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A SURVEY OF *Legionella pneumophila* AMONG PNEUMONIA PATIENTS AT KENYATTA NATIONAL HOSPITAL.

BY

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DECLARATION

I, Susan Odera, do hereby declare that all the work submitted is original except where otherwise acknowledged and it has not been presented either wholly or in part to this or any other university for the award of any degree.

Susan Akinyi Odera

Signed.....Date.....4th July 2008.....

APPROVAL BY SUPERVISOR

This is to certify that this is bonafide research work carried out independently by Susan Odera under my guidance and supervision.

Signed..... Date.....

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APPROVAL BY DEPARTMENT

This is to certify that this study was registered and carried out under the department of medical microbiology. The results have been analyzed and document forwarded as proof of work done leading to an award of a master's degree.

Signed..... Date.....

Prof. Walter Jaoko

Chairman,

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DEDICATION

This work is dedicated to my parents for their patience and encouragement during the course of this work.

May the Almighty God bless you.

ACKNOWLEDGMENT

I would like to express my sincere appreciation to all those who assisted me in one way or the other during my study period. Special thanks to:

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LIST OF ABBREVIATIONS

KNH Kenyatta National Hospital

WHO World Health Organization

CDC Center for Disease Control

EWGLI European Working Group for Legionella Infections

BCYE Buffered Charcoal Yeast Extract Agar

DFA Direct Fluorescent Antibody Staining

PCR Polymerase Chain Reaction

DNA Deoxyribonucleic Acid

RNA Ribonucleic Acid

IFA Immunofluorescence Assay

EIA Enzyme Immunoassay

ELISA Enzyme Linked Immunosorbent Assay

HIV Human Immunodeficiency Virus

MA Micro agglutination

IgG Immunoglobulin G

IgM Immunoglobulin M

ABSTRACT

Legionellae has a worldwide distribution though the actual statistics especially in the tropics has not been established. In human history, numerous infectious diseases have emerged and re-emerged. Understanding the dynamics of emerging and reemerging infections is critical to efforts to reduce the morbidity and mortality of such infections, to establish policy related to preparedness for infectious threats, and for decisions on where to use limited resources in the fight against infections. Reports of legionnaires disease in developing countries especially from Africa have been sparse. The real magnitude of the problem is therefore unknown and it may be responsible for some of the cases of pneumonia than is generally recognized. Lack of appropriate legionella diagnostic and surveillance systems, and limited resources in Kenya and other African countries contribute to the situation.

Initiation of appropriate therapy has always been associated with improved outcome therefore discovery of even a single case would be an important sentinel of indicating the likelihood of detecting undiscovered cases. This study investigated the occurrence of *L.pneumophila* in pneumonia patients and possible association with risk factors in the group that is positive for infection

Research Hypothesis:

There are cases of *Legionella pneumophila* infection that go undiagnosed among patients presenting at Kenyatta National Hospital with signs and symptoms suggestive of pneumonia.

Objectives

1. To determine the occurrence of *L.pneumophila* among patients admitted at Kenyatta National Hospital medical ward with signs and symptoms suggestive of pneumonia.
2. To determine possible risk factors for those who are infected with *L.pneumophila.*
3. To determine the association between risk factors and infection with *L.pneumophila.*

Study Design: A cross-sectional descriptive study was carried out between March and June, 2007.

Setting: The medical ward of Kenyatta National Hospital, Nairobi.

Study population: The study population comprised of patients admitted with a provisional diagnosis for pneumonia.

Sample size: One hundred and twenty pneumonia patients who satisfied the inclusion criteria were obtained by consecutive sampling

Ethical Consideration: Informed consent was obtained from the participants. Approval to carry out the study was obtained from the Kenyatta National Hospital Research and Ethics Committee, and the Chairman of the Department of Internal Medicine, Kenyatta National Hospital.

Methodology: The ELISA technique was used to analyze the urine samples for the presence of *L. pneumophila* antigen..

Results

The findings indicated that up to 9.2% (11 out of 120) of patients admitted at the medical ward of Kenyatta National Hospital due to signs and symptoms suggestive pneumonia between March and June were infected with *L. pneumophila.*

At a confidence limit of 0.05, there was statistical significance in the number of pneumonia patients infected with *L. pneumophila* and exposure to air conditioners (p= 0.003). 22.58% of patients who were exposed to air conditioners were positive for *L. pneumophila* urinary antigen. There was a statistical significance between exposure to air conditioners and location of work area (p= 0.001)). 38.46% of those who worked indoors were exposed to air conditioners.

There was also statistical significance in the number of pneumonia patients infected with *L. pneumophila* and a history of a past or concurrent respiratory illness (p= 0.021). 14.2% of all respondents who had a history of past or concurrent respiratory illness were positive for *L. pneumophila* urinary antigen.

Exposure to air conditioners is a key predisposing factor to infection with *L.pneumophila* and there is need for public health education on routine inspection and maintenance of air conditioners and hot water systems.

CHAPTER ONE

INTRODUCTION

Since the epidemic of legionnaires disease in Philadelphia in 1976 and the description of the causative agent *Legionella pneumophila*, there have been several reports of outbreaks and single cases throughout the world.

Legionella pneumophila is recognized as one of the causes of atypical pneumonia, both community acquired and nosocomial[1]. The incidence of community acquired Legionnaires disease varies widely according to the setting investigated and the diagnostic methodology applied. Since many countries lack appropriate methods of diagnosing the infection or surveillance systems capable of monitoring the situation, the real magnitude of the problem is unknown and it may be responsible for more of the pneumonia occurring in the tropics than is generally recognized. Infections of any kind can be recognized more accurately with increased physician awareness and availability of diagnostic tools. Serological tests are mainly applied as epidemiological tools and can only be useful in diagnosis of disease when the background prevalence of antibody to *L.pneumophila* within the local community is established thereby providing a correct guide for interpretation of serological tests. However reliability of serological testing is hampered by several limitations including cross reactions due to antibodies to *Pseudomonas aeruginosa* and *Campylobacter spp.* The need for testing of paired serum samples collected 3 to 6 weeks apart also diminishes the use of antibody testing in serology tests. Urine antigen is now the most frequently used diagnostic test permitting early diagnosis, initiation of appropriate therapy and a rapid public health response.

Prevalence surveys have been conducted in several countries all over the world. In the developed countries concrete systems of surveillance of legionella in the environment and monitoring of reports of legionellosis outbreaks have been established though the Center for Disease Control (in USA) and the European Working Group for Legionella Infections (EWGLI). The disease is a major concern of public health professionals and

individuals involved in maintaining building water systems. Studies have estimated that between 8000 and 18000 persons are hospitalized with legionellosis annually in the United States of America[1]. In 2003, 34 countries (population: 467.76 million) out of the 36 in the EWGLI reported a total of 4578 cases, meaning an average rate across Europe of 9.8 per million populations. Reports of legionnaire's disease in developing countries especially from Africa have been sparse. Studies of antibody prevalence across the continent are few and legionellosis is hardly considered during differential diagnosis of respiratory infections. Failure to diagnose legionellosis is largely due to lack of clinical awareness.

The factors that lead to outbreaks or cases of Legionnaires' disease are not completely understood, but certain events are considered prerequisites for infection. These include the presence of the bacterium in an aquatic environment, amplification of the bacterium to an unknown infectious dose, and transmission of the bacteria to a human host that is susceptible to infection[2].

Kenya has witnessed an expansion of its urban centers in the last decade, with increased usage of facilities such as air conditioners and hot water systems both of which are main man-made habitats of legionella. Factors that have been observed in other places to predispose one to infection with legionella such as cigarette smoking are also present in our setting making it possible that legionellosis could be an under-reported and under-diagnosed disease. The aim of this study was to investigate what proportion of pneumonia patients at the medical ward are excreting *L.pneumophila* antigen and evaluating for possible risk factors in the positive group using demographic and clinical data. The study also generated useful data on socio-demographic factors of pneumonia patients in general.

CHAPTER TWO

LITERATURE REVIEW

Despite a substantial disease burden, there is little descriptive epidemiology of acute pneumonia in sub-Saharan Africa.

Pneumonia in general

Pneumonia is an inflammation of the lung that is most often caused by infection with bacteria, viruses, or other organisms. Occasionally, inhaled chemicals that irritate the lungs can cause pneumonia. Healthy people can usually fight off pneumonia infections. However, people who are sick have weakened immune systems that make it easier for bacteria to grow in their lungs[4]. Symptoms of pneumonia are shortness of breathe, shallow breathing, chest pain, coughing, fever and chills.

Pneumonia may be defined according to its location in the lung- either as lobar pneumonia, which occurs in a lobe of the lung, or as bronchopneumonia which tends to be scattered throughout the lung.

One may also classify pneumonia based on where the disease is contracted. This helps predict which organisms are most likely responsible for the illness and, therefore, which treatment is most likely to be effective. It can be Community-Acquired Pneumonia[3] (CAP) in which infection is contracted outside a hospital setting. One of the most common causes of bacterial CAP is *Streptococcus pneumoniae.* Other causes include *Haemophilus influenzae, Mycoplasma pneumoniae, and Chlamydia spp.* The pneumonia can be hospital-acquired (nosocomial) in which infection of the lungs is contracted during a hospital stay. This type of pneumonia tends to be more severe, because hospital patients already have weakened defense mechanisms. Hospital patients are particularly vulnerable to Gram-negative bacteria like *Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Moraxella catarrhalis, Neisseria meningitidis. Staphylococcus aureus* has also been implicated as a cause. A subgroup of hospital-acquired pneumonia is ventilator-associated pneumonia (VAP), a highly lethal form contracted by patients on ventilators in hospitals and long-term nursing facilities.

Pneumonia-causing agents reach the lungs through different routes. In most cases, a person breathes in the infectious organism, which then travels through the airways to the lungs. Sometimes, the normally harmless bacteria in the mouth, or on items placed in the mouth, can enter the lungs. This usually happens if the body's "gag reflex," an extreme throat contraction that keeps substances out of the lungs, is not working properly. Infections can also spread through the bloodstream from other organs to the lungs[4].

Atypical pneumonias are generally caused by: *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionellae pneumophila*. *Mycoplasma pneumoniae* often affects younger people and may be associated with symptoms outside of the lungs (such as anemia and rashes), and neurological syndromes (such as meningitis, myelitis, and encephalitis). Severe forms of *Mycoplasma* pneumonia have been described in all age groups. Pneumonia due *Chlamydia* occurs year round and accounts for 5-15% of all pneumonias. It is usually mild with a low mortality rate. In contrast, atypical pneumonia due to *Legionellae* accounts for 2-6% of pneumonias and has a higher mortality rate. Elderly individuals, smokers, and people with chronic illnesses and weakened immune systems are at higher risk for this type of pneumonia. Contact with contaminated aerosol systems (like infected air conditioning systems) has also been associated with pneumonia due to *Legionellae*[4].

A number of viruses can cause pneumonia either directly or indirectly, and include the following: Influenza virus, Respiratory syncytial virus (RSV), severe acute respiratory syndrome (SARS), Adenoviruses and Herpes viruses. In adults, herpes simplex virus and varicella-zoster (the cause of chicken pox) are generally causes of pneumonia only in people with impaired immune systems.

The mouth harbors a mixture of bacteria that is harmless in its normal location but can cause a serious condition called aspiration pneumonia if it reaches the lung. This can happen during periods of altered consciousness, often when a patient is affected by drugs or alcohol, or after head injury or anesthesia. In such cases, the gag reflex is diminished, allowing these bacteria to enter the airways to the lung. These organisms are generally

different from the usual microbes that enter the lung by inhalation. Many are often anaerobic.

Impaired immunity leaves patients vulnerable to serious, even life-threatening, pneumonias known as opportunistic pneumonias[4]. They are caused by microbes that are harmless to people with healthy immune systems. Infecting organisms include the following: *Pneumocystis jiroveci,* an atypical organism that is very common and generally harmless in people with healthy immune systems. It is one of the most common causes of pneumonia in AIDS patients. Bacteria, such as *Mycobacterium avium* and viruses, such as cytomegalovirus (CMV) have also been implicated. In addition to AIDS patients, other conditions also put patients at risk for opportunistic pneumonia. They include lymphomas, leukemia, and other cancers. Long-term use of corticosteroids and immuno-suppressants increase the susceptibility to these pneumonias.

Exposure to chemicals can also cause inflammation and pneumonia. A number of people are exposed to pneumonia-causing organisms specific to particular occupations for example:

- Cattle rearing: Risk of pulmonary anthrax due to *Bacillus anthracis*.
- Agricultural and construction work: Coocidioidomycosis and histoplasmosis due to *Coccidiodes imitis* and *Histoplasma capsulatum* respectively.
- Bird rearing: Psittacosis due to *Chlamydia psitacci*.

Historical Background of *Legionellae*

The first outbreak of legionnaire's disease occurred in 1976[2], at Bellevue Stratford Hotel that was hosting a convention of the Pennsylvania department of the American Legion. Approximately 221 people contracted the disease, 34 of whom died. The source of the bacteria was found to be contaminated water used to cool air in the hotel's air conditioning system.

In January 18th 1977, Joseph McDade and Charles Shepard working at CDC, Atlanta discovered that the causative organism of the Philadelphia outbreak was a previously

unknown organism[65]. Their findings opened doors to a broad range of studies that are continuing to this day.

The newly discovered bacterium was assigned to the family *Legionellaceae,* the genus *Legionellae* (for legion), and the species *pneumophila* ('lung loving'): causative agent *Legionellae pneumophila.* Ninety percent of infections attributed to *Legionellae* are caused by this particular species and illnesses by serogroup 1 and in some cases 4 and 6[6]. Since all *Legionellae* are presumed to be capable of intracellular growth in some host cell[7] it is likely that most *Legionellae* can cause human disease under appropriate conditions[7]. Infections due to the less common strains of *Legionellae* are infrequently reported because of their rarity and lack of diagnostic reagents[8]. The first strains of *Legonellae* were isolated in guinea pigs by using procedures for isolation of *Rickettsia*[9]. The first was isolated by Tatlock in 1943, while another strain was isolated in 1947 by Jackson et al[10]. In 1954, Drozanski isolated a bacterium that infected free living amoebae from soil in Poland[11]. This organism was classified as a species of *Legionellae* in 1996[12].

Description of the genus.

The genus *Legionellae* was established in 1979 after a large outbreak of pneumonia among members of the American legion that had occurred 3 years earlier and was traced to a previously unrecognized bacterium, *Legionellae pneumophila. Legionellae* are intracellular parasites of freshwater protozoa and use a similar mechanism to multiply within mammalian cells. Currently, there are 48 species comprising of 70 distinct serogroups in the genus *Legionellae* [13,14,15,16]. Although there are now 15 serogroups of *L.pneumophila*, 79% of all culture confirmed or urine antigen confirmed cases are caused by *L.pneumophila* serogroup 1[17].

Legionellae are obligate aerobic gram-negative rods that stain faintly with the standard gram stain. Morphologically, they appear as coccobacilli in infected tissues and secretions. Long filamentous forms can be seen in culture media. *Legionellae* are distinguished from other saccharolytic bacteria by their requirement for cysteine and iron

salts for primary isolation on solid media and their unique cellular fatty acids and ubiquinones[18]. Gas liquid chromatography shows large amounts of cellular branched-chain fatty acids and respiratory ubiquinones with more than10 isoprene units.

Habitats of *Legionellae*

Water is the major reservoir for *Legionellae*, and the bacteria are found in fresh water environments worldwide[19]. A single exception to this observation is *Legionellae longbaechae*, a frequent isolate from potting soil[20]. This species is the leading cause of legionellosis in Australia and occurs in gardeners and those exposed to commercial potting soil.[21]. *Legionellae* survive in a wide range of conditions like p^h of 5 to 8.5, dissolved oxygen concentrations of 0.2 to 15ppmm in water and multiply at temperatures between 25°C and 42°C, with optimal growth at 35°C. Outbreaks of pneumonia in hospitals have been attributed to the presence of the organism in potable water systems i.e. building plumbing systems distributing water for direct human contact e.g. sinks and showers[23]. Thermally altered aquatic environments can shift the balance between protozoa and bacteria, resulting in rapid multiplication of *Legionellae*, which can translate into human disease. Legionellosis is a disease that has emerged in the last half of the 20th century because of human alteration to the environment. Left in their natural state, *Legionellae* would be an extremely rare cause of human disease, as natural fresh water environments have not been implicated as reservoirs of outbreaks of legionellosis[24].

Some outbreaks of legionellosis have been associated with construction, and it was originally believed that the bacteria could survive and be transmitted to humans via soil. However, *L.pneumophila* does not survive in dry environments, and these outbreaks are more likely the result of massive descalement of plumbing systems due to changes in water pressure during construction [22, 25]. Aerosolization or aspiration of contaminated water is a major route of transmission to patients in nosocomial legionellosis. Colonization of hot water tanks occurs at temperatures conducive for proliferation of the organism, usually at 40- 50°C. *Legionellae* and other microorganisms attach to and colonize surfaces in aquatic environments forming a biofilm.

Man made habitats of *Legionellae* include cooling towers, evaporative condensers, whirlpool spas, decorative fountains and potable water distribution systems, where sediment and scaling of biofilms are present. The bacteria are more easily detected from swab samples of biofilm than from flowing water, suggesting that the majority of *Legionellae* are biofilm associated[26]. The complex nutrients available with biofilms have led some researches to propose that biofilms support the survival and multiplication of *Legionellae* outside a host cell[27]. The control of biofilm-associated *Legionellae* may lead to the most effective control measures to prevent legionellosis. Institutions that have experienced outbreaks of legionellosis are all too aware of how tenacious the bacteria can be within building water system biofilms[24].

Acquisition of legionnaire's disease has been linked to contamination of water supplies in residences, rehabilitation centers, nursing homes and industrial buildings. British Communicable Disease Surveillance Center reported that 19 out of 20 hospital outbreaks of Legionnaires disease in United Kingdom from 1980-1992 were attributed to hospital potable water distribution systems[28].

Legionellae survive in aquatic and moist soil environments as intracellular parasites of free living protozoa[7, 29]. These bacteria have been shown to multiply in 14 species of amoebae, two species of ciliated protozoa, and one species of slime mould, while growth of *Legionellae* in the absence of protozoa has been documented only on laboratory media[7, 30, 31]. Acanthamoeba spp and Naegleria spp harboring *Legionellae* are chlorine resistant infection reservoirs for the organism in water supplies. It has been suggested that *Legionellae* can be transmitted to humans via inhalation of amoebic vesicles. Pontiac fever (humidifier fever) is linked to hypersensitivity reactions to these free living amoeba. While protozoa are the natural hosts of *Legionellae*, the infection of human phagocytic cells is opportunistic[24].

Clinical Significance

Legionellosis classically presents as two distinct clinical entities, Legionnaires' disease, a severe multisystem disease mainly presenting as pneumonia[32], and Pontiac fever, a self-limited flu-like illness[33]. Additionally, some people may be asymptomatic.

Pontiac fever is an acute self-limiting flu like illness without any pneumonia. Its incubation period is 24 to 48 hours and complete recovery occurs within a week without antimicrobial therapy. Legionnaires' disease has an incubation period of two to ten days (but up to 16 days has been documented in some recent well documented outbreaks). Initial symptoms are fever, loss of appetite, headache, malaise and lethargy. Some patients may also have muscle pain, diarrhea and confusion. Blood- streaked phlegm occurs in about one third of patients. The severity of the disease ranges from a mild cough to a rapidly fatal pneumonia. Although no chest X-ray pattern can distinguish this infection from other types of pneumonia, alveolar infiltrates are more common with Legionnaires' disease[34].The key to diagnosis is performing appropriate microbiologic testing when a patient is in a high risk category. Death occurs through progressive pneumonia with respiratory failure and/or shock and multiorgan failure[35]. The most frequent complications are respiratory failure, shock and acute kidney failure. Recovery always requires antibiotic treatment.

After the bacteria enter the upper respiratory tract, the organism is cleared by cilia on respiratory epithelial cells and the normal pulmonary immune system. Impaired mucocilliary clearance followed by aspiration can increase risk of infection. Some populations have an increased risk to developing severe legionella infections[36]. Risk factors for community-acquired and travel-associated legionellosis include: being a male, the elderly (>65years), cigarette smokers, history of heavy drinking, pulmonary related illnesses, immunosuppresion, and chronic debilitating illnesses e.g. hematological malignancies. Risk factors for hospital acquired pneumonia in the host are: tracheal intubations, mechanical ventilation, aspiration, presence of nasogastric tubes, and the use of respiratory therapy equipment. Surgery is a major predisposing factor in nosocomial

infection with transplant recipients at the highest risk. The most susceptible hosts are immuno-compromised patients, including organ transplant recipients and those receiving corticosteroid treatment. The disease is rare among children. . Potential virulence factors include several cytotoxins, heat shock proteins; phospholipases, lipopolysaccharides and metalloproteases[37].Genes regulate most of these virulence factors. Delay in diagnosis and administration of appropriate antibiotic treatment, increasing age and presence of co-existing diseases are predictors of death from Legionnaires' disease.

Pathogenesis and Immune Response

In lung air spaces, *Legionellae* is ingested spontaneously by resident alveolar macrophages. The bacteria enter the cells by phagocytosis and once phagocytosed, the bacteria reside within a unique phagosome that does not fuse with lysosomes or become highly acidic[38, 39, 40]. Phagocytosis in human monocytes has been shown to be partly mediated by a three-component system composed of complement receptors CR1 and CR3[41]. Horwitz also described the interaction of the phagosome with mitochondria and ribosome studded vesicles[42, 43]. The ribosome associates first with other cellular organelles like the mitochondria and later with the rough endoplasmic reticulum. Within the resulting endosome, bacterial multiplication proceeds until the host cell is packed with bacteria. Eventually the cell dies and ruptures releasing the progeny to other cells. *L.pneumophila* kills its' host cells either by apoptosis or necrosis mediated by a pore forming activity or both. In macrophages and alveolar epithelial cells, *L.pneumophila* induces apoptosis during the early stages of infection[44, 45]. A second phase of necrosis induced by a pore-forming activity takes place in infected human phagocytes. In contrast, death of host amoeba cells has not been associated with apoptosis in studies utilizing *Acanthamoeba castellani* and *Acanthamoeba polyphaga.*

Macrophages infected with *Legionellae* release cytokines that contribute to influx of blood monocytes and neutrophils into lung airspaces. Nodular areas of infection enlarge and become visible as infiltrates on chest X-rays. These areas develop into micro abscesses and may coalesce to form cavities. Bronchi and bronchioles are not affected. Much damage is attributed to vigorous host inflammatory response. Illness begins with

flu like complaints, fever and typical development of clinical features of pneumonia-cough, shortness of breathe and chest pain. Patients rarely have grossly purulent sputum. Nausea, vomiting and abdominal pain may be present[46].

The host may inhibit intracellular growth of the organism by non-specific immune mechanisms. After contact with the organism, macrophages secrete tumor necrosis factor alpha (TNF) that inhibits intracellular bacterial growth. Once infection is established however, specific immune responses only can clear the infection. Specific antibodies may play a role in containing the infection but recovery needs cell- mediated response. Antibodies bind to the surface and enhance uptake of bacteria by neutrophils. The cell mediated immunity response limits growth in macrophages. Legionella immune lymphocytes proliferate and secrete cytokines after contact with cells presenting legionella antigens with class 2 histocompatibility molecules. One cytokine, gamma interferon, suppresses growth within macrophages by inducing the cells to limit availability of iron to intracellular bacteria.

Laboratory Diagnosis

Specialized laboratory tests are necessary for identification of legionella. A study done at the Scottish Legionella Reference Laboratory[66] to compare the phenotypic and genotypic methods of diagnosis showed that the relative sensitivity and specificity of the urinary antigen Enzyme Linked Immunosorbent Assay (ELISA) and the serum Polymerase Chain Reaction (PCR) was found to be 100%. The Immunofluorescent Antibody (IFA) Test gave relative sensitivity and specificity values of 93.8% and 95%. Direct Fluorescent Antibody (IFA) and culture although 100% specific had low sensitivities of 19% and 42% respectively. The study concluded that urinary antigen and serum PCR were the most valuable tests with excellent sensitivity and specificity values[66]. Some of these tests have been discussed below:

Culture on Media

Of the various methods available, culture is the most specific and is usually accepted as the gold standard. However, in routine and clinical laboratory work, legionellosis is rarely proven by culture whereas detection of urinary antigen is now common.

Legionellae was first isolated by using Mueller-Hinton agar supplemented with hemoglobin and IsoVitaleX (MH-IH) [47].These refinements with a soluble form of iron and cysteine led to the development of Feeley-Gorman agar, which provides better recovery of the organ from tissue[47]. Later starch was replaced with charcoal to detoxify the medium and the amino acid source was changed to yeast extract agar.

The medium currently being used is buffered charcoal yeast extract (BCYE) agar α-ketoglutarate and other selective agents. Although the majority of *Legionellae spp.* grow readily on BCYE agar, some require supplementation with bovine serum albumin to enhance growth[48].

Direct Fluorescent Antibody (DFA) Detection

Microscopic examination of specimens using DFA staining was the first method used to detect *Legionellae* from lung tissue (from autopsy or biopsy specimens) and respiratory secretions[24].

Polymerase Chain Reaction

Various Polymerase Chain Reaction (PCR) tests that have been developed for *Legionellae* target either random DNA sequences for *L.pneumophila*, the 5S rRNA gene, the 16S rRNA gene or the mip gene[49,50]. Recently several researchers have reported on the use of real time PCR combined with the use of a hybridization probe to confirm the product identity for rapid detection of *Legionellae* in clinical specimens[51].

Serology

Serological investigation for *Legionellae* is the most widely used technique in epidemiological studies. It involves detection of either antibodies or antigens.

Antisera produced in rabbits have been prepared against all species and serogroups of *Legionellae* and have been used in the Center for Disease Control and Prevention (CDC) laboratory to identify most strains in slide agglutination tests[52].

Antibody serology tests are presumptive only if results are available for a single specimen. Definitive diagnosis requires a fourfold rise between the acute and convalescent phase titres. If the seroprevalence of legionella pneumonia antibody titres within a community is known to be low, a single elevated titre may possibly indicate presence of an acute disease. The use of indirect immunofluorescence assay (IFA) was used to detect antibodies from the Philadelphia outbreak and was instrumental in determining the cause of the illness. Other antibody tests that have been developed include enzyme immunoassay and microagglutination[53, 54] and indirect hemagglutination. Stanek et al.[55] have carried out studies comparing the sentivities and specificities of indirect immunofluorescenceassay (IFA), micro agglutination test (MA) and enzyme linked immunosorbent assay (ELISA) in diagnosis of legionellosis. However, false positive titres to *Legionellae spp* have been observed in several infections including *Bacteroides fragilis, C. psittaci, Pseudomonas pseudomallei, Haemophilas influenzae, Coxiella burnetti, Rickettsia typhi* and *Proteus vulgaris*.

Antigens are generally detectable in urine within a few days of illness onset and can remain so for several weeks after initiation of antimicrobial therapy. Shortly after the outbreak of Legionnaires ' disease at the American Legion Convention in Philadelphia, two investigators reported the ability to detect antigen in the urine of serologically confirmed Legionnaires' disease patients by ELISA[56]. Due to the high specificity of commercially available legionella urine antigen assays, Plouffe et al[3] proposed that the case definition for definitive Legionnaires ' disease be expanded to include diagnosis by detection of urinary antigen. Urine is a convenient sample, being obtained in a non-invasive way. Also the ELISA technique employed to detect the antigens in the urine gives rapid results.

Treatment

There is a growing spectrum of antibiotics available for the treatment of Legionella infections. The macrolides, such as erythromycin have been used most consistently but newer agents, such as azithromycin have become available. Tetracyclines may have a role in mild infection. Studies have demonstrated fluoroquinolone resistance in

Legionella pneumophila[67]Rifampicin tends to be reserved for more serious infections combined with other agents. All these agents have a reasonable capacity to inhibit the multiplication of intracellular organisms. However, their efficacy is dependent upon heightened clinical suspicion and the early collection of appropriate specimens for the definitive diagnosis of Legionella.

Supportive therapy includes administration of antipyretics, fluid replacement, circulatory support and if necessary oxygen administration by mask.

Past Studies

Several epidemiological surveys for *Legionellae spp.* have employed serology in investigating the occurrence of the bacteria in the environment or general population.

M. Rolfe[57] did a study in Zambia in 1986. Following a case of pneumonia which was suggestive of Legionnaires' disease, a serological survey of people in the same community showed a titre of 1: 256 or more in 10% of 73 sera examined by the indirect fluorescent antibody test using heat-inactivated antigen. A further study of 105 patients with pneumonia failed to show a significant rise in antibody titre using acute and convalescent serum.

In South Africa in 1987-1988, Maartens et al[58] at Groote Schuur Hospital assessed the proportion of cases of community acquired pneumonia caused by atypical bacteria. Acute and convalescent sera were tested in batches for antibodies and the two most common organisms were found to be *C. pneumoniae* (20.7%) and *L. pneumophila* (8.7%).

In a study carried out in Kenya in 1990 by Phakkey et al[59], the prevalence of legionella antibodies was investigated in domestic and wild animal sera, as well as healthy blood donor sera using microagglutination. The study concluded that *Legionellae* exists in the environment. However, Farshay et al.[60] in studying the immunoglobulin specificity of the micro agglutination test for the Legionnaires' disease bacterium suggested that like other agglutination tests, micro agglutination is heavily dependant on the presence of IgM antibody.

PROBLEM STAEMENT

Reports of legionnaire's disease in developing countries especially from Africa have been sparse and unlike Europe and USA, these countries including Kenya lack a surveillance system with data on the current state of affairs. It would be true to say that the real magnitude of the problem is unknown. Moreover, people here are also exposed to factors that have been identified in temperate regions to predispose one to infection with legionella. With the increased causes of immunosuppresion, particularly due to Human Immunodeficiency Virus, there is a likelihood of emerging and re-emerging infections. There has been no study done so far on the prevalence of *Legionellae pneumophila* at Kenyatta National Hospital and the level of infection, if any, among pneumonia patients.

Under diagnosis is a major bias in computing the incidence of any disease. Laboratory investigations carried out on pneumonia patients at the hospital never investigate for infection with legionella, and very rarely identification of the actual causative agent of the pneumonia. Although the patients receive antibiotics as part of their management, identification of causative microbial agents and possible co-infection would definitely improve prognosis, especially with regard to antibiotic sensitivity testing. Delay in diagnosis and lack of appropriate treatment could actually result in death. This study was to investigate the occurrence of legionella among pneumonia patients at the hospital and possible risk factors in the positive group. Demographic data obtained from the pneumonia patients also give an opportunity to study their lifestyle history with a main interest in smoking and alcohol consumption.

RESEARCH HYPOTHESIS

There are cases of *Legionella pneumophila* infection that go undiagnosed among patients presenting with signs and symptoms suggestive of pneumonia at Kenyatta National Hospital.

RESEARCH QUESTIONS

1. How many cases of patients admitted at the Kenyatta National Hospital medical ward with signs and symptoms suggestive of pneumonia test positive for infection with *L. pneumophila*?
2. Are there any risk factors in the group that tests positive for infection with *L. pneumophila*?
3. Is there any association between the risk factors and presence of *L.pneumophila*?

OBJECTIVES

1. To determine the occurrence of *L.pneumophila* among patients admitted at Kenyatta National Hospital medical ward with signs and symptoms suggestive of pneumonia.
2. To determine possible risk factors for those who are infected with *L.pneumophila.*
3. To determine the association between risk factors and infection with *L. pneumophila.*

JUSTIFICATION

Early initiation of appropriate therapy has always been associated with improved outcome. Discovery of even a single case would be an important sentinel of other undiscovered cases. Using the data obtained from questionnaires possible risk factors or common variables can be determined from those who test positive.

Very few studies have explored this pathogen in Africa. Only two studies have been done in Kenya so far. Phakkey et al[59] in 1990 used micro agglutination to investigate the presence of antibodies against *L.pneumophila* in animals and the second, unpublished data by Dr. Revathi et al which investigated the presence of *Str. Pneumoniae* and *L. pneumophila* antigens in urine of patients and blood donors at Aga Khan Hospital, Nairobi. This was going to be the 3rd study in Kenya and the first at Kenyatta National Hospital.

Although legionella culture is usually accepted as the gold standard for laboratory diagnosis, legionellosis is rarely proved by culture due to the difficulty in obtaining bronchioalveolar lavage fluid. Presence of the antigen in the urine of legionellosis patients was noted way back in 1976 during the Philadelphia outbreak. Over the last few years urinary antigen has been increasingly used as an in-house monitoring tool for legionellosis and is without doubt a primary diagnostic tool. Studies of healthy adults and children have failed to detect any antigen in their urine. Unlike broncho-alveolar lavage (BAL) for culture or blood for antibody detection, urine is obtained in a non-invasive way and is therefore a suitable specimen for a study survey. Furthermore the test used gives rapid results.

The findings of this study are expected to raise the index of suspicion among clinicians treating pneumonia patients. They could guide policy makers in making recommendations on the need for routine inspection and maintenance of hot water systems and air conditioners. Knowledge of the aetiological agent of pneumonia in a patient will influence the choice of antibiotics for treatment.

CHAPTER THREE

STUDY METHODS

Study Design

A cross-sectional descriptive study was carried out. The study entailed consecutive sampling of patients admitted with a provisional diagnosis for pneumonia and investigating for the presence or absence of infection with legionella. Their demographic and clinical data was also evaluated.

Study Site

The study was conducted at the medical ward of Kenyatta National Hospital, a university affiliated hospital located in Nairobi, Kenya. It is a primary and secondary health care facility. Analysis of samples was done at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) serology laboratories.

Study Population

The study population comprised of patients admitted with pneumonia at the medical ward of the hospital. Urine samples were obtained from patients who consented to the study. Demographic data and other vital patient details were obtained using questionnaires and patient files.

Inclusion Criteria

- All adult patients (above18 years), hospitalized due to initial presumptive diagnosis of pneumonia were eligible for the study.
- The patients who consented to the study were recruited and urine samples obtained within three days of admission.

Exclusion Criteria

- Any patient who had been admitted for more than three days was excluded.
- Any patient who had been admitted at another hospital then transferred to Kenyatta National hospital was excluded.
- Children who were admitted due to pneumonia were not included in the study.
- Patients who declined to give their consent were excluded.

Sampling Method

All patients who were admitted at the medical ward with a provisional diagnosis for pneumonia and satisfied the inclusion criteria were recruited into the study by consecutive sampling. This entailed sampling every patient who met the defined eligibility criteria until the predetermined sample size was achieved.

Sample Size

A total of 120 patients were selected for the study as estimated using the formular by Fisher et al[64] sample size calculation.

$N= z^2pq/d^2$

Where z is the desired sample size, p is the prevalence of legionella infection estimated at 8.7%, based on a study done in South Africa[58], q is 1-p and d is the confidence limit of the prevalence which is 0.05. This represented the desired confidence interval for the study.

Seventeen patients admitted within the same ward but due to illnesses other than pneumonia were also included as negative controls for the urine assay.

Data Collection

A structured questionnaire written in English was administered to collect demographic and qualitative data from study subjects. Patients who did not understand English were questioned in Kiswahili. One research assistant assisted in administering the questionnaires. Information obtained from the medical records was filled out on the clinical data forms. The laboratory results for each patient were recorded on the questionnaire.

Laboratory Methods

Specimen Collection- Urine samples were collected in standard sterile containers and transported to the laboratory within one hour. The urine was alliquoted into smaller

sterile containers and frozen at -20°C. Before processing the urine was thawed and warmed to 37°C using a water bath.

The urine was then analyzed for the presence of *L. pneumophila* urinary antigen using ELISA according to the manufacturer's (Binax) [68] instructions.

The kit that was used was the Binax Legionella Urinary Antigen Enzyme Immunoassay. It is a test system intended for in vitro diagnostic use to qualitatively detect the presence of *Legionella pneumophila* serogroup 1 antigen. It uses the microtiter ELISA methodology for the detection of soluble antigen in urine from patients with with *Legionella pneumophila* serogroup 1 infections.

Materials provided in the kit included:

- Microtitre Wells- Each kit contains 96 micotitre wells, coated with purified rabbit anti-*L. pneumophila* serogroup 1 IgG.
- Wash Concentrate- One vial with 40ml of 0.1 M phosphate buffered saline, detergent and preservative. The contents were diluted to 400ml with distilled water.
- Positive control urine- One vial with 2ml of human urine containing *L. pneumophila* serogroup 1 antigen and preservative.
- Negative control urine- One vial with 2ml of normal human urine and preservative.
- HRP Conjugate- One vial with 15ml of purified rabbit anti-*L. pneumophila* serogroup1 IgG conjugated to horseradish peroxidase (HRP) in a Tris buffer with a protein stabilizer.
- Colour Developer- One vial with 25ml of chromogenic substrate solution containing tetramethylbenzidine (TMB) and hydrogen peroxide.
- Stop solution- One vial with 10ml of 1N H_2SO_4.

Materials required but not provided in the kit included:

- Pipettors and pipets that can accurately deliver 50, 100, 200, 250 ul volumes.
- Microtiter plate reader at 450nm.
- Graduated cylinder to dilute wash concentrate to 400ml.
- Stock bottle to store diluted wash solution.
- Distilled or deionised water to dilute wash concentrate.

Principle of the Test- The strips of a microplate are coated with polyclonal rabbit antibody which reacts with *L. pneumophila* antigen. Patient urine was added to the wells of the microplate and any legionella antigen present bound to the specific antibody at the solid phase. Following the first incubation, the wells were washed and a peroxidase-labelled antibody, which reacts with *L.pneumophila* antigen, was added and it binds to free binding sites on the antigen during a second incubation. After a further washing stage, the presence of bound peroxidase was demonstrated in a colour reaction with a substrate. The reaction was stopped by adding sulphuric acid and the optical density measured with a spectrophotometer at 450nm.

The performance characteristics of this test have been evaluated by the manufacturer[68]. It has a sensitivity of 97.7% and a specificity of 100%.

Assay Procedure:

1. Reserve well A1 for substrate blank.
2. Pipette 100ul of well mixed positive control, negative control and patient specimens into the appropriately labeled wells.
3. Pipette 100ul of rabbit anti- legionella HRP conjugate into all previously pipetted wells, except A1.
4. Gently tap the plate to mix the reagents within wells, being careful to avoid splashing and cross-contamination of wells.
5. Incubate at room temperature (20-25°C) for 2 hours.
6. After incubation, aspirate sample and conjugate mixture from wells.
7. Add 250ul per well of wash solution. Aspirate. Repeat wash steps for a minimum of three washes.
8. Pipet 200ul of colour developer to all previously pipetted wells, including A1 (substrate blank).
9. Incubate in the dark at room temperature (20-25°C) for 15 minutes. Covering the plate is sufficient.
10. After incubation, stop colour development by pipetting 50ul of stop solution to each previously pipetted well, including A1.
11. Gently tap plates to mix reagents. The plates should appear yellow.Green colour indicates insufficient mixing.

12. Read absorbances immediately at 450nm on a microtitre plate reader, blanking against well A1 (substrate blank).

Calculation and Interpretation of Results:

An example of a layout of the micotitre plate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B												
C												
D												
E												
F												
G												
H												

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

The microtitre plate above shows the layout of positive and negative controls, non-pneumonia patient controls and sample distribution.

The absorbance of well A1 (substrate blank) was subtracted from the absorbance of each well, to obtain a corrected absorbance for use in the calculation of patient results.

Calculation of mean absorbance:

The mean absorbance of the duplicate positive control urine and negative control urine was determined by:

Mean absorbance = $\frac{\text{Absorbance (1) + Absorbance (2)}}{2}$

Calculation of RATIO to Negative:

The ratio of the positive control urine absorbance to the negative control urine absorbance, and of patient urine absorbance to negative control urine absorbance was determined by:

$$\text{RATIO} = \frac{\text{Mean positive control or Patient urine absorbance}}{\text{Mean negative control absorbance}}$$

Interpretation:

Urine samples that had a RATIO value greater than or equal to 3 were considered positive for the presence of *L. pneumophila* serogroup 1 antigen.

Urine samples that had a ratio value less than 3 were considered negative.

Quality Control

- Laboratory data was generated using the proper protocol and standards to ensure effectiveness.
- Positive and Negative urine controls supplied in the kit were run in duplicate in every batch of tests.
- The average absorbance of all negative controls fit the required criteria of an absorbance of less than or equal to 0.100.
- The average absorbance of all positive controls also fit the required criteria of greater than or equal to three times that of the negative controls.
- Urine samples from 17 patients admitted in the medical ward for conditions other than pneumonia were also included as controls. All of them tested negative.
- Coding of data was done accurately
- All samples were obtained within three days of admission.

Data Analysis

A computer based file was developed using SPSS. The results were then presented in descriptive statistics using frequency tables, cross tabulation and pie charts. Frequencies of various parameters were obtained. Chi square and Fishers' Exact tests for significance

were used to analyze the risk factors. The level of significance for this study had been set at 0.05.

Minimizing of errors and biases

The research assistant was trained on the contents of the questionnaire, aim of the study and how to fill the questionnaires, as the principle investigator supervised collection of Data. Laboratory data was generated using the proper protocol and standards to ensure effectiveness. Coding of data was done accurately.

Ethical Issues

Informed consent was obtained from the participants. Approval to carry out the study was obtained from the Kenyatta National Hospital Research and Ethics Committee, and the Chairman of the Department of Internal Medicine, Kenyatta National Hospital. The approval was on the agreement that participants anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. The clinicians were notified of positive findings for consideration during treatment.

Study Limitations

Some of the patients had difficulties in recalling or expressing information.

The researcher would have liked to expand the study area to include patients from other hospitals but due to limited time and resources, this was not possible.

CHAPTER FOUR

RESULTS

This chapter reports on the study findings based on quantitative and qualitative data obtained from 120 patients using a structured questionnaire as well as from laboratory analysis of samples. Laboratory analysis was carried out at the University of Nairobi's Institute of Tropical and Infectious Diseases serology laboratories.

1. Laboratory Results

The samples were processed in a total of three batches.

1. First run- 21 samples

Binax Legionella Urinary Antigen Microplate 1: **Sample distribution**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	4	12	20								
B	Neg	5	13	21								
C	Neg	6	14	C1								
D	Pos	7	15	C2								
E	Pos	8	16									
F	1	9	17									
G	2	10	18									
H	3	11	19									

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

Total number of patient samples processed- 21

Controls used-kit positive controls-2

-kit negative controls-2

-non-pneumonia patient controls-C1-2.

Binax Legionella Urinary Antigen Microplate 1: Optical Densities at 450nm corrected with blank.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.095	0.277	0.217	0.162								
B	0.101	0.149	0.205	0.182								
C	0.105	0.208	0.228	0.232								
D	2.51	0.150	0.212	0.196								
E	2.172	0.231	0.365									
F	0.154	0.291	0.193									
G	0.24	0.176	0.127									
H	0.152	0.268	0.133									

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

Mean negative absorbance- 0.103. Fits required criteria of less than 0.100

Mean positive absorbance- 2.341. Fits required criteria of >3 times negative control absorbance.

Binax Legionella Urinary Antigen Microplate 1: **Calculated Ratio Values**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	2.69	2.11	1.57								
B	1.0	1.45	1.99	1.77								
C	1.0	2.02	2.21	2.25								
D	24.36	1.45	2.06	1.09								
E	21.09	2.24	3.54									
F	1.49	2.83	1.87									
G	2.33	1.71	1.23									
H	1.47	2.60	1.29									

Key

Positive for *L.pneumophila* antigen
Negative for *L. pneumophila* antigen
Empty wells

Ratio Values = Mean positive control OR Patient sample absorbance
Mean negative control absorbance

Interpretation: >3 presumptive positive for the presence of *L. pneumophila* serogroup 1 antigen in urine, suggesting current or past infection.

<3 presumptive negative for *L. pneumophila* serogroup 1 antigen in urine, suggesting no recent or current infection.

Positive samples: E3- 1 sample- Sample 16

2. Second Run

Binax Legionella Urinary Antigen Microplate 2: **Sample distribution**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	24	30	C9								
B	Neg	25	C6	C10								
C	Neg	C4	31	C11								
D	Pos	26	32	35								
E	Pos	27	C7									
F	22	C5	33									
G	23	28	34									
H	C 3	29	C8									

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

Binax Legionella Urinary Antigen Microplate 2: **Optical Densities at 450nm corrected with blank.**

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.012	0.113	0.265	0.178								
B	0.104	0.08	0.289	0.113								
C	0.10	0.084	0.257	0.13								
D	1.099	0.113	0.201	0.147								
E	1.065	0.109	0.167									
F	0.090	0.091	0.2									
G	0.064	0.123	0.049									
H	0.06	0.058	0.034									

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

Mean negative absorbance- 0.102. Fits required criteria of less than 0.100

Mean positive absorbance-1.082. Fits required criteria of >3 times negative control absorbance.

Binax Legionella Urinary Antigen Microplate 2: **Calculated Ratio Values**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1.10	2.59	1.75								
B	1.0	0.78	2.83	1.11								
C	1.0	0.82	2.52	1.27								
D	10.77	1.11	1.97	1.44								
E	10.44	1.07	1.63									
F	0.88	0.892	1.96									
G	0.63	1.20	0.48									
H	0.59	0.57	0.33									

Key

Positive for *L.pneumophila* antigen
Negative for *L. pneumophila* antigen
Empty wells

Ratio Values = Mean positive control OR Patient sample absorbance

Mean negative control absorbance

Interpretation: >3 presumptive positive for the presence of *L. pneumophila* serogroup 1 antigen in urine, suggesting current or past infection.

<3 presumptive negative for *L. pneumophila* serogroup 1 antigen in urine, suggesting no recent or current infection.

Positive samples: None

3. Third Run

Binax Legionella Urinary Antigen Microplate 3: **Sample distribution**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	39	46	53	60	68	76	84	C16	98	106	114
B	Neg	40	47	54	61	69	77	85	91	99	107	115
C	Neg	C12	48	55	62	70	78	86	92	700	108	116
D	Pos	41	49	56	63	71	79	87	93	101	109	117
E	Pos	42	C13	57	64	72	80	C15	94	102	110	118
F	36	43	50	58	65	73	81	88	95	103	111	C17
G	37	44	51	59	66	74	82	89	96	104	112	119
H	38	45	52	C14	67	75	83	90	97	105	113	120

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

Binax Legionella Urinary Antigen Microplate 3: **Optical Densities at 450nm corrected with blank.**

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.079	0.043	0.045	0.043	0.079	0.087	0.053	0.065	0.052	0.129	0.153	0.026
B	0.045	0.089	0.082	0.049	0.099	0.112	0.231	0.081	0.073	0.131	0.098	0.039
C	0.054	0.06	0.06	0.038	0.074	0.178	0.148	0.083	0.406	0.13	0.125	0.13
D	1.618	0.085	0.043	0.072	0.072	0.167	0.061	0.19	0.127	0.094	0.247	0.054
E	1.689	0.111	0.049	0.082	0.082	0.106	0.06	0.091	0.057	0.077	0.123	0.083
F	0.043	0.049	0.069	0.08	0.075	0.077	0.088	0.122	0.104	0.084	0.243	0.043
G	0.051	0.061	0.084	0.217	0.106	0.089	0.083	0.311	0.105	0.077	0.061	0.049
H	0.1	0.083	0.053	0.064	0.06	0.081	0.072	0.067	0.062	0.138	0.12	0.052

Key:

Negative controls
Positive controls
Pneumonia patient samples
Non-pneumonia patient samples
Empty wells

Mean negative absorbance- 0.045. Fits required criteria of less than 0.100

Mean positive absorbance-1.65. Fits required criteria of >3 times negative control absorbance.

Binax Legionella Urinary Antigen Microplate3: **Calculated Ratio Values**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	0.8	0.91	0.87	1.59	1.76	1.07	1.3	1.15	2.6	3.09	0.52
B	1.0	1.79	1.66	0.98	2.0	2.24	5.13	1.64	1.48	2.64	1.98	0.78
C	1.0	1.21	1.21	0.77	1.49	3.59	2.98	1.67	8.20	2.62	2.5	2.62
D	32.7	1.71	0.87	1.45	1.45	3.3	1.21	4.22	2.5	1.89	5.48	0.9
E	32.7	2.24	0.98	1.66	1.66	2.1	1.2	1.84	1.15	1.55	2.48	1.67
F	0.8	0.98	1.39	1.63	1.51	1.56	1.77	2.46	2.10	1.69	4.9	0.86
G	1.1	1.23	1.69	4.38	2.14	1.79	1.67	6.91	2.14	1.15	1.23	0.98
H	2.02	1.69	1.07	1.29	1.21	1.63	1.45	1.35	1.24	2.7	2.42	1.05

Key

Positive for *L.pneumophila* antigen
Negative for *L. pneumophila* antigen
Empty wells

Ratio Values = Mean positive control OR Patient sample absorbance

Mean negative control absorbance

Interpretation: >3 presumptive positive for the presence of *L. pneumophila* serogroup 1 antigen in urine, suggesting current or past infection.

<3 presumptive negative for *L. pneumophila* serogroup 1 antigen in urine, suggesting no recent or current infection.

Positive samples: 10 samples- Samples 59, 70, 71, 77, 87, 89, 92, 106, 109, 111.

Summary

- 11 out of a total of 120 respondents tested positive for *L. pneumophila* serogroup 1 antigen in their urine.
- All 17 non- pneumonia patients tested negative for the antigen.
- All positive and negative kit controls fitted the required criteria.

2. Statistical Analysis:

1.Socio- Demographic and Clinical factors

Table 1: Summary of Demographic, Socio-economic and Clinical Characteristics of respondents

N= 120

Variable		Frequency	Percentages (%)
Gender:	Female	47	39.2
	Male	73	60.8
Age:	Mean	38.68	
Work Area:	N/A	31	25.8
	Outdoors	27	22.5
	Indoors	62	51.7
Air Conditioners:			
	Exposed	31	25.8
	Unexposed	89	74.2
Residence:	Urban	85	70.8
	Rural	35	29.2
Piped Hot water:			
	Exposed	66	55
	Unexposed	54	45
Alcohol:	Consumes	57	47.5
	Stopped <1year prior	19	15.8
	Never Consumed	44	36.7
Smoking:	Smokes	40	33.3
	Stopped <5years prior	15	12.5
	Never Smoked	65	54.2
Admission Period prior to Sample Collection:			
	1 day	41	34.2
	2 days	39	32.5
	3 days	40	33.3
HIV Status:	Positive	75	62.5
	Negative	43	35.8
	Unknown	2	1.7
History of past/ concurrent Respiratory Illness (Pneumonia, TB)			
	Positive	70	58.3
	Negative	50	41.7

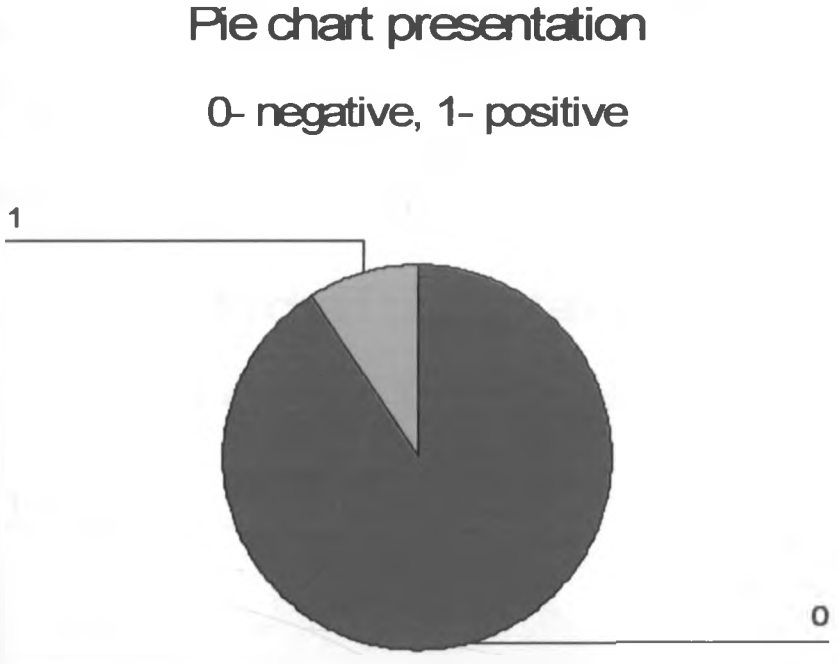
2. Proportion of Legionella positive patients

Table 2: Proportion of *L. pneumophila* positive pneumonia patients

N = 120

Test Result	Frequency of *L. pneumohila* infection	Percentage of *L. pneumophila* infection
Negative	109	90.8
Positive	11	9.2
TOTAL	120	100

1. Figure 1: Proportion of L. pneumophila positive patients.



The findings indicated that 9.2% of the pneumonia patients recruited in the study tested positive for the presence of *L. pneumophila* serogroup 1 urinary antigen.

3. Occurrence of *L. pneumophila* and its association with risk factors

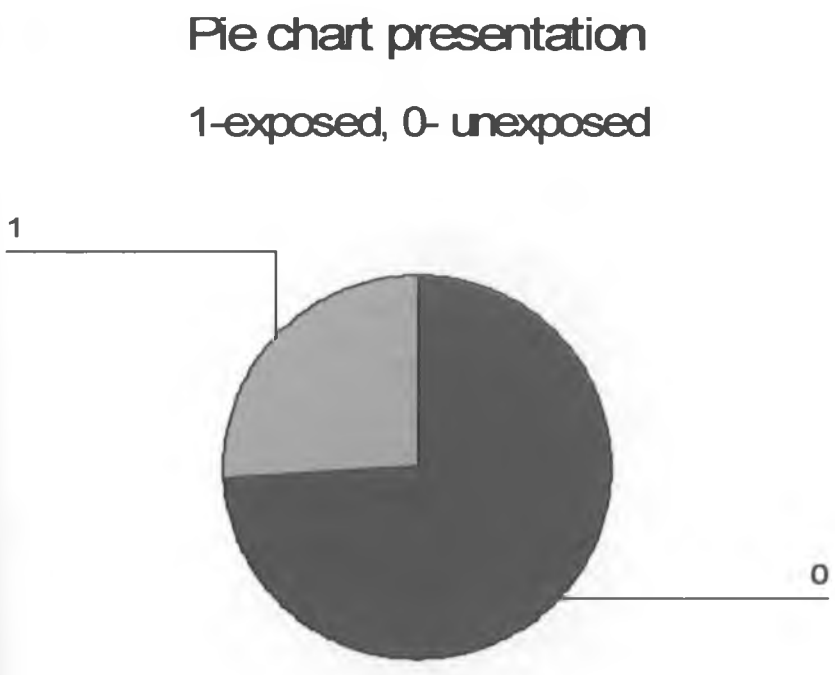
1. Air Conditioners

Table 3: Exposure to air conditioners

N = 120

L.pneumophila test	Exposure to air conditioners		Total
	Exposed	Not Exposed	
Negative	85	24	109
Positive	4	7	11
Total	89	31	120

Figure 2: Exposure to air conditioners



Of all pneumonia patients, 25.8% were exposed to air conditioners and of these 22.58% were positive for *L. pneumophila* urinary antigen.

There was statistical significance in the number of pneumonia patients infected with *L. pneumophila* and exposure to air conditioners (P= 0.003).

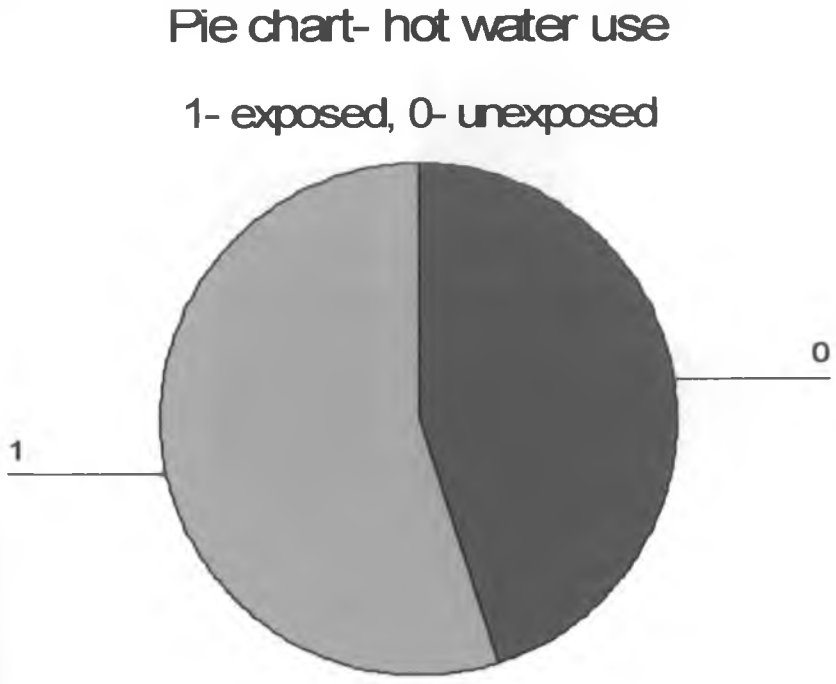
2. Piped Hot Water

Table 4: Exposure to Piped Hot Water

N = 120

L. pneumophila urinary antigen test	Exposure to piped hot water		Total
	Exposed	Not Exposed	
Negative	50	59	109
Positive	4	7	11
Total	54	66	120

Figure 3: Use of Piped hot water



Among the pneumonia patients 55% were exposed to piped hot water systems and of these, 10.6% were positive for *L. pneumophila* urinary antigen There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and exposure to piped hot water systems (p = 0.546).

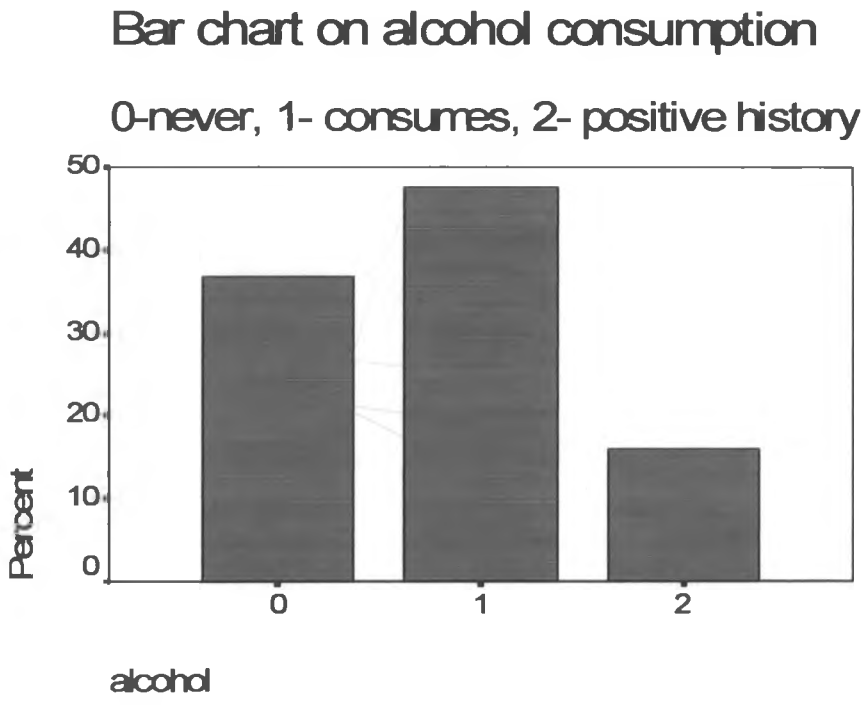
4. Alcohol Consumption

Table 5: Consumption of Alcohol

N = 120

Alcohol / *L. pneumophila*	Never Consumed	Currently Consumes	Stopped <1 year prior	Total
Negative	42	50	17	109
Positive	2	7	2	11
Total	44	57	19	120

Figure 4: Alcohol Consumption



Among the respondents 47.5% currently consume alcohol and of these, 12.3% were positive for *L. pneumophila* urinary antigen.

Out of the respondents 12.8% stopped consuming alcohol<1year prior to admission due to pneumonia and of these 10.5% were positive for *L. pneumophila* urinary antigen. 36.7% of all respondents have never consumed alcohol and of these, 4.5% were positive for *L. pneumophila* urinary antigen.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and consumption of alcohol ($p = 0.4$).

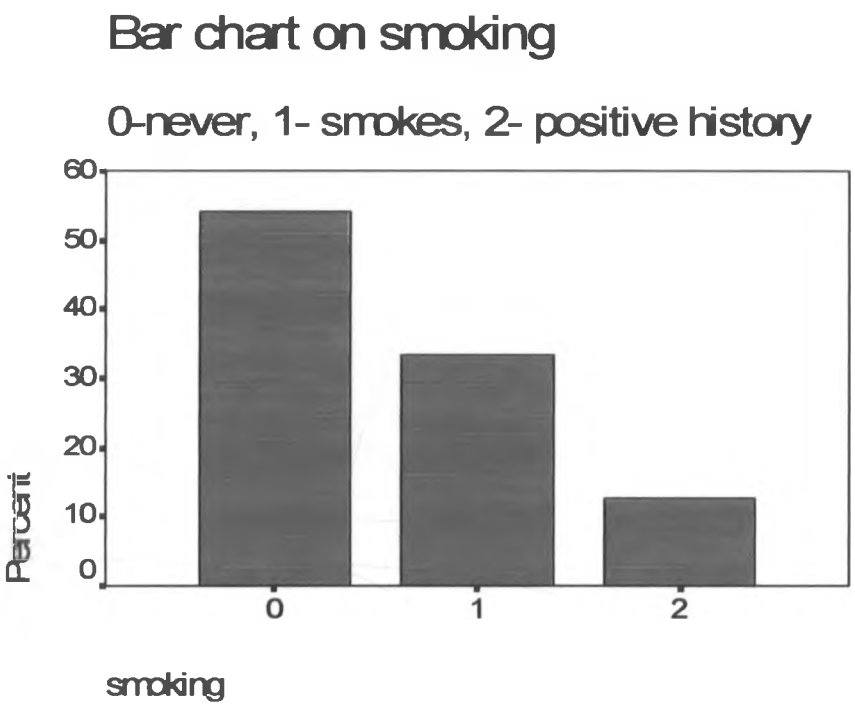
5. Smoking

Table 6: History of Smoking

N = 120

Smoking / *L. pneumophila*	Never Smoked	Currently Smokes	Stopped <5 years prior	Total
Negative	60	34	15	109
Positive	5	6		11
Total	65	40	15	120

Figure 5: Smoking of cigarettes



Thirty three percent of all respondents currently smoke and of these, 15% were positive for *L. pneumophila* urinary antigen.
Twelve percent of all respondents stopped smoking <5 years prior to admission and of these, none were positive for *L. pneumophila* urinary antigen.
Fifty four percent of all respondents have never smoked and of these, 7.7% were positive for *L. pneumophila* urinary antigen.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and history of smoking (P= 0.190).

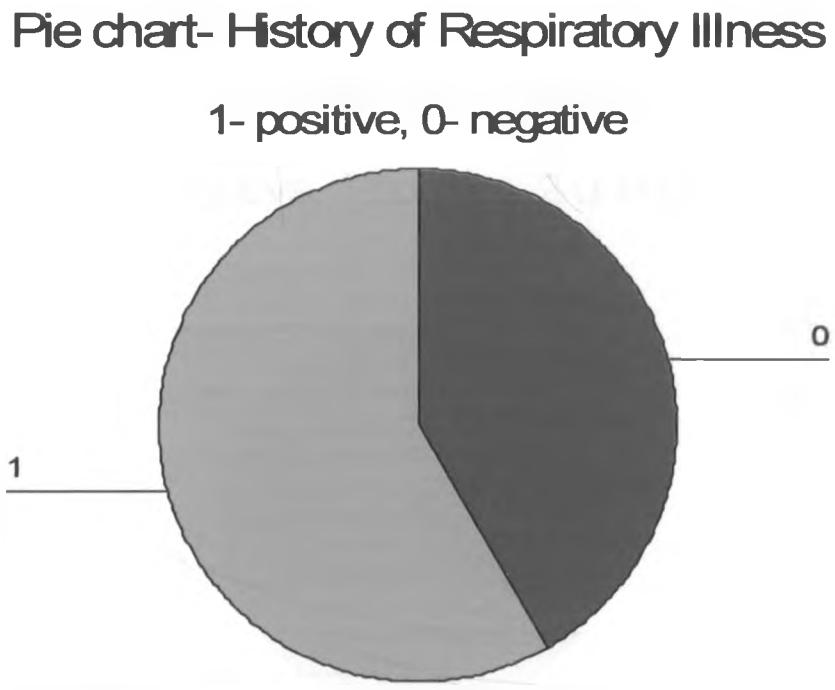
History of Respiratory Illness

Table 7: History of Respiratory Illness

N = 120

H_x of Illness / *L. pneumophila*	Positive	Negative	Total
Negative	60	49	109
Positive	10	1	11
Total	70	50	120

Figure 6: Respiratory Illness



Fifty eight percent of all respondents have a history of past or concurrent respiratory illness, and of these 14.2% were positive for *L. pneumophila* urinary antigen.

There was statistical significance in the number of pneumonia patients infected with *L. pneumophila* and a history of a past or concurrent respiratory illness (P= 0.021).

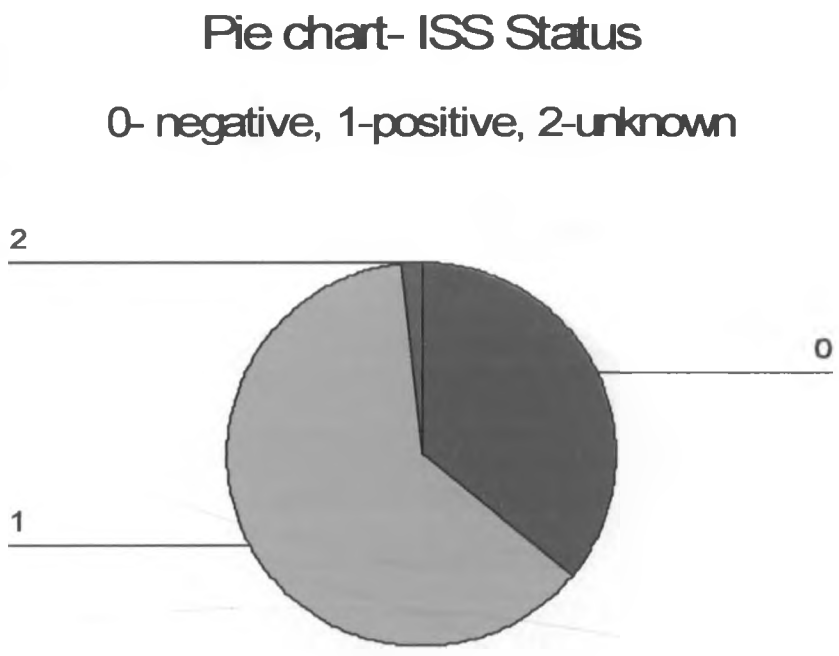
6. HIV Status

Table 8: HIV Status

N = 120

HIV status / *L. pneumophila*	Negative	Positive	Unknown	Total
Negative	41	66	2	109
Positive	2	9		11
Total	43	75	2	120

Figure 7: HIV Status



Sixty two percent of all respondents were HIV positive and of these, 12% were positive for *L. pneumophila* urinary antigen.

Thirty five percent of all respondents were HIV negative and of these 4.6% were positive for *L. pneumophila* urinary antigen.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and HIV status (p= 0.577).

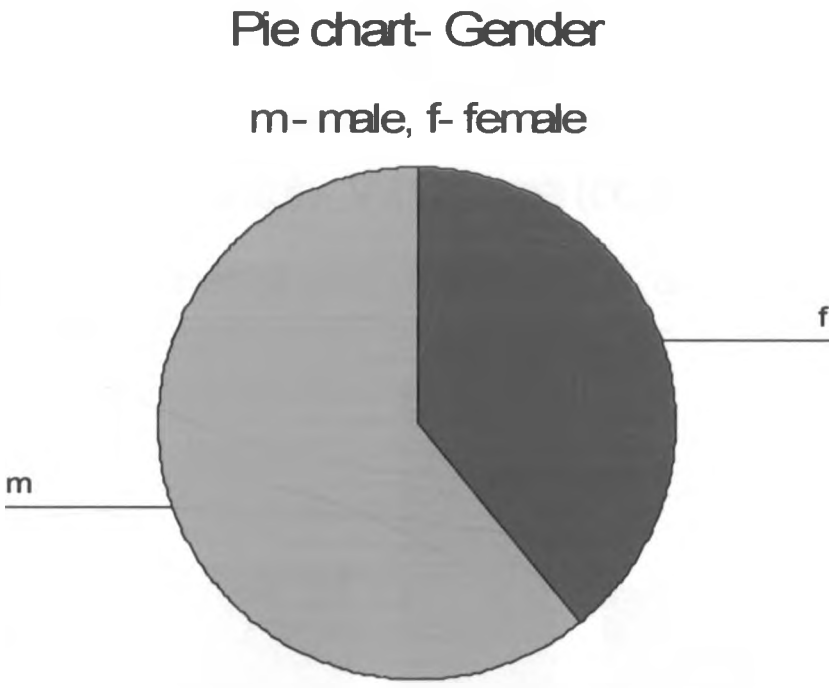
8. Gender

Table 9: Gender of Respondents

N = 120

Gender / *L.pneumophila*	Female	Male	Total
Negative	45	64	109
Positive	2	9	11
Total	47	73	120

Figure 8: Gender of respondents



Sixty percent of all respondents were male and of these, 12.3% were positive for *L. pneumophila* urinary antigen while 4.2% of the females were positive.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and gender. (p = 0.135).

9. Age

The mean age of respondents was 38 years. There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and age. (p = 0.492).

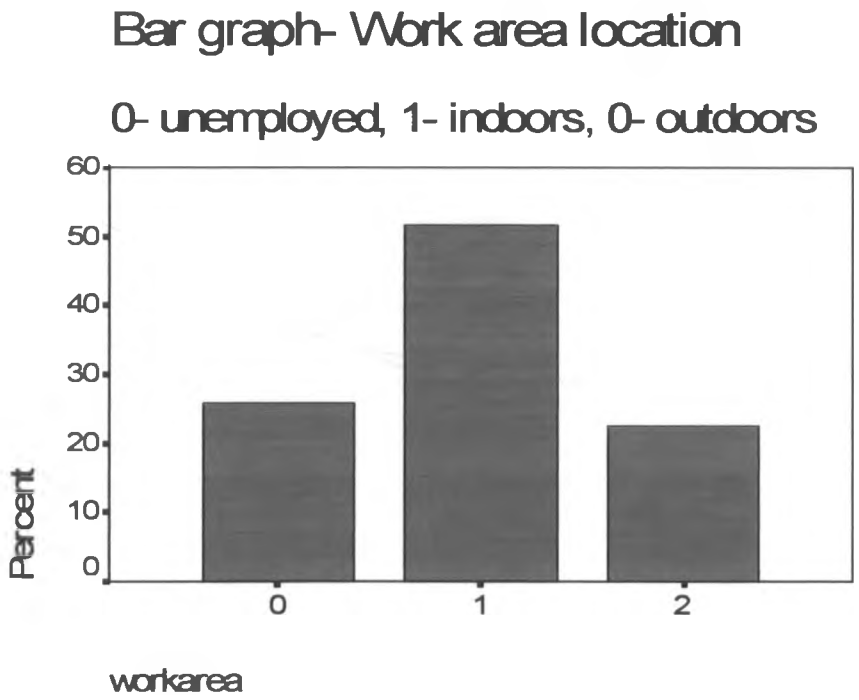
10. Employment/ Work Area

Table 10: Employment or Work Area

N = 120

Work area / *L. pneumophila*	Unemployed	Outdoors	Indoors	Total
Negative	31	25	53	109
Positive		2	9	11
Total	31	27	62	120

Figure 9: Work Area Location



Fifty one percent of total respondents work outdoors and of these, 7.4% were positive for *L. pneumophila* urinary antigen.

Twenty two percent of total respondents work indoors and of these, 14.5% were positive for *L. pneumophila* urinary antigen.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and their place of work. ($p = 0.069$).

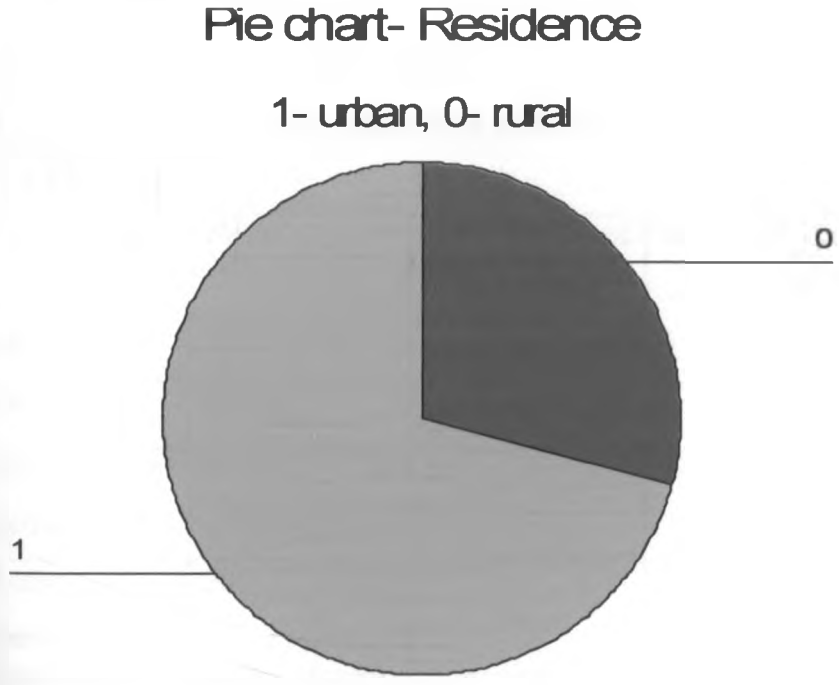
11. Residence

Table 11: Area of residence

N = 120

Residence / *L. pneumophila*	Rural	Urban	Total
Negative	34	75	109
Positive	1	10	11
Total	35	85	120

Figure 10: Residence



Seventy percent of all respondents resided in an urban setting and of these, 11.77% were positive for *L. pneumophila* urinary antigen.

Twenty nine percent of all respondents resided in a rural setting and of these, 2.8% were positive for *L. pneumophila* urinary antigen.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and area of residence. (p = 0.124).

12. Air Conditioners and Work Area

Table 12: Exposure to Air Conditioners and the Work Place

N = 120

Workplace / Air conditioners	Unemployed	Indoors	Outdoors	Total
Negative	28	37	24	89
Positive	3	25	3	31
Total	31	62	27	120

Thirty eight percent of those who work indoors are exposed to air conditioners.

There is a statistical significance between exposure to air conditioners and location of work area (p = 0.001)).

13. Alcohol Consumption and Smoking

Table 13: Alcohol Consumption and Smoking

N = 120

Smoking / Alcohol	Never	History of smoking	Active smoker	Total
Never	37	2	5	44
History of alcohol	8	6	5	19
Active consumer	20	7	30	57
Total	65	15	40	120

There is a statistical significance between alcohol consumption and smoking (p = 0.000)

Consumption of alcohol and smoking has a likely increased predisposition to *L. pneumophila* infection.

3. Summary of association between risk factors and occurrence of *Legionellae.*

Table 2: Occurrence of *L. pneumphila* and its association with Risk factors

Variable	Calculated p value
Air conditioners	0.003*
Piped Hot Water	0.546
Alcohol	0.4
Smoking	0.190
History of past/ concurrent Respiratory Illness	0.021*
ISS Status	0.577
Gender	0.135
Age	0.492
Employment/ Work area	0.069
Residence	0.124
Air conditioners and Work Area	0.001*
Alcohol and Smoking	0.000*

- The frequencies of Socio Demographic factors obtained in Table one above was cross-tabulated with the occurrence of *L. pneumophila* to test for possible association.
- Fishers Exact Test and Chi square tests for significance were used.
- The study had been set within a 95% confidence interval. The level of significance was 0.05.
- *- Indicates the variables that showed possible association between their occurrence and infection with *L. pneumophila.*

CHAPTER FIVE

DISCUSSION

This study revealed that 9.2% of pneumonia patients admitted at the medical ward of Kenyatta National Hospital between March and June were infected with *L. pneumophila* serogroup 1. These findings correlate with those of a study done in South Africa[58] on atypical causes of community-acquired pneumonia in which 8.7% of pneumonia patients tested positive for *L. pneumophila.* A serological study conducted in Zambia[57] revealed that 10% of the pneumonia patients had been exposed to *L. pneumophila* although a further study failed to indicate an increase in antibody titre.

Studies done elsewhere have shown that some populations have an increased risk to developing severe legionella infections. Some of the risk factors for community-acquired and travel-associated legionellosis include: being a male, the elderly (>65years), cigarette smokers, history of heavy drinking, pulmonary related illnesses, immunosuppresion, and chronic debilitating illnesses e.g. hematological malignancies[36].

According to this study, 22.58% of the pneumonia patients who were exposed to air conditioners tested positive for *L. pneumophila.* A dirty air filter can harbor pollen, fungi, and bacteria and allow microorganisms into the room, possibly triggering an asthma attack, irritation of the eyes, nose, and throat - even flu like illness. Air conditioning systems have been documented as one of the man-made habitats of *Legionellae*.Thirty eight percent of those who work indoors in this study are exposed to air conditioners. The study also showed statistical significance between exposure to air conditioners and infection (p= 0.003), as well as between those who work indoors and exposure to air conditioners (p= 0.001). Other studies have also shown that contact with contaminated aerosol systems (like infected air conditioning systems) is associated with pneumonia due to *Legionellae*[4]. Air conditioners in large buildings can pose a more serious threat because they use reservoirs of water that can harbor harmful bacteria. Air filters should be vacuumed periodically and washed with a disinfectant to prevent mildew. The filter

should be left to dry completely before reinstalling, while disposable filters should be replaced at recommended intervals.

Hot water systems have also been shown to be man-made habitats of *L. pneumophila.* The bacteria are more easily detected from swab samples of biofilm than from flowing water, suggesting that the majority of *Legionellae* are biofilm associated[27]. Aerosolization or aspiration of contaminated water is a major route of transmission. However there was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and exposure to piped hot water systems (p= 0.546). This could be explained by the fact that most patients who confirmed using hot water only did so at their places of work, for washing hands or teacups. Most of them did not have hot water systems at home for showering, which would probably be a major source of infection.

The study also showed statistical significance between a history of respiratory illness and infection with *L.pneumophila* (p= 0.021). History of respiratory illness in this study included a past history of pneumonia and tuberculosis. Fifty eight percent of all respondents have a history of past or concurrent respiratory illness, and of these 14.2% were positive for *L. pneumophila* urinary antigen. Pulmonary related illnesses weaken the immune system thereby weakening the body's ability to fight of infection[62]. Patients with defective immune systems are susceptible to *Legionellae* infection, especially when the defect involves cell-mediated immunity. Patients who have the Human Immunodeficiency Virus may be at risk for relapsing infections[61]. In this study 61.7% of all respondents were HIV positive and of these 12% were positive for *L. pneumophila* urinary antigen.

Fourty seven percent of all respondents were active consumers alcohol and of these, 12.3% were positive for *L. pneumophila* urinary antigen. Studies have shown that excessive alcohol consumption can contribute to contraction of community-acquired pneumonia. The increased risk of suffering from pneumonia in alcoholic patients exists due to the fact that the activity of their immune system decreases. Alcohol acts as a

sedative and can diminish the reflexes that trigger coughing and sneezing. It also interferes with the action of macrophages. A study conducted at Lousiana State University Health Sciences Center involving mice showed that it suppresses an immune system protein- interleukin- 17 but its effect in humans is yet to be confirmed[63]. Although this study showed that there was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and consumption of alcohol (p= 0.4), the high percentage of a positive history of alcohol consumption among pneumonia patients in general is worth investigating increased risk. The amount of alcohol consumed may also be a factor in its effect on the immune response of an individual.

The study found that 33.3% of the pneumonia patients were exposed to cigarette smoking. Just like alcohol, smoking is also known to interfere with the pulmonary immune response. Other studies have shown that chronic exposure to cigarette smoke can result in injury to the airways and damage of the cilia. It alters the efficiency of their beating so that bacteria entering the trachea have an increased likelihood of entering the lungs. Although this study showed that there was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and history of smoking (p= 0.190), smoking may predispose the individual to infection with other pneumonia causing pathogens. Just like in previous studies, this study has also revealed a statistical significance between alcohol consumption and cigarette smoking (p= 0.000). The potential combined adverse effects of alcoholism and cigarette smoking on lung defenses against pathogen infection probably increases the risk of developing serious disease.

There was no statistical significance in the number of pneumonia patients infected with *L. pneumophila* and gender. (p= 0.135). Studies have shown however that the male sex is at more risk of infection with *L. pneumophila* than their female counterparts[36]. In this study out of the 11 patients who tested positive for the antigen 9 were male and 2 were female. The reasons why the male have been found to be more susceptible is yet to be established, but it could be due to more exposure to environmental pollutants based on their lifestyle.

The mean age of patients admitted due to pneumonia was found to be 38 years. This finding is significant because this falls within the productive age group, both economically and socially.

CONCLUSION

The hypothesis set at the beginning of the study that there are cases of *Legionella pneumophila* infection among pneumonia patients was approved. Among the patients recruited in the study, 9.2% tested positive for infection *Legionella pneumophila* serogroup 1.

Exposure to air conditioners and a history of past or concurrent respiratory illness have been found to predispose one to infection with the bacteria.

Most of those exposed to air conditioners are exposed at their places of work and there is need for routine inspection and maintenance.

RECOMMENDATIONS

- Exposure to air conditioners is a key predisposing factor to infection with *L.pneumophila* and this should raise the index of suspicion among clinicians as they obtain a patient's medical history.
- There is need for Public Health education on:
  - Routine inspection and maintenance of air conditioners and hot water systems
  - Habitats and possible sources of infection with *Legionella pneumophila.*
  - Socio- demographic factors increasing risk of contracting pneumonia.
- There is need for a larger multi-center study on the prevalence of infection by *L. pneumophila* in pneumonia patients (both community acquired and nosocomial), existence of co- infection and the antibiotic susceptibility of isolated organisms.
- There is a need to carry out studies on other causes of atypical pneumonia to provide information on the local epidemiological picture

REFERNCES

1. Marston, B. J., J. F. Plouffe, T. M. File, B. A. Hackman, S. J. Salstrom, H. B. Lipman, M. S. Kolezak, and R. F. Breiman. 1997. Incidence of community acquired pneumonia requiring hospitalization- results of a population based active surveillance study in Ohio. Arch. Intern. Med. 157: 1709-1718.

2. Fraser, D. W. 1984. Sources of legionellosis, pg.277- 280. A. Balows, C. Thornsberry, J. C. Feeley, and W. Jakubowski (ed.), Proceedings of the Second International Symposium on Legionella. American Society for Microbiology, Washigton, D.C.

3. Mandell LA, Wunderink RG, Anzueto A, et al. 2007. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis*.1;44 Suppl 2:S27-72.

4. Cohen J, Powderly WG. *Infectious Diseases*. 2nd ed. New York, NY: Elsevier, 2004.

5. Abu Kwaik, Y. 1996. The phagosome containing *Legionella pneumophila* within the protozoan Hartmannella vermiformis is surrounded by the rough endoplasmic reticulum. Appl. Environ. Microbiol. 62: 2022-2028.

6. Reingold, A. L, Thompson, B. M., Brake, B. J. L 1984. *Legionella pneumonia* in the United States: The distribution of serogroups and species causing human illness.J. Infect. Dis. 149-219.

7. Fields, B. S. 1996. The molecular ecology of legionellae. Trends Microbiol. 4: 286-290.

8. Fields, B. S. 1997. Legionellae and legionnaieres' disease, pg 666-675. Manual of Environmental Microbiology, vol.1. American Society for Microbiology, Washington, D. C.

9. McDade, J. E.,C. C. Shepard, D. W. Frase, T. R. Tsai, M. A. Redus, and W. R. Dowdle. 1977. Legionnaires' disease: isolation of a bacterium and demonstration of its' role in other respiratory diseases. N. Engl. J. Med. 297: 1197-1203.

10. Tatlock, H. 1944. A Rickettsia- like organism recovered from guinea pigs. Proc. Soc. Exp. Biol. Med. 57: 95-99.

11. Drozanski, W. 1956. Fatal bacterial infection in soil amoebae. Acta Microbiol. Pol. 5: 315-317.

12.Hookey, J. V., N. A. Saunders, N. K. Fry, R. J Birtles, and T. G. Harrison. 1996. Phylogeny of Legionellaceae based on small subunit ribosomal DNA sequences and proposal of *Legionellae lytica* comb. Nov. for Legionella- like amoebal pathogens. Int. J. Syst. Bacteriol. 46: 526-531.

13. Adeleke, A.A., B.S. Fields, R.F. Benson, M. I. Daneshvar, J. M. Purckler, R.M.Ratcliff, T. G. Harrison, R. S. Weyant, R. J. Birtles, D. Raoult, and M. A. Halablab.2001. *Legionellae drozanskii* sp. Nov., *Legionellae rowbothamii sp*. Nov. and *Legionellae fallonii sp*. Nov: three unusual new Legionella species. Int. J. Syst. E vol. Microbiol. 5: 1151- 1160.

14. Benson, R.F. and B. S. Fields. 1998. Classification of the genus Legionellae. Semin. Respir. Infect. 13: 90-99.

15. Lo Presti, F., S. Riffard,, H. Meugnier, M. Reyrolle, Y. Lasne, P. A. Grimont, F. Benson, D. J. Brenner,, A. G Steigerwalt, J. Etienne, and J. Freney. 2001. *Legionella*

*gresilensis sp.*nov. and *Legionella beliardenesis sp.* Nov., isolated from water in France. Int. J. Syst. E vol. Microbiol. 51: 1949-1957.

16. Lo Presti, F., S. Riffard,, H. Meugnier, M. Reyrolle, Y. Lasne, P. A. Grimont, F. Vandenessch, J. Etienne, J. Fleurette, and J. Freney. 1999. *Legionella taurinensis sp.* Nov., a new species antigenically similar to *Legionella spiritensis*. Int. J. Syst. Bacteriol. 49: 397-403.

17. Marston, B. J., H. B. Lipman,, and R. F. Breiman. 1994. Surveillance for Legionnaires ' disease. Risk factors for morbidity and mortality. Arch. Intern. Med. 154: 2417-2422.

18. Murray, P. Legionella. In Manual of Clinical Microbiology, 8th Ed. Baron, E. J: Jorgensen, J. H., Pfaller, M., Yolken, R.H. Washigton D. C., American Society for Microbiology 2000: 809-823.

19. Fliermans, C. B., W. B. Cherry, L. H. Orrison, S. J. Smith, D. L. Tison, and D. H. Pope. 1981. Ecological distribution of *Legionella pneumophila.* Appl. Environ. Microbiol. 41:9-16.

20. Steele, T. W. 1989. Legionnaires' disease in South Australia, 1979-1988. Med. J. Austr. 151: 322, 325-326, 328.

21. Ruehlemann, S. A., and G. R. Crawford. 1996. Panic in the potting shed: the association between *Legionellae longbeachae* serogroup 1 and potting soils in Australia. Med. J. Austr. 164: 36-38.

22. Katz, S. M., and J.M. Hammel. 1987. The effect of drying, heat, and ph on the survival of Legionella pneumophila. Ann. Clin. Lab. Sci. 17:150-156.

23. Hanrahan. J. P., Morse.D. L., Scharf, V. B., et al.1987. A community hospital outbreak of legionellosis: transmission by potable water. Am J Epidemiol. 125: 639-649.

24. Barry S, Fieds, Robert F. Benson, and Richard E. Besser. 2002. Legionellae and legionnaires' disease: 25 years of investigation. Clin Microbiol Rev. 15(3): 506-526.

25. Mermel, L. A., S. L. Josephson, C. H. Giorgio, J. Dempey, and S. Parenteau. 1995. Association of Legionnaires' disease with construction: contamination of potablewater? Infect. Control Hosp. Epidemiol. 16: 76-81.

26. Ratcliff, R. M.,J. A. Lanser, P.A. Manning, and M. W. Heuzenroeder. 1998. Sequence based classification scheme for the genus Legionella targeting the mip gene. J. Clin. Microbiol. 36: 1560-1567.

27. Rogers, J., and C. W. Keevil. 1992. Immunogold and fluorescein immunolabelling of Legionella pneumophila within an aquatic biofilm visualized by using episcopic differential interference contrast microscopy. Appl. Environ. Microbiol. 58: 2326-2330.

28. Joseph, C. A., Watson J. M., Harrison, T. G, and Bartlett, C. L. R. 1994. Nosocomial legionnaires' disease in England and Wales. Epidemiol. Infect.112: 329-345.

29. Rowbotham, T. J. 1980. Preliminary report finding on the pathogenicity of Legionellae pneumophila for freshwater and soil amoebae. J. Clin. Pathol. 33: 1179-1183.

30. Halele, S., R. Kohler, H. Merkert, M. Schleicher, J. Hacker, and M. Steinert. 2000.Dictyostelium discoideum: a new host model system for intracellular pathogens of the genus Legionella. Cell. Microbiol. 2: 165-171.

31. **Solomon, J. M.,A. Rupper, J. A. Cardelli, and R. R. Isberg**. 2000. Intracellular growth of Legionella pneumophila in Dictyostelium discoideum, a system for genetic analysis of host pathogen interactions. Infect. Immun. 68: 2939- 2947.

32. **Fraser, D.W., T. R. Tsai, W. Orenstein, W. E. Parkin, H. J. Beecham, R. G. Sharrar, J. Harris, G. F. Mallison, S. M. Martin, J. E. McDade, C. C. Shepard, and P. S. Brachman.** 1997. Legionnaires' disease: description of an epidemic pneumonia. N. Engl. J. Med. 297: 1189-1197.

33. **Glick, T. H., M. B. Gregg, B. Berman, G. Mallison, W. W. Rhodes, Jr., and 1. Kassanoff.** 1978. Pontiac fever. An epidemic of unknown etiology in a health department. I. Clinical and epidemiologic aspects. Am. J. Epidemiol. 107:149-160.

34. **Macfarlane, J. T., A. C. Miller, W. H. Roderick Smith, A. H. Morris, anti D. H. Rose**. 1984. Comparative radiographic features of community acquired Legionnaires' disease. pneumococcal pneumonia. mycoplasma pneumonia, and psittacosis. Thorax 39:28-33.

35. **Stout, J.E. and Yu, V.** L. 1997. Legionellosis. N. Engl. J. Med. 337: 682-687.

36. **Straus, W.L; Plouffe, J.F; File,T.M; et al.** 1996.Risk factors for domestic acquisition of legionnaries disease. Arch. Intern. Med. 156: 1685-1692.

37. **Swanson, M. S., and B. K. Hammer**. 2000. *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. Annu. Rev. Microbiol. 54:567-613.

38. **Gao, L. Y., O.S. Harb, and Y. Abu Kwaik**. 1997. Utilization of similar mechanisms by *Legionella pneumophila to* parasitize to evolutionarily distant host cells, mammalian macrophages and protozoa. Infect. Immun. 65: 4738-4746.

39. **Horwitz, M. A., and F. R. Maxfield.** 1984. Legionella pneumophila inhibits acidification of its phagosome in human monocytes. J. Cell Biol. 99:1936-1943.

40. **Horwitz, M. A., and S. C. Silverstein.** 1980. Legionnaires' disease bacterium (Legionella pneumophila) multiplies intracellularly in human monocytes J. Clin. lnvestig. 66:441-450.

41. **Bellinger-Kawahara, C., and M. A. Horwitz.** 1990. Complement component *C3* fixes selectively to the major outer membrane protein (MOMP) of *Legionella pnemophila* and mediates phagocytosis of liposome-MOMP complexes by human monocytes. J. Exp. Med. 172:1201-1210.

42. **Horwitz, M. A.** 1983. The Legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome –lysosome fusion in human monocytes. J. Exp. Med. 158: 2108-2126.

43. **Horwitz, M. A.** 1983. Formation of a novel phagosome by the Legionnaires' disease bacterium *(Legionella pneumophila)* in human monocytes. J. Exp. Med. 158:13 19-1331

44. **Gao, L. Y., and Y. Abu Kwaik.** 1999. Apoptosis in macrophages and alveolar epithelial cells during early stages of infection by *Legionella pneumophila* and its role in cytopathogenicity. Infect. Immun. 67:862-870.

45. **Hagele, S., J. Hacker, and B. C. Brand.** 1998. *Legionella pneumophila* kills human phagocytes but not protozoan host cells by inducing apoptotic cell death. FEMS Microbiol. Lett. 169:51-58.

46. **Shaechter, M; Engleberg, N.C; Eisenstein, B; Medoff, G.** Mechanisms of Microbial Disease. 3rd Edi.

47. **Feeley. J. C., G. W. Gorman, R. E. Weaver, D.C. Mackel, and H. W. Smith.** 1978. Primary isolation media for Legionnaires' disease bacterium. J. Clin. Microbiol. 8:320-325.

48. Feelev, **J. C., R. J. Gibson, G. W. Gorman, N. C. Langford, J. K.** Rasheed, D. C. **Mackel, and W. B. Baine.** 1979. Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila.* J. Clin. Microbiol. 10:37-441.

49. **Starnbach, M.** N., S. **Falkow, and L. S. Tompkins.** 1989. Species-specific detection of *Legionella pneumophila* in water by DNA amplification and hybridization. J. Clin. Microbiol. 27: 1257-1261.

50. **Jonas, D., A. Rosenbaum, S. Weyrich, and S. Bhakdi.** 1995. Enzyme-linked immunoassay for detection of PCR-amplified DNA of legionellae in bronchoalveolar

fluid. J.
Clin. Mcrobiol. 33:1247-1252.

51. **Ballard, A. L., N. K. Fry, L. Chan, S. B. Surman, J. V. Lee, T. G. Harrison, and K. J. Towner.** 2000. Detection of *Legionella pneumophila* using a real-time PCR hybridization assay. .J. Clin. Microbiol. *38:4215-4218.*

52. **Thacker, W. L., B. B. Plikaytis, and H.** V. **Wilkinson.** 1985. Identification of 22 *Legionella* species and 33 serogroups with the slide agglutination test .1. Clin. Mcrobiol. 21:779-782.

53. **Farshy, C. E., D. D. Cruce, G. C. Klein,** H. **W. Wilkinson, and J.** C. **Feeley.** 1979. Immunoglobulin specificity of the microagglutination test for the Legionnaires disease bacterium. Ann. Intern. Med. 90:690.

54. **Farshy, C. E., C. C. Klein, and J. C. Feeley.** 1978. Detection of antibodies to legionnaires disease organism by microagglutination and micro-enzyme-linked immunosorbent assay tests. J. Clin. Microbiol. 7:327-331

55. **Stanek G, Hirschl A,** Lessky F, **Wewalka F. Ruckdeschel G, Wewalka G.** 1983. Indirect immunofluorescence assay (lFA). microagglutinaqtion test (MA) and enzyme linked immunoassay (ELISA) in diagnosis of legionellosis. Zentralbl Bakteriol Mikrobiol Hyg (A). *255*(1): 108-14

56. Berdal, B. P., C. E. **Farshy, and J. C. Feeley.** 1979. Detection *of Legionella pneumophila* antigen in urine by enzy me-linked immunospecific assay. J. Clin. Microbiol. 9:575-578.

57. **M. Rolfe**. 1986. A study of legionnaries disease in Zambia. Annals of Tropical Medicine and Parasitology.80: (3) *325-328.*

58. **Maartens G, Lewis SJ, de Goveia C, Bartie C, Roditi D, Kiugman KP**.1994. A typical' bacteria are a common cause of community acquired pneumonia in hospitalized adults S Afr Med J. 84(l0):678-82.

59. **Phakkey A, Lindqvist KJ, Omland T, Berdal BP..** 1990Legionella antibodies in human and animal populations in Kenya. Acta Pathologica Microbiologica et Immunologica Scandinavica. 98(1): 43-9.

60. **Farshv CE, Cruce DE, Klein GC, Wilkinson HW, Feely JC.** Immunoglohulin specificity of the microagglutination test for the Legionnaires disease bacterium. Ann Intern Med. 1979. April; 90(4):690

61. **Pedro-Botet ML, Sopena N, Garcia-Cruz A**, et al.2007 *Streptococcus pneumoniae* and *Legionella pneumophila* pneumonia in HIV-infected patients. Scand J Infect Dis. 39(2):122-128.

62. **Schlossberg D, Bonoan J.** 1998.Legionella and Immunosuppresion. Semin Respir Infect. Jun; 13(2):128-31.

63. **Judd Shellito, Zheng Min, Ye Peng, Ruan, Sanbao, Shean, Mary K, Kolls Jay.** 2001. Effect of alcohol consumption on host release of interleuikin -17 during pulmonary infection with *Klebsiella pneumoniae.* Clinical and Experimental Research ; 25: 872-881.

64. **Fisher A, Laing J, Stoeckel J. E, et al.** Handbook for Family Planning Operations Research Design. 2nd edition. Population Council, New York.

65. **McDade , J. E.,Shepard , C. C.,Fraser, D.W.,Tsai, T.P.,Redus, M.A.,Dowdle, W.R.** Legionnaires Disease: Isolation of a bacterium and demonstration of its role in other respiratory diseases. N. Engl. J. Med. 297:1197-1203.

66. **Diane S. Lindsay, William Abraham, William Findlay, Peter Christie, Fiona Johnstone and Giles Edwards.** 2004. Laboratory Diagnosis of Legionnaires' Disease due to *L. pneumophila* serogroup 1: comparison of phenotypic and genotypic methods. J. Med. Microbiol. 53: 183-187.

67. **Daniel Jonas, Inge Engels, Dons Hartung, Jan Bayersmann, Uwe Frank and Franze D. Daschner.** 2003. Development and Mechanism of fluoroquinolone resistance in *Legionella pneumophila*. Antimicrobial Chemother.,51: 275-280.

68. Binax, Inc. 10. Southgate Road, Scarborough, Maine 04074, USA. US 1-800-257-9525. Outside US 1-609-627-8000. Fax: 207-730-5710

APPENDIX 1

CONSENT FORM

Title of the Study: A survey of the occurrence of L. pneumophila among pneumonia patients at the medical ward of Kenyatta National Hospital.

My name is Susan Odera and I am undertaking a postgraduate study at the Department of Medical Micribiology, University of Nairobi. I would like to request you to participate in a medical research study.

The objective of this study is to determine the level of infection, if any, with pneumonia causing bacteria, *L. pneumophila*. The information obtained from you will assist me in obtaining the study objectives. Any positive finding from the study will be brought to the attention of your physician and therefore better management of your condition, and will also alert policy makers on the need for routine inspection and maintenance of potable water systems and air conditioners. You will be required to give only one sample of urine.

Please understand that the following principles apply to all participants of the study:

1. Participation is entirely voluntary.
2. Your confidentiality will be safeguarded. Names of participants will not appear in any final report or publication resulting from the study.
3. Refusal to participate will involve no penalty.
4. No risks will be incurred in participating in the study.

In case of any problem or concern you may contact my supervisor or I through this number: 0723470211 or the KNH Research and Ethics Committee, P. O. Box 20273, Nairobi.

I..... have fully understood the objectives of the research and hereby show a willingness to participate in the study.

Signature..... Date.....

APPENDIX TWO

QUESTIONNAIRE

DEMOGRAPHIC DATA

Age in Completed years……..

Sex…….Male……Female……..

Occupation……

Place of Work……

Are Air Conditioners used at your work place?.....

Do you use hot water at your work place……

Residence……Rural……Urban……..

Do you have air conditioners at home?.....

What is your source of water at home?.....

Do you have: Hot Water Tanks?.....

Shower with hot water?.....

Alcohol Consumption: Do you drink? Yes…. How often per week?.....

No…… When did you stop?.....

Never drank……..

Smoking status: Do you smoke……. Yes…. How often per week?.....

No…… When did you stop?.....

Never smoked……

Have you been admitted before?.....No……

Yes……..

Where……

Why?……..

APPENDIX THREE

CLINICAL DATA

Duration of Admission.....

Duration of symptoms.....

X-Ray.....

History of Previous Antibiotics.....

Antibiotics Prescribed.....

Laboratory Investigations.....

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: "MEDSUP", Nairobi.
Email: **KNHplan@Ken.Healthnet.org**

Ref: KNH-ERC/01/3612

Date: 10th July 2006

Susan Akinyi Odera
Dept. of Medical Microbiology
Faculty of Medicine
University of Nairobi

Dear Susan

RESEARCH PROPOSAL: "A SURVEY OF *LEGIONELLA PNEUMOPHILIA* AMONG PNEUMONIA PATIENTS AT THE MEDICAL WARD OF K.N.H." P86/5/2006)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** revised version of your research proposal for the period 10th July 2006 – 9th July 2007.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

c.c. Prof. K.M.Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean Faculty of Medicine, UON
The Chairman, Dept. of Medical Microbiology, UON
The Supervisor: Dr. O. Anzala, Dept.of Med. Microbiology, UON

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Email: knhadmin@knh.or.ke

REF: KNH/MED/08

Date: 29TH January, 2006

Consultant in- charge

WARD 7A

WARD 7B

WARD 7D

WARD 8A

WARD 8B

WARD 8C

WARD 8D

PERMISSION TO CARRY OUT RESEARCH – SUSAN AKINY ODERA

The above named student from University of Nairobi has been allowed to collect data from the Hospital Medical wards for preparation of her thesis.

Please accord her the necessary assistance.

DR. N. K. MBOLOI
HOD, MEDICINE