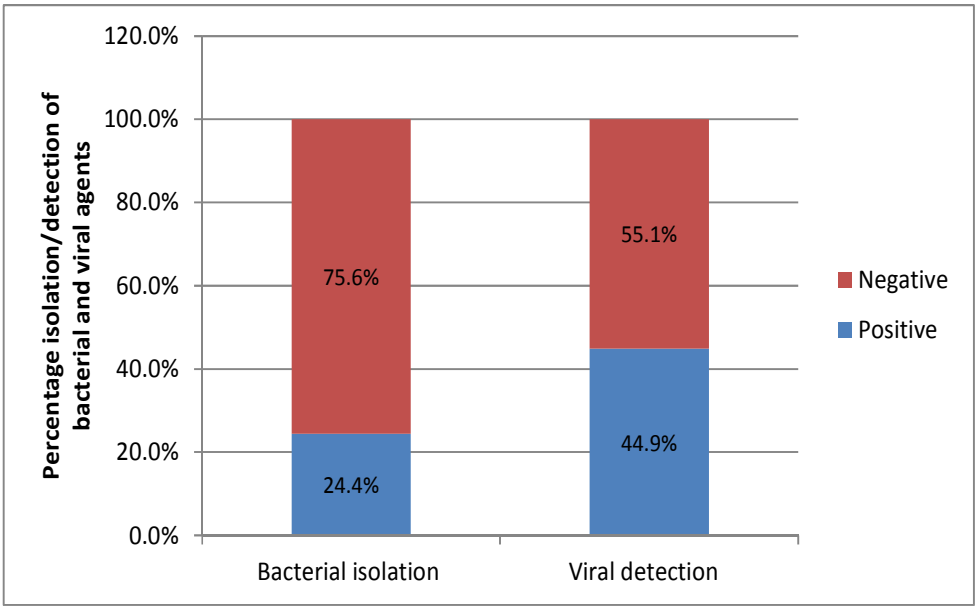

Figure 4.1: Distribution of aetiological agents among the study subjects

Among the bacteria isolated, *Streptococcus pyogenes* was most common, isolated in 13 out of the 78 (15.4%) throat swabs followed by *Streptococcus viridans,* 5 (6.4%). Other bacteria isolated were *Streptococcus pneumoniae and Staphylococcus aureus* (Figure 4.2).

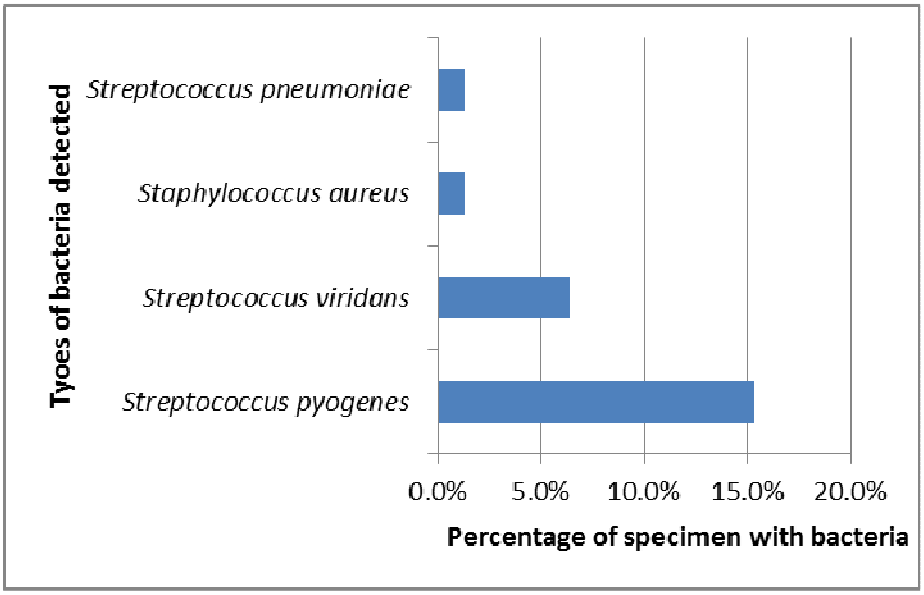

Figure 4.2: Frequency of isolation of bacteria in clinical specimen from the study participants

Influenza Type A virus was more commonly isolated than other viruses, detected in 20.5% specimen followed by RSV (16.7%) and influenza type B (10.3%). Among the influenza Type A viruses, seasonal influenza A (H3N5) was more common, 13 (16.7%) than pandemic influenza A (H1N1) which was detected in 3 (3.8%) of all the clinical specimen. Other viruses included parainfluenza Type 1 and parainfluenza Type 3 detected in two and one clinical specimen respectively specimen while parainfluenza Type 2 and hMPV viruses were not detected in any of the specimen (Figure 4.3). Representative photographic agarose electrophoresis display of multiplex PCR products parainfluenza viruses, RSV and hMPV are shown in Figure 4.4 and Figure 4.5 respectively, below and a pictorial representation of influenza viruses detection by real time PCR is shown in Figure 4.6.

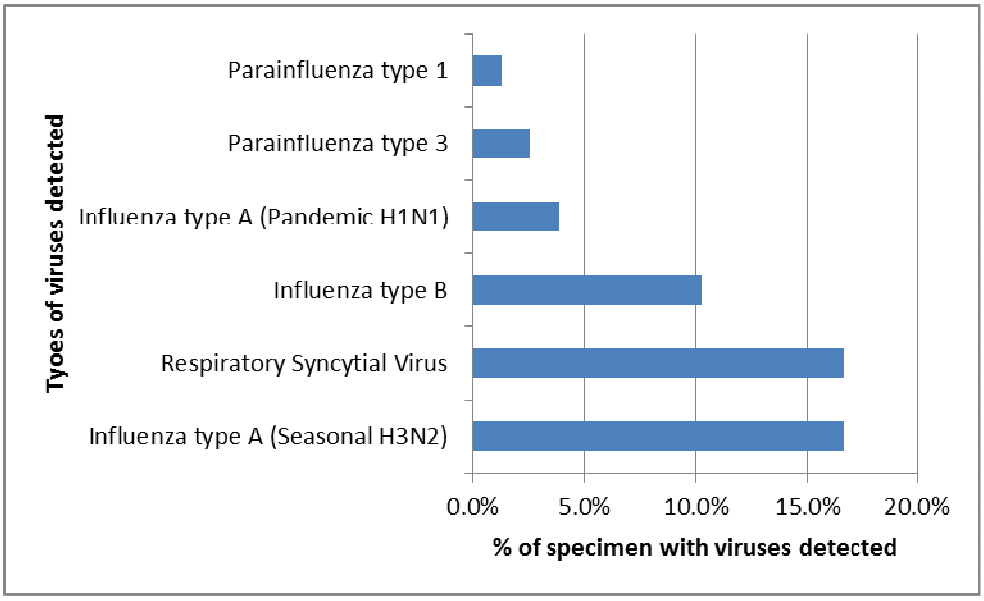

Figure 4.3: Frequency of detection of viral agents in clinical specimen from the study participants

Influenza A was detected in 16 (20.5%) samples which included 13(16.7%) season influenza A (H3N2) and 3 (3.8%) pandemic influenza A (H1N1); hMPV and Parainfluenza type 2 were not detected in any of the clinical specimen

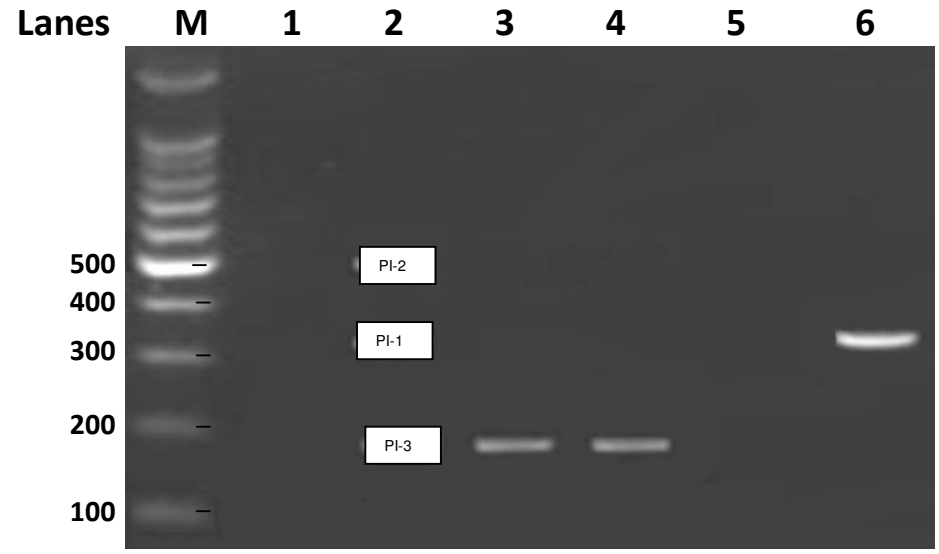

Figure 4.4: Detection of parainfluenza viruses by multiplex reverse transcriptase polymerase chain reaction

This assay was to differentiate parainfluenza types 1, 2 and 3 from the clinical specimen. Lane M = 100bp DNA marker (Invitogen Cat74602-250). Lane 1 is an internal negative control, lane 2 has - PI-1: parainfluenza 1 (317bp), PI-1; PI-2: parainfluenza 2 (507bp), and PI-3: parainfluenza 3 (PI-3; 189bp) positive controls. Lanes 3 to 6 represents field samples used in this study- samples in lanes 3 and 4 were positive for parainfluenza 1 but negative for parainfluenza 1 and 2; the sample in lane 6 was positive for parainfluenza 1 but negative for RSV and parainfluenza 2.

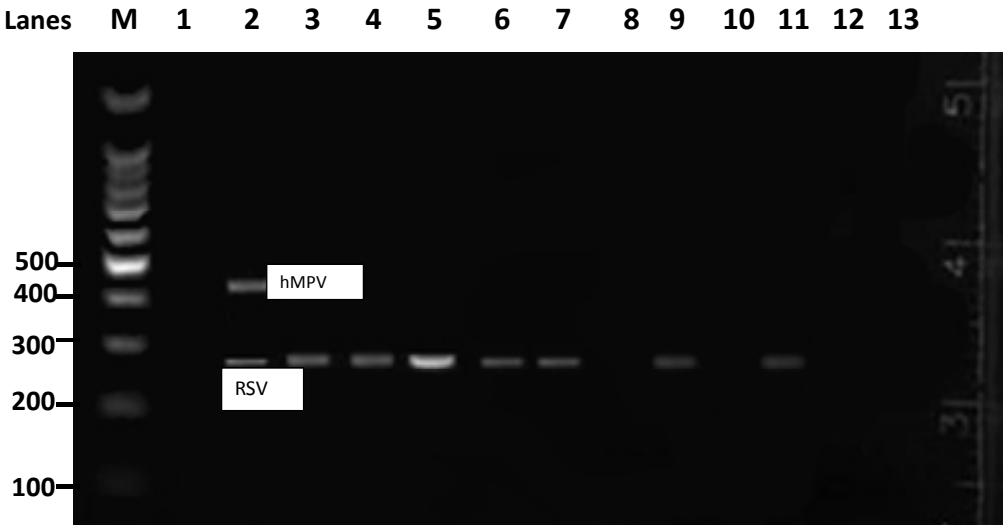

Figure 4.5: Detection of human metapneumovirus and respiratory syncytial virus by multiplex polymerase chain reaction

Lane M = 100bp DNA marker (Invitogen Cat74602-250). Lane 1 contained an internal negative control; lane 2 contained hMPV (416bp) and hRSV (279bp) positive controls. Lanes 3 to 13 represents field samples used in this study-non of the samples contained hMPV; samples in lanes 3 to 7, lane 9 and 11 were positive for RSV, samples in lanes 8, 10, 12 and 13 were negative for RSV.

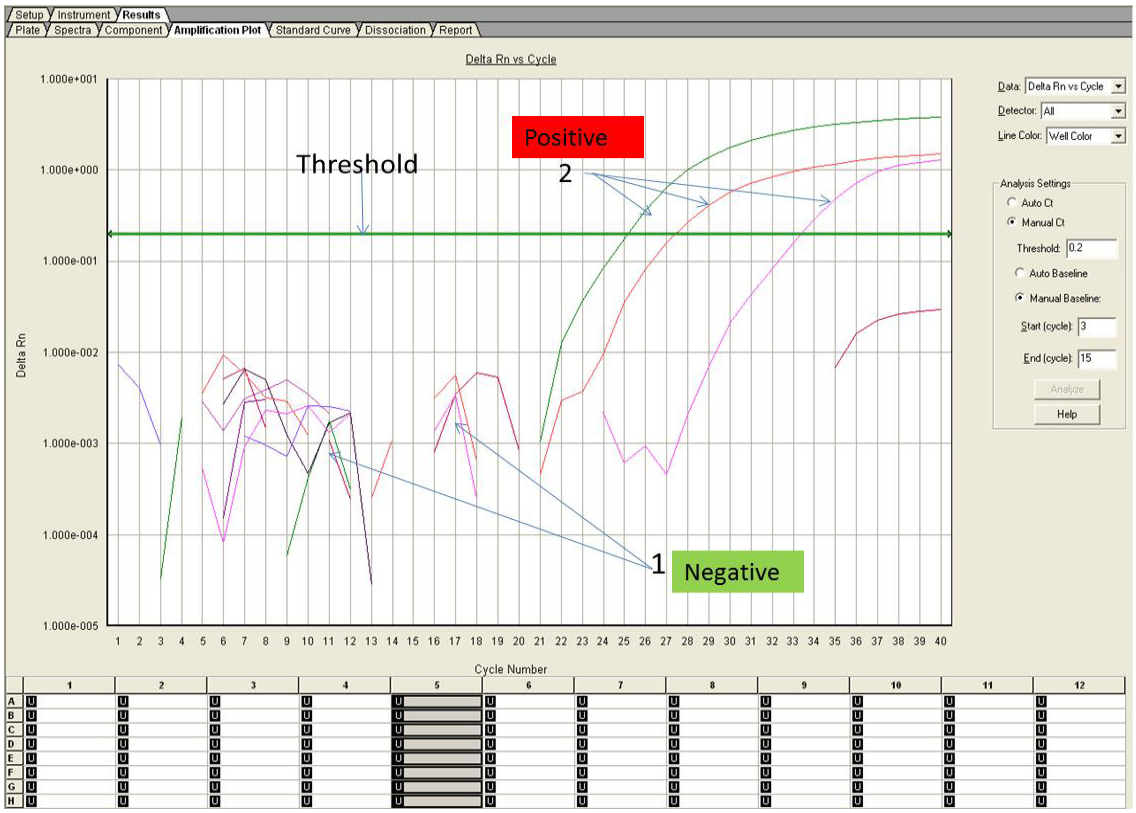

Figure 4.6: Pictorial representation of virus detection of influenza viruses in clinical specimen of the participants by real time polymerase chain reaction

At the top are different menu to switch between different test program and results displays. Amplification plots are created when the fluorescent signal from each sample is plotted against cycle number; therefore, amplification plots represent the accumulation of product over the duration of the real-time PCR experiment. The X-axis shows the number of cycles while the X-axis and Y-axis shows the florescent signal. Beneath the chart is a representation of the plate layout that is adapted to the plate size (96 well layout i.e. wells 1 to 12 running from A to H). Wells in column 5 have been selected (appearing greyish) to display the status of the amplification in the specific wells to display the status curves. This view is helpful to evaluate the performance of the PCR for each well and is useful to perform a quick quality check of the conducted real-time PCR run. In this example, the selected wells in column 5 display (1) curves that do not cross the threshold line which shows wells with negative results i.e. negative for influenza A (2) three curves that have crossed the threshold line that shows the wells with positive results for influenza A (as described in the methodology section wells 1-5 contained influenza A primers while 7 to 11 contained primers for influenza B).

4.2.1. Multiple viruses and bacteria detection

Mixed agents were detected in 23 out of the 78 (29.5%) specimen. A mixture of bacteria and single virus was detected in 18 out of the 23 (78.3%) specimen. One specimen contained a mixture of bacteria and two viruses (RSV and influenza B) while others contained a mixture of influenza A and RSV, influenza B and RSV, parainfluenza 1 and RSV and parainfluenza type 3 and RSV (Table 4.5)

Table 4.5: Detection of mixed agents in clinical specimen of the study subjects

	Co-infected specimen	
Type of agents	**No.**	**%**
Bacteria and single virus*	18	78.30%
Bacteria and more than one virus**	1	4.34%
Influenza type A and RSV	1	4.34%
Influenza type B and RSV	1	4.34%
Parainfluenza type 1 and RSV	1	4.34%
Parainfluenza type 3 and RSV	1	4.34%
Total number of co-infected specimen	**23**	**100.00%**

*Co-infected with bacteria and one viral agent; **Co-infected with bacteria and two viruses i.e Respiratory Syncytial Virus and influenza B

4.2.2. Comparison of bacterial and viral agents children with and without acute respiratory infections

A mixture of bacteria and viruses were isolated in 19 out of 78 (24.4%) children; among them were 14 (22.2%) cases and 5 (15.2%) controls. Although not statistically significant, cases were 2.5 more likely to have bacteria isolated than controls (OR=2.53, 95% CI: 0.81-7.92). All the children who had bacteria isolated from their clinical specimen had viral pathogens detected as well (Table 4.6). *Streptococcus pyogenes* (Group A streptococcus) was isolated in significantly more cases than controls (OR=11.6, 95% CI: 1.43-94.7; p=0.02) while Streptococcus viridans were more common in control children than cases, although this did not reach statistical significance (OR=0.16, 95% CI: 0.018-1.5; p=0.1).

Among the 35 study subjects who had at least one virus detected, 25 (32.1%) had viruses only (without bacteria) among them were 16 (35.6%) of the cases and 9 (27.3%) of the controls. As shown in Table 4.6 below, viruses were more likely to be detected in cases than in controls, although the difference was not significant (OR=1.5, 95% CI: 0.6-3.9; p=0.5). Influenza A viruses were detected in significantly more cases than controls (OR=16, 95% CI: 1.98-128; p=0.001). Athough not statistically significant, influenza B detected in more cases than controls (OR=2.4, 95% CI: 0.45-12.7; p=0.296; Table 4.6).

Table 4.6: Association of isolation rate of bacterial and viral agents among cases and controls

			Patient type			
Agents detected	**Status**	**n=78**	**Cases n(%)**	**Controls n(%)**	**OR (95%CI)**	**p**
Bacteria and viruses (mixed)*	Yes	19 (24.4)	14 (22.2)	5 (15.2)	1	0.17
	No	59 (75.6)	31 (68.8)	28 (84.8)	2.53 (0.81-7.92)	
Bacteria isolated#						
Streptococcus pyogenes	Yes	13 (16.7)	12 (26.7)	1 (3.0)	1	
	No	65 (83.3)	33 (73.3)	32 (97)	11.6 (1.43-94.7)	0.02
Streptococcus viridans	Yes	5 (6.4)	1 (2.2)	4 (12.1)	1	
	No	73 (93.6)	44 (97.8)	29 (87.9)	0.16 (0.018-1.5)	0.1
Viruses Only	Yes	25 (32.1)	16 (35.6)	9 (27.3)	1	0.47
	No	53 (67.9)	29 (64.4)	24 (72.7)	1.5 (0.6-3.9)	
Viral pathogens**						
Influenza type A	Yes	16 (20.6)	15 (33.3)	1 (3)	1	
	No	62 (79.4)	30 (66.7)	32 (97)	16 (1.98-128)	0.001
Influenza type B	Yes	8 (10.2)	6 (13.3)	2 (6.1)	1	
	No	70 (89.8)	39 (86.7)	31 (93.9)	2.4 (0.45-12.7)	0.296
RSV	Yes	13 (16.7)	6 (13.3)	7 (21.2)	1	
	No	65 (83.3)	39 (86.7)	26 (78.8)	0.57 (0.17-1.89)	0.356

*All children with bacteria isolated had viruses as well; **hMPV and parainfluenza type 2 were not detected in any specimen while parainfluenza type 1 and 3 were detected in specimen of one and two participants respectively and hence exempted from this analysis; # other bacteria species were isolated in small proportions and were therefore exempted from the analysis; OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, $p<0.05$ was considered significant.

4.3. Risk factors for acute respiratory infections

4.3.1. Types of cooking fuels among study subjects

A number of potential risk factors were assessed to determine association with the ARI. Among the potential risk factors was indoor air pollution contributed by the type of fuel used by the study respondents. Solid fuels were the most prevalent sources of fuel for cooking among the study participants which included charcoal (48.4%) and wood (43.3%). Other types of fuel used less commonly were gas (6.7%) and paraffin stove (3.1%; Figure 4.7).

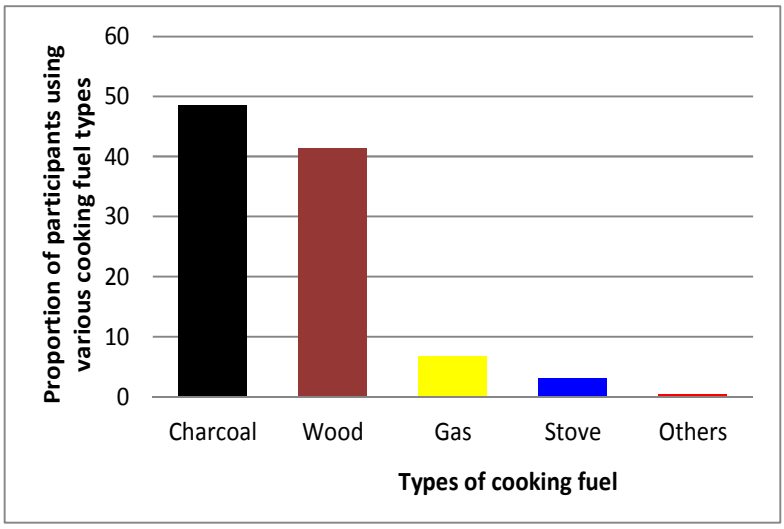

Figure 4.7: Types of fuels used for cooking by the study respondents

4.3.2. Association between indoor pollution and the risk of acute respiratory infections

Table 4.7 shows the potential indoor pollution related risk factors for ARI among the study subjects. Among the types of cooking fuels used, charcoal and wood were the most common type of fuel used by cases, 59 (46.5%) and 56 (44.1%); and controls, 64 (50.4%) and 49 (38.6%) for the two types of fuel source, respectively. There was a slightly increased odds with the use of charcoal (OR=1.24, 95% CI: 0.74 - 2.09) and gas (OR=1.63, 95% CI: 0.578-4.616). The association between fuel types used among the cases and controls was not statistically significant (p=0.699).

One hundred and forty seven (58.1%) participants lived in houses made of stones or bricks, followed by mud, 88 (34.8%). Among these, 72 (56.7%) and 48 (37.8%) cases compared to 75 (59.5%) and 40 (31.7%) of the controls lived in houses made of stones or bricks and mud, respectively. Other types of housing reported by the study respondents included timber and iron sheets. There was an increased odds of ARI among those living in houses made of timber and iron sheets (OR=1.89, 95% CI: 0.69-5.32). However, this was not significantly associated with risk of ARI (p=0.237; Table 4.7).

The number of house occupants living within the same houses as the study subjects was categorized as shown in Table 4.7 with 45.3% of the participants living in houses occupied by 3-5 people. Of these, 61 (47.7%) were cases and 55 (43.0%) were controls.

Although not statistically significant, there was a trend of reduced odds among respondents living in houses occupied by less than 8 people (Table 4.7). Sixty nine (27%) of all the households had a member smoking within the home. Among these were 43 (33.6%) cases and 26 (20.3%) controls. A significant association was found between smoking and risk of ARI by univariate analysis (OR=1.98, 95% CI: 1.13-3.49; p=0.017). The association was not significant after adjusting for related factors (Adjusted OR =1.6, 95% CI: 0.79-3.3).

Majority (83.3%) of the participants had cooking place inside the house; among these were 99 (82.5%) cases and 101 (84.2%) controls. Although, the association between the cooking place and the participant types was not statistically significant (p=0.729), there were reduced odds among those cooking from outside the house (OR=0.88, 95% CI: 0.45-1.75). One hundred and forty three (56.3%) had kitchen located outside the main living room among them were 44 (36.4%) cases and 58 (46.8%) controls. Although there were reduced odds among those with kitchen located outside the living room (OR=0.65, 95% CI: 0.38-1.08), the association between the location of the cooking place and the risk of ARI was not statistically significant (p=0.98).

Majority of the respondents indicated that they never carried the children while cooking, 116 (46.4%), while 89 (35.6%) and 45 (18%) indicated they sometimes and always carry the children respectively. There were reduced odds among those who never carried their children while cooking, though this was not statistically significant (OR=0.66, 95% CI: 0.329-1.32; Table 4.7).

As shown in table 4.7, 116 (46.0%) participants lived in single roomed houses among them were 57 (45.2%) and 59 (46.8%) cases and controls respectively. There were reduced odds of living in houses with more than three bedrooms (OR=0.54, 95% CI: 0.17-1.7). The differences between the number of rooms and risk for ARI was not statistically significant (p=0.597; Table 4.7).

Table 4.7: Association of risk factors related to indoor pollution among the cases and controls

Possible risk factors	Participant type		Total	OR (95%CI)	P
	Cases (%)	Controls (%)			
Fuel type*					
Wood	56 (44.1)	49 (38.6)	105	1	
Gas	7 (5.5)	10 (7.9)	17	1.63 (0.578-4.616)	
Charcoal	59 (46.5)	64 (50.4)	123	1.24 (0.74 - 2.09)	0.699
Stove	5	4 (3.1)	9	0.91 (0.23-3.6)	
House make*					
Mud	48 (37.8)	40 (31.7)	88	1	
Stones and bricks	72 (56.7)	75 (59.5)	147	1.25 (0.74-2.12)	0.237
Timber and ironsheets	7 (5.5)	11 (8.7)	18	1.89 (0.69-5.32)	
Occupants					
>8	2 (1.6)	4 (3.0)	6	1	
>5-8	34 (26.5)	24 (18.8)	58	0.35 (0.05-2.08)	0.152
3-5	61 (47.7)	55 (43.0)	116	0.45 (0.08-2.56)	
1-3	31 (24.2)	45 (35.2)	76	0.73 (0.13-4.21)	
Smoking by a member of the household*					
Yes	43 (33.6)	26 (20.3)	69	1	
No	85 (66.4)	102 (79.7)	187	1.98 (1.13-3.49)	0.017
Cooking place*					
Inside the house	99 (82.5)	101 (84.2)	200	1	
Outside the house	21 (17.5)	19 (15.8)	40	0.88 (0.45-1.75)	0.98
Child carried on the back while cooking*					
Always	19 (15.3)	26 (20.6)	45	1	
Sometimes	44 (35.5)	45 (35.7)	89	0.75 (0.36-1.54)	0.498
Never	61 (49.2)	55 (43.7)	116	0.66 (0.329-1.32)	
Kitchen is inside the living room*					
Yes	44 (36.4)	58 (46.8)	102	1	0.98
No	77 (63.6)	66 (53.2)	143	0.65 (0.38-1.08)	
House size*					
Single room	57 (45.2)	59 (46.8)	116	1	
One bedroom	33 (26.2)	33 (26.2)	66	0.97 (0.53-1.79)	
Two bedroom	27 (21.4)	29 (23.0)	56	1.04 (0.55-1.96)	0.92
Thee bedroom or more bedrooms	9 (7.1)	5 (4.0)	14	0.54 (0.17-1.7)	

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, p<0.05 was considered significant; *Adjusted OR=1.6, 95% CI: 0.79-3.3 (logistic regression analysis). *Few participants declined to respond to the respective questions indicated.

4.4. Caregiver's knowledge and practices on transmission and prevention of acute respiratory infections

4.4.1. Caregiver's awareness of danger signs of childhood illness

The mothers' awareness about the danger signs of childhood illness was poor as none of the caretaker was aware of all the danger signs of childhood illness. Fever was the most commonly identified danger sign by 199 (77.7%) but the responses were not significantly different among the cases and controls (p=0.453). As shown in table 4.8, many

parents/guardians were not aware of the following danger signs; child drinking poorly (95.7%), child not able to drink or breastfeed (90.2%), fast breathing (93.8%), difficulty breathing (78.1%) and blood in stool (97.7%). There was no difference among the cases and controls on the awareness of danger signs (Table 4.8).

Table 4.8: Parental awareness of danger signs

		Participant type				
Danger signs	**Parents aware**	**Total (%)**	**Cases (%)**	**Controls (%)**	**OR (95%CI)**	**p**
Child develops fever	No	57 (22.3)	26 (45.6)	31 (54.4)	1	
	Yes	199 (77.7)	102 (51.3)	97 (48.7)	0.8 (0.44-1.44)	0.453
Child is drinking poorly	No	245 (95.7)	122 (49.8)	123 (50.2)	1	
	Yes	11 (4.3)	6 (54.5)	5 (45.5)	0.8 (0.25-2.78)	0.758
Child is not able to drink or breastfeed	No	231 (90.2)	112 (48.5)	119 (51.5)	1	
	Yes	25 (9.8)	16 (64.0)	9 (36.0)	0.5 (0.22-1.25)	0.141
Child has fast breathing	No	240 (93.8)	117 (48.8)	123 (51.3)	1	
	Yes	16 (6.3)	11 (68.8)	5 (31.3)	0.4 (0.15-1.28)	0.121
Child has difficulty in breathing	No	200 (78.1)	100 (50	101 (50)	1	
	Yes	56 (21.9)	28 (50)	28 (50)	0.99 (0.55-1.79)	1.000
Child has blood in stool	No	250 (97.7)	125 (50)	126 (50)	1	
	Yes	6 (2.3)	3 (50)	3 (50)	0.99 (0.2-5.01)	1.000

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using Fisher's test, $p<0.05$ was considered significant

4.4.2. Knowledge of acute respiratory infections clinical signs

When the respondents were requested to identify the signs of ARI, 161 (62.9%) of the respondents did not indicate cough as one of the signs of ARI. There was no significant difference in the correct response of cough as a sign to seek medical care for the ARI children (p=0.5). Similarly, among the rural and urban participants the difference in the responses on cough being a sign for ARI was not significantly different (p=0.069). Majority of participants did not correctly identify difficulty breathing, 159 (62.1%), wheezing, 128 (89.1%), chest in-drawing, 171 (66.8%) and convulsions, 252 (98.4%) as danger signs of ARI (Table 4.9). There was no statistical difference in the responses among the cases and controls on the above clinical signs. However, significantly more participants from the urban site, 78 (80.4%) indicated difficulty in breathing as a sign for ARI compared to those from the rural site 19 (19.6%) (p=001) while more participants

from the rural site, 56 (65.9%) indicated chest in-drawing as a sign for ARI compared to those from the urban site 29 (34.1%; p=001) indicating differences in level of knowledge between rural and urban participants on disease signs (Table 4.9).

Table 4.9: Parental knowledge on signs of acute respiratory infections

			Participant type		
ARI symptom	Response	Total (%)	Cases (%)	Controls (%)	OR (95%CI)
Cough	No	161 (62.9)	80 (49.7)	81 (50.3)	1
	Yes	95 (37.1)	47 (49.5)	48 (50.5)	1.0 (0.6-1.68)
Difficulty to breathing	No	159 (62.1)	80 (50.3)	79 (49.7)	1
	Yes	97 (37.9)	48 (49.5)	49 (50.5)	1.0 (0.62-1.71)
Wheezing	No	128 (89.1)	114(50.0)	114 (50.0)	1
	Yes	28 (10.9)	14 (50)	14 (50.0)	1.0 (0.46-2.19)
Chest in-drawing	No	171 (66.8)	83 (48.5)	88 (51.5)	1
	Yes	85 (33.2)	45 (52.9)	40 (47.1)	0.84 (0.5-1.41)
Convulsions	No	252 (98.4)	126 (50.0)	127 (50.0)	1
	Yes	4 (1.6)	2 (50.0)	3 (50.0)	1.48(0.24-9.06)

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using Fisher's test, p<0.05 was considered significant; Urban (FITC); Rural (Mangu health centre).

4.4.3. Knowledge about modes of transmission and disease perceived severity of acute respiratory infections

Eighty three point six percent considered ARI in children as highly fatal, among them were, 107 (84.9%) cases and 107 (85.6%) controls. Twenty seven (10.5%) indicated that ARI can cause severe irreversible bodily damage while 10 (3.9%) indicated that ARI was not severe. The differences in responses among cases and controls were not significantly different (p=0.694). Of all respondents, 41 (16%) incorrectly indicated that ARI could be transmitted through through water sources such as reservoirs, among these were, 22 (17.2%) and 19 (14.8%) cases and controls respectively (p=0.609). Seven (2.7%) incorrectly answered that ARI is transmitted through insect bites; among them were, 4 (3.1%) cases and 3 (2.3%; p=0.702). Eighty (31.3%) including 40 (31.3%) and 40 (31.3%) cases and controls respectively believed that ARI can be transmitted by eating inadequately cooked pork or chicken (p=1.0; Table 4.10).

Respectively, 240 (93.8%), 19 (7.4%) and 20 (7.8%) of the respondents correctly knew that ARI is transmittable through air droplets such as sneezing, touching the body of infected person and contaminated objects. As shown in Table 4.10 the differences in the responses between the cases and controls on the three modes of transmission of ARI were not significantly different (p= 0.302, p=0.233 and p=0.641 respectively).

Out of 254 who responded to the question on perceived availability of treatment and preventive vaccines in the government health facilities, 218 (85.2%) and 206 (80.5%) were confident that effective medicine is available to treat ARI and an effective vaccine was available to prevent people from acquiring ARI. There was no significant difference in the responses between the cases and controls with regard to availability of effective medicine (p= 1.00) and vaccine (p=0.150; Table 4.10).

Majority of the participants, 149 (58.2%) believed that injection was the most effective approach for treating ARI in children, others believed that use of syrups, 108 (42.2%) and tablets, (31 (12.1%) was equally effective. A few believed in other approaches for treatment including use of home remedies such as tea, ointment and traditional medicine were effective. One of the respondents indicated that they did not think there was an effective drug that was capable of treating ARI in children (Table 4.10).

Table 4.10: Knowledge on transmission and management of acute respiratory infections

Characteristics			Total n(%)	Cases n (%)	Controls n (%)	OR (95%CI)	p
Perceived severity of ARI in children*							
Not severe	n=251		10 (3.9)	4 (3.2)	6 (4.8)	1	
Highly fatal			214 (83.6)	107 (84.9)	107 (85.6)	0.67 (0.2-2.4)	0.74
Can cause severe irreversible bodily damage			27 (10.5)	15 (11.9)	12 (9.6)	0.5 (.1-2.3)	
Knowledge on modes of transmission							
Through water sources (e.g. reservoirs)	n=256	No	215 (84)	106 (83.8)	109 (85.2)	1	0.609
		Yes	41 (16)	22 (17.2)	19 (14.8)	0.84 (0.43-1.64)	
Through insect bites	n=256	No	249 (97.3)	124 (96.9)	125 (97.7)	1	
		Yes	7 (2.7)	4 (3.1)	3 (2.3)	0.74 (0.16-3.4)	0.702
Through un-well cooked pork or chicken	n=256	No	176 (68.7)	88 (68.7)	88 (68.7)	1	
		Yes	80 (31.3)	40 (31.3)	40 (31.3)	1 (0.59-1.7)	1.000
Through droplets (e.g. sneeze)	n=256	No	16 (6.2)	10 (7.2)	6 (4.7)	1	
		Yes	240 (93.8)	118 (92.2)	122	0.84 (0.43-1.64)	0.302
Through touching body of infected persons	n=256	No	237 (92.6)	121 (94.5)	116	1	
		Yes	19 (7.4)	7 (5.5)	12 (9.4)	1.7 (0.68-4.7)	0.233
Through touching contaminated objects	n=256	No	236 (92.2)	117 (91.4)	119 (93)	1	
		Yes	20 (7.8)	11 (8.6)	9 (7.0)	0.8 (0.321-2.0)	0.641
Perceived availability of treatment and preventive vaccine*							
Effective medicine available	n=254	No	36 (14.8)	14 (55.5)	22 (58.7)	1	
		Yes	218 (85.2)	113 (44.5)	105	1.6 (0.8-3.4)	1.00
Effective vaccine available	n=254	No	48 (19.5)	24 (59.4)	24 (59.4)	1	
		Yes	206 (80.5)	103 (40.6)	103 (40.6)	1 (0.53-1.9)	0.150
Appropriate medicines for treating ARI in children							
Syrups	n=256	No	148 (57.8)	72 (56.2)	76 (59.4)	1	
		Yes	108 (42.2)	56 (43.8)	52 (40.6)	1.1 (0.69-1.9)	0.613
Tablets	n=256	No	225 (87.9)	114 (89.1)	111	1	
		Yes	31 (12.1)	14 (10.9)	17 (13.3)	0.8 (0.38-1.7)	0.565
Injection	n=256	No	107 (41.8)	55 (43)	52 (40.6)	1	
		Yes	149 (58.2)	73 (57.0)	76 (59.4)	0.9 (0.55-1.5)	0.704
Tea	n=256	No	255 (99.6)	127 (99.2)	128 (100)	1	
		Yes	1 (0.4)	1 (0.8)	0	2 (1.8-2.3)	0.316
Traditional medications	n=256	No	251 (98)	124 (96.9)	127 (99.2)	1	
		Yes	5 (2.0)	4 (3.1)	1 (0.8)	4.0 (0.45-37.2)	0.175
Ointment	n=256	No	255 (99.6)	127 (99.2)	128 (100)	1	
		Yes	1 (0.4)	1 (0.8)	0	2 (1.8-2.3)	0.316
There is no effective drug for the treatment of ARI	n=256	No	255 (99.6)	128 (100)	127 (99.2)	1	
		Yes	1 (0.4)	0	1 (0.8)	2 (1.8-2.3)	0.316

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, $p<0.05$ was considered significant; * a few participants did not respond to the question; % - percentage.

4.4.4. Responses on public health preventive and avoidance behaviours for acute respiratory infections

Mixed responses were obtained with regard to wearing of face mask to prevent ARI transmission. Respondents believed that wearing face masks was; not effective at all, 97 (38.2%), not very effective, 90 (35.4%), quite effective, 45 (17.7%) and very effective, 22

(8.7%). No significant differences in the responses was found between cases and controls (p=0.170; Table 4.11). Among the respondents, 23 (9.1%) indicated that hand washing was not effective at all, 82 (32.6%), 64 (25.4%) was quite effective and 83 (32.9%) thought that hand washing was very effective. No significant differences in the responses was found between cases and controls (p=0.987; Table 4.11).

The respondents felt that other preventive measures such as declaration of ARI symptoms at border health checkpoints, 22 (8.6%) and seeking of medical consultation immediately after onset of a fever, 167 (65.2%) would be effective in early management and in controlling spread of ARI. Table 4.11 below reports the avoidance behavior of the respondents in preventing acquisition of ARI. Respectively, 212 (82.8%), 40 (15.6%) and 7 (2.7%) of the respondents indicated they would avoid going to crowded places, avoid going out unless necessary or avoid visiting hospitals. The responses between the cases and controls on avoidance behaviors was not significant for each of the avoidance behaviour indicated (Table 4.11).

Table 4.11: Prevalence of knowledge on preventive and avoidance behaviors for acute respiratory infections

Characteristics			Total n(%)	Cases n (%)	Controls n (%)	OR (95%CI)	p
Efficacy of public health measures							
Wearing face masks in public areas*							
Not effective at all	n=254		97 (38.2)	42 (33.3)	55 (42.9)	2.2 (0.8-5.9)	0.10
Not very effective			90 (35.4)	50 (39.7)	40 (31.3)	1.4 (0.5-3.7)	0.60
Quite effective			45 (17.7)	20 (15.9)	25 (19.5)	2.2 (0.8-6.2)	0.19
Very effective			22 (8.7)	14 (11.1)	8 (6.3)	1	
Washing hands frequently*							
Not effective at all	n=252		23 (9.1)	12 (9.7)	11 (8.6)	0.85 (0.3-2.1)	0.8
Not very effective			82 (32.6)	40 (32.3)	42 (32.8)	0.9 (0.53-1.8)	1
Quite effective			64 (25.4)	32 (25.8)	32 (25.0)	0.9 (0.48-1.7)	0.86
Very effective			83 (32.9)	40 (32.2)	43 (33.6)	1	
Other preventive measures							
Declaration of ARI symptoms at border health checkpoints	n=256	No	234 (91.4)	120 (93.7)	114 (89.1)	1	
		Yes	22 (8.6)	8 (6.3)	14 (10.9)	0.5 (0.22-1.3)	0.181
Seeking of medical consultation immediately with	n=256	No	89 (34.8)	45 (35.2)	44 (34.6)	1	
		Yes	167 (65.2)	83 (64.8)	84 (65.6)	0.9 (0.6-1.6)	0.896
Avoidance behaviours							
Avoid going to crowed places	n=256	No	44 (17.2)	24 (18.7)	20 (85.6)	1	
		Yes	212 (82.8)	104 (81.3)	108 (84.4)	0.8 (0.42-1.5)	0.508
Avoid going out unless necessary	n=256	No	216 (84.4)	110 (85.9)	106 (82.8)	1	
		Yes	40 (15.6)	18 (14.1)	22 (17.2)	0.8 (0.4-1.5)	0.491
Avoid going to hospitals	n=256	No	249 (97.3)	124 (96.9)	125 (97.7)	1	
		Yes	7 (2.7)	4 (3.1)	3 (2.3)	1.3 (0.95-6.1)	0.702

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, $p<0.05$ was considered significant; * a few participants did not respond to the question; % - percentage.

4.5. Care seeking behaviors among the study respondents for childhood illness

4.5.1. Alternative care sought by study participants in rural and urban sites

One hundred twenty two of the 128 (95.3%) care takers of children with signs of ARI provided information on whether they had sought treatment elsewhere for ARI prior visiting the health facility. Out of these, 49 (40.2%) indicated that they had sought alternative treatment prior to visiting the health facility, among them were 25 (51%) from the urban health facility and 24, (49%) from the rural health facility. Seventy three out of 122 (59.8%) indicated that they had not sought treatment elsewhere, 40 (54.8%) from urban facility compared to 33 (45.2%) from a rural health facility (Figure 4.8). The

distribution of the participants who sought alternative care from rural and urban sites was not significant (p=0.682).

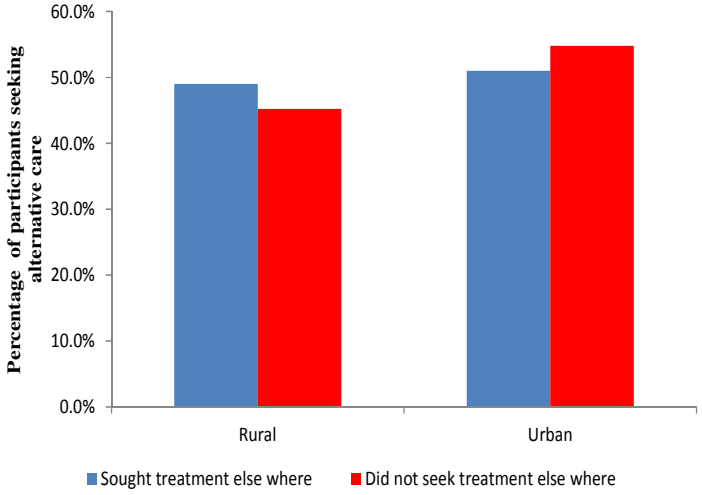

Figure 4.8: Proportion of participants from rural and urban centers seeking alternative care for acute respiratory infections in children

4.5.2. Care seeking practices among the study participants for childhood illnesses

All the 256 participants (caretakers of both cases and controls) were interviewed on the health care sought prior to visiting the health facilities for various childhood illnesses. The health care practices were categorized into; 'appropriate care' where health care was sought from qualified medical professionals in government health facilities and private hospitals/clinics and other types of care such as purchasing medicines from pharmacy, home remedies, religious/faith healing and traditional healers which was defined as inappropriate care. Majority, 231 (92.8%) indicated seeking health care from a health facility (appropriate care) among these were, 117 (94.4%) of the cases and 114 (90.5% of the controls. Inappropriate care was also sought from traditional doctors, 6 (2.4%), direct purchase of drugs from pharmacies, 8 (3.2%) and home remedies, 5 (2.0%). There was no significant difference in the care sought among the respondents. None of the participants indicated that they waited for the illness to clear or took the children to religious leaders for prayers (Table 4.12).

Table 4.12: Health care seeking for acute respiratory infections and other childhood illness by participant type and location

Type of care sought	Patient type				
	Total n (%)	Cases n (%) n=123*	Controls n (%) n=126**		p
Home remedies	5 (2.0)	2 (1.6)	3 (2.4)	1	
Hospital	231 (92.4)	117 (94.4)	114 (90.5)	0.64 (0.12-3.9)	0.7
Traditional doctors	6 (2.4)	4 (3.2)	2 (1.6)	0.3 (0.02-3.9)	0.6
Purchased drugs from phamacy	8 (3.2)	1 (0.8)	7 (5.6)	4.7 (0.29-73.3)	0.5

No parent indicated that they wait for illness to subside or take them for prayers by religious leaders; *5 cases and 2 control caretakers declined to give the alternative care sought; OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using chi-square test, p<0.05 was considered significant.

4.5.3. Reasons for seeking different heath care services among the cases and controls

Various reasons informed the type of care to be sought by the study participants. The most prevalent reason for seeking care from a health facility (appropriate care) was the perception that the health facilities offered better treatment, 93 (40.8%); among these were 40 (35.4%) controls and 53 (46.1%) cases. Hospital being close to the participants residents was another major factor that promoted appropriate care seeking behavior with 47 (20.6%) participants citing close proximity to the hospital a promoting factor; 27 (23.9%) controls and 20 (17.4%) cases. The fear of the disease worsening was cited by 42 (18.4%) participants among them 20 (17.7%) controls and 22 (19.1%) cases. Twenty four participants (10.5%) considered that proper diagnosis (investigations) is conducted at the health facilities for better treatment. This acted as an inducing factor to seek appropriate care among 15 (13.3%) controls and 9 (7.8%) cases. There was no significant difference between the participants giving different reasons for seeking appropriate care among the cases and controls in the study (p=0.335). Other reasons motivating the participants to seek appropriate care were the perception that the health facilities had qualified staff or the children were seriously sick (Figure 4.9).

Among the participants who reported seeking inappropriate health care, majority, 8 (44.4%) sought such health care because the pharmacy was closer, among these were 7 (58.3%) controls and 1 (16.7%) case. Other major reasons for seeking inappropriate health care were the fear of disease worsening, 6 (33.3%) and they needed to seek care to support the sick children urgently. One case indicated that she had no money to pay doctors consultation while 2 (11.1%) felt that the services outside hospital were better. There was no significant difference between the different reasons for seeking appropriate care among the study subjects (p=0.289; Figure 4.9).

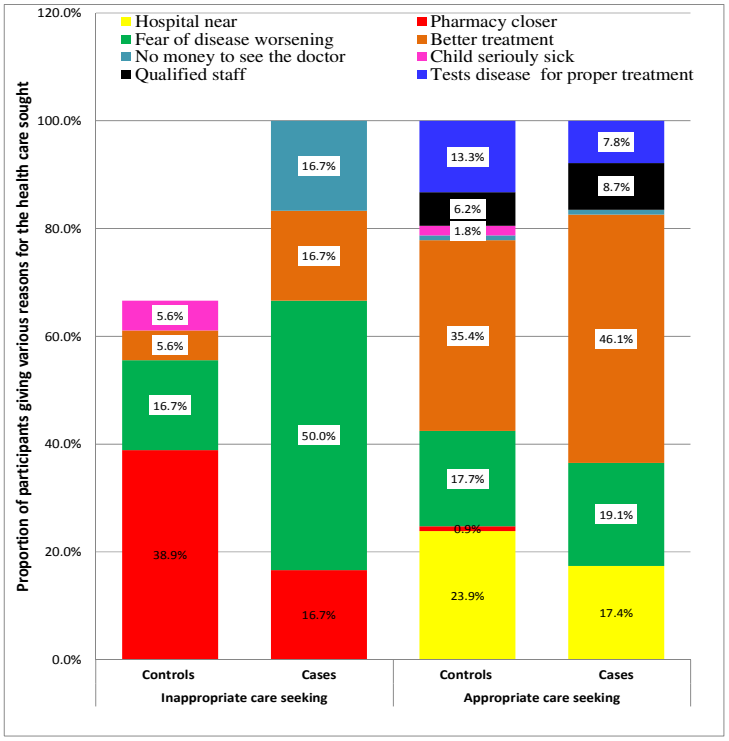

Figure 4.9: Reasons for different care seeking patterns

4.5.4. Predictors of health care seeking behaviors of the study subjects

4.5.4.1. Contribution of parental factors in health care seeking

Table 4.13 shows the various parental factors associated with seeking behavior among the all respondents. Respondents from families with less than five persons (Adjusted OR=3.89, 95% CI: 2.16-5.1; p=0.007), normally deliver at a health facility (Adjusted OR=88.1, 95% CI: 6.5-11.88; 0.001) and those with education level above primary school (OR=3.497, 95% CI: 1.30-9.39; 0.017) were significantly associated with appropriate care seeking behavior. The education level was not significant after adjusting for other factors (Adjusted OR=1.9, 95% CI: 0.29-12.9; p=0.503). Other factors with increased odds although not significant included; married couples (OR=1.7, 95% CI: 0.61-4.78), had formal occupation (OR=1.2, 95% CI: 0.148-9.573) and those with less than three children (OR=1.27, 95% CI: 0.34-4.75). Factors that were not positively associated with the appropriate care seeking behavior included religion (OR=0.9, 95% CI: 0.05-17.1), shorter distance to health facility (OR=0.72, 95% CI: 0.25-1.9), being older than 30 years (OR=0.8, 95% CI: 0.29-2.2), income higher than kshs 10,000 (equivalent to US$120; OR=0.689, 95% CI: 0.147-3.24) and residents of the rural site (OR=0.55, 95% CI: 0.2-1.52).

4.5.4.2. Contribution of child factors in health care seeking

A logic regression model applied to determine the child related predictors of health care seeking behaviour among the study respondents showed increased odds of seeking appropriate care in participants with male children (OR=1.484, 95% CI: 0.568-3.879), participants with children aged two years and less (OR=2.017, 95% CI: 0.7321-5.559). Perception that the illness was severe contributed to participants' likelihood of seeking appropriate care (OR=4.787, 95% CI: 0.274-83.7). These factors were, however, not statistically significant. Significantly, respondents with children who had two or less sibling sought appropriate care (OR=3.87, 95% CI: 1.78-8.33; p=0.001). Other factors such as child birth order were not significantly related to care sought (OR=1.2, 95% CI: 0.62-2.33; Table 4.14).

Table 4.13: Comparison of caregiver's related factors of care seeking behaviors appropriate care and inappropriate care

	Type of health care			
Predicting factors	**Inappropriate n (%)**	**Appropriate n (%)**	**Odds ratio (95%CI)**	**p**
Residence				
Rural	6 (5.2)	110 (94.8)	1	0.3278
Urban	12 (9.0)	121 (91)	0.55 (0.1996-1.516)	
Parent/guardian age				
≤30 years	12 (6.8)	165 (93.2)	1	0.7875
>30 years	6 (8.3)	66 (91.7)	0.8 (0.288-2.221)	
Family size				
>5 persons	24 (37.5)	40 (62.5)	1	
≤5persons	25 (13.2)	164 (86.8)	3.94 (2.038-7.6)	< 0.0001
Parent/guardian education				
Primary and below	11 (13.4)	71 (86.6)	1	
Above primary	7 (4.2)	158 (95.8)	3.497(1.30-9.39)	0.0166
Family income				
Low (≤Kshs10000)	16 (7.1)	209 (92.9)	1	
High (>Kshs 10000)	2 (10)	18 (90)	0.689 (0.147-3.24)	0.6475
Marital status				
Single/not currently married	6 (10.3)	52 (89.7)	1	
Married	12 (6.3)	178 (93.7)	1.7 (0.61-4.78)	0.382
Occupation				
Non-formal	17 (7.4)	214 (92.6)	1	
Formal	1 (6.3)	15 (93.8)	1.2 (0.148-9.573)	1.000
No. of children				
>3	3 (8.1)	34 (91.9)	1	
≤3	12 (6.5)	173 (93.5)	1.27 (0.34-4.75)	0.7206
Distance from Health facility				
Long (>1km)	7 (6.1)	108 (93.9)	1	
Short (≤1km)	10 (8.4)	109 (91.6)	0.71 (0.25-1.9)	0.6167
Religion				
Christian	18 (7.5)	223 (92.5)	1	1.000
Other religion	0	5 (100)	0.9105 (0.0484-17.126)	
Place of delivery				
Home	49 (100)	0	1	
Hospital	0	204 (100)	Infinity	< 0.0001

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using Fisher's exact test, $p<0.05$ was considered significant.

Table 4.14: Comparison of child related predictors of care seeking behaviors appropriate care and inappropriate care by logistic regression analysis

	Type of health care			
Potential risk factors	Inappropriate n (%)	Appropriate n (%)	Odds ratio (95%CI)	p
Child sex				
Female	9 (8.8)	93 (91.2)	1	0.4613
Male	9 (6.1)	138 (93.9)	1.484 (0.568-3.879)	
Participant type				
Case	6 (4.9)	117 (95.1)	1	0.221
Control	12 (9.5)	114 (90.5)	0.4872 (0.1768-1.342)	
Child age				
≤ 2 years	12 (9.4)	115 (90.6)	1	0.222
> 2 years	6 (4.9)	116 (95.1)	2.017 (0.7321-5.559)	
Siblings				
>2	15 (38.5)	24 (61.5)	1	0.001
≤ 2	26 (13.9)	161 (86.1)	3.87 (1.78-8.33)	
Child order				
Not first born	33 (20.6)	127 (79.4)	1	0.623
First born	16 (17.8)	74 (82.2)	1.2 (0.62-2.33)	
Perceived severity of illness				
Not severe	0	9 (100)	1	0.213
Severe	48 (20)	192 (80)	4.787 (0.274-83.748)	

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using Fisher's exact test, $p<0.05$ was considered significant.

4.5.4.3. Comparison of child predicting factors of health seeking behavior among the study subjects

Among those who reported seeking inappropriate, parents with control children aged less than 2 years were more likely to seek inappropriate care (OR=5.333, 95% CI: 0.62-46.02). Those with children who had less than 2 siblings were less likely to seek inappropriate care than those with more than two siblings (OR=0.22, 95% CI: 0.019-46.02). However, no significant difference was found between the number of siblings and

whether the participant was case or a control (p=0.517). Other factors were not related with health care sought among cases and controls as shown in table 4.15.

Among those who sought appropriate care, significantly more of the controls who perceived that the illness in the children was severe, were more likely to seek appropriate care than cases (OR=6.673, 95% CI: 1.91-23.354; p=0.001). Among the parents who sought appropriate care, there was a slightly higher proportion of male cases, 72 (61.5%; p=0.670). Other factors shown in Table 4.15 did not show strong association in seeking appropriate care among the cases and controls.

Table 4.15: Comparison of child factors associated with care seeking patterns among cases and controls

	Type of health care							
	Inappropriate				Appropriate			
Potential risk factor	Cases n (%)	Controls n (%)	Odds ratio (95%CI)	p	Cases n (%)	Controls n (%)	Odds ratio (95%CI)	p
Child sex								
Female	5 (83.3)	4 (33.3)	1	0.131	45 (38.5)	48 (42.1)	1	0.670
Male	1 (16.7)	8 (66.7)	0.1 (0.009-1.171)		72 (61.5)	66 (57.9)	1.164 (0.6874-1.970)	
Child age								
> 2 years	4 (66.7)	3 (27.3)	1		62 (53.0)	61 (53.5)	1	
≤ 2 years	2 (33.3)	8 (72.7)	5.333 (0.618-46.02)	0.161	55 (47.0)	53 (46.5)	0.979 (0.584-1.643)	1.000
Siblings								
>2	2 (50.0)	9 (81.8)	1		51 (47.7)	47 (47.0)	1	
≤ 2	2 (50.0)	2 (18.2)	0.22 (0.019-2.67)	0.517	56 (52.3)	53 (53.0)	1.027 (0.59-1.77)	1.000
Child order								
Not first born	3 (50.0)	9 (75.0)	1		75 (65.2)	70 (61.9)	1	
First born	3 (50.0)	3 (25.0)	0.3 (0.42-2.6)	0.344	40 (34.8)	43 (38.1)	1.15 (0.67-1.98)	0.680
Perceived severity of illness								
Not severe	0	2 (16.7)	1	0.529	18 (15.5)	3 (2.7)	1	0.001
Severe	6 (100)	10 (83.3)	0.323 (0.013-7.85)		98 (84.5)	109 (97.3)	6.673 (1.91-23.354)	

OR = Odds Ratio; 95% CI = 95% Confidence Interval; p = level of significance using Fisher's exact test, p<0.05 was considered significant.

4.6. Estimates for the financial/economic burden for the acute respiratory illnesses

4.6.1. Cost of managing acute respiratory infections episode per child

The mean cost per child per episode of different resources for management of the ARI is given in table 4.16. These included initial consultation costs (any prior treatment) and treatment during the period prior to cough resolution, the total mean cost of various resources per child was Kenya shillings 1,504 ($17.70; exchange rate 1US$=Kshs85)

with a total mean cost of Kshs 1,828 ($21.51) in the urban facility and Kshs 1,117 ($13.14) in the rural facility. The difference was due to significantly lower consultation fees in the rural facility. Clinician's (general practitioner, GP) consultation and cost of prescribed medicine constituted the highest cost drivers for the ARI management, mean±SD was $ 6.5±4.7 and $ 5.2±17.6 respectively. The consultation cost was significantly higher in urban mean±SD was $ 6.5±4.7 compared to rural facility, mean±SD was $1.6 (±3.1) (p=0.001). The cost of consultation ranged from Kshs 50 ($0.59) to $11.8 (equivalent to Kshs 1000). Only participants from the urban health facility indicated utilizing laboratory investigations for diagnosis, the mean cost for laboratory testing was $ 1.2±1.0 with the costs ranging from $0.6 - $2.4 for each child managed. Similarly, only patients from the urban facility had follow up for the child management (Table 4.16). The mean cost of prescribed medicine was $ 5.2±17.6 and significant differences were found between the costs of medicine between the rural and urban health facility (p=003). The participants incurred other indirect costs mainly related to travel to the health facility, the total mean costs of the travel was $ 1.3 (±1.4) ranging from $0.23 (equivalent of kshs 20) to $11.8 (equivalent of kshs 1000) probably for participants who occasionally used taxi for transport. The mean cost of transport was significantly higher in urban participants compared to their rural counterparts (p=0.0001). The participants used an average of 2.4 (SD=1.2) days off work taking care of sick child with ARI, this period ranged from 1 to 7 days. Table 4.16 indicates that mean number of days spent in taking care of the sick child by rural and urban participants was not significantly different (p=0.156).

Table 4.16: Mean resource use per episode of acute respiratory infections per participant

	Total		Urban facility		Rural facility		
Item of resource use	**Mean (±SD)**	**Range**	**Mean (±SD**	**Range**	**Mean (±SD)**	**Range**	**p**
Total cost (US$)	$ 17.70 (±2.3)		$21.51 (±6.3)		$ 13.14 (±2.4)		0.99
Primary Care							
GP consultations	$ 6.5 (±4.7)	$0.2 - 11.8	$ 6.3 (±4.6)	0.6 - 11.8	$ 1.6 (±3.1)	0- 11.8	0.001
Investigations							
Laboratory diagnosis	$ 1.2 (±1.0)	0.6 - 2.4	$ 1.2 (±1.0)	0.6 - 2.4	-	-	
Secondary Care							
Follow up visits consultation	$ 2.9 (±2.6)	$1.2 - 5.9	$ 2.9 (±2.6)	$1.2 - 5.9	-	-	
Prescriptions							
Cost of prescribed medicine	$ 5.2 (±17.6)	$0.6 - 17.6	$ 6.2 (±8.0)	0.6 - 17.6	$ 2.4 (±2.9)	0.6 - 5.7	0.03
Indirect parental resource use							
Transport costs	$ 1.3 (±1.4)	$0.2 - 11.8	$ 1.9 (±2.0)	0.2 - 11.8	$ 0.8 (±0.4)	0.2 - 1.8	0.0001
Days off work (Mean working days)	2.4 (1.2)	1 - 7	2.8 (±1.6)	1 - 7	2.4 (±0.9)	1 - 4	0.156

An exchange rate of 85 Kenya shillings (Kshs) per one United States dollar (US$) was applied in the conversion of shillings to US dollars; SD-Standard Deviation; GP-General Practitioner (clinician).

CHAPTER FIVE

5.0. DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

Acute respiratory tract infections are one of the most common illnesses in all individuals, regardless of age or gender. Epidemiologic surveys and community based studies conducted since the beginning of the 20th century have determined the rates of illness and the pathogens involved in such infections (Emmelin and Stig, 2007). Of all acute illnesses, respiratory conditions are the most common, generally occurring twice as frequently as the next most common condition. Acute respiratory infections (ARIs) continue to be one of the leading cause of acute illnesses worldwide and remain an important cause of infant and young children mortality, accounting for about two million deaths each year (Emmelin and Stig, 2007). Although the frequency of ARI is similar in both developed and developing countries; mortality due to ARI is 10–50 times higher in developing countries (Broor *et al.*, 2007). Success of public health preventive interventions to reduce the transmission and incidence of ARI as well as improve care seeking behavior and management of ARI depends on understanding of the etiology, risk factors associated with ARI and care seeking behaviours among caregivers.

5.1.1. Aetiology of acute respiratory infections

This study detected at least one ARI respiratory associated virus in 44.9% of the specimen from the children. The study findings compares to work of previous studies conducted in Kenya and in other parts of the continent. Studies conducted elsewhere in the World revealed that respiratory viruses were common in children with ARI. A study conducted in Kilifi, Kenya, by Munywoki *et al.* (2011) found that 66.6% children had at least one respiratory virus detected. Ahmed *et al.* (2012) detected respiratory viruses in 49.8% in a study conducted at a refugee camp in Kenya. Bharaj *et al.* (2009) in a paediatric study in India reported a viral detection rate of 35.2% while Louie *et al.* (2005) in study in San Fransisco, California in the United States found a respiratory viral detection rate of 38%. Another study conducted in Australia reported detection rate of viruses in 69% of ARI suspected patients (Kusel *et al.,* 2006*).* A study conducted in children under five in Brazil reported the highest virus detection rate of 85.5% (Bezerra *et al.,* 2011). The above studies report respiratory viruses' detection rate of 35% to 86%,

indicating the role of respiratory viruses as etiological agents of acute respiratory infections. The findings of the current study are consistent with findings found in other studies conducted within the country, in developing and in developed world. Compared to bacterial agents, viruses were more commonly isolated among the study subjects. These findings are in agreement with the conclusions made by van Gageldonk-Lafeber *et al.* (2005) that most ARI are viral.

Various respiratory viruses were detected in this study which included; influenza A which was detected in majority (20.5%) of the specimen, followed by RSV (16.7%) and influenza B detected in 10.3% of the samples. Human parainfluenza 1 and parainfluenza 3 were detected in minority of the samples, 2.6% and 1.3% specimen respectively. Parainfluenza 2 and hMPV viruses were not detected in any of the patient samples. Gageldonk-Lafeber *et al.* (2005) predominantly detected influenza and rhino viruses in a study in Netherlands. Findings of Ahmed *et al.* (2012) indicated that the viruses predominantly detected were RSV (12.5%) and other viruses detected were hMPV (5.7%), parainfluenza (9.4%), influenza A (9.7%) and influenza B (2.6%). Bezerra *et al.* (2011) in another study reported detection of RSV in 37% of the subjects and hMPV in 10% while Bharaj *et al.* (2009) reported that out of 103 clinical specimen positive for respiratory viruses, RSV contributed the majority (59.2%). In other related studies, Kusel *et al.* (2006) found RSV (10.9%) to be among the most common viruses while Munywoki *et al.* (2011) reported RSV being detected in 24.4% of the study subjects.

Contrary to the findings of the current study which detected parainfluenza viruses in only 3.8% of the clinical samples and did not detect hMPV among the study participants, several previous studies reported parainfluenza virus and hMPV in over 10% and over 5.0% subjects respectively and found little involvement of influenza A (Munywoki *et al.,* 2011; Bezerra *et al.,* 2011; Bharaj *et al.,* 2009). The current study found a strong involvement of influenza A, RSV and influenza B among the study subjects. Among the influenza viruses, influenza A accounted for 66.7% compared to influenza type B, 33.3%. These findings are in concurrence with the report of the WHO Global Influenza Surveillance and Response System (GISRS). According to GISRS, during the epidemiological period January–September 2013, the period during which this study was conducted, among ILI cases reported, influenza A accounted for 86% of all influenza

viruses detected (728/845) and influenza B represented 13% (117/845) (www.who.int/flunet). The current study found that among the influenza type A detected, seasonal influenza type A (H3N2) was more predominant, 81.2% (13/16) compared to pandemic influenza type A (H1N1). The Global Influenza Surveillance and Response System (GISRS) report indicated that influenza A (H1N1) pdm09 transmission peaked around 9 weeks earlier than A(H3N2), resulting in a marked predominance of A(H1N1) pdm09 in the first 3 quarters of the season, with transition to A(H3N2) in the final quarter of the season. The findings of this study were consistent with the reports of the WHO GISRS in the southern hemisphere where more Seasonal H3N2 was reported compared to pandemic H1N1 and influenza B after the first three quarters of the season (www.who.int/flunet).

The study reported mixed infection of viruses and bacterial pathogens. A mixed infection was defined as the presence of a virus in combination another with one or more other viruses or with one or more bacterial isolates. Mixed infections were reported in 29.5% of the patients out of which bacterial and viral combination (78.3%) was predominant. These findings are in agreement with reports of previous studies which found mixed infection of viral and bacterial pathogens associated with ARI (Gageldonk-Lafeber *et al.,* 2005). These findings indicate that a good proportion of patients presenting with ARI may be co-infected with viral and bacterial agents and hence management may require multi-agent screening to ensure proper management and successful resolution of ARI is achieved where multiple etiological agents are involved. Potential mechanisms of interaction of viral and bacterial pathogens have been reported in literature. Infection with a viral pathogen may predispose an individual to bacterial infection through a number of viral-bacterial infections, a few of which are explained below (Bosch *et al.,* 2013; McCullers and Bartmess, 2003). McCullers (2006) explains one of the mechanisms on viral–bacterial interaction on synergism between influenza virus and *S. pneumonia.* Although an influenza virus infection alone can be fatal, mortality increases dramatically when a bacterial super-infection occurs, as in the case of the ‘‘Spanish flu’’ pandemic in 1918–1919 when millions of people died, most from secondary pneumococcal pneumonia. This is further underlined by animal experiments showing that death occurred in 35% and 15% of mice infected with either influenza virus or pneumococcus, respectively, whereas 100% of mice infected with both pathogens simultaneously

succumbed to infection within one day (McCullers and Rehg, 2002). Another mechanism of viral-bacterial interaction is viral predisposition and bacterial adherence which explains that since attachment of a pathogen to mucosal surfaces is the first step towards respiratory disease, and viral infection alters the defense of the host epithelium in general, it has been postulated that viral presence may render the epithelium more susceptible to bacterial colonization (Bogaert *et al.,* 2004). Respiratory viruses may also directly affect the immune system, for example by impairment of neutrophil function, decreased oxidative burst and enhanced neutrophil apoptosis, thereby increasing susceptibility to bacterial super-infection (McNamee and Harmsen, 2006). Additionally, some strains of influenza virus infection may predispose to superinfection by *S. aureus* due to ineffective natural killer (NK) cell recruitment and activation (Small *et al.,* 2010).

A breakdown of the most common pathogens in case patients and control subjects revealed that influenza A viruses were the most common pathogens in case patients (55.6%; p=001) while RSV was the most common pathogen among the control subjects (53.8%). Findings of the study are in agreement with the study by Mandell (2005) who reported influenza type A virus to be the most predominant virus in causing ARI (42%) while RSV was the most common viral agent in control (asymptomatic) subjects (17%). The detection of RSV in study subjects without symptoms is suggestive that asymptomatic subjects may harbor pathogens and could be a potential source of transmission of respiratory pathogens. Asymptomatic persons may act as unsuspected sources of infection.

5.1.2. Risk factors for acute respiratory infections

This study found a significant association of malnutrition with ARI (Adjusted OR=2.8; p=0.001). Malnutrition (severe and moderate) was found in 27.8% of the children in the study. Findings of this study compared with data from a population-based survey, conducted in Brazil by Cunha (2000) who reported that current and past malnutrition were associated with ARI in children under five. Earlier study by Deb (1998) also reported that ARI was more common among malnourished children compared to well-nourished children (52.2% vs. 28.8%; p = 0.001) with increased incidence of ARI related to deteriorating nutritional status (P = 0.05). Decreasing mid-arm circumference (p = 0.001) was reported to be associated with ARI by Kaushik *et al.* (1995). In other studies,

severe malnutrition increased the risk of acquiring ARI by 1.85 folds (Saeed and Bani, 2000) and in the absence of other factors malnutrition alone significantly influenced the ARI in children younger than two years old (Cunha, 2000). Chalabi (2013) in a recent case-control study among young children aged < 5 years reported that ARI was more common among children with indicators of malnutrition using two measures of malnutrition i.e. Welcome (p=0.007) and Gomez criteria (P< 0.001). In Gomez criteria, the average ''theoretical weight'' found by Gomez and colleagues among Mexican children normal children of different ages (weight for age) was used to calculate the severity of malnutrition. The severity of malnutrition was classified into three groups based on percentage of expected weight for age, first degree (mild) malnutrition where the patient has 76–90% of the ''theoretical weight'' average for the child's age, second degree (moderate) malnutrition (61–75%), and third degree (severe) malnurition (60% and less) while over 90% is considered normal (Gomez *et al.,* 1955). Wellcome classification evaluates the child for oedema and with the Gomez classification system. In this criterion malnutrition is classified based on percentage of expected weight-for-age and the presence or absence of oedema. Between 60 and 80% of expected weight is underweight in the absence of oedema, and kwashiorkor if oedema is present; under 60% of expected weight is marasmus in the absence of oedema, and marasmic kwashiorkor if oedema is present (Waterlow *et al.,* 1977).

Other studies have reported association between malnutrition and ARI (Savitha *et al.,* 2007; Mwiru *et al.,* 2013). All these studies underscored the role of nutrition in ARI. It has been earlier reported that impaired cellular immunity in malnourished children makes them more prone to ARI (Chalabi, 2013; Yellanthoor and Shah, 2013). Inadequate nutrition *in-utero* and during infancy and early childhood is closely linked to lifelong immune deficiencies and acute respiratory infections. Nutritional deficits may result from any combination of insufficient caloric intake, lack of protein, and inadequate levels of micronutrients. Acute Respiratory Infections generally occur more frequently, last longer, and are more severe in malnourished children, typically because the mucous membranes and other mechanical structures designed to keep the respiratory tract clear are impaired, and the immune system has not developed properly. Being underweight is especially dangerous: Worldwide, childhood underweight is responsible for more poor health than any other single factor, and in low-income countries it is one of the leading risk factor for

morbidity and mortality in children. Rodriguez *et al.* (2011) reported increasing evidence suggesting that protein-calorie malnutrition is the underlying reason for the increased susceptibility to infections observed. Moreover, he indicated that certain infectious diseases also cause malnutrition, which can result in a vicious cycle. Measures aimed at reducing malnutrition would significantly reduce the incidence of ARI which is one of the leading causes of morbidity and mortality in children.

It has been reported in literature that a child when fully immunized is protected against respiratory infections (Prajapati *et al.,* 2012). According to immunization status, 24 (9%) children were reported not to have completed childhood immunization compared to 143 (56%) who had completed and 88 (35%) who were still continuing with the programme. Completion of immunization was associated with reduced odds of ARI (OR=0.88, 95% CI: 0.37-2.09). A study by Prajapati *et al.* (2012) reported that 43.8% were fully immunized, 33.8% were not immunized and 22.4% were partially immunized children. The study reported direct correlation between immunization status of children and occurrence of ARI and that ARI incidence was least in fully immunized children ($p<0.001$). Other studies from developing region reported an association between incomplete immunization and severe ARI (Broor *et al.,* 2001; Savitha *et al.,* 2007). Completion of immunization should be encouraged to parents with young children to protect them from the common childhood infections.

Although not statistically significant, duration of breastfeeding was found to be associated with reduced odds of ARI in children breastfed for 4-6 months (OR=0.61) and over six months (OR=0.76). A study in Tanzania by Mwiru *et al.* (2013) showed that breastfeeding was associated with 52% reduction in risk of ARI (95% CI: 0.35–0.64) in the first 12 months of life. In a study conducted in Bagdad and Iraq, Al-Sharbatti and AlJumaa (2012) found that infants who had undergone a short duration of breastfeeding (<3 months) had a 1.4 times increased risk of ARI (CI: 0.89-2.23). Other studies have reported increased risk of ARI with reduced period of breastfeeding (Shah *et al.,* 1994; Arifeen *et al.,* 2001; Sinha *et al.,* 2003; Koch *et al.,* 2003).

Authors have suggested mechanisms by which breastfeeding can lower the risk of ARI. Arya *et al.* (1987) reported a mechanism that breastfeeding transfers to infants maternal

innate immune components such as lactoferrin, lysozyme and secretory IgA; influences of breast milk on immune-system maturation, and enhancement of the antibody response to pathogens may play a role in protecting children against ARI. However other epidemiological studies argue that the apparent protection of breastfed infants could reflect better overall nutrition for breastfed infants or reduced exposure to infectious agents rather than specific anti-infective substances in breast milk (Leon-Cava *et al.,* 2002). While breastfeeding is shown to be protective against ARI, it remains unclear how it protects infants against acute respiratory infection (ARI). Studies investigating the protective mechanisms of breastfeeding to ARI may provide explanations on the actual role of breastfeeding in reducing risk for ARI.

Several epidemiological studies, using different measures of crowding such as total number of residents in the home, number of siblings, number of persons sharing the bed, room occupancy, and population density, have reported an association between crowding and respiratory diseases (Ballard and Neumann, 1995; Cardoso *et al.*, 2004; Koch *et al.,* 2003; Prietsch *et al.,* 2008). When using the number of residents in the house and number of siblings as indicators for crowding, this study found increased odds of ARI in households with more than eight occupants and that households of children with more than five siblings living in the house were more likely to be cases than controls. These observations support the findings of previous research documenting a link between overcrowding and ARI. Banerji *et al.* (2009) reported ARI to be significantly associated with ARI acquisition (OR = 2.5, 95% CI: 1.1-6.1). Murray *et al.* (2011) found evidence of an increased strength of association as household crowding increased (≥3 people/room, OR 3.31, 95% CI 2.03-5.38). Okiro *et al.* (2008) in another study in Kenya found that houses with occupants more than three had more ARI cases than those with less. Living in close proximity to others, typically in overcrowded housing, is associated with higher levels of acute respiratory infections. In the growing cities of developing countries, slum communities that stack neighbors closely together allow pathogens to spread rapidly, especially in combination with inadequate ventilation, poor sanitation, and other toxic effects of poverty. Conditions within individual homes such as number of residents, number of siblings, and number of people who share a bed or a room add further risk. Crowding may plausibly increase the risk of respiratory infection by increasing the opportunity for cross infection among the family and chances for re-infection from

previously sick household members. The agents of such infections are readily transmitted, usually through air by droplets or aerosols, in crowded and ill-ventilated rooms where people are sneezing, coughing or simply talking and this is enhanced in crowded environment.

Although not statistically significant, there were reduced odds of ARI among parents with advanced education level. Okiro *et al.* (2008) found education higher than secondary school to be associated with protection. The study found that a caretaker with a college education (>12 years of schooling) was associated with reduced odds of ARI. An earlier study investigating risk factors for ARI in Kenyan children found parental literacy to be associated with increased risk of ARI (Ballard and Neumann, 1995). Other studies have reported a relationship between parental education and risk of ARI (Saeed and Bani, 2000; Prietsch *et al.,* 2008). A possible explanation for such observation is that better educated mothers may have higher income to be able to provide for a cleaner environment, better housing and better knowledge on ARI preventive measures than less well educated parents.

The study did not find significant association of parental age with ARI. However, parents aged more than 40 had reduced odds of ARI (OR=0.32). Few studies have reported an association of ARI with age. Saeed and Bani, (2000) reported increased prevalence of ARI mostly in children whose mothers aged 35 years. Contrary, a separate study reported that children of younger mothers (aged 15–34) were more likely to have suffered from ARI than other children (Mishra, 2003). Prietsch *et al.* (2008) reported that mothers aged 30 years or older were identified as a protective factor. It is expected that older parents may have more experience in managing various diseases and events especially if they have other children. This may also be attributed to higher education and income which provide better capacity to parents to take care of the ill children much more promptly. Children in families earning a monthly income of more than Kshs 15,000 (~US$176) were found to have reduced odds of ARI (OR=0.6, 95% CI: 0.14-2.63). Zimbabwe study found that children from households with higher standards of living were considerably less likely to have had ARI than those from low or medium standard of living households (Mishra, 2003). Prietsch *et al.* (2008) in a study in Brazil found that monthly family income less than US$ 200 was associated with increased risk of ARI.

The study found almost equivalent number of cases, 76 (59.4) and controls, (58.6%) among the male children and a similar distribution among females with 52 (40.6%) cases and 53 (41.1%) controls. There was no significant association of sex and being a case or control (p=0.957). Additionally, although this study found a slightly higher proportion of female children (32.1%) with bacteria isolated than males (20%) and a reverse trend for virus detection with males having a slightly higher proportion (48%) than females (42.9%), association of sex and pathogens detection among the children was not statistically significant. Similar findings were reported in a study in Zimbabwe which did not find prevalence of ARI to vary significantly with the sex of child (Mishra, 2003). On the contrary, Prajapati *et al.* 2012 reported more males (56.3%) with ARI than females (43.7%). However, in each of these studies no strong relationship was established between sex and ARI. A study of children in India, noted that the prevalence of ARI was high among boys than girls (Mishra and Retherford, 1997). The authors concluded this was due to strong preference of sons; the study reported that mothers in India are more likely to carry young boys than girls or keep them in the kitchen area while cooking. Due to this practice boys are likely to be exposed to smoke more than girls (Mishra and Retherford, 1997).

The current study found majority of the children enrolled were less than 1 year (31.6%) with a slightly higher proportion of cases (32.8%) than controls (30.5%). The association between risk of ARI and children age was not significant. Contrasting results have been reported on association of age and risk of ARI by various authors. Okiro *et al.* (2008) reported current age of the child as an independent predictor of ARI with increased risk of ARI found in children aged 12–17 months. Koch *et al.* (2003) in another study found age as a strong risk factor for both upper and lower respiratory tract infections, with the highest risk found among children aged 6–11 months. In another study by Prajapati *et al.* (2012), more ARI cases were seen in children aged 4-5 years (48-60 months). Viruses were detected in significantly higher proportion of children aged up to one year, 16 (61.5%, $p<0.03$). The possible explanation could be that the age category less than 18 months is more prone to infections due to under developed immunity.

This study found no significant association of birth order with risk of ARI, although it was observed that being last born had reduced odds of ARI (OR=0.47, 95% CI: 0.17-1.27). This contradicts the findings of Prajapati *et al.* (2012) who reported that occurrence of ARI was lowest among children who were first born while it was highest in 5th birth order. The possible explanation for the observation in this study may be that the parent of a last born may have more experiences from previous births and able to initiate preventive measures for ARI.

Univariate analysis found significantly more cases than controls belonged to homes where a member of the family was smoking (OR=1.89). These findings were in agreement with reports from previous studies that have associated smoking with increased risk of ARI infection (Banerji *et al.,* 2009; Okiro *et al.,* 2008; Koch *et al.,* 2013). A number of pollutants commonly found in indoor have been shown to adversely affect components of the defense mechanisms against infectious organisms. For example, the particulate phase of cigarette smoke and gas phase components have been found to adversely affect mucociliary function in *in vitro* models. Gaseous components that appear to be important include nitrogen dioxide, ammonia, cyanides, aldehydes, ketones, acrolein, and acids. Nitrogen dioxide has been shown to adversely affect both the mucociliary apparatus and humoral and cellular immune defenses. The complex mixture of sulphur dioxide and particulates may reduce the efficacy of host defenses against microbial agents and respiratory tract inflammation.

Other factors that may be associated with increased risk odds of ARI in this study although not significant were; living in houses made of timber or iron sheets (OR=1.89). Okiro *et al.* (2008) reported that living in a mud-walled house was associated with increased risk of ARI. Factors associated with protection were cooking from outside the house (OR=0.88, 95% CI: 0.45-1.75), not carrying the child while cooking (OR=0.66, 95% CI: 0.329-1.32). This situation put the child to less risk of exposure to smoke from cooking fuels which as explained below are thought to affect components of the defense mechanisms against infectious organisms by affecting the mucociliary function and exposing the person to risk of infection. Living in houses that had more than three bedrooms also presented trends of reduced odds (OR=0.54, 95% CI: 0.17-1.7) as well as living in houses with few occupants and where cooking place that was outside the main

living room (OR=0.65, 95% CI: 0.38-1.08). The increased rate of ARI may be related to contact frequency with previously sick people and exposure to more inocula with sequential inoculation from numerous contacts which increases their chance of severe disease where the room is crowded. Majority of the participants used the charcoal (48.5%) and wood (41.3%) as the main fuel for cooking. Although the study did not show strong relationship of cooking fuel with risk of ARI in general, there were increased odds of ARI among children from homes that were using solid fuels. Previous studies have reported association of cooking fuel with risk of ARI. Bhat *et al.* (2012) reported that cooking fuel other than liquid petroleum gas (LPG) was a significant risk factor for developing ARI (OR 4.73, 95% CI: 1.67-13.45). Okiro *et al.* (2008) reported that using firewood as the main cooking fuel was associated with increased risk of ARI. Another study that supported the role of cooking fuels in risk of ARI was by Bhat and Manjunath, (2013) who indicated that cooking fuel other than LPG (OR 3.58, 95% CI: 1.23-10.45) were found to be significant risk factors for ARI ($p < 0.05$).

5.1.3. Caregiver's knowledge and practices on transmission and prevention of acute respiratory infections

Majority of the respondents (77.7%) identified fever as a danger sign while the proportion that identified other common danger signs ranged from 6% - 56%. The caretakers' awareness about the danger signs of childhood illnesses was therefore poor as none of the caretakers were aware of all the danger signs of childhood illnesses. This would call for public health education especially during antenatal and post natal care as well as during community public health activities. In this study, majority of the respondents (83.6%) perceived ARI to be a serious disease and this did not differ significantly between cases and controls (p=0.694). According to most models of health behavior, perception of being at risk is a prerequisite for behavior change, a supposition supported by empirical studies (Brewer *et al.,* 2004). These models endorse the belief that a high perceived risk of harm encourages persons to take action to reduce their risk. However, the direction of the association between risk perception and behavior in empirical studies varies positively, negatively, or not at all (Brewer *et al.,* 2004; Brewer *et al.,* 2007).

Majority of the respondents correctly identified one mode of transmission was through air droplets (93.8%), however only a few were able to identify other possible modes of

transmission of ARI such as touching body of infected persons (7.4%) and touching contaminated objects (7.8%). Lau *et al.* (2009) reported the prevalence of respondents not knowing that the virus is transmittable via droplets, contact with affected persons and contact with contaminated objects were, respectively, 2.0%, 24.8% and 21.1%. Although the studies were conducted among geographically different populations, there was high level of knowledge that ARI transmission was via droplets among the respondents in the two studies. However, further health education to inform of other modes of transmission as well as preventive measures is important to the community in this area.

In this study about one third (32.9%) of the respondents indicated that hand washing was very effective in preventing the transmission and acquisition of ARI. Hand washing has been previously reported to be efficacious in preventing influenza (Balicer *et al.,* 2006; Abdullah *et al.,* 2004). In the study of Sadique *et al.* (2007) vast majority of the respondents (94%) believed that handwashing would be efficacious in preventing human-to human avian influenza. Such a belief was, in turn, associated with anticipated higher frequency of hand washing. Other studies have demonstrated association of hand washing with lowered risk of respiratory infection (Luby *et al.,* 2005; Rabie and Curtis, 2006). Hand washing may become very useful if commonly accepted as means of preventing infectious respiratory diseases. As the perception efficacy of hand washing was low among the study respondents, this community may be a good target for health education to encourage hand washing as a means of infection control for ARI and other childhood illnesses such as diarrhea.

Only 38.2% of the respondents indicated wearing face masks in public areas as highly effective in controlling ARI. A study in Hong Kong during SARS outbreak reported that over 75% of the population indicated wearing face masks in 2003 (Leung *et al.,* 2003). Another study in Hong Kong found that approximately half of the population reported that they would don a surgical mask in public places if they developed flu symptoms (Lau *et al.,* 2009). However, there are limited data to support the effectiveness of face masks to prevent infection in public settings (Cowling *et al.,* 2009; Cowling *et al.,* 2010). While the wearing of facemasks may help prevent infection transmission and acquisition in a clinical setting it is unclear whether they may confer the same preventive effect in

community settings and even if they do, their use may be unpopular owing to discomfort of wearing masks in public and costs may be prohibitive to motivate their use.

The risk of acquiring an infectious disease may stimulate persons to take precautionary actions to reduce this risk as they perceive it. The potential effect of this perceived risk induced behavior was apparent during the outbreak of severe acute respiratory syndrome (SARS) in 2003 (Bell, 2004). For example, use of public transportation in affected areas and international flights to these areas were reduced dramatically (Bell, 2004; Abdullah *et al.,* 2004). In this study 82.8% of respondents indicated they would avoid going to crowded places, 15.6% would avoid going out where they may contact infected persons unless necessary and 2.7% would avoid going to hospitals where they would potentially acquire the disease. These findings support the study by Sadique *et al.* (2007) who reported that about 75% of respondents reported that they would avoid public transportation and 20%–30% would try to stay indoors. These reported actions are also in agreement with those reported in similar hypothetical studies and recorded behavior in the face of an epidemic (Balicer *et al.,* 2006). Precautionary actions, such as avoiding public transportation or avoiding situations in which persons congregate, may have potential epidemiologic effects and would be expected to have economic consequences. The demand for certain goods and services may decline, and output may be reduced if persons avoid work or social interactions and associated purchase of goods (Sadique *et al.,* 2007). Depending on different lifestyles and socio-cultural variations these avoidance factors may differ with regional differences.

Over half of the respondents were confident with government's preparedness in case of a serious outbreak of ARI. Eighty point nine percent were confident that local health system has enough medication for treating ARI while 55.9% believed there were enough vaccine for preventing ARI and 51.2% were confident the country will be able to control an outbreak. However, only a small proportion of respondents (13.3%) thought that the local hospitals have enough personal protection equipment for preventing ARI. Perception of government capacity to control and prevent diseases is likely to increase the confidence of the population to seeking services from government institutions and this may influence their actions in case of occurrence of events such as outbreaks. Building

confidence during public health education may influence the health seeking behavior and preventive actions and responses of the people during outbreaks.

5.1.4. Health care seeking behaviors for childhood illnesses

Attainment of the Millennium Development Goal (MDG) 4, i.e. reducing under-five mortality by two-thirds by 2015, requires improvements in the quality of care provided to children and in healthcare-seeking behaviours of their families. Care seeking interventions have the potential to substantially reduce child mortality, in the country where common childhood illnesses are a major problem. Prompt and appropriate care seeking practices have importance to avoid many deaths attributed to delays and not seeking care particularly in developing countries. A better understanding of the factors that influence healthcare-seeking is critical to ensure that policies and programmes effectively address the constraints families face and build upon the enabling factors that promote appropriate healthcare-seeking.

Majority (92.8%) of the study respondents indicated seeking health care from a health facility (appropriate care) with a small proportion of participants mentioning that they commonly seek inappropriate care from traditional doctors (2.4%), direct purchase of drugs from pharmacies (2.8%) or use home remedies (2.0%). These findings were in agreement with reports of an earlier study documenting health seeking behavior which indicated that among the alternative care sought traditional healers topped the list (Jimba *et al.,* 2003). Another study in Nepal found that medical shop and traditional healers were common sources of alternative medical care (Shankar *et al.,* 2003). Among the respondents, inappropriate care seeking behavior among the cases (50%) was mainly motivated by the fear of disease worsening while among the controls (38.9%) close proximity of a pharmacy was their main reason for the seeking inappropriate care.

The main motivating factor for appropriate care seeking behavior was the parental perception that care given at the health facilities was superior (cases, 46.1%; controls, 35.4%) to other treatment options**.** Patients' trust in the health providers is an important consideration for utilization of health services. Increasing trust to the health care seekers increases the likelihood of choosing healthcare from health facilities relative to alternative inappropriate treatment. Muriithi (2013) in a study on care seeking behavior in Kibera

slums in Kenya observed that the more trusting the relationship that the provider builds with their patients, the higher the probability of a visit to that provider in the event of illness or injury relative to going for self-treatment.

Family sizes with less than five persons were more likely to seek appropriate care than those with more than 5 persons (p=0.0001). The study further observed that children with less than two siblings were more likely to seek appropriate care than those with more (p=0.001). Earlier studies indicated that having a large family increases the probability of visiting both public and private health facilities compared to self-treatment. Sahn *et al.* (2003) found household size to be positively related to probability of seeking health care from the formal health care system. Muriithi (2013) was in agreement with this observation that larger family size is related to better health care seeking. Bolduc *et al.* (1996) who argued that the greater the number of members of the family who were employed, the less likely individuals seeks self-medication. Finding of this study contradicts this observation as larger family size does not necessarily mean members are working or educated to provide support for appropriate health care seeking. The possible explanation for the observation in the current study is that in smaller sized families, there is less likelihood of having competition for resources and parents may tend to pay more attention to seeking appropriate health care. Another possible reason is that in a large household there may be less attention to members of the household in terms of their nutritional needs and the income may be limited to meeting critical needs, thereby reducing their probability of using medical care where it is likely to cost more. This reasoning can however be affected by other factors such as education, formal occupation and socio-economic status.

Parental education above primary school was positively associated with seeking appropriate care (p=0.0166). This result supports the belief that educated individuals are more likely to seek out professional heath care relative to self-treatment. These results are consistent with previous findings reporting that education positively affects demand for health care (Sahn *et al.,* 2003). Hutchinson (1999) found that more educated women had a higher likelihood of seeking health care than less educated ones. These findings are also in line with the general notion that the pattern of reporting morbidity and contacting a health professional tends to increase with the level of education owing to their

understanding of the importance of seeking appropriate care. Additionally people who are more educated are more likely to be employed and likely to have higher income which would encourage them to seek health care from a health facility.

Delivering from a health facility was found to be strongly associated with seeking health care at health facilities than home delivery ($p<0.0001$). This finding supports the report by Pfeiffer and Mwaipopo (2013) who found that women who delivered at health facility especially from urban site have high level of awareness on importance of seeking health services from qualified medical services. This would explain their preference of seeking appropriate care at a health care facility than those who deliver at home and have limited access to health information. Lack of adequate health information has been associated with variations in health care utilization at various health facilities (Thompson *et al.,* 2003)

Parents with higher income, married and had formal occupation were more likely to seek appropriate care. Rahman *et al.* (2010) demonstrated the role of income and occupation in influencing medical care seeking. Other studies have supported this observation (Sreeramareddy *et al.,* 2006). Muriithi (2013) on the other hand found that occupation did not have a significant impact on the choice of health facility. Findings of this study are in consistent with the widely held assumption that those who are formally employed are likely to have higher income and hence finances available to seeking of health services.

The study found that caretakers who perceived that the disease was severe were more likely to seek health care than those who felt it was not serious (OR=4.787). This finding is consistent with findings of several studies that found that the perceived severity of illness influences decision to seek health care (Goldman *et al.,* 2002; Pillai *et al.,* 2003; Sreeramareddy *et al.,* 2006; Taffa and Chepngeno, 2005; Tsion *et al.,* 2008; Magu *et al.,* 2011). It is assumed that maternal perceived illness severity would influence decisions to seek appropriate health care. Where the perceived severity of the disease was high, caretakers may fear that the disease may develop complications that may be difficult to handle if they sought alternative health care away from appropriate health facilities.

Although this study did not find an association of distance to the health facility with choice of health care (distance 0.71, 0.25-1.9) previous reports shows that distance has a significant and negative impact on the choice of a health facility. Increasing distance would increase the likelihood of a household opting for self-treatment rather any of the formal health providers (Sharkey *et al.,* 2011; Miriithi, 2013). An increase in distance implies paying some extra cost to travel to the source of treatment as opposed to seeking self-treatment. There is a sense in which distance adds an extra burden to the monetary cost of treatment. This study did not find shorter distance to influence appropriate care seeking probably because many caretakers especially from rural areas generally walk long distances to the health facility and whether the distance is short or long would not add monetary burden to the cost of treatment, although on the other hand long distance to health facility may reduce the motivation to seek appropriate care.

5.1.5. Estimates of cost of illness for acute respiratory illnesses

The total mean cost of managing ARI was $17.70 (urban=$21.51; rural=$13.41) with consultation expenses and cost of prescribed medicine constituting the highest cost drivers for management of ARI accounting to about 37% of the total mean cost ($ 6.5±4.7) and 29.3% ($5.2±17.6) of the total costs respectively. Monte *et al.* (2008) in a study in USA found that antibiotics were responsible for 15% of payer costs. Hollinghurst *et al.* (2008) conducted a cost of illness study in preschool going children in United Kingdom (UK) and found that average cost per episode was £27.43 (US$52) with costs related to consultations with general practitioners accounting for accounted for 93% of this cost (£22.91; US$43), cost per episode to parents and caregivers was £14.77 (US$28) and mean expenditure per child on over-the-counter preparations was £1.32 (US$2.5). Lambert *et al.* (2008) in a separate study in Australia, found an average cost of AU$309 (US$293) to manage all ARI's with a range of AU$180 to AU$553 (US$171-525) per episode. As would be expected, there are cost differences between these studies done elsewhere in the world owing to differences in cost of healthcare and economic differences between developing and the developed nations. However, the studies were in agreement on the consultation costs and cost of medication being the major cost drivers. The study also found differences in the costs between the rural and urban health sites possibly due to lower transport cost, cost of medicine and consultation costs at the rural

site. Nevertheless, the estimates may provide an indication of the episode cost for planning purposes in management of ARI in similar settings.

5.2. Conclusions

Following results of this study, the following conclusions are made:

i. Acute respiratory infections in children are broadly of viral etiology with influenza type A, RSV and influenza type B being more predominant.
ii. Mixed bacterial and viral etiology was common among the children.
iii. Malnutrition was was found to the main be modifiable factor associated with risk of ARI in children under 5 years of age.
iv. Smoking was the major the environmental factor found to be associated with ARI.
v. Knowledge on the major route of transmission of ARI was high among the study respondents but knowledge on common prevention measures for ARI need to be enhanced.
vi. Seeking health care from health facilities was found to be high among the respondents mainly motivated by caretakers' perception that services provided at health facilities were superior to alternative care. Parental education, delivering at health facilities, hospital being near and fear of complications related to the disease were positively associated with appropriate health care seeking behavior among the study subjects.
vii. The average cost of managing ARI in this population was about $18 and was higher in the urban than rural health facility. The greatest cost drivers were clinicians' consultation and cost of prescribed medicine.

5.3. Recommendations

i. Development of ARI vaccine should mainly cover influenza type A, RSV and influenza type B which were the main predominant viral pathogens found in this study and in similar studies conducted elsewhere in the country.
ii. There is need for community-based interventions directed towards improved diet, supplementation (vitamin supplements or fortified milk) and parental education (promoting breastfeeding) to have significant positive benefits in reducing malnutrition.

iii. Accelerating mandatory implementation of the Kenya's new legislation on boosting the nutritional value of basic staples such as flour and cooking fat through fortification could be a significant step towards reducing malnutrition rates in the study area and the country and potentially reduce the incidence of ARI and other childhood diseases.

iv. In management of ARI, diagnosis should cover both viral and bacterial pathogens.

v. Efforts should continue to promote indoor air pollution reduction in the community to reduce the health effects especially smoking and other environmental risk factors associated with ARI. It is recommended that health education activities be enhanced to promote improved environmental sanitation and to reduce crowding. The interventions may include; promotion for cleaner burning fuels, improved cooking stoves, housing design and reduction of crowding. Designing public information campaigns to inform people about the health risks of exposure to indoor environmental pollution involving communities should be given priority. In the long term, the country and national government should work towards facilitating affordable houses with good ventilation and bigger sized rooms especially in the urban lower resource settings in Nakuru.

vi. Smoking in the household should be strongly discouraged to reduce the incidence of ARI and other smoking related risks.

vii. Government efforts to reduce cost of management of ARI and improve on the care seeking behavior should mainly target these two prime cost drivers i.e. clinician's consultation and cost of medicine.

Generally, it is recommended that the government and academicians should work jointly to create a communication platform with the general public, through which scientific knowledge and guidelines for adopting particular preventive measures for ARI are disseminated. Since community responses to the ARI epidemic are fluid and dynamic, continual surveillance of community responses is valuable and would facilitate relevant governmental risk communication and health education efforts.

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APPENDICES

Appendix 1: Informed Consent Document

Aetiology, risk factors and community economic burden of Acute Respiratory Infections in children under five year selected facilities in Nakuru district, Kenya

Introduction

We are conducting a study to investigate the issues that pose danger in children (risk factors) to acute respiratory infections in order to look for solutions of reducing the disease burden in the community. In order to be sure that you are informed about being in this research, we are asking you to read (or will read to you) this consent form. The purpose of this consent form is to give you the information you will need to help you decide whether or not to participate in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. Before you decide if you wish to be in this study, you need to know about any good or bad things that may arise if you decide to join. This form tells you about the study. This consent form may contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Being in the study is your choice

When we have answered all your questions, you can decide if you want to participate in the study or not. This process is called 'informed consent. This consent form gives you information about the study and the risks were explained to you. Once you understand the study, and if you agree to take part, you were asked to sign your name or make your mark on this form in the presence of a witness. We will give you a copy of this form for your records.

Before you learn about the study, it is important that you know the following:

- Your participation in this study is entirely out of your choice (voluntary)
- You may decide not to answer questions, give any specimens or even withdraw from the study at any time.

Purpose for the Research

We are asking you to participate in this study to help us assess the viruses or bacteria that causative agents, issues that put children at risk for acute respiratory infections (ARI) in this region. We would also like to get information on the cost of managing this disease to you and the community in children who are under the age of 5 years. We would also like to know the costs related to managing the ARI including the doctors' fees, travelling, days off work among others. In addition, we would like to establish the causative agent of ARI and hence we may request for your consent to collect a throat specimen (swab) and nasal specimen (nasopharyngeal swab) should your child be selected to participate in this part of the study.

Study groups

The study groups will comprise of adult women and men who are parents or guardians of children under the age of five years and their children. All groups of people mentioned here are very important to this study.

Procedures

If you agree to participate in this study by signing at the end of this form, you will participate in the following activities: You will be questioned about your personal life related to this study such as your education background, income and sources of income. You will also be asked questions to assess your knowledge on prevention of acute respiratory infections (ARI) as well as questions regarding your lifestyle issues that may be potentially put your child to risk for ARI. We shall also request you to provide us with details of length of the ARI in your child and the cost related to management. This questionnaire will take approximately 30 – 45 minutes of your time. This were a one-time assessment.

We will select some children and take nasal specimen to help us in investigation the cause of ARI. If your child is selected, we also request that you consent to remove the nasal sample from him/her for further investigations in the laboratory. This selection were done in a way that ensures that every child has equal chance of being included into the study and you will receive the medical services as usual regardless of whether your child was selected or not. In case your child is selected in this group, you were required to visit the

facility to obtain the results of the laboratory investigations after two weeks. The specific dates were provided by the study clinician.

Precautions

Your child might feel a little discomfort when the nasal specimen is removed, however, there are no other expected complications associated with this exercise. The team is well trained and experienced staff will guide you through this exercise and will take necessary precaution to ensure minimum discomfort.

Possible Risks/discomfort

There are no disturbing procedures that were carried out on you. You may feel uncomfortable during the interview due to the sensitive nature of the some questions including loss of privacy, but safeguards were implemented to minimize this risk. We will minimize risk and discomfort from the interview by using a trained staff to place you at ease during the interview. You may skip any questions that you do not want to answer and may terminate the interview at any time without consequence. You will also be free to withdraw from the study any time you feel like.

Data security and Confidentiality

All the information gathered by the research team were used in confidence for the sole purpose of this research only. Any records relating to your identity and test results will remain confidential. Your name will not be put (divulged) in any report of the results, and you will receive a copy of this consent form. No one will have access to the interviews except the researchers and supervisors. The study team will provide you with examination results immediately for the tests carried out in KEMRI. The information obtained from KEMRI laboratories were put (pooled) together with those of other individuals participating in this study. Strict data management procedures are intended to ensure confidentiality of the study subjects.

New findings

Results were distributed/ disseminated to the relevant health ministries in Kenya, the district and other stakeholders in the country. The findings were used to provide

information to be used for improving management and help to identify ways of reducing the burden of ARI in all communities in the country.

Benefits

Results obtained will aid in making recommendations of taking on (employing) new public health approaches/methods to reduce the burden of ARI in the community.

Costs to you

There is no cost to you for participating in the study.

Reimbursement

A participant is this study you were reimbursed for out of pocket expenses spent to visit the study site. The compensation will include return bus ticket at a rate of Kshs 250 per visit related to the study.

If You Decide Not to Be in the Research

You are free to decide if you want to be in this research. Your decision will not affect the health care/service you would normally receive. You will therefore receive the usual treatment you deserve within the health facility.

Leaving the Research

If you choose to be in the study, you can still decide not to complete the interview. If you leave the study, please tell the interviewer why you are leaving so that this information can be used to improve our work and provide more support if possible.

Problems and questions

If you ever have questions about this study, you should contact: Martin Matu, Study Principal Investigator, (Mobile: +254 721 374 830).

Your rights as a Participant

This research has been reviewed and approved by the Ethical Review Committee of the Kenyan Medical Research Institute (KEMRI), if you have any questions about your rights

as a research participant you may contact the secretary of the KEMRI ERC (a group of people who review the research to protect your rights) at 020-272-2541, or 020-272-6781.

Your statement of consent and signature

If you have read the informed consent, or had it read and explained to you, and you understand the information and voluntarily agree to join this study, please carefully read the statements below and think about your choice before signing your name or making your mark below. No matter what you decide, it will not affect your rights in anyway:

- The risks and benefits involved in this study have been read and explained to me.
- I have been given the chance to ask any questions I may have and I am content with the answers to all of my questions.
- I know that my records were kept confidential and that I may leave this study at any time
- The name, phone number and address of whom to contact in case of an emergency has been told to me, and has also been given to me in writing.
- I agree to take part in this study as a volunteer, and were given a copy of this informed consent form to keep.

_____

Participant's Name (printed)

_____ _____

Signature or Participant or thumb print (for those who cannot sign) Date

If volunteers cannot read the form themselves, a witness must sign here:

I was present throughout the entire informed consent process with the participant. All questions from the subject were answered and the participant has agreed to take part in the research.

_____

Printed Name of Witness

_____ _____

Signature of Witness Date

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____

Printed Name of Person Who Obtained Consent (Study staff)

_____ _____

Signature of Person Who Obtained Consent Date

NOTE: You are not giving up any of your legal rights by signing this informed consent document.

Appendix 2: Kiambatisho 2 - Khabari Idhini Kudhibiti

Utafiti kuhusu "Viini husika, sababu za hatari, mzigo wa kiuchumi kwa jamii, wa maambukizi ya magonjwa ya sehemu za kupumua (ARI) katika watoto waliochini ya miaka tano katika baadhi ya vituo vya afya, Wilaya ya Nakuru, Kenya.

Utangulizi

Sisi tunafanya utafiti ili tuchunguze ni vitu gani vinavyohusika na maambukizi ya magonjwa ya sehemu za kupumua (ARI) katika watoto ili kutafuta suluhisho za kupunguza magonjwa haya katika jamii. Ili kuwa na uhakika kwamba wewe umepata taarifa zote kuhusiana na utafiti huu, sisi tunakuuliza wewe usome (au pia usomewe) fomu hii ya idhini. Madhumuni kuu ya fomu hii ya idhini ni kukupa wewe habari unayohitaji kukusaidia kuamua kama utashiriki ama hautashiriki katika utafiti. Tafadhali soma fomu hii kwa makini. Unaweza kuuliza maswali kuhusu madhumuni ya utafiti, nini tunataka kukuuliza wewe kufanya, hatari, faida, haki zako kama mhusika wa kujitolea, na kitu kingine chochote kuhusu utafiti au fomu hii ambayo hukuelewa. Fomu hii inkuelezea kuhusu utafiti. Fomu hii ya idhini inaweza ikawa na maneno ambayo usiyoelewa wewe. Tafadhali kama hukuelewa maneno hayo tuulize sisi tukueleze.

Kuwa katika utafiti huu ni kwa hiari yako

Wakati tumeyajibu maswali yako yote, unaweza kuamua kama unataka kushiriki katika utafiti au la. Shughuli hii unaitwa 'ridhaa'. Fomu hii ya idhini inakupa habari kuhusu utafiti huu na hatari za kuhusika. Mara baada ya kuelewa utafiti, na kama wewe unakubali kushiriki, utaombwa uweke sahihi/saini au uweke alama yako ya kidole katika fomu hii mbele ya shahidi. Tutakupa nakala ya fomu hii kwa kumbukumbu zako.

Kabla ya kujifunza juu ya utafiti huu, ni muhimu kwamba wewe kujua yafuatayo:

- Ushiriki wako katika utafiti huu ni hiari yako kabisa
- Unaweza kuamua kutokujibu maswali, kutokupeana sampuli yoyote au hata kuondoka kutoka utafiti wakati wowote.

Kusudi la Utafiti

Tunakuuliza wewe ushiriki kwenye utafiti huu ilituweze kuchunguza ni viini vipi, hatari ya maambukizi ya ARI katika mkoa huu. Tungependa pia kupata maelezo juu ya gharama ya matibabu ya magonjwa haya kwako wewe na kwa jamii kwa ujumla, zaidi kwa watoto

ambao wako chini ya umri wa miaka 5. Tungependa pia kujua gharama kuhusiana na kudhibiti ARI ikiwa ni pamoja na ada ya madaktari, kusafiri, siku ngapi za 'ofu' uliyochukua kutoka kazi ili kumwangali mtoto, miongoni mwa wengine. Aidha, tungependa pia kujua ni viini gani vinahusika na ugonjwa wa ARI na hivyo sisi tunaoomba pia kupata sampuli za pua na koo kwa uchunguzi huo, hii ni kama mtoto wako atachaguliwa kushiriki katika hii sehemu ya utafiti.

Vikundi vya Utafiti

Vikundi vya utafiti vitawahusisha kina mama na wanaume ambao ni wazazi na pia walezi wa watoto pamoja na watoto wao waliochini ya miaka tano. Vikundi hivi vyote vilivyotajwa hapa ndio muhimu sana kwenye utafiti huu.

Taratibu

Kama unakubali kushiriki katika utafiti huu na kuweka saini/sahihi mwishoni mwa fomu hii, utashiriki katika shughuli zifuatazo: Wewe utahojiwa kuhusu maisha yako binafsi kuhusiana na utafiti huu kama vile elimu yako, mapato na vyanzo vya mapato. Unaweza pia kuulizwa maswali ya kutathmini maarifa yako juu ya kuzuia maambukizi ya ARI, na pia maswali kuhusu maisha yako ya kawaidi ambayo inaweza changia mtoto kushikwa na magonjwa haya ya ARI. Tutakuomba pia utupatie maelezo kuhusu muda mtoto alivyouguwa, na gharama kuhusiana na usimamizi. Maswali haya yatachukua takriban dakika kama 30 – 45 ya muda wako. Hii itafanyika mara moja tu.

Sisi tutawachagua baadhi ya watoto na kuchukua sampuli za koo na pua ili tuchunguze viini vinavyosababisha ARI. Kama mtoto wako atchaguliwa, tutakuomba pia utupe ruhusa ya kuchukua sampuli kutoka kwake kwa uchunguzi zaidi katika maabara. Madhumuni ya uteuzi huu ni kuhakikisha kuwa kila mtoto ana nafasi sawa ya kuwa pamoja na kupokea huduma ya matibabu kama kawaida bila ya kujali kama mtoto wako alichaguliwa au la. Mtoto wako akichaguliwa katika kundi hili, utahitajika kutembelea kituo ili kupata matokeo ya uchunguzi wa maabara baada ya wiki mbili. Utaelezwa tarehe maalum ya kuja kituoni na daktari wa utafiti huu.

Tahadhari

Mtoto wako anaweza kuhisi usumbufu kidogo wakati sampuli ya koo na pua inapochukuliwa, hata hivyo, hakuna usumbufu mwingine utatarajiwa baada ya zoezi hili.

Wahusika wa utafiti wamefunzwa kabisa jinsi ya kuchuka sampuli na pia wako na maarifa ama uzoefu. Kwa hiyo watawaongoza katika zoezi hili na kuchukua tahadhari ya muhimu ili kuhakikisha usumbufu niwakiwango cha chini.

Usumbufu/ Hatari zinazowezekana

Hakuna taratibu za kumwingilia/kumvamia mgonjwa utakaotumiwa. Unaweza kujisikia na wasiwasi wakati wa mahojiano kutokana na undani wa baadhi ya maswali, lakini kanuni zimewekwa ili kupunguza hatari hii. Hivyo basi tutapunguza hatari na usumbufu wakati wa mahojiano kwa kutumia wafanyakazi wenye ujuzi. Sio lazima ujibu maswali yote na unaweza kukomesha mahojiano wakati wowote. Unauhuru wa kujiondoa kutoka utafiti kwa wakati wowote.

Usalama wa Taarifa na Usiri

Habari zote zitakazokusanywa na timu ya utafiti zitatumika kwa madhumuni pekee ya utafiti huu. Habari zinazihusika na wewe na matokeo ya upelelezi wa maabara zitahifadhiwa kwa siri. Majina yako haitatumika katika ripoti yoyote ya matokeo, na utapokea nakala ya fomu hii ya ridhaa. Hakuna mtu yeyote wa nje atakuwa na ruhusa ya kuona ripoti ya maswali, ila wasimamizi wa utafiti huu. Timu ya utafiti itakupa matokeo ya vipimo mara moja baada ya kufanywa huko KEMRI. Ripoti/matokeo ya upelelezi kutoka kwa maabara ya KEMRI zitajumwishwa pamoja na za watu wengine katika utafiti huu. Mikakati kali itawekwa ili kudumisha usiri wa wahusika utafiti.

Matokeo Mapya

Matokeo itasambazwa kwa wizara ya afya nchini Kenya, wilaya na washikadau wengine katika kwa madhumuni ya kuanzisha juhudi za kuzuia magonjwa ya ARI nchini. Matokeo ya utafiti wa mradi huu zitatumika kutoa taarifa ya kutumika kwa ajili ya kuboresha usimamizi na kusaidia kubainisha njia ya kupunguza viwango vya magonjwa ya ARI katika jamii zote nchini.

Faida

Matokeo ya utafiti huu itasaidia katika kutoa mapendekezo ya kuajiri mbinu mpya ya afya ya umma na kupunguza viwango vya magonjwa ya ARI katika jamii.

Gharama kwako

Hakuna gharama yoyote utakayotozwa wewe kama mshirika katika utafiti.

Kurudishiwa gharama

Mshirika wa utafiti huu atarudishiwa gharama za mfukoni alizotumia kutembelea kliniki/hospitali ya utafiti. Ukihusika na utafiti, utapokea fidia ambayo itasimamia tiketi ya basi na niya kiwango cha shilingi 250.

Kama utaamuwa kutokushiriki na Utafiti.

Wewe uko huru kuamua kama wanataka kuwa katika utafiti huu. Uamuzi wako hautaathiri huduma za afya utakazopokea kwa kawaida. Wewe utapokea huduma na matibabu ya kawaida unayostahili hapo kwenye kituo cha afya.

Kuondoka Utafiti

Kama wewe utaamua kuwa katika utafiti, basi unaweza kuamua kutokukamilisha mahojiano. Na ukiondoka kwenye utafiti, tafadhali mweleze anayekuhoji kwa nini wewe unaondoka ili habari hii iweze kutumika kuboresha kazi zetu na kutoa msaada zaidi kama inawezekana.

Matatizo na maswali

Kama utakuwa na maswali kuhusu utafiti huu, unapaswa kuwasiliana na: Martin Matu, Mpelelezi Mkuu, kupitia namba hii ya simu +254 721 374 830.

Haki yako kama Mshiriki wa Utafiti

Utafiti huu umekaguliwaa na kupitishwa na Kamati ya Maadili ya Shirika la Utafiti wa Kiafya (KEMRI). Kama utakuwa na maswali yoyote kuhusu haki zako kama mshiriki wa utafiti unaweza kuwasiliana katibu wa ERC KEMRI (kikundi cha kukagua tafiti mbalimbali ili kulinda haki yako) kupitia 020-272-2541, au 020-272-6781.

Taarifa yako ya ridhaa na saini

Kama umesoma ridhaa hii, au ulisomewa na kuelezwa, na ukaelewa habari iliyohumu ndani na kwa hiari kukubaliana kujiunga utafiti huu. Tafadhali kwa makini soma kauli

chini na kufikiri kuhusu uchaguzi wako kabla ya kusaini jina yako au kuweka alama ya kidole yako. Uamuzi wako, vyovyote vile, hauta adhiri haki zako:

- Nimesoma nakuelezewa hatari na faida zote za kushiriki katika utafiti huu.
- Nimepewa nafasi ya kuuliza maswali niliyokuwa nayo na nimetosheka na majibu yote ya maswali yangu.
- Najua kwamba kumbukumbu yangu itakuwa siri na naweza kuondoka utafiti huu wakati wowote.
- Wakati wa dharura, majina, namba za simu na anuani za mplelezi mkuu nazifahamu.
- Kwa hiari yangu nakubali kuhusika na utafiti huu, na nimepewa nakala ya fomu hii ridhaa ya kutunza.

_____

Jina la mshiriki (Andika)

_____ _____

Sahihi/Saini au alama ya kidole Mhusika Tarehe

Kama mhusika wa kujitolea hawezi kusoma, basi shahidi apige sahihi/saini hapa:
Nilikuwepo wakati wote fomu ya ridhaa ilipoelezwa kwa mhusika. Mswali yote kutoka kwa mhusika yalijibiwa na amekubali kujiunga na utafiti huu.

_____

Andika Jina la Shahidi

_____ _____

Sahihi/Saini ya Shahidi Tarehe

Nahakikisha ya kwamba mhusika ameelezewa kuhusu hali, umuhimu, faida na hatari zinazohusika na utafiti huu.

_____

Jina la aliyepokea makubaliano (Mtafiti)

_____ _____

Sahihi/Saini ya aliyepokea ridhaa Tarehe

KUMBUKA: Ukiweka sahihi/saini yako hapa, hautapoteza haki zako za kupata matibabu yanayofaa.

Appendix 3: Standard operating procedures for patients recruitment and enrolment

Patient consenting and recruitment

Recruitment of cases

Materials

1. **RANDOM SAMPLING CARDS** – Cards to help in random selection of children to be sampled for collecting specimen for laboratory investigation of causative agent marked 'NO' or 'YES'.
    a. NO card – child not included for specimen collection;
    b. YES card – child included for specimen collection

Inclusion criteria

iv. Parents or guardians with children under 5 who have suspected ARI at enrolment consulting the clinician for the first time in that episode
v. Over 18 years of age
vi. Accept to give consent to participate and consent to their children to be sampled for ARI aetiology confirmation

Note: The study will sample 30% of the cases i.e. children diagnosed with ARI to test for viral and bacterial aetiology of ARI.

Exclusion criteria

iii. Those who fail to consent
iv. Those with children above 5 years of age
v. Complaints of an ARTI in the last two weeks, belonging to the same household as the case,
vi. Use of antibiotics or anti-viral medication in the last two weeks

1. From the outpatient clinic, the clinicians identified children who meet the case definition of **Acute Respiratory Infections** (ARI) in children as follows:
   A child with sudden onset of fever with cough and /or sore throat in the absence of other diagnoses. Fever considered as elevated temperature above expected ranges i.e. rectal temperature above 38ºC; oral temperature above 37.8ºC; axillary (armpit) temperature above 37.2ºC; ear (tympanic membrane) temperature above 38ºC in rectal mode or 99.5ºF (37.5ºC) in oral mode or forehead (temporal artery)

temperature above 100.4ºF (38ºC). Fever will be considered as axillary temperature of above 37.2^{o}C. Children will also be defined as febrile if they reported recent history of fever and had taken medication to relieve the fever (including ibuprofen or paracetamol) during the previous 12 hours.

2. The clinician reviewed the patients (under five children with suspected ARI) and provided the necessary care and management as per the hospital guidelines
3. Following this provision of services the clinician requested the participants (guardians/parents of the children with ARI) to join the study. They explained the objectives of the study and what the study involves and refered them to the study research assistants for more details.
4. The study research assistants explained in details the benefits of the study to the participants and the procedures involved as outlined in the informed consent document and request them to join the study and consent for their children's specimen collection should they be selected for specimen collection to identify the causative agents for ARI.
5. Those who were willing to join the study were requested to give the consent by signing the consent document (**Appendix 1 and Appendix 2**).
6. The participants were given Study Identification Numbers to be used on the questionnaires for anonymity.
    a. The study numbers followed the following sequence – First letter of the study site, followed whether the participants represents a case (CS) or Control (CTS) then the numerical order in which the participants are recruited.
    b. For example the first participant in FITC representing a case was given the number 'FITCS001'.
    c. The first control from the same facility had 'FITCTS001' but the form also indicated the number of the case for which the control is matched i.e. FITCS001 (matched for FITCS001) if the control number 1 was the matching for the first case recruited.
7. The research assistants then administered the questionnaire to the study subjects **(Appendix 4 and 5)**; for those who prefered to fill in the questionnaire were be given the questionnaire to complete.

8. Following the administration of the questionnaire; the research assistant requested the participants (guardians/parents) to pick one RANDOM SAMPLING CARD through the mouth of a closed opaque box
    a. *To ensure that the participants randomly select the cards the participants dipped in the hands in the opaque closed box so that the card they pick is selected by chance.*
    b. *For participants who pick a "YES", specimen were collected from their children for further investigations while for those who pick a 'NO' specimen were not be collected from their children for the purpose of this study.*
    c. *However, for those who requested that the study team collect specimen from their children for investigations despite picking a 'NO', then the study team collected the samples and label them accordingly for the benefit of the patient.*
    d. *GREEN cards will constituted 33% of the total cards per site*
9. Participants who selected a green card were requested to allow the clinician to collect throat swab specimen and nasopharyngeal specimen for further investigation for the causative bacterial and viral agent for ARI **(Appendix 11).**
10. After collection of the specimen, the clinician will measure the mid upper arm circumference (MUAC) for determination of the children nutritional status as described in **Appendix 8** and compare any relationship with ARI infection.
11. Any child found to be severely malnourished; the clinician provided the necessary support to the parents/guardian to manage the problem as per the facility protocols.

Recruitment of Control subjects

1. The study research assistants were provided details of all the recruited participants and their children to the hospital/clinic study clinician every end of the day
2. The clinicians helped in the identification of the matching controls for each case i.e. those who match with the recruited cases age (children within the same anniversary) and sex i.e. males matched with males of the same age and females matched with females of the same age.
3. The controls were enrolled on meeting the definition of controls in this study as follow:

   Control subjects were defined as children who had complaints other than respiratory complaints, who had no complaints of an ARI in the prior 2 weeks,

who donot belong to the same household as the case patient, and who has not used antibiotics or antivirals in the previous 2 weeks.

4. The recruitment of the cases with be done as for the cases as outlined in the above procedure. The research assistant will used the questionnaires designed for controls –**Appendix 6 and 7.**
5. Similarly, control patients will be sampled to obtain throat and nasopharyngeal specimen to compare the causative agent profiles with those of the cases.

Appendix 4: Semi - Structured Interview Questionnaire
(Cases)

Aetiology and risk factors of Acute Respiratory Infections in children under five year selected facilities in Nakuru County, Kenya

Date of visit

Day		Month		Year	

Study ID_____

_____

Gender:_____

A. Demographic and socio-economic details

1. What is your year of birth?

   _____

2. What is your marital status?
   - Single
   - Married/cohabited
   - Divorced/separated
   - Widowed
3. What is your level of education?
   - No formal education
   - Primary school
   - Secondary education
   - College
   - University
4. What is your religion?
   - Christian
   - Muslim
   - Hindu
   - Other (specify)_____
5. What is your tribe?_____
6. What is your family monthly income in Kshs?
   - <5000
   - 5000-10000
   - 10001-15000
   - >15000 (specify how much)

     _____
7. What is you occupation?
   - Farming
   - Business
   - Housewife
   - Permanent employment
   - Casual employment
   - Hawking
   - Other (Specify) _____
8. What is the occupation of your spouse (if applicable)
   - Hawking
   - Farming
   - Business
   - Casual employment
   - Permanent employment

- ☐ Other (Specify)

9. Where do you live?

   _____

10. What is the estimated distance from the health facility to your home?
    - ☐ Less than half kilometre
    - ☐ About 1kilometer
    - ☐ Two kilometres and above

Child's details

1. Date of birth of your child? (Day/month/year)_____ _
2. What is the sex of your child?
   - ☐ Male
   - ☐ Female
3. What is the child order?
   - ☐ First born
   - ☐ Second born
   - ☐ Between second and last born
   - ☐ Last born
4. What was the birth weight of your child? *(you can check from the immunization card)* _____kgs
5. Has the child completed all the immunizations? *(you can check from the immunization card)*
   - ☐ Yes
   - ☐ Continuing
   - ☐ No
6. Has your child been vaccinated for seasonal influenza in the last one year?
   - ☐ Yes
   - ☐ No
7. For how long has your child breastfed
   - ☐ Not breast fed
   - ☐ Less than 4 months
   - ☐ 4-6 months
   - ☐ 6months and above
   - ☐ Continuing *(tick this if the child is still breastfeeding)*

Predictors of care seeking behaviour for child hood illnesses

1. How many other children do you have?
   - ☐ 1
   - ☐ 2-3
   - ☐ 4-5
   - ☐ More than 5
2. Where do you commonly deliver your children?
   - ☐ Hospital
   - ☐ At home
   - ☐ Other (specify)_____
3. What is the reason for the answer above?
   - ☐ They are experienced
   - ☐ They have better equipment
   - ☐ They are able to support you in case of emergency
   - ☐ Their cost is low
   - ☐ Other (specify)_____
4. Where do you commonly seek medical care when your children are sick?

- Take children to hospital
- Take children to traditional doctors
- Buy drugs from pharmacy
- Home treatment with home remedies
- Wait until illness subsides
- Religious minister for healing prayers

5. What is the reason for your choice of seeking health care from the above mentioned? *(Select all that is applicable)*
   - Hospital is nearby
   - Pharmacy is closer
   - Fear the illness may get worse
   - They give better treatment
   - No money to see doctor
   - When child is seriously sick
   - There are qualified medical staff
   - They test for disease to give better treatment
   - Staff are friendly
   - Others (Specify)_____ _____ _

Potential risk factors for ARI in the region

1. What kind of fuel do you use for cooking?
   - Charcoal
   - Wood
   - Stove
   - Gas
   - Electricity
   - Other (specify)
2. Where do you cook from?
   - Inside house
   - Outside the house
3. How often do you carry the baby while cooking?
   - Always
   - Sometimes
   - Never
4. Is the kitchen inside the main living room?
   - Yes
   - No
5. What is the size of your house?
   - Single room
   - One bed room
   - Two bedrooms
   - Three bedrooms
   - Four or more bedrooms
6. What is the material that has constructed your house?
   - Stone or bricks house
   - Mud
   - Timber
   - Iron sheet
   - Other (specify)_____
7. What is the number of people living in the house? (*Including children)*

□ 1-3

□ 3-5

□ 5-8

□ More than 8

8. Does anybody smoke cigarettes in the home?

□ Yes

□ No

B. **Knowledge on transmission and preventive measures for ARI**

1. How do you notice the child is sick so that you can seek medical care?

□ Child develops fever □ Yes □ No

□ Child is drinking poorly □ Yes □ No

□ Child is not able to drink or breastfeed □ Yes □ No

□ Child has fast breathing □ Yes □ No

□ Child has difficulty breathing □ Yes □ No

□ Child has blood in stool □ Yes □ No

□ Child has severe diarrhea □ Yes □ No

□ Other (specify)_____

2. What are the signs of Acute respiratory illness (ARI) in a child? *(Indicate yes or no in each of the signs)*

□ Cold, cough, catarrh □ Yes □ No

□ Difficulty to breath □ Yes □ No

□ Wheezing □ Yes □ No

□ Chest in-drawing □ Yes □ No

□ Convulsions □ Yes □ No

□ Inability to drink □ Yes □ No

□ Difficulty to wake up □ Yes □ No

□ Others (please list)

_____

_____

__

3. How is ARI transmitted? *(tick all that is applicable)*

□ Through droplets (e.g. sneeze) □Yes □ No

□ Through water sources □ Yes □ No

□ Through insect bites □ Yes □ No

□ Through un-well cooked pork or chicken □ Yes □ No

□ Through touching body of infected persons □ Yes □ No

□ Through touching contaminated objects □ Yes □ No

4. What is the severity of ARI in a child according to your views?
   - □ High fatal
   - □ Can cause severe irreversible bodily damage
   - □ Not severe
5. Do you think there is an effective vaccine for influenza and ARI?
   - □ Yes
   - □ No
6. Do you think there is an effective medicine to treat influenza and ARI?
   - □ Yes
   - □ No
7. Which medicines do you think are effective for treating ARI for children?
   - □ Syrups □Yes □ No
   - □ Tablets □Yes □ No
   - □ Injection □Yes □ No
   - □ Tea □Yes □ No
   - □ Traditional medications □Yes □ No
   - □ Ointment □Yes □ No
   - □ There is no effective drug for the treatment of ARI □Yes □ No
8. What are your views regarding the following preventive measures for preventing ARI transmission?

   Wearing face masks in public areas
   - □ Not effective at all
   - □ Not very effective
   - □ Quite effective
   - □ Very effective

   Washing hands frequently
   - □ Not effective at all
   - □ Not very effective
   - □ Quite effective
   - □ Very effective
9. What are other preventive measures among the listed do you think could for preventing ARI transmission? (tick all that is applicable)
   - □ Avoid going to crowed places □Yes □ No
   - □ Avoid going out unless necessary □Yes □ No
   - □ Avoid going to hospitals □Yes □ No
   - □ Face mask use in public venues □Yes □ No
   - □ Declaration of ILI symptoms at border health checkpoints □Yes □ No
   - □ Seeking of medical consultation immediately with the onset of a fever □Yes □ No
10. How do you think the government and health facilities in this area are prepared in case of an outbreak with ARI? (select all that is applicable)
    - □ The local health system have enough medication for treating ARI □Yes □ No

- The local health system have enough vaccine for preventing ARI □Yes □ No
- Hospitals have enough personal □Yes □ No
- Protection equipment for preventing ARI □Yes □ No
- The country will be able to control an outbreak □Yes □ No

Estimated financial burden of ARI (cost of illness)

1. For how long has the child been sick
   - Less than 1 day
   - 2 – 3 days
   - 4 -5 days
   - 1 week (7days)
   - More than 1 week (>7 days)
2. What are the signs/sympotoms exhibited by the child (to be filled by the clinician)
   - Fever □Yes □ No
   - High respiratory rate □Yes □ No
   - Wheezing □Yes □ No
   - Tachypnea □Yes □ No
   - Cough □Yes □ No
   - Sore throat □Yes □ No
   - Stridor □Yes □ No
   - Dysponea □Yes □ No
   - Earache □Yes □ No
   - Rhinorrhea □Yes □ No
3. Had you sought treatment elsewhere for the child.
   - Yes
   - NO
4. If yes, indicate the treatment
   - Had gone to a private clinic
   - Bought medicine from a pharmacy
   - From a traditional healer
   - The priest prayed for the child
   - Other (specify)_____
5. Approximately how much money have you spent for the following health services for the child during this episode (in Kshs)
   - Clinician fee (consultation) – Kshs_____
   - Follow up visits (consultation) Ksh_____
   - Medicines - Kshs_____
   - Laboratory tests Kshs _____
6. Indicate approximately the mode of

transport and amount spent on transport when taking the child to hospital

- Bus - Kshs_____
- Taxi Kshs_____
- Motocycle (Bodaboda) – Kshs_____
- Minibus/van (Matatu) Kshs_____
- Personal car (cost of fuel) Kshs_____
- Other means (specify)_____ Kshs_____

7. Indicate if the mother, father, guardian or caretaker missed work to take the child to hospital and take care of the enrolled child at home (circle as appropriate)
   - Mother - Yes No Days _____
   - Father - Yes No Days _____
   - Guardian Yes No Days _____
   - Caretaker Yes No Days _____

Appendix 5: Kiambatisho - Kidadisi cha mahojiano Muondo-nusu

Cases

Utafiti kuhusu viini husika, sababu za hatari, mzigo wa kiuchumi kwa jamii, wa maambukizi ya magonjwa ya sehemu za kupumua katika watoto waliochini ya miaka tano katika baadhi ya vituo vya afya, Kauti ya Nakuru, Kenya

Tarehe ya ziara

Siku		Mwezi		Mwaka	

Namba ya utafiti

ID_____

Jinsia: _____

A. Maelezo ya kidemografia, jamii na uchumi

1. Je, uzaliwa mwaka gani?

_____

2. Hali yako ya ndoa/Je umeolewa?

□ Sijaolewa

□ Nimeolewa / Tunaishi pamoja

□ Talaka / tumetengana

□ Mjane

3. Unakiwango kipi cha masomo?

□ Sijaenda shule

□ Elimu ya msingi

□ Elimu ya sekondari

□ Chuo cha ufundi

□ Chuo Kikuu

4. Je, unatumikia dini gani?

□ Mkristo

□ Mwisilamu

□ Dini ya kibaniyani

□ nyingine (taja) _____

5. Je, kabila lako ni lipi?

_____

6. Je, mapato ya familia yako ya kila mwezi ni kiasi gani?

□ Chini ya 5,000

□ Kati ya 5,000-10,000

□ Kati ya 10,001-15,000

□ Zaidi ya 15,000 (taja ni kiasi gani)

_____

7. Je, wewe unafanya kazi gani?

□ Kilimo

□ Biashara

□ Mke wa nyumba

□ Nimeajiriwa

□ Nafanya kibarua

□ Mchuuzi

□ Nyingine (Taja)

_____

8. Je, mwenzako anafanya kazi gani (ikiwa umeolewa/mnaishi pamoja)

□ Mchhuzi

□ Kilimo

□ Biashara

□ Anafanfya kibarua

□ Ameajiriwa

□ Nyingine (Taja)

9. Unaishi wapi? _____

10. Je, ukikadiri kuna umbali wa kiasi gani kutoka kwako nyumbani hadi kituo cha afya?

□ Chini ya kilomita nusu

□ Kama kilomita 1 hivi

□ Kama kilomita mbili au zaidi

Maelezo yote ya mtoto

1. Tarehe ya kuzaliwa ya mtoto wako? (Siku / mwezi / mwaka) _____

2. Mtoto wako ni wa jinsia gani?

□ Kiume

□ Kike

3. Mtoto wako amezaliwa kwa nafasi ipi?

□ Ndio wa kwanza

□ Ndio wa pili

□ Amezaliwa kakati ya mtoto wa pili na wa mwisho (Kitinda mimba)

□ Wa mwisho (Kitinda mimba)

4. Mtoto wako alizaliwa na uzito upi? (*Unaweza kuangalia kadi ya chanjo*) _____ kilo

5. Je, mtoto amemaliza chanjo zote? (*Unaweza kuangalia kadi ya chanjo*)

□ Ndiyo

□ Anaendelea

□ La

6. Mtoto wako amepokea chanjo ya virusi vya influenza kwa mwaka uliopita?

□ Ndiyo

□ La

7. Umemnyonyesha mtoto wako kwa muda gani?

□ Sijamnyonyesha

□ Chini ya miezi 4

□ Kati ya miezi 4-6

□ Zaidi ya miezi 6

□ Anaendelea (Jibu hapa kama mtoto bado ananyonya)

Utabiri wa Tabia za Kutafuta Huduma kwa ajili ya Magonjwa Watoto

1. Je, una watoto wangapi?

□ 1

□ 2-3

□ 4-5

□ Zaidi ya 5

2. Je, kwa kawaida umewazalia watoto wako wapi?

□ Hospitali

□ Nyumbani

□ Nyingine (taja) _____

3. Sababu ya jibu lako la hapo juu?

□ Wao ni wazoefu

□ Wao wana vifaa bora

□ Wanauwezo wa kukusaidia wakati wa dharura

□ Gharama yao ni ya chini

□ Nyingine (taja) _____

4. Unawapeleka wapi mtoto wako kwa kawaida kutafuta huduma ya matibabu wakati ni wa mgonjwa?

□ Nawapeleka watoto hospitalini

□ Nawapeleka watoto kwa waganga

□ Nanunua dawa kutoka duka la dawa

□ Nawatibu nyumbani kwa kutumia matibabu ya nyumbani

□ Nasubiri hadi ugonjwa upunguke

□ Nawapeleka kwenye sala za uponyaji

5. Ni sababu gani zilizofanya uchague jibu lako la kutafuta huduma ya afya kutoka zilizotajwa hapo juu? (Chagua zote zinazofaa)

□ Hospitali ni jirani/iko karibu

□ Duka la dawa liko karibu

□ Naogopa ugonjwa utamzidi mtoto

□ Wao hutoa matibabu bora

□ Hakuna fedha za kuona daktari

□ Wakati mtoto ni mgonjwa sana

□ Wafanyakazi wa matibabu wamehitimu

□ Wao wanapima ugonjwa ili wakupe matibabu mazuri

□ Watumishi ni wa kirafiki

□ Nyingine (Taja) _____

Uwezekano wa hatari wa magonjwa ya ARI katika sehemu hii

1. Ni aina gani ya mafuta unayotumia kwa ajili ya kupikia?

□ Mkaa

□ Kuni

□ Jiko

□ Gesi

□ Umeme

□ Nyingine (taja)

_____

2. Je, ni wapi wewe hupikia hapo nyumbani?

□ Ndani nyumba

□ Nje nyumba

3. Ni mara ngapi wewe hubeba mtoto wakati unapika?

□ Daima?kila wakati

□ Wakati mwingine

□ La simbebi

4. Je sehemu ya kupikia iko ndani ya chumba unayokaliya?

□ Ndiyo

□ La

5. Je, nyumba yako unaukubwa wa kiasi gani?

□ Chumba 1 pekee

□ Kuna chumba 1 ya kulala

□Kuna vyumba viwili vya kulala

□ Kuna vyumba vitatu vya kulala

□ Kuna vyumba vinne au zaidi vya kulala

6. Nini ni nyenzo kwamba ina ujenzi nyumba yako?

□ Mawe au matofali

□ Udongo

□ Mbao

□ Mabati

□ Nyingine (taja) _____

7. Je, kuna idadi ya watu wangapi wanaoishi katika nyumba yako? (Ikiwa ni pamoja na watoto)

□ 1-3

□ 3-5

□ 5-8

□ Zaidi ya 8

8. Je, hapo nyumbani kuna mtu anayevuta sigara?

□ Ndiyo

□ La/ Hakuna

B. Maarifa juu ya maambukizi na hatua za kuzuia ARI

1. Je, unagunduaje kuwa mtoto wako ni mgonjwa ili uweze kumtafutia huduma ya matibabu?

□ Mtoto anapata joto mwilini □Ndio □ la

□ Mtoto anashidwa kunywa □Ndio □ la

□ Mtoto hawezi kunywa au kunyonya □Ndio □ la

□ Mtoto ana pumuwa kwa haraka □Ndio □ la

□ Mtoto ana shida ya kupumua □Ndio □ la

□ Mtoto ana damu kwenye kinyesi □Ndio □ la

□ Mtoto ana hararisha □Ndio □ la

□ Nyingine (taja) _____

2. Je, ni ishara zipi zinazoambatana na ugonjwa ARI katika mtoto?

□ Baridi, kukohoa □Ndio □ la

□ Ugumu wa kupumua □Ndio □ la

□ Uingizaji □Ndio □ la

□ Kifua kubana □Ndio □ la

□ Degedege □Ndio □ la

□ Kutoweza kunywa □Ndio □ la

□ Ugumu kuamka □Ndio □ la

□ Wengine (tafadhali orodhesha)

_____

3. Je, magonjwa ya ARI yanasambazwaje? (*Weka alama kwa zote zinazofaa*)

□ Kupitia vitone tone (mfano kuchafya) □Ndio □ la

□ Kupitia vyanzo vya maji (mfano hifadhi) □Ndio □ la

□ Kupitia kuumwa na wadudu □Ndio □ la

□ Kupitia nyama (nguruwe au kuku) isiyopikwa vizuri □Ndio □ la

□ Kupitia kugusa mwili wa watu aliyeaambukizwa □Ndio □ la

□ Kupitia kugusa vitu/vifaa vilivyo chafuka □Ndio □ la

4. Je kulingana na maoni yako, ugonjwa wa ARI una ukali wa aina gani kwa mtoto?

□ Ina mauti mengi/mbaya

□ Inaweza kusababisha uharibifu mkali mwilini

□ Si kali

5. Unadhani kuna chanjo madhubuti dhidi ya virusi vya influenza na ugonjwa wa

ARI?

□ Ndiyo

□ La/Hakuna

6. Je, unadhani kuna dawa madhubuti ya kutibu ugonjwa wa ARI?

□ Ndiyo

□ La/Hakuna

7. Je unafikiri madawa zipi ni madhubuti kwa ajili ya kutibu ARI kwa watoto?

□ Dawa ya aina ya maji (syrup) □Ndio □ la

□ Dawa ya aina ya vidonge (tablets) □Ndio □ la

□ Sindano □Ndio □ la

□ Chai □Ndio □ la

□ Madawa ya kienyeji □Ndio □ la

□ Mafuta ya kupaka □Ndio □ la

□ Hakuna madawa ya kutibu ARI □Ndio □ la

8. Je, kwa maoni yako kuhusu hatua za kuzuia maambukizi ARI?

Kuvaa vifunika uso hadharani

- □ Haifai hata kidogo
- □ Inafaa kwa kiasi kidogo
- □ Inafaa kwa kiasi fulani
- □ Inafaa sana

Kuosha mikono mara kwa mara

- □ Haifai hata kidogo
- □ Inafaa kwa kiasi kidogo
- □ Inafaa kwa kiasi fulani
- □ Inafaa sana

9. Je, ni hatua nyingine ya kuzuia kati ya waliotajwa unafikiri inaweza kwa ajili ya kuzuia maambukizi ARI? (Weka alama kwa zote zinazofaa)

□ Epuka kwenda maeneo yaliyokuwa na watu wengi □Ndio □ la

□ Epuka kwenda nje isipokuwa muhimu □Ndio □ la

□ Epuka kwenda hospitali □Ndio □ la

□ Matumizi ya vifunika uso katika kumbi za umma □Ndio □ la

□ Kukiri kuwa mgonjwa ana ishara za magonjwa ya ARI katika vituo vya ukaguzi wa afya mipakani □Ndio □ la

□ Kutafuta mashauri ya matibabu mara moja na mwanzo wa homa □Ndio □ la

10. Je, unadhani serikali na vituo afya katika eneo hili ziko tayari kudhabiti hali kama kukizuka ugonjwa wa ARI? (Kuchagua zote majibu zinazofaa)

□ Utaratibu wa kiafya hapa uko na dawa za kutosha dhidi ya ugonjwa wa ARI □Ndio □ la

- Utaratibu wa kiafya hapa uko na chanjo za kutosha dhidi ya ugonjwa wa ARI
  Ndio la
- Hospitali ziko za vifaa vya kujikinga dhidi ya ugonjwa wa ARI
  Ndio la
- Nchi itaweza kudhibiti mkurupuko wa ugonjwa huu wa ARI

Makadiriyo ya matumizi ya fedha wakati wa ARI (Gharama ya ugonjwa)

1. Mtoto amkuwa mgonjwa kwa muda gani?

 Chini ya siku 1

 Kati ya siku 2-3

 Kati ya siku 4 -5

 Kama wiki 1 (siku 7)

 Zaidi ya wiki 1

2. Mtoto alikuwa anaonyesha dalili gani (*Weka alama kwa zote zinazofaa*)

 Homa

 Kukokota pumzi

 Kukosa pumzi

 Msongamano kwenye mapafu

 Kikohozi nyevu

 Tungwa (nimonia)

 Mafua

 Msongamano puani

 Kuumwa koo

 Kuwashwa

 Udhaifu

 Kutapika

3. Je, ulimtafutia mtoto matibabu mahali pengine?

 Ndiyo

 La

4. Kama ndiyo, hebu zionyeshe

 Nilienda kwa kliniki ya kibinafsi

 Nilimnunulia dawa kutoka duka la dawa

 Kutoka mganga

 Kasisi alimwombea mtoto

 Nyingine (taja)

_____

5. Takriban umetumia kiasi gani cha fedha kwa ajili ya huduma ya afya ya mtoto katika sehemu hii

 daktari ada (mashauriano) - Kshs_____

 Kufuatilia ziara (mashauriano) Ksh_____

 Madawa - Kshs_____

 Vipimo vya maabara Kshs _____

6. Takriban umetumia kiasi gani cha fedha kwa usafiri ukimleta kuchukua mtoto kwa hospitali

 Basi - Kshs_____

 Teksi Kshs_____

 Pikipiki (Bodaboda) - Kshs_____

 Matatu Kshs_____

 Gari ya kibinafsi (gharama za mafuta) Kshs_____

 Njia nyingine (taja) _____ Kshs_____

7. Hebu eleza kama mama, baba au mlezi

alikosa kwenda kazini ndio amlete mtoto hospitalini na pia kumlinda mtoto nyumbani (Weka alama panapofaa)

□ Mama – Ndiyo Hapana siku _____

□ Baba – Ndiyo Hapana siku _____

□ Mlezi – Ndiyo Hapana siku _____

Appendix 6: Semi - Structured Interview Questionnaire

Controls

Aetiology and risk factors of Acute Respiratory Infections in children under five year selected facilities in Nakuru County, Kenya

Date of visit

Day		Month		Year	

Study ID_____

Gender:_____

A. Demographic and socio-economic details

1. What is your year of birth?
   _____
2. What is your marital status?
   - Single
   - Married/cohabited
   - Divorced/separated
   - Widowed
3. What is your level of education?
   - No formal education
   - Primary school
   - Secondary education
   - College
   - University
4. What is your religion?
   - Christian
   - Muslim
   - Hindu
   - Other (specify)_____
5. What is your tribe?_____
6. What is your family monthly income in Kshs?
   - <5000
   - 5000-10000
   - 10001-15000
   - >15000 (specify how much)_____
7. What is you occupation?
   - Farming
   - Business
   - Housewife
   - Permanent employment
   - Casual employment
   - Hawking
   - Other (Specify)
     _____
8. What is the occupation of your spouse (if applicable)
   - Hawking
   - Farming
   - Business
   - Casual employment
   - Permanent employment

- Other (Specify)

9. Where do you live?

   _____

10. What is the estimated distance from the health facility to your home?
    - Less than half kilometre
    - About 1kilometer
    - Two kilometres and above

Child's details

8. Date of birth of your child? (Day/month/year)_____ _
9. What is the sex of your child?
    - Male
    - Female
10. What is the child order?
    - First born
    - Second born
    - Between second and last born
    - Last born
11. What was the birth weight of your child? *(you can check from the immunization card)* _____kgs
12. Has the child completed all the immunizations? *(you can check from the immunization card)*
    - Yes
    - Continuing
    - No
13. Has your child been vaccinated for seasonal influenza in the last one year?
    - Yes
    - No
14. For how long has your child breastfed
    - Not breast fed
    - Less than 4 months
    - 4-6 months
    - 6months and above
    - Continuing *(tick this if the child is still breastfeeding)*

Predictors of care seeking behaviour for child hood illnesses

6. How many other children do you have?
    - 1
    - 2-3
    - 4-5
    - More than 5
7. Where do you commonly deliver your children?
    - Hospital
    - At home
    - Other (specify)_____
8. What is the reason for the answer above?
    - They are experienced
    - They have better equipment
    - They are able to support you in case of emergency
    - Their cost is low
    - Other (specify)_____
9. Where do you commonly seek medical care when your children are sick?
    - Take children to hospital
    - Take children to traditional doctors
    - Buy drugs from pharmacy

- Home treatment with home remedies
- Wait until illness subsides
- Religious minister for healing prayers

10. What is the reason for your choice of seeking health care from the above mentioned? *(Select all that is applicable)*
    - Hospital is nearby
    - Pharmacy is closer
    - Fear the illness may get worse
    - They give better treatment
    - No money to see doctor
    - When child is seriously sick
    - There are qualified medical staff
    - They test for disease to give better treatment
    - Staff are friendly
    - Others (Specify)_____

Potential risk factors for ARI in the region

9. What kind of fuel do you use for cooking?
    - Charcoal
    - Wood
    - Stove
    - Gas
    - Electricity
    - Other (specify)

10. Where do you cook from?
    - Inside house
    - Outside the house

11. How often do you carry the baby while cooking?
    - Always
    - Sometimes
    - Never

12. Is the kitchen inside the main living room?
    - Yes
    - No

13. What is the size of your house?
    - Single room
    - One bed room
    - Two bedrooms
    - Three bedrooms
    - Four or more bedrooms

14. What is the material that has constructed your house?
    - Stone or bricks house
    - Mud
    - Timber
    - Iron sheet
    - Other (specify)_____

15. What is the number of people living in the house? (*Including children)*
    - 1-3
    - 3-5
    - 5-8
    - More than 8

16. Does anybody smoke cigarettes in the home?

□ Yes

□ No

C. **Knowledge on transmission and preventive measures for ARI**

1. How do you notice the child is sick so that you can seek medical care?
   - □ Child develops fever □ Yes □ No
   - □ Child is drinking poorly □ Yes □ No
   - □ Child is not able to drink or breastfeed □ Yes □ No
   - □ Child has fast breathing □ Yes □ No
   - □ Child has difficulty breathing □ Yes □ No
   - □ Child has blood in stool □ Yes □ No
   - □ Child has severe diarrhea □ Yes □ No
   - □ Other (specify)_____
2. What are the signs of Acute respiratory illness (ARI) in a child? *(Indicate yes or no in each of the signs)*
   - □ Cold, cough, catarrh □ Yes □ No
   - □ Difficulty to breath □ Yes □ No
   - □ Wheezing □ Yes □ No
   - □ Chest in-drawing □ Yes □ No
   - □ Convulsions □ Yes □ No
   - □ Inability to drink □ Yes □ No
   - □ Difficulty to wake up □ Yes □ No
   - □ Others (please list)

     _____
3. How is ARI transmitted? *(tick all that is applicable)*
   - □ Through droplets (e.g. sneeze) □Yes □ No
   - □ Through water sources □ Yes □ No
   - □ Through insect bites □ Yes □ No
   - □ Through un-well cooked pork or chicken □ Yes □ No
   - □ Through touching body of infected persons □ Yes □ No
   - □ Through touching contaminated objects □ Yes □ No
4. What is the severity of ARI in a child according to your views?
   - □ High fatal
   - □ Can cause severe irreversible bodily damage
   - □ Not severe
5. Do you think there is an effective vaccine for influenza and ARI?

□ Yes

□ No

6. Do you think there is an effective medicine to treat influenza and ARI?

□ Yes

□ No

7. Which medicines do you think are effective for treating ARI for children?

□ Syrups □Yes □ No

□ Tablets □Yes □ No

□ Injection □Yes □ No

□ Tea □Yes □ No

□ Traditional medications □Yes □ No

□ Ointment □Yes □ No

□ There is no effective drug for the treatment of ARI □Yes □ No

8. What are your views regarding the following preventive measures for preventing ARI transmission?

Wearing face masks in public areas

□ Not effective at all

□ Not very effective

□ Quite effective

□ Very effective

Washing hands frequently

□ Not effective at all

□ Not very effective

□ Quite effective

□ Very effective

9. What are other preventive measures among the listed do you think could for preventing ARI transmission? (tick all that is applicable)

□ Avoid going to crowed places □Yes □ No

□ Avoid going out unless necessary □Yes □ No

□ Avoid going to hospitals □Yes □ No

□ Face mask use in public venues □Yes □ No

□ Declaration of ILI symptoms at border health checkpoints □Yes □ No

□ Seeking of medical consultation immediately with the onset of a fever □Yes □ No

10. How do you think the government and health facilities in this area are prepared in case of an outbreak with ARI? (select all that is applicable)

□ The local health system have enough medication for treating ARI □Yes □ No

□ The local health system have enough vaccine for preventing ARI □Yes □ No

□ Hospitals have enough personal □Yes □ No

□ Protection equipment for preventing ARI □Yes □ No

□ The country will be able to control an outbreak □Yes □ No

Appendix 7: Kiambatisho - Kidadisi cha mahojiano Muondo-nusu (Controls)

Utafiti kuhusu Viini husika, sababu za hatari, mzigo wa kiuchumi kwa jamii, wa maambukizi ya magonjwa ya sehemu za kupumua katika watoto waliochini ya miaka tano katika baadhi ya vituo vya afya, Wilaya ya Nakuru, Kenya

Tarehe ya ziara					
Siku		Mwezi		Mwaka	

Namba ya utafiti

ID_____

Jinsia: _____

A. Maelezo ya kidemografia, jamii na uchumi

1. Je, uzaliwa mwaka gani?

_____

2. Hali yako ya ndoa/Je umeolewa?

□ Sijaolewa

□ Nimeolewa / Tunaishi pamoja

□ Talaka / tumetengana

□ Mjane

3. Unakiwango kipi cha masomo?

□ Sijaenda shule

□ Elimu ya msingi

□ Elimu ya sekondari

□ Chuo cha ufundi

□ Chuo Kikuu

4. Je, unatumikia dini gani?

□ Mkristo

□ Mwisilamu

□ Dini ya kibaniyani

□ nyingine (taja) _____

5. Je, kabila lako ni lipi? _____

6. Je, mapato ya familia yako ya kila mwezi ni kiasi gani?

□ Chini ya 5,000

□ Kati ya 5,000-10,000

□ Kati ya 10,001-15,000

□ Zaidi ya 15,000 (taja ni kiasi gani)_____

7. Je, wewe unafanya kazi gani?

□ Kilimo

□ Biashara

□ Mke wa nyumba

□ Nimeajiriwa

□ Nafanya kibarua

□ Mchuuzi

□ Nyingine (Taja)

_____

8. Je, mwenzako anafanya kazi gani (ikiwa umeolewa/mnaishi pamoja)

□ Mchhuzi

□ Kilimo

□ Biashara

□ Anafanfya kibarua

□ Ameajiriwa

□ Nyingine (Taja)

9. Unaishi wapi? _____

10. Je, ukikadiri kuna umbali wa kiasi gani kutoka kwako nyumbani hadi kituo cha afya?

□ Chini ya kilomita nusu

□ Kama kilomita 1 hivi

□ Kama kilomita mbili au zaidi

Maelezo yote ya mtoto

1. Tarehe ya kuzaliwa ya mtoto wako? (Siku / mwezi / mwaka) _____

2. Mtoto wako ni wa jinsia gani?

□ Kiume

□ Kike

3. Mtoto wako amezaliwa kwa nafasi ipi?

□ Ndio wa kwanza

□ Ndio wa pili

□ Amezaliwa kakati ya mtoto wa pili na wa mwisho (Kitinda mimba)

□ Wa mwisho (Kitinda mimba)

4. Mtoto wako alizaliwa na uzito upi? (*Unaweza kuangalia kadi ya chanjo*)

_____ kilo

5. Je, mtoto amemaliza chanjo zote? (*Unaweza kuangalia kadi ya chanjo*)

□ Ndiyo

□ Anaendelea

□ La

6. Mtoto wako amepokea chanjo ya virusi vya influenza kwa mwaka uliopita?

□ Ndiyo

□ La

7. Umemnyonyesha mtoto wako kwa muda gani?

□ Sijamnyonyesha

□ Chini ya miezi 4

□ Kati ya miezi 4-6

□ Zaidi ya miezi 6

□ Anaendelea (Jibu hapa kama mtoto bado ananyonya)

Utabiri wa Tabia za Kutafuta Huduma kwa ajili ya Magonjwa Watoto

1. Je, una watoto wangapi?

□ 1

□ 2-3

□ 4-5

□ Zaidi ya 5

2. Je, kwa kawaida umewazalia watoto wako wapi?

□ Hospitali

□ Nyumbani

□ Nyingine (taja) _____

3. Sababu ya jibu lako la hapo juu?

□ Wao ni wazoefu

□ Wao wana vifaa bora

□ Wanauwezo wa kukusaidia wakati wa dharura

 Gharama yao ni ya chini

 Nyingine (taja)

_____

4. Unawapeleka wapi mtoto wako kwa kawaida kutafuta huduma ya matibabu wakati ni wa mgonjwa?

 Nawapeleka watoto hospitalini

 Nawapeleka watoto kwa waganga

 Nanunua dawa kutoka duka la dawa

 Nawatibu nyumbani kwa kutumia matibabu ya nyumbani

 Nasubiri hadi ugonjwa upunguke

 Nawapeleka kwenye sala za uponyaji

5. Ni sababu gani zilizofanya uchague jibu lako la kutafuta huduma ya afya kutoka zilizotajwa hapo juu? (Chagua zote zinazofaa)

 Hospitali ni jirani/iko karibu

 Duka la dawa liko karibu

 Naogopa ugonjwa utamzidi mtoto

 Wao hutoa matibabu bora

 Hakuna fedha za kuona daktari

 Wakati mtoto ni mgonjwa sana

 Wafanyakazi wa matibabu wamehitimu

 Wao wanapima ugonjwa ili wakupe matibabu mazuri

 Watumishi ni wa kirafiki

 Nyingine (Taja) _____

Uwezekano wa hatari wa magonjwa ya ARI katika sehemu hii

1. Ni aina gani ya mafuta unayotumia kwa ajili ya kupikia?

 Mkaa

 Kuni

 Jiko

 Gesi

 Umeme

 Nyingine (taja) _____

2. Je, ni wapi wewe hupikia hapo nyumbani?

 Ndani nyumba

 Nje nyumba

3. Ni mara ngapi wewe hubeba mtoto wakati unapika?

 Daima?kila wakati

 Wakati mwingine

 La simbebi

4. Je sehemu ya kupikia iko ndani ya chumba unayokaliya?

 Ndiyo

 La

5. Je, nyumba yako unaukubwa wa kiasi gani?

 Chumba 1 pekee

 Kuna chumba 1 ya kulala

Kuna vyumba viwili vya kulala

 Kuna vyumba vitatu vya kulala

 Kuna vyumba vinne au zaidi vya kulala

6. Nini ni nyenzo kwamba ina ujenzi nyumba yako?

□ Mawe au matofali

□ Udongo

□ Mbao

□ Mabati

□ Nyingine (taja) _____

7. Je, kuna idadi ya watu wangapi wanaoishi katika nyumba yako? (Ikiwa ni pamoja na watoto)

□ 1-3

□ 3-5

□ 5-8

□ Zaidi ya 8

8. Je, hapo nyumbani kuna mtu anayevuta sigara?

□ Ndiyo

□ La/ Hakuna

B. B. Maarifa juu ya maambukizi na hatua za kuzuia ARI

1. Je, unagunduaje kuwa mtoto wako ni mgonjwa ili uweze kumtafutia huduma ya matibabu?

□ Mtoto anapata joto mwilini □Ndio □ la

□ Mtoto anashidwa kunywa □Ndio □ la

□ Mtoto hawezi kunywa au kunyonya □Ndio □ la

□ Mtoto ana pumuwa kwa haraka □Ndio □ la

□ Mtoto ana shida ya kupumua □Ndio □ la

□ Mtoto ana damu kwenye kinyesi □Ndio □ la

□ Mtoto ana hararisha □Ndio □ la

□ Nyingine (taja) _____

2. Je, ni ishara zipi zinazoambatana na ugonjwa ARI katika mtoto?

□ Baridi, kukohoa □Ndio □ la

□ Ugumu wa kupumua □Ndio □ la

□ Uingizaji □Ndio □ la

□ Kifua kubana □Ndio □ la

□ Degedege □Ndio □ la

□ Kutoweza kunywa □Ndio □ la

□ Ugumu kuamka □Ndio □ la

□ Wengine (tafadhali orodhesha)

_____

3. Je, magonjwa ya ARI yanasambazwaje? (*Weka alama kwa zote zinazofaa*)

□ Kupitia vitone tone (mfano kuchafya) □Ndio □ la

□ Kupitia vyanzo vya maji (mfano hifadhi) □Ndio □ la

□ Kupitia kuumwa na wadudu □Ndio □ la

□ Kupitia nyama (nguruwe au kuku) isiyopikwa vizuri □Ndio □ la

□ Kupitia kugusa mwili wa watu aliyeaambukizwa □Ndio □ la

□ Kupitia kugusa vitu/vifaa vilivyo chafuka

□Ndio □ la

4. Je kulingana na maoni yako, ugonjwa wa ARI una ukali wa aina gani kwa mtoto?

□ Ina mauti mengi/mbaya

□ Inaweza kusababisha uharibifu mkali mwilini

□ Si kali

5. Unadhani kuna chanjo madhubuti dhidi ya virusi vya influenza na ugonjwa wa ARI?

□ Ndiyo

□ La/Hakuna

6. Je, unadhani kuna dawa madhubuti ya kutibu ugonjwa wa ARI?

□ Ndiyo

□ La/Hakuna

7. Je unafikiri madawa zipi ni madhubuti kwa ajili ya kutibu ARI kwa watoto?

□ Dawa ya aina ya maji (syrup) □Ndio □ la

□ Dawa ya aina ya vidonge □Ndio □ la

□ Sindano □Ndio □ la

□ Chai □Ndio □ la

□ Madawa ya kienyeji □Ndio □ la

□ Mafuta ya kupaka □Ndio □ la

□ Hakuna madawa ya kutibu ARI

□Ndio □ la

8. Je, kwa maoni yako kuhusu hatua za kuzuia maambukizi ARI?

Kuvaa vifunika uso hadharani

□ Haifai hata kidogo

□ Inafaa kwa kiasi kidogo

□ Inafaa kwa kiasi fulani

□ Inafaa sana

Kuosha mikono mara kwa mara

□ Haifai hata kidogo

□ Inafaa kwa kiasi kidogo

□ Inafaa kwa kiasi Fulani

□ Inafaa sana

9. Je, ni hatua nyingine ya kuzuia kati ya waliotajwa unafikiri inaweza kwa ajili ya kuzuia maambukizi ARI? (Weka alama kwa zote zinazofaa)

□ Epuka kwenda maeneo yaliyokuwa na watu wengi □Ndio

□ la

□ Epuka kwenda nje isipokuwa muhimu

□Ndio □ la

□ Epuka kwenda hospitali □Ndio □ la

□ Matumizi ya vifunika uso katika kumbi za umma

□Ndio □ la

□ Kukiri kuwa mgonjwa ana ishara za magonjwa ya ARI katika vituo vya ukaguzi wa afya mipakani

□Ndio □ la

□ Kutafuta mashauri ya matibabu mara moja na mwanzo wa homa

□Ndio □ la

10. Je, unadhani serikali na vituo afya katika eneo hili ziko tayari kudhabiti hali kama kukizuka ugonjwa wa ARI? (Kuchagua zote majibu zinazofaa)

- Utaratibu wa kiafya hapa uko na dawa za kutosha dhidi ya ugonjwa wa ARI □Ndio □ la
- Utaratibu wa kiafya hapa uko na chanjo za kutosha dhidi ya ugonjwa wa ARI □Ndio □ la
- Hospitali ziko za vifaa vya kujikinga dhidi ya ugonjwa wa ARI □Ndio □ la
- Nchi itaweza kudhibiti mkurupuko wa ugonjwa huu wa ARI

Appendix 8: Screening for malnutrition using Mid-Upper Arm Circumference

Background: Acute malnutrition is a result of recent (short-term) deficiency of protein, energy together with minerals and vitamins leading to loss of body fats and muscle tissues. Acute malnutrition presents with wasting (low weight-for-height) and /or presence of pitting oedema of both feet. Screening for Acute Malnutrition should be done at any contact points; children wards, immunization points, community out-reaches, ART sites, young child clinics, counselling units and psycho social groups. Community-based service providers can also perform malnutrition screening provided that they are adequately trained and equipped.

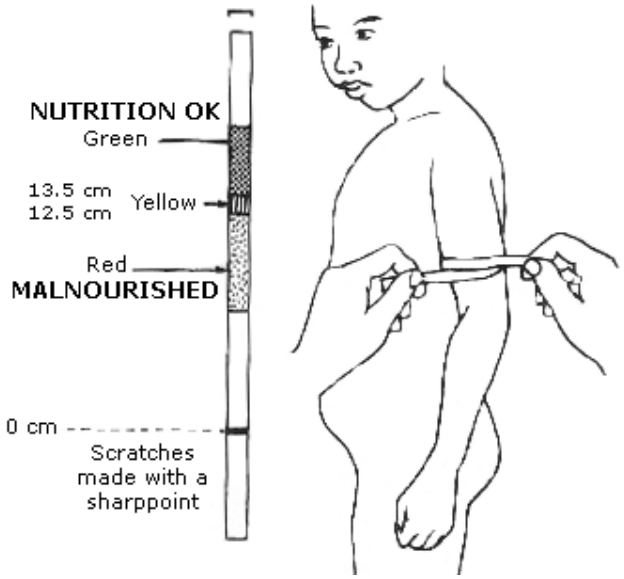
Principle: MUAC is a quick and simple way to determine whether or not a child is malnourished using a simple colored plastic strip. MUAC is suitable to use on children from the age of 12 months up to the age of 59 months. However, it can also be used for children over six months with length above 65 cm.

Materials

1. MUAC 4 colour Tape

Steps for taking the MUAC measurement of a child

1. Determine the mid-point between the elbow and the shoulder (acromion and olecranon) as shown on the picture below.
2. Place the tape measure around the LEFT arm (the arm should be relaxed and hang down the side of the body).
3. Measure the MUAC while ensuring that the tape neither pinches the arm nor is left loose.
4. Read the measurement from the window of the tape or from the tape.
5. Record the MUAC to the nearest 0.1 cm or 1mm in the interview form**.**



Interpretation

Using a 4-colour tape:

- Measurement in the green zone means the child is properly nourished (>13.5cm)**;**
- Measurement in the yellow zone means that the child is at risk of malnutrition (between 12.5 – 13.5)**;**
- Measurement in the orange zone means that the child is moderately malnourished (between 11-12.5cm);
- Measurement in the red zone means that the child is severely malnourished (<11cm).

Note: Repeat the measurement two times to ensure an accurate interpretation.

Appendix 9: Approval letter from Scientific Steering Committee of the Kenya Medical Research Institute

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

15 AUG 2012

ESACIPAC/SSC/100727 **14th August, 2012**

Martin Matu

Thro'
Director, CPHR
NAIROBI

forwarded 15/8/12

REF: SSC No. 2282 (Revised) – Aetiology, risk factors and community economic burden of acute respiratory infections in children under five years in selected facilities in Nakuru District, Kenya.

Thank you for your letter dated 7th August, 2012 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval

Sammy Njenga, PhD
SECRETARY, SSC

Appendix 10: Approval letter from Ethical Research Committee of the Kenya Medical Research Institute

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **March 11, 2013**

TO: **MARTIN MATU**
PRINCIPAL INVESTIGATOR

THROUGH **DR. YERI KOMBE**
THE DIRECTOR, CPHR
NAIROBI

Forwarded
15/03/2013

Dear Sir,

RE: SSC NO. 2282- (REV): AETIOLOGY, RISK FACTORS AND COMMUNITY ECONOMIC BURDEN OF ACUTE RESPIRATORY INFECTIONS IN CHILDREN UNDER FIVE YEARS SELECTED FACILITIES IN NAKURU DISTRICT, KENYA.

This is to inform you that during the 212th meeting of the KEMRI/ERC held on 26th February 2013, the above referenced study was reviewed.

The Committee concluded that due consideration has been given to the ethical issues that may arise from the conduct of the study and granted approval for implementation effective the **26th February 2013**

Please note that the authorization to conduct this study will automatically expire on **25th February 2014.** If you plan to continue with the study beyond this date please submit an application for continuation approval to the ERC secretariat by **15th January 2014**

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC and ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours sincerely,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

Appendix 11: Collection and transportation specimen for isolation and testing of viral and bacterial respiratory tract pathogens

Collection of Nasopharyngeal swab

Principle: Nasopharyngeal (NP) swabs can be used to collect an appropriate specimen for influenza testing by RT PCR. Specimen must be immediately placed in 1 - 3 ml of viral transport medium (VTM). If an NP swab kit with VTM is not used, VTM* can be made in house. Specimens should be collected within the first 24-72 hours of onset of symptoms, and no later than 5 days after onset of symptoms.

Indications for Testing:

Collect specimens from patients presenting with Influenza-like illness within 72 hours of onset of symptoms. Specimens from severely ill patients and children are acceptable after 72 hours of symptom onset.

Materials

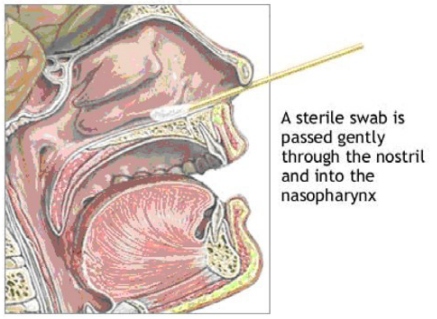
- Tongue depressor (spatula)
- Swab sticks ("orange" stick with cotton swab)
- Viral Transport Medium (VTM)
- Ziplock bag
- Surgical mask
- Gloves
- Soap/alcohol based hand gel

Infection control precautions:

- Personal protective equipment: wear a surgical mask and disposable gloves.
- When completed, dispose of all PPE and other contaminated materials in the trash.
- Wash hands thoroughly with soap and water or alcohol-based hand gel before and after the procedure.

Procedure

- Use a flexible fine-shafted aluminum swab with a polyester (dacron or rayon, not cotton or calcium alginate) tip.



- Incline the patient's head as required and insert the cotton swab along the base of the nasal cavity to a depth of about 2-4 cm into the nostril. Insert swab into one nostril straight back (not upwards) and back to the nasopharynx
- Swab around the inside of the nostril and along the nasal septum by rotating the swab between fingers
- Slowly withdraw swab with a rotating motion.
  Patients with copious discharge should be requested to gently clean their nose by washing or with tissue.
- P
  lace tip of the swab into a vial containing 2–3 ml of Viral Transport Medium (VTM), cut the shaft and tighten the lid securely.
- **Complete the specimen referral form one per patient**
- **Label** the container with the participants (parent/guardian) **Study number** and **date of birth**. For example, the first case from FITC will be labeled FITCS001 and date of birth (e.g. 01/Jan/2013)

It is essential that the nasal passage be swabbed sufficiently firmly to collect infected cells rich in virus. Nasopharyngeal swabs inserted along the base of the nasal cavity (6cm or deeper) are excellent but may be more traumatic to the patient. Mucous discharge and throat swabs contain fewer viruses and are discouraged.

Transport

1. Ensure the VTM is lid is tightly secured
2. Place the sample in a ziplock specimen transfer bag
3. Place in a cool box and tighten the cool box lid
4. Send the specimen to the laboratory for processing. Use the address below
   Center for Microbiology Research, KEMRI
   Kenyatta National Hospital Grounds,
   Next to KNH post office
   P.O. Box ……..KNH, Nairobi
   Contact person: Sam Symekher or John Kiiru

Note: If the specimen are not submitted the same day, please keep them in a refrigerator at 4^{o}C for up to 48 hours

Collection of Throat Swab

Materials

- Tongue depressor (spatula)
- Swab sticks ("orange" stick with cotton swab)
- Amies transport media (ATM)

Procedure

1. Provide explanation and obtain consent from parents/guardian as described in SOP no. SSC2282-SOP1.
2. Get a swab stick for use readily.
3. Ask the parent/guardian of the child to help him/her to open the mouth widely. If the child can receive instructions, ask them to open the mouth widely
4. Use tongue depressor to depress the tongue slightly to allow easier access to pharynx.

   If the patient is cooperative, may try to get him to say "ah"
5. Put a swab stick into oral cavity and swab the lateral wall of pharynx (i.e "tonsillar" area) without touching the buccal mucosa or tongue
6. Remove the swab stick from the oral cavity carefully without touching the buccal mucosa or tongue.
7. Put the swab stick immediately into ATM

   Note: use one ATM for one swab stick only
8. Immerse cotton end (cotton swab) completely in ATM and break the swab stick adequately to fit into the ATM tube
9. Seal ATM tube properly to avoid leakage and transport to laboratory as soon as possible

   • If unable to send to laboratory immediately, keep specimen at 4 °C
10. Complete the specimen referral form one per patient
11. **Label** the container with the participants (parent/guardian) **Study number** and **date of birth**. For example, the first case from FITC will be labeled FITCS001 and date of birth (e.g. 01/Jan/2013)

Transport

1. Ensure the ATM is lid is tightly secured
2. Place the sample in a ziplock specimen transfer bag
3. Place in a cool box and tighten the cool box lid

4. Send the specimen to the laboratory for processing. Use the address below

   Center for Microbiology Research, KEMRI

   Kenyatta National Hospital Grounds,

   Next to KNH post office

   P.O. Box ……..KNH, Nairobi

   Contact person: Sam Symekher or John Kiiru

Note: If the specimens are not submitted the same day, please keep them in a refrigerator at 4°C for up to 48 hours

Specimen processing for isolation bacterial respiratory tract pathogens
Inoculation of specimen

1. Inoculate the following media with the swab
   a. Blood agar (incubate at 35-37°C in CO_2)
   b. Chocolate agar/GC agar (incubate at 35-37°C in CO_2)
   c. MacConkey (incubate at 35-37°C in air)
2. Initial inoculum should cover between a quarter and a third of the plate to be used. The swab should be rolled over the inoculation area to maximise transfer of organisms, taking care to avoid the edges of the plate.
3. Use the swab to prepare a gram smear for direct examination. Allow to dry, stain and examine the smear and record on the work sheet
4. Incubate the plates under the conditions indicated in 1 above for each plate type for 24-48 hours (more time if necessary especially for slowly growing organisms in culture)
5. Examine the plates and describe the growth i.e the size, color and texture of the colonies and record the description of each colony type separately. Give a specific identification for each colony type which should be traceable to the original patient e.g. if the sample was labelled **FITCT001, the first colony can be labeled FITCT001#1 and followed to identify the pathogen.**
6. Subculture the colonies to fresh plate to allow accurate identification
7. For each isolate (pure colony) record the description on the worksheet, gram stain and follow the flow charts below for isolates identification

Reporting

a) Direct sample Gram stains: *Describe the specimen – organisms seen, cells types and numbers per field*

b) Culture:

Negative report: Indicate no pathogen isolated if no clinically important organisms is isolated

Positive report: Indicated the organism isolated and the antimicrobial susceptibility profile. Clinically significant organisms include "Group A streptococcus", "*Neisseria gonorrhoeae,* "*Neisseria meningitidis*", "*Corynebacterium diphtheriae"*

Telephone all positive *N. gonorrhoeae*, *N. meningitidis* and Group A streptococci isolates to health facility for immediate management before submitting the report form within a week of receiving the specimen.

Appendix 12: Standard operating procedures for preparation of reagents and culture media for microbiological tests

A. Preparation of Gram stain Reagents

Ammonium oxalate-crystal violet

Crystal violet powder	20 grams
Methylated Spirit	200 mls
Ammonium oxalate	8 grams
Distilled water	800 mls

Dissolve the crystal violet powder with the methylated spirit. Then add the ammonium oxalate solution. Stir to mix thoroughly and then filter the solution into a clean bottle. Make a new label recording the date of preparation and your initials. Store at room temperature for 1 year.

Iodine solution

Iodine	10 grams
Potassium iodide	20 grams
Distilled water	1000 mls

Dissolve the iodine and potassium iodide in the water. Mix thoroughly before filtering into a clean bottle. Make a new label recording the date of preparation and your initials. Store at room temperature for 1 year.

Liquor iodifortis (BP)

Iodine	10 grams
Potassium iodide	6 grams
Methylated spirits	90 mls
Distilled water	10 mls

Dissolve the iodine and potassium iodide in the methylated spirits. Add the water and mix thoroughly. Make a label recording the date of preparation and your initials. Store in a clean bottle at room temperature for 1 year.

Iodine-acetone

Liquor iodifortis	35 mls
Acetone	965 mls

Mix the solutions well. Make a label recording the date of preparation and your initials. Store in a clean bottle for 6 months.

Basic Fuchsin stain

Basic fuchsin 1.0 grams

Distilled water 100 mls

Dissolve the basic fuchsin in the water. Mix thoroughly before filtering into a clean bottle. Make a label recording the preparation date and your initials. Store at room temperature for 1 year.

Quality Control

Organism	**desired result**	**culture number**
Staphylococcus aureus	gram positive	ATCC 25923
Escherichia coli	gram positive	*ATCC* 25922

B. Preparation of Bile Esculin Agar

To prepare Bile Esculin agar base

1. Prepare Bile Esculin agar base by adding the specified amounts of Bile Esculin agar base to the specified volume of distilled water.
2. Mix thoroughly and heat with frequent agitation
3. Boil for one minute to ensure complete solution of ingredients
4. Adjust volume of finished product as needed using distilled water
5. Dispense 4ml amounts into 13x100mm screw cap tubes
6. Autoclave at 121 ^{o}C for 15 minutes
7. After autoclaving, place tubes in a slanted position and allow to cool

Storage instructions

Prepared media may be stored at 2-8 ^{o}C for up to 6 months in screw caps. Each lot should be dated

Quality control

Check performance of the complete medium with pure cultures of stable control organisms producing known desired reactions. Inoculate one tube for each of the following stock cultures. Incubate tubes as directed for 24-48 hours at 35 ^{o}C

Atmosphere	**Organism**	**Culture #**	**Desired result**
Room air	*E.faecalis*	ATCC 29212	Growth, BE positive (black)
Room air	*Group A Streptococcus*	ATCC 19615	Inhibited; BE negative

Incubate one un-inoculated tube to serve as sterile control.

C. Preparation of Blood Agar (BA)

Procedure

1. Weigh 500ml of distilled water using a measuring cylinder.
2. Transfer the distilled water into a 1 litre cornical flask.
3. Weigh 20g of Tryptic Soy Blood Agar Base (TSBA) using a weighing balance.
4. Suspend the measured TSBA into the 500ml of distilled water.
5. Mix thoroughly (dissolving occurs during autoclaving).
6. Autoclave at 121°C for 15 minutes.
7. Allow the autoclaved TSBA to cool to 45-50°C and then aseptically add 25ml of sterile defibrinated blood. Mix thoroughly.
8. Arrange the petri-dishes onto the clean safety hood and then gently pour the warm blood agar onto the plates.
9. Using a bunsen burner gently invert and pass the flame over the poured blood agar in the plate such that the air bubbles are removed.
10. Cover the petri-dishes and allow the blood agar to coagulate before storage in a refrigerator.
11. Label on the bottom top of the blood agar plates the batch number, date prepared and expiration date, and tech. initials.

Quality Control

a. Sterility Check

1. Randomly select 2 blood agar plates and incubate them at 37°C for 24 hours.
2. If there is no visible growth or haemolysis of the media then the blood agar is sterile and ready for use.

b. Test to support growth of bacteria

1. After sterility check, inoculate two BA plates with a strain of *Staphylococcus aureus.*
2. Incubate the plates at 37°C for 24 hours.
3. Observe for a luxurious growth of *S. aureus* on both plates.
4. If only one plate shows growth, repeat QC with two other plates.
5. If there is no growth or only one plate shows growth, then QC fails.

D. Preparation of GC agar

Preparation of GC agar base

1. Prepare double strength base by suspending the indicated amount of GC agar base in purified distilled water using a flask that will contain the final volume of the media.
2. Add extra agar and glucose
3. Mix thoroughly and heat with frequent agitation.
4. Boil for 1 minute to assure complete solution of ingredients.

To prepare hemoglobin solution

1. Prepare double haemoglobin solution by suspending the indicated amount of haemoglobin in a small volume of purified distilled water using a flask that will contain the final volume of the media.
2. Mix until a smooth paste is achieved.
3. Gradually add the balance of the water until the solution is homogenous.

Autoclave both solutions separately at 121 ^{o}C for 15 min, cool to 50 ^{o}C.

To prepare Isovitalex ™

1. Reconstitute Isovitalex by adding 10ml of the diluent provided to the Isovitalex.
2. Shake to ensure complete solution.
3. Use immediately or store at 2-8 ^{o}C for up to 2 weeks.

To make the GC agar

1. Add cooled haemoglobin to the cooled GC agar base. Avoid creating bubbles if possible.
2. Pour into petridishes and let harden before storing in plastic bags.
3. Store at 2-8 ^{o}C for up to 4 weeks

Quality control

Check the performance of the complete medium with pure culture of stable control organisms producing known desired reactions. Use the following stock cultures and incubate plates as directed for 24-48 hrs at 35 ^{o}C in 5% CO_2 atmosphere.

Organism	**desired result**	**culture number**
Neisseria gonorrhoeae	growth	ATCC 31426
Haemophilus influenzae	growth	

Incubate one additional plate per batch to determine the sterility of the media.

E. Preparation of MacConkey agar

Procedure

1. Prepare the MacConkey agar base by adding the specified amount of MacConkey agar base to the specified volume of distilled water.
2. Mix thoroughly, and heat with frequent agitation.
3. Boil for 1 minute to ensure complete solution of ingredients. Adjust volume of finished product as needed using distilled water.
4. Dispense in 5ml amounts into 13x100mm screw top tubes. Cap and autoclave at 121^{o}C for 15 minutes.
5. After autoclaving, cool to 50^{o}Cand dispense into plates.

Storage instructions

1. Prepared media may be stored at 2-8 oC for up to4 weeks if stored in plastic bags. Each lot should be dated.

Quality control

Check performance of the complete medium with pure cultures of stable control organisms producing known desired reactions. Divide plates into four quadrants. Use the following stock cultures to inoculate each of the quadrants. Incubate plates as directed for 24-48 hours at 35^{o}C. Leave one quadrant inoculated to serve as sterile control.

Atmosphere	Organism	Culture No	Desired Result
Room air	*Proteus mirabilis*	ATCC 12453	Growth; Lactose negative
Room air	*Escherichia coli*	ATCC 25922	Growth; lactose positive
Room air	*Staphylococcus aureus*	ATCC25923	Inhibited

Appendix 13: Standard operating procedures for bacterial identification tests

A. Gram staining technique

Principle

The gram stain is used to differentiate between gram-positive and gram-negative bacteria. Cellular morphology can also be determined. Gram-positive and gram-negative bacteria are both stained by crystal violet. The addition of iodine forms a complex within the cell wall. Addition of a decolorizer removes the stain from gram-negative organisms due to their increased lipid content. These cells are stained pink with the counter stain safranin.

Specimen

The gram stain can be performed on the growth of any strain grown on any type of media. However, for this group of bacteria (gram-positive cocci), it is best performed on the growth of bacteria in thioglycolate broth at 24h incubation. The staining procedure is modified when preparing the smear from thioglycolate broth. The smear cannot be fixed to the slide with hear but must be fixed with methanol.

Reagents and Material (Store at room temperature)

i. Crystal Violet Stain
ii. Gram Iodine (Combine Gram Iodine Concentrate to Gram Iodine Diluent)
iii. Decolorizer Solution
iv. Methanol
v. Slides
vi. Inoculating loop
vii. Microscope with Immersion Objective

Procedure

1. **Spread single loop of culture from the thioglycolate broth to a microscope slide.** Spread the culture over $^{1}/_{3}$ to ½ to the total area of the slide.
2. Allow the smear to air dry. This may take up to 1 hour depending on the temperature and humidity of the room.
3. Cover the entire bacterial smear with 3 or 4 drops of methanol to fix the smear and allow to air dry. Again this may take up to an hour.
4. Cover the bacterial smear with crystal violet stain and allow to stand 1 minute. Gently was the stain off with cool tap water and drain water from slide.
5. Cover the smear with grams iodine and allow to stand 1 minute. Gently wash the iodine off with water and drain the water from the slide.
6. Rinse the bacterial smear with decolorizer solution for 10 seconds; decolorization is complete when the solution runs clear from the slide. Gently rinse with water and drain the slide.
7. Cover the bacterial smear with safranin stain, and allow to stand for 1 minute, then gently wash the stain from the slide.
8. Blot the slide dry with absorbent paper and examine the slide under oil immersion lens.

Reading and Interpretation

The gram stain is used to aid in the differentiation of the gram positive cocci. The arrangement of the cells is what helps to differentiate the genera. Bacteria that divide on random planes form grape-like clusters of cells. This is the type of arrangement commonly observed with staphylococci. Bacteria that divide on one plane form pairs and eventually form chains if the cells remain attached to each other. This type of cellular morphology is observed with streptococci. Bacteria that divide on two planes at right angles form packets of fours or tetrads. This type of arrangement is observed with the aerococci.

One of the most difficult tasks that microbiologist have is determining whether or not the cellular morphology of the cells are actually cocci or short rods. Since many of the lactobacilli are gram positive short rods in chains, they are sometimes confused with the streptococci. The clinical sources and colonial morphology on blood agar plates of the lactobacilli are also similar to the streptococci, especially members of the viridans streptococci. When reading the gram stain, remember that the cellular arrangement is never 100% chains, pairs, tetrads, or clusters. The microbiologist must determine the most common cellular arrangement. For example, for the *Gemella* species, one might observe some pairs and short chains as well as tetrads. If tetrads are observed in most fields under observation, then the strain is dividing on two planes and this should be recorded.

Quality Control

The gram stain quality control is performed once per week.Inoculate *Streptococcus sanguinis* strain SS910 and *Escherichia coli* 25922 into thioglycolate broth medium and incubate overnight at 35˚ C ambient air. Prepare the slide using 1 loopful of each culture on the same slide. Slides may be fixed in advance and stored. The completed procedure should show gram-positive cocci in chains and gram- negative rods. Record results in QC maual.

B. Catalase Test

Principle

Hydrogen peroxide is used (H_2O_2) to determine if bacteria produce the enzyme catalase.

Specimen

Cultures that are grown on a blood free media or a colony grown on a blood agar plate that is carefully transferred to a slide without carry-over of any of the erythrocytes. Cultures are typically grown overnight at 35˚C in CO_2.

Reagents and Materials

1. Three percent hydrogen peroxide is obtained from a commercial drug store.
2. Pipet
3. Slides

Procedure

1. The catalase test is best performed by flooding the growth of the bacteria (usually on an agar slant but blood free agar plates can be used) in question with 1.0 ml of 3% hydrogen peroxide and observing for effervescence (bubbling) which indicates a positive test. The bacteria must be grown on blood free medium.
2. Modifications of the catalase test may be performed by very carefully removing a colony of growth from a blood agar plate with a plastic needle or wooden applicator stick and transferring the colony to a glass slide. A drop of 3% hydrogen peroxide is added to the colony on the slide and observed for effervescence.

Reading and Interpretation

Any sign of bubbling is interpreted as **a positive test**. The absence of bubbling is interpreted as negative.

Quality control

The catalase quality control is performed once per lot and shipment. For positive reaction use a blood- free culture of *Staphylococcus aureus*: i. e., Cowen strain I but other confirmed Staphylococcal cultures can be used. For negative reaction use *Streptococcus sanguinis* strain SS-910 (ATCC-10556). Record in QC manual.

C. Coagulase test

Principle

Coagulase exists in two forms, bound coagulase and free coagulase. Bound coagulase is bound to the cell wall of *S. aureus* and is detected with the slide test. Free coagulase is excreted by the cell and is present in culture filtrates. Free coagulase (or extra cellular

coagulase) is detected with a tube test. The coagulase test is used to distinguish between *Staphylococcus aureus* and species of coagulase-negative *Staphylococcus*.

Equipment and materials

Slide Test

1. Glass slide
2. Sterile wooden sticks
3. Distilled water
4. Coagulase solution: (see preparation below)
5. Sterile inoculating loops
6. Fresh cultures of organisms to be tested

Tube Test

1. Coagulase solution: (see preparation below)
2. Sterile inoculating loops
3. 12x75mm tubes
4. Fresh cultures of organisms to be tested
5. 35^{o}C aerobic incubator or heating block

Preparation of required reagents

Coagulase: To prepare rabbit plasma reagent, rehydrate commercially packed EDTA-treated rabbit plasma by adding 5 mls of sterile distilled water to the lyophilized reagent. Shake well to ensure complete solution of reagent before use. Date each vial when reconstituted. The expiration date after reconsttuiton is the same as the expiration date printed on the vial. Discrad if the vial becomes cloudy or if obvious growth of organisms has occurred in the vial.

Procedure for slide test

1. Use 24-72hr growth and emulsify a heavy suspension of the organism in a drop of sterile water on clean glass slide. If auto agglutination occurs, the tube test must be used.
2. Place a drop of plasma next to the organism suspension.
3. Use a sterile wooden stick to mix the coagulase solution into the organism suspension.

4. Observe for a white, flaky fibrin precipitate. Development of a precipitate constitutes a positive test. If desired, confirm negative results with the tube test. Delayed results should be confirmed with the tube test.

Interpretation

Positive: Development of precipitate within 20 seconds

Weak: Development of a precipitate after more than 20 seconds

Negative: No precipitate

Procedure for tube test

1. Pipette 0.5mls of coagulase reagent into a 13x100mm screw cap tube.
2. Emulsify several colonies of the suspected organism in the reagent to form a milky suspension.
3. Incubate the test at 35^{o}C in ambient air for 4 hours.
4. Check the tube for clot formation. Compare the test against the positive control.
5. If the tube is negative at 4 hours, continue the incubation overnight and check again for clot formation.

Interpretation

Positive: Development of a clot; any clot is considered a positive test. However Flocculent or fibrous precipitate is not a true clot and should be recorded as negative

Negative: No clot is formed.

Quality control

Positive: *Staphylococcus aureus* ATCC 27923

Negative: *Staphylococcus epidermidis* ATCC 14990

D. Bacitracin Test

Principle

The bacitracin disk is sensitivity test used to differentiate the beta- hemolytic *Streptococcus*.

Inoculum

An overnight culture grown on 5% sheep blood agar incubated 35˚C in CO_2.

Reagents and Materials

1. Bacitracin "A" disk (BBL)

Procedure

1. Select a beta-hemolytic colony and heavily inoculate a quadrant of a 5% sheep blood agar plate.
2. Drop an "A" disk in the heaviest zone of inoculation.
3. Tap disk lightly to ensure that it adheres to the agar.
4. Incubate plate overnight in CO_2 at 35˚C.

Reading and Interpretation

Any zone of inhibition is considered a positive test or sensitive test.

Growth to the edge of the disk is interpreted as a negative test or resistant test.

Quality Control

Quality Control is performed on each lot of bacitracin disk. *Streptococcus pyogenes* is the positive (sensitive control) and *Enterococcus faecalis* SS1273 is the resistant or negative control. Results are recorded in the QC book.

E. Bile Esculin Test

Principle

A selective and differential medium used in the identification of catalase-negative bacteria. The selective agent bile, inhibits most gram positive bacteria. The enterococci and *Streptococcus bovis* will grow.

Esculin in the medium is hydrolyzed to esculetin and dextrose. The esculetin reacts with ferric chloride in the media to form a black-brown color.

Inoculum

An overnight culture in Todd Hewitt broth incubated over night at 35˚ C or a fresh bacterial suspension in Todd Hewitt broth may be used as the inoculum. An inoculating loopful of culture may also be used.

Reagents and Materials

1. Bile esculin slant (Remel)

Procedure

1. Inoculate tube with a loopful of growth from a blood agar plate.
2. The slant is then incubated at 35^{o}C for 2 days in ambient air.

   Fastidious organisms may be held up to 14days.

Reading and Interpretation

Positive: The bile esculin test is positive when a black color forms over one-half or more of the slant.

Negative: If no blackening occurs the test is negative.

Quality Control

Positive and negative reactions are determined on each new lot of media. *Enterococcus faecalis* strain SS-1273 is used for positive control reactions and *Streptococcus sanguinis* strain SS-910 is used for negative control reactions. Results are recorded in the QC book.

F. Optochin Test

Principle

The purpose of the optochin test is to confirm the identification *S. pneumoniae* before serotyping and to aid in the differentiation of *S. pneumoniae* from viridans streptococci during surveillance studies.

Inoculum

Isolated alpha-hemolytic colonies suspected of being pneumococci

Reagents and Materials

1. Optochin^{R} disks purchased from Becton Dickinson Microbiology Systems, Cockeysville, Md.
2. blood agar plates

Procedure

1. Transfer an isolated colony and streak to a quarter of a blood agar plate.
2. Place the optochin "P" disk in the upper third of the inoculum. Tap the disk to insure that it stays on the media after the plate is inverted.
3. The plate is incubated overnight at 35-37^{o}C in a candle extinction jar or carbon dioxide incubator.

Reading and Interpretation

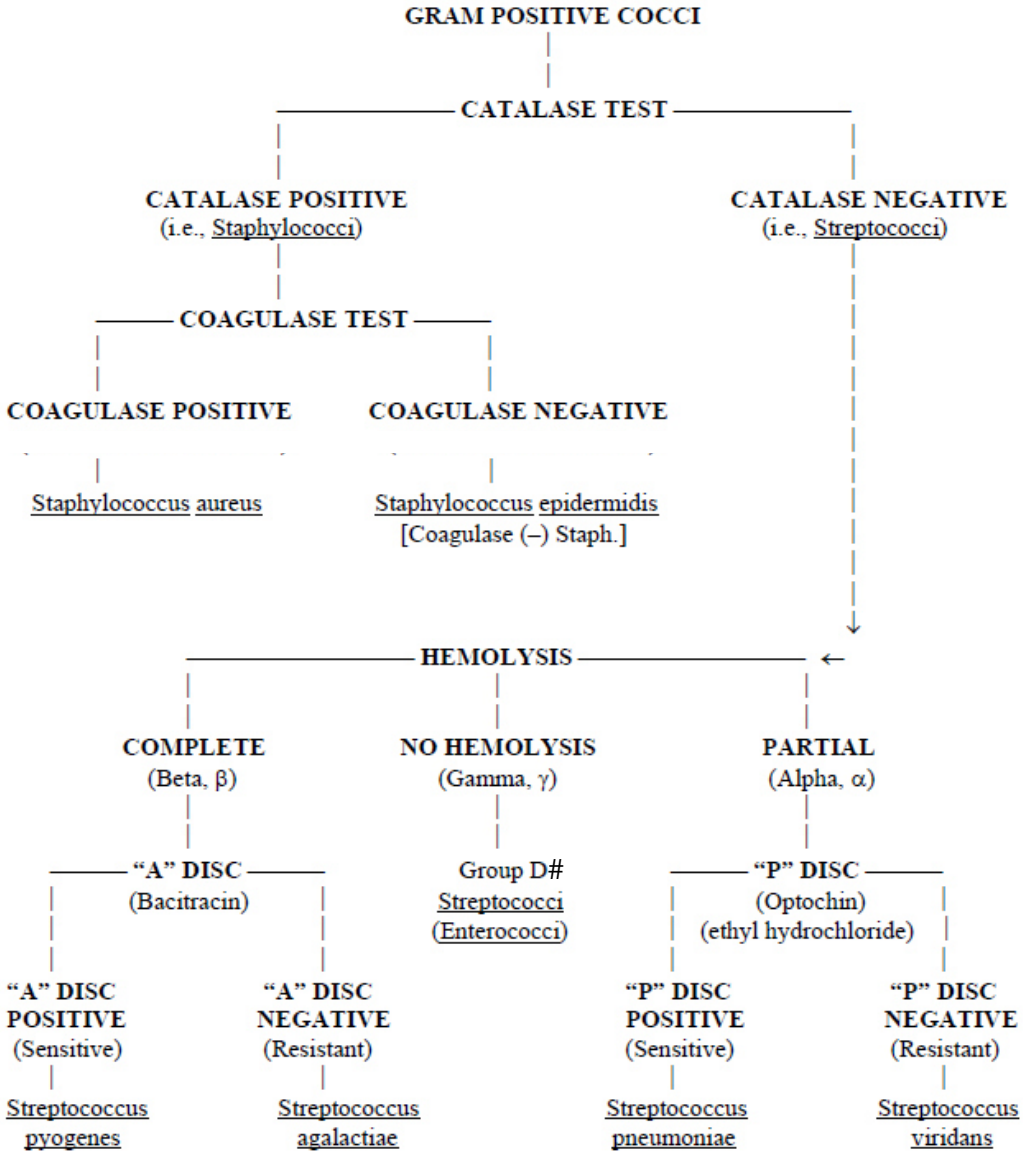
If a 6 mm disk is used, a zone of inhibition of at least 14 mm in diameter is considered positive for identification of pneumococci. A zone of inhibition between 6 and 14 mm in diameter is considered questionable for identification of pneumococci and a bile solubility test should be performed. Bile soluble strains with optochin zones of inhibition between 6 and 14 mm are considered pneumococci, those strains that are not bile soluble with the same zone sizes are not considered pneumococci.

Quality Control

Each new lot and shipment of optochin disks is tested for positive and negative reactions. *S. pneumoniae* strain ATCC-49619 is inhibited by optochin (positive reaction) and *S. mitis*

strain SS-429 is not inhibited by optochin (negative reaction). Results are recorded in the QC log book.

Bacteria identification flow chart



 # Group D (Enterococci are confirmed by positive bile esculin test

Appendix 14: Laboratory microbiology identification worksheet

Specimen No. _____(Separate sheet for every specimen) Sheet No._____ Microbiology Worksheet

Specimen No.	Specimen G/stain	Media used	Isolate #	Colony Description	Isolate Gram stain	Catalase	Coagulas	Optorchin	Bacitracin	**Other Biochem**							
					API20E	Indole	Nitrate	VP	ADH	ODC	Urea	OX	ONPG	LDC	CIT	H2S	
Susceptibility	**Drug**											**Final Identification**			Stock	Date	Tech. Initials
	Susceptibility																
	Other tests														Apprv	Date	Tech. Initials

Specimen No.	Specimen G/stain	Media used	Isolate #	Colony Description	Isolate Gram stain	Catalase	Coagulas	Optorchin	Bacitracin	**Other Biochem**							
					API20E	Indole	Nitrate	VP	ADH	ODC	Urea	OX	ONPG	LDC	CIT	H2S	
Susceptibility	**Drug**											**Final Identification**			Stock	Date	Initials
	Susceptibility																
	Other tests														Apprv	Date	Tech. Initials

Specimen No.	Specimen G/stain	Media used	Isolate #	Colony Description	Isolate Gram stain	Catalase	Coagulas	Optorchin	Bacitracin	**Other Biochem**							
					API20E	Indole	Nitrate	VP	ADH	ODC	Urea	OX	ONPG	LDC	CIT	H2S	
Susceptibility	**Drug**											**Final Identification**			Stock	Date	Initials
	Susceptibility																
	Other tests														Apprv	Date	Tech. Initials

Microbiology Worksheet *REC 021* *14-4-2013*

Appendix 15: Procedures for Ribonucleic Acid extraction

RNA extraction and purification by Qiagen QIAamp Viral RNA Mini kit cat# 52904

1. Add 560 µL Buffer AVL containing carrier RNA to 140 µl sample. Mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the tube to remove drops from inside the lid. *Note: Prepare lysis buffer in clean reagent preparation area to prevent carry over contamination.*
2. Prepare individual 560 µl aliquots of AVL lysis buffer into 1.4 ml or 2.0 ml microcentrifuge tubes. Incubate at room temperature (15-25ºC) for 10 min.
3. Add 560 µl (equal volume) of 96-100% ethanol to the sample and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
4. Apply 630 µl onto QIAamp Mini spin column in a 2 ml collection tube. Close the cap and centrifuge for 1 min at 6,000 rpm. Place the QIAamp spin column into a clean 2 ml collection tube and discard the tube containing the filtrate.
5. Carefully apply the rest of the lysate into the same spin column and repeat step 4.
6. Add 500 µl of Wash Buffer AW1 to spin column. Close the cap and centrifuge for 1 min at 8,000rpm. Transfer column into a new 2ml collection tube. Discard flow-through and collection tube.
7. Add 500 µl Wash Buffer AW2. Close the cap and centrifuge for 3 min at 20,000rpm. Discard flow-through and collection tube.Optional: to eliminate the chance of possible Buffer AW2 carryover, place column in a new 2ml collection tube and centrifuge at full speed for 1 min.
8. To elute the RNA, transfer column to a new 1.5 ml tube and add 60 µl buffer AVE. Be careful to add buffer to the center of the filter membrane. Do not touch the tip to the filter. Close the tube. Incubate at room temperature for 1 min.
9. Centrifuge 1 minute at 6,000 rpm. Eluted RNA is contained in the flow-through fraction of water.
10. Keep on ice if the RNA is to be used immediately. Viral RNA is stable for up to one year when stored at 80°C.

Appendix 16: Standard operating procedure for real-time polymerase chain reaction protocol for detection and characterization of influenza viruses

Materials

Equipment

1. Real-time PCR detection system with a 96-well format thermocycler reaction block such as Applied Biosystems™ real-time PCR systems (7000, 7300, 7500, etc.), BioRad real-time PCR detection system (iQ™ or iQ5™) or Stratagene QPCR instruments (MX4000®, MX3000® or MX3005®).
2. Microcentrifuge
3. Vortex

Supplies

1. Laboratory marking pen
2. Cooler racks for 1.5 microcentrifuge tubes and 96-well 0.2ml PCR reaction tubes
3. 20µl and 200µl adjustable pipettes and aerosol barrier tips
4. 0.2ml PCR reaction tube strips or plates
5. Optical strip caps
6. Sterile, nuclease free 1.5 ml microcentrifuge tubes
7. Disposable powder-free gloves

Reagents

1. The Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (CDC; catalog #KT0078).
2. One-step quantitative RT-PCR probe hydrolysis kit
3. Molecular grade sterile distilled water (RNase and DNase free)
4. Forward and reverse primers (40µM)
5. Dual-labeled probes (10µM)
6. Positive control RNAs

Procedure

1. Reagent preparation

NOTE: Keep all reagents on cold rack during assay set up.

(a) Primers and probes

- Thaw frozen aliquots of primer and probes (Thawed aliquots of probes may be stored in the dark up to 3 months at 2-8°C. Do not re-freeze probes).

- Vortex all primers and probes.
- Flash centrifuge all primers and probes and then place in cold rack.

(b) Realtime RTPCR reagents

- Place Master Mix and enzyme in cold rack
- Thaw the 2X Reaction Mix vial.
- Mix the 2X Reaction Mix by inversion.
- Flash centrifuge 2x Reaction Mix and enzyme then place in cold rack

Tests for each RT-PCR run

1. Each sample RNA extract is tested by separate primer/probe sets: Influenza A, Influenza A seasonal (H3N2), Influenza A pandemic (H1N1)2009 and Influenza B.
2. No template controls (NTC) and positive template controls for all primer/probe sets are included in each run.

Type/subtype	Gene fragme	Primer	Sequence (5'- 3')
Influenza type A	Matrix (M)	FLUA-1Forward	AAGACCAATCCTGTCACCTCTGA
		FLUA-1Reverse	CAA AGCGTCTACGCTGCAGTCC
		FLUA-1Probe	TTTGTGTTCACGCTCACCGT
Influenza A (H1N1)2009 (Pandemic influenza)	HA	PndmFA-Forward	GACAAAATAACAAACGAAGCAACTGG
		PndmFA- Reverse	GGGAGGCTGGTGTTTATAGCACC
		PndmFA- Probe	GCATTCGCAA"t"GGAAAGAAATGCTGG
Influenza A(H3N2) (Seasonal influenza)	HA	SeaFluA-Forward	AGCAAAGCCTACAGCAA
		SeaFluA-Reverse	GACCTAAGGGAGGCATAA
		SeaFluA-Probe	5'-Fam-CCGGCACATCATAAGGGTAACA 3'-BHQ
Influenza B	HA	FluB – Forward	AGACCAGAGGGAAACTATGCCC
		FluB-Reverse	TCCGGATGTAACAGGTCTGACTT
		FluB-Probe	Fam-5' CAGRCCAATGTGTGTGGGGAYCACACC-3'-BHQ

Reaction setup

Reaction assay mixtures are made as a cocktail and dispensed into the 96-well reaction plate. Water, extracted nucleic acid or positive template controls, are then added to the appropriate test reactions and controls.

1. Label one 1.5 ml microcentrifuge tube for each primer/probe set.
2. Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction cocktail to allow for the NTC and positive controls. See below:

- If number of samples (n) including controls = 1 to 14, then N = n + 1
- If number of samples (n) including controls > 15, then N = n + 2

3. Master Mix: calculate the amount of each reagent to be added for each primer/probe set reaction master mix. The calculations are as follows:

Reagent	**Volume**
Nuclease free water	N x 6.0 µl
Forward primer (0.8 µM final concentration)	N x 0.25 µl
Reverse primer (0.8 µM final concentration)	N x 0.25 µl
Probe (0.2 µM final concentration)	N x 0.25 µl
Enzyme (RT) Mix	N x 0.75 µl
2X PCR Master Mix	N x 12.5 µl
Total volume	N x 20.0 µl

4. In the assay set up area, dispense reagents into labeled 1.5 ml microcentrifuge tubes. After addition of the water, mix reaction mixtures by pipetting up and down. Do not vortex.

5. Centrifuge for 5 sec to collect contents at bottom of the tube, and then place the tube in cold rack.

6. Set up reaction strip tubes or plates in 96-well cooler rack.

7. Dispense 20µl of each master mix into each well going across the row as shown below: Example Test setup:

	1	2	3	4	5	6	7	8	9	10	11	12
	Influenza A reaction mix in all						Influenza B reaction mix in all					
A	S1	S2	S3	S4	S5		S1	S2	S3	S4	S5	FluA +control
B	S6	S7	S8	S9	S10		S6	S7	S8	S9	S10	Empty
C	S11	S12	S13	S14	S15		S11	S12	S13	S14	S15	FluA -control
D	S16	S17	S18	S19	S20	EMPTY	S16	S17	S18	S19	S20	Empty
E	S21	S22	S23	S24	S25		S21	S22	S23	S24	S25	FluB +control
F	S26	S27	S28	S29	S30		S26	S27	S28	S29	S30	Empty
G	S31	S32	S33	S34	S35		S31	S32	S33	S34	S35	FluA +control
H	S36	S37	S38	S39	S40		S36	S37	S38	S39	S40	Empty

8. Before moving the plate to the nucleic acid handling area, set up the NTC reactions for column 1 in the assay set-up area. As shown above, samples can be added by column.

9. Pipette 5 µl of nuclease free water into the negative template control wells. Cap NTC wells.

10. Cover the reaction plate and move the reaction plate to the nucleic acid handling area.

11. Vortex for 5 sec the tubes containing the samples. Centrifuge tubes for 5 sec.

12. Set up the extracted nucleic acid samples in the cold rack.

13. As shown above, samples can be added by well. Pipette 5 µl of the first sample into the wells labeled for that sample (for example, Sample "S1" as shown above). Change tips after each addition

14. Cap the column to which the sample has been added. This will help to prevent sample cross contamination and enable you to keep track of where you are on the plate.

15. Change gloves when necessary to avoid contamination.

16. Repeat steps **13.** through **15.** for the remaining samples.

17. Finally, pipette 5µl of positive control RNA into respective wells for influenza A and B. Cap the wells

18. Centrifuge the plates at 500 x g for 30 seconds at 4°C. Return to cold rack.

RT-PCR amplification conditions:

The reaction volume is 25µl. Program the thermocycler as follows:

Step	**Conditions**
Reverse Transcription	40°C for 30 min
Taq inhibitor inactivation	95°C for 10 min
PCR amplification (45 cycles)	95°C for 15 sec 55°C for 30 sec*

* Fluorescence data (FAM) should be collected during the 55°C incubation step.

Interpretation/examination

1. The positive and negative controls are examined to ensure they meet the condition prior to interpretation of the results. The negative controls are not expected to exhibit fluorescence growth curves that cross the threshold line while positive template control is expected to produce positive reactions between 20 and 35 cycles. The results are interpreted as positive if growth curves crossed the threshold line within 35 cycles in each of the sample wells and negative if growth curves for neither Influenza A nor Influenza B crossed the threshold line within 35 cycles.

2. When all controls meet stated requirements, a specimen is considered

presumptive positive for influenza A virus if the influenza A reaction growth curves cross the threshold line within 40 cycles. If the reaction for influenza A is positive,

3. When all controls meet stated requirements, a specimen is considered presumptive positive for influenza B virus if influenza B reaction growth curves cross the threshold line within 40 cycles.
4. When all controls meet the stated requirements, a specimen is considered negative for influenza virus if growth curves for neither Influenza A nor Influenza B cross the threshold within 40 cycles.

Appendix 17: Standard operating procedure for conventional polymerase chain reaction protocol for detection and characterization of non-influenza viruses

Materials

Equipment

1. Microcentrifuge
2. Vortex
3. PCR thermocycler system with a 96-well format.

Supplies

i. Laboratory marking pen
ii. Cooler racks for 1.5 microcentrifuge tubes and 96-well 0.2ml PCR reaction tubes
iii. 20µl and 200µl adjustable pipettes and aerosol barrier tips
iv. 0.2ml PCR reaction tube strips or plates
v. Strip caps
vi. Sterile, nuclease free 1.5 ml microcentrifuge tubes
vii. Disposable powder-free gloves

Reagents

5. One-step RT-PCR kit

    - Qiagen OneStep RT-PCR (cat# 210210 for 25 rxns, cat# 210212 for 100 rxns)

6. Molecular grade sterile distilled water (RNase and DNase free)
7. Forward and reverse primers (40µM)
8. Positive control RNAs

Procedure

Reagent preparation

NOTE:Keep all reagents on cold rack during assay set up.

- Primers
    - Thaw frozen aliquots of primers.
    - Vortex all primers.
    - Flash centrifuge all primers and then place in cold rack.
- RT-PCR reagents
    - Place Master Mix and enzyme in cold rack
    - Thaw the 5X Reaction Mix vial.
    - Mix the 5X Reaction Mix by inversion.
    - Flash centrifuge 5X Reaction Mix and enzyme then place in cold rack

Tests for each RT-PCR run

I. Each sample RNA extract is tested by primer sets below in multiplex PCR:

Virus	Primers	Sequence (5' – 3')	Gene	Amplicon size (bp)	Melting temp (°C)
hRSV	vrs 1	GGA ACA AGT TGT TGA GGT TTA TGA ATA TGC	Nucleocapsid	279	60
	vrs P2	TTC TGC TGT CAA GTC TAG TAC ACT GTA GT ACT TG			55
hMPV	hmpv 1	CCC TTT GTT TCA GGC CAA	Matrix protein	416	54
	hmpv 2	GCA GCT TCA ACA GTA GCT G			58
Parainfluenza virus 1	PIS1+	CCG GTA ATT TCT CAT ACC TAT G	Hemagglutinin-	317	48
	PIS1−	CCT TGG AGC GGA GTT GTT AAG	Neuraminidase		51
Parainfluenza virus 2	PIP2+	AAC AAT CTG CTG CAGCAT TT	Hemagglutinin-	507	56
	PIP2−	ATG TCA GAC AAT GGG CAA AT	Neuraminidase		56
Parainfluenza virus 3	Para3.1	CTC GAG GTT GTC AGG ATA TAG	Hemagglutinin-	189	46
	Para3.2	CTT TGG GAG TTG AAC ACA GTT	Neuraminidase		48
Internal control	GAPDH1	TCA TCC ATG ACA ACT TTG GTA TCG TG	GAPDH	564	59
	GAPDH2	CTC TTC CTC TTG TGC TCT TG			60

II. No template controls (NTC) and positive template controls (hMPV, RSV) one run and another run (parainfluenza 1, 2 and 3) for all primer sets are included in each run.

Reaction setup

Reaction assay mixtures are made as a cocktail and dispensed into the 96-well reaction plate. Water, extracted nucleic acid or positive template controls, are then added to the appropriate test reactions and controls.

1. Label one 1.5 ml microcentrifuge tube for each primer set.

2. Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction cocktail to allow for the NTC, positive template controls. See below:

- If number of samples (n) including controls = 1 to 14, then N = n + 1
- If number of samples (n) including controls > 15, then N = n + 2

3. Master Mix: calculate the amount of each reagent to be added for each primer set reaction master mix. The calculations are as follows:

Reagent	Volume
Nuclease free water	N x 13 µl
Forward primer (0.8 µM final concentration)	N x 1.25 µl
Reverse primer (0.8 µM final concentration)	N x 1.25 µl
dNTPs (0.4 mM)	N x 1.0 µl
Enzyme Mix	N x 1.0 µl
5X PCR Master Mix	N x 5 µl
Total volume	N x 22.5 µl

4. In the assay set up area, dispense reagents into labeled 1.5 ml microcentrifuge tubes. After addition of the water, mix reaction mixtures by pipetting up and down. Do not vortex.
5. Centrifuge for 5 sec to collect contents at bottom of the tube, and then place the tube in cold rack.
6. Set up reaction strip tubes or plates in 96-well cooler rack.
7. Dispense 20µl of each master mix into each well going across the row as shown below: Example Test setup:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
B	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
C	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36
D	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48
E	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60
F	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71	S72
G	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83	S84
H	S85	S86	S87	S88	S89	S90	S91	S92	-Control	-Control	+Control	+Control

8. Before moving the plate to the nucleic acid handling area, set up the NTC reactions for column 1 in the assay set-up area. As shown above, samples can be added by column.
9. Pipette 5 µl of nuclease free water into the NTC wells. Cap NTC wells.
10. Cover the reaction plate and move the reaction plate to the nucleic acid handling area.
11. Vortex for 5 sec the tubes containing the samples. Centrifuge tubes for 5 sec.
12. Set up the extracted nucleic acid samples in the cold rack.
13. As shown above, samples can be added by column. Pipette 5 µl of the first sample into the well labeled for that sample (for example, Sample "S1" as shown above). Change tips after each addition
14. Cap the column to which the sample has been added. This will help to prevent sample cross contamination and enable you to keep track of where you are on

the plate.

15. Change gloves when necessary to avoid contamination.

16. Repeat steps **13.** through **15.** for the remaining samples.

17. Finally, pipette 5 µl of positive template control RNA into the respective wells. Cap the wells

18. If using plates, centrifuge at 500 x g for 30 seconds at 4°C. Return to cold rack.

RT-PCR amplification conditions:

The reaction volume is 25µl. Program the thermocycler as follows:

	Qiagen
Reverse Transcription	50°C for 30 min
Taq inhibitor inactivation	95°C for 15 min
PCRamplification (45 cycles)	94°C for 30 sec 55°C for 30 sec
Final extension	720C for 10 min

Interpretation/examination

Electrophoresis of all reactions including all test samples and controls must be performed on a 2% agarose gel (0.5X TBE or 1X TAE) containing ethidium bromide at a final concentration of 0.5 ug/ml. Mix 10ul of each reaction with equal volume of loading buffer containing 0.25% xylene cyanol but does not contain Bromphenol Blue (this may obscure visualization of DNA fragments of 100-300 bp). Each agarose gel should include a DNA size standard range of 50-1000 bp. Standard gel electrophoresis should be performed at 150V for 30 minutes.

1. The NTC reactions for all primer sets should not exhibit presence of amplified DNA products of size similar to that of the positive control reaction. If a false positive occurs with one or more of the primer NTC reactions, sample contamination may have occurred. Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.

2. Positive template control reactions should produce a positive result with the respective virus hMPV, RSV, parainfluenza 1,2 and 3 as demonstrated by presence of amplified DNA products of appropriate size.

If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines. Determine the cause of failed

POSITIVE TEMPLATE CONTROL reactivity, implement corrective actions, and document results of the investigation and corrective actions. Do not use POSITIVE TEMPLATE CONTROL reagents that do not generate expected result.

3. Specimen are considered to be positive if they exhibited presence of amplified DNA products of corresponding to the respective positive control as follows: hMPV (416bp) and hRSV (279bp) and the second reaction, parainfluenza 1 (317bp), parainfluenza 2 (507bp) and parainfluenza 3 (189bp).

4. Specimen are considered negative if the reactions do not exhibit presence of amplified DNA products of size equivalent to the positive control of the virus targeted.