T.C.

ISTANBUL MEDIPOL UNIVERSITY

HEALTH SCIENCES INSTITUTE

MASTER THESIS

LOWER RESPIRATORY SYSTEM INFECTIONS BACTERIAL AGENTS OF ADULTS AND CHILDREN IN ISTANBUL, A CASE STUDY BY MEDIPOL MEGA HOSPITAL

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ISTANBUL - 2017

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Level of Programme	:	Master (X) Doctorate ( )
Department	:	Microbiology
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Title	:	Lower Respiratory System Infections Bacterial Agents of Adults and Children in Istanbul, A Case Study of Medipol Mega Hospital
Place	:	Medipol Mega University Hospital
Examination date	:	11.08.2017

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DECLARATION

I hereby declare that this thesis is my own study, in all steps of the thesis from planning to writing, I have not done anything out of the ethical behaviors, I have obtained all of the information of this thesis inside the academic and ethical principles, I have shown references to all of the information and comments that were reached other out of this thesis study and have shown these references at the section of references, also I have not done any behavior infringing patent and copyright during the performing and writing of this thesis.

MOHAMMED MOHAMMED AHMED

ACKNOWLEDGEMENT

I would like to thank Almighty Allah for bestowing upon me an opportunity and strength to follow my education. It is His plenty elegance that has brought this research project to a success. I also would like to thank my supervisor Yard.Doç.Dr. Ayşe İSTANBULLU TOSUN who gave me brilliant advise to complete my research project. Without her fully support and encouragement I wouldn't be the one I am now.

I would like to thank Head Department of Microbiology Prof.Dr.Ahmet Zeki ŞENGİL, Associate Professor Süleyman YILDIRIM and Assistant Professor Deniz DURALI.

I would also like to thank my professors Doç.Dr. Sevgi ERGİN, Yard.Doç.Dr.Özlem GÜVEN, for their support, encouragement and advices.

Especial thanks is due to the Medipol Mega University hospital administrations who have contributed to provide us a very unforgettable and conducive environment for better applied learning in to the modern technology practice. I am deeply indebted to thank Yard Doç.Dr.Canan SEVER for her undivided effort in supporting me to pursue my education of this level. I am deeply indebted to thank to Dr Omar Abdulle Omar for his undivided effort in supporting me to pursue my education at this level.

I would like to thank all my friends Abdulshakur Mohamed, Gözde Inci, Kalif, Hatice, Selcuk, Ahmed, Gözde, Leyla, Müge, Mücahit, Cemile, Ezgi, and especially Fadumo Yusuf.I would like to thank my classmates, friends who have supported me and encouraged me to pursue my education.

Having this opportunity i would like to express my sincere gratitude and thank to my beloved parents for their countless support and also to my brothers and sisters for their encouragement and love.

Finally I would like to thank İHH and Zamzam foundations for providing scholarship and financial support. Thank you all.

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LIST OF ABBREVIATIONS

BAL: Bronchoalveolar Lavage

LRTIs: Lower Respiratory Tract Infections

WHO: World Health Organization

RTIs: Respiratory Tract Infections

URI: Upper Respiratory Infections

TB: Tuberculosis

ILDs: Interstitial Lung Diseases

MDRO: Multiple Drug Resistant Organism

ERS: European Respiratory Society

OPD: Outpatient Department

IPD: Inpatient Department

ICU: Intensive Care Unit

PSA: Pseudomonas aeruginosa

MIO: Motility Indole Ornithine

MHA: Mueller Hinton Agar

TSI: Triple Sugar Iron

CHOC: Chocolate Agar

NaCl: Sodium Chloride

PBB: persistent bacterial bronchitis

NAD: Nicotinamide adenine dinucleotide

CLSI: Clinical Laboratory Standards Institute

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1- ABSTRACT

LOWER RESPIRATORY SYSTEM INFECTIONS BACTERIAL AGENTS OF ADULTS AND CHILDREN IN ISTANBUL, A CASE STUDY BY MEDIPOL MEGA HOSPITAL

The general objectives of the study were to explore the incidence of lower respiratory infections due to bacterial pneumonias and to determine antimicrobial susceptibility patterns of infectious agents. The study participants were 200 patients included between January-March, 2017, mainly children and adults which were divided into two age groups 0-17 and those aged >18 years. The samples were collected from both inpatients and outpatients. The studied samples were; sputum, broncheoalveolar lavage (BAL) and tracheal aspirate. After proper incubation time, significant bacteria were identified and antimicrobial susceptibility tests were done.

According to findings of the study, 86% of the samples were Gram-negative bacilli while 14% of the samples showed to be Gram positive. Based on the causative agents of lower respiratory tract infections following findings were discovered 41.1% *Pseudomonas aeruginosa*, 29.9% *Klebsiella* spp., 15% *Escherichia coli*, 8% *Staphylococcus aureus* while 5.6% were shown to be *Streptococcus pneumoni*ae.

In conclusion, although some antibiotics were found to be effective, the study findings were indicative of increased resistance to some common antibiotics that are widely prescribed by doctors for treatment of patients with lower respiratory system infections. There were also signs of growing overuse of such antibiotics, which can sadly lead to greater antibiotic resistance in the near future.

Key words: Lower Respiratory System Infections, Bacterial Agents, and Antimicrobial Resistance.

2- ÖZET

ERİŞKİN VE ÇOCUK HASTALARDA ALT SOLUNUM YOLU İNFEKSİYONLARINDA İZOLE EDİLEN MİKROORGANİZMALAR, MEDİPOL MEGA HASTANESİ VAKA ÇALIŞMASI

Bu çalışmanın genel hedefleri, alt solunum yolları enfeksiyonlarından pnömonilerin erişkin ve cocuk hastalardaki yoğunluğunu ve infeksiyon etkeni olan mikroorganizmaların antimikrobiyal duyarlılıklarını araştırmaktır. Araştırmamızda Ocak – Mart 2017 arasında hastanemize alt solunum yolu enfeksiyonu şüphesi ile başvuran 200 hastadan alınan alt solunum yolu örnekleri kullanılmıştır. Hastalardan 0-17 yaş arasında olanlar çocuk, 18 yaş ve üstü olanlar erişkin olarak gruplandırılmıştır. Numuneler hem yatan hem de ayaktan hastalardan alınmıştır. İncelemeye alınan numuneler balgam, bronkoalveoler lavaj ve trakeal aspirat olarak ayrı ayrı incelenmiştir. Gerekli inkübasyon dönemi sonrası anlamlı üremeler tiplendirilerek antimikrobiyel duyarlılık testleri yapılmıştır.

Elde edilen örneklerin %86`sı Gram negatif çomak olarak belirlenirken %14`ü Gram pozitif olduğu görüldü. Alt solunum yolu enfeksiyonlarında organizmalar arasında şu etkenler saptandı; *Pseudomonas* spp. 41,1%, *Klebsiella* spp. 29,9%, *Escherichia coli* 15%, *Staphylococcus aureus* 8%, *Streptococcus pneumoniae* 5%. Kimi antibiyotiklere karşı duyarlılık görülse de, çalışmamızda hekimler tarafından alt solunum yolları enfeksiyonları tedavisi için çok kullanılan kimi antibiyotiklere karşı da yükselen direnç oranları saptandı. Bu antibiyotiklerin kontrolsüz ve yaygın kullanımından dolayı gelecekte daha da yüksek oranda bir direnç tablosu ile karşı karşıya gelmemiz malesef beklenmektedir.

Anahtar Kelimeler: Alt Solunum Yolu İnfeksiyonları, Bakteriyel Etkenler, Antimikrobiyal Direnç.

3. INTRODUCTION AND PURPOSE

Respiratory tract infection is any kind of transmittable disease concerning the respiratory tract. An infection of this sort is generally categorized more detailed as an upper respiratory tract infection (URI or URTI) or a lower respiratory tract infection (LRI or LRTI). Lower respiratory infections, like pneumonia are more likely to be more severe than upper respiratory infections, for instance the common cold.

The upper respiratory tract is normally regarded to be the airway above the glottis or vocal cords together with the nose, sinuses, pharynx, and larynx despite the dispute among scientists on the precise border between the upper and lower respiratory tracts. The lower respiratory tract incorporates the trachea, bronchial tubes, the bronchioles and the lungs. LRIs have been the most important cause of death in the midst of all contagious illnesses; the two widespread LRIs are bronchitis and *pneumonia* (1).

The upper respiratory tract infection (URI) is a imprecise expression used to explain serious infections relating to the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi (2). The prototype is the disease identified as the common cold, which is further explained in this research in adding together with pharyngitis, sinusitis and tracheobronchitis. Influenza is a systemic disease that engages the upper respiratory tract and must be distinguished from other URIs (3).

The inflammation of the throat that is brought about by a respiratory virus (Rhinovirus, coronavirus, adenovirus, influenza virus, parainfluenza viruses, respiratory syncytial virus, epstein–barr virus or coxsackievirus is considered to be Pharyngitis (4). Bacterial pharyngitis also is not as common and its only main regular cause is *S. pyogenes, Neisseria meningitidis, Mycoplasma pneumoniae, C.diphtheria* and *Arcanobacterium haemolyticum* are other uncommon bacterial causes of URI or LRI (5).

Topmost occurrence is between autumn and spring in moderate climates, and during the rainy season in tropical areas. Nevertheless, the progress of transmission is speedy between societies sharing populated living areas or districts because of the widespread transmission by droplets or direct diffusion. The discovery of *S. pyogene*s the exploration of most regularly demanded for pharyngitis (4).

This species is identified either by culture on blood agar and subsequent latex agglutination reaction for group-specific polysaccharide, or by direct antigen detection. Thus, there is no method that can differentiate oropharyngeal colonization from actual infection except the culture, which permits antibiotic susceptibility testing. Suspicion of infection with *N.gonorrhaea*, *Mycoplasma* spp., *Arcanobacterium* spp. *or Corynebacterium* spp. must be informed to the laboratory so that specialized non-routine culture media can be utilized. To cure Streptococcal pharyngitis, an oral penicillin or erythromycin is utilized (6).

Medication may not change the course of the primary pharyngeal infection, but it is supposed to decrease the risk of main non-infective sequelae like rheumatic heart disease, post streptococcal glomerulonephritis and Sydenham's chorea. The demand for antibiotic medication of streptococcal pharyngitis has been controversial issue in developed countries, since the non-infective sequelae of streptococcal infections are all scarce. However, the current rise in streptococcal infection rates in Europe and North America might advocate this point (7). The other problems of streptococcal pharyngitis comprise of scarlet fever (less common than in the past in developed countries), streptococcal toxic shock syndrome (brought about by toxins) and quinsy (paratonsillar abscess).

In quinsy, there might be minor infection with oral anaerobic bacteria, but these are often penicillin susceptible. Moreover, the drainage of purulent foci is demanded. Pathogens (viruses and bacteria) must fight a number of physical and immunologic barriers in order for pathogens to attack the mucus membranes of the upper airways.

General lower RTIs include flu (this can affect both the upper and lower respiratory tract), bronchitis, pneumonia, bronchiolitis and tuberculosis 8). The major sign of a lower RTI is cough; it is typically serious and might create phlegm and mucus. Other probable signs are a tense feeling in the chest, increased rate of breathing, breathlessness and wheezing. RTIs can be expressed in many ways. LRTI is one of the foremost sources of morbidity and death throughout the world (9). In rising countries, the condition is more complex and management is often complicated because of the troubles related to the recognition of the etiological agents and the management of suitable medication in cases antibiotic therapy is needed (10). LRTI is not a group of definite infections with diverse epidemiology's, pathogeneses, clinical presentations, and outcomes.

Variation of the etiology and symptomatology of respiratory diseases are attributed to, but not limited to the disparity of the following factors; age, gender, season, the type of population at risk. LRTIs are commonly the first infection to happen after birth and pneumonia is often too the final illness to happen before death (11). The etiological agents of LRTIs cannot be identified clinically and distinguished from zone to zone (12).

To identify suitable antimicrobial therapy, the recognition of bacteria bringing about lower-airway infections is significant. Despite the possibility of easy sputum collection in adults, it is complicated to gather sputum from children who usually cannot expectorate. Thus, flexible bronchoscopy with broncheoalveolar lavage (BAL) is frequently utilized to get a lower-airway specimen for culture of microorganisms in children. The European Respiratory Society (ERS) Task Force has made available advices and guiding principles for the performance of BAL in children and processing the return fluid. Typically BAL is conducted in the main affected zone, or the right middle lobe in case of diffuse lung disease (13).

BAL fluid gathered during bronchoscopy is normally chronological with two or more lavages carried out. The ERS suggests that the first lavage (lavage-1) is utilized for bacterial culture and any following lavage is used for cytology and non-cellular studies such as immunology-based work (14). Lavage-1 is regarded to be more reflective of bronchial airways, while the second (lavage-2) is reflective of distal airways (bronchioles and alveoli), and cellularity findings from the two lavages are dissimilar (15). Therefore, it is biologically reasonable that cultivable bacteria also vary between lavage-1 and lavage-2. Gram-positive bacteria, for instance *Staphylococcus aureus, Streptococcus pneumoniae,* etc. in addition to Gram negative bacteria like *Haemophilus influenzae, Pseudomonas*, *Acinetobacter*, and *Klebsiella species* are recovered from LRTIs (16).

Supervising and observing the antimicrobial resistance samples of the etiological agents is required not only to direct the clinician when running cases requiring antibiotic therapy but also to observe the development of these infections. Bacteria are recognized to bring about primary infection or superinfection, and in majority of the cases, they need aimed antimicrobial therapy (17).

Pneumonia keeps on to be a main health problem although there are developments in the determination of causal microbes and the accessibility of new antimicrobial drugs. Additionally, there are still many arguments concerning diagnostic methods and medication preferences. Pneumonia is the sixth most important reason of death in the United States and the main general cause of mortality from infectious disease. Moreover, pneumonia is the foremost cause of death in hospital-acquired infections. Left untreated and relying on the causal microbe and population, bacterial pneumonia has a mortality rate that may extend 30%. Every year more than 60,000 Americans die from pneumonia. Pneumococcal pneumonia alone represents probably 40,000 deaths yearly.

The illness is a special worry for older adults and people with chronic diseases or harmed immune system, but it can also strike previously healthy, young people. Worldwide, pneumonia is a main cause of mortality in children, many of whom are younger than 1 year of age. Bronchiectasis is a main problem of bacterial pneumonia where damaged alveoli and bronchioles are replaced by small sacs filled with infected debris. A low-grade, smoldering infection devastates more lung tissue with time. Bacterial pneumonia may also cause destruction of lung tissue with succeeding scarring, a permanent reduction in gas exchange, and a loss of respiratory reserve. The lungs also turn to be less elastic and inflation of the lungs need more energy and work for the duration of the aspiratory phase of respiration.

Bacterial pneumonia can evolve deadly when fluid loads the air sacs and obstructs the capability to swap oxygen and carbon dioxide. Circulating oxygen levels reduces (i.e., hypoxemia) and circulating carbon dioxide concentrations rise (i.e., hypercapnia), causing eventually to respiratory breakdown and mortality. In some situations, microorganisms get access to the blood spread quickly to other organs, and leading life-threatening sepsis or septic shock (18).

The aim of the study is to explore the burden of lower respiratory infection due to bacterial pneumonias and govern children and adult people with lower respiratory infections. The study was based in Istanbul Medipol University Hospital where the quantitatively data is collected and data from the hospital registers as well as the experiences of the medical doctors and laboratory results was used. And also the study will further examine resistances of bacterial species isolates to antibiotics including amikacin, ampicillin, piperacillin-tazobactam, amoxicillin-clavulanate, ampicillin-sulbactam, ciprofloxacin, ceftazidime, trimethoprim-sulfamethoxazole, gentamicin, imipenem, meropenem, oxacillin, ceftriaxone, and vancomycin. The samples of the study have been analyzed for three months from January to March, 2017.

4. GENERAL INFORMATION

4.1 Introduction

Lower respiratory tract infections (LRTIs) are described respiratory diseases involving the lower sections of the respiratory system. These infections have an effect on the respiratory system from the bronchi down and circumstances are classified into acute or chronic bronchitis according to the period of the signs.

4.2 Acute Bronchitis

To express diseases exemplified by cough: bronchitis, wheezy bronchitis, asthmatic bronchitis, and tracheobronchitis, there are many terms used regarding to these diseases. The lack of clinical definitions, disagreement of cough illnesses and bronchitis are attributed to the complexities in evaluating findings from bronchitis or cough disease researches as well as the lack of a solid agreement on diagnosis and medication (19). Acute bronchitis is an acute or sub-acute cough disease enduring fewer than 2-3 weeks, with or without phlegm production, commonly related to other upper respiratory tract and constitutional signs, the cough is the mainly often declared sign calling for office assessment; so, acute bronchitis is one of the top 10 analyses in ambulatory care medicine (20).

Physicians reveal wide inconsistency in diagnostic necessities and curing, because the diagnosis is clinical, without standardized diagnostic symptoms and sensitive or precise confirmatory laboratory examinations (21). Diagnosis of bronchitis frequently results in a prescription for an antimicrobial agent, displaying the physicians' belief of bacterial disease, although the term bronchitis does not mean definite etiology and is most generally brought about by viral pathogens.

Pathophysiology and etiology: Acute bronchitis is described as inflammation of the bronchial respiratory mucosa, resulting in production of cough. For nearly all clinicians, bronchitis is a disease clinically distinguished by cough, along with or with no fever or sputum production (19).

Bronchial epithelial injury is brought by transmittable or non-transmittable triggers, which cause an inflammatory reply with subsequent airway hyper reaction and mucus production. International literature proposes that clinical features of unsophisticated acute bronchitis expand in chronological phases: an acute infection phase, ensuing from direct inoculation by the contagious virus of the tracheobronchial epithelium, causing cytokine discharge and inflammatory cell commencement (22).

In this phase there are inconsistent constitutional signs, such as fever, myalgia, and malaise, which last 1 to 5 days depending on the infectious agent. The protracted phase results from hypersensitivity of the tracheobronchial epithelium and airway receptors (bronchial hyper responsiveness), portrayed mainly by cough, frequently come with phlegm emission and wheezing, and typically continues 1 to 3 weeks. Respiratory epithelial cell function plays a vital position in airway inflammation, and vagal-mediated airway hyper responsiveness has been revealed to agree with repair of the bronchial epithelial surface. Other mechanisms of bronchial hyper responsiveness might also be existent, such as adrenergic-cholinergic tone imbalance and IgE-mediated histamine production (20).

Chosen triggers that can start the cascade causing acute bronchitis are:

✓**Viruses**: Adenovirus, coronavirus, coxsackievirus, enterovirus, influenza virus, parainfluenza virus, respiratory syncytial virus, rhinovirus, and human metapneumovirus.

✓**Bacteria**: *Bordetella pertussis, Bordetella parapertussis, Moraxella catarrhalis, Haemophilus influenzae, Streptococcus pneumoniae*, atypical bacteria (e.g., *Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella species*).

✓**Yeast and fungi**: *Blastomyces dermatitidis, Candida albicans, Candida tropicalis, Coccidioides* immitis, *Cryptococcus neoformans, Histoplasma capsulatum.*

✓**Noninfectious triggers**: Asthma, air pollutants, ammonia, cannabis, tobacco, trace metals.

- ✓ **Acute bronchitis** is typically brought about by a viral infection (23).

In patients whose age is less one year, the causative agent is commonly respiratory syncytial virus, parainfluenza virus, and coronavirus; among patients whose ages are in between one to 10 years, parainfluenza virus, enterovirus, respiratory syncytial virus, and rhinovirus predominate; among patients whose age is more than 10 years, the most frequent agents are influenza virus, respiratory syncytial virus, and adenovirus. There is also a seasonal variability in the etiology with most commonly parainfluenza virus, enterovirus, and rhinovirus infections in the fall, while influenza virus, respiratory syncytial virus, and coronavirus infections are most frequent in the winter and spring (22).

In addition to that, there are other viral triggers such as influenza A and B and human metapneumovirus, while bacterial pathogens involved can be *Bordetella pertussis, Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, "No isolated pathogen" is also a regular result, possibly representing viral infections for which studies commonly do not perform appropriate analyses (20). Indications: Cough is the most generally detected sign of acute bronchitis, starting inside 2 days of infection in 85% of patients, continuing in nearly all of patients for fewer than 2 weeks; however, 26% are still coughing after 2 weeks, and a few cough for up to 6 to 8 weeks (24). Other indications may include dyspnea, wheezing, sputum production, chest pain, fever, hoarseness, malaise, rhonchi and rales in different degrees. Sputum might be obvious, white, yellow, green, or even colored with blood.

Diagnosis: There are different recommendations on the use of Gram staining and sputum culture in acute bronchitis therapy; especially the usefulness of these tests in the outpatient treatment is questionable, because they often show no growth or only normal respiratory flora (25).

Chest radiography must be kept for patients in which pneumonia is alleged or involved by heart failure, and in high risk patients with older age, chronic obstructive pulmonary illness, lately recognized pneumonia, malignancy, tuberculosis, and immunocompromised or debilitated status (22). Pulmonary function testing as spirometry is not regularly employed in the acute bronchitis diagnosis, but conducted only when underlying disruptive pathology is assumed or if there are frequent incidences of bronchitis. Pulse oximetry might assist to identify the seriousness of the disease, but findings do not verify or rule out bronchitis, asthma, pneumonia, or other definite diagnoses.

Differential diagnosis: The disparity of diagnosis comprises the main widespread triggers of acute cough:

✓Acute bronchitis

✓ Allergic rhinitis

✓ Asthma

✓Chronic obstructive pulmonary disease exacerbation

✓ Congestive heart failure exacerbation

✓Gastroesophageal reflux disease

✓ Malignancy

✓ Pneumonia

✓ Post-infectious cough

✓ Postnasal drip, sinusitis and viral syndrome

4.3 Chronic Bronchitis:

Despite the ambiguity regarding the description of chronic bronchitis, chronic bronchitis in adults is obvious and can be expressed: “the existence of chronic productive cough for 3 months in each of 2 consecutive years, in a patient under whom other factors of chronic cough have been left out”.

The possibility of applying this definition to childhood chronic bronchitis still stays in an ambiguity (26). The diagnosis of chronic bronchitis is supposed to take place in two phases. The first one is consideration and determination of many well-identified respiratory problems based on a staged administration protocol. The second but concurrent phase is removal or alteration of exogenous aspects that generate or sustain the child’s disease. However, this diagnosis has the potential to switch the pediatrician from discovering a more definite respiratory condition (26).

Although there is noticeably small in the literature concerning etiology, examination and organization of chronic cough in childhood, coughing in childhood is general. Current repots have highlighted the significance of making a exact diagnosis in children with a chronic cough (>3 weeks) (27). Juvenile chronic bronchitis with persistent end bronchial disease (lately considered persistent bacterial bronchitis) has been explained for many decades. The persistent bacterial bronchitis (PBB) is, according to some authors, the main reason of a chronic cough (28).

A variety of diagnostic terms have been applied to define this situation such as chronic suppurative lung disease (29). Persistent end bronchial infection and PBB describe the pathological process and site of infection, while terms like ‘‘chronic bronchitis’’ FIELD CE (1949 or ‘‘protracted bronchitis’’ Chang AB (2005) describe the clinical phenotype. Others have recommended using the label ‘‘pre-bronchiectasis’’ to emphasize the situation’s possible function in causing injured airways, as evident on high-resolution computed tomography or at bronchography (30).

PBB is a pediatric condition illustrated by the existence of an isolated moist or wet cough, lasting more than 4 weeks in the lack of other exact causes. It typically have an effect on children who are younger than 5 years and it has been determined more by pediatric pulmonologists who resolve it with antibiotic treatment. The most well-known organisms engaged in infants and children are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*. PBB is under diagnosed and frequently misdiagnosed as asthma (31).

Differential diagnosis: The term of "chronic bronchitis" should only be employed after underlying causes have been excluded.

4.4 Influenza

Influenza and pneumonia are closely linked conditions, and for this reason often considered together. Both affect the lungs and are usually short-lived, lasting less than three months. Influenza is exclusively a viral condition, whereas pneumonia can be viral and/ or bacterial. Influenza, also known as the flu, is caused by a number of viruses of three types - influenza A, influenza B, and influenza C (32). These viruses can occasionally cause serious complications - such as pneumonia (which sometimes can be life-threatening). General flu symptoms include fever, cough, headache, tiredness, inflamed respiratory mucous membranes, and head cold symptoms such as a runny nose and watery eyes. Occasionally, a person may experience nausea and vomiting. The majority of people recover from the flu in 1 to 2 weeks and, being a viral condition, it doesn't need antibiotics. Many of the complications and illnesses caused by the flu can now be prevented by influenza vaccinations, and it is specifically recommended that certain groups of people (such as older people) are vaccinated annually.

4.5 Pneumonia

Pneumonia, an inflammation of one or both of the lungs may affect an entire lobe (lobar pneumonia), a segment of a lobe, alveoli contiguous to bronchi (bronchopneumonia), or interstitial tissue C (33). It can be caused as a result of a previous respiratory infection, or develop in conjunction with other respiratory diseases. Symptoms of pneumonia include fever, chills, cough with mucus production, and sometimes pleuritic chest pain and shortness of breath. Symptoms can sometimes be mistaken initially for the common cold, but pneumonia usually develops over days or weeks and lasts longer than a cold. Pneumonia can be bacterial, viral, fungal or parasitic, but bacterial and viral pneumonia are the most common (34).

The type of pneumonia is usually named and diagnosed according to the pneumonia-causing organism. Bacteria are the most common cause of pneumonia in adults aged 30 years or older, with *Streptococcus pneumoniae* (also known as pneumococcal disease) being the most common pneumonia causing organism, National Heart Land Blood Institute (2003). Some bacterial pneumonia can be avoided, as there are now a wide variety of pneumonia vaccines available. However, when a person has already developed pneumonia, the usual treatment is with antibiotics. Occasionally, pneumonia-causing bacteria can become resistant to a number of antibiotics, which then makes the condition much harder the treat. Sometimes, pneumonia-causing bacteria can cause a number of conditions more serious than pneumonia. Examples are *Burkholderia pseudomallei* bacterium; and *Mycobacterium tuberculosis*. Viruses are the most common cause of pneumonia in children, but the influenza A and B viruses and rarely varicella-zoster virus have been known to cause pneumonia in adults. Viral pneumonia develops when viruses invade the tissues of the bronchioles causing bronchiolitis and occasionally affect the alveoli. The common symptoms of viral pneumonia are headache, fever, tiredness, coughing, and mucus production (35).

4.5.1 Pneumonia in immunocompromised hosts

Pneumonia is one of the most life-threatening infections in the immunocompromised host. A broad range of pathogens needs to be excluded; and the infectious agents to be considered vary depending upon the type and duration of immunosuppression, past exposures, geographic location, and the nature of the treatments administered. Less controversial than the diagnostic utility of ventilator associated pneumonia is perhaps the diagnostic utility of fiberoptic bronchoscopy in this setting. BAL protocols which process samples for both viral and bacterial pathogens, pneumocystis, legionella, fungi, and mycobacteria as well as cytologic analysis for noninfectious causes may be appropriate. Such protocols require communication between the clinical microbiology laboratory, infectious diseases specialists, pulmonologists, and transplant teams (36).

In summary, lower respiratory tract infections are among the most commonly encountered infectious diseases causing significant morbidity and mortality. The role of the microbiology laboratory in diagnosis remains controversial until better standardization of methods and outcomes data are generated. Empirical treatment approaches are recommended for bronchitis and community acquired pneumonia **(**CAP) not requiring hospitalization. In the hospitalized patient, although diagnostic tests are imperfect, they are suggested. This is particularly true for the immunocompromised host, for whom invasive procedures guided by clinical and epidemiological data may reveal unsuspected opportunistic pathogens (37).

Community acquired pneumonia (CAP) is described as a diagnosis of pneumonia in patients who did not satisfy any of the measure for hospital acquired pneumonia **(**HAP). The clinical diagnoses of CAP and HAP must be established within 48 h of hospitalization so that confirmatory respiratory cultures can be gained. Two of the following clinical measures are needed: fever (>38.3°C) or hypothermia (≤36.0°C), leukocytosis (>10 × 10^9 cells/liter) or leukopenia (≤4 × 10^9 cells/liter), or purulent tracheal aspirate or sputum. HAP is expressed as a diagnosis of pneumonia in patients accepted to the hospital who met at least one of the following measures: admission from a nursing home, rehabilitation hospital, or other long-term nursing care facility; earlier hospitalization within the instantly preceding 12 months; receiving outpatient hemodialysis, peritoneal dialysis, or infusion therapy requiring regular visits to a hospital-based clinic; or (having an immunocompromised state). This definition for HAP was founded on prior experience with health care-related diseases (38).

4.6 BAL

Bronchoalveolar lavage (BAL) is a saline wash of the bronchial tree introduced in 1970. It is an investigative technique. It became a diagnostic tool in India in 1994. BAL material has a very important role in diagnosis of infections and malignancies. It is a relatively safe procedure and is well tolerated. BAL provides material for various microbiological tests. One major limitation of BAL is a large range of normal values. An American thoracic society guideline for clinical practice has given the normal ranges for healthy adult nonsmokers (39).

4.7 Sputum

The sputum Gram stain which is a standard procedure in clinical microbiology is utilized for evaluation of specimen quality, for preliminary, quick diagnostic information, and for laboratory quality assurance. Many systems are used to evaluate specimen quality including the sputum Gram stain. A number of quantitative criteria have been developed to screen for specimen quality, all of which are based on the foundation that an abundance of squamous epithelial cells is pinpointing of superficial oropharyngeal contamination, Samples judged by Gram stain to consist principally of upper respiratory tract material are refused for usual bacterial culture (40).

In this condition the Gram stain has two roles: cost-effectiveness and prevention of reporting of false information to the clinician, which may cause misdiagnosis, resulting in either wrong or pointless medication. Reporting of deceptive clinical information is also evaded by refusing sputa for culture that is infected with upper respiratory flora because many of the potential pathogens which cause pneumonia may also settle the upper respiratory tract. Establish the value of the sputum Gram stain for preliminary diagnosis of respiratory illness is well instituted. Of importance, measure for explanation and giving information of microorganisms in Gram-stained smears of lower respiratory tract (LRT) secretions are changeable. As well, suggestions have been provided that sputum culture findings to be associated with direct Gram stain findings, so as to give more clinically applicable information in view of the restrictions of culture (41).

5. MATERIAL AND METHOD

5.1 Culture Media

5.1.1Blood Agar (Becton Dickenson, USA):

This media is integrated into agar. We obtained a blood agar which would improve the development of medically fundamental finicky bacteria. Blood agar consists of a base containing a protein source (tryptones), soybean protein digest, sodium chloride (NaCl), agar and 5% sheep blood. Four types of hemolysis are produced in sheep blood agar by Streptococci namely; alpha hemolysis, beta hemolysis, gamma hemolysis and alpha prime or wide zone alpha hemolysis (42).

Alpha hemolysis: Partial lysis of the red blood cells to produce a greenish-gray or brownish discoloration around the colony. α hemolysis is due to the reduction of red blood cells hemoglobin to methemoglobin in the medium surrounding the colony. Many of the alpha hemolytic streptococci are part of the normal body flora. *Streptococcus pneumoniae* which is also alpha hemolytic causes serious pneumonia and other deadly infectious disease (42).

Beta hemolysis: Complete lysis of red blood cells, causing a clearing of blood from the medium under and surrounding the colonies e.g. Group A beta hemolytic streptococci (*Streptococcus pyogenes*) and Group B, beta hemolytic streptococci (*Streptococcus agalactiae*). For group A streptococci maximal activity of both the hemolysins; oxygen labile (SLO) and oxygen stable (SLS) hemolysins is observed only in anaerobic conditions (42).

Gamma hemolysis: Some other bacteria do not act in response with the red blood cells, drastically parting them untouched. The medium demonstrates no discoloration or clearance because of the growth. These bacteria are categorized as gamma hemolytic bacteria. eg: *Enterococcus faecalis* (42).

Photo 5.1.1.1 Type of Hemolysis (43)

5.1.2Chocolate Agar (Becton Dickenson, USA):

Chocolate blood agar (CHOC) is an improved growth medium and is significantly non-choosy. This media's content is same as blood agar. It is a substitute of the blood agar Petri plate, encompassing of lysed red blood cells. This is reached by slowly heating the plate to 80^{O}C. Fastidious respiratory bacteria like *Hemophilus influenzae* and *Nesisseria meningitidis* necessitate chocolate agar for their appropriate growth. In addition to this certain bacteria, noticeably *H.influenzae*, need growth aspects such NAD (Nicotinamide adenine dinucleotide) (factor V) and hemin (factor X) which are found within red blood cells. Thus, a crucial standard for such bacterial growth is reliant on the lysis of the red blood corpuscles. Degradation of NAD (Nicotinamide adenine dinucleotide) is banned by the inactivation of the enzymes because of the high temperature. The agar medium is given name according to its color and encompasses of no authentic chocolate (42).

5.1.3 CHROMagar Orientation (Becton Dickenson, USA):

For the discovery and segregation of Gram positive and Gram negative pathogenic microorganisms CHROMagar Orientation was assessed.

BBL™ CHROMagar™ Orientation medium(Becton Dickenson,USA) can also be defined as a nonchoosy distinguished standard for the segregation, distinguishing and naming and listing of urinary tract pathogens separately. It is a superior to commonly used differential media for the isolation, differentiation and counting of UTI pathogens (42).

Photo 5.1.3.1 Different bacteria on Chromagar Orientation (44)

Table 5.1.3.1 Typical colony appearance of Gram (-) microorganisms (45)

Microorganism	**Typical colony appearance Chromagar Orientation**
E.coli	Dark pink to reddish
Klebsiella,Enterobacter,Citrobacter, Serratia	Metallic blue (+/- reddish halo)
Proteus,Morganella,Providencia	Brown halo
Proteus vulgaris	Blue with brown halo
Pseudomonas	Translucent (+/- natural pigmentation cream to green)
Acinetobacter	Cream
Stenotrophomonas	Colourless

Table 5.1.3.2 Typical colony appearance of Gram (+) microorganisms (45)

Microorganism	**Typical colony appearance Gram (+)**
Enterococcus	Turquoise blue
S.aureus	Golden, opaque, small
S.epidermidis	Cream, pinpoint colonies
S.saprophyticus	Pink, opaque, small
Streptococcus agalactiae	Light blue

5.1.4 BBL CHROMagar Candida Medium (Becton Dickenson, USA):

This media is a choosy and distinguishable medium for the separation of fungi. With the addition of chromogenic substrates in the medium, the colonies of C.albicans, *C.tropicalis and C.krusei* generate dissimilar colors, thus permitting the straight discovery of these yeast species on the separation plate. 1-6 Colonies of C.albicans emerge light to medium green, C.tropicalis colonies come into view blue-greenish to metallic-blue, and C.krusei colonies appear light rose with a whitish border (42).

Photo: 5.1.4.1 Different types of candida on Chromagar Candida (46).

Table 5.1.4 .1Typical colony appearance of candida species for microorganisms (47)

Microorganism	**Typical colony appearance**
C.albicans	Green
C.tropicalis	Metallic blue
C.krusei	Pink, fuzzy
C.kefyr, C.glabrata	Mauve-brown
Other species	White to mauve

5.2Biochemical Tests

5.2.1 Catalase test (Osel, Turkey)

To determine organisms that produce catalase enzyme, Catalase test was applied. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. The bubbles appearing from production of oxygen gas obviously indicate a catalase positive result. The sample on the right below shows catalase positive. The *Staphylococcus* spp. and the *Micrococcus* spp. are catalase positive. The *Streptococcus* spp. and *Enterococcus* spp. are catalase negative (48).

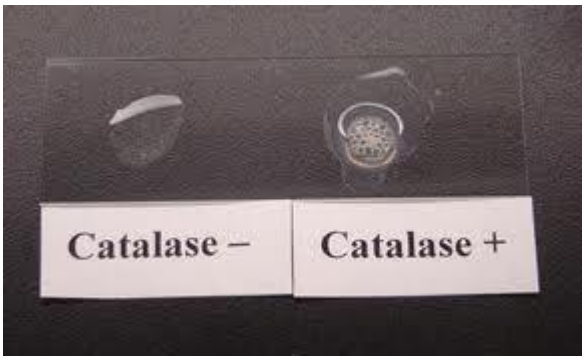

Photo: 5.2.1.1 Catalase Test (49).

5.2.2 Bile Esculin Agar (BEA) (Becton Dickenson, USA):

BEA is a choosy and differential medium which is presumptively utilized to determine Enterococci and group D Streptococci founded on the capability of an organism to hydrolyze esculin. Bile esculin agar includes oxygall (bile salts, first selective ingredients) to restrain from the growth of other Gram-positive organisms excluding Enterococci and group D Streptococci. Bile esculin disk is exercised for the quick finding of esculin hydrolysis in existence of bile for differentiating group D Streptococci from non-group D Streptococci (50)

Photo: 5.2.2.1 Bile esculin disk (51).

5.2.3 Coagulase test (Oxoid Limited, USA)

Coagulase test is one of the utilized biochemical tests. It is extremely fundamental test in the microbiology. The coagulase test recognizes whether an organism gives the exoenzyme coagulase, which brings about the fibrin of blood plasma to clot. Coagulase responds with prothrombin in the blood. It causes blood to clot by alerting fibrinogen to fibrin. The coagulase test was exploited to distinguish the essentially pathogenic species *Staphylococcus aureus* from the regularly non-pathogenic species *Staphylococcus epidermis*. The *S.aureus* potentially pathogenic in human beings and animals, but *S. epidermis* is commonly not pathogenic (48).

5.2.4 Cytochrome Oxidase test (Oxoid Limited, USA)

The study also utilized the oxidase test. It is the final enzyme in the respiratory electron transport chain of mitochondria that spots the existence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-*p*-phenylene-diamine. The dye was reduced to deep purple color. This test was employed to help the determination of *Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella* and *Pasteurella,* all of which produce the enzyme cytochrome oxidase. *Acinetobacter* and *Enterobacteriaceae* family do not produce this enzyme (48).

Its major function is to convert molecular oxygen to water and aid in establishing mitochondrial membrane potential. Cytochrome c oxidase locates to the inner membrane which segregates the mitochondrial matrix from the intermembrane space. This colorimetric assay is founded on surveillance of the reduction in absorbance at 550 nm of Ferro cytochrome c brought about by its oxidation to ferricytochrome c by cytochrome c oxidase (48).

5.2.5 MIO (Motility, Indole, Ornithine) Medium (Salubris Inc.,Turkey)

We utilized MIO (Motility, indole production, and ornithine decarboxylation) which are three distinguishing tests that are supplied in the one culture tube. It is recommended to use in testing motility, indole production, and ornithine-decarboxylase activity of enteric bacilli. We employed this test to differentiate the motility of the diverse *Enterobacteria* that bring about the lower respiratory infections. MIO medium is a semisolid medium utilized in the differentiation of the *Enterobacter*iaceae group by motility, ornithine decarboxylase activity and indole production (52).

Gelatin and casein peptones offer nitrogen, vitamins, minerals and amino acids vital for growth. They also give tryptophan required for the creation of indole. Yeast extract is a source of vitamins, especially of the B-group; Dextrose is the fermentable carbohydrate providing carbon and energy. L-ornithine is added to test the existence of the enzyme ornithine decarboxylase. If the organisms have such enzyme, it will be activated in an acid environment created by the initial fermentation of dextrose. Once the amino acid is decarboxylated, diamine putescine is produced. The result is an alkalinization of the medium, which turns it a dark blue. Organisms without the enzyme will stay acidic because of the fermentation, resulting in a yellow color in the medium. Bromocresol purple is a pH indicator to specify decarboxylase activity; the low concentration of bacteriological agar is for motility (52).

The bacteria are inoculated by stabbing the MIO medium and incubated in an aerobic atmosphere for 18 - 24 hours at 35 ± 2°C. If the indole reaction is negative, it is incubator for additional 24 hours. The motility and ornithine decarboxylase reactions were read before adding the Kovac's Reagent for the indole test. The motility was pointed out by cloudiness in the media or growth extending away from the line of inoculation. Ornithine decarboxylation is specified by a purple color in the medium. A negative ornithine reaction produces a yellow color at the bottom of the tube (52).

For the indole test, 3 to 4 drops of Kovac's Reagent was supplemented and the tube shaken carefully. The appearance of a red or pink color in the reagent layer is a positive indication of indole. Kovac's Reagent finds out the microorganism competent of cleaving the tryptophan. When these microorganisms are found in the medium, they liberate indole that responds with 4-dimethylaminobenzaldehyde to shape a dark red dye (52).

5.2.6 Citrate utilization test (Salubris Inc., Turkey)

Simmon's citrate media is composed of citrate only. If the bacteria grows in this media this means that bacteria can utilize the citrate to obtain from it all its requirements (Carbon + Nitrogen). It presents in bottles or tubes as slope media (48).

The purpose of slope is to obtain large area of surface for the bacteria, it contains indicator (Bromothymol blue). The color of media is green. If the bacteria utilizes the citrate, it produces alkaline sub of the pH of media will change. The media thus changes from green to blue (48).

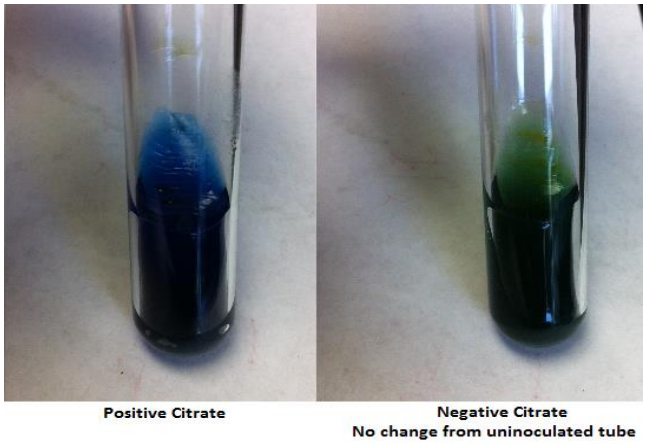

Photo: 5.2.6.1 Citrate Test (53).

5.2.7Urea test (Salubris Inc., Turkey)

It contains urea and the indicator is phenol red. If the bacteria secrete the urease enzyme that break down the urea in the medium, the pH of media will change to alkaline and color of media will change from yellow or orange to pink (Neutral or acid to alkaline). This media is also presented in bottles or tubes (48).

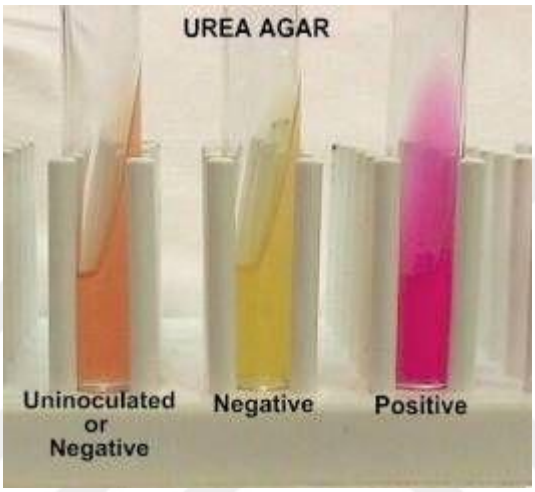

Photo: 5.2.7.1 Urease Test (54).

5.2.8 Triple Sugar Iron Agar (TSI) (Salubris Inc., Turkey)

TSI Contain: Glucose + lactose + Sucrose + Iron

Indicator: - They contain two types of indicator:

Phenol red: Fermentation

Ferric ammonium citrate: H_2S Production

In this medium the bacteria begin to take Glucose then lactose then sucrose then protein.

Mostly the *Enterobacteriaceae* break down the glucose.

Break down of sugar: Acidic (Low pH)

Break down of protein: Alkaline (High pH)

The color of medium after preparation is red (Alkaline) when the bacteria utilize the sugar it produce acid, the color of media will change from Red (alkaline) to yellow (acidic)

1. A/A
2. K/A
3. K/No change

A: Acidic (Yellow)

K: Alkaline (Red)

A/A: - The color of whole blood is yellow

It's mean glucose fermenter & lactose fermenter.

K/A: - The color of slope is red (No change)

The color of butt is yellow (48).

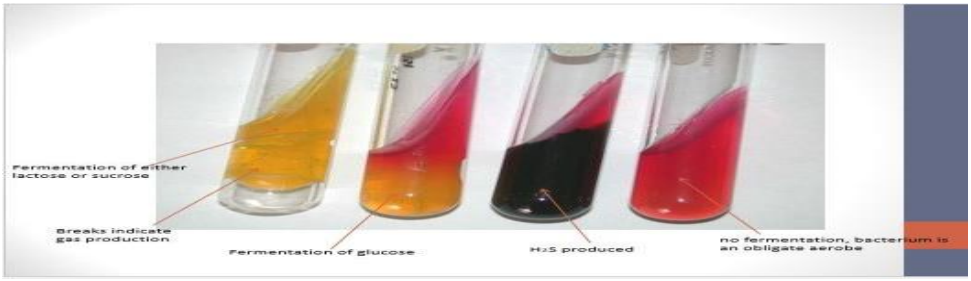

Photo: 5.2.8.1 TSI Media (55).

A) Non-lactose fermenter:

This is the case that non-lactose fermenter represents 90% of the possibilities; this means that the bacteria fermented the glucose and did not ferment the lactose and then transferred directly to the protein and produced K (48).

B) Quick-lactose fermenter (L.F) Such as *Klebsiella* spp.

This kind of bacteria ferments the glucose and lactose and transfers directly to the protein (48).

C) Late lactose fermenter: Such as *Shigella sonnei.*

The lactose is regarded to be difficulty to ferment for the reason of these bacteria. K/No change

The color of whole media is red

Not break down for any sugar

The bacteria break down protein directly

In this media we read:

1. Fermentation: - A/A or K/A or K/no change

2. Gas Production: - Crack or bubble

Results: - Gas positive or Gas negative

3. H_2S Production:-

-We can detect the H_2S production by the indicator (Ferric ammonium)

-By the presence of black color in the media.

- H_2S production produce in acidic media K/A

- Results: - H_2S Positive or H_2S Negative (48).

5.2.9 Mueller Hinton Agar Medium (Becton Dickenson, USA):

This media includes beef infusion, casamino acids, and starch. The levels of tetracycline and magnesium, thymidine, thymine, sulfonamide inhibitors, and calcium ions, are controlled so in order not to get in the way susceptibility testing and to yield good growth. The Kirby-Bauer antimicrobial disk diffusion procedure was used with Mueller Hinton Agar plates (48).

5.2.10 Antimicrobial Susceptibility Testing (Kirby Bauer Method)

Antimicrobial susceptibility testing (AST) is pointed out for pathogens contributing to an infectious process that warrants antimicrobial therapy. Otherwise susceptibility to antimicrobials could not be discovered consistently based on knowledge of their identity. Such tests are majorly employed when the etiologic agents are members of species competent of demonstrating resistance to generally prearranged antibiotics. Some organisms have predictable susceptibility to antimicrobial agents (ie, *Streptococcus pyogenes* to penicillin), and empirical therapy for these organisms is usually utilized. Thus, AST for such pathogens is seldom needed or conducted. Many laboratory approaches are available to characterize the in vitro susceptibility of bacteria to antimicrobial agents (48).

Phenotypic methods for discovering precise antimicrobial resistance mechanisms are mainly being used to complement AST (ie, inducible clindamycin resistance among several Gram-positive bacteria) and to supply clinicians with preliminary direction for antibiotic selection pending results generated from standardized AST (ie, β-lactamase tests) (48).

The Kirby-Bauer (K-B) test employs small filter disks impregnated with a recognized concentration of antibiotic. The disks are put on a Mueller-Hinton agar plate that is inoculated with the test microorganism. Upon incubation, antibiotic diffuses from the disk into the surrounding agar. If susceptible to the antibiotic, the test organism will be incapable to develop in the spot straight away surrounding the disk, exhibiting an area of inhibition (see figure below). The size of this area is reliant on a number of aspects, including the sensitivity of the microbe to the antibiotic, the rate of diffusion of the antibiotic through the agar, and the depth of the agar. Microorganisms that are resistant to an antibiotic will not display an area of inhibition (growing right up to the disk itself) or show a comparatively small zone (48).

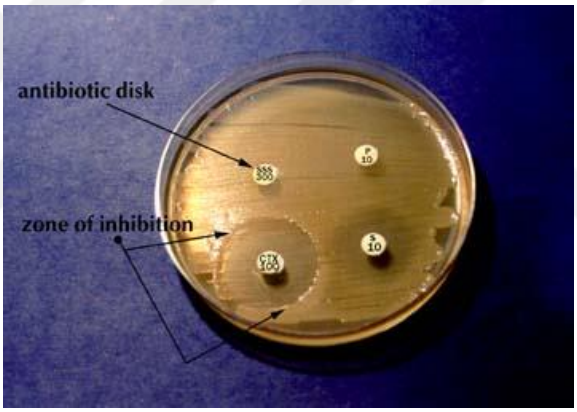

Photo: 5.2.10.1 Antimicrobial Susceptibility Testing (56).

Results

Results may be read after 18-24 hours of incubation. Subsequent incubation, the zone sizes are evaluated to the nearest millimeter (mm) utilizing a ruler or caliper; including the diameter of the disk in the measurement. We used Clinical Laboratory Standards Institute (CLSI) criteria for AST evaluation, CLSI 2016 (57).

5.3 Used Instruments (VITEK) (BioMerieux, France)

The VITEK 2 is an automated microbial identification system which gives greatly precise and reproducible findings as displayed in several independent researches, with its colorimetric reagent cards, and related hardware and software advances.

They also offer an alternative of automatic pipetting and dilution for antimicrobial susceptibility testing (58).

5.4 Specimen Collection and Handling:

1. Sputum, tracheal aspirate, broncheoalveolar lavage (BAL) fluid:

a. **Optimal timing:** Specimens were preferably acquired before commencement of antimicrobial therapy although they might be gained at any time throughout the clinical course.

b. Specimen types: The study included the following satisfactory lower respiratory tract specimens such as sputum, tracheal aspirate, BAL fluid. Specimens with possibility for upper airway contamination (i.e., BAL fluid, pleural fluid, lung biopsy) are preferred.

c. Specimen collection:

i. BAL fluid, tracheal aspirate: The study used sterile containers in which specimens were collected. Each of these specimen containers was labeled with the patient's name, ID number, the specimen type, and the date when theses specimens were collected.

ii. Sputum: Patients were precisely informed regarding the distinction between sputum and oral emissions, after that they were instructed to wash the mouth with water and then expectorate deep cough sputum exactly into a sterile screw-cap collection cup or sterile dry container.

Specimen rejection criteria:

The following samples were rejected based on the following criteria:

- ✓ Without barcode specimens
- ✓ No source on List
- ✓ Leaked specimens
- ✓ Non sterile cup
- ✓ Inappropriate storage conditions (More than 1 hour and >4+^{O}C)
- ✓ Same day more than one specimen

5.5 Specimen Inoculation

Specimen processes were worked out in biological safety cabinet, as aerosols can result in laboratory acquired respiratory infections.

1. All specimen processed were rapidly as possible to maintain viability of pathogen and avoid putting the patient at risk for repeated procedure.

2. We selected the purulent or most blood- tinged portion of the specimen.

3. Prepared Gram stain for details on preparation and reading of smears.

4. Used sterile swap, stick, loop or pipette.

5. An optochin disk was added to media.

Streak Plate:

Sputum and tracheal aspiration specimens were inoculated on the Medias with streaking method with 10µl sterile loop.

1. Specimen was spread over a portion of the culture media surface wisely.

2. The loop dragged from the inoculated section and spread it out into a second section.

3. The loop dragged again from the section 2 and then spread out into the third section. The same was done for the third and the fourth section. Sections 1 and 4 were ensured that were not overlapped. The inoculation loop used was disposed into a suitable container.

4. The lid was replaced and the streaked agar plate incubated at the appropriate temperature in an inverted position to avoid condensation.

5. Over the agar surface the inoculum was streaked in such a way that it “thins out” the bacteria.

6. Streaked plates were incubated at **37°C** for 24 hours and examined the colonies grown in the plate carefully.

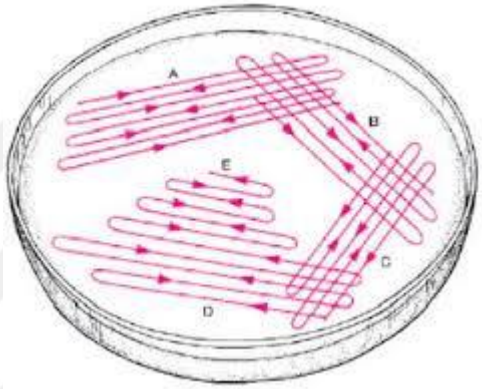

Photo: 5.5.1 Streaking Technique (59).

5.5.1 BAL Inoculation: Dilution method

BAL: Utilizing a sterile loop each agar plate is inoculated with the deposit of the centrifuge sample. Centrifuged BAL is re-suspended in the fluid and three serial dilutions are made (1/10, 1/1000 and 1/100,000) 0.1mL of each dilutions is plated out. Optionally a calibrated loop is employed. For BAL fluids tests, quantitative calibrated loops intended for the conveyance of 0.010 and 0.001 µL are made use of. The colonies are calculated on the plates following incubation, and the number of CFU per milliliter is identified by multiplying the number of colonies by the dilution factor and 100. Diagnostic threshold is 10^4cfu/mL for BAL. Specimens from patients who have got antibiotics may also provide false-negative findings. Finding: Number of colony x10^4 CFU/mL.

5.5.2 TA and sputum inoculation:

TA and sputum samples were inoculated on the following media: Chrome agar (Becton Dickenson, USA), Blood agar (Becton Dickenson, USA), Chocolate agar (Becton Dickenson, USA), Chrome candida agar (Becton Dickenson, USA).

According to specimens, the study used colony counting but according to chocolate agar it was added optochin disk and incubated aerobically and made on Gram stain. 24 h later we observed the colony counting, if the amount of colony counting is less than 1000 CFU/mL, it is considered not significant but if the amount of colony is 10^4 or more than it is considered significant.

The study also used subculture and biochemical tests then put incubation with 24 h after this time it was read again and used antibiogram test to obtain specific type of bacteria. Incubation Plates were incubated at 35 to 37^OC in 5% CO_2 for a minimum of 48 h to 72 h as it is preferred.

5.6 Investigation of Gram Stain and Culture Results

The study employed a Gram stain (Salubris Inc., Turkey) technique which is a method that distinguishes Gram positive from Gram negative bacteria where the former showed purple color and the latter exhibited pink color.

Gram positive bacteria *(thick layer of peptidoglycan-90% of cell wall*) **stains purple**

Gram negative bacteria *(thin layer of peptidoglycan-10% of cell wall and high lipid content)* **stains red/pink**

Gram staining technique: Crystal violet stain, lugol's iodine (mordant), acetone-alcohol decolorizer (more rapid, not over decolorize smear), fuchsin (counter stain)

Report of Gram Reaction

1. Number of bacteria present (scanty – few - moderate- many)

2. Gram reaction of bacteria (Gram positive or Gram negative)

3. Morphology of bacteria (Cocci- diplococcic- rod – coccobacilli-bacilli)

Investigation of Bronchoalveolar Lavage

Streaked plates were incubated at **37°C** for 24 hours and the colonies examined for grown in the plate carefully the next day. If the amount of colony count is less than 1000 CFU/mL, it is considered not significant but if the amount of colony is 10^4 or more than it is considered significant. Then we decided to do subculture and biochemical tests applied from Isenberg guide.

Investigation of Tracheal Aspirate

Streaked plates were incubated at **37°C** for 24 hours before we examined the colonies grown on the plate carefully the next day.

Subsequent to incubation, the colonies were calculated on the plates and the number of CFU per milliliter is identified by multiplying the number of colonies. Predominantly if we get 10^4 CFU/mL we decided infection for determination of sub culture, biochemical tests, AB, and Vitek to obtain the exact of bacteria.

Investigation of Sputum

Streaked plates were incubated at **37°C** for 24 hours and examined for the colonies grown in the plate carefully the next day. After incubation the dominantly growth colonies were identified.

Interpretation and Reporting of the Results

Employing the published CLSI guiding principles, the susceptibility or resistance of the organism to each drug tested is determined.

One the recording sheet for each drug, it is displayed whether the area size is susceptible (S), intermediate (I), or resistant (R) according to the explanation chart.

The findings of the Kirby-Bauer disk diffusion susceptibility test are stated only as susceptible, intermediate, or resistant.

5.7 Bacterial Identification

5.7.1 Gram negative identification flowchart

Gram negative report is divided into two categories:

a. Lactose fermentative

b. Non lactose fermentative

a. Lactose fermentative: *Klebsiella* spp., *Escherichia coli, Enterobacter* spp. Then we used indole media to distinguish between positive *E.coli* and *K.oxytoca* in one side, producing negative *E.coli* and *K.oxytoca* by using citrate, and the other side negative *K. pneumonia* and *Enterobacter* spp., breaking down by using urease into positive *K. pneumonia* and negative *Enterobacter* spp.

b- Non lactose fermentative: *Proteus* spp., *Morganella morganii, P.aeruginosa.* According to these three species we used oxidase test to differentiate positive PSA on one side while negative *Proteus* spp. and *Morganella morganii* on the other side. Then we used citrate to produce *Proteus* spp. which was positive and *Morganella morganii* which was negative. Then we used indole to classify *Proteus* spp. into *P .vulgaris* which was positive while *P. mirabilis* was negative.

5.7.2 Gram positive coccus identification flowchart

We used catalase test to generate positive catalase *Staphylococcus* spp. and negative hemolysis. On the side of positive catalase *Staphylococcus* spp. we used coagulase to break down it into *Staphylococcus aureus* which was positive and *Staphylococcus epidermidis* was negative.

On the other side of negative hemolysis we classified into beta, gamma and alpha. Then we used bacitracin sensitivity in order to differentiate beta hemolysis into sensitive which represented *S. pyogenes* and resistant which represented *S. agalactiae.*

In the case of gamma hemolysis, we used bile esculin to separate positive salt tolerant which in turn becomes *S.bovis* if it has tolerance or may become *Enterococcus faecalis* if it does not have tolerance. According to alpha hemolysis, we used optochin disk to segregate between *S.pneumoniae* which was sensitive and *S.sanguinis* which was resistant.

5.7.3 Gram negative coccus/coccobacilli identification flowchart

Gram negative cocci are divided into coccobacilli and diplo coccus. In the situation of coccobacilli, it is examined the growth on CAP which shows large, round, colorless to grey, opaque colonies. Then we used Kovac's oxidase test to differentiate the specific type of bacteria. If the oxidase is positive, growth factor requirement testing will be performed to identify whether it requires both hemin (X factor BD) and NAD (Nicotinamide adenine dinucleotide) (V factor BD)for growth which represents *H.influenzae* or does not require both hemin (X factor) and NAD (V factor) for growth will not be *H.influenzae.* On the other hand with diplo coccus, we used Kovac's oxidase test. If the oxidase becomes positive, it is a Moraxella spp and if it becomes negative, it is not *Moraxella* spp.

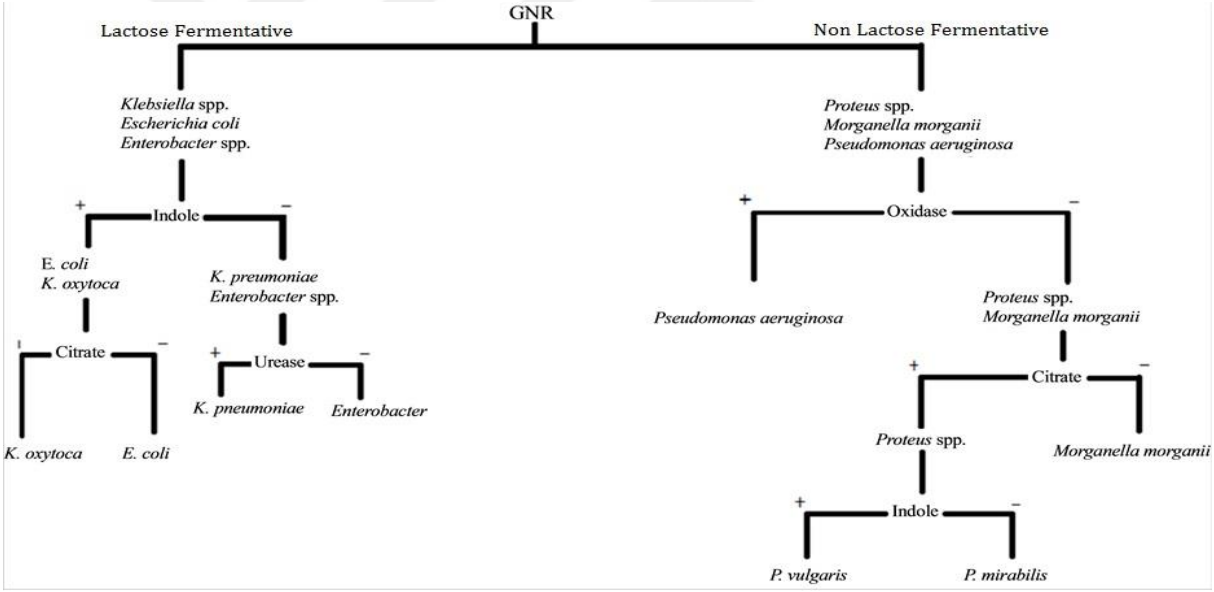

Figure 5.7.3.1 Gram negative rods identification flowchart (60).

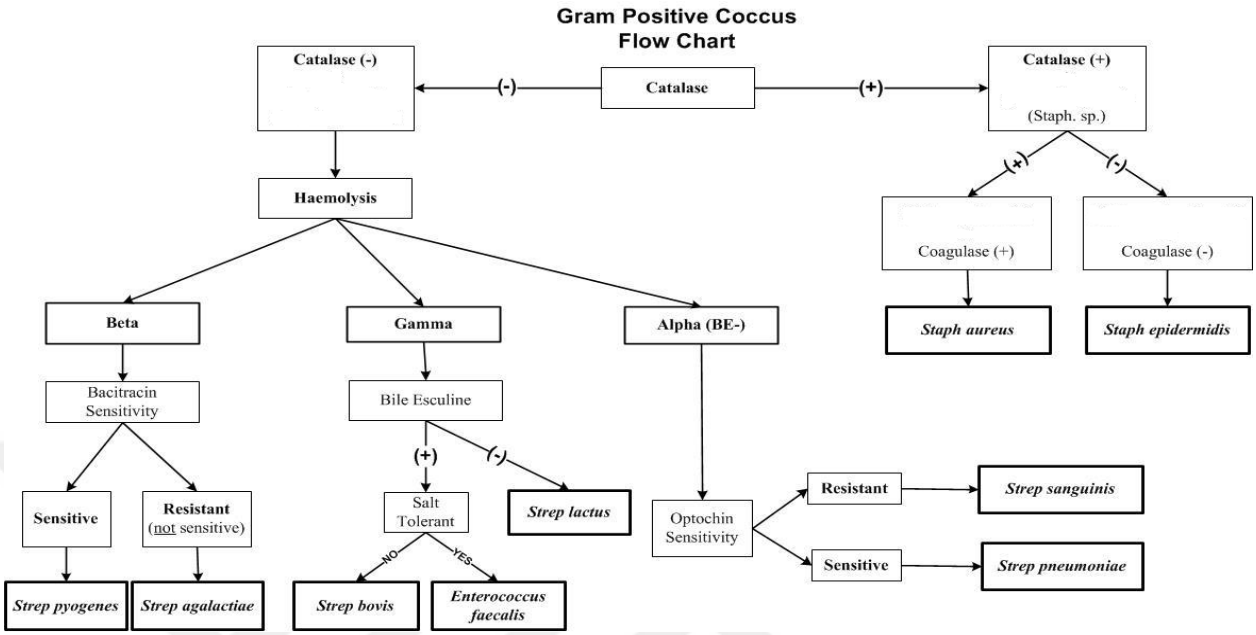

Figure: 5.7.3.2 Gram positive coccus identification flowchart (62).

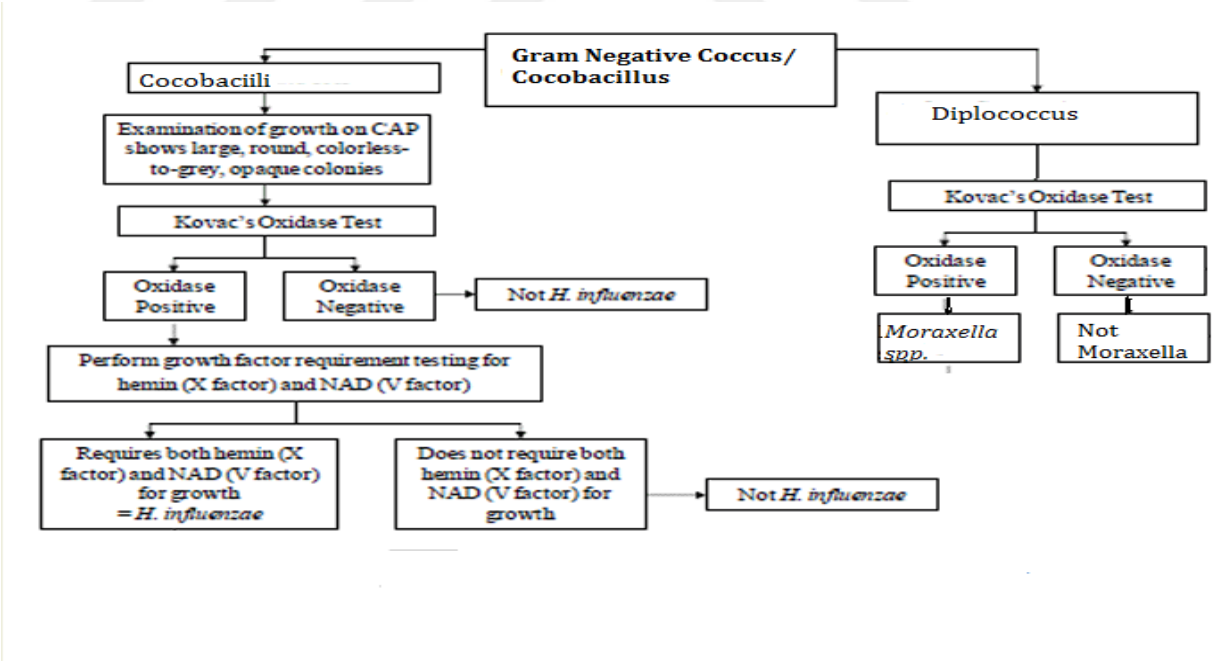

Figure 5.7.3.3 Gram negative coccus/coccobacilli identification flowchart (63).

6. RESULTS

A total of 200 inpatient and outpatients with respiratory infection was included from different departments of the Medipol Mega University hospital. Three different specimens were collected namely BAL (36), sputum (90) and tracheal aspirates (74) to a total of 200 samples.

117 were male patients and 83 were female patients. Based on the objective, which was to determine the causative agents and their antimicrobial resistance patterns in the two groups; namely children and adults, the tables and figures below demonstrate our findings.

6.1 Patient Differentiation by Gender and Origin

Table 6.1.1 Patient differentiation by gender and origin

	Inpatient	Outpatient	Total
Male	99	18	**117**
Female	71	12	**83**
Total	**170**	**30**	**200**

Table 6.1 shows the numbers of cases based on IPD versus OPD. The IPD samples were 170, meanwhile 30 samples were from OPD. Making 85% of the participants from IPD and 15% from OPD. When comparing genders, we found 58.5%, to be female while 41.5% accounted to be male participants.

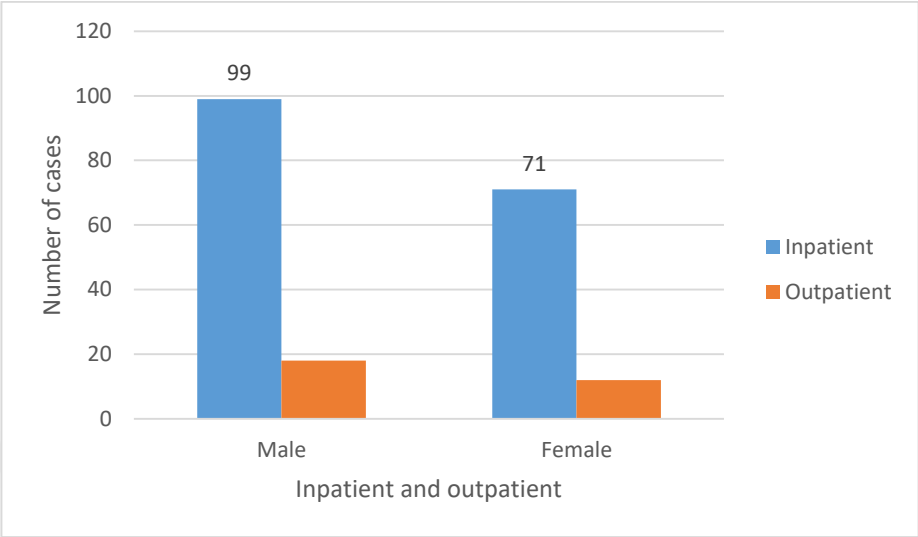

Figure 6.1.1 Patient differentiation by the gender and origin

6.2 BAL, Sputum and Tracheal Samples of Adult Patients

Table 6.2.1BAL, sputum and tracheal samples of adult patients classified as inpatient and outpatient.

	Inpatient	**Outpatient**	**Total**
Male	66	14	**80**
Female	49	7	**56**
Total	**115**	**21**	**136**

This table shows the distribution of sputum, BAL and tracheal samples collected from adult patients. 115 samples were IPD while 21 cases were from OPD, making 84.5% of the samples from IPD and 15.4% from OPD. Here in terms of comparing gender, male representation was 58.8% of the total participants meanwhile 41% demonstrated to be female samples.

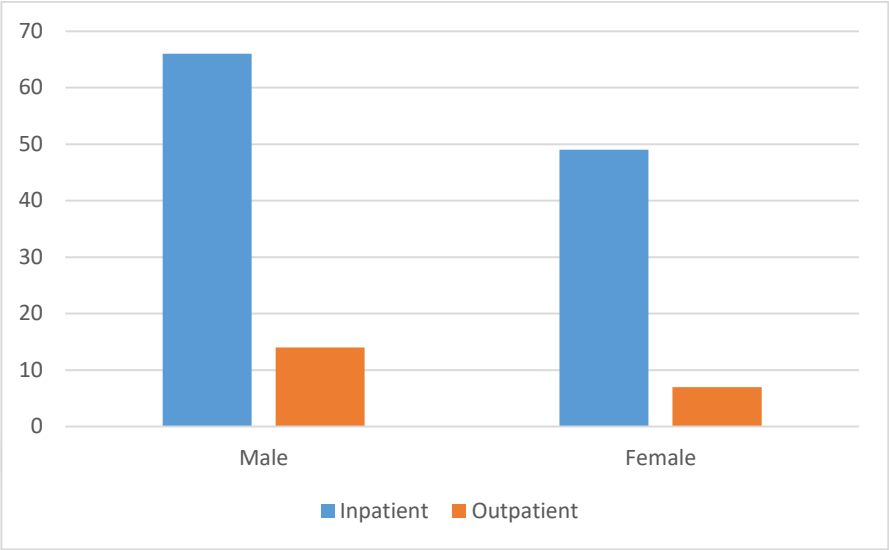

Figure 6.2.1BAL, sputum and tracheal samples of adult patient's samples according to their gender and IPD or OPD.

6.3 BAL, Sputum and Tracheal Samples of Pediatric Patients

Table 6.3.1 BAL, Sputum and tracheal samples of pediatric patients classified as inpatient and outpatient:

	Inpatient	**Outpatient**	**Total**
Male	33	4	**37**
Female	22	5	**27**
Total	**55**	**9**	**64**

Here the patients from IPD represent 86%, while OPD patients represent 14% this means that most of the samples were from IPD, on the other hand male represented 58% and female 42% here it means that male gender and IPD samples were the leading participants of the specimen collected here below is the figure demonstration.

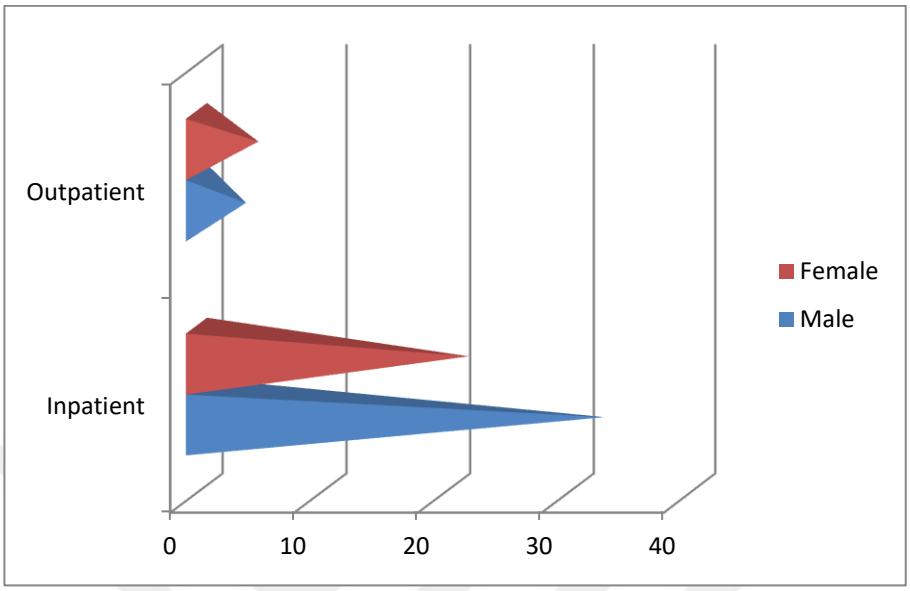

Figure 6.3.1BAL, sputum and tracheal specimens of pediatric patients classified as inpatient and outpatient.

6.4 Demographic Distribution of the Participants

Table 6.4.1 Respondents distribution by age

	Pediatric	**Adult**	**Total**
Male	37	80	**117**
Female	27	56	**83**
Total	**64**	**136**	**200**

The table above shows the data splitted into two categories pediatric (those below 17 years) and adult (those ≥17 years) Pediatric samples represented 32%, while adult cases were 68%. Male pediatric samples represented 58.5%, while female samples represented 14.5%.

The overall picture is that adult participants were twice the number of pediatric patients as well as male participants were thrice more than female participants.

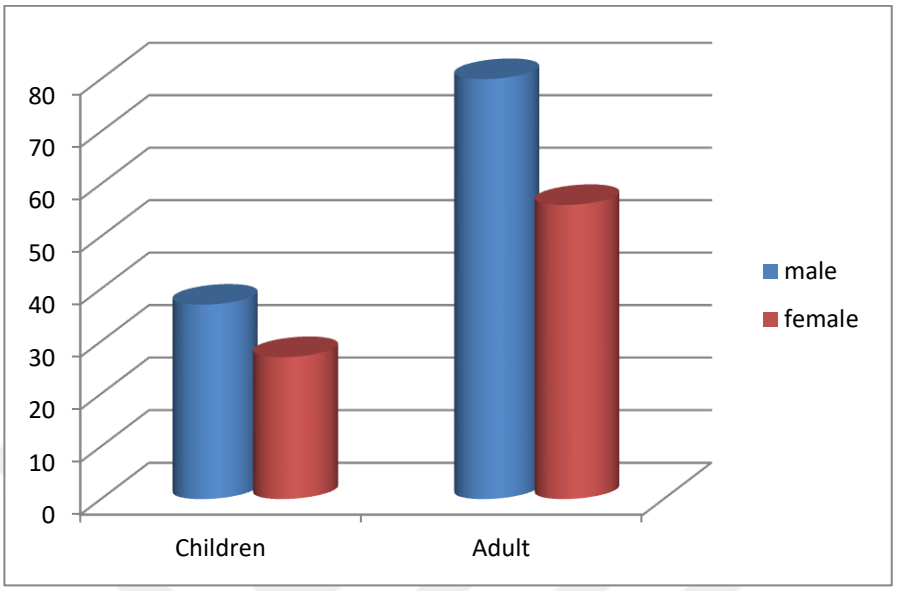

Figure 6.4.1 Participants categorized according to adult- and childhood

6.5 Department versus Type of Procedure Used

Table 6.5.1 Number of BAL samples by department

Department	**BAL Samples**	**Percent %**
Adult Chest Diseases	15	42
Pediatric Chest Diseases	11	31
Child Health and Diseases	4	11
Newborn Intensive Care Unit	3	8
Adult Intensive Care Unit	2	6
General Surgery	1	3
Total	**36**	**100**

The table above shows the amount of BAL samples collected from different departments. A total of 36 samples, from which 42% were from adult chest diseases, 31% from pediatric chest diseases, 11% from child health diseases, 8% from newborn intensive care unit department, 6% from adult intensive care unit, and 3% from general surgery.

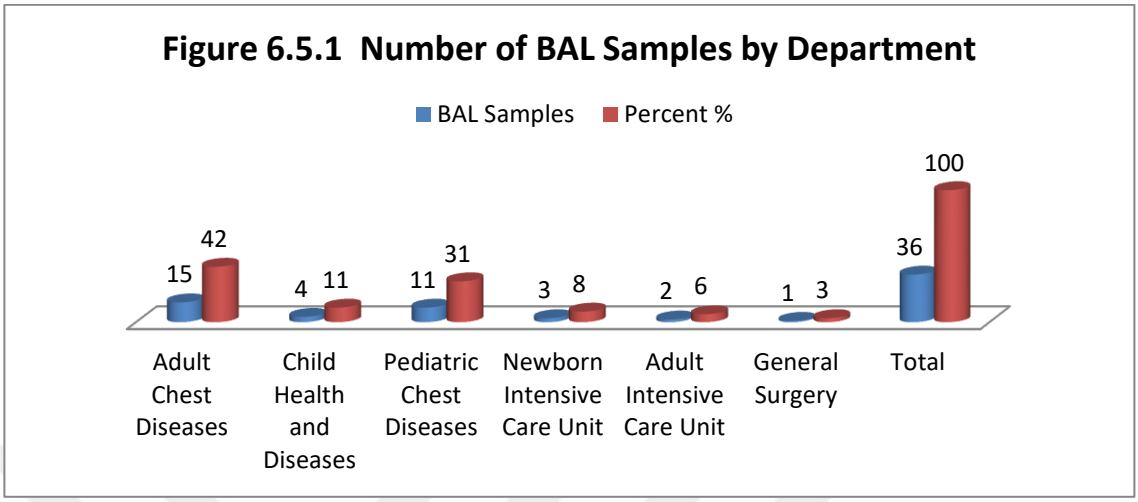

Figure 6.5.1 Department versus number of BAL samples

6.6 Departments versus Sputum Specimen

Table 6.6.1 Department versus number of sputum samples collected

Department	Sputum samples	Percent %
Medical Oncology	13	20
Adult Chest Diseases	12	19
Pediatric Chest Diseases	11	17
Adult Intensive Care Unit	9	14
Hematology	6	9
Internal Medicine	3	5
Child Health and Diseases	3	5
Gastroenterology	2	3
Cardiology	1	2
Rheumatology	1	2
Organ Transplantation	1	2
Infectious Diseases and Clinical Microbiology	1	2
Thoracic Surgery	1	2
TOTAL	**64**	**100**

The table above shows 64 sputum samples collected and presented according to their department, 20% were from the medical oncology department, 19% from the adult chest diseases, 17% were from the pediatric chest diseases department, % of the samples were from the adult intensive care unit, 9% came from the hematology department, 5% were from internal medicine, 5% from child health and diseases, 3% from gastroenterology and the rest which constituted 2% came from cardiology, rheumatology, organ transplantation, infectious diseases and clinical microbiology, and thoracic surgery. This shows that the majority of the samples came from, medical oncology, adult chest diseases, pediatric chest diseases as well as the adult intensive care unit departments.

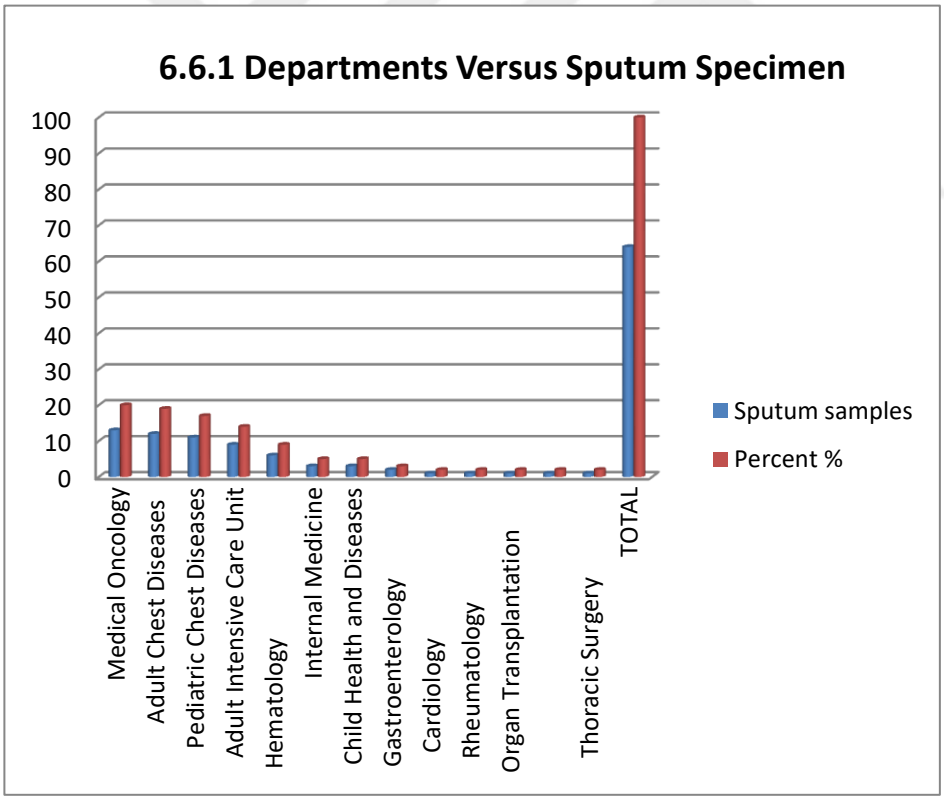

Figure 6.6.1 Departments versus cases with sputum specimen collected

6.7 Department versus Tracheal Specimen

Table 6.7.1 Number of tracheal aspirate samples collected

Department	Tracheal samples	Percent %
Adult Intensive Care Unit	44	63
Pediatric Chest Diseases	8	11
Cardiovascular Surgery	6	9
Child Health and Diseases	6	9
Newborn Intensive Care Unit	2	3
Brain and Nerve Surgery	2	3
Physical Medicine and Rehabilitation	1	1
Organ Transplantation	1	1
Total	**70**	**100**

The departments where tracheal aspirates were collected included adult intensive care unit, cardiovascular surgery, pediatric chest diseases, child health and diseases, newborn intensive care unit, brain and nerve surgery, physical medicine and rehabilitation and organ transplantation. Percentage distributions of the tracheal specimens were as follows; 63% of the samples were from the adult intensive care unit, 11% of the cases were from pediatric chest diseases, while 9% of the samples came from both child health and cardiovascular surgery departments, 3% of the samples came from both newborn intensive care unit and brain and nerve surgery and 1% of the samples came from physical medicine and rehabilitation and organ transplantation .This means that most of the tracheal specimens were from the adult intensive care unit.

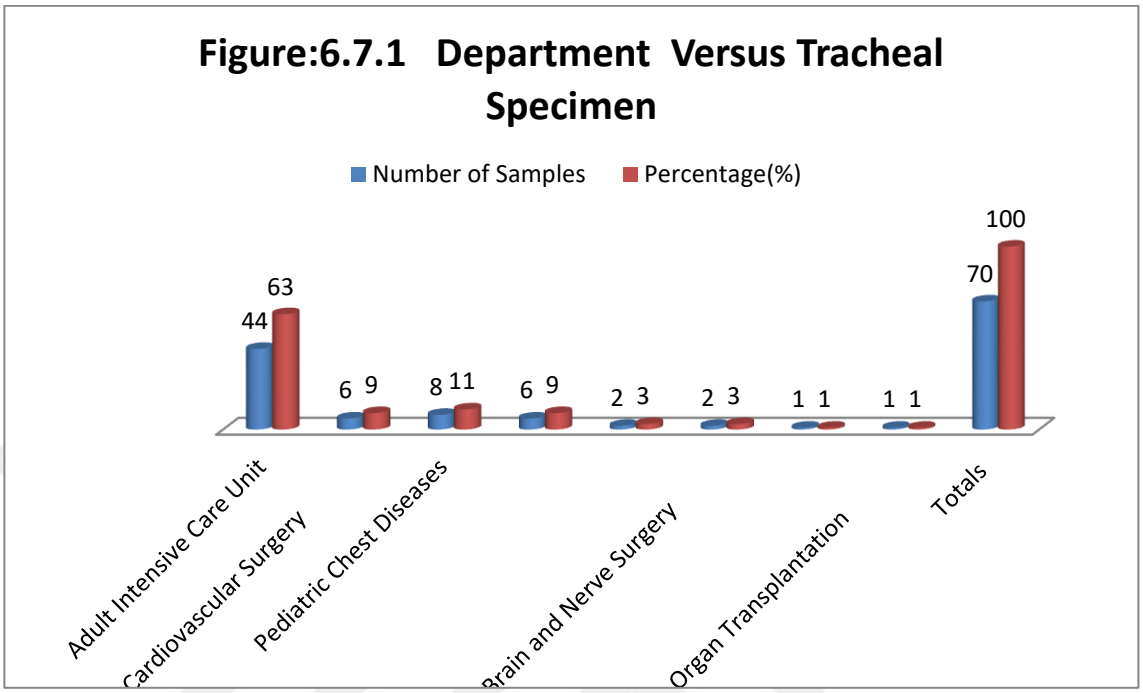

Figure 6.7.1 Department versus tracheal specimen collected

6.8 Outpatient Department Distribution of Tracheal and Sputum Samples

Table 6.8.1 Distribution of tracheal and sputum samples according to department

Cases seen In Outpatient Department	**No. of Samples**	**Percent %**
Adult Chest Diseases	10	33
Pediatric Chest Diseases	6	20
Hematology	3	10
Infectious Diseases and Clinical Microbiology	3	10
Child Health and Diseases	2	7
Internal Diseases	2	7
Medical Oncology	1	3
Child Immunology and Allergies Diseases	1	3
Ear, Nose and Throat Diseases	1	3
Total	**30**	**100**

A total of 30 cases were included irrespectively of sputum or tracheal aspirate. There were no BAL in the outpatient group. Here, comparisons of the number of cases from the different OPDs are shown. Number of cases from adult chest diseases department accounted for 33% of the samples, followed by pediatric department from which 20% of the cases came from, while 10% of the cases came from the department's hematology and infectious diseases units. 7% of the samples came from the child health and disease department and from internal medicine. 3% of the samples came from the departments of medical oncology, child immunology, allergic diseases and ear-, nose- and throat diseases. This shows that most cases were from the department of adult chest diseases, followed by pediatric chest department.

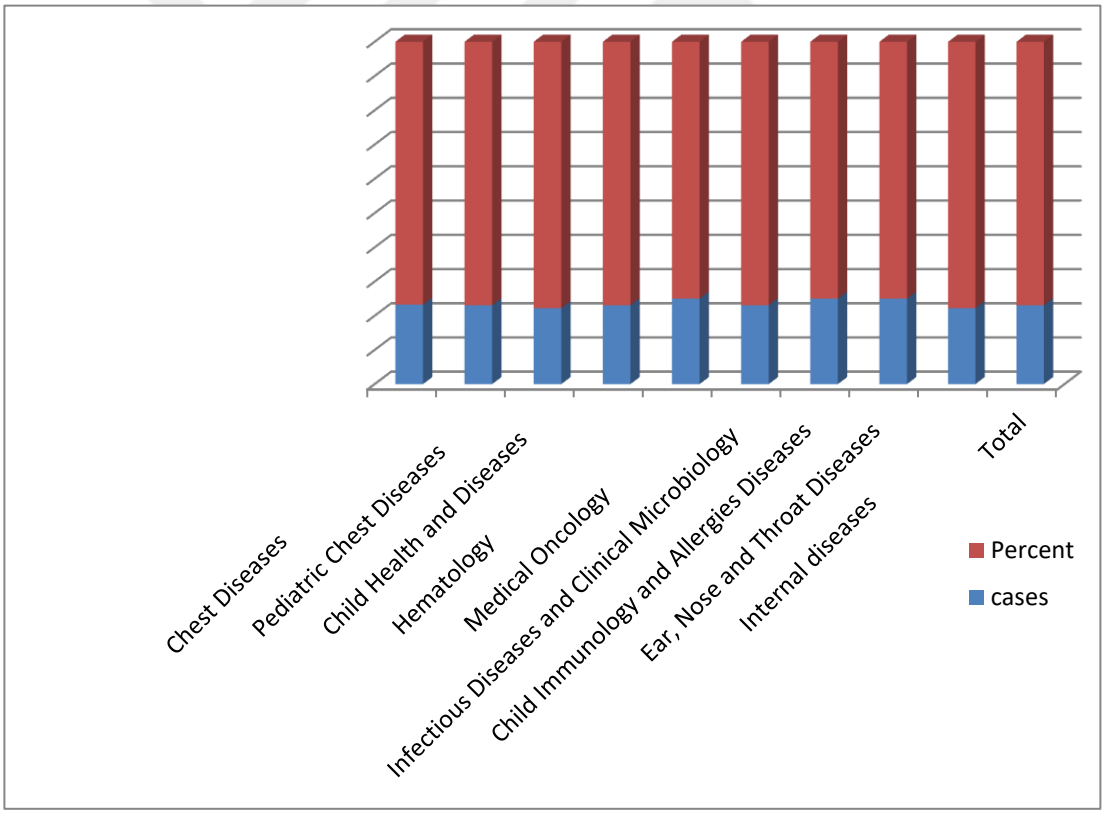

Figure 6.8 Outpatient department distributions

6.9 Samples of BAL, Sputum and Tracheal

Table 6.9.1 Number of samples collected from BAL, sputum and tracheal aspirate.

Specimens	No. of Samples	Pathogenic Growth Observed	Percentage (%)
Sputum	90	58	64.4%
BAL	36	18	50.0%
Tracheal	74	50	67.6%

The table above shows the percentage of growth observed from samples collected from LRTS by different mechanisms including BAL, sputum, and tracheal aspirate. The results show 18 cases of growth out of 36 BAL samples which is about 50% of growth. 58 out of 90 sputum samples showed growth, which is about 64.4%. 50 out of 74 samples collected from tracheal aspirates also showed growth, which makes about 67.6%

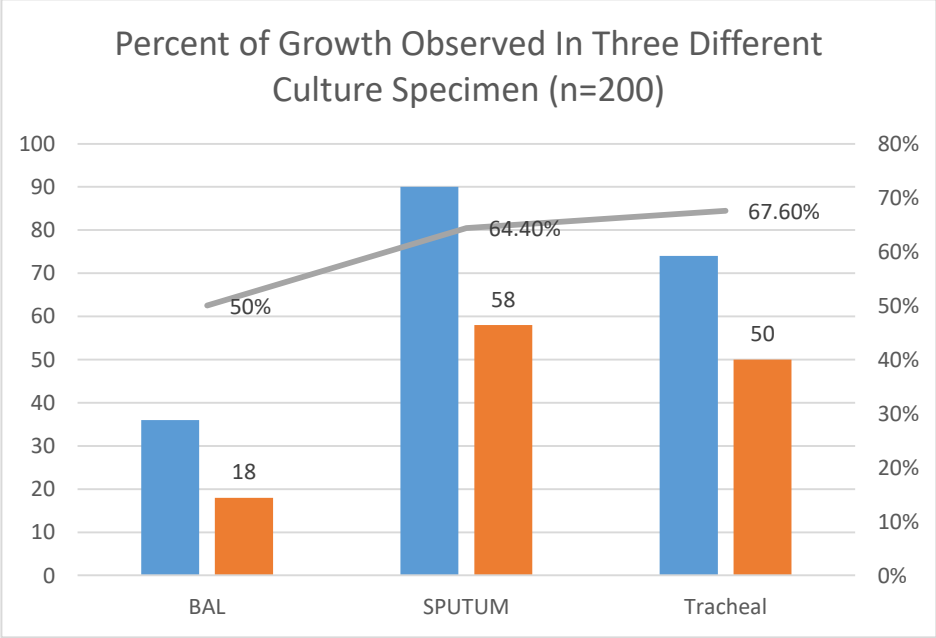

Figure 6.9.1 Bacterial species detected from BAL, sputum and tracheal aspirate of patients with LRTS

6.10 Causative pathogen versus sample frequency prevalence of the causative agents

Inpatient

Table 6.10.1.A Percentage of bacterial species detected from BAL, sputum and tracheal aspirate of inpatients with LRTS (n=112)

Causative Pathogens	**No. of Samples**	**Percentage (%)**
Pseudomonas aeruginosa	37	33.04
Klebsiella spp.	30	26.79
Escherichia coli	14	12.50
Staphylococcus aureus	7	6.25
Streptococcus pneumoniae	5	4.46
Enterobacter spp.	8	7.14
Haemophilus influenzae	8	7.14
Acinetobacter spp.	3	2.68
Total	**112**	**100**

The above table compares the results from 112 sample specimen collected from the inpatients with lower respiratory tract infections. The table has demonstrated *Pseudomonas aeruginosa* (33.04%), *Klebsiella* spp. *(*26.79%), *Escherichia coli* (12.50 %), *Staphylococcus aureus* (6.25%), *Streptococcus pneumoniae* (4.46%), *Enterobacter* spp.(7.14%), *Haemophilus influenza* (7.14%), *Acinetobacter* (2.68%).

Outpatient

Table 6.10.1.2B Percentage of bacterial species detected from BAL, sputum and tracheal aspirate of outpatients with LRTS (n=18)

Causative Pathogens	**No. of Samples**	**Percentage (%)**
Pseudomonas aeruginosa	7	38.89
Klebsiella spp.	2	11.11
Escherichia coli	2	11.11
Staphylococcus aureus	2	11.11
Streptococcus pneumoniae	1	5.56
Enterobacter spp.	1	5.56
Haemophilus influenzae	3	16.67
Acinetobacter spp.	0	0.00
Total	**18**	**100**

The above table compares the results from 18 sample specimen collected from the outpatients with lower respiratory tract infections. The table has displayed *Pseudomonas aeruginosa* (39.89%) *Klebsiella* spp. (11.11%), *Escherichia coli* (11.11%) *Staphylococcus aureus* (11.11%), *Streptococcus pneumoniae* (5.56%), *Enterobacter* spp. (5.56%), *Haemophilus influenza* (16.67%), *Acinetobacter* spp. (0.00%).

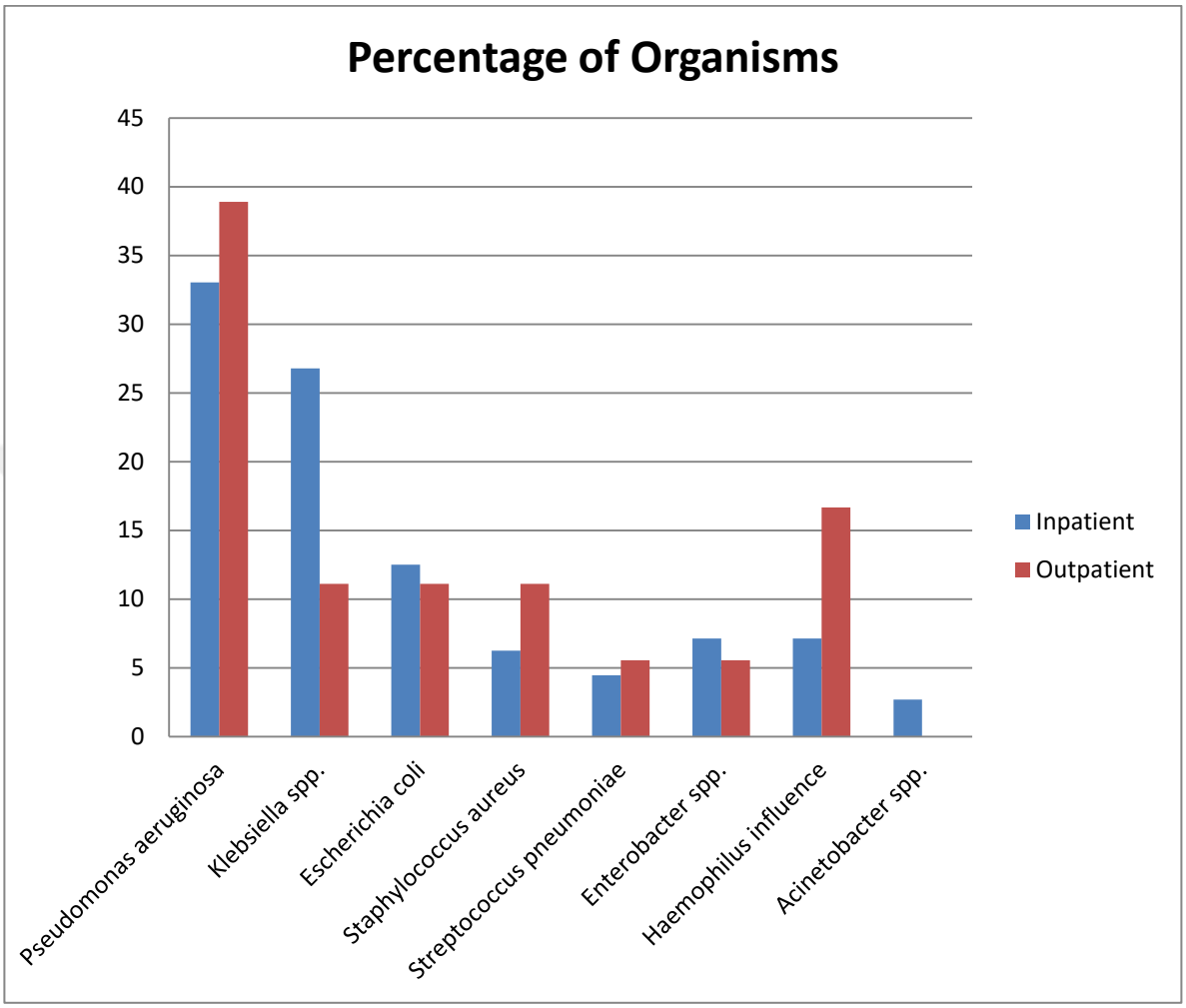

Figure: 6.10.1A& 6.10.1.2 B Percentage of bacterial species detected from BAL, sputum and tracheal aspirate of inpatients and outpatients with LRTS.

6.11 Gram Negative Bacilli and Gram Positive Cocci

Table 6.11.1 Percentage of Gram negative bacilli and Gram positive cocci

Pathogens	**Percentage (%)**
Gram Negative Bacilli	86 %
Gram Positive Cocci	14 %

Among the cases examined with Gram stain for positive and negative organisms, it was observed that 86% of the samples were Gram negative bacilli, while 14% of the samples showed to be Gram positive cocci. This means most of the samples were Gram negative.

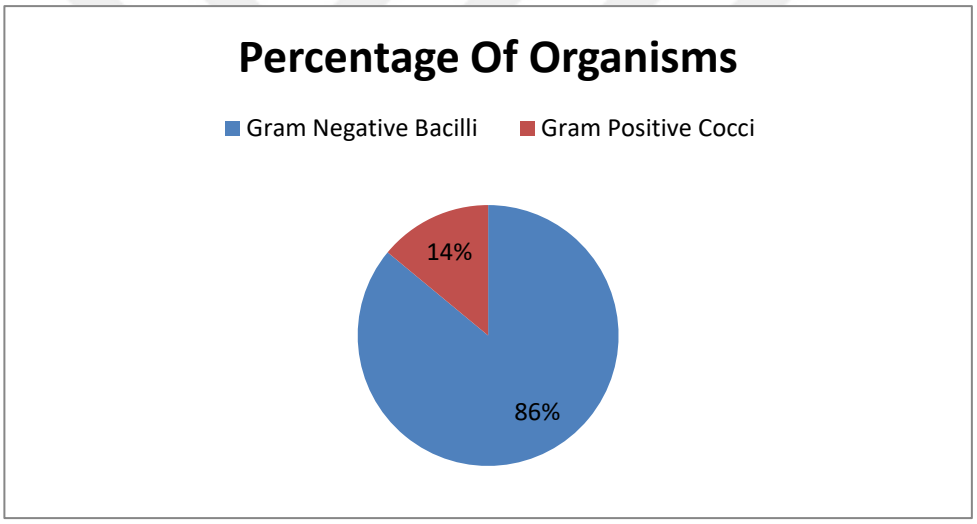

Figure 6.11.1 Percentage of Gram negative bacilli and Gram positive cocci

6.12 Drug Sensitivity Tests Results

Table 6.12.1 Antibiotic susceptibility test results of *Pseudomonas species*.

***Pseudomonas species* (n: 37)**

ANTIBIOTICS	SENSITIVE		RESISTANT	
	Number	**Rates**	**Number**	**Rates**
Amikacin	31	83.78	6	16.22
Gentamicin	28	75.67	9	24.32
Imipenem	25	67.56	12	32.43
Levofloxacin	26	70.27	11	29.73
Meropenem	27	72.97	10	27.03
Piperacillin-Tazobactam	25	67.56	12	32.43
Cefepime	31	83.78	6	16.22
Ceftazidime	27	72.97	10	27.03
Ciprofloxacin	28	75.67	9	24.32

6 out of 37 samples were found to be resistant to amikacin, yielding a resistance rate of 16.22%, 9 out of 37 samples were resistant to gentamycin, which means 24.32% resistance. 12 out of 37 samples were found to be resistant to imipenem, which means 32.43% resistance. 11 out of 37 samples were resistant to levofloxacin, which means 29.73% resistance, 10 out of 37 samples demonstrated resistance against meropenem, meaning 27.03% resistance. 12 of 37 samples were resistant to piperacillin-tazobactam,, meaning 32.43%resistance.

6 of 37 samples were resistant to cefepime, meaning 12% resistance. Ceftazidime resistance was seen in 27.03% of cases which is 10 out of 37 samples. 9 of 37 samples were resistant to ciprofloxacin, meaning 24.32% resistance.

The overall resistance rate observed was 25.5%. This is very close to the antibiotics resistance threshold of 25%. It can be seen the red line below figure the resistance rate.

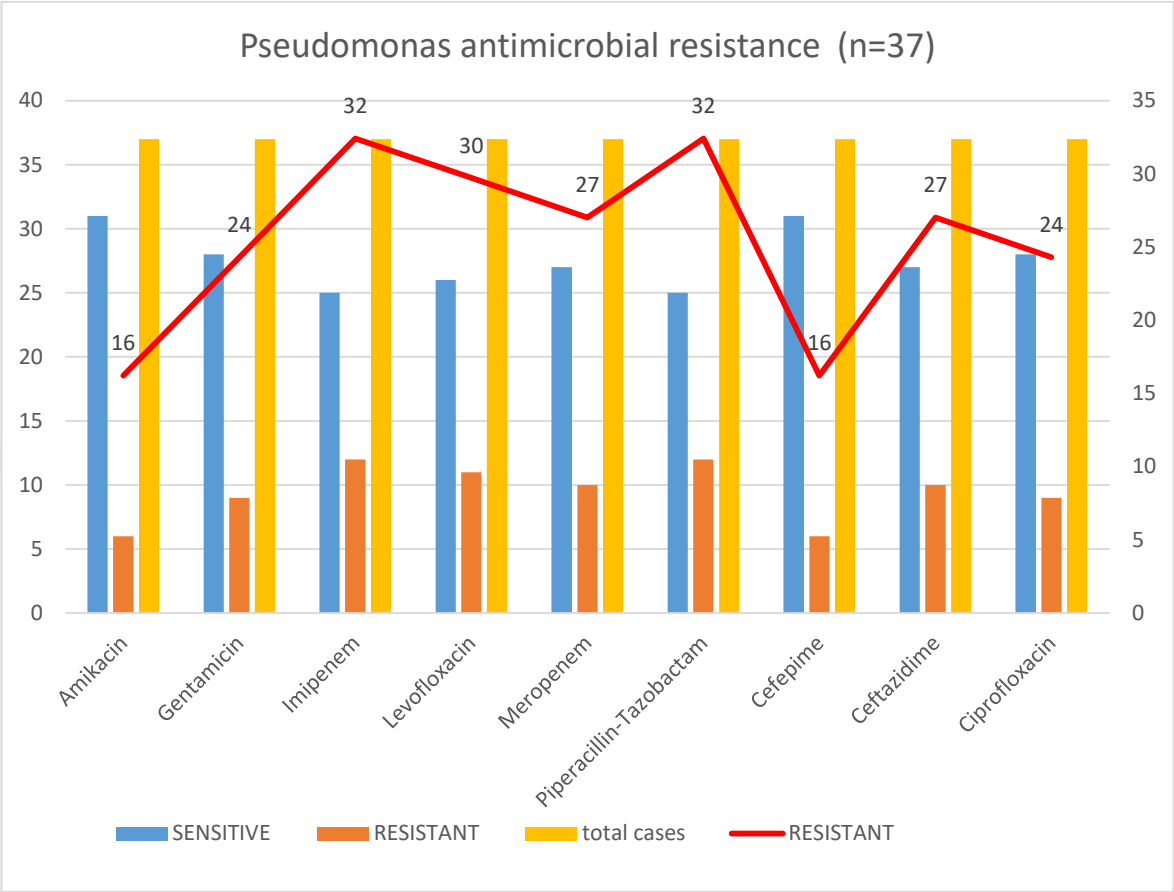

Figure**6.12.1** Antibiotic susceptibility test results of *Pseudomonas species*.

Table: 6.13 Antibiotic susceptibility test results of *Klebsiella species*

Klebsiella species **(n: 29)**

ANTIBIOTICS	SENSITIVE		RESISTANT	
	Number	**Rates**	**Number**	**Rates**
Amikacin	25	86.21	4	13.79
Amoxicillin-Clavulanic Acid	13	44.83	16	55.17
Ampicillin-Sulbactam	10	34.48	19	65.52
Gentamicin	19	65.52	10	34.48
Imipenem	23	79.31	6	20.69
Levofloxacin	21	72.41	8	27.59
Meropenem	23	79.31	6	20.69
Piperacillin-Tazobactam	13	44.83	16	55.17
Cefepime	18	62.07	11	37.93
Cefixime	15	51.72	14	48.28
Cefotaxime	16	55.17	13	44.83
Ceftazidime	16	55.17	13	44.83
Ceftriaxone	16	55.17	13	44.83
Cefuroxime	14	48.28	15	51.72
Ciprofloxacin	18	62.07	11	37.93
Trimethoprim-Sulfamethoxazole	17	58.62	12	41.38

Based on these results, very high resistance rates to some certain types of antibiotics are seen. This is very alarming including amoxicillin-clavulanic acid, ampicillin-sulbactam, cefuroxime which were all above 50% resistance rate. So here in this table 6.13 *Klebsiella* spp. have shown to be very resistant to most of the antimicrobials with the overall resistance rate of 42.6% which is very high.

Table 6.14 Antibiotic susceptibility test results of *E. coli*

E. coli (n: 15)

ANTIBIOTICS	SENSITIVE		RESISTANT	
	NUMBER	RATES	NUMBER	RATES
Amikacin	15	100	0	0.00
Amoxicillin-Clavulanic Acid	7	46.66	8	53.33
Ampicillin	0	0.00	15	100.00
Ampicillin-Sulbactam	4	26.66	11	73.33
Gentamicin	6	40.00	9	60.00
Imipenem	14	93.33	1	6.67
Levofloxacin	7	46.66	8	53.33
Meropenem	14	93.33	1	6.67
Piperacillin-Tazobactam	9	60.00	6	40.00
Cefepime	9	60.00	6	40.00
Cefixime	5	33.33	10	66.67
Cefotaxime	5	33.33	10	66.67
Ceftazidime	8	53.33	7	46.67
Ceftriaxone	5	33.33	10	66.67
Cefuroxime	4	26.66	11	73.33
Ciprofloxacin	6	40.00	9	60.00
Trimethoprim-Sulfamethoxazole	6	40.00	9	60.00

This table demonstrates sensitivity rates of *E. coli*. Some samples were shown to be very resistant. In the case of ampicillin, we found 100% resistance. This means that ampicillin cannot be used in the treatment of infections with the *E. coli*. *E.coli* also showed very high resistance rates towards amoxicillin-clavulanic acid and ampicillin-sulbactam with 53.33% and 73.33% respectively, similar with gentamicin 60%, levofloxacin 53.33%, cefepime, and cefixime both at 60%

The overall resistance rate for all of the listed antimicrobials was 51.4%. This means patients receiving any of the listed antibiotics against *E.coli* only have a chance of recovery in 50% of the cases and 50% may not response to the treatment.

Table 6.15 Antibiotic susceptibility test results of *Enterobacter species*

***Enterobacter* spp. (n:9)**

ANTIBIOTICS	SENSITIVE		RESISTANT	
	NUMBER	RATE	NUMBER	RATE
Amikacin	8	88.88	1	11.12
Amoxicillin-Clavulanic Acid	1	11.11	8	88.89
Ampicillin-Sulbactam	3	33.33	6	66.67
Gentamicin	8	88.88	1	11.12
Imipenem	8	88.88	1	11.12
Levofloxacin	8	88.88	1	11.12
Meropenem	8	88.88	1	11.12
Piperacillin-Tazobactam	7	77.77	2	22.23
Cefepime	8	88.88	1	11.12
Cefixime	6	66.66	3	33.33
Cefotaxime	6	66.66	3	33.33
Ceftazidime	7	77.77	2	22.23
Ceftriaxone	7	77.77	2	22.23
Cefuroxime	2	22.22	7	77.78
Ciprofloxacin	8	88.88	1	11.12
Trimethoprim-Sulfamethoxazole	7	77.77	2	22.23

Enterobacter sensitivity is demonstrated in table 6.15. Amoxicillin-clavulanic acid was 88.89%, ampicillin-sulbactam resistance was 66.67%. Cefixime and cefotaxime resistance was shown in 33.33% in *Enterobacter* species. The remaining antimicrobials are shown to be below the antibiotic resistance threshold of 25%.The overall resistance to the listed antimicrobials in the table above, have shown to be 33.3%.

Table 6.16. Antibiotic susceptibility test results of *Moraxella species*

***Moraxella* spp (n: 9)**

ANTIBIOTICS	**SENSITIVE**		**RESISTANT**	
	NUMBER	**RATE**	**NUMBER**	**RATE**
Amoxicillin-Clavulanic Acid	9	100	0	0.00
Ampicillin	5	55.55	4	44.44
Ampicillin-Sulbactam	9	100	0	0.00
Azithromycin	9	100	0	0.00
Imipenem	9	100	0	0.00
Levofloxacin	9	100	0	0.00
Meropenem	9	100	0	0.00
Cefixime	9	100	0	0.00
Cefotaxime	8	88.88	1	11.11
Ceftazidime	7	77.77	2	22.22
Ciprofloxacin	9	100	0	0.00
Cefuroxime	8	88.88	1	11.11
Ceftriaxone	9	100	0	0.00
Trimethoprim-Sulfamethoxazole	6	66.66	3	33.33
Tetracycline	7	77.77	2	22.22

Moraxella spp. sensitivity are shown in table 6.16, which indicates that only six antimicrobials were seen to be resistant, namely ampicillin 44.44%, ceftazidime 22.22%, cefuroxime11.11%, trimethoprim-sulfamethoxazole 33.33%, cefotaxime 11.11% and tetracycline 22.22% resistance rates. *Moraxella* spp. was sensitive to rest of the antimicrobials shown.

Table 6.17 Antibiotic susceptibility test results of *Acinetobacter species*.

Acinetobacter species (n: 8)

ANTIBIOTICS	**SENSITIVE**		**RESISTANT**	
	NUMBER	**RATE**	**NUMBER**	**RATE**
Amikacin	3	37.5	5	62.50
Ampicillin-Sulbactam	1	12.5	7	87.50
Gentamicin	1	12.5	7	87.50
Imipenem	1	12.5	7	87.50
Levofloxacin	1	12.5	7	87.50
Meropenem	1	12.5	7	87.50
Piperacillin-Tazobactam	1	12.5	7	87.50
Cefepime	1	12.5	7	87.50
Cefotaxime	1	12.5	7	87.50
Ceftazidime	1	12.5	7	87.50
Ceftriaxone	1	12.5	7	87.50
Ciprofloxacin	1	12.5	7	87.50
Trimethoprim-Sulfamethoxazole	3	37.5	5	62.50

Eight samples were collected and susceptibility tests were undergone against the list of the antibiotics in the right column.

It was found that *Acinetobacter* spp. were resistant against, the following antibiotics; amikacin, ampicillin-sulbactam, gentamicin, imipenem, levofloxacin, meropenem, piperacillin-tazobactam, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin with resistance rates of 87.50%. This means that they have less than 12.50% effect. Trimethoprim-sulfamethoxazole demonstrated resistance at a rate of 62.50%. These drugs cannot be used in the treatment of patients with *Acinetobacter* spp. related infections.

Table 6.18 Antibiotic susceptibility test results of *Haemophilus species*

Haemophilus species (n: 10)

ANTIBIOTICS	**SENSITIVE**		**RESISTANT**	
	NUMBER	**RATE**	**NUMBER**	**RATE**
Amoxicillin-Clavulanic Acid	6	60.00	4	40.00
Ampicillin	6	60.00	4	40.00
Ampicillin-Sulbactam	8	80.00	2	20.00
Imipenem	8	80.00	2	20.00
Levofloxacin	9	90.00	1	10.00
Meropenem	8	80.00	2	20.00
Cefixime	5	50.00	5	50.00
Cefotaxime	7	70.00	3	30.00
Ceftazidime	7	70.00	3	30.00
Ceftriaxone	7	70.00	3	30.00
Cefuroxime	5	50.00	5	50.00
Ciprofloxacin	8	80.00	2	20.00
Tetracycline	6	60.00	4	40.00
Trimethoprim-Sulfamethoxazole	4	40.00	6	60.00
Azithromycin	8	80.00	2	20.00

Table 6.18 indicates the susceptibility results of *Haemophilus species.* Based on antimicrobial tests of 10 samples, 32% overall resistance is shown.

As an example, amoxicillin-clavulanic was tested against *Haemophilus species*. As shown in the table, *Haemophilus* showed a resistance rate of 40% against the amoxicillin-clavulanic acid, and the same result was found against ampicillin and tetracycline. *Haemophilus* was shown to be resistant to amoxicillin-sulbactam, imipenem, meropenem, ciprofloxacin and azithromycin in 20% of the cases with 4 out of 6 samples resistant.

Haemophilus showed 30% resistance against cefotaxime, ceftazidime and ceftriaxone. Against trimethoprim–sulfamethoxazole *Haemophilus* indicated 60% resistance. *Haemophilus* was shown to be resistant to most of the antibiotics listed above.

Table 6.19 Antibiotic susceptibility test results of *S.pneumoniae*

***S. pneumoniae* (n: 5)**

ANTIBIOTICS	SENSITIVE		RESISTANT	
	NUMBER	RATE	NUMBER	RATE
Amoxicillin-Clavulanic Acid	5	100	0	0
Erythromycin	4	80.00	1	20.00
Imipenem	5	100	0	0
Clindamycin	4	80.00	1	20.00
Chloramphenicol	5	100	0	0
Levofloxacin	5	100	0	0
Meropenem	5	100	0	0
Penicillin	5	100	0	0
Cefixime	5	100	0	0
Cefotaxime	5	100	0	0
Ceftriaxone	5	100	0	0
Cefuroxime	5	100	0	0
Tetracycline	4	80.00	1	20.00
Trimethoprim-Sulfamethoxazole	1	20.00	4	80.00
Vancomycin	5	100	0	0

Drugs were tested on 5 samples of *S. pneumoniae* and they were found sensitive to most of the drugs except the following; erythromycin, clindamycin and tetracycline which indicated 20% resistance and trimethoprim-sulfamethoxazole which demonstrated 80% resistance. Meaning that *S.pneumoniae* was resistant against the four mentioned drugs. The overall resistance rate of *S.pneumoniae* against the listed antibiotics was 9.3%.

Table 6.20 Antibiotic susceptibility test results of *Staphylococcus aureus*

S.aureus **(n: 8)**

ANTIBIOTICS	SENSITIVE		RESISTANT	
	NUMBER	RATE	NUMBER	RATE
Azithromycin	7	87.50	1	12.50
Chloramphenicol	7	87.50	1	12.50
Levofloxacin	8	100.00	0	0.00
Penicillin	1	12.50	7	87.50
Ciprofloxacin	8	100.00	0	0.00
Tetracycline	7	87.50	1	12.50
Trimethoprim-Sulfamethoxazole	8	100.00	0	0.00
Teikoplanin	8	100.00	0	0.00
Clindamycin	7	87.50	1	12.50
Vancomycin	8	100.00	0	0.00
Gentamycin	7	87.50	1	12.50

We carried out drug sensitivity testing against *S.aureus* and the 11 antibiotics in table 6.20 was examined. Of the 11 antibiotics *S.aereus* was resistant to 6 and sensitive to 5. Those antibiotics that *S.aereus* was resistant to include: azithromycin with 12.50% resistance, chloramphenicol with12.50% resistance, penicillin with 87% resistance. The latter being very significant as it has virtually no effect on *S.aereus*. Tetracycline showed 12.50% resistance, clindamycin and gentamycin both showed 12.50% resistance rates.

7. DISCUSSION

The study represents numbers of cases based on IPD versus OPD, whose respiratory specimens have been received. The study showed that the majority of the patients were from IPD compared with OPD. In addition to this, based on gender, female gender accounted to be more than that of the male.

Other studies have shown that LRTI `s are higher in males than in females due to more prevalent associated risk factors (e.g. smoking and chronic alcoholism) of respiratory infections in males than females (63).

Our study showed the percentage of growth observed from specimen samples collected from LRTS by different mechanisms including BAL, sputum, and tracheal. Based on the results from the culture, 18 cases out of 36 samples with BAL specimens showed growth, which is about 50% of growth. 58 samples were observed showing fine growth out of 90 samples with sputum specimens collected, about 64.4% of growth was observed from those specimens, meanwhile 50 cases showed fine growth out of 74 samples of the samples collected from tracheal aspirates, about 67.6% of samples with growth. On the other hand the opposite percentage showed no growth.

Looking closer at antimicrobial susceptibility test results, the following is observed; on our *E. coli* strains; some samples had very high resistance rates. In the case of ampicillin, we found 100% resistance. This means that ampicillin cannot be used in the treatment of infections with *E. coli*. *E.coli* also showed very high resistance rates towards amoxicillin-clavulanic acid and ampicillin-sulbactam with 53.33% and 73.33% respectively.

E. coli was resistant to gentamicin in 60%, levofloxacin53.33%, cefepime, and cefixime 60%. This means patients receiving any of the listed antibiotics against *E. coli* only have a chance of recovery in 50% of the cases and 50% may not respond to the treatment. Carbapenems are the choice of treatment in infections caused by multidrug resistant *Enterobacter*iaceae. In recent years carbapenem-resistant *Enterobacteriaceae* isolates due to carbapenemases have been increasingly reported worldwide. Multicenter studies on carbapenemases are scarce in Turkey (64).

Observing the antimicrobial susceptibility test results, the following is observed on our *Klebsiella species;* some samples had very high resistance rates to some certain types of antibiotics such as amoxicillin-clavulanic acid (55.17%), ampicillin-sulbactam (65.57%), cefuroxime (51.72%), which were all above 50. In addition to that, carbapenem demonstrated a total resistance rate of 41.38% (Imipenem, 20.69% and meropenem, 20.69%).

A study intended to assess retrospectively the occurrence of carbapenemase-producing *Enterobacteriaceae* in the Medipol University Hospital in Istanbul, Turkey, found a great number of carbapenem-resistant isolates gained from patients hospitalized in the neonatal intensive care unit (NICU) for a short period. Different studies have concluded that the pervasiveness of NDM-1 Producers might be more significant than expected. However, the first case of NDM-1-producing *K.pneumoniae* in Turkey was attributed to come from Iraq. In addition to that, it revealed the outbreak of NDM-1-producing *K.pneumoniae* isolates in a Turkish hospital situated in Kayseri, Turkey, which is 800 km from Istanbul determining a probable concealed reservoir for those multidrug-resistant isolates (65).

In accordance with the antibiotic susceptibility tests made on *Klebsiella species*, our study revealed that the most of the antibiotics such as amoxicillin-clavulanic acid, ampicillin, ampicillin-sulbactam, amikacin, imipenem, and meropenem cannot be used in the treatment of *Klebsiella* spp.

Regarding the undertaken *Enterobacter* spp. sensitivity tests, amoxicillin-clavulanic and ampicillin-sulbactam demonstrated high resistance with a resistant rate of 88.8%, 66.67% accordingly while cefixime and cefotaxime showed a resistance of 33.33% for each. Moreover, the study found a total resistance rate of carbapenem which is 22.24% (Imipenem, 11.12% and meropenem, 11.12%). On the other hand, the remaining antimicrobials including imipenem and levofloxacin demonstrated high level of sensitivity.

Our findings suggest the tendency of carbapemen resistant strains to spread in Turkey as well. It is clear that more comprehensive infection control precautions and antibiotic usage control precautions should be implemented in hospitals (66). A similar study carried out by Acharya et al, looked into antibiotic susceptibility test of *Enterobacter* spp. and displayed that the whole antibiotics tested including cephotaxime and gentamicin had an absolute resistance of 100% against *Enterobacter* spp. (67).

Taking into account the presented results, it is clear that most of the tested antibiotics such as amoxicillin-clavulanic, ampicillin-sulbactam, amikacin, and meropenem cannot be used as empiric therapy in dealing with infections of *Enterobacter* spp. except imipenem and levofloxacin.

In line with the antibiotic susceptibility tests made on *Moraxella* spp., the study pointed out that six out of the fifteen antimicrobials such as ampicillin, ceftazidime, cefuroxime, cefotaxime, trimethoprim-sulfamethoxazole and tetracycline with a resistance rates 44.44%, 22.22%, 11.11%, 11.11%, 22 and 33.33%, and 22.22% accordingly were seen to be resistant whereas the rest proved complete sensitivity against *Moraxella* like amoxicillin-clavulanic acid, ampicillin-sulbactam, azithromycin, imipenem, levofloxacin, meropenem, and cefixime. On the other hand, a study carried out in Taiwan based on *Moraxella* spp. sensitivity contrasted a data between 1993–1994 and 2001–2004 showed that a rise in resistance of cefuroxime and tetracycline over the years. Comparing between the results it seems that most of the antibiotics are more likely to be used in healing patients infected with *Moraxella* spp.

It was found that *Acinetobacter* spp. were resistant against the following antibiotics; amikacin, ampicillin-sulbactam, gentamicin, imipenem, levofloxacin, meropenem,, piperacillin-tazobactam, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin with resistance rates of 87.50%. This means that they have less than 12.50% effect. Both trimethoprim-sulfamethoxazole and amikacin demonstrated resistance at a rate of 62.50%. These drugs cannot be used in the treatment of patients with *Acinetobacter* spp. related infections.

According to Sohail at el, in their antimicrobial susceptibility profile, antibiotic resistance profile of *Acinetobacter* indicated that cefotaxime and ceftazidime had a resistance rate of 99.2%, gentamicin 93.6%, amikacin 51%, and imipenem 90.9%. As both findings show high resistance rate we highly recommend to be avoided these antibiotics in treatment of *Acinetobacter* spp. (68).

Based on the findings of antibiotic susceptibility tests on *Haemophilus* spp., the study indicated that *Haemophilus* spp. demonstrated a resistance rate of 40% against amoxicillin-clavulanic acid, ampicillin and tetracycline antibiotics, while it represented a resistance rate of 20% according to the subsequent antibiotics; amoxicillin-sulbactam, imipenem, meropenem, ciprofloxacin and azithromycin. In addition to that, it showed a resistance rate of 30% against cefotaxime, ceftazidime and ceftriaxone whereas it had a 60% resistance alongside with trimethoprim-sulfamethoxazole against *Haemophilus* spp. This reveals that *Haemophilus* spp. species were shown to be resistant to the most of antibiotics discussed above.

Okesola and Ige found in their study made in Nigeria that some resistance exists in a number of generally utilized antibiotics and another study specified that amoxicillin clavulanate (73.9%) and ceftriaxone (87.0%) represented high susceptibility against *Haemophilus* spp. *(*69). In accordance with the above results, these antibiotics can probably be used in curing *Haemophilus* spp. infections although there is some extant of resistance.

According to *S.pneumoniae,* the study discovered that most of the tested antibiotics apart from erythromycin and clindamycin, which both demonstrated 20% of resistance, verified complete sensitivity against *S.pneumoniae* such as amoxicillin-clavulanic acid, imipenem, chloramphenicol, levofloxacin, meropenem, pencillin, cefixime, cefotaxime, ceftriaxone, and cefuroxime. Tetracycline and trimethoprim-sulfamethoxazole showed the following resistance rate 20% and 80% respectively.

Karcic et al concluded that erythromycin, clindamycin and trimethoprim-sulfamethoxazole presented the highest resistance rates against *S.pneumoniae* and recommended these to be stayed away from in the treatment, whereas tetracycline and chloramphenicol displayed the least resistance (70).

It can obviously be seen that most of the antibiotics showed absolute sensitivity including amoxicillin-clavulanic acid, imipenem, chloramphenicol, levofloxacin, meropenem, pencillin, cefixime, cefotaxime, ceftriaxone, and cefuroxime, so they are suitable in treatment of *S.pneumoniae* infected patients.

In keeping with *S.aureus* and the examined antibiotic sensitivity, the study presented that five out of the eleven tested antibiotics such as trimethoprim-sulfamethoxazole, teikoplanin, vancomycin, levofloxacin, and ciprofloxacin showed complete sensitivity against *S.aureus* whereas the penicillin accounted for high resistance rate of 87.50% and azithromycin, chloramphenicol, tetracycline, clindamycin and gentamycin with the resistance rate of 12.50% for each.

In relation to a study made in Uganda specified that trimethoprim-sulfamethoxazole scored a rate of resistance of 44.7% in both inpatient and outpatient departments respectively. However, gentamycin verified no resistance (71).

Thus, observing the above results, it is evident that all the examined antibiotics are applicable to be used in the treatment of *S.aureus* bacteria apart from penicillin, which demonstrated a resistance rate of 87.50%.

In relation with the infected patients and the departments they come from, findings showed that the most infected patients with *Pseudomonas aeruginosa, Klebsiella spp. Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Enterobacter* spp. and *Haemophilus* spp. were inpatients and those infected with *Acinetobacter* spp .who were all inpatients.

8. CONCLUSION

Resistance to carbapenems is of great concern as carbapenems are considered to be antibiotics of last resort to combat infections by multidrug resistant bacteria, especially in ICU and high-risk wards. While carbapenem resistance in *Pseudomonas* spp. and *Acinetobacter* spp. is well known, resistance among *Enterobacter*iaceae is increasing.

Turkey is recently and increasingly benefiting from medical tourism. In particular, some hospitals in Istanbul admit a great number of foreign patients, and such resistance emerging from patients admitted to the intensive care units of these hospitals is easily spreading throughout the country. Moreover, thousands of medical tourists come to Turkey from different parts of the world.

Carbapenem resistance was more likely to be seen in patients from organ transplantation department of our hospital. This is not surprising considering the fact that these patients frequently have to use broad-spectrum antibiotics to combat opportunistic and potentially lethal infections due to impairment of their immune system by their underlying malignant condition as well as the profound immunosuppression they have to undergo to avoid organ rejection.

Care should be taken to make a correct etiological diagnosis of the cause of respiratory infections as to give adequate treatment to patients, preventing unnecessary morbidity and mortality. A correct identification of the causative organism will also enable the physician to taper down unnecessary broad-spectrum antibiotics thus preventing any overuse, which in turn is known to elevate the resistance rates to antibiotics.

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10. APPROVAL Of ETHİCAL COMMİTTEE

MEDİPOL UNV İSTANBUL MEDİPOL ÜNİVERSİTESİ

E-İmzalıdır

T.C.
İSTANBUL MEDİPOL ÜNİVERSİTESİ
Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu Başkanlığı

Sayı : 10840098-604.01.01-E.1612
Konu : Etik Kurulu Kararı

19/01/2017

Sayın Yrd. Doç. Dr. Ayşe İstanbullu Tosun

Üniversitemiz Girişimsel Olmayan Klinik Araştırmalar Etik Kuruluna yapmış olduğunuz "Erişkin ve çocuk hastalarda alt solunum yolu infeksiyonlarında izole edilen mikroorganizmalar.Medipol Mega Hastanesi Vaka Çalışması (Lower Respiratory system infections bacterial agents of adults and children in Istanbul, A case study of Medipol Mega Hospital)" isimli başvurunuz incelenmiş olup, etik kurulu kararı ekte sunulmuştur.

Bilgilerinize rica ederim.

Prof. Dr. Hanefi ÖZBEK
Girişimsel Olmayan Klinik Araştırmalar
Etik Kurulu Başkanı

Ek:
-Karar Formu (2 sayfa)

Bu belge 5070 sayılı e-İmza Kanununa gore Prof. Dr. Hanefi OZBEK tarafından 19.01.2017 tarihinde e-imzalanmıştır.
Evrağınızı https://ebys.medipol.edu.tr/e-imza linkinden 123F7BE5X9 kodu ile dogrulayabilirsiniz.

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BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	Erişkin ve çocuk hastalarda alt solunum yolu infeksiyonlarında izole edilen mikroorganizmalar.Medipol Mega Hastanesi Vaka Çalışması" (Lower Respiratory system infections bacterial agents of adults and children in Istanbul, A case study of Medipol Mega Hospital)			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	Yrd. Doç. Dr. Ayşe İstanbullu Tosun			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI				
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ BULUNDUĞU MERKEZ	İstanbul			
	DESTEKLEYİCİ	-			
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☐	ÇOK MERKEZLİ ☒	ULUSAL ☒	ULUSLARARASI ☐

İSTANBUL MEDİPOL ÜNİVERSİTESİ GİRİŞİMSEL OLMAYAN KLİNİK ARAŞTIRMALAR ETİK KURULU KARAR FORMU

	Belge Adı	Tarihi	Versiyon Numarası	Dili
Değerlendirilen Belgeler	ARAŞTIRMA PROTOKOLÜ/PLANI	17.01. 2017		Türkçe ☒ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU	17.01.2017		Türkçe ☒ İngilizce ☐ Diğer ☐
Karar Bilgileri	**Karar No: 07**	**Tarih: 18/01/2017**		
	Yukarıda bilgileri verilen Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu başvuru dosyası ile ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve araştırmanın etik ve bilimsel yönden uygun olduğuna **"oybirliği"** ile karar verilmiştir.			

İSTANBUL MEDİPOL ÜNİVERSİTESİ GİRİŞİMSEL OLMAYAN KLİNİK ARAŞTIRMALAR ETİK KURULU	
BAŞKANIN UNVANI / ADI / SOYADI	Prof. Dr. Hanefi ÖZBEK

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		Araştırma ile ilişki		Katılım *		İmza
Prof. Dr. Şeref DEMİRAYAK	Eczacılık	İstanbul Medipol Üniversitesi	E☒	K☐	E☐	H☒	E☐	H☐	
Prof. Dr. Hanefi ÖZBEK	Farmakoloji	İstanbul Medipol Üniversitesi	E☒	K☐	E☐	H☒	E☐	H☐	
Yrd. Doç. Dr. Sibel DOĞAN	Psiko-onkoloji	İstanbul Medipol Üniversitesi	E☐	K☒	E☐	H☒	E☐	H☐	
Yrd. Doç. Dr. Devrim TARAKCI	Ergoterapi	İstanbul Medipol Üniversitesi	E☒	K☐	E☐	H☒	E☐	H☐	
Yrd. Doç. Dr. İlknur KESKİN	Histoloji ve Embriyoloji	İstanbul Medipol Üniversitesi	E☐	K☒	E☐	H☒	E☐	H☐	
Yrd. Doç. Dr. Mehmet Hikmet ÜÇIŞIK	Biyoteknoloji	İstanbul Medipol Üniversitesi	E☒	K☐	E☐	H☒	E☐	H☐	

* :Toplantıda Bulunma

11. CURRICULUM VITAE

Personal Information

Name	Mohammed Mohammed	**Last Name**	Ahmed
Place of birth	BELEDWEYN	**Date of birth**	1.1.1987
Nationality	SOMALI		
E-mail	halgan007@gmail.com		

Education Level

	Name of the Institute	**Year of Graduation**
PhD		
MSc	MICROBIOLOGY Medipol University	2016-2017
Undergraduate	Al-Nasser University San'a, Yemen	2010-2011
High School	Mujamac dibah taclimi	2005-2006

Work Experience

	Title	**Name of Organization**	**Duration (Year- Year)**
1.	**Lecturer**	Mogadishu University	2013-2015
2.	Head of Laboratory	Al-Birri General Hospital	2013-2015
3.	**Laboratory**	Madina Hospital	2012-2013

Foreign Languages	**Reading**	**Speaking**	**Writing**
ENGLISH	Very Good	Very Good	Very Good
ARABIC	Very Good	Very Good	Good

Computer Skills

Software	**Skill**
Micosoft Word	Good
Microsoft Exel	Good
Microsoft Power point	Good