PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF BACTERIAL PATHOGENS ISOLATED FROM OUTPATIENTS WITH UPPER RESPIRATORY TRACT INFECTIONS IN KITUI DISTRICT HOSPITAL, KENYA

MUTINDA JOSPHAT

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MAY, 2015
DECLARATION

I declare that this thesis is my original work and has not been presented for a degree in any other University or for any other award.

Mutinda Josphat
156/CE/22251/2010

Signature Date 29/5/2015

Supervisors’ approval

This thesis has been submitted for examination with our approval as university supervisors.

Dr. John Maingi
Department of Microbiology
Kenyatta University

Signature Date 29/5/2015

Dr. Anthony Kebira
Department of Microbiology
Kenyatta University

Signature Date 29/5/2015
This work is dedicated to my parents Mr. Anthony Mutinda Kiteme and Mrs. Mary Mutinda who set me on the right path for life.
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# Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CBPs</td>
<td>Choline binding proteins</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical Laboratory Standards Institute</td>
</tr>
<tr>
<td>GABHS</td>
<td>Group A Beta Hemolytic <em>Streptococcus</em></td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Human Immune Virus/ Acquired Immuno-Deficiency Syndrome</td>
</tr>
<tr>
<td>JICA</td>
<td>Japan International Co-operation Agency</td>
</tr>
<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Statistics</td>
</tr>
<tr>
<td>LOS</td>
<td>Lipooligosaccharide</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin Resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee on Clinical Laboratory Standards</td>
</tr>
<tr>
<td>PVL</td>
<td>Panton valentine leukocidin</td>
</tr>
<tr>
<td>RSV</td>
<td>Respiratory syncitial virus</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythromatosus</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SSSS</td>
<td>Staphylococcal scalded skin syndrome</td>
</tr>
<tr>
<td>TAA</td>
<td>Trimeric autotransporter adhesin</td>
</tr>
<tr>
<td>TSS</td>
<td>Toxic shock syndrome</td>
</tr>
<tr>
<td>URTIs</td>
<td>Upper Respiratory Tract Infections</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
Respiratory tract infections, such as pharyngitis, laryngitis, common cold, sinusitis and tonsillitis are the most frequently occurring infections of all human diseases. Since these infections often seem minor, they are more commonly discounted as temporary inconveniences that cause transient discomfort. However, limited information has been documented in Kenya in relation to occurrence and prevalence of the etiological agents causing upper respiratory tract infections. Therefore, this study was aimed at determining the prevalence of bacterial agents causing upper respiratory tract infections and their susceptibility patterns to commonly used antibiotics among outpatients in Kitui district hospital. The objectives of this study were; To determine the prevalence of upper respiratory tract infections in patients of different age groups and sexes, to isolate, identify and assess the distribution of bacterial pathogens associated with upper respiratory tract infections, to determine the prevalence of pathogenic bacteria of the upper respiratory in different age groups and sexes and to determine antimicrobial susceptibility profiles of the isolated bacterial pathogens. A total of 237 throat swabs were collected during the period between November, 2012 to April, 2013 and incubated onto Blood agar, MacCkonkey agar and Chocolate agar then incubated at 37 °C for 24 hours. Colony morphology and standard biochemical tests; Gram staining, catalase test, coagulase test, Mannitol fermentation test, bacitracin sensitivity test, bile solubility, hippurates test, optochin sensitivity test and germ tube test were performed for identification and confirmation of the isolates based on their Gram staining and cultural characteristics. Kirby-Bauer disc diffusion technique was used to determine antimicrobial sensitivity patterns of the bacteria to antibiotics. P values of ≤ 0.05 were considered to have clinical and epidemiological significance. The sample consisted of 36.7 % males and 63.3 % females. The age between 1 and 5 years was a risk factor for these infections with clinical and epidemiological significance. Bacteria isolated were *S. aureus* (44.3 %), viridans group streptococci (32.5 %), *S. pyogenes* (13.5 %) and 5 % were mixed cultures involving *C. albicans* and either viridans group streptococci or *S. aureus*. Resistance of bacterial pathogens to at least one antibiotic in Kitui district hospital was; viridans group streptococci (48.2 %), *S. aureus* (40.5 %), *S. pyogenes* (28.1 %) and there were no cases of multi-drug resistance. *S. aureus* was the most prevalent isolate. Proper interventions should therefore be put in place to prevent young people from contracting and transmitting upper respiratory tract infections.
CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Upper respiratory tract infections can be described as acute infections involving one or
more parts of the upper respiratory tract which comprises of sinuses, nasal passages,
pharynx and larynx (Bisno, 2001; Boon et al., 2013). They include; tonsillitis,
pharyngitis, laryngitis and sinusitis (Eccles et al., 2007). It has been widely reported that
the respiratory tract is the most frequent site of infection because it comes into direct
contact with the physical environment and is exposed to airborne microorganisms such as
viruses, bacteria, fungi and parasites (El-Mahmood et al., 2010).

Studies have shown that upper respiratory tract infections are the most common bacterial
diseases encountered in medical practice today and they affect people of all ages leading
to high morbidity and mortality rates around the world (Kunin, 1994; Raju and Tiwari,
2004; Zafar et al., 2008). For instance, the incidence of these infections has been reported
to be between two and five cases every year in children while among adults, one or two
infections occur per year (Cotton et al., 2008). Such infections have been described as
mild and self limiting, however, they may lead to life threatening complications if left
untreated (Torralba and Quismorio, 2009).

Viruses are the leading cause of URTIs that lead to inflammation of the nose and throat
(Cotton et al., 2008), however, some studies have associated them with bacterial etiology.
Viruses which have been implicated in most URTIs include rhinovirus, parainfluenza
virus, coronavirus, adenovirus, respiratory syncytial virus, coxsackievirus and influenza virus (Cotton et al., 2008; Rohilla et al., 2013), whereas beta-hemolytic streptococci, Corynebacterium diphtheriae, Neisseria gonorrhoeae, Arcanobacterium haemolyticum, Chlamydia pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Bordetella pertussis and Moraxella catarrhalis are the most common bacteria associated with URTIs (Poole and Portugal, 2005). These pathogens invade the mucosal lining of the upper respiratory tract leading to a disease condition (Imani et al., 2005).

The incubation period of most URTIs last from a few hours to 3 days after exposure while the symptoms have been known to last from 7-10 days or even longer (Boon et al., 2013). Some of the clinical signs and symptoms of URTIs which have been reported include; stuffy and runny nose, sneezing, coughing, sore throat, fever, vomiting, irritability, loss of appetite and watery eyes. Proper judgment is required in differential diagnosis of URTIs since there is a wide range of related conditions that may have similar or overlapping clinical signs and symptoms (Boon et al., 2013).

Information about prevention, management or treatment of URTIs is rather inadequate and mostly unconfirmed, this has led to the patients taking preventive measures based on their own experience or preferences in most parts of the globe (Rohilla et al., 2013). Various studies have shown that antibiotic use improves the health of patients with upper respiratory tract infections (Woodhead et al., 2005; Mandell et al., 2007). Moreover, antibiotics seem to increase cure rates and reduce duration of some upper respiratory tract
infections whose microbiological diagnosis suggest bacterial etiology (Wasserfallen et al., 2004).

Boon et al. (2013) reported an increasing trend in prescription of antibiotics for upper respiratory tract infections. However, the quality and necessity of antibiotic therapy has been questioned with various studies confirming that over 50% of antibiotic use in treatment of upper respiratory tract infections is unnecessary and may not improve the clinical outcomes (Dunagan et al., 1991; Boon et al., 2013). Self prescription of antibiotics often lead to increased resistance to commonly used antibiotics by bacterial pathogens, worsen the clinical outcomes and lead to high treatment cost (Laupland et al., 2006).

Due to scarcity in health care resources, policy makers, medical practitioners and the patients themselves focus on cost and effectiveness of antimicrobial therapy in treatment of upper respiratory tract infections (Baltimore, 2010). It is important for medical practitioners to re-examine their prescription or conduct antimicrobial resistance profiling for URTI pathogens to confirm whether it is consistent with current guidelines. This will lead to provision of rational prescription and improve the quality of treatment, which is part of the professional role of the medical practitioners and also curb the emergence of antibiotic resistance by these pathogens (Craig et al., 2003). This study was carried out to determine the prevalence and antimicrobial susceptibility patterns of bacterial pathogens associated with upper respiratory tract infections to commonly prescribed antibiotics among patients visiting Kitui district hospital, Kenya.
1.2 Problem statement and justification

Upper respiratory tract infections are the leading cause of morbidity in both adults and children which may be associated with absenteeism from school and work (Wat, 2004). Moreover, pneumonia may occur due to secondary bacterial infections as a result of these infections (Ndip et al., 2008).

Clinical differentiation of viral and bacterial URTIs using signs and symptoms is often ineffective and unreliable (Choby, 2009). Microbiological analysis and antibiotic susceptibility testing should be done to determine the prevalent organisms and their current sensitivity before antibiotic therapy is administered, however, this is often not possible and antibiotic choice is commonly empirical (El-Mahmood et al., 2010). Furthermore, the increased use of broader spectrum and more expensive antimicrobial drugs have implications for all patients due to the impact on health care costs and the increasing emergence of antimicrobial resistance. In Kenya, most upper respiratory tract infections are treated empirically possibly due to lack of laboratory services or high cost of these services where available. Empirical treatment has been complicated by the emergence of antimicrobial resistance among bacterial pathogens (Aydemir et al., 2006). This challenge coupled with other socio-economic problems like self prescription, high levels of ignorance, poverty, poor hygienic practices and a high prevalence of fake and spurious drugs of questionable quality in circulation in the developing nations as a result of poor governance and regulation of drugs worsens the situation (El-Mahmood et al., 2010).
Previous studies have only focused on describing upper respiratory tract infections in children under the age of 5 years since they are a leading cause of morbidity and mortality in this age group (Wald et al., 1992; Kogan et al., 1994; Snodgrass, 2001; Chien et al., 2003). However, Koch et al. (2003) suggested that symptomatic and asymptomatic adult carriers may be a source of these infections to children. Currently, no report has been documented about the prevalence of etiological agents of upper respiratory tract infections in Kitui among the general population. The antimicrobial susceptibility profiles of these bacterial pathogens are also largely unknown in Kitui.

1.3 Research questions

i. What is the distribution of outpatients with upper respiratory tract infections in terms of age and sex in Kitui District Hospital?

ii. What is the distribution and types of pathogenic bacteria associated with upper respiratory tract in outpatients in Kitui District Hospital?

iii. What is the prevalence of bacterial infections of the upper respiratory tract in different age groups and sexes in outpatients in Kitui District Hospital?

iv. What is the response of bacterial pathogens isolated from upper respiratory tract of outpatients in Kitui district hospital to conventional antibiotics?

1.4 Research hypotheses

i. The prevalence of upper respiratory tract infections in different age groups and sexes is the same in Kitui District Hospital.
ii. The prevalence of different bacteria associated with upper respiratory tract infections is the same in Kitui District Hospital.

iii. The distribution of pathogens isolated in cases of upper respiratory tract infections according to age and sex in outpatients is uniform in Kitui District Hospital.

iv. All pathogens isolated from the upper respiratory tract of outpatients in Kitui District Hospital are sensitive to various antibiotics used for their treatment.

1.5 Objectives of the study

1.5.1 General objective

To determine the prevalence and antimicrobial susceptibility profiles of bacterial pathogens of upper respiratory tract infections in Kitui District Hospital.

1.5.2 Specific objectives

i. To determine the prevalence of upper respiratory tract infections in patients of different age groups and sexes in outpatients in Kitui District Hospital.

ii. To isolate, identify and assess the distribution of bacterial pathogens associated with upper respiratory tract infections in outpatients in Kitui District Hospital.

iii. To determine the prevalence of pathogenic bacteria isolated from the upper respiratory tract in outpatients of different age groups and sexes in Kitui District Hospital.

iv. To determine antimicrobial susceptibility profiles of the bacterial pathogens isolated from outpatients with upper respiratory tract infections in Kitui District Hospital.
1.6 significance of the study

In this study, the occurrence, prevalence and antimicrobial susceptibility profiles of bacterial pathogens isolated from outpatients with upper respiratory tract infections in Kitui District Hospital has been described. Further, the study identifies special groups at high risk of contracting and transmitting these infections and thus forms a foundation for formulation and implementation of preventive strategies. Screening of population at increased risk of infection is a cost effective strategy for clinical application. This information updates the existing knowledge and informs the basis for management of these infections in Kitui District Hospital. Describing antimicrobial susceptibility profiles of these bacteria will help the clinicians in formulating the correct treatment regimens for these infections.
CHAPTER TWO
LITERATURE REVIEW

2.1 Upper respiratory tract infections
The upper respiratory tract infections (URTIs) are the most commonly observed infections in daily practice and therefore it is essential to differentiate between viral and bacterial causes to establish a logical and satisfactory treatment. The frequent use of antibiotics and the easy transmission of bacterial agents that cause upper respiratory tract infections often lead to increased antimicrobial resistance of these pathogens (Laupland et al., 2006), in addition to this, failure to direct the correct and adequate therapy against these pathogens may lead to clinical failures (Brook, 2002).

It has been reported that anaerobes are the most predominant components of the normal human oropharyngeal bacterial flora, however it has also been shown that they can cause bacterial infections of the upper respiratory tract that are of endogenous origin (Brook, 2002). In addition to their direct pathogenicity in these infections, they possess an indirect role through their ability to produce the enzyme beta-lactamase. In this fashion, they are capable of "shielding" non-beta-lactamase-producing bacteria from penicillins (Brook, 2007). In a similar study conducted in Brazil, Mouro et al. (2010) reported that etiologic agents of upper respiratory tract infections vary with age, for instance, *S. pyogenes* was reported to be predominant in children aged between 3 and 12 years.
2.2 Transmission

Transmission of organisms causing upper respiratory tract infections may occur by aerosol droplets or direct hand-to-hand contact with infected secretions, with subsequent passage to the ears or eyes (Musher, 2003). Direct invasion by the pathogens in the respiratory epithelium results in symptoms corresponding to the area(s) involved (Imani et al., 2005). Since these infections are spread by direct or close contact with an infected person, crowding as may be found in military camps and schools may predispose one to infection (Hayes and Williamson, 2001; Baltimore, 2010). It has also been established that dried bacteria in dust are not infectious, although moist bacteria on toothbrushes or similar items can persist for up to fifteen days and in rare cases outbreaks can be caused by contaminated food (Hayes and Williamson, 2001).

2.3 Risk factors associated with upper respiratory tract infections

A noble approach towards prevention of upper respiratory tract infections is to intervene in risk factors contributing to these infections since proper identification of the risk factors may be important in efforts to interrupt transmission. Studies in developing countries have identified risk factors such as overcrowding, nutritional factors and parental smoking (Campell et al., 1989; Aziz et al., 1995). Known risk factors for children include young age (Koch et al., 2003) environmental tobacco smoke (Hajnal et al., 1999) home-dampness (Rylander and Megevand, 2000) and attending day-care centres. Other risk factors associated with upper respiratory tract infections include low socio-economic status, occupational exposure and exposure to air pollution (Hedlund et
al., 2006; Jaen et al., 2006). These factors are endemic in the area of study according to KDHS, 2003.

2.4 Types of upper respiratory tract infections

URTIs are generally characterized by an inflammation which significantly occurs in upper respiratory tract and may include common cold, pharyngitis, tonsillitis, epiglottitis, sinusitis, bronchitis, rhinitis and nasopharyngitis (Rohilla et al., 2013). This study is focusing on bacteria that have strongly been associated with URTIs.

2.4.1 Pharyngitis

Pharyngitis is an inflammation of the throat or pharynx which is manifested by symptoms like enlargement of tonsils, cough, fever and enlargement of cervical lymph nodes (Choby, 2009). The main bacterial pathogen associated with pharyngitis is Group A beta-hemolytic Streptococcus (Shaikh et al., 2010). Others like Neisseria meningitidis, Chlamyphila pneumoniae and Mycoplasma pneumoniae have also been implicated in some cases of URTIs (Bisno, 2001). Findings of a study carried out by Shaikh et al. (2010) also reported that, 12 % of children with no signs or symptoms of URTIs are carriers of Group A beta-hemolytic Streptococcus in their pharynx. Previous studies have shown that, it is often hard to differentiate between a viral and a bacterial cause of a sore throat based on clinical signs and symptoms alone, thus a throat swab should be carried out to rule out a bacterial cause (Choby, 2009).
2.4.2 Diphtheria

Diphtheria is an upper respiratory tract infection caused by *Corynebacterium diphtheriae* and is characterized by sore throat, low fever and an adherent membrane on the tonsils, pharynx and the nasal cavity; however a milder form of diphtheria also known as cutaneous diphtheria may be restricted to the skin (Atkinson *et al.*, 2007). There are less common complications which occur as a result of diphtheria such as myocarditis which occurs in about 20% of reported cases (Havalder *et al.*, 2000) and peripheral neuropathy which occurs in about 10% of cases (Solders *et al.*, 1989).

The infection which has proved to be highly contagious is spread by direct physical contact or breathing the aerosolized secretions from infected individuals, but has significantly decreased in developed nations due to widespread vaccination (Atkinson *et al.*, 2007). Foodborne transmission of *C. Diphtheriae* has been reported (Linda *et al.*, 2000). In diphtheria endemic areas, a high prevalence of cutaneous diphtheria is associated with a low prevalence of respiratory diphtheria due to the high rate of natural immunity attained through exposure to cutaneous diphtheria (Bergamini *et al.*, 2000).

2.4.3 Tonsillitis

Tonsillitis is an inflammation of the tonsils usually associated with symptoms such as sore throat, fever, lethargy, headache, earache, difficulties in swallowing, voice complications and halitosis (Kempen, 2000). The most common bacterial cause of this infection is Group A β-hemolytic *Streptococcus* (GABHS) which causes sore throat (Brook, 1981) however, less common bacteria such as *Staphylococcus aureus*,
Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae and Fusobacterium have also been implicated (Brook, 1981). Bacterial causes of tonsillitis are treatable with antibiotics.

The pathophysiological mechanisms of pathogenesis of tonsillitis involve the entry of these pathogens into the tonsils and destruction of the defense mechanisms of leukocytes accompanied by the release of inflammatory mediators like phospholipase A2 leading to appearance of the symptoms of the disease (Rohilla et al., 2013). Complications like rheumatic fever (Del ma et al., 2004) and glomerulonephritis (Zoch-zwierz et al., 2001) have been reported in some cases of tonsillitis especially in developing countries, but they are extremely rare in developed nations (Ohlsson and Clark, 2004).

2.4.4 Sinusitis

Sinusitis is an inflammation of the mucous membrane lining the paranasal sinuses which is a common cause of complications in about 0.5 % in children and 5 % - 10 % in adults with upper respiratory tract infections (Wald, 1992). It has also been implicated as a major cause of mortality and morbidity in immune compromised people such as those undergoing bone marrow transplant and HIV/AIDS patients (Brook, 2009). The common bacterial pathogens isolated in cases of sinusitis infection include H. influenzae, S. pneumoniae, S. pyogenes and M. catarrhalis (Leung and Katial, 2008).
2.5 Major upper respiratory tract bacterial pathogens

2.5.1 *Haemophilus influenzae*

Most strains of *H. influenzae* are opportunistic pathogens which have been known to infect humans only (Chang et al., 2010; Roberts et al., 2011). In infants and young children, *H. influenzae* may cause bacteremia, pneumonia and acute bacterial meningitis especially in developing countries (John et al., 1991; Slack, 1998; Kennedy et al., 2007). Non-encapsulated strains of *H. influenzae* have been isolated in cases of otitis media, conjunctivitis, sinusitis and pneumonia in children (John et al., 1991).

Clinical diagnosis of *H. influenzae* is carried out by bacterial culture or latex particle agglutination test (Kennedy et al., 2007) and is only considered confirmed when the organism is isolated from a sterile body site like cerebrospinal fluid or blood. In this regard, *H. influenzae* isolated from the nasopharyngeal cavity or sputum may not necessarily indicate *H. influenzae* disease because the organism has been reported to colonize these sites in individuals without signs and symptoms of infection (Puri et al., 1999). Bacterial culture of *H. influenzae* is performed on chocolate agar at 37 °C in a CO₂-enriched incubator (John et al., 1991; NCCLS, 2003). Colonies of *H. influenzae* appear as convex, smooth, pale, grey or transparent colonies. Gram-stained and microscopic observation of a specimen of *H. influenzae* will show Gram-negative, rod-shaped bacteria, with no specific arrangement. The cultured organism can be further characterized using catalase and oxidase tests, both of which the bacteria is positive (CLSI, 2008).
2.5.2 *Streptococcus pneumoniae*

*Streptococcus pneumoniae* are Gram-positive bacteria in the shape of slightly pointed cocci which cause alpha-hemolysis on red blood cells, do not form spores and are non-motile, though they sometimes have pili which they use for adherence (Barocchi et al., 2006). They are found as normal flora in the upper respiratory tract including the throat and nasal passages (Lanie et al., 2007).

*S. pneumoniae* played a significant role in the history of molecular genetics being the subject of the experiments that gave birth to the field. In 1928, Frederick Griffith was able to transform live, harmless *S. pneumoniae* into a deadly strain by combining them with an extract from heat-killed, virulent *S. pneumoniae*. Later, in 1944, Avery, MacLeod and McCarthy proved that genetic material is DNA by showing that the transforming factor in Griffith's experiments was not protein but DNA (Lederberg and Gotschlich, 2005).

*Streptococcus pneumoniae* is characterized by a polysaccharide capsule that completely encloses the cell and plays a key role in its virulence and survival (Yother, 2011). The cell wall of *S. pneumoniae* is composed of peptidoglycan, teichoic acid and lipoteichoic acid which are attached to the membrane via a lipid moiety. Both teichoic and lipoteichoic acid contain phosphorylcholine. Two choline residues may exist on each carbohydrate repeat, which is important to *S. pneumoniae* because the choline adheres to choline-binding receptors located on human cells (Barocchi et al., 2006). The bacteria contain a family of choline-binding proteins (CBPs), twelve of which are bound to the
choline moiety of the cell wall and assist in attaching various functional elements onto the surface of the cell. Among the CBPs are found important determinants of virulence including protective antigen, autolysins A, B, C and adhesins (Lanie et al., 2007).

2.5.3 *Streptococcus pyogenes*

*Streptococcus pyogenes* is a spherical, Gram-positive bacterium that causes group A streptococcal infections. It is so called because it displays streptococcal group A antigen on its cell wall (Cunningham, 2000). The bacteria typically produces large zones of beta-hemolysis on blood agar plates and are therefore also called Group A beta-hemolytic *Streptococcus* (GABHS). It is an infrequent part of the skin flora which is estimated to cause more than 700 million infections worldwide each year with over 650,000 cases of severe, invasive infections leading to a mortality rate of about 25% (Aziz, 2010).

*S. pyogenes* is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases (Carapetis et al., 2005; Cohen et al., 2005). Examples of mild *S. pyogenes* infections include pharyngitis and impetigo (Cohen et al., 2005). Other infections of *S. pyogenes* include erysipelas, cellulitis and necrotizing fasciitis, a life-threatening condition that may require surgery (Torralba and Quismorio, 2009).

Infections by certain strains of *S. pyogenes* can be associated with the release of bacterial toxins. Throat infections associated with release of pyogenic exotoxin A may lead to scarlet fever and streptococcal toxic shock syndrome which can be life-threatening
(Krause, 2002). It can also cause post-infectious syndromes such as rheumatic fever and endocarditis several weeks following the initial streptococcal infection (Cohen, 2005).

*S. pyogenes* has several virulence factors that enable it to attach to host tissues, evade the immune response and spread by penetrating host tissue layers (Cunningham, 2000). A carbohydrate-based bacterial capsule composed of hyaluronic acid surrounds the bacterium protecting it from phagocytosis by neutrophils (Starr and Engleberg, 2006). The capsule and several factors embedded in the cell wall such as M-protein, lipoteichoic acid and protein F facilitate attachment to various host cells (Bisno et al., 2003). The M-protein also inhibits opsonization by the alternative complement pathway by binding to host complement regulators or in some serotypes by binding to fibrinogen. However, the M protein is also the weakest point in this pathogen's defense since antibodies produced by the immune system against M-protein target the bacteria for engulfment by phagocytes (Baruah, 2012). M proteins are unique to each strain, and identification can be used clinically to confirm the strain causing an infection (McGregor et al., 2004). The pathogen can also release a number of virulence factors into its host such as streptolysin O, streptolysin S, pyogenic exotoxin A and C, streptokinase, hyaluronidase, C5a peptidase and chemokine protease (Wexler et al., 1985; Hidalgo et al., 2004; Buchanan et al., 2006; Hidalgo et al., 2006; Starr and Engleberg 2006).

2.5.4 *Staphylococcus aureus*

*Staphylococcus aureus* is a facultatively anaerobic, Gram-positive coccal bacterium, which appears as grape-like clusters when viewed under a microscope and has large,
round, golden-yellow colonies, often with alpha-hemolysis when grown on blood agar plates (NCCLS, 2003). It is responsible for many infections but it may also occur as a commensal and thus the presence of *S. aureus* does not always indicate infection (Cole *et al.*, 2001), however, this bacterium can infect tissues when the skin or mucosal barriers have been breached. It has been reported that some strains of *S. aureus* can survive from hours to several weeks or even months on dry environmental surfaces (Cimolai, 2008). In infants, *S. aureus* infection is associated with staphylococcal scalded skin syndrome (SSSS) (Curran and Al-Salihi, 1980) while deeply penetrating *S. aureus* infections may cause arthritis, endocarditis (Bayer *et al.*, 1998) and pneumonia (Klytmans *et al.*, 1997) which can be very severe (Aires and Lencastre, 2004).

Virulence of *S. aureus* is associated with production of enzymes such as coagulase which clots plasma and coats the bacterial cell thus preventing it from phagocytosis; in addition, it produces hyaluronidase which breaks down hyaluronic acid and helps in tissue destruction hence spreading of the pathogen (Dinges *et al.*, 2000). Depending on the strain, *S. aureus* is capable of secreting exotoxins such superantigens which induce toxic shock syndrome (TSS) (Holtfreter and Broker, 2005). Other strains of *S. aureus* can produce an enterotoxin which leads to *S. aureus* gastroenteritis (Cenci-Goga *et al.*, 2003). Exfoliative toxins produced by some strains of the bacterium have been implicated in staphylococcal scalded-skin syndrome (SSSS) which occurs most commonly in infants and young children (Curran and Al-Salihi, 1980).
Other staphyloccocal toxins that act on cell membranes include alpha toxin, beta toxin, delta toxin and several bi-component toxins including Panton-Valentine leukocidin (PVL) which is associated with severe necrotizing pneumonia in children (Blot et al., 2002; Kaneko and Kamio, 2004). The genes encoding the components of PVL have been reported as being encoded on a bacteriophage found in community-associated methicillin-resistant *S. aureus* (MRSA) strains (Blot et al., 2002). Other immune-evasive factors include protein A which is anchored to staphyloccocal peptidoglycan pentaglycine bridges by the transpeptidase sortase A (Schneewind et al., 1995). Studies involving mutation of genes coding for protein A which is an IgG-binding protein that binds to the Fe region of the antibody resulted to a lowered virulence of *S. aureus* as measured by survival in blood (Patel et al., 1987; Zhu et al., 2008). Some strains of *S. aureus* are also capable of producing staphyloxanthin which helps the microbe to evade the reactive oxygen species which the host immune system uses to kill pathogens (Liu et al., 2005; Clauditz et al., 2006).

### 2.5.5 *Corynebacterium diphtheriae*

*Corynebacterium diphtheriae* is a non-motile, non-capsulated, club-shaped, Gram-positive bacillus that infects the nasopharynx or skin. Strains that are lysogenic to a family of corynebacteriophages carrying the gene for diphtheria toxin secrete a potent exotoxin which may cause diphtheria (Atkinson et al., 2007). Although asymptomatic nasopharyngeal carriage of the organism is common in regions where diphtheria is endemic, in susceptible individuals, toxigenic strains cause disease by multiplying and secreting diphtheria toxin in either nasopharyngeal or skin lesions (Bergamini et al.,
Clinical diagnosis for the disease depends upon culture-proven toxigenic *C. diphtheriae* infection of the skin, nose, or throat combined with clinical signs of nasopharyngeal infection. For primary isolation, Loeffler’s agar is used (Prasad *et al.*, 2005) followed by Elek’s immunodiffusion test to determine the toxigenicity of the isolates (Hadfield *et al.*, 2000).

### 2.5.6 *Neisseria meningitidis*

*N. meningitidis* is a major cause of morbidity and mortality during childhood in industrialized countries and has been responsible for epidemics in Africa and Asia (Rouphael and Stephens, 2012). It exists as normal flora in the nasopharynx of up to 5-15% of adults and can only infect humans since the bacterium cannot get iron other than from human sources (Larson *et al.*, 2002). Attachment of the bacterium to the epithelial cells of the nasopharynx is mediated by the presence of fimbriae (Pujol *et al.*, 1999). Trimeric Autotransporter Adhesins (TAA) are used by the pathogen to stick to the host cell and hence infecting them while Lipooligosaccharide (LOS) is a component of the outer membrane of *N. meningitidis* which acts as an endotoxin and is responsible for septic shock and hemorrhage due to the destruction of red blood cells (Griffiss *et al.*, 1988). The bacterium also has a polysaccharide capsule which prevents host phagocytosis and aids in evasion of the host immune response (Griffiss *et al.*, 1988).
2.6 Complications as a result of upper respiratory tract infections

2.6.1 Scarlet fever
Scarlet fever is an infectious disease which is usually spread by inhalation and most commonly affects 4-8 year old children with symptoms such as sore throat, fever, bright-red tongue, swollen glands in the neck, nausea, vomiting, loss of appetite and a characteristic red rash on the skin (Czarkowski et al., 2011). Most of the clinical features are caused by an erythrogenic toxin produced by *Streptococcus pyogenes* when infected by a T12 phage which carries the gene for production of the toxin (Yu and Ferretti, 1991; Krause, 2002). The bacteriophage T12 integrates into the serine tRNA gene of the streptococcal genome from where the toxin is transcribed (McShan and Ferretti, 1997).

2.6.2 Glomerulonephritis
Glomerulonephritis is a renal disease characterized by inflammation of the glomeruli with symptoms such as isolated hematuria, proteinuria or nephritic syndrome which may lead to acute or chronic renal failure (Brandy et al., 2005). Post-streptococcal glomerulonephritis has been described as the leading cause of acute nephritic syndrome (Ricardo et al., 2005). While most cases are sporadic, the disease has been known to occur in epidemic form especially in children between two and six years of age with a recent history of pharyngitis in a winter setting or a rash in a warmer climate (Couser, 1999). It has not been clearly explained whether antibiotic treatment of the primary infection provides protection from the development of post-streptococcal glomerulonephritis (Delmar et al., 2004).
2.6.3 Endocarditis

Endocarditis is an inflammation of the endocardium which usually involves the heart valves, the inter-ventricular septum, the mural endocardium or even the intra-cardiac devices. It is characterized by a prototypic lesion containing a mass of platelets, fibrin, micro-colonies of microorganisms and scant inflammatory cells. It occurs when the bacteria enter the bloodstream with subsequent attachment to a damaged portion of the inner lining of the heart or abnormal heart valves (Christopher et al., 2003). The ability of these bacteria to stick to the surface lining is aided by a preexisting microscopic clot that often forms at these abnormal sites or a preexisting heart disease (Strom et al., 1998). Diagnosis of endocarditis is based on clinical investigations such as an echo-cardiogram and blood cultures demonstrating the presence of endocarditis causing microorganisms (Bayer et al., 1998).

2.6.4 Rheumatic fever

Rheumatic fever is a systemic disease affecting the peri-arteriolar connective tissue occurring after an untreated Group A Beta-hemolytic streptococcal pharyngeal infection which is believed to be caused by antibody cross-reactivity (Dinkla, 2009). During a *Streptococcus* infection, mature antigen presenting cells such as B cells present the bacterial antigen to CD4-T cells which differentiate into helper T2 cells with subsequent activation of the B cells to become plasma cells and induce the production of antibodies against the cell wall of *Streptococcus* (Dinkla, 2009). However, these antibodies also react against the myocardium and joints producing the symptoms of rheumatic fever (Faë et al., 2006; Dinkla, 2009).
Group A *streptococcus pyogenes* has a cell wall composed of branched polymers which contain M protein that are highly antigenic (McGregor *et al.*, 2004). The antibodies which the immune system generates against the M protein may cross react with cardiac myofibre protein, heart muscle glycogen and smooth muscle cells of arteries, inducing cytokine release and tissue destruction (Fae *et al.*, 2006).

### 2.7 Management of upper respiratory tract infections

#### 2.7.1 Non-pharmacological therapy

Infections of upper respiratory tract (URT) are among the commonest of infections, and account for a large percentage of the consultations in general practice, particularly in the case of neonates, infants and children. These infections, though not life threatening, require proper selection of drugs (Teng *et al.*, 2004).

Non-pharmacological management of upper respiratory tract infections include taking adequate rest, drinking a lot of fluids, regular handwashing (Luby *et al.*, 2005) and avoidance of cigarette smoking (Peat *et al.*, 2001). Educating the patients about the infections and the correct indications for their treatment especially the use of antibiotics has also been described as an intervention strategy to satisfy the patients rather than directly prescribing the antibiotics (Teng *et al.*, 2004; Green, 2006).
2.7.2 Pharmacological therapy

2.7.2a Symptomatic relief

The main emphasis of management is symptom relief of fever, nasal congestion and coughing. A variety of adrenergic agonists, anticholinergics, antihistamine preparations, antitussives and expectorants are used. Common constituents of such medication include first generation antihistamines, antipyretics or anti-inflammatory agents, cough suppressants, expectorants and decongestants (Green, 2006).

2.7.2a Antibacterial therapy

While antibiotic use is credited with the dramatic reduction in the morbidity and mortality associated with many bacterial infections, its abuse has resulted in the rapid emergence of resistant strains that reduce the effectiveness of many antibiotics (Lim, 2001). It has been recognized that the problem of antibiotic abuse is a result of a complex interplay of various socio-cultural, economic and cognitive factors at the level of the patients, the prescribers and the drug industry (Okeke et al., 1999).

Most URTIs are viral in origin and require symptomatic treatment only (Middleton, 1991; Mainous et al., 1996; Boon et al., 2013). Antibiotic intervention should be based on a knowledge of likely prevalent organisms and their current sensitivity, however, this is often not possible and antibiotic choice is commonly empirical, a situation which is further compounded by the increasing self-prescription of antibiotics (El-Mahmood et al., 2010). This may lead to selection of resistant strains of pathogens hence posing an obstacle in the control and treatment of such pathogens (Ndip et al., 2001). The current increase in the rates of antibiotic resistance in community pathogens has made
researchers and clinicians to focus on this public health problem (Jernigan et al., 1996). Furthermore, variation of the susceptibility profiles of these pathogens to antibiotics has largely been associated with time and geographical location (El-sheikh, 1998; Ndip et al., 2002).

Common antibiotics used to treat URTIs include oral amoxicillin, trimethoprim-sulfamethoxazole or doxycycline given for 3 to 10 days which is the recommended first-line antibiotics for the treatment of these infections (Piccirillo et al., 2001; Poole and Portugal, 2005). Although, the prevalence of penicillin-resistant and beta-lactamase producing organisms causing these infections has steadily increased in the community, larger doses of amoxicillin of up to 3 mg daily or a combination of amoxicillin and clavunate remain effective in most cases caused by resistant organisms (Piccirillo, 2004; Aagard and Gonzales, 2004). Second-line, broad-spectrum, and more expensive agents such as the newer macrolides (clarithromycin and azithromycin) and the fluoroquinolones (levofloxacin, gatifloxacin, and moxifloxacin) are no more effective than amoxicillin and therefore should be reserved for use in individuals who are allergic or intolerant to first-line agents, those who do not respond to first-line agents within 3 days or in cases of confirmed antimicrobial resistance (Casey, 2007; Marcy, 2007). This approach would help contain the ever-increasing health care costs and most importantly curtail the emergence of resistant organisms as a result of selection pressure.
2.8 Prevalence of upper respiratory tract infections

Cotton et al. (2008) reported that upper respiratory tract infections are common both in children and adults and is a major cause of mild morbidity. There is sufficient literature to prove that upper respiratory tract infections are the leading cause of mild morbidity and even mortality globally (Palmer and Bauchner, 1997; Zafar et al., 2008). In Malaysia, Teng et al. (2004) highlighted that these infections accounted for 27% of all clinical encounters. URTIs have also been implicated for a large proportion of medical consultations in children (Bhasin et al., 2002). The incidence of these infections has been reported to be between 3 – 8 times per year in all children with 10 – 15% of children experiencing at least 12 episodes annually (West, 2002) in United Kingdom. Mortality associated with URTIs has been estimated at 1.9 million children each year out of which about 70% occur in Africa and South East Asia (Teng et al., 2004).

Mbonye (2004) reported a prevalence of 37% of upper respiratory tract infections among children in Uganda, Symekher et al. (2009) a prevalence rate of twenty six percent at Kenyatta National Hospital, Kenya and Sikolia et al. (2002) seventy percent in Kibera, Nairobi, Kenya. However, there exists limited information on the prevalence of bacterial pathogens causing upper respiratory tract infections in Kenya (Cotton et al., 2008; Symekher et al., 2009). Therefore, this study was conducted in Kitui District hospital to determine the prevalence and antimicrobial susceptibility profiles of bacterial pathogens associated with upper respiratory tract infections.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area
The study was carried out in Kitui District Hospital, in Kitui County, Kenya. Kitui District Hospital is the largest and the busiest health facility in the county. It is located in Kitui town, which lies 1.37° South, 38.02° East and about 1152 meters above the sea level. The town is 130 kilometers east of Nairobi and 75 kilometers east of Machakos. Kitui town has the following main residential estates: Kunda Kindu, Kalundu, Majengo, Bondeni, California, Mjini, JICA and Valley view. The population of the town is now about 150,000 people (KNBS, 2009).

3.2 Study population
The study targeted outpatients of all ages and sexes visiting Kitui district hospital with upper respiratory tract infections from November, 2012 to April, 2013.

3.2.1 Inclusion criteria
The criteria for inclusion in the study were that the patient must be suffering from upper respiratory tract infections, must not have taken antibiotics of any kind for at least one week before the clinical visit and must consent voluntarily to participate in the study.

3.2.2 Exclusion criteria
Young children aged below 1 year, those who had taken antibiotics within one week before the clinical visit and inpatients were excluded from the study.
3.2.3 Sample size

Samples were collected from 237 patients visiting the hospital according to the formula:

\[ n = \frac{Z^2 \times pq}{d^2} \], (Naing et al., 2006) using upper respiratory tract infection prevalence rate of 19 % (Kenya demographic and health survey, 2003) where; \( n \) = desired minimal sample size, \( Z \) = standard normal deviate = 1.96, \( p \) = prevalence of condition under study, \( q = 1-p \), \( d \) = precision required for the study at 95 % confidence level = 0.05.

\[ n = (1.96)^2 \times (0.19) (0.81)/0.05^2 = 237 \]

3.3 Study design

This was a cross-sectional study and samples were collected from outpatients visiting Kitui district hospital with upper respiratory tract infections. All media, reagents, sterile swabs, Petri dishes and any other materials used in this study were supplied be the Kenya Medical Supplies Agency, Kenya (KEMSA).

3.4 Sample collection

Clinical samples of throat swabs were collected using sterile swabs and transported to the hospital’s bacteriology laboratory using Amies transport medium for bacteriological analysis. Age and sex of the patient was indicated on the swabs. The samples were cultured within two hours after collection.

3.5 Isolation of pathogens

Isolation of bacterial pathogens was carried out as described by CLSI (2008). A throat swab was obtained by rubbing the swab firmly over the back of the throat, tonsils and any inflamed area, exudation or ulceration. This was done carefully to avoid touching the
tongue, checks or lips with the swab. A loopful of each sample was then inoculated onto sterile Blood agar, Chocolate agar, Sabouroud's agar and MacConkey agar immediately after collection and then incubated aerobically at 37 °C for 24 hours except for Chocolate agar which was incubated under anaerobic conditions. Sabouroud's agar was used to isolate *Candida* spp. After incubation period, macroscopic and microscopic examinations of colonies on the media were carried out based on growth patterns, colony morphology, colour and gram staining characteristics while colonies on blood agar were also examined for hemolysis (CLSI, 2008).

3.6 Growth and confirmation of isolates

The colonies obtained were then examined by Gram staining and the appropriate biochemical tests employed to confirm their identity. Standard microbiological techniques and biochemical tests were performed to confirm the isolates; catalase test, coagulase test, Mannitol fermentation test, bile solubility test, bacitracin test, hippurates test, optochin test and germ tube test (NCCLS, 2003; CLSI, 2008) were performed on the isolates depending on their Gram staining characteristics to confirm the presence of bacterial pathogens based on biochemical reactions. Germ tube test was used to confirm large gram positive oval or spherical cells suspected to be *Candida* spp. (CLSI, 2008).

3.6.1 Gram staining

A loopful of sterile normal saline was placed on a clean slide and a colony of the isolate transferred onto the drop from the growth media using a sterile cool loop. The colony was emulsified in it and the film allowed to air dry. It was then fixed by briefly passing it
through a Bunsen flame two to three times without exposing the dried film directly to the
flame; this was done gently to ensure that the slide is not so hot as to be uncomfortable to
touch. The slide was then flooded with crystal violet solution for up to one minute and
the stain washed off briefly with tap water. This was followed by flooding the slide again
with Gram’s Iodine for another one minute then washing off with tap water. After that, it
was flooded with 95% alcohol for ten seconds followed by tap water then counterstained
with neutral red solution for thirty seconds and washed again with tap water. The slide
was allowed to air dry and examined under the microscope using oil immersion lens
(CLSI, 2008).

3.6.2 Catalase test
This test was performed on Gram positive cocci which occurred in clusters or in chains
after Gram’s stain. The test was used to differentiate bacteria that produce the enzyme
catalase, such as *Staphylococcus* from non catalase producing bacteria such as
streptococci. A sterile bacteriological loop was used to pick a colony of the test organism
from the Chocolate agar plate and immerse it into a test tube containing 3% hydrogen
peroxide (CLSI, 2008).

3.6.3 Coagulase test
This test was performed on Gram positive and catalase positive cocci occurring in
clusters. It was used to differentiate between coagulase positive *Staphylococcus* and
coagulase negative *Staphylococcus*. The suspected colonies were emulsified in a few
drops of normal saline placed on a clean slide. A few drops of plasma were added to the emulsified colonies and then the slide was rotated for one minute (NCCLS, 2003).

3.6.4 Mannitol fermentation test

This test was performed on organisms which were Gram positive cocci in clusters after Gram’s stain and both catalase and coagulase positive. This is used to positively identify *S. aureus* which has the ability to grow on this media and ferment mannitol. A small colony of the isolated bacteria was emulsified in 3 ml sterile normal saline in bijou bottles according to 0.5 McFarland’s standard. The bacterial inoculum was then inoculated onto mannitol salt agar plate under sterile conditions and incubated aerobically at 37 °C for 24 hours. Change of colour from red to yellow on the media around the colonies indicated the presence of *S. aureus* which ferments mannitol resulting to a pH drop in medium (CLSI, 2008).

3.6.5 Bile solubility test

This was carried out on Gram positive cocci which produced alpha hemolysis on blood agar but did not grow on MacConkey agar to differentiate between *Streptococcus pneumoniae* and Viridans streptococci. A drop of the reagent was placed directly on a colony of the isolate placing the plate level to prevent the reagent from running and washing the colony away. The plate was then incubated at room temperature for 30 minutes until the reagent dried (CLSI, 2008).
3.6.6 Optochin sensitivity test

This was carried out on colonies which produced alpha hemolysis on blood agar but did not grow on MacConkey agar. It was also performed on Gram positive cocci in chains to differentiate between *Streptococcus pneumoniae* and Viridans streptococci. A small colony of the isolated bacteria was emulsified in 3 ml sterile normal saline in bijou bottles according to 0.5 McFarland’s standard. The bacterial inoculum was then inoculated in blood agar to form a lawn of bacteria under sterile conditions. An optochin disc was placed directly on the surface of the agar plate and incubated for 24 hours (CLSI, 2008).

3.6.7 Hippurates hydrolysis test

This test was carried out on isolates which produced beta-hemolysis on blood agar and did not grow on MacConkey agar. It was also performed on Gram positive cocci which were catalase negative to differentiate group A *Streptococcus* from group B *Streptococcus* which are hippurates positive. Three drops of distilled water were added into a sterile test tube; then a colony was picked from the growth media using a sterile cool wire loop and placed into the test tube to form a heavy suspension of the organism to be tested. A rapid hippurates disk was placed into the mixture using sterile forceps; then the test tube was capped and incubated for 2 hours at 37 °C after which 4 drops of ninhydrin reagent were added to the mixture of hippurates reagent and the organism. The resultant mixture was then re-incubated for 30 minutes at 37 °C and observation of any colour change done at intervals of 10 minutes during this period of incubation (CLSI, 2008).
3.6.8 Bacitracin sensitivity test

This was carried out on colonies which produced beta hemolysis on blood agar but did not grow on MacConkey agar. Again it was performed on Gram positive cocci in chains to differentiate between *Streptococcus pyogenes* and other non group A beta hemolytic streptococci. A small colony of the isolated bacteria was emulsified in 3 ml sterile normal saline in bijou bottles according to 0.5 McFarland’s standard. The bacterial inoculum was then inoculated in blood agar to form a lawn of bacteria under sterile conditions. A bacitracin sensitivity disc was placed directly on the surface of the agar plate. This was incubated for 24 hours at 37 °C. After incubation, the plates were examined for zones of clearance around the bacitracin discs (CLSI, 2008).

3.6.9 Germ tube test

This was performed on large oval, Gram positive isolates from Sabouroud’s agar to confirm the identity of *C. albicans* from other types of yeast which are negative for this test. About 0.5 mm of human serum was put in a small test tube then a light suspension of the suspect yeast colonies was made by touching 2 colonies with a sterile wooden applicator stick and inoculating in the human serum. The tube was then incubated for 3 hours at 37 °C. After incubation, a drop of the suspension was placed on a slide using a Pasteur pipette and examined microscopically for growth of germ tubes (CLSI, 2008).
Throat swab to microbiology laboratory

Inoculated on Blood agar, MacConkey agar, Sabouroud’s agar and Chocolate agar (in CO₂ jar) and incubate at 37 °C for 24 hours.

Gram positive cocci

Catalase test

Catalase positive

Catalase negative

Germ tube test

Germ tube test positive

Coagulase test

α-hemolytic

β-hemolytic

Bacitracin test

Optochin test

Optochin negative

S. aureus

S. pyogenes

viridans streptococci

Figure 1: Summary of biochemical tests
3.7 Antibiotic susceptibility testing

All bacterial isolates were subjected to *in vitro* sensitivity tests for the following antibiotics amoxiclav (30 mcg), Ceftriaxone (30 mcg), piperacillin (100 mcg), gentamicin (10 mcg), amikacin (30 mcg), meropenem (10 mcg), tazobactam (10 mcg) and ofloxacin (10 mcg). This was carried out using Kirby-Bauer method of antibiotic susceptibility testing (Bauer *et al.*, 1966; NCCLS, 2003). A small inoculum of the isolated bacteria was emulsified in 3 ml sterile normal saline in bijou bottles according to 0.5 McFarland's standard. The bacterial inoculum was inoculated in Muller-Hinton agar to form a lawn of bacteria under sterile conditions. A paper disc impregnated with the specific concentration of the antibiotics was then placed on the surface of the inoculated bacteria and incubated at 37 °C for 24 hours. Diameters of formed zones of inhibition were measured in milimetres and compared with recorded diameters of the control organisms. *Staphylococcus aureus* ATCC 25923 was used as a control for Gram positive bacteria to determine the breaking-even points of susceptibility or resistance (NCCLS, 2003).

3.8 Data analysis

All data was analyzed with SPSS, version 20.0 statistical software (SPSS Inc. Chicago III, USA). Chi-square test was used to compare categorical data while adjusted residual was computed to measure deviation from the expected Mean values. T-test was used to compare the mean ages of males and females while one sample Chi square test was used to assess the distribution of patients with URTIs according to age and sex as well as the significance of differences in the prevalence of different pathogens. P values of \( \leq 0.05 \) were considered to be statistically significant.
3.9 Ethical consideration

The research protocol was approved by the ethics review committee at Kenyatta University and research permit obtained from National Commission for Science, Technology and Innovation. The participants were informed of the aim of the study and assured that all the information supplied by them will remain confidential. Their verbal and written consent and cooperation to participate in the study was solicited through the hospital management. For minors, an informed consent was obtained from their parents or guardians.
CHAPTER FOUR
RESULTS

4.1 Patients demographic information

A total of 237 patients were screened for bacterial pathogens in their upper respiratory tract between November 2012 and April 2013. The Mean age of the patients was 22.64 years with a Standard deviation of 19.76 while the Mode was 3 accounting for 11% of the total sample, however, the distribution of age was not normal based on that Mean and Standard deviation ($p = 0.015$). The percentage of females with URTIs was significantly higher than that of males in this study ($p = 0.007$). Out of the 237 subjects recruited, 36.7% with a Mean age of 19.5 years, Standard deviation of 19.8 and mode of 3 were males while females accounted for 63.3% with a Mean age of 23.97 years, Standard deviation of 19.39 and Mode of 1. Nevertheless, the difference in the mean ages for males and females was not statistically significant ($p = 0.227$).

The distribution of patients with upper respiratory tract infections according to sex in different age groups was not uniform ($p = 0.011$). Males aged between 1 – 5 years were more compared to females of the same age with a percentage of 17.7% (adjusted residual = 3.3). Again, the percentage of females aged between 56 – 65 years was significantly higher than that of their male counterparts at 3.4% (adjusted residual = 2.2). This information is summarized in table 4.1 below.
Table 4.1: Demographic characteristics of patients with URTIs

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex % (Adjusted residual)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1 – 5</td>
<td>17.7 (3.3)*</td>
<td>17.3 (-3.3)*</td>
</tr>
<tr>
<td>6 – 14</td>
<td>3.4 (-1.5)</td>
<td>10.1 (1.5)</td>
</tr>
<tr>
<td>15 – 25</td>
<td>4.2 (-1.0)</td>
<td>10.1 (1.0)</td>
</tr>
<tr>
<td>26 – 35</td>
<td>5.9 (0.6)</td>
<td>8.4 (-0.6)</td>
</tr>
<tr>
<td>36 – 45</td>
<td>2.1 (-1.7)</td>
<td>8.0 (1.7)</td>
</tr>
<tr>
<td>46 – 55</td>
<td>1.7 (-0.7)</td>
<td>4.2 (0.7)</td>
</tr>
<tr>
<td>56 – 65</td>
<td>0.0 (-2.2)*</td>
<td>3.4 (2.2)*</td>
</tr>
<tr>
<td>Above 65</td>
<td>1.7 (0.8)</td>
<td>1.7 (-0.8)</td>
</tr>
<tr>
<td>Total</td>
<td>36.7 (-2.5)*</td>
<td>63.3 (2.5)*</td>
</tr>
</tbody>
</table>

Measures of central tendency

Mean     19.5  23.97  22.64  0.227
SD       19.895  19.34  19.76  
Range    1 – 85  1 – 84  1 - 85  
SEM      3.157  2.356  3.198  
Mode     3          1        3    

Key

* - shows significant difference, SD- standard deviation, SEM- standard error of Mean.

4.2 Prevalence of URTIs in different age groups and sexes

Upper respiratory tract infections that were reported in this study were; tonsillitis, pharyngitis, sinusitis and uvulitis. Tonsillitis (46.4 %) was the most prevalent URTI followed by pharyngitis (27.8 %), sinusitis (22.5 %) while uvulitis (2.8 %) was least encountered. The distribution of different types of upper respiratory tract infections in different age groups was similar (p = 1.000). However this was different for sex (p = 0.011), for instance, pharyngitis was significantly more prevalent in males at 10.1 % (adjusted residual = 2.0) than in females while the prevalence of sinusitis in females
stood at 14.3 % (adjusted residual = 3.2) which was higher than that of males. The age and sex distribution of outpatients with different types of upper respiratory tract infections is shown in table 4.2.

Table 4.2: Prevalence of URIs in different age groups and sexes

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Tonsillitis</th>
<th>Pharyngitis</th>
<th>Sinusitis</th>
<th>Uvulitis</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - 5</td>
<td>15.6 (-0.4)</td>
<td>10.1 (0.3)</td>
<td>8.0 (0.0)</td>
<td>1.2 (0.4)</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>6 - 14</td>
<td>6.3 (0.1)</td>
<td>3.7 (0.0)</td>
<td>2.9 (-0.1)</td>
<td>0.42 (0.1)</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>15 - 25</td>
<td>6.7 (0.1)</td>
<td>3.3 (-0.6)</td>
<td>3.7 (0.6)</td>
<td>0.42 (0.0)</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>26 - 35</td>
<td>6.7 (0.1)</td>
<td>3.7 (-0.2)</td>
<td>3.3 (-0.1)</td>
<td>0.42 (0.0)</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>36 - 45</td>
<td>4.6 (-0.1)</td>
<td>2.9 (0.2)</td>
<td>2.1 (-0.2)</td>
<td>0.42 (0.4)</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>46 - 55</td>
<td>2.9 (0.3)</td>
<td>1.7 (0.1)</td>
<td>1.2 (-0.1)</td>
<td>0.0 (-0.7)</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>56 - 65</td>
<td>1.7 (0.2)</td>
<td>1.2 (0.6)</td>
<td>0.42 (-0.7)</td>
<td>0.0 (-0.5)</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Above 65</td>
<td>1.7 (0.2)</td>
<td>0.84 (-0.2)</td>
<td>0.84 (0.2)</td>
<td>0.0 (-0.5)</td>
<td>3.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16.8 (0.7)</td>
<td>10.1 (2.0)*</td>
<td>8.4 (-3.2)*</td>
<td>1.2 (0.3)</td>
<td>36.7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>29.5 (-0.7)</td>
<td>17.7 (-2.0)*</td>
<td>14.3 (3.2)*</td>
<td>1.7 (-0.3)</td>
<td>63.3</td>
<td></td>
</tr>
</tbody>
</table>

4.2 Isolation and identification of pathogens

Growth occurred only on blood agar, chocolate agar and Sabouroud's agar. There was appearance of a purple blue colour after Gram staining in the 226 (95.4 %) isolates obtained. Dome-shaped and grey colonies which were circular with entire margins on blood agar exhibiting beta-hemolysis on red blood cells were observed in 32 (13.5 %) of the isolates, Golden brown, round and convex colonies with a sharp border on blood agar accounted for 105 (44.3 %) of the total isolates while creamy colonies surrounded by a
greenish colouration of alpha-hemolysis were 77 (32.5 %). Another 12 (5 %) of the total isolates gave white, smooth and glabrous colonies on Sabouroud’s agar.

In 105 (44.3 %) of the isolates there was production of gas bubbles from the hydrogen peroxide within one minute in catalase test, appearance of clumps on the slide in coagulase test and colour change from red to yellow on the media in mannitol fermentation test. These isolates were identified as \textit{S. aureus}. All \(\alpha\)-hemolytic isolates which did not produce bubbles of hydrogen in catalase test were resistant to optochin and were not lysed by bile, they accounted for 77 (32.5 %) of the total isolates thus identified as viridans group streptococci. For the \(\beta\)-hemolytic isolates, there was no production of bubbles in catalase test, a yellow appearance was observed in hippurates test and there was formation of a clear zone of inhibition around the bacitracin discs, these isolates were 32 (13.5 %). \textbf{They were confirmed to be} \textit{S. pyogenes}. In the isolates growing on Sabouroud’s agar, there was growth of a germ tube in all of them after the germ tube test and they were \textit{C. albicans}. 

Plate 4.2 Growth of isolates on Blood agar

Plate 4.2 a

Plate 4.2 b

Plate 4.2 c

Key:
Plate 4.2 a: Growth of *S. aureus*
Plate 4.2 b: Growth of *S. pyogenes*
Plate 4.2 c: Growth of viridans group streptococci
4.2.1 Prevalence of different pathogens

Pathogens were isolated in 95.4% of the subjects screened. The isolated pathogens were *Staphylococcus aureus* (44.3%), viridans group streptococci (32.5%) and *Streptococcus pyogenes* (13.5%). Co-infections were found in 5% of the patients consisting of 2.5% co-infections of viridans group streptococci and *C. albicans* and 2.5% co-infections of *S. aureus* and *C. albicans*. Growth did not occur in 4.7% of the samples. The difference in prevalence of these isolates was statistically significant (p = 0.0001).

4.2.2 Prevalence of bacterial pathogens in different age groups

Majority of the pathogens were isolated in patients aged between 1 – 5 years which accounted for 34.7% (adjusted residual = 2.1), however no pathogen was significantly more prevalent than the others in this age group. There were statistically significant differences in the prevalence of different pathogens in different age groups (P = 0.015) as depicted in table 4.2. For example, viridans group streptococci was not isolated in patients aged between 56 – 65 years and this was significant compared to the distribution of the pathogen in other age groups (adjusted residual = -2.0). Similarly, *S. pyogenes* was not isolated from patients aged between 36 – 45 years and this was significantly low compared to its prevalence in other age groups (adjusted residual = -2.0). It was also observed that the prevalence of *S. aureus* was significantly high in patients aged between 36 - 45 years with a percentage of 7.6 (adjusted residual = 3.2).
4.2.3 Prevalence of mixed infection in different age groups

Mixed infections of *C. albicans* and viridans group streptococci were only isolated in the age groups of between 6 – 14 years and 15 – 25 years with each one of them accounting for 1.3 % (adjusted residuals = 2.6 and 2.5 respectively). These percentages were significantly high in these age groups. *S. aureus* and *C. albicans* with a prevalence of 0.8 % in each category were isolated in the age groups of between 1 – 5 years, 6 – 14 years and 15 – 25 years, the infection was not discovered in any other age group but this was not statistically significant (adjusted residuals = -0.1, 1.4 and 1.3 respectively).

4.2.4 Distribution of bacterial pathogens in different sexes

The difference in prevalence of different pathogens between males and females was statistically significant only in *S. aureus* and *S. pyogenes* (P = 0.0001). *S. pyogenes* was more prevalent in males than in females with a prevalence of 8.9 % (adjusted residual = 3.6) against 4.6 % (adjusted residual = -3.7) of females. For *S. aureus*, the prevalence was higher in females at 32.1 % (adjusted residual = 2.7) compared to 12.2 % (adjusted residual = -2.5) isolated in males. Viridans group streptococci isolated from males accounted for 12.7 % and those from females 19.8 % but this difference was not statistically significant.

4.2.5 Prevalence of co-infections in different sexes

The prevalence of mixed infections in different sexes was not different statistically. For instance, the prevalence of mixed infections of viridans group streptococci and *C. albicans* in males was 0.8 % while 1.7 % was isolated from females but the difference
was not statistically significant (adjusted residual = 0.2 and -0.2 respectively). Again, 0.4% of mixed infections of *S. aureus* and *C. albicans* were isolated from male patients and 2.1% from female patients. This difference was also not statistically significant (adjusted residual = -1.0 and 1.0 respectively).

**Table 4.3: Prevalence of isolates in different age groups and sexes**

<table>
<thead>
<tr>
<th>Risk</th>
<th>Proportion of isolates % (adjusted residual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
<td>VGS</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>1 – 5</td>
<td>13.9 (1.8)</td>
</tr>
<tr>
<td>6 – 14</td>
<td>3.0 (-1.4)</td>
</tr>
<tr>
<td>15 – 25</td>
<td>4.2 (-0.4)</td>
</tr>
<tr>
<td>26 – 35</td>
<td>5.9 (1.2)</td>
</tr>
<tr>
<td>36 – 45</td>
<td>2.5 (-0.8)</td>
</tr>
<tr>
<td>46 – 55</td>
<td>2.1 (0.3)</td>
</tr>
<tr>
<td>56 – 65</td>
<td>0.0 (-2.0)*</td>
</tr>
<tr>
<td>Above 65</td>
<td>0.8 (-0.5)</td>
</tr>
</tbody>
</table>

**Sex**

<table>
<thead>
<tr>
<th>Risk</th>
<th>Proportion of isolates % (adjusted residual)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VGS</td>
</tr>
<tr>
<td>Male</td>
<td>12.7 (0.5)</td>
</tr>
<tr>
<td>Female</td>
<td>19.8 (-0.4)</td>
</tr>
<tr>
<td>Total (n)</td>
<td>32.5</td>
</tr>
</tbody>
</table>

**Key:**

(VGS – Viridans group streptococci, SP – *S. pyogenes*, SA – *S. aureus*, CA – *C. albicans*)

* - shows significant difference

### 4.3 Sensitivity of pathogens to antibiotics

In this section, the sensitivity patterns of the 3 bacterial pathogens isolated in this study to antibiotics is described.
4.3.1 sensitivity of viridans streptococci

There was no case of resistance of viridans streptococci to ofloxacin, however 2.1 % were intermediately susceptible and only 97.9 % were sensitive to ofloxacin. This pathogen was more sensitive to gentamicin (82.6 %, adjusted residual = 2.2) and ofloxacin (97.9 and adjusted residual = 2.0) compared with either the other isolates. Sensitivity of this pathogen was significantly low for piperacillin 77.7 % (adjusted residual = - 3.8) and tazobactam (63.1 %, adjusted residual = -3.2) compared to the sensitivity of the other isolates to this antibiotic.

4.3.2 Sensitivity of Streptococcus pyogenes

Sensitivity of *Streptococcus pyogenes* was 100 % for ofloxacin, although this was not statistically different from the sensitivities of the other pathogens to this antibiotic (adjusted residual = 1.0). Notably, this pathogen did not display any significant difference in its sensitivity to any antibiotic compared with the other pathogens (Adjusted residuals between 0.8 – 1.7).

4.3.3 Sensitivity of *Staphylococcus aureus*

The pathogen was more sensitive to piperacillin (97.1 %, adjusted residual = 2.9) and tazobactam (81.3 %, adjusted residual = 2.4) compared to the other isolates. Sensitivity of this pathogen was significantly low for gentamicin (71.1 %, adjusted residual = -3.3) and ofloxacin (93.1 %, adjusted residual = -2.4) in comparison with that of the other pathogens to the same antibiotics.
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Amox</th>
<th>Cftr</th>
<th>Pip</th>
<th>Gen</th>
<th>Amk</th>
<th>Mer</th>
<th>Tzb</th>
<th>Ofl</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGS (n = 83)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>68.9</td>
<td>88.2</td>
<td>77.7</td>
<td>-3.8</td>
<td>82.6</td>
<td>2.2</td>
<td>91.3</td>
<td>63.1</td>
</tr>
<tr>
<td>I</td>
<td>12.3</td>
<td>3.4</td>
<td>4.2</td>
<td>0.9</td>
<td>5.3</td>
<td>-0.2</td>
<td>3.7</td>
<td>3.2</td>
</tr>
<tr>
<td>R</td>
<td>18.8</td>
<td>8.4</td>
<td>18.1</td>
<td>2.9</td>
<td>12.1</td>
<td>-2.0</td>
<td>6.0</td>
<td>0.0</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>90.7</td>
<td>94.3</td>
<td>93.2</td>
<td>1.1</td>
<td>93.4</td>
<td>1.7</td>
<td>94.2</td>
<td>93.8</td>
</tr>
<tr>
<td>I</td>
<td>3.1</td>
<td>3.1</td>
<td>3.7</td>
<td>-0.6</td>
<td>1.7</td>
<td>-0.6</td>
<td>5.8</td>
<td>3.1</td>
</tr>
<tr>
<td>R</td>
<td>6.2</td>
<td>3.1</td>
<td>3.1</td>
<td>-0.5</td>
<td>6.2</td>
<td>-1.1</td>
<td>0.0</td>
<td>3.1</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 111)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>83.5</td>
<td>93.5</td>
<td>97.1</td>
<td>2.9</td>
<td>71.1</td>
<td>-3.3</td>
<td>89.2</td>
<td>96.3</td>
</tr>
<tr>
<td>I</td>
<td>5.7</td>
<td>5.6</td>
<td>2.0</td>
<td>-2.0</td>
<td>4.6</td>
<td>0.9</td>
<td>5.4</td>
<td>2.8</td>
</tr>
<tr>
<td>R</td>
<td>10.8</td>
<td>0.9</td>
<td>0.9</td>
<td>-0.8</td>
<td>24.3</td>
<td>2.4</td>
<td>5.4</td>
<td>0.9</td>
</tr>
<tr>
<td>P – Value</td>
<td>0.564</td>
<td>0.360</td>
<td>0.008</td>
<td></td>
<td>0.0001</td>
<td>0.142</td>
<td>0.877</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Key**

4.3.2 Resistance of isolates to antibiotics

Viridans streptococci demonstrated a high level of resistance to piperacillin ($p = 0.008$, adjusted residual $= 2.9$) and tazobactam ($p = 0.0001$, adjusted residual $= 3.2$). Resistance of *S. pyogenes* was not significantly high in any of the antibiotics tested compared with the others. Isolates of *S. aureus* showed high levels of resistance in comparison with viridans streptococci and *S. pyogenes* to gentamicin ($p = 0.001$, adjusted residual $= 3.3$) and ofloxacin ($p = 0.001$, adjusted residual $= 2.0$). This information is presented in Table 4.4 above.

Out of all viridans group streptococci isolated, 51.8% were susceptible to all antibiotics, 48.2% were resistant to $\leq 3$ antibiotics tested and none was resistant to more than 4 antibiotics. For *S. pyogenes* 71.9% were sensitive to all antibiotics while 28.1% were resistant to $\leq 3$ antibiotics and none was resistant to more than 4 antibiotics. In the isolates of *S. aureus* 59.5% were susceptible to all antibiotics, 40.5% were resistant to $\leq 3$ antibiotics and none was resistant to more than 4 antibiotics. In these findings, trends of resistance of the isolates to several antibiotics followed similar patterns as ($p = \geq 0.05$) depicted in Table 4.5 below.
Table 4.5 Resistance of isolates to several antibiotics

<table>
<thead>
<tr>
<th>Proportion of isolates</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VGS (n = 83)</strong></td>
<td></td>
</tr>
<tr>
<td>Sensitive to 8</td>
<td>51.8</td>
</tr>
<tr>
<td>Resistant to ≤ 3</td>
<td>48.2</td>
</tr>
<tr>
<td>Resistant to ≥ 3</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>S. pyogenes (n = 32)</strong></td>
<td>1.00</td>
</tr>
<tr>
<td>Sensitive to 8</td>
<td>71.9</td>
</tr>
<tr>
<td>Resistant to ≤ 3</td>
<td>28.1</td>
</tr>
<tr>
<td>Resistant to ≥ 3</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>S. aureus (n = 111)</strong></td>
<td>0.100</td>
</tr>
<tr>
<td>Sensitive to 8</td>
<td>59.5</td>
</tr>
<tr>
<td>Resistant to ≤ 3</td>
<td>40.5</td>
</tr>
<tr>
<td>Resistant to ≥ 3</td>
<td>0.0</td>
</tr>
</tbody>
</table>
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The results of this study are discussed in this section which has been broken down to sub-topics based on the specific objectives.

5.1.1 Distribution of patients with URTIs according to age and sex

The study was carried out from November, 2012 to April, 2013 in which 237 patients were screened for bacterial pathogens in their upper respiratory tract. The sample consisted of more females than males; this may be linked to the general population of the area which consists of more females than males according to KNBS census report of 2009 and therefore may not have clinical or epidemiological significance.

In this study, the prevalence of upper respiratory tract infections was highest in patients aged between 1 – 5 years than in any other age group which is consistent with other studies (Koch et al., 2003; Zafar et al., 2008; Symekher et al., 2009; Mouro et al., 2010). Males aged between 1 – 5 years were at a higher risk of getting these URTIs compared with females in the same age group. A study conducted by Koch et al. (2003) also reported a higher prevalence of upper respiratory tract infections including otitis media in young boys than girls. Young age has also been found to be a significant risk factor for acquisition and transmission of these infections in other studies. Ndip et al. (2008) reported a higher prevalence of these infections in Cameroon; this could be attributed to exposure to these infections in the learning institutions. In a community-based study in
Gambia, Hill *et al.* (2006) also documented a higher prevalence of respiratory pathogens in young children than in adults.

This increased risk may be due to degradation of maternal antibodies, immaturity of the adaptive immune system, cessation of breastfeeding and attendance to childcare centers (Koch *et al.*, 2003). According to Sazawal *et al.* (2003), young children are the most vulnerable to upper respiratory tract infections, and therefore, these diseases usually spread rapidly through schools and households with youngsters.

The differences in the prevalence of different URTIs in different sexes and age groups observed in this study may not be conclusively explained. However, these may suggest a complex inter-relationship between several factors that lead to consultation for URTIs in general practice such as socio-economic status and occupation of the patients as reported by Cheong *et al.* (2004).

### 5.1.2 Isolation and confirmation of pathogens

Growth of organisms occurred only on blood agar, chocolate agar and Sabouroud’s agar because blood and chocolate agar can support the growth of both Gram positive and Gram negative organisms, MacConkey agar is both selective and differential for Gram negative bacteria while Sabouroud’s agar is selective for *Candida* spp. (CLSI, 2008). This indicates that all the isolates in this study were Gram positive. Isolates of *S. aureus*, *S. pyogenes*, viridans group streptococci and *C. albicans* are all Gram positive and represent clinically significant pathogens.
5.1.3 Prevalence of isolates

Isolation of pathogens in 95.4% of the patients is consistent with a study done in Nigeria by El-mahmood et al. (2010) in which the isolation rate was 92.8%, however, Ndip et al., (2008) reported an isolation rate of 56% in a similar study conducted in Cameroon. Viridans group streptococci though a normal flora in the oral cavity has been reported as an important pathogen in various studies. For instance, Brook (2002) reported that these organisms can be a source of upper respiratory tract infections of endogenous origin. Oral viridans group streptococci have also been reported as an important cause of streptococcal endocarditis (Young, 1987; McCartney, 1992; Manford et al., 1992).

Isolation of S. pyogenes was within the range reported in most studies. Mouro et al. (2010) reported a prevalence of 14.7% of this pathogen in Brazil although the pathogen was found to be predominant in children between 3 – 12 years which is contrary to these findings. Magnúsdóttir et al. (2008) also reported a prevalence of 22% of S. pyogenes in Iceland, Akoachere et al. (2002) a prevalence of 20.1% in Buea, Cameroon and van Gageldonk-Lafeber et al. (2007) a prevalence of 8% in Netherlands. It is not clear from this study why this pathogen was more prevalent in males than in females. Notably, this also coincides with a higher prevalence of pharyngitis in males than in females in this study. This pathogen has been cited as the leading cause of pharyngitis. Previous studies have emphasized the importance of establishing the appropriate treatment for this infection to decrease chances of complications such as rheumatic fever, endocarditis and scarlet fever (McGregor et al., 2004; Choby, 2009).
The prevalence of *S. aureus* compares with what Biedenbach *et al.* (2004) and Styers *et al.* (2006) reported in United States and Europe respectively. However, Heijer *et al.* (2013) reported a prevalence rate of 21.6 % in Europe, Choi *et al.* (2006) a prevalence of 23.4 % in Malaysia and Rebecca (2012) a prevalence of 26 % in Thirroul, New South Wales. The high prevalence of this pathogen reported in this study in patients aged between 36 and 45 and in females may not be absolutely ascertained; however nasal carriage has been reported previously as a major source of these infections.

Co-infections involving *C. albicans* and bacterial pathogens have also been reported in other studies. Bii *et al.* (2002) reported a prevalence of 13 % of *Candida* spp. in Mbagathi district hospital, Nairobi, Kenya. The higher risk of contracting infections of *C. albicans* in children below 5 years of age has been reported in other studies (Hoppe, 1997; Goins *et al.*, 2002; Su *et al*., 2008). This may be as a result of a weak immune system which is not fully developed while in adolescents and young adults, it could be caused by hormonal imbalances which may affect the immune system thus predisposing them to infections (Zhang, 2000; Brusca, 2010). Imbalance of the oral microbiota as a result of Broad-spectrum antibiotics which eliminate the competing bacteria and disrupt the normally balanced ecology of oral micro-organisms or medication with corticosteroids may also predispose patients to these infections (Williams and Lewis, 2011). It is possible for candidiasis to spread from the mouth to other sites such as the pharynx, oesophagus, lungs, liver, ano-genital region, skin or even the nails especially in debilitated individuals (Naglik *et al*., 2011; Williams and Lewis, 2011) and this may explain the isolation of this pathogen from the oropharynx. The role of oral thrush in the
hospital and ventilated patients has not been clearly described; however it has been proposed that, there is a risk of positive interaction of *Candida* spp. with topical bacteria (Peleg *et al.*, 2010). This interaction could be associated with increased risk of ventilator associated pneumonia (Kourkoumpetis and Themistoklis, 2010) and therefore necessitates the establishment of proper treatment of these infections to reduce such risks. The *Candida* load in the mouth which is thought to be the source of these infections can also be reduced by improving oral hygiene measures such as regular brushing of teeth and use of antimicrobial mouthwashes (Williams and Lewis, 2011).

Failure to isolate pathogens in 4.7 % of the samples could be attributed to the patients’ use of antibiotics prior to visiting the hospital or viral etiology of these infections. Furthermore, other pathogens such as *H. influenzae, S. pneumoniae, K. pneumoniae* and *P. aeruginosa* which have been incriminated for causing these infections in other studies (Larsson *et al.*, 2000; Ndip *et al.*, 2008; Zafar *et al.*, 2008; El-Mahmood *et al.*, 2010) were not isolated. Although it may not be possible to conclusively explain the non-isolation of these pathogens in the present study, it can be suspected that the patients had taken antibiotics although the inclusion criteria excluded patients who were on antibiotics one week prior to sampling or the infections were associated with viral etiology.

5.1.4 Antibiotic susceptibility patterns of the isolates

Resistance of viridans group streptococci to at least one antibiotic was the highest as observed in this study, this is important particularly considering that these bacteria are considered as part of the normal flora. Resistance of this pathogens has also been
reported in other studies (Kastner and Guggenbichler, 2001; Bruckner et al., 2002; Tazumi et al., 2009). Brook (2002) reported that these could be a source of infection of upper respiratory tract. It has further been shown that this resistance of viridans streptococci to antibiotics could be passed on to other sensitive pathogens through sharing of resistance markers (Brook, 2007).

The sensitivity of isolates of *Streptococcus pyogenes* was consistent with what El-mahmood et al. (2010) reported in Nigeria. Previously, resistance of this pathogen to penicillin has also been reported in other studies (Mary et al., 1999; Passali et al., 2007; Nevio, 2009). Internalization and intracellular survival of this pathogen has been suggested as an explanation to this resistance (Passali et al., 2007).

There was no antibiotic to which *Staphylococcus aureus* demonstrated 100% sensitivity although the resistance was generally low. This low rate of antibiotic resistance by *S. aureus* has also been reported by Heijer et al. (2013) across nine European countries. This pathogen has been reported to be a β-lactamase producer which has the ability to cause therapeutic failures by mediating antibiotic resistance. The occurrence of bacterial resistance mediated by β-lactamases has been reported in Nigeria and South Africa (Onanuga et al., 2005; Zeba, 2005; Nwanze et al., 2007). In a similar study in Vietnam, Larsson et al., (2000) reported that 90% of *S. aureus* were resistant to at least one antibiotic. In Nigeria, El-Mahmood et al. (2010) reported that 87.7% of *S. aureus* were resistant to at least one antibiotic with 41.2% being multi-drug resistant. However in this study, only 40.5% of *S. aureus* were resistant to at least one antibiotic and there was no
case of multi-drug resistance since no organism was resistant to more than three antibiotics.

The differences in susceptibility patterns of the bacteria to several antibiotics were not different statistically. This may be as a result of the choice of antibiotics selected for sensitivity testing in this study because all of them are normally preserved for 2nd or 3rd line of treatment for complicated bacterial infections and are not likely to be easily abused (Owens and Ambrose, 2005; Bilgrami et al., 2010; Guleria et al., 2013).

The differences noted in resistance rates between isolates in this study and others may be due to variations of time and geographical location as previously documented (Ndip et al., 2002). Further, the choice of antibiotics in this study may have influenced the rates of resistance. The low rate of antibiotic resistance could be attributed to the fact that these antibiotics are likely to be less abused due to their prohibitive cost and their mode of administration which is specifically intravenous or intramuscular except for amoxiclav which can be administered orally.

The low rate of resistance to amoxiclav by isolates could be because it is a combination of two antibiotics which may bring about a synergistic effect (Harr and French, 2010). In addition, the overall resistance rates were generally low for meropenem, ceftriaxone, ofloxacin and amikacin because these antibiotics are usually preserved for 2nd or 3rd line of treatment for complicated bacterial infections. According to a study carried out in South Africa, 91% of all bacterial isolates were susceptible to ceftriaxone (Samie et al.,
2009), El-Mahmood et al. (2010) also reported that all isolates were susceptible to both ofloxacin and ceftriaxone and therefore these studies are in harmony with findings of this study. However, tazobactam had the highest resistance possibly because it is just a β-lactamase inhibitor and is usually administered together with piperacillin and not independently suggesting that it lacks other antibacterial properties (Yang et al., 1999).

5.2 Conclusion

Young patients aged between 1 and 5 years are at a higher risk of contracting and transmitting these infections compared with patients in other age groups. Therefore the distribution of upper respiratory tract infection according to age is not normal based on the Mean and the Standard deviation. Again, the distribution of patients with upper respiratory tract infections according to sex in different age groups was significantly different since males aged between 1 – 5 years were more compared to females of the same age group. The null hypothesis which states that the distribution of patients with upper respiratory tract infections is uniform in different age groups and sexes is therefore rejected.

Pathogens isolated were *S. aureus*, *S. pyogenes*, viridans group streptococci and *C. albicans*. *S. aureus* was the most prevalent pathogen in this study, followed by viridans group streptococci while *S. pyogenes* was the least encountered bacteria. The prevalence of these isolates was significantly different and therefore the null hypothesis which states that the prevalence of bacterial pathogens associated with upper respiratory tract infections is the same in Kitui District Hospital is rejected.
Majority of the pathogens were isolated in patients aged between 1 – 5 years. Notably, no pathogen was significantly more prevalent than the others in this age group. Again, Patients aged between 6 – 14 and 15 -25 are at a higher risk of getting mixed infections of *C. albicans* and viridans streptococci compared to those in other age groups. *S. pyogenes* was more prevalent in males than in females while *S. aureus* was more prevalent in females than in males. Based on these findings, it can be conclusively reported that the prevalence of different types of bacteria associated with upper respiratory tract infections is not uniform in all the age groups and sexes therefore the null hypothesis is rejected.

Viridans streptococci were more sensitive to gentamicin and ofloxacin and less sensitive to piperacillin and tazobactam compared with the other isolates. Isolates of *S. aureus* were more sensitive to piperacillin and tazobactam and less sensitive to gentamicin and ofloxacin compared to the other isolates. Sensitivity of *S. pyogenes* was within the range of the others for all antibiotics tested. Resistance of bacterial pathogens to at least one antibiotic was highest in viridans group streptococci, followed by *S. aureus* while *S. pyogenes* had the least resistance among the isolates and there was no case of multi-drug resistance. The null hypothesis is therefore rejected based on these findings since there were statistically significant levels of resistance in viridans streptococci and *S. aureus*.

5.3 Recommendations

i. Proper interventions should be put in place to prevent young children aged between 1 and 5 years from contracting and transmitting upper respiratory tract
infections because they are at a higher risk of getting these infections than the other age groups.

ii. Continuous surveillance to monitor the prevailing pathogens associated with upper respiratory tract infections and their antimicrobial susceptibility patterns should be carried out from time to time.

iii. Molecular characterization and typing of viridians streptococci should be carried out to identify the species involved in upper respiratory tract infections and their resistance markers.

iv. This study further recommends that, more studies to be done to determine the prevalence of other pathogens such as viruses and fungi that are associated with these infections and their interactions with bacterial pathogens.

v. More studies need to be carried out to assess the rate of asymptomatic carriage of these pathogens in the upper respiratory tract of healthy persons since this is a major source infection by these pathogens.
REFERENCES


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Tazumi A., Maeda Y., Goldsmith C.E., Coulter W.A., Mason C., Millar B.C., McCalmont M., Rendall J., Elborn J.S., Matsuda M. and Moore J.E. (2009). Molecular characterization of macrolide resistance determinants (erm(B) and mef(A) in
Streptococcus pneumoniae and viridans group streptococci (VGS) isolated from adult patients with cystic fibrosis (CF). *Journal of Antimicrobial Chemotherapy*. 64: 501-506.


Appendix I: Culture media preparation

1. Blood agar

This is an enriched medium.

**Preparation:** 40g of the powder was suspended in 1 litre of distilled water. It was boiled to dissolve completely. Sterilization was done by autoclaving at 121 °C for 15 minutes. The solution was then cooled to 50 °C and 7 % of human blood added. It was mixed thoroughly and poured into Petri dishes.

2. Chocolate agar

It is an enriched medium for fastidious organisms.

**Preparation:** 40g of the powder was suspended in 1 litre of distilled water. It was boiled to dissolve completely. Sterilization was done by autoclaving at 121 °C for 15 minutes. 7 % of human blood added immediately. It was mixed thoroughly and poured into Petri dishes.

3. MacConkey agar

This is a selective and differential medium for the isolation and differentiation of *Enterobacteriaceae*. Swarming of *Proteus* is prevented.

**Preparation:** This was prepared by suspending 48.5 grams in 1 litre of distilled water. It was then boiled to dissolve completely. Sterilization was done by autoclaving at 121 °C for 15 minutes. The medium was allowed to cool to about 50 °C and poured onto sterile petri dishes.
4. Mueller-Hinton Agar

This is an antimicrobial susceptibility testing medium.

**Preparation:** To prepare the medium, 38 g of the powder was dissolved in 1 litre of distilled water. It was boiled to dissolve the medium completely. It was sterilized by autoclaving at 121 °C for 15 minutes. The medium was allowed to cool to about 50 °C and poured onto sterile petri dishes.
Appendix 11: A study questionnaire for patients with upper respiratory tract infections in Kitui District Hospital, Kenya

Section 1: Introduction

a. Preamble.

I am Josphat Mutinda, a student at Kenyatta University taking Master of Science (Microbiology). I am carrying out a research on the Prevalence and antimicrobial susceptibility profiles of bacterial pathogens isolated from outpatients with upper respiratory tract infections in Kitui District Hospital, Kenya. I request you to assist me by filling in this questionnaire to make the study a success. Kindly note that all the information provided in this questionnaire is purely for research purpose and will remain confidential.

b. Instructions for filling the questionnaire

1. Do not write your name.

2. Read all the questions carefully before answering them.

3. Select the answer that best suits your situation.

4. Mark with a tick in the appropriate block to indicate your choice.
Section 2: Demographic information of the patients

1. What is your sex?
   a. Male
   b. Female

2. What is your age?

3. Have you taken antibiotics of any kind for the last one week?
   a. Yes
   b. No
Appendix III: Clinical breakpoint values for inhibition zone diameters of CLSI, 2008 for antibiotic susceptibility testing for gram positive bacteria using disk diffusion method

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inhibition zone diameter (mm)</th>
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<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>≥21</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≥23</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥22</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥17</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>≥22</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>≥18</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>≥21</td>
</tr>
</tbody>
</table>
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.

Date: 13th November, 2014

NACOSTI/P/14/7578/3361

Mutinda Josipat
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Prevalence and antimicrobial susceptibility profiles of bacterial pathogens of upper respiratory tract in Kitui District Hospital, Kenya,” I am pleased to inform you that you have been authorized to undertake research in Kitui County for a period ending 10th December, 2014.

You are advised to report to the Medical Superintendent, Kitui District Hospital, the County Commissioner, the County Director of Education and the County Coordinator of Health, Kitui County before embarking on the research project.

On completion of the research, you are expected to submit two hard copies and one soft copy in pdf of the research report/thesis to our office.

SAID HUSSEIN
FOR: SECRETARY/CEO

Copy to:

The Medical Superintendent
Kitui District Hospital.

The County Commissioner
Kitui County.