STUDY OF BACTERIAL AND FUNGAL PROFILE IN BRONCHOALVEOLAR LAVAGE FROM CHRONIC RESPIRATORY DISEASES PATIENTS ATTENDING A TERTIARY CARE CENTRE IN THANJAVUR AND MOLECULAR CHARACTERISATION FOR THE COMMONEST ORGANISM ISOLATED.

Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the regulations
for the award of the degree of

M.D (MICROBIOLOGY)

BRANCH – IV

THANJAVUR MEDICAL COLLEGE,
THANJAVUR – 613004

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600032

APRIL 2017

CERTIFICATE

I hereby certify that the dissertation entitled, **" STUDY OF BACTERIAL AND FUNGAL PROFILE IN BRONCHOALVEOLAR LAVAGE FROM CHRONIC RESPIRATORY DISEASES PATIENTS ATTENDING A TERTIARY CARE CENTRE IN THANJAVUR AND MOLECULAR CHARACTERISATION FOR THE COMMONEST ORGANISM ISOLATED "** submitted to THE TAMLNADU Dr. M.G.R. MEDICAL UNIVERSITY , in partial fulfillment of regulations required for the award of M.D Degree in microbiology is a record of original research done by **Dr.M.BASHEER AHAMED,** carried out in the Department of Microbiology, Thanjavur Medical College, Thanjavur during the period from May 2015 to April 2016 under my guidance and supervision and the conclusions reached in this study are his own.

Dr.M. VANITHAMANI M.S.M.Ch,
The Dean,
Thanjavur Medical College,
Thanjavur – 613004.

Dr. EUNICE SWARNA JACOB,M.D.,
The Professor and HOD,
Department of Microbiology,
Thanjavur Medical College,
Thanjavur – 613004.

DECLARATION

I **DR.M.BASHEER AHAMED,** solemnly declare that this dissertation "**STUDY OF BACTERIAL AND FUNGAL PROFILE IN BRONCHOALVEOLAR LAVAGE FROM CHRONIC RESPIRATORY DISEASES PATIENTS ATTENDING A TERTIARY CARE CENTRE IN THANJAVUR AND MOLECULAR CHARACTERISATION FOR THE COMMONEST ORGANISM ISOLATED"** is a bonafide record of work done by me in the Department of Microbiology, Thanjavur Medical College, Thanjavur under the Guidance and Supervision of my Professor **Dr. EUNICE SWARNA JACOB,M.D.,**The Head of the Department, Department of Microbiology, Thanjavur Medical College, Thanjavur between May 2015 and April 2016.

This dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University , Chennai in partial fulfillment of University regulations for the award of M.D Degree (Branch – IV) in Microbiology to be held in April 2017.

Place:
Date:

Dr.M.BASHEER AHAMED,
Postgraduate Student,
Thanjavur Medical College,
Thanjavur.

Thanjavur Medical College

THANJAVUR, TAMILNADU, INDIA - 613001

(Affiliated to the T.N.Dr.MGR Medical University, Chennai)

INSTITUTIONAL ETHICAL COMMITTEE

CERTIFICATE

Approval No. : 158

This is to certify that The Research Proposal / Project titled

STUDY OF BACTERIAL AND FUNGAL PROFILE IN BRONCHOALVEOLAR LAVAGE FROM CHRONIC RESPIRATORY DISEASES PATIENTS ATTENDING A TERTIARY CARE CENTRE IN THANJAVUR AND MOLECULAR CHARACTERIZATION FOR THE COMMONEST ORGANISM ISOLATED

submitted by Dr. M. BASHEER AHAMED **of**

Dept. of MICROBIOLOGY **Thanjavur Medical College, Thanjavur**

was approved by the Ethical Committee.

Thanjavur

Dated : 10.04.2015

THANJAVUR MEDICAL COLLEGE M.E UNIT THANJAVUR-4

Secretary
Ethical Committee
TMC, Thanjavur.

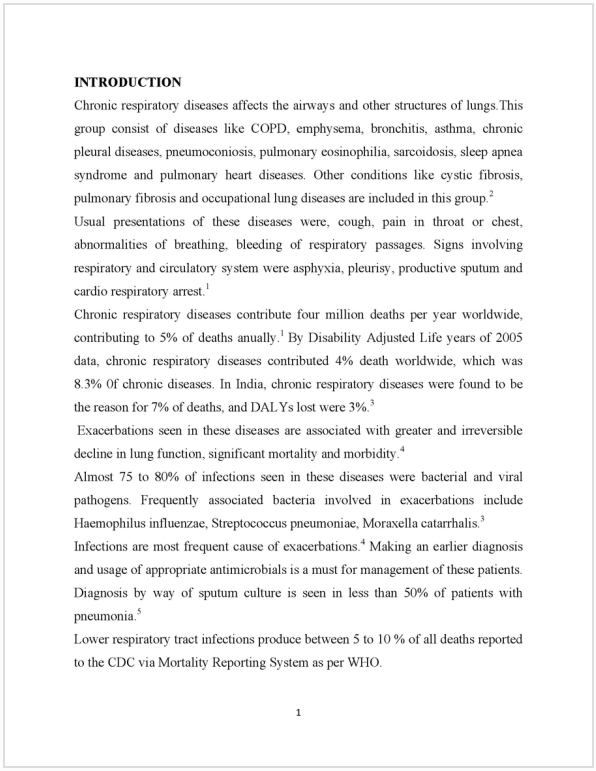
THE SECRETARY
INSTITUTIONAL ETHICAL COMMITTEE
THANJAVUR MEDICAL COLLEGE,
THANJAVUR.

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	201414201 Md Microbiology M. BA...
Assignment title:	2015-2015 plagiarism
Submission title:	Study of Bacterial and Fungal Prof...
File name:	Dr._Basheer_Dissertation_April_20..
File size:	2.07M
Page count:	87
Word count:	11,194
Character count:	66,244
Submission date:	29-Sep-2016 02:56PM
Submission ID:	710400042



INTRODUCTION

Chronic respiratory diseases affects the airways and other structures of lungs.This group consist of diseases like COPD, emphysema, bronchitis, asthma, chronic pleural diseases, pneumoconiosis, pulmonary eosinophilia, sarcoidosis, sleep apnea syndrome and pulmonary heart diseases. Other conditions like cystic fibrosis, pulmonary fibrosis and occupational lung diseases are included in this group.[2]

Usual presentations of these diseases were, cough, pain in throat or chest, abnormalities of breathing, bleeding of respiratory passages. Signs involving respiratory and circulatory system were asphyxia, pleurisy, productive sputum and cardio respiratory arrest.[1]

Chronic respiratory diseases contribute four million deaths per year worldwide, contributing to 5% of deaths anually.[1] By Disability Adjusted Life years of 2005 data, chronic respiratory diseases contributed 4% death worldwide, which was 8.3% 0f chronic diseases. In India, chronic respiratory diseases were found to be the reason for 7% of deaths, and DALYs lost were 3%.[3]

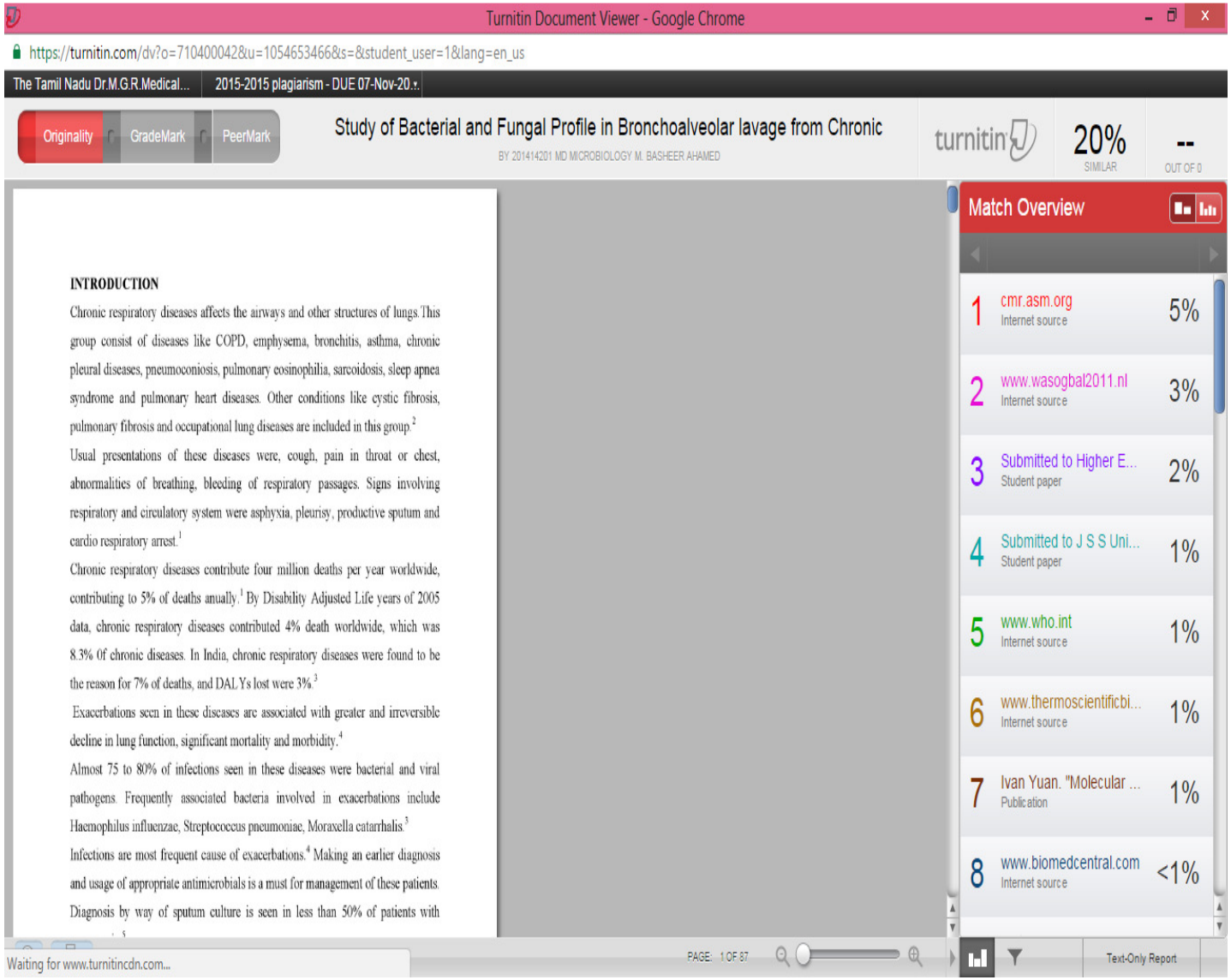
Exacerbations seen in these diseases are associated with greater and irreversible decline in lung function, significant mortality and morbidity.[4]

Almost 75 to 80% of infections seen in these diseases were bacterial and viral pathogens. Frequently associated bacteria involved in exacerbations include Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis.[3]

Infections are most frequent cause of exacerbations.[4] Making an earlier diagnosis and usage of appropriate antimicrobials is a must for management of these patients. Diagnosis by way of sputum culture is seen in less than 50% of patients with pneumonia.[5]

Lower respiratory tract infections produce between 5 to 10 % of all deaths reported to the CDC via Mortality Reporting System as per WHO.

1


Turnitin Document Viewer - Google Chrome
https://turnitin.com/dv?o=710400042&u=1054653466&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical...
2015-2015 plagiarism - DUE 07-Nov-20..
Originality
GradeMark
PeerMark
Study of Bacterial and Fungal Profile in Bronchoalveolar lavage from Chronic
BY 201414201 MD MICROBIOLOGY M. BASHEER AHAMED
turnitin
20%
SIMILAR
--
OUT OF 0
INTRODUCTION
Chronic respiratory diseases affects the airways and other structures of lungs.This group consist of diseases like COPD, emphysema, bronchitis, asthma, chronic pleural diseases, pneumoconiosis, pulmonary eosinophilia, sarcoidosis, sleep apnea syndrome and pulmonary heart diseases. Other conditions like cystic fibrosis, pulmonary fibrosis and occupational lung diseases are included in this group.[2]
Usual presentations of these diseases were, cough, pain in throat or chest, abnormalities of breathing, bleeding of respiratory passages. Signs involving respiratory and circulatory system were asphyxia, pleurisy, productive sputum and cardio respiratory arrest.[1]
Chronic respiratory diseases contribute four million deaths per year worldwide, contributing to 5% of deaths anually.[1] By Disability Adjusted Life years of 2005 data, chronic respiratory diseases contributed 4% death worldwide, which was 8.3% 0f chronic diseases. In India, chronic respiratory diseases were found to be the reason for 7% of deaths, and DALYs lost were 3%.[3]
Exacerbations seen in these diseases are associated with greater and irreversible decline in lung function, significant mortality and morbidity.[4]
Almost 75 to 80% of infections seen in these diseases were bacterial and viral pathogens. Frequently associated bacteria involved in exacerbations include Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis.[3]
Infections are most frequent cause of exacerbations.[4] Making an earlier diagnosis and usage of appropriate antimicrobials is a must for management of these patients.
Diagnosis by way of sputum culture is seen in less than 50% of patients with
Match Overview
1 cmr.asm.org Internet source 5%
2 www.wasogbal2011.nl Internet source 3%
3 Submitted to Higher E... Student paper 2%
4 Submitted to J S S Uni... Student paper 1%
5 www.who.int Internet source 1%
6 www.thermoscientificbi... Internet source 1%
7 Ivan Yuan. "Molecular ... Publication 1%
8 www.biomedcentral.com Internet source <1%
PAGE: 1 OF 87
Text-Only Report
Waiting for www.turnitincdn.com...

ACKNOWLEDGEMENT

I am immensely grateful to our Honourable Dean, **Dr.M. VANITHAMANI M.S.M.Ch** for permitting me to conduct the study.

I am also thankful to **Prof.Dr.M.ELANGOVAN,M.S.**,**F.I.C.S.,**Medical Superintendant, who allowed me to collect samples from bronchoscopy patients.

I am thankful to **Dr. SIVAKAMI, MS.,** Vice Principal, Thanjavur Medical College, by giving me the moral support for the project.

My sincere thanks to our Professor and Head of Department, **Dr.EUNICE SWARNA JACOB,M.D.,** who helped me to choose the topic and for guiding me throughout my work.

I am extremely grateful to my co-guide **Dr.C.K BHUVANESWARI,M.D.,** who was always been patient, encouraging and giving valuable suggestions to improve my work.

I take this opportunity to express my sincere gratitude to our Assistant Professors **Dr.D.AYISHA**,**M.D.,Dr.S.P.SHANMUGAPRIYA,M.D.,**whose optimism and encouragement was of immense help in caring out my dissertation work.

I express my sincere thanks to **Dr. A.MANIMARAN,M.D.,** Department of Thoracic Medicine and the Post Graduates for their kind cooperation and help.

I am thankful to all my Co-PGs for their kind support during the study.

I extend my thanks to laboratory technicians for their suggestions with regard to the practical aspects.

I also thank my family for their moral support and help.

Finally I also thank all the patients in this study.

LIST OF ABBREVIATIONS

BAL – Bronchoalveolar lavage..

FOB – Fibreoptic bronchoscopy.

CFU – Colony forming units.

LRTI – Lower Respiratory Tract Infection.

CDC – Centre for Disease Control and Prevention.

IMVIC – Indole/Methyl red/Voges-Proskauer/Citrate.

ATCC – American type culture collection.

CLSI – Clinical Laboratory Standards Institution.

MIC – Minimal inhibitory concentration.

WHO – World Health Organisation.

VAP – Ventilator associated pneumonia

TBB- Transbronchial biopsy.

PSB- Protected specimen brush.

PCP- Pneumocystis Pneumonia.

CMV- Cytomegalovirus.

DFA- Direct Fluoresent antibodies.

CONTENTS

INTRODUCTION

Chronic respiratory diseases affects the airways and other structures of lungs.This group consist of diseases like COPD, emphysema, bronchitis, asthma, chronic pleural diseases, pneumoconiosis, pulmonary eosinophilia, sarcoidosis, sleep apnea syndrome and pulmonary heart diseases. Other conditions like cystic fibrosis, pulmonary fibrosis and occupational lung diseases are included in this group.[2]

Usual presentations of these diseases were, cough, pain in throat or chest, abnormalities of breathing, bleeding of respiratory passages. Signs involving respiratory and circulatory system were asphyxia, pleurisy, productive sputum and cardio respiratory arrest.[1]

Chronic respiratory diseases contribute four million deaths per year worldwide, contributing to 5% of deaths anually.[1] By Disability Adjusted Life years of 2005 data, chronic respiratory diseases contributed 4% death worldwide, which was 8.3% 0f chronic diseases. In India, chronic respiratory diseases were found to be the reason for 7% of deaths, and DALYs lost were 3%.[3]

Exacerbations seen in these diseases are associated with greater and irreversible decline in lung function, significant mortality and morbidity.[4]

Almost 75 to 80% of infections seen in these diseases were bacterial and viral pathogens. Frequently associated bacteria involved in exacerbations include Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis.[3]

Infections are most frequent cause of exacerbations.[4] Making an earlier diagnosis and usage of appropriate antimicrobials is a must for management of these patients. Diagnosis by way of sputum culture is seen in less than 50% of patients with pneumonia.[5]

Lower respiratory tract infections produce between 5 to 10 % of all deaths reported to the CDC via Mortality Reporting System as per WHO.

Clinically visible chronic respiratory diseases are the ones usually diagnosed rather than the hidden entities. This can hamper the exact prevalence and morbidity data, which can show underestimated values of these diseases. Thereby it is difficult to formulate policies of prevention, diagnosis and treatment of chronic respiratory diseases in the population. So we need to standardize disease definition, have improved monitoring and a time bound proper surveillance.

Bacterial colonization of the distal airways, though poorly understood, can worsen the underlying disease. The constant presence of microorganisms in the distal airways can lead to severe progression of the conditions. We need to know the type of colonizing microbial agents, which can help in devising antibiotic protocols for treating these conditions affecting the lower respiratory tract. This has envisaged the importance of acquiring the quantitative invasive procedure like BAL for identifying the pathogen and aiding in their diagnosis and management.

With the arrival of bronchoscopy and quantitative invasive techniques like Bronchoalveolar lavage showed a marked improvement in the sensitivity and specificity of these diagnostic techniques in detection of pulmonary pathogens.[6]

BAL has the dual advantage of being the most appropriate one for most of the microbiological procedures, and usually has adequate volume to perform multiple tests. The procedure has become easily accessible to the community and is available at an affordable cost.

This study was done on patients of chronic respiratory diseases who underwent BAL, which was processed in the central diagnostic laboratory, department of microbiology and the results obtained were analyzed.

AIM OF STUDY

- To detect pathogenic organism by microscopical examination of Bronchoalveolar lavage specimens.
- To isolate and identify aerobic bacteria and fungus from Bronchoalveolar lavage specimens.
- To determine antimicrobial susceptibility pattern of the bacterial isolates.
- To determine the molecular characterization of the commonest isolated organism by Polymerase Chain Reaction.

REVIEW OF LITERATURE

Historical review:

BAL is an important procedure in identifying pulmonary diseases, ever since rigid bronchoscopy was done by Jackson in the year 1904. Initially BAL was used to treat patients with diseases causing productive secretions like alveolar proteinosis, cystic fibrosis and bacterial pneumonia.

BAL had two types, one was large volume and the other was small volume. Large volume was made up of saline 15 to 30 liters which was instilled in a two to three hour period of time to clear the lungs of its secretion. This was called as bronchioalveolar debridement. Small volume was instilling 300 ml of saline and clearing the lung. This was introduced in 1960s. It was a safe method and found to provide good information about the cellularity of the fluid content.

Flexible bronchoscope came in the 1970s, following which there was a high level of usage of the equipment and BAL. This helped in lot of research and numerous publications followed this between 1970 and 1990. Currently research activities are undertaken with publications of approximately 500 in number per year.

CHRONIC RESPIRATORY DISEASES[1]

Chronic diseases contributes to a huge share of premature adult deaths all over the world. Inspite of the magnitude of the problems these conditions are not very importance in the global arena. WHO's data on chronic diseases indicates urgent need for prevention and control.

The economic burden caused by chronic respiratory disease is a matter of concern due to its effect on occupational lung diseases. Diseases like asthma, respiratory allergies, COPD, occupational lung disease, sleep apnea are categorized as preventable chronic respiratory diseases. They cause a major health problem especially in low and middle income countries.

MAJOR RISK FACTORS[1]

1. Tobacco smoking.
2. Air pollution.
3. Allergens.
4. Occupational agents.

LIST OF CHRONIC RESPIRATORY DISEASES[1]

- Asthma
- COPD
- Chronic bronchitis
- Emphysema
- Bronchiectasis
- Hypersensitivity pneumonitis
- Chronic rhinosinusitis
- Lung carcinoma

- Neoplasm's of respiratory and intra thoracic organs.
- Lung fibrosis
- Chronic pleural diseases
- Pulmonary eosinophilia
- Pneumoconiosis
- Pulmonary heart disease
- Pulmonary embolism
- Pulmonary hypertension
- Cor pulmonale
- Rhinitis
- Sarcoidosis
- Sleep apnea syndrome

SIGNS AND SYMPTOMS

Haemorrhage from the airway passage

- Haemoptysis
- Epistaxis
- Cough
- Abnormal sputum
- Dyspnoea
- Abnormal breathing
- Stridor
- Wheezing
- Sneezing
- Hyperventilation
- Pain in the chest
- Pleurisy
- Respiratory arrest

Asthma[1]

Asthma is a chronic inflammatory condition of the airway associated with airway hyper-response and variable airflow obstruction that is reversible either spontaneously or under treatment. Allergen sensitization is an important risk factor for asthma and it is associated with rhinitis.

Chronic Obstructive Pulmonary Disease.[1]

COPD is a heterogeneous disease with various clinical presentations. The basic abnormality in all patients with COPD is airflow limitation. Global Initiative for Obstructive Lung Diseases have defined the disease based on spirometric criteria by using the post-bronchodilator forced expiratory volume in one second and its ratio to the forced vital capacity. The main criterion for COPD is a FEV1/FVC ratio <70%. At present chronic bronchitis and emphysema are different entities and not part of COPD.

Chronic Bronchitis[1]

Clinical definition

Chronic productive cough for three months in each of two consecutive years in a patient in whom other causes of productive chronic cough have been excluded.

Emphysema[1]

Anatomical definition

Permanent enlargement of the air spaces distal to the terminal bronchioles, accompanied by destruction of their walls without obvious fibrosis.

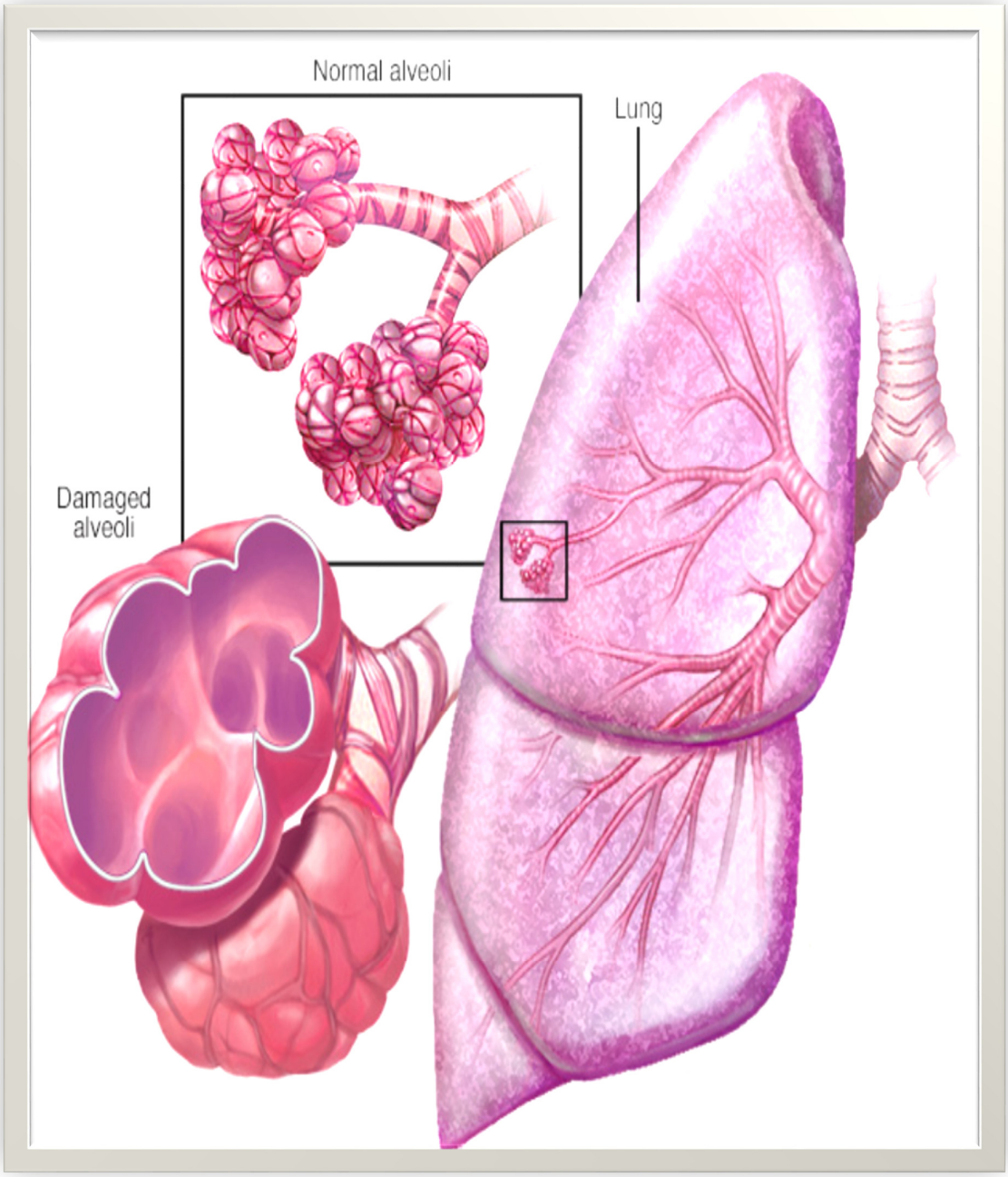
Pulmonary Hypertension[1]

Pulmonary hypertension is defined as a mean pulmonary artery pressure above 25 mm Hg. If untreated, this condition has a poor prognosis. This condition complicates other lung conditions like bronchiectasis, cystic fibrosis and COPD.

Epidemiology[1]

Chronic respiratory diseases affect less than 25 % of the population globally. This data is obtained mainly from high income countries. It is expected that number of cases will increase in the low and middle income countries. At present the available data on risk factors, burden and surveillance of chronic respiratory diseases are incomplete and fragmented.

Figure I



Infections of the Respiratory Tract[31]

The respiratory tract is divided into Upper Respiratory Tract and Lower Respiratory Tract. The upper airway starts from nose, throat, oropharynx and nasopharynx. The lower airway begins from larynx, trachea, bronchi, bronchioles and alveolar air sacs.

Infections of the Upper Respiratory Tract[31]

The oropharyngeal flora of normal individual is comprised of

Bacteria

Viridans streptococci,

B-hemolytic streptococci,

Staphylococcus aureus,

Haemophilus influenza,

Streptococcus pneumonia,

Moraxella catarrhalis,

Fusobacterium spp,

Actinomyces israelii,

Fungi

Candidia albicans.

Virus

Adenoviruses,

Herpes simplex virus.

In patient who are sick and hospitalized the indigenous flora switches from gram-positive to gram-negative in the upper airway.

Infection of the Lower Respiratory Tract.[31]

The lower respiratory tract consists of all structures below the larynx.

Trachea (tracheitis).

Bronchi (Bronchitis).

Bronchioles (Bronchiolitis) and

Distal air spaces (Pneumonia).

Pneumonia

The most serious infection of the respiratory tract is pneumonia, which is centered on the distal air spaces from the alveolar ducts to the alveolar sacs.

Definition

"Pneumonia is defined as a new, progressive or persistent (more than 24 hours) infiltrate on a chest radiograph when two or more of the following criteria were met.

macroscopic purulent sputum, temperature of more than 38.5°C or less than 36.5°C, leukocytosis of 10,000 cells/μL or more, or leukopenia of less than 4,000 cells/μl and dyspnea or worsening of respiratory status."

Symptoms

Fever,

Cough,

Sputum production and

Chest pain

Types

Atypical pneumonia.

Acute pneumonia and

Chronic pneumonia.

Specimens to Culture

Sputum.

Blood.

Bronchoscopy secretions.

Transtracheal aspirate.

Lung aspirates .

Biopsy.

Bacterial species potentially associated with infections[31]

Streptococcus pneumonia

Haemophilus influnzae

Staphylococcus aureus

Klebsiella pneumonia and other Enterobacteriaceae

Moraxella catarrhalis

Legionella spp

Mycobacterium spp

Fusobacterium nucleatum

Prevotella melaninogenicus

Bordetella species.

BRONCHOSCOPY

Definition:

It is a technique of visualizing the inside of the airways for diagnostic and therapeutic purposes by using a bronchoscope.[34]

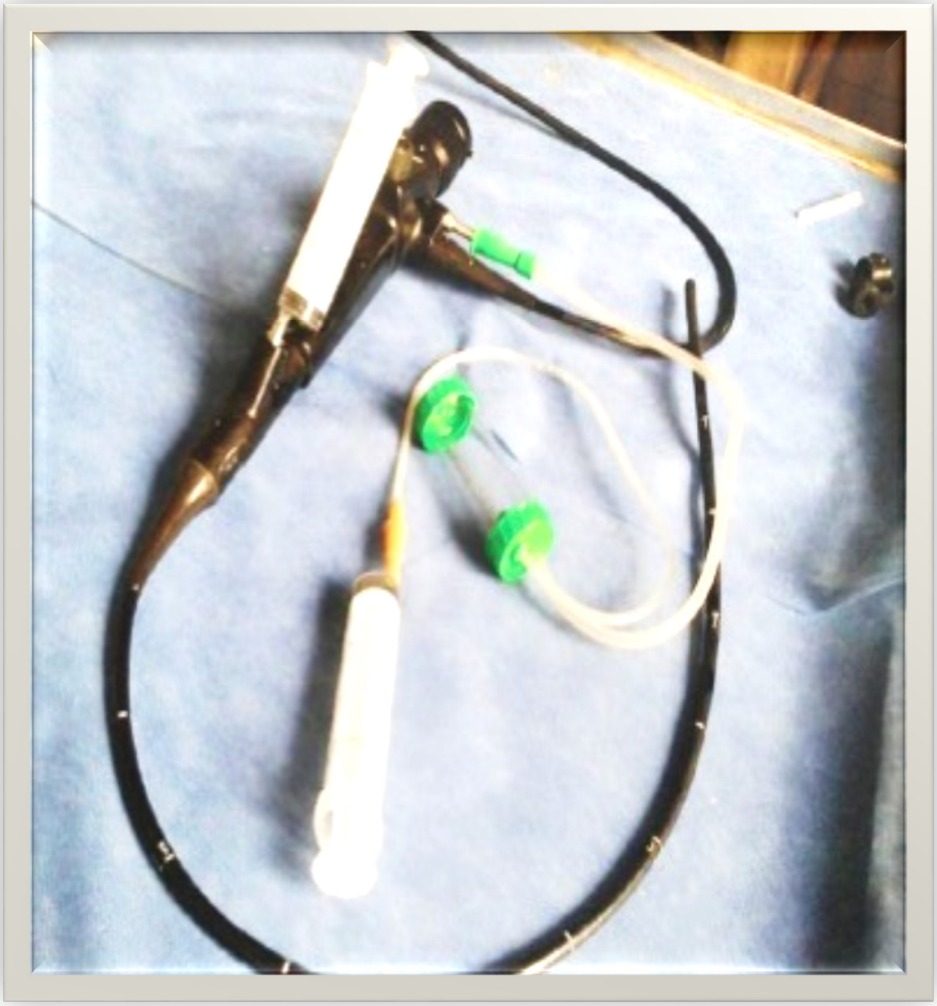
There are basically two types of bronchoscopy. First one is a rigid bronchoscopy and associated with a lot of complications. The second one is the flexible fibre optic bronchoscopy. This type is flexible and has fewer complications.

Bronchoscopy has both diagnostic and therapeutic uses in the field of medicine. Some of the diagnostic uses are to find the cause of haemoptysis, wheezing and uuexplained cough of more than 4 weeks duration. If radiological image is suggestive of atelectasis of segment, lobe or the entire lung. If there is a radiological opacity which is localized to a segment or lobe of the lung. It is indicated in obstructive emphysema to rule out any forgien bodies in the lung. To diagnose the cause of hilar and mediastinal shadows. To find out the reason for vocal palsy. Another main use is collecting bronchial secretions for bacterial and fungal culture and antimicrobial susceptibility testing. It is therapeutically used in the removal of foreign bodies, secretions and mucus.

Bronchoscopy is contraindicated in some condition. The procedure cannot be performed when proper oxygenation is not possible during scopy. It cannot be performed when the patient suffers from non treatable coagulation disorders. In conditions like marked kyphosis and aneurysm of thoracic aorta. New diagnosed myocardial infarction and unstable angina patients cannot undergo bronchoscopy. Lastly bronchoscopy cannot be done on respiratory failure patients requiring assisted ventilation.

Complication related to bronchoscopy are dental injuries, edema of the larynx, bleeding from the site of biopsy and lastly hypoxia and cardiac arrest.

FIGURE II - Flexible fibre optic bronchoscopy



Advantages of Flexible Fibre Optic Bronchoscopy

- Provides magnification and better illumination.
- Smaller size permits examination of subsegmental bronchi.
- Easy to use in patients with neck or jaw abnormalities.
- Can be performed under topical anaesthesia and useful for bedside examination of critically ill patients.
- Suction and biopsy channel provided helps to remove secretions, mucus plugs and small foreign bodies.
- Can be easily passed through endotracheal tube and in tracheostomy opening.

BRONCHOSCOPY SPECIMENS.[35]

Bronchial washing

Bronchial washings are the secretions aspirated at the back channel of the bronchoscope, after instillation of saline into a major respiratory passage.

Bronchial brushings

Bronchial brushings are exclusively used for exfoliative cytology which aids in the diagnosis of malignancy. Bronchial brush is prone for contamination during insertion into the bronchoscope, hence not an ideal material for bacterial cultures.

Bronchoalveolar lavage

BAL requires careful manipulation of the bronchoscope into the airways. Thereby it can reach the particular airway with ease. Here a large volume usually 140 milliliters provided in 3 to 4 aliquots is pushed into the lumen of the airway and the aspirated large volume can be used to sample the secretions and fluid of the distal segments of the airway.[36,37]

Commensal Flora of Respiratory tract.[31]

α/γ-Hemolytic streptococci

β-Hemolytic Steptococci, other than group A

Candida spp.

Coagulase-negative staphylococcus.

Corynebacterium spp. (diphtheroids)

Haemophilus parainfluenza

Neisseria spp.

Pathogens of Respiratory tract.[31]

Adeno virus

Anaerobes (as part of mixed infection)

Bordetella pertussis

Chlamydia pneumonia

Chlamydia psittaci

Corynebacterium diptheriae

Cryptococcus neoformans

Cytomegalovirus

Enterobacteriaceae

Haemophilus influenza

Herpes simplex virus

Legionella spp.

Moraxella catarrhalis

Mycobacterium spp.

Mycoplasma pneumonia

Myxoviruses and paramyxoviruses

Neisseria gonorrhoeae

Neisseria meningitides

Pneumocystis jivovecii

Pseudomonas aeruginosa

Stahylococcus aureus

Streptococcus pneumoniae

Streptococcus pyogenes (group A)

BRONCHOSCOPIC SPECIMENS

General characteristics.

Bronchoscopy produces several types of specimens for analysis. Here qualitatively and quantatively they must be taken into consideration for handing in a appropriate way for each. They is a high possibility of contaminations while passing through different anatomical site right from the upper respiratory tract to the lower respiratory tract.[59]

There is a possibility for yielding a true pathogen even in a mixed secretion which can pose a problem in analysis and interpretation of the specimens. Brushing samples are very limited, so the number of test performed on it are also less. Whereas with large volume lavage the samples are usually sufficient for multiple tests and analysis.[63]

Multiple types of specimen can be obtained by a single bronchoscopic procedure and the information obtained may complement each other. Types of specimens differ based on the clinical conditions suspected.[64]

In HIV positive cases commonly obtained specimen is BAL, TBB can add some more information in this sutiation. When bacterial pneumonia is suspected PBS samples is obtained. In other immunocompromised conditions BAL with PBS is obtained. TBB plays a vital role in proving tissue invasion of herpesviruses and opportunistic fungus. Bilateral BAL increases the sensitivity of identification of pathogens like P. jirovecii and CMV.[83,85]

When VAP is suspected BAL and PBS are obtained from the subsegment of the lung that is affected. But there is disagree in selecting a specific subsegment for diagnosis. In non ventilated patients with severe pneumonia both BAL and PSB specimens are collected.

BRONCHOALVEOLAR LAVAGE

Definition:

"BAL is a method for the recovery of cellular and non-cellular components from the lower respiratory tract (e.g. alveoli)."

In BAL procedure bronchoscopic tip is carefully wedged into an airway lumen which is isolated from the rest of the central airway. Greater than 140 milli liters of saline is injected into the lumen from 3 to 4 different aliquots. It is designed to sample, fluid and secretions of distal airway bronchioles and alveoli. An estimate of 1 million alveoli are sampled for 1 ml of actual pulmonary secretions returned in the total BAL fluid. There is a huge difference in the total return volume which ranges from 10 to100 ml.[36]

Due to the large volume available BAL is appropriate for microbiological procedures which involves multiple tests. Protected BAL catheter helps in reducing contamination of the upper airway.[37]

COMPLICATIONS OF BRONCHOSCOPY

The complication of bronchoscopy varies with condition of the illness. Respiratory arrest is a complication in severely ill patients with impending respiratory failure.[38]

Other major complications of BAL are hypoxemia, pneumothorax, cardiac compromise and hemorrhage. The is a rare possibility of transbronchial spread of infection in patient undergoing BAL.[39]

Indications for Bronchoalveolar Lavage

BAL is a critical tools for the work up of infectious diseases. It also has its application on diagnostic, therapeutic and research lines. BAL is performed in immune competent patients suffering from chronic pneumonia, tuberculosis and cystic fibrosis. It can also be performed in immune compromised conditions like HIV infection, organ transplantation and chemotherapy.[42]

BAL is also useful in evaluating non specific chronic respiratory symptoms, in clinical presentation suggestive of chronic interstitial lung disease, non specific radiological findings and diffuse parenchymal lung disease.

Uses of BAL in diagnosis of Infections

The conditions are as follows

- Ventilator associated pneumonia[40]
- Community acquired pneumonia[41]
- Hospital acquired pneumonia
- Mycobacterial infection of the lung
- Aspergillus infection of the lung
- Pneumocystis pneumonia.
- Toxoplasma pneumonia

- Mycoplasma pneumonia
- Legionella pneumonia
- Chlamydia pneumonia
- Viral pneumonia

Site of lavage

In the case of localized lesion like in lung infection the affected segment is lavaged. Sampling is influenced by radiologically apparent infiltration indicating the segment involved. The middle lobe or lingual is the most preferred site for lavage in diffused lung disease. The reasons are that the site is anatomically more accessible and the fluid of this one site represents the whole lung. Using this method, approximately 1.5 – 3 % of the lung are sampled. This amounts to sampling 100000 alveoli.

Fluid used

Sterile normal saline is used in performing lavage. It is preferable to used the saline at 37˚C which is equal to our body temperature. This precaution helps prevent cough in patients. The volume of sterile saline varies between 100 ml and 300 ml in aliquots of 20 to 50 ml capacity.

Fluid instillation and recovery

The fibro optic bronchoscope is wedged into a sub segmental bronchus. The fluid is instilled through the bronchoscope and almost immediately recovered by applying suction of 25-100 mmHg. Each aliquot is aspired into a separate syringe. Recovered aliquots are consecutively numbered.

In health individuals 60-70 % of the volume may be recovered. In patients with underlying pulmonary disease, the recovery rate is lower.

Factors affecting composition of BAL

Many factors can influence the quality and composition of BAL samples, including the total volume of saline instilled and the length of the dwell time between saline instillation and withdrawal, because ELF can be diluted by fluid exchange occurring between alveolar, vascular, and interstitial compartments.

The BAL sample should be considered adequate if there is 40% recovery of instilled fluid, ,5% epithelial cells and minimal amounts of mucus after filtering.

There is no reliable indicator to calculate the proportion of BAL fluid that represents ELF, which makes comparison between research studies difficult.

Guidelines for transport BAL.[43]

Saline solution of BAL should be transported in sterile, leak proof and non adherent container.[44] Potential use of sample is to identify strict pathogens of the lower respiratory tract. Designated personal in a laboratory should be responsible for dividing the sample aseptically for appropriate tests. BAL should be transported in specific transport media for diagnosis of mycoplasma, chalmydiae and viruses.[46]

Undue delay in transport should be avoided to prevent over growth of colonizing and contaminating organisms.[48]This will also help prevent decline of fastidious pathogens. 30 minutes is the optimal time and 2 hours at room temperature is the upper limit for transporting respiratory samples for processing bacterial pathogens excluding anaerobes. For isolation of anaerobes, BAL should be transported in anaerobic media and processed within 30 minutes.[45]

For fungal and mycobacterium, refrigeration for prolonged time is acceptable.[47]

Specimen Handling

Bronchoscopic specimens should be handled with clearly defined guidelines. Guidelines should cover specimen preparation, staining techniques, smear interpretation, culture techniques and culture interpretation.[52]

Minimum accepted volume for BAL is 10 ml for comprehensive microbiological analysis. Quantitative culture is performed directly from the fluid. BAL is centrifuged for other tests.[49]

Processing of BAL

BAL fluid is processed immediately upon arrival at the laboratory. Since the first aliquot is usually poorly recovered and reflects a disproportionate amount of bronchial material, this fraction is used for mycobacterial investigation.[50]

Other fractions are pooled and processed. Equal parts of fractions are pooled and used for further investigation, such as total cell count, cytospin preparations and quantitative culture.

When indicated additional tests can be included like detection of fungi, Legionella pneumophillia, viruses, detection of Mycoplasma pneumonia and Chlamydia pneumonia by means of polymerase chain reaction.[51]

Quality control of BAL

To ensure that the obtained material represents the situation in the alveoli, a number of criteria have been established.

A BAL fluid is regarded non-representative if one of the following criteria is seen:

- Volume less than 20 ml.
- Total cell count less than 60,000 cells/ml.
- Presence of more than 1% squamous epithelial cells.
- Presence of more 5 % bronchial epithelial cells.
- Presence of extensive amounts of debris.
- Severely damaged cell morphology.

DIRECT MICROSCOPY OF BAL

Direct microscopy may be viewed as three distinct categories:[49]

- Stains for cytological assessment,
- Stains for general organism categories and
- Stains for specific organism types.

Cytological assessment.

BAL sample histopathology detects cell changes due to viral infection and invasive fungal infections. To assess oropharyngeal contamination of BAL the relative numbers of inflammatory cells and squamous epithelial cells are examined by Gram stain. Increase in the percentage of neurophils and lymphocytes is noted in infectious conditions. Elastin fibers are noted in necrotizing pneumonia.[53]

Organism detection

Different staining procedures are used in detection of organism. Gram staining is frequently employed for bacterias. It provides quick information on morphology of the bacteria which provides valuable information in the initial

selection of antibiotics.[54] For the detection of Mycobacterium spp. Stains like carbol fuchsin based acid fast stain and auramine rhodamine are used. Modified acid fast stain is used in the identification Nocardia spp. Cell wall stains like methenamine silver and calcofluor white is used in the identification of Pneumocystic jirovecii and fungus.[55]

Specific stains

DFA staining is used for detection of Legionella, Pneumocystis jirovecii, respiratory viruses, CMV and Herpes simplex virus.[57]

OVERVIEW OF LABORATORY METHODS

Bronchoscopic specimens microbiological analysis is made complicated due to reasons like pathology of infectious lung diseases and practical difficulties of specimen handling.[58]

Firstly the etiological agents belong to a large group, comprising of all major categories of microorganism. So the laboratory must be prepared to identify the vast number of organisms by employing a number of different procedures. Many infections are polymicrobial in nature and necessitates to perform multiple analysis on the specimen.[60]

Secondly, even when careful bronchoscopic techniques are used, the analysis of specimens is confounded by the inevitable presence of colonizing organisms that may also be etiologic agents of disease in the respiratory tract of seriously ill patients. To differentiate colonization from infection, the laboratory must employ quantitative culture techniques.[59]

Thirdly, because of the clinical need for timely institution of specific therapy, special emphasis is placed on the availability of tests with rapid turnaround times, especially direct microscopy.[68]

Rapid processing of specimens for culture is also desirable to prevent loss of viability of pathogens or overgrowth of contaminants in these unpreserved specimen types.[67]

Finally, the renewed emphasis on cost containment in the era of managed competition challenges the laboratory to accomplish all of these tasks in a cost effective manner.[56]

Microbiologic Spectrum

An overview of the microorganisms that may be etiologic agents of lower respiratory tract infection in adults in the clinical settings in which bronchoscopy is frequently performed is given below as a table.

Gram positive aerobic bacteria	Staphylococcus aureus, Streptococcus pneumonia and Viridans group
Gram negative aerobic bacteria	Pseudomonas aeruginosa, Acinetobacter spp., Enterobacter spp., Klebsiella spp., Moraxella catarrhalis, Haemophilus influenza.
Viruses	Herpes virus, Respiratory viruses.
Fungi	Cryptococcus neoformans, Candida spp., Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis, Sporothrix schenkii, Aspergillus spp., Pneumocystis jirovecii
Protozoa	Toxoplasma gondii, Leishmania spp., Microspordia, Cryptosporidium parvam,
Helminths	Strongyloides stercoralis.

Organisms of the lung are classified into two groups. Some organisms are considered pathogens of the lung and the others are considered as colonizing flora of the respiratory system. Later organisms present in the respiratory secretions pose technical difficult in detection. This difficult can be minimized by careful selection of specimens, collection and critical interpretation of the findings.

Strict pathogens which does not pose difficult in diagnosis are Legionella spp., Mycoplasma pneumoniae, Chlamydia spp., Nocardia spp., Mycobacterium tuberculosis, Francisella spp., Bordetella spp., systemic dimorphic fungi, Seasonal respiratory viruses, Protozoans and helminthes.[61]

Careful clinical assessment at times can predict the etiologically agent in a given patient. But there is significant overlaps in the clinical features imposing the need of laboratory processing of respiratory samples for detection of the pathological agents.[59]

In 30 to 60 % of cases suspected of pneumonia no specific etiological organism is identified even with an extensive battery of non invasive tests done. In this scenario, if bronchoscopy is incorporated with well defined diagnostic protocol than the percentage of identification of pathogens rise to 80 to 90 %. So total diagnostic yield of bronchoscopy is greater in comparison with non invasive methods.[62]

.

Stains performed on BAL fluid

Application of BAL fluid in the diagnosis of pulmonary infections has been proven to be very useful. A number of stains are routinely performed on BAL fluid samples. These includes the Gram-stain and May-Grunwald Giemsa stain.

Besides the standard stains, a number of stains can be added to the investigation upon clinical indication.

For example:

Grocott methenamine-silver for fungi and pneumocystis jiroveci.

Auramine-Rhodamine, Ziehl-Neelsen for acid-fast bacteria.

Legionella immunofluorescence for Legionella spp.

Acridine-organe for microorganism in general.

BAL culture procedures

For most organisms, culture remains the definitive diagnostic method.

Basis of quantitative culture

Quantitative culture method is employed there to differentiate pathogens from contaminants as it is difficult to avoid oropharyngeal contamination during collection of BAL.[37,67]

Pathogens	More than 10^5 CFU/ml
Contaminants	Less than 10^4 CFU/ml

Quantitative culture methods

It is the gold standard method in the diagnosis of pneumonia in BAL sample. BAL fluid represents the situation of alveoli by 10 to 100 times.[49]

Two methods are employed for quantitative culture of BAL. One is the serial dilution method where two 100 fold dilution from 0.1 ml sample are spread on surface of the agar plate. Counts are obtained from the plate with more number of colonies and without confluent growth. Reported as cfu per

ml.[7] Second method is calibrated loop method and this method is very easy and practical. This method is similar to urine culture and 0.001 or 0.01 ml is suitable for BAL sample. Results are given as $\log_{10}$ ranges.[70]

Quantitative culture interpretation

In both type of procedures, each colony types should be separately quantitated and reported. The identification of the organisms and antimicrobial susceptibility testing should be decided based on the quantitaion of the organisms. Colonies which are less than the cut off value should be considered contaminants.[69]

Positive quantitative bacterial culture from BAL fluid

A positive quantitative culture was defined as more than10^5 cfu/ml of Bacteria from BAL sample.[37]

Diagnostic methods employed in detection of various microorganisms in BAL fluid

For the identifications of Bacteria Gram's staining and Quantitative bacterial cultures are used. Chlamydial infections are detected by direct immunoassay, culture and molecular methods. Fungi like pneumocystis jiverocii is identified by staining methods like Gomori-Grocott, Toludine blue and Wright-Giemsa stains. Other methods which are of use are direct immunoassay and Molecular methods.

Other fungus are identified by Gram's stain, Gomori-Grocott, culture and direct immunoassay.

Legionella infections are diagnosed by Gomori-Grocott staining, direct immunoassay, culture and molecular methods. Infections caused by mycobacterium are detected by Auramine-rhodamine staining, Zielh-Neelsen

staining, culture and molecular methods. Mycoplasma is identified by Immunoassay, culture and molecular methods.

Viruses like Cytomegalovirus, Herpes simplex virus are identified by cytopathic effect in tissue culture, inclusion bodies, direct immunoassay, culture, electron microscopy and molecular methods.

Disc diffusion methods of antimicrobial sensitivity testing.[33]

These methods are suitable for organisms that grow rapidly overnight at 35-37˚C. The antibiotic-impregnated disc adsorbs moisture from the agar and antibiotic diffuses into the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance increases, there is a logarithmic reduction in the antibiotic concentration.

The extent of antibiotic diffusion is affected by the depth of the agar. Visible growth of the bacteria occurs on the surface of the agar where the concentration of the antibiotic has fallen below its inhibitory level for the test strain.

The time required to reach the critical cell mass (4 to 10 hours) is characteristic of each species but is influenced by the media composition and incubation time. The point at which the critical cell mass is reached appears as a circle of bacterial growth, with the middle of the antibiotic disc forming the centre of the circle.

There is no single internationally accepted method of disc diffusion testing. In the USA a modification of the Kirby-Bauer (Bauer et al 1966) method is used. This method is recommended by the CLSI and WHO. Standardization of the technique controls variation, and interpretation is by comparison of inhibition zones with published tables of critical zone diameter.

In Europe, guidelines for diffusion methods are available from the European Committee for Clinical Laboratory Standards. Rigid standardization of technique is published regression lines that plot MICs for strains of different species against the zone diameter produced by a disc of the strength used in the test. The validity of this method is controversial.

Specific etiologic agents

Based on the specific agents of etiology, bronchoscopy as a varying indication in the process of diagnosis. Bronchoscopy is the first line procedure in some cases and bronchoscopy plays a secondary role when there is a failure in empirical therapy and inability to identify the causative agent by non invasive techniques.

Bacterial Pathogens

Aerobic bacteria

Gram staining and quantitative culture of BAL has become an important tool for the diagnosis of pneumonia caused by aerobic bacteria. When 10^6 cfu/ml is used as the threshold for BAL quantitative culture the sensitivity increases.[71]

Anaerobes

Role of bronchoscopy for diagnosis of anaerobic infections is not established beyond doubt. Studies are needed in this area to establish the significance of bronchoscopy.[72,73]

Legionella spp.

In conditions like hospital acquired pneumonia, CAP and transplant cases Legionella has been identified as important lung pathogen. Bronchoscopy is used in this conditions due to absence of sputum production.[74] Increased number of polymorphonuclear cells in respiratory secretions with the absence of recognizable morphotype is indicative of legionellosis. BAL specimen is useful in rapid diagnosis of Legionella by DFA staining.[75]

Nocardia spp

BAL is identified as a useful specimen in the diagnosis of Nocardia as important lung pathogen in groups like immunocompromised and organ transplantation cases.[76] The organism is identified microscopically gram positive, branching with beaded filaments. They are acid fast to weak acid decolorizer.Charcoal-yeast extract medium is used as culture media for primary isolation of Nocardia.[77]

Mycoplasmas

Knowledge in use of bronchoscopic samples for diagnosis of mycoplasma pneumonia is very much limited due to reasons like diffuiculty in culturing the organism and lack of alternative methods ilke direct specimen. BAL sample isolation of Mycoplasma pneumonia is seen in conditions like Community acquired pneumonia and pneumonia in immune suppressed patients.[79]

The commonest specimen used are swabs of upper respiratory system. There is no study comparing BAL and upper respiratory swab.[78]

Chlamydia spp

BAL isolation of Chlamydia pneumonia in clinical setting like hospital acquired infection and HIV cases has been successful. Culture of this organism is difficult due to the need of special transport media, suitable cell culture and specific reagents. So serological diagnosis can be used as an additive to culture. Study by Gaydos et al reported 12 PCR positive from 132 culture negative BAL specimens from immune suppressed cases. This observation proves PCR is more sensitive than culture.[80]

Mycobacterial Infections

Bronchoscopy has proven useful in sputum smear and culture negative with atypical presentation where the clinical suspicion is very high like in the case of military disease. BAL and Bronchial washing were observed to have similar sensitivity in the diannosis. Since new culture methods provide rapid results than conventional culture methods, it s unclear when to perform bronchoscopy as a diagnostic procedure.[81]

Mycobacteria other than M. tuberculosis

Mycobacterium kansasii is clinically significant and difficult to isolate form respiratory secretions. Upper airway colonization is present with Mycobacterium avium complex in HIV patients and Mycobacterium fortuitum in intensive care unit patients. BAL with TBB if performed gives maximum diagnostic yield. Studies show BACTEC system for isolation of Mycobacterium from bronchoscopy specimen provides increase in the isolation.[82]

Pneumocystis jirovecii

Bronschoscopy has been accepted as a definitve diagnostic method for Pneumocystic jirovecii due to AIDS epidemic and recognition of Pneumocystis jirovecii as lung pathogen. BAL had 82% sensitivity when compared with brushing and washing which was only 53% sensitive. BAL sample of bilateral lavage procedure has greater sensitivity when compared with unilateral Bal sample. Induced sputum a non invasive alternative to BAL had sensitive ranging from 15 % to 100 %.[83]

Common staining techniques of Bronchoscopic specimens used in the detection of Pneunocystic jiverocii cyst are Gomori's methenamine silver, Gram-Wiegert, Toluidine blue O and calcofluor white. For the detection of trophozoites and intracycstic bodies modified Wright-Giemsa method is used.

Viral Infections

Common respiratory viruses.

A role for bronchoscopy in detection of common, seasonal respiratory viruses like respiratory syncytial virus, parainfluenza viruses, and influenza viruses is not well established. Nasopharyngeal, tracheal swab and aspirates are preferred respiratory samples for culture and antidetection in viral infection.

Viruses can be detected in bronchoscopic samples of severely ill patients suspected with suspection of non bacterial pneumonia. Laboratories should be equipped to detect viruses from bronchoscopic samples in epidemic suitations. Viruses can be identified from BAL by culture, DFA and antigen detection.[84]

Cytopathological changes of BAL cells may help in the recognition of respiratory syncytial virus. Cytopathological changes and culture of bronchoscopic samples have demonstrated adenovirus and measles virus.

Latent viruses

It is most challenging to diagnose latent viruses like herpes and the interpretation of the lab results becomes moer difficult in immune compromised setting. In bronchoscopic specimens CMV poses challenges, due to immune suppression of cell mediated immunity reactivates latent infections. CMV is reported to be frequently associated with pneumonia in organ transplantation and HIV infection.[85]

CMV.

CMV pneumonitis manifest as a mild focal interstitial process to severe diffuse alveolar damage. Bilateral BAL sample increases the sensitivity of diagnosing CMV infection. BAL offers addition advantage of diagnosing other opportunistic pathogens since CMV coexists with other pathogens. Since CMV frequently coexists with other pathogens, BAL offers the added advantage of the opportunity to detect other pathogens. Methods like cytopathology, DFA, culture and in situ hybridization are employed to detect CMV for BAL. Of the above methods culture is more sensitive.

HSV.

HSV is present in respiratory secretions of immune suppressed individuals and oropharynx of severely ill cases with pneumonia. Pneumonia in HSV spreads from contiguous mode and less common by hematogenous route to lung. Due to the above reasons HSV may be a pathogen or contaminant in bronchoscopic samples.

Other latent viruses

Lymphocytic interstial pneumonitis is associated with HIV and Epstein-Barr virus.

Bronchoscopic samples role is not well documented in the diagnosis of these conditions.[85]

Protozoans and Helminths

Bronchoscopic samples demonstrates unusal parasites occasional in immune compromised host. The diagnostic ability of the microbiologist paly an important role in identifying such parasites. Since pneumonia is associated with disseminated infection in such conditions other lab investigations like stool examination and serology should be done as indicated.

Toxoplasma gondii

BAL sample proves to be useful in the diagnosis of pulmonary toxoplasmosis. Staining methods like Wright-Giemsa are used to demonstrate intra and extracellular tachyzoites. As the organism as can be easily missed to it important to examine under oil immersion. Most of the disease in immunocompromised hosts is due to reactivation odf the organism dormant in cysts, serological tests can be made use for screening in high risk cases.[86]

Other protozoans

Many other protozoans of opportunistic nature has been noted in bronchoscopic samples. In HIV-infected cases intracellular Leishmania amastigotes were demonstrated in alveolar macrophages of BAL samples for endemic areas. By using acid-fast and specific DFA stains Cryptosporidium cysts was identified in bronchoscopic samples. Large numbers of microsporidia was demonstrated in BAL samples by special chromotrope stain in HIV cases.[87]

Strongyloides stercoralis

Immunocompromised cases with hyperinfection syndrome may present with Strongyloides stercoralis filariform larvae in respiratory secretions. Low power examination of BAL sample with any stained material can demonstrate the larvae.[88]

Other helminths

Ovas Paragonimus westermani has been noticed in the sputa of suspected causes of tuberculosis in south East Asia. Rarely other helminthes have been reported in respiratory samples.[89]

FUNGAL INFECTIONS

Systemic fungal infection

In respiratory secretions isolations of any systemic or dimorphic fungus has clinical significations. If possible, first non invasive specimens should be evaluated. If results are negative than bronchoscopic specimen can be test if there is a strong clinical suspicion.[91]

Sputum has a low sensitivity in detection of fungi in smear and culture when compaired with bronchoscopic specimen. Tissue diagnosis may be required to confirm systemic fungal infection. BAL in predominantly immuncocompromised conditions yields 85 to 100 % sensitive in recent studies. This make BAL a preferred bronchoscopic specimen.[90]

In any types of respiratory secretion, the microbiologist should be aware of distinctive microscopic morphologies of the tissue form of the fungi and report accordingly. As microscopic finding of fungus is not specific and sensitive, the following methods like isolation with appropriate selective media and rapid identification methods such as nucleic acid probe testings and exoantigen should be performed.[92]

Opportunistic fungi

Bronchoscopic samples pose a number of interpretive problems in diagnosing infections produced by opportunistic yeast and filmentous fungi. Diagnosing filamentous fungi like aspergillus spp in bronchoscopic samples is a challenge due to lack of sensitivity. For the diagnosis of Aspergillus in BAL samples, Aspergillus antigen test and PCR test shows promising result due to increased sensitivity.[93]

Major problems in diagnosis of Candida spp in bronchoscopic sample is lack of specificity and asymptomatic colonization. So 255 of all bronchoscopic specimens may show candid spp on smear and culture. Demonstration of yeast and pseudohyphae improves the microscopic specificity for candida spp.But final diagnosis is by demonstration of organism in tissue. Promising results are obtained when antigen test for candida spp is use for BAL specimens.[94]

Identification of Crytococcus neoforms is more significant than other yeasts in bronchoscopic sample. BAL is the most useful sample for smear and culture of this yeast. Latex agglutination test is useful in rapid identification of this yeast.

Molecular techniques in BAL fluid work-up

The introduction of molecular diagnostic techniques like PCR in the work up of respiratory infectious diseases has increased in the recent years. These procedures have increased sensitivity where compared to conventional methods like culture and serology as a diagnostic tool.

This property of PCR is helpful in cases where the microorganisms load is very low and in organisms which cab not be easily cultured. PCR is specific in the diagnosis due to the used of specific primers and probes. The technique is

universal and method is rapid when compared with culture and serology. In pulmonary infectious diseases different types of PCR can be employed for the diagnosis.

The post PCR product in a conventional PCR are analyzed by gel electrophoresis, dot blot hybridization and enzyme immunoassay. In today's time conventional PCR is replaced by Real time PCR. The advantage of real time PCR is that there is no post PCR analysis due to which results are obtained quickly and there is less chance of contamination.

Real time PCR method measures the amount of amplified PCR product during each cycle, by which quantitative results can be obtained. This helps in identifying carrier state and infection. Multiple organisms can be diagnosed by multiplex PCR methods in a single test because respiratory pathogens cause same clinical symptoms. This method is cost effective but sensitivity is less compared to mono specific PCR. Causative organisms of pneumonia can be diagnosed by the above PCR methods.

The Table below gives a list of causative organism of pneumonia for which PCR is described in the literature

Bacteria	**Fungus**	**Virus**
Streptococcus pneumonia	Pneumocystis jirovecii	Herpes simplex virus 1 and 2
Haemophilus influenza	Aspergillus fumigates	Respiratory syncytial virus
Mycoplasma pneumonia		
Chlamydia pneumonia		
Legionella pneumophila		

Molecular Characterizations of Klebsiella pneumoniae

The majority of proteins are involved in metabolic processes, such as energy metabolism and transporters, supporting the general concept that the core genome encompasses essential functions required for survival of the microorganism.

The Klebsiella pneumoniae core genome comprised several sets of genes whose functions are related to bacterial survival in the environment or interaction with its host,and possibly virulence.

Haemolysin gene of Klebsiella pneumoniae has been cloned and sequenced. This peptide of approximately 200 basepair was identified.

MATERIALS AND METHODS

APPROVAL OF THE ETHICAL COMMITTEE

The ethical committee of this institution approved the study and informed consent was obtained from all patients undergoing the study.

PERIOD OF STUDY

This study was conducted over a period of one year from May 2015 to April 2016.

PLACE OF STUDY

It was carried out in the Department of Microbiology, Thanjavur Medical College.

COLLABORATING DEPARTMENT

Department of Thoracic Medicine, Thanjavur Medical College.

DESIGN OF STUDY

It was a prospective observation study.

STATISTICAL ANALYSIS

Data collected will be analyzed using descriptive statistical methods by computing Percentage, mean and standard deviation. Wherever necessary the results were depicted in the form of percentages.

MATERIALS

This study included 100 patients (men and women above 18 years) who underwent bronchoscopy.

INCLUSION CRITERIA

1. Adult patients with Chronic Respiratory Diseases undergoing Bronchoalveolar lavage. (Age above 19 years).
2. To identify aerobic bacteria and antimicrobial sensitive of the bacterial isolates.
3. To identify fungal isolates.

EXCLUSION CRITERIA

1. Patients with unstable cardiac conditions (recent myocardial infarction, cardiac arrhythmia, etc.)
2. Pregnant women.
3. Mycobacterial culture.
4. Patients who do not give consent for the procedure.

Examination of Patients.

In the Thoracic Medicine ward, patients with Chronic respiratory diseases where asked for history of presenting complaints like fever, cough and production of sputum. Duration of illness. History of any cardiac problems.

Specimen Collection.

Patients were instructed to stop smoking and fast for a minimum of 12 hrs before sample collection.

Bronchoscopy with BAL was performed according to standardized procedures designed to minimize oral contamination.

BAL was performed by sequentially instilling and then withdrawing 50 ml aliquots of sterile normal saline.

Bronchoalveolar lavage fluid specimens collected under aseptic precautions was immediately transported to the laboratory for bacterial and fungal processing.

FIGURE –III SPEICMEN OF BAL FLUID.

Direct microscopy

Centrifugation of the sample is performed for detection of bacteria and fungi by direct microscopy.

Sample is subjected for direct microscopy with Wet Mount preparation (10 % Potassium Hydroxide).

Smears are prepared and air dried for use of special stains like Gram's stain for Bacteria and Fungus, Acid Fast staining techniques like Ziehl-Neelsen stain to identify Mycobacteria, Kinyoun's technique for Nocardia, and May-Grunwald Giemsa stain to identify Pneumocystis and fungus.

Bacterial culture

For bacterial culture uncentrifuged samples were used. The sample was inoculated for quantitative bacterial culture using standard laboratory techniques on Blood agar, Chocolate agar and Mac conkey agar using a sterile 4mm nichrome loop (0.01 ml) and incubated at 37°C for 72 hours. Colony count of $>10^5$ colony forming units per milliliter of BAL fluid was taken as the cutoff for bacterial infection.

Fungal culture

For fungal culture centrifuged samples were used. Sample were inoculated on Sabouraud's dextrose agar for fungal culture and incubated for 4 weeks at 37°C.

FIGURE IV

Bacterial growth on Mac conkey agar by Semi quantitative culture method.

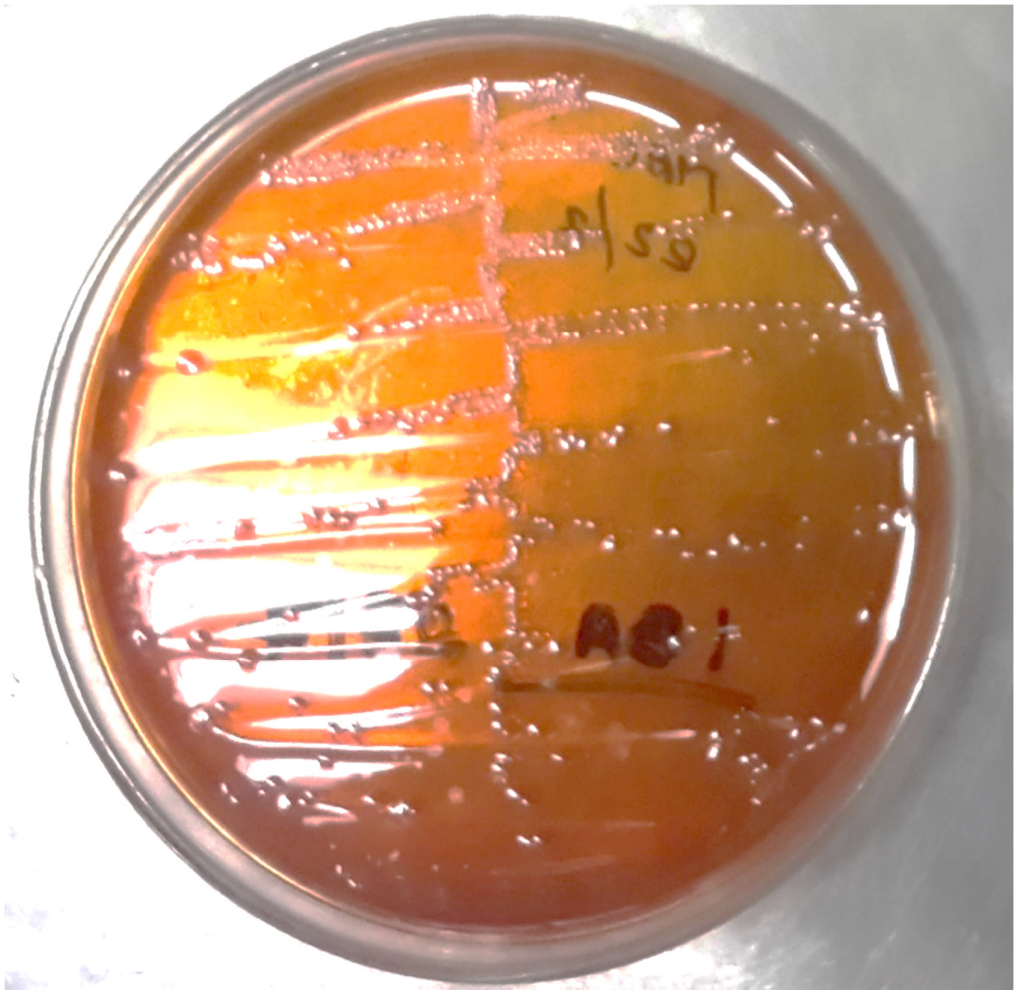


FIGURE V

Bacterial growth on Nutrient agar plate by Semi quantitative method.

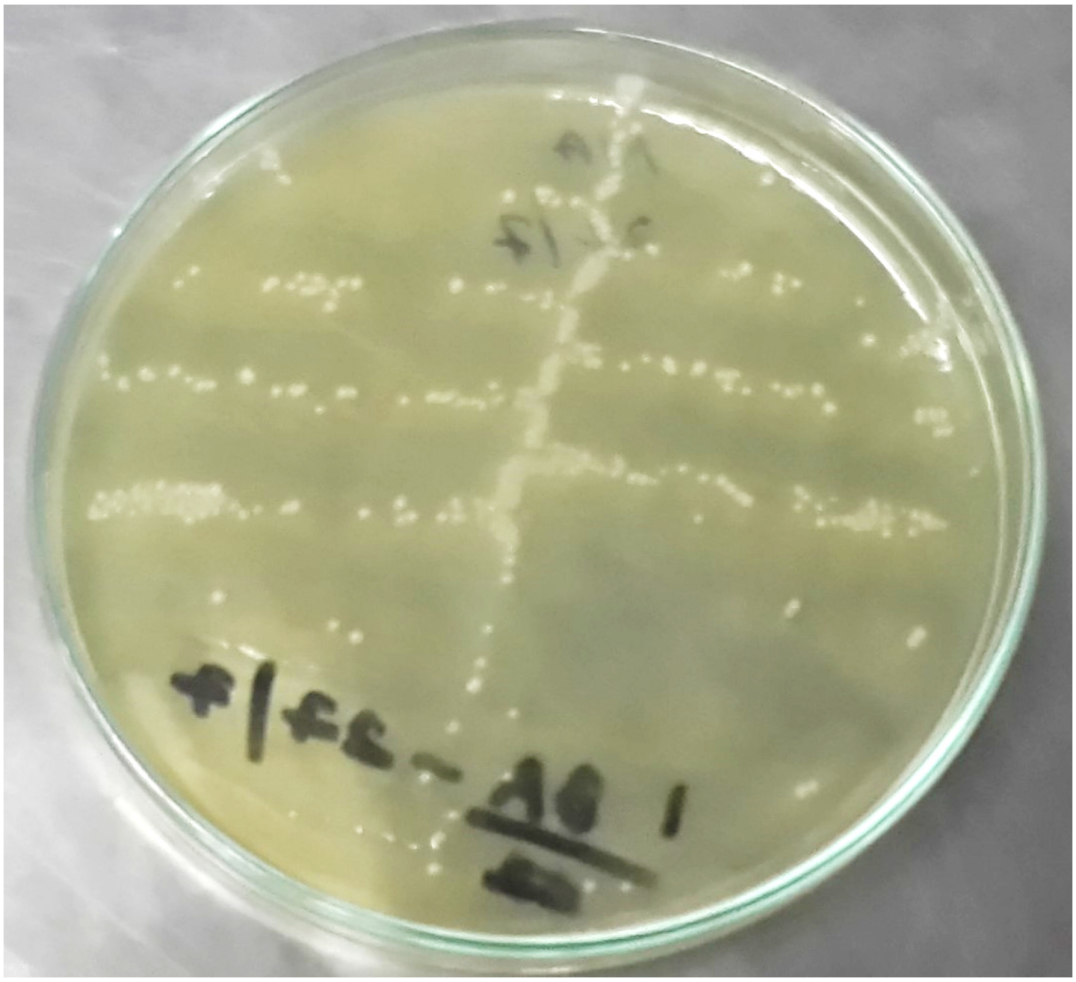


FIGURE VI

Growth of Candida on Sabouraud dextrose agar.

FIGURE VII

Growth of Aspergillus niger on Sabouraud dextrose agar.

FIGURE VIII

Growth of Aspergillus flavus on Sabouraud dextrose agar.

Identification of Bacteria

The culture plates were examined for the presence of bacterial colony and the growth was identified with Gram's staining, Catalase test, oxidase test, hanging drop test, Biochemical reaction and carbohydrate fermentation.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done for bacterial isolates by Kirby-Bauer's disc diffusion method on Muller-hinton agar plate.

Antimicrobial susceptibility testing was performed by agar disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

The following antimicrobial agents were tested:

Aminoglycosides- amikacin (30µg),

β Lactamase inhibitor combination –

Amoxicillin/Clavulanic acid (20/10µg), Piperacillin/tazobactam (100/10µg)

Lincosamides- Clindamycin (2µg),

Cephalosporins- Cefoperazone (75µg), Cefepime (30µg),

Carbapenems- Imipenem (10µg),

Oxazolidinones- Linezolid (30µg),

Glycopeptides- Vancomycin (30µg).

Storage of antimicrobial discs

Container of antimicrobial disc was refrigerated at 4 - 8˚C. β lactam antibiotics were stored in the freezer compartment. Imipenem when stored frozen remains stable til the day of use. Disc containers were brought to room temperature before use. After the use for antimicrobial sensitivity the antibiotic discs were tightly sealed and stored in a dry container.

Preparation of turbidity standard

Commonly used is the McFarland 0.5 standard. This contains 99.5 ml of 1 % sulphuric acid and 0.5 ml of 1.175 % barium chloride. The solution is poured in the tube, tightly sealed and stored at room temperature at a dark place. This is comparable with bacterial suspension of 1.5 X 10^8 colony forming unit/ml.

Preparation of Inoculum

For preparation of the inoculum, 3 to 5 colonies were lifted and inoculated in 5 ml of peptone water and incubated at 37˚C for 2 to 6 hours in order to obtain 0.5 McFarland standard which corresponds to 150 million organism per ml. If more turbidity was noticed, peptone water was added to adjust it to 0.5 McFarland's standard.

Inoculation of MHA plates

A sterile cotton swab was dipped and rotated several times within 15 minutes of adjusting the turbidity of the inoculum. To be remove excess fluid the swab was pressed firmly on the inner side of the wall above the level of the fluid. By streaking method the dry surface of the Muller-hinton agar plate was swabbed. This method was repeated 2 or more times by rotating the plate to 60˚to cover the entire surface of the plate. Finally the rim of the agar plate was swabbed. The plate was closed and allowed to wait for 3 t0 5 minutes for excess moisture to be absorbed before applying drug impregnated discs.

Application of discs to inoculated agar plate

A set of predetermined battery of antimicrobial discs were tested on all isolates. The whole disc was placed on the agar surface and pressed to make sure the entire surface of the disc comes in contact with the agar. Even distribution of the discs were make to ensure 25 mm distance from centre to centre of discs. This was followed by incubation at 37˚C for 16 – 18 hrs.

Reading and interpretation of results

Each plates were examined after 16 – 18 hours for satisfactory streaking with uniform circular zones of and confluent lawn of growth. The diameter of the zones of complete inhibition including the diameter of the discs were

measured. The zone was measured to the nearest millimeter using a ruler which was held on the back of the agar plate. The zone of inhibition size was interpreted by referring to CLSI guidelines and reported the organism as resistant, intermediate or susceptible.

QUALITY CONTROL RECOMMENDATION BY CLSI

Enterobacteriaceae

Testing condition

Media: Muellar-Hinton Agar by disk diffusion method.

Inoculum: Direct colony suspension equivalent to 0.5 McFarland standards.

Incubation : 35˚C ± 2˚C in ambient air for 16-18 hours.

Routine Quality Control: Escherichia coli ATCC 25922

Pseudomonas aeruginosa

Testing conditions

Media: Muellur-Hinton Agar by disk diffusion method.

Inoculum: Direct colony suspension equivalent to 0.5 McFarland standards.

Incubation: 35˚C ± 2˚C in ambient air for 16-18 hours.

Routine quality control: Pseudomonas aeruginosa ATCC 27853

Acinetobacter spp.

Testing conditions

Media: Muellur-Hinton Agar by disk diffusion method.

Inoculum: Direct colony suspension equivalent to 0.5 Mc Farland standards.

Incubation: 35˚C ± 2˚C in ambient air for 16-18 hours.

Routine quality control: Pseudomonas aeruginosa ATCC 27853

Staphylococcus spp.

Testing condition

Media: Muellar-Hinton Agar by disk diffusion method.

Inoculum: Direct colony suspension equivalent to 0.5 McFarland standards.

Incubation: 35˚C ± 2˚C in ambient air for 16-18 hours.

Routine Quality control: Staphylococcus aureus ATCC 25923

Enterococcus spp

Testing conditions

Media: Muellar-Hinton agar by disk diffusion method.

Inoculum: Direct colony suspension equivalent to 0.5 McFarland standards.

Incubation: 35˚C ± 2˚C in ambient air for 16-18 hours.

Routine Quality control: Staphylococcus aureus ATCC 25923

Streptococcus pneumonia

Testing conditions

Media: Muellar-Hinton Agar with 5 % sheep blood.

Inoculation: Direct colony suspension equivalent to 0.5 McFarland standards.

Incubation: 35˚C ± 2˚C with 5% CO_2 for 20-24 hours.

ZONE SIZE INTERPRETATIVE CHART IN ACCORDING TO CLSI.[32]

Sl. No.	Antimicrobial agent	Symbol	Drug concentration (µg)	Zone size in mm		
				Resistant	Intermediate	Sensitive
A. Aminoglycosides						
1.	Amikacin	AK	30	≤14	15-16	≥17
B. β-Lactam/β-Lactamase inhibitor combination						
1.	Amoxicillin/ Clavulanic acid	AU	20/10	≤17	14-17	≥18
2.	piperacillin/ tazobactam	PT	100/10	≤14	15-20	≥21
C. Lincosamides						
1.	Clindamycin	CY	2	≤14	15-20	≥21
				Staphylococcus aureus		
				≤15	16-18	≥19
				Strepotococcus pneumoniae		
D. Cephalosporins						
1.	Cefoperazone	CF	75	≤15	16-20	≥21
2.	Cefepime	CPM	30	≤14	15-17	≥18
E. Carbapenems						
1.	Imipenem	I	10	≤19	20-22	≥23
F. Oxazolidinones						
1.	Linezolid	LZ	30	≤20	21-22	≥23
G. Glycopeptides						
1.	Vancomycin	VA	30	≤14	15-17	≥17

FIGURE IX

Antimicrobioal Sensitivity Test on Meuller-Hinton Agar.

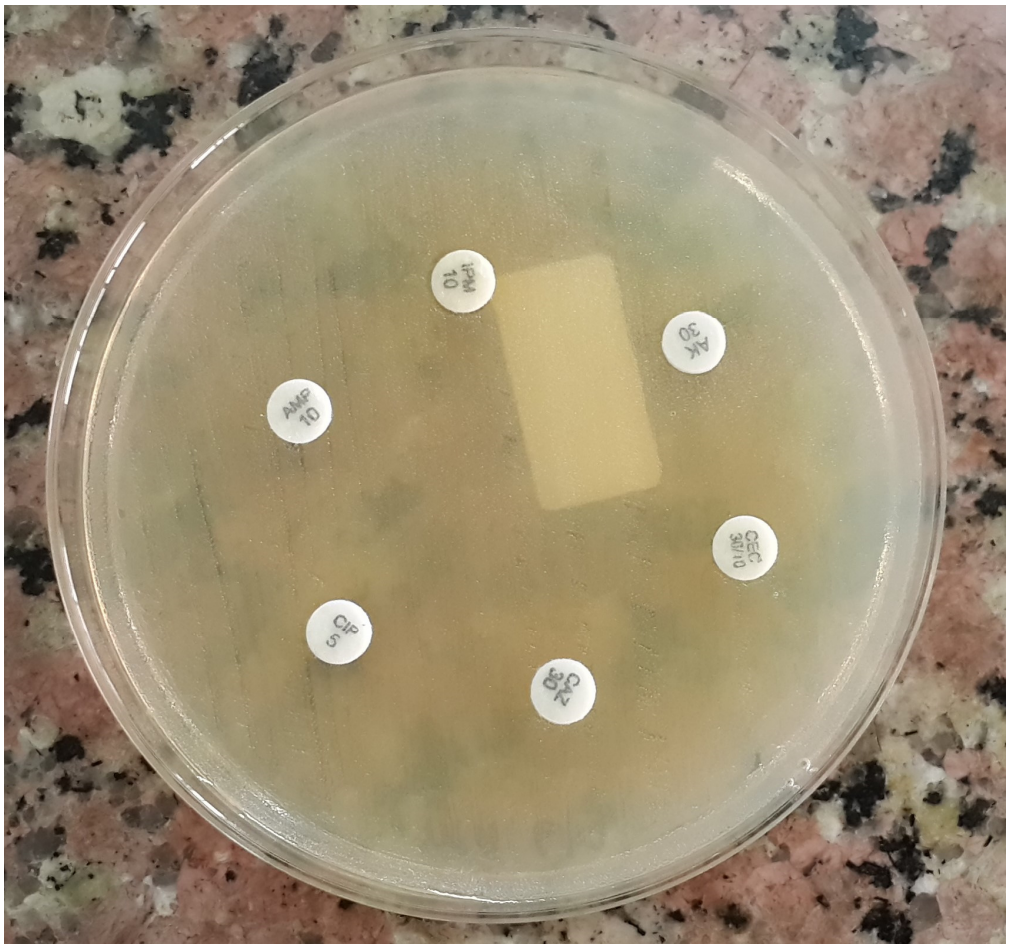


FIGURE – X

Antimicrobial sensitivity test on Meullar-Hinton Blood agar for fastidious organisms.

Molecular identification

DNA extraction

All the isolates were grown on Muller Hinton Agar and were incubated for 48 hours at 37°C under aerobic conditions and the bacterial colonies were then used for DNA extraction.

Molecular characterisation was done for the commonest isolated organism by Polymerase Chain Reaction.

Kit used for PCR

HELINI™ Purefast Bacterial Genomic DNA Minispin prep Kit [from Bacterial cultures]

Kit components

No. of reactions **Catalogue Number**	**25** **2007**
Lysoyme	0.5 ml
Proteinase	0.5 ml
Digestion buffer	5 ml
Binding buffer	5 ml
Elution buffer	2.5 ml
Wash buffer-1	9 ml
Wash buffer-2	6 ml
Spin column with collection tube	25
Hand book	1

Storage and Stability

HELINI™ Purefast Bacterial Genomic DNA Minispin prep Kit can be stored for up to 6 months at room temperature (+15-+25°C). For longer storage, it is recommended to keep the kit at 4°C. If precipitate forms in the buffers during storage, it should be re-dissolved by incubating the buffers at 37°C before use.

Proteinase K and Lysozyme should be stored at -20ºC.

Description

HELINI Purefast Bacterial Genomic DNA Minispin prep Kit is designed for rapid and cost-effective small-scale preparation of high quality genomic DNA from both gram negative and positive bacteria. The kit utilizes an exclusive silica based membrane technology in the form of a convenient spin column. Each Purefast® spin column can recover up to 10µg of DNA.

The standard procedure takes less than 30 minutes following cell lysis and yields purified DNA of more than 30 kb in size. Isolated DNA can be used directly in PCR, Southern blotting and enzymatic reactions.

Principle

Cells are lysed during a short incubation with Proteinase K in the presence of chaotropic salt, which immediately inactivates all nucleases. Cellular nucleic acids bind selectively to special glass fibres pre-packed in the Purefast purification filter tube. Bound nucleic acids are purified in a series of rapid “wash and spin” steps to remove contaminating cellular components.

Finally low salt elution releases the Nucleic acids from the glass fibre. This sample method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

Material Required

- Micro Pipettes Variable Volume 0.5-10µl, 10-100µl, and 100-1000µl
- Sterile pipette tips with aerosol barrier 2-20µl, 10-100µl, and 100-1000µl
- Disposable powder-free gloves
- Vortex mixer / Water bath
- Centrifuge with rotor for 1.5 ml reaction tubes
- 1.5ml/2ml centrifuge tubes
- Water bath
- 100% ethanol

Reagent Preparation

Add the indicated volume of ethanol (96-100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

	Cat.No: 2007-25 prep	
	Wash buffer-1	Wash buffer-2
Concentrated buffer	9 ml	6 ml
Ethanol (96-100%)	6 ml	24 ml
Total volume	15 ml	30 ml

DNA Purification – Procedure

Before starting the purification reaction, set water bath to 56ºC and warm up the Elution buffer to 56ºC.

1. Suspend bacterial pellet by vortexing.
2. Add 180µl of Digestion buffer and 20µl of Lysozyme. Gently vortex for 10 seconds and briefly centrifuge to remove drops from inside the lid of the tube.
3. Incubate at 37ºC for 15min.
4. Add 200µl of Binding Buffer and 20µl of Proteinase K, Mix well by pulse vortex.

5. Briefly centrifuge to remove drops from inside the lid of the tube.
6. Incubate at 56ºC for 15min.
7. Add 200µl absolute ethanol [100%] and mix well by inverting several times.
8. Pipette entire sample into the Purefast spin column. Centrifuge at 8000rpm for 1 min. Discard the flow-through and place the column back into the same collection tube.
9. Add 500µl of Wash buffer-1 [ethanol added] to the Purefast spin column. Centrifuge at 10000rpm for 1 min and discard the flow-through. Place the column back into the same collection tube.
10. Add 500µl of Wash buffer-2 [ethanol added] to the Purefast spin column. Centrifuge at 10000rpm for 1 min and discard the flow-through. Place the column back into the same collection tube.
11. Repeat wash buffer-2 once.
12. Empty centrifuge at 12000rpm - Spin column with collection tube for an additional 2 min. This step is essential to avoid residual ethanol.
13. Discard collection tube and transfer the Purefast spin column into a fresh 1.5 ml micro centrifuge tube.
14. Add 100µl of the pre-warmed Elution Buffer to the centre of Purefast spin column membrane. Take care not to contact the membrane with the pipette tip.
15. Incubate for 2 min at room temperature and Centrifuge at 13000rpm for 1 min. Discard the spin column and store the purified DNA at -20°C. Note: Elution volume can be adjusted from 30µl to 100µl.

PCR

HELINI Ready to use Klebsiella pneumoniae Primer mix - 5µl/reaction

PCR Product: 200bp

Gene: hemolysin gene

HELINI-F: TTCATCTACGTGCTGGAGGG

HELINI-R: AGCCTGGATTGAGCGGATAA

RESULTS AND INTERPRETATION

In this study 100 samples of BAL from chronic respiratory diseases cases were microbiologically analyzed for bacterial and fungal profile and the results are given below.

TABLE 1- AGE AND SEX DISTRIBUTION IN CHRONIC RESPIRATORY DISESASE.

Age	Male	%	Female	%
< 20	1	2%	0	0
21-40	2	5%	13	26%
41-60	27	68%	24	47%
61 and above	10	25%	14	27%
Total	**49**		**51**	
Mean	**10**		**12.75**	
SD	**12.02775**		**9.844626**	

The age and sex distribution of the study population is shown in the above table. In this study female population had a slight increase in incidence 51% than male population which was 49 %. The commonest age group affected in the study was between 41 and 60. In this group male population was affected 68 % more than the female population which was only 47 %.

FIGURE-1 AGE AND SEX DISTRIBUTION IN CHRONIC RESPIRATORY DISEASES.

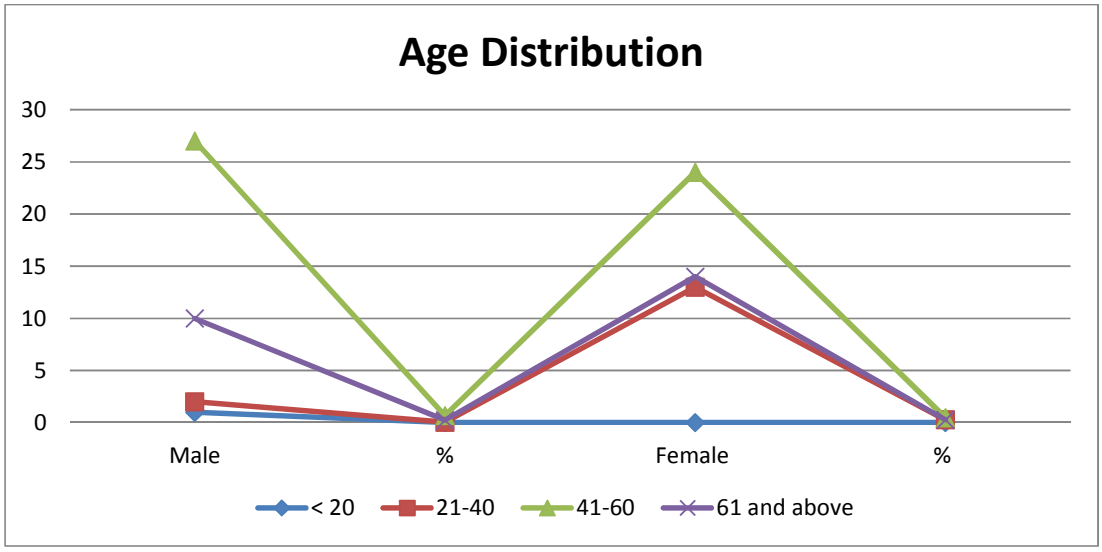


FIGURE- 2 GENDER DISTRIBUTION IN CHRONIC RESPIRATORY DISEASES.

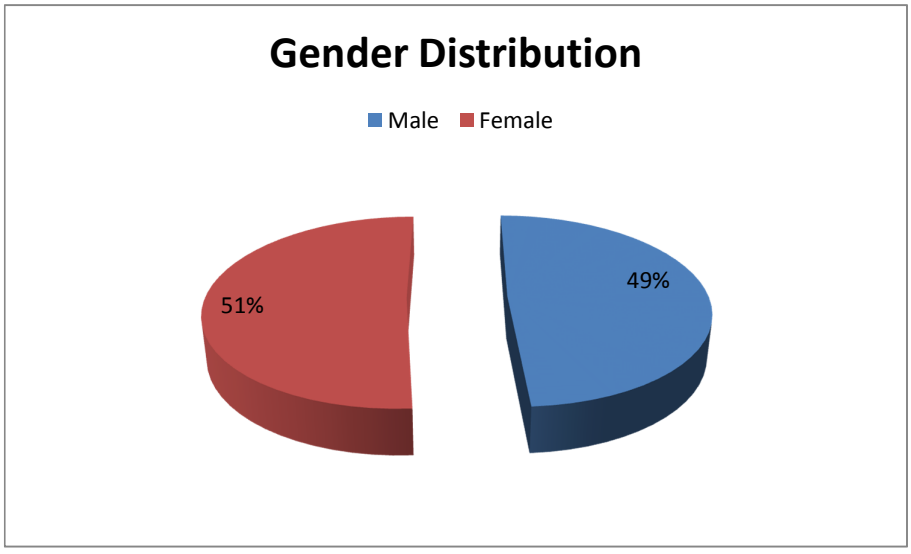


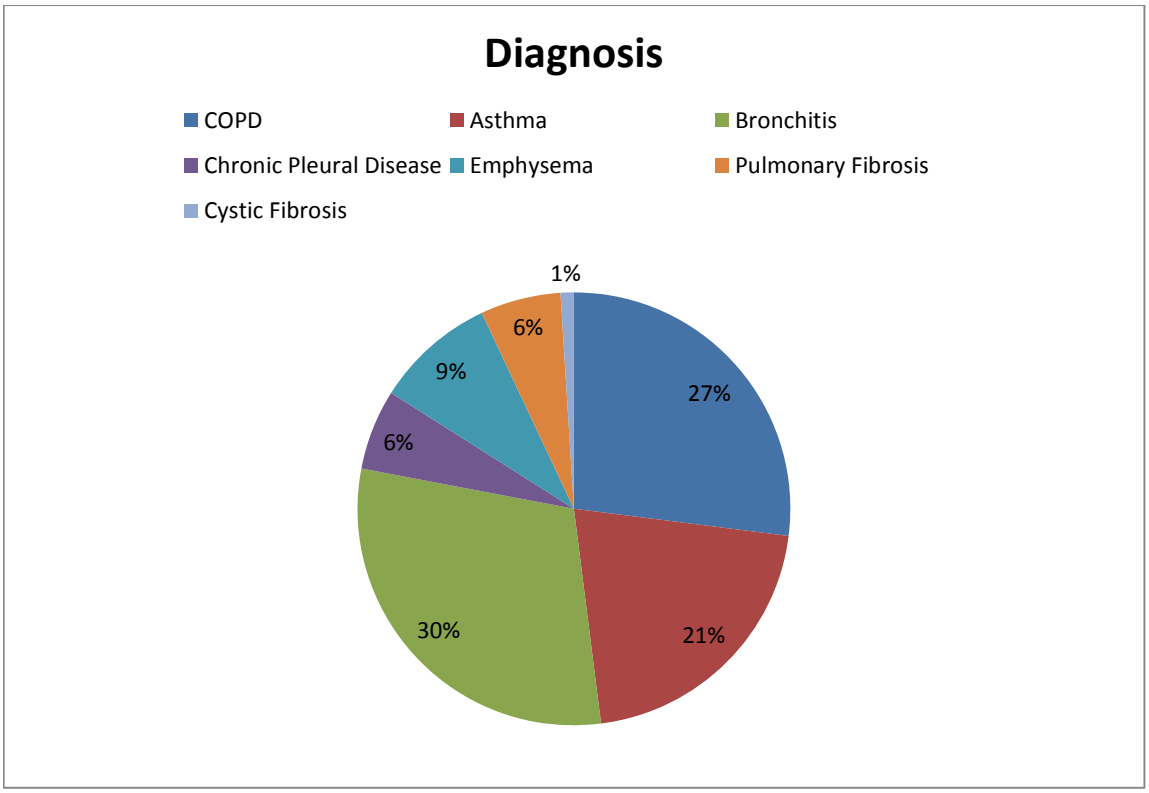
TABLE 2- PERCENTAGE OF GRAM POSITIVE AND GRAM NEGATIVE INFECTION AMONG CLINICAL CONDITIONS

	Cases	G (+)	G (+) %	G (-)	G (-) %
COPD	27	6	38%	6	43%
Asthma	21	3	19%	1	7%
Chronic Bronchitis	30	4	25%	4	29%
Chronic Pleural Disease	6	1	6%	-	-
Emphysema	9	1	6%	3	21%
Pulmonary Fibrosis	6	1	6%	-	-
Cystic Fibrosis	1	-	-	-	-
Total	**100**	**16**		**14**	

G (+)- Gram positive; G (-)- Gram negative

Among the Chronic respiratory diseases the common occurrence was Chronic Bronchitis 30% followed by COPD 27% and Asthma amounting for 21%. Lower respiratory tract infection was observed more among COPD in which Gram negative organism was 43 % and Gram positive organism was 38%. This was followed by Chronic bronchitis in which Gram negative organism was 29 % and Gram positive organism was 25 %. Asthma cases had infection associated more with Gram positive organisms 19% than Gram negative organism 7%.

FIGURE 3- DISTRIBUTION OF DISEASES AMONG CHRONIC RESPIRATORY DISEASES CASES



In our study chronic bronchitis accounts to 30%, followed by COPD 27% and Asthma 21% and chronic respiratory diseases.

TABLE 3- PROFILE OF THE PATHOGENS

Type of Organisms	No of Cases	Percentage (%)
Staphylococcus aureus	5	14 %
Acinetobactor baumanii	5	14 %
Enterococcus faecalis	2	6 %
Streptococcus pneumoniae	3	8 %
Pseudomonas aeuroginosa	1	3 %
Escherichia coli	2	6 %
Klebsiella pneumoniae	7	20 %
Candida albicans	5	14 %
Aspergillus flavus	2	6 %
Aspergillus niger	2	6 %
Aspergillus fumigatus	1	3 %
Total	**35**	

In this study a total of 35 organisms were isolated. Out of which 25 isolates were bacteria and 10 isolates were fungus. Among bacterias gram negative organisms were isolated more than gram positive organisms. Klebsiella pneumoniae was the commonest bacteria isolated accounting to 20%. It was also noted that Klebsiella pneumoniae was also the commonest gram negative bacteria isolated. Staphylococcus aureus was the commonest positive bacteria isolated. Among the fungus Candida albicans was the commonest isolate accounting to 14 %.

FIGURE 4- DISTRIBUTION OF BACTERIAL AND FUNGAL PATHOGENS.

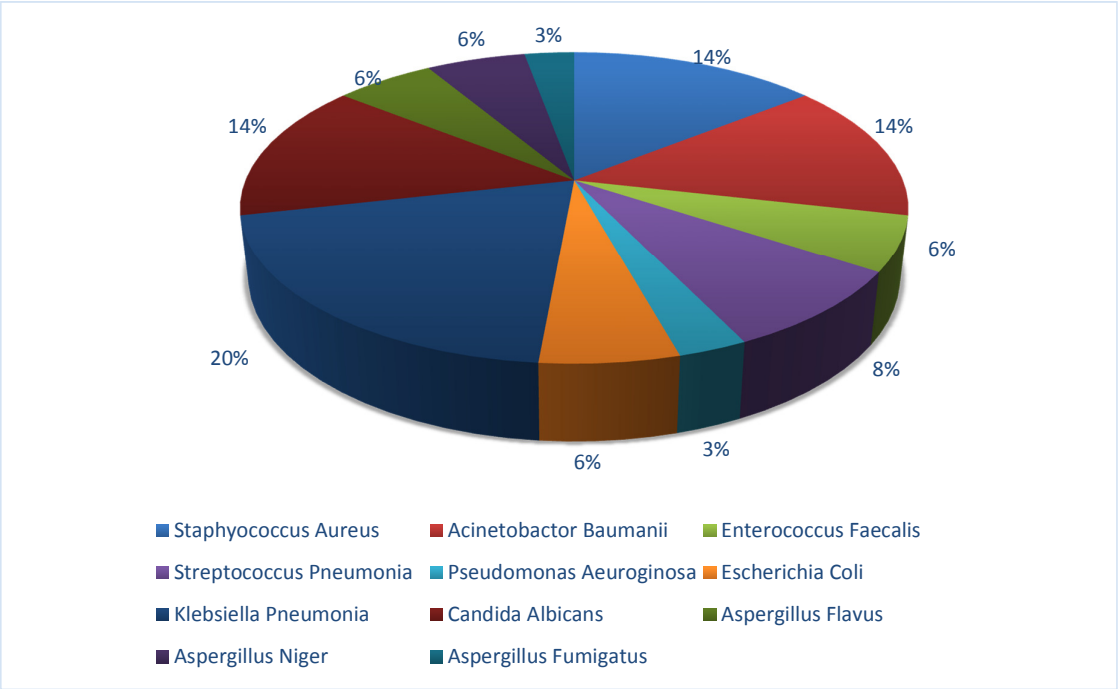


TABLE 4- DISTRIBUTION OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIAL PATHOGENS.

Types of Bacteria	Gram (+)	Gram (-)	Total No.Cases	Percentage %
Klebsiella pneumoniae	-	7 (47%)	7	28%
Acinetobacter baumanii	-	5(33%)	5	20%
Enterococcus faecalis	2(20%)	-	2	8%
Escherichia coli	-	2 (13%)	2	8%
Streptococcus pneumoniae	3(30%)	-	3	12%
Staphylococcus aureus	5(50%)	-	5	20%
Pseudomonas aeuroginosa	-	1(7%)	1	4%
Total	**10**	**15**	**25**	

The above table shows Gram negative infections are common in chronic respiratory disease which is 68% of bacterial infections. Klebsiella pneumoniae is the commonest among Gram negative bacteria followed by Acinetobacter baumanii.

FIGURE 5- PERCENTAGE AND DISTRIBUTION OF BACTERIAL ISOLATES.

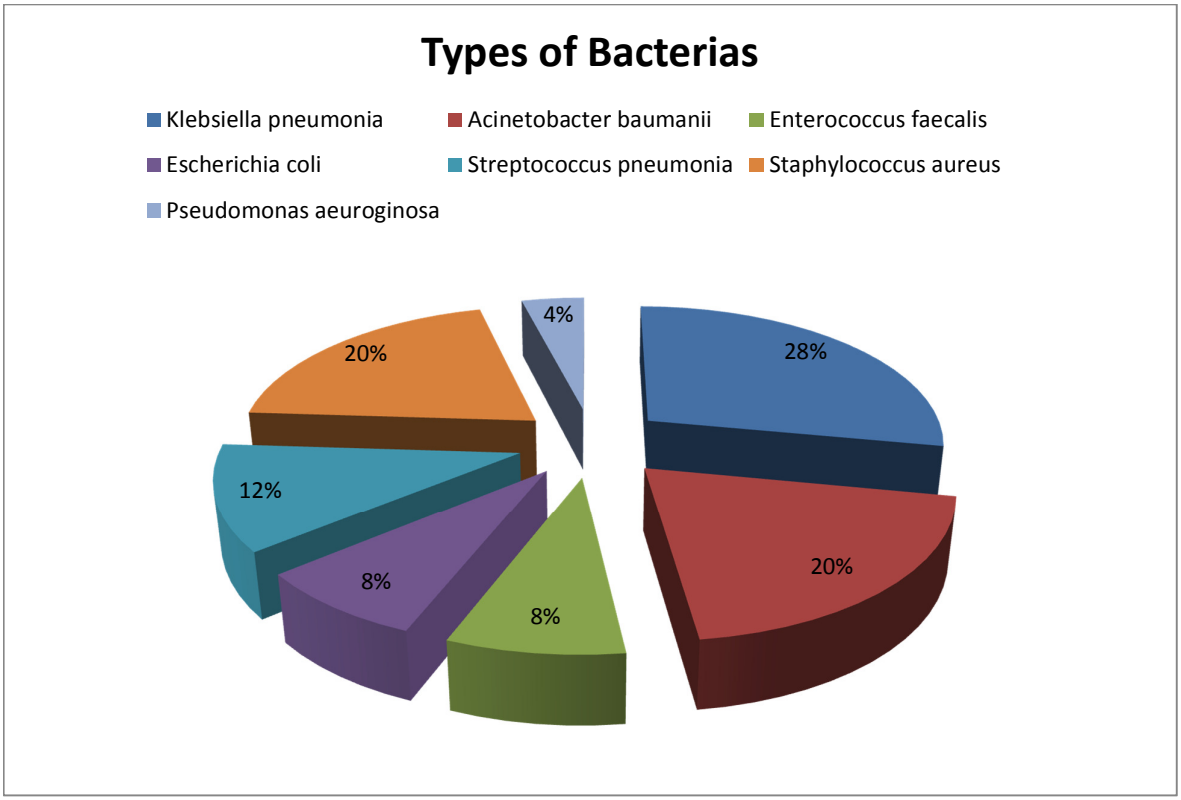


TABLE 5: PERCENTAGE AND DISTRIBUTION OF FUNGAL ISOLATES.

Type of Fungus	No of Cases	Percentage (%)
Candida albicans	5	50%
Aspergillus flavus	2	20%
Aspergillus niger	2	20%
Aspergillus fumigatus	1	10%
Total	**10**	

Out of the 100 sample 10 samples showed positive for fungal culture. Out of the fungal culture positive samples Candida albicans was 50% , Aspergillus flavus 20%, Aspergillus niger 20% and Aspergillus fumigates 10%.

FIGURE 6- PERCENTAGE OF FUNGAL ISOLATES.

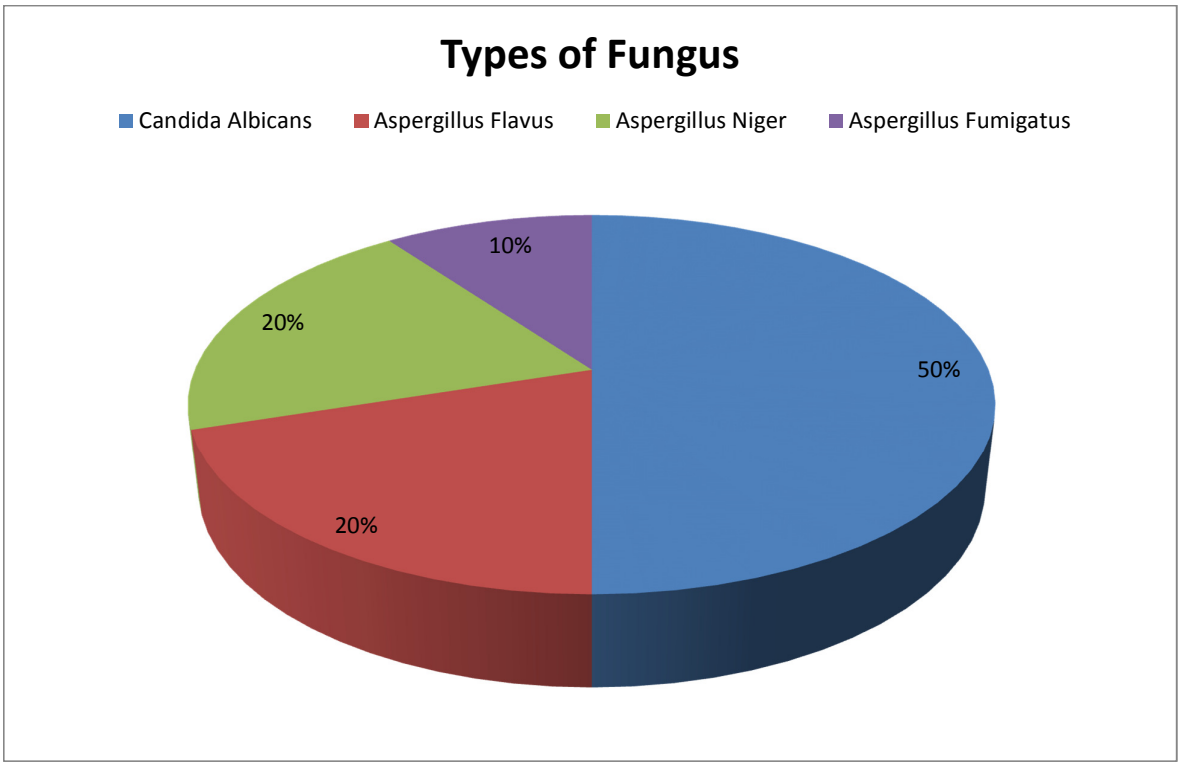


TABLE 6-PREVALENCE OF GRAM POSITIVE, GRAM NEGATIVE ORGANISMS AND FUNGUS.

GRAM NEGATIVE BACTERIA				GRAM POSITIVE BACTERIA			FUNGUS			
KLEB. PNEUM	ACINE. BAUNAMII	ESHER. COLI	PSEUD. AEURO	STAPHY. AUREUS	STREPT. PNEUMO	ENTERO. FAECALI	CANDIDA ALBICAN	ASPER. FLAVUS	ASPER NIGER	ASPER. FUMI
7	5	2	1	5	3	2	5	2	2	1

Gram negative bacterias were isolated more than Gram positive bacterias. In fungus candida and aspergillus isolates were equal in number.

FIGURE 7-PREVALENCE OF GRAM POSITIVE ORGANISMS

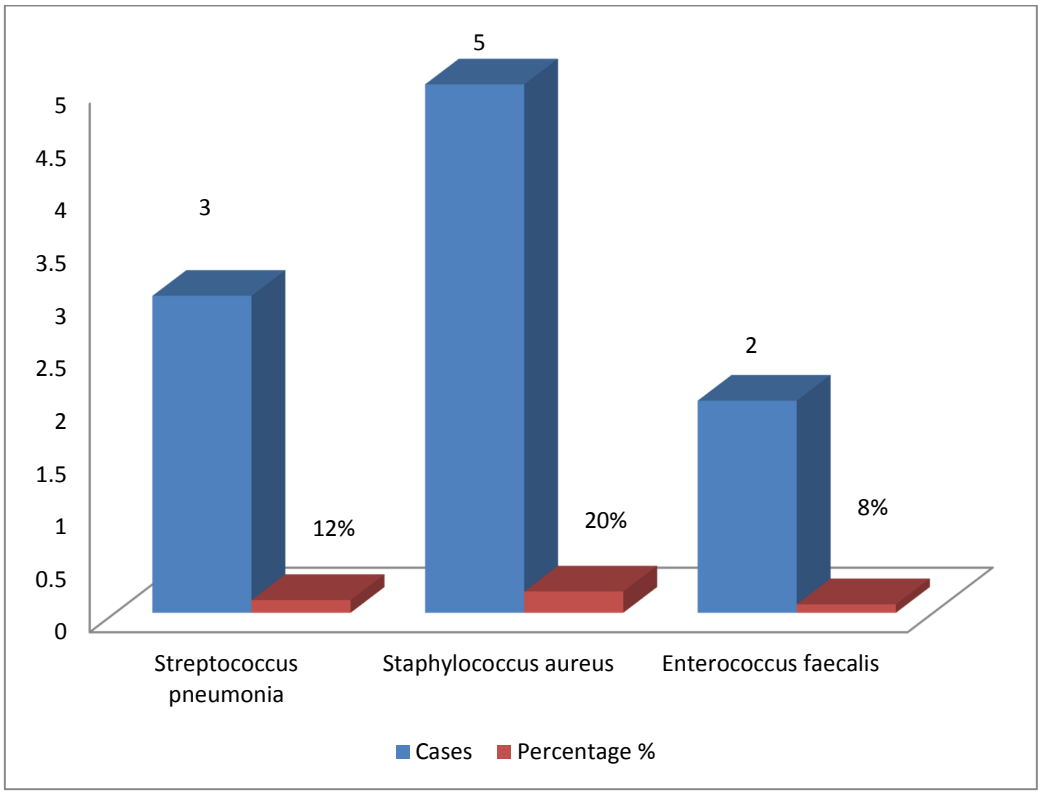


FIGURE 8- PREVALENCE OF GRAM NEGATIVE ORGANISMS

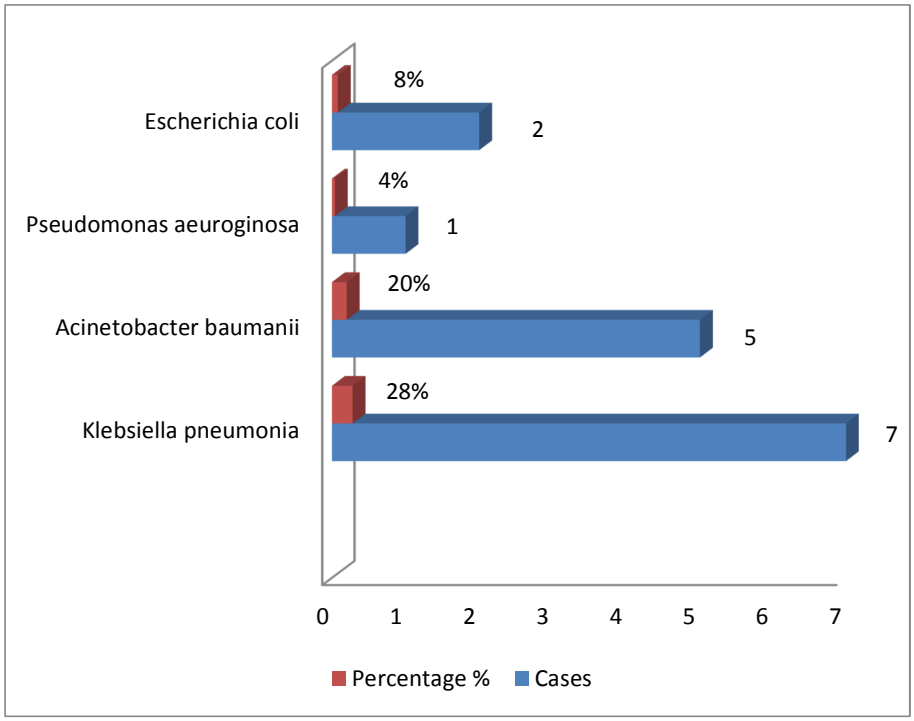


TABLE 7-

ANTIBIOTICS RESISTANCE PATTERN GRAM NEGATIVE & POSITIVE PATHEGONS

Antibiotics	Klebsiella pneumoniae (7)	Acinetobater baumanii (5)	Escherichia coli (2)	Pseudomonas aeruginosa (1)	Staphylococcus aureus (5)	Streptococcus pneumonia (3)	Enterococcus faecalis (2)
Amikacin	0	1(20%)	1(50%)	0	0	0	0
Amoxicillin/ Clavulanic	3(43%)	3(60%)	2(100%)	0	3(60%)	1(33%)	2(100%)
Clindamycin	0	0	0	0	0	1(33%)	0
Cefoperazoine	1(14%)	2(40%)	1(50%)	0	0	1(33%)	0
Cefepime	1(14%)	2(40%)	1(50%)	0	0	1(33%)	1(50%)
Imipenem	0	2(40%)	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0
Vancomycin	0	0	0	0	0	0	0
Piperacillin /Tazobactam	0	1(20%)	0	0	0	0	0

Antimicrobial resistance is noted more among Gram negative bacterias than Gram positive bacterias in lower respiratory tract infections. There is a high amount of resistance to Amoxicillin/Clavulanic acid for both Gram positive and Gram Negative bacterias. Among all bacterias Pseudomonas aeruginosa shows least resistance and Acinetobacter baumanii shows highest resistance pattern.

FIGURE 9- ANTIBIOTIC RESISTANCE PATTERNS

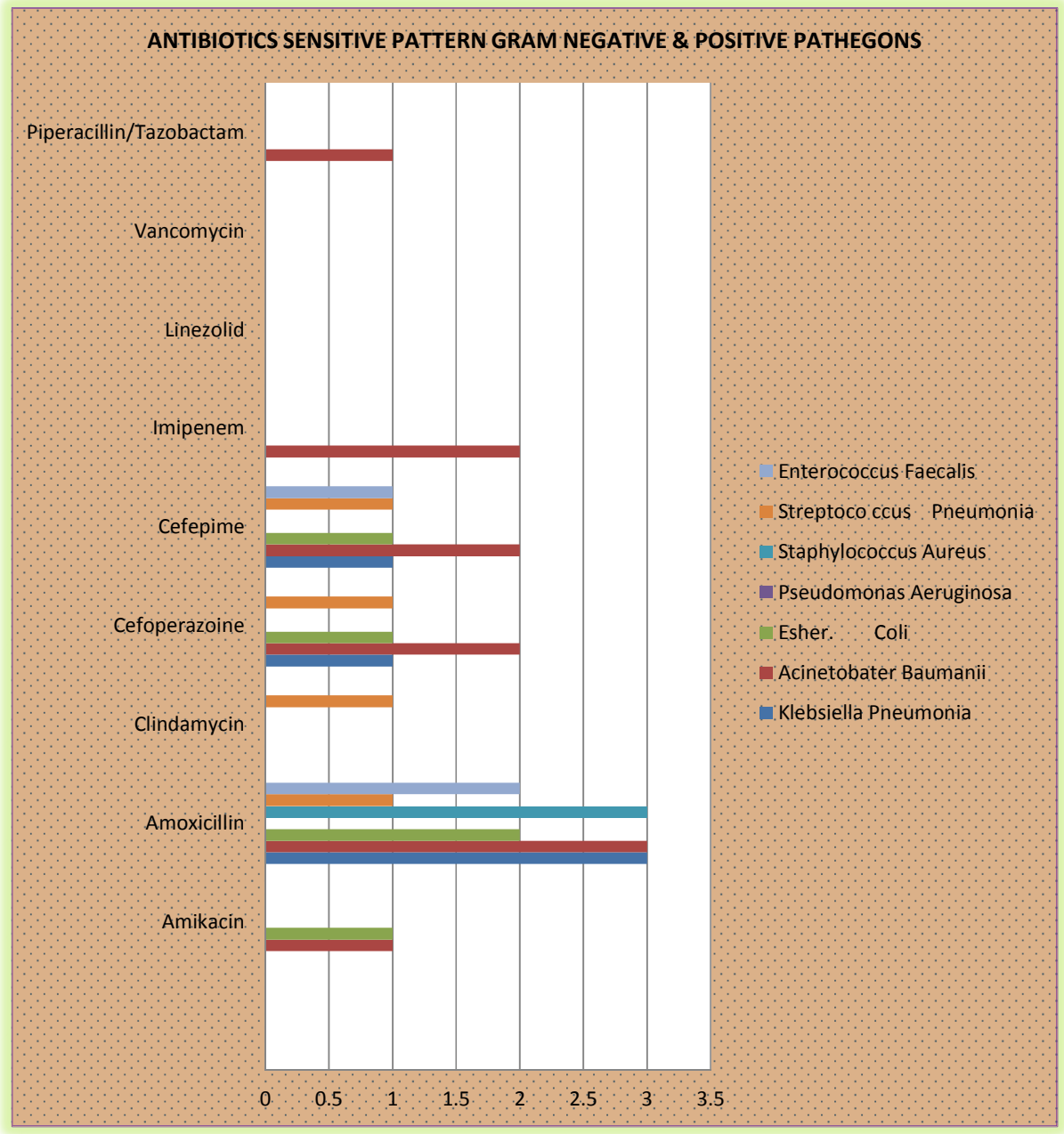


FIGURE 10- ANTIBIOTIC SENSITIVITY PATTERN

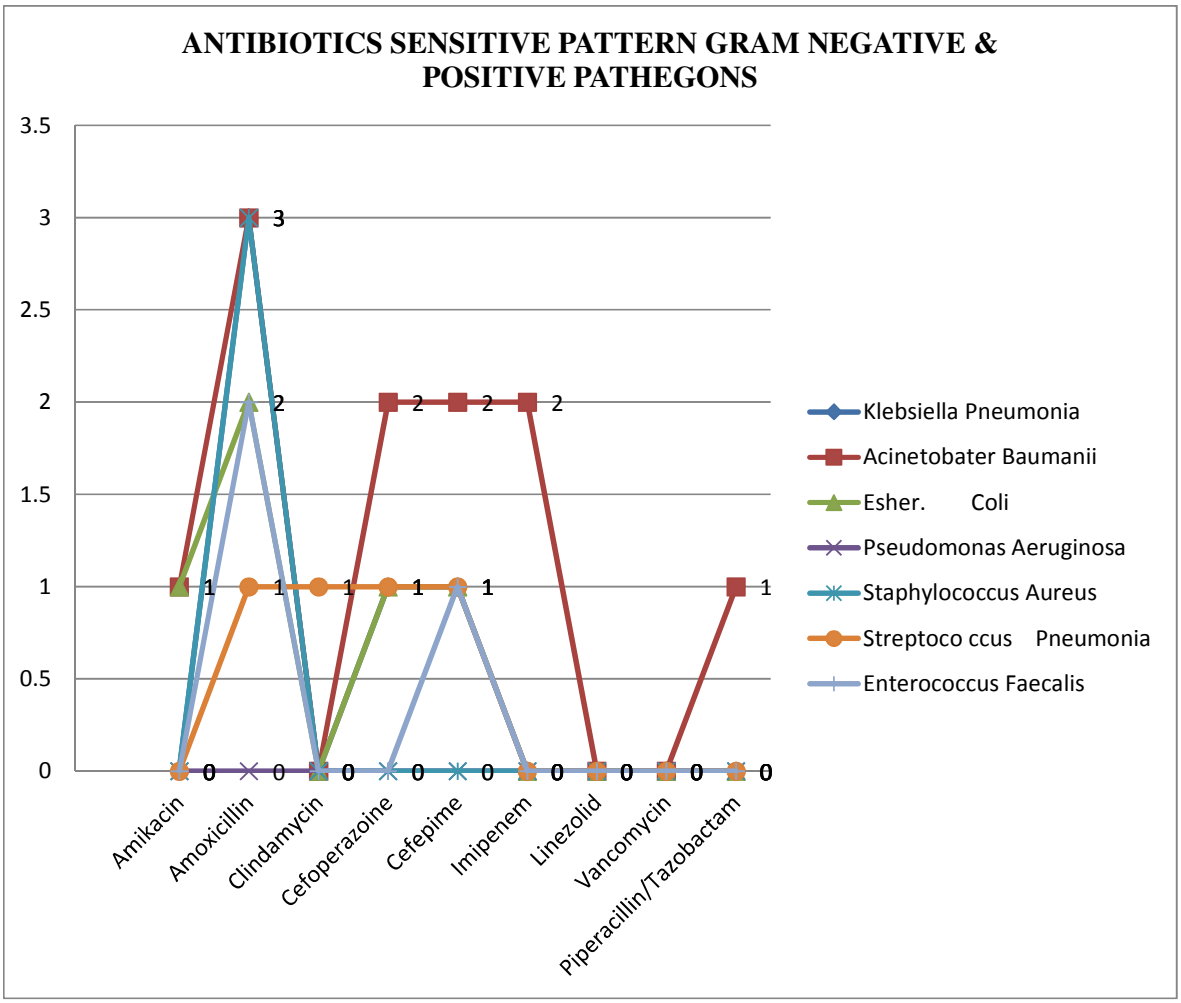


TABLE 8- AGE PREVELANCE AMONG DIFFERENT PATHOGENS

ISOLATES / AGE	Staphylococcus aureus	Enterococcus faecalis	Streptococcus pneumoniae	Klebsiella pneumoniae	Pseudomonas aeuroginosa	Acinetobactor baumanii	Escherichia coli	Candida albicans	Aspergillus flavus	Aspergillus niger	Aspergillusfumigatus
1-30	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**
31-60	**4**	**1**	**0**	**5**	**0**	**4**	**1**	**3**	**1**	**0**	**1**
61-90	**1**	**1**	**3**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**0**
Total	**5**	**2**	**3**	**7**	**1**	**5**	**2**	**5**	**2**	**2**	**1**

From this table we observe that pulmonary infection are more common among 31-60 age group and less common among below 30 years of age. Bacterial infections are more common in the age group of 31-60 years with predominant Gram negative infections. In the age group above 60 years bacterial infections occurs equally with both Gram positive and Gram negative bacterias .

PCR RESULT

HELINI Ready to use Klebsiella pneumoniae Primer mix - 5µl/reaction

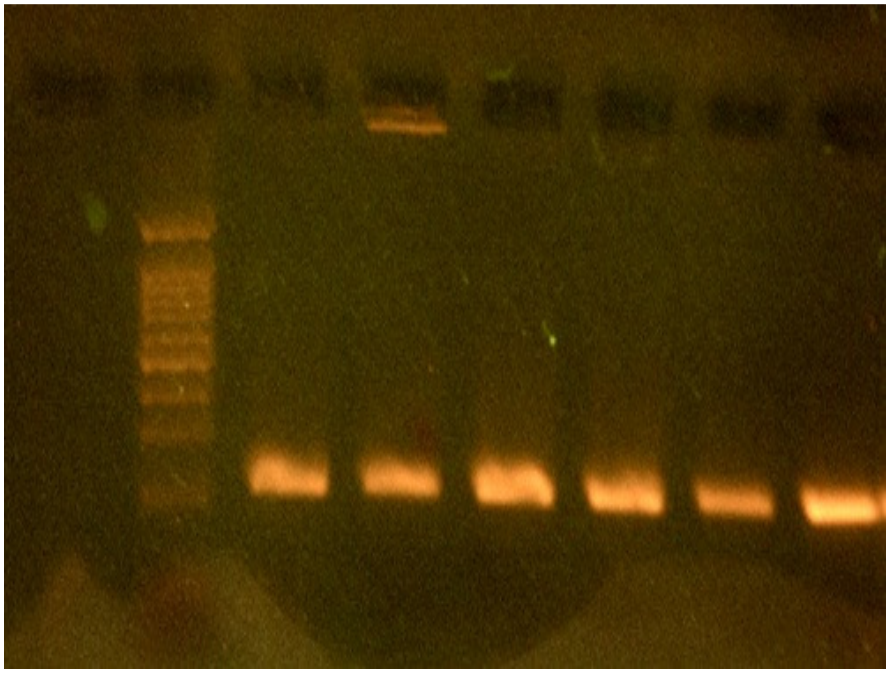
PCR Product: 200bp

Gene: hemolysin gene

HELINI-F: TTCATCTACGTGCTGGAGGG

HELINI-R: AGCCTGGATTGAGCGGATAA

FIGURE 11 : GEL ELECTROPHOROSIS



1st well- Negative control.

2nd well- Reference ladder.

3rd well onwards sample wells- All the samples have been noted at 200 base pairs expected of Klebsiella pneumoniae.

DISCUSSION

Bacterial infections are generally considered to be the most common cause of lower respiratory tract infection in chronic lung disease patients.

Proper identification of respiratory pathogen from BAL is central for the treatment of pulmonary infections.

Use of invasive techniques like bronchoscopic specimens like BAL fluid has led to better diagnosis of the lower respiratory tract infection.

In non-ventilated patient, semi quantitative culture of BAL fluid have shown to have significiant predicative value for lower respiratory tract infection.

Klebsillea pneumoniae is one of the important Gram-negative bacteria in nosocomial infections. Antibiotic resistant isolates make therapeutic challenge in treatment of the patient.

Acinetobacter baumanni is another Gram negative bacteria in hospital acquired infections and particularly pose problems due to large numbers of Multi drug resistant strains. This is a matter of great concern.

Candida spp and Aspergillus spp contribute to the large bulk of pulmonary mycoses in fungal reporting.

Chronic respiratory diseases are public health problems due to their frequency of occurrence and financial impact on both developed and developing countries like ours. It is also a major contributor of motility and morbidity worldwide. Our study was conducted with the aim of determining the bacterial and fungal causative agents associated with chronic respiratory

disease and also to estimate the antimicrobial sensitivity for the bacterial isolates.

Chronic respiratory disease cases were more among the age group of above 40 years in our study which correlates with studies like *Mullerova et al* (45%)[12] and Merino- Sanchez et al (60%).[13]

In lower respiratory tract infections BAL is a very useful diagnostic tool. This study showed a positive bacterial culture in 35 % of the cases of lung infection. This percentage is lower than when compared with studies like *Velez et al* (51.6%)[14], *Kottmann et al* (55.6%)[15] as they were done on immunocompromised patients. This study is in consistant with *Vivek KU*, *Nutan Kumar* study (38 %)[30] as both of the studies were carried out on general population.

In our study, aerobic gram negative bacterias were 42 % and aerobic gram positive bacterias were 28 %. The percentage of gram negative bacterias are lower when compared with studies like Groenewegen and Wouters (71%)[16] with suspected pneumonia in COPD and *Vivek KU* and *Nutan Kumar* (65.7%). On the other hand our study is in consistence with gram positive bacterias 27% and 34.5% respectively.

We observed klebisellia as the commonest pathogen isolated in this study which was 20%. This is in correlation with studies undertaken by *Lin SH et al* (19.6%) [17]and *Vivek KU* and *Nutan Kumar (*26%)[30]. Klebsiella being part of normal flora of mouth and commonly associated with pneumonia in elderly and hospitalized patients may been the reason of more isolation of the organism in our study.

Comparison of bacterial pathogens isolated in chronic respiratory disease from different studies.

Study	Total isolates	Klebsiella	Acinetobacter	S. aureus	S. pneumoniae	Pseudomonas aeroginosa
Torres et al (1998)[19]	73	-	-	-	43%	-
Lin SH et al (2007)[17]	328	19.6%	6.9%	6.1%	2.4%	16.8%
Bari et al (2010)[20]	60	13.3%	6%	-	3%	25%
Vivek KU (2016)[30]	42	26%	14.2%	11.9 %	9.5%	2%
This study	25	20%	14%	14%	8%	3%

In this study Gram negative bacterias shows more antibiotic resistance when compared with Gram positive bacterias. Among Gram negative bacterisa Acenitobacter baumanii exhibits highest resistance followed by Klebsiella pneumoniae and E. coli. Least resistance was show by Pseudonomas aeruginosa.

In Gram positive bacteria Staphylococcus aureus are resistant to Amoxicillin/clavulanic acid and exhibit sensitivity to other antibiotic. Streptococcus pneumonia shows 33% resistance to Amoxicillin/Clavulanic acid, clindamycin and cephalosporins. Enterococcus exhibits high resistance to Amoxicillin/clavulanic acid. All bacterial isolates were sensitive to Linezolid and vancomycin.

Low number of bacterial isolates may be due to prior use of antibiotics.

Comparison of fungal isolates in various studies of chronic respiratory diseases.

Study	No of isolates	Candida albicans	Aspergillus niger	Aspergillus flavus	Aspergillus fumigates
Vivek KU[30]	27	10 %	2 %	1 %	1 %
Our study	10	14 %	6 %	6 %	3 %

Our study correlates with Vivek where the commonest fungus associated with lower respiratory tract infection was candida albicans. Followed by Aspergillus species.

Conventional PCR test performed on samples of commonest isolate was tested for hemolysin gene for Klebsiella pneumoniae and were noted on 200 base pairs for Klebsiella pneumoniae in the post PCR product. PCR test was concordant with the phenotypic test.

SUMMARY

This one year prospective study was conducted among chronic respiratory diseases patients undergoing bronchoscopy admitted in the Department of Thoracic Medicine, Thanjavur Medical College and hospital.

A total of 100 BAL samples which was processed for Bacterial and fungal agents from May 2015 to April 2016 at Department of Microbiology, Thanjavur Medical College was included in this study.

The BAL sample was examined macroscopically for appearance of visible blood and microscopically for the presence of bacteria and fungus by KOH mount and Grams stain. Subsequently the sample was inoculated on Mac conkey, Blood, Chocolate ager by semi quantitative method for bacterial growth. It was also inoculated on SDA for fungal growth. All bacterial growth were biochemically test for identification of the organism. Antimicrobial sensitivity was done on the bacterial isolates and the results noted. Klebsiella pneumoniae were isolated in 7 samples and the commonest organism. These samples were send for conventional PCR testing for identification of hemolysin gene for confirmation.

- Out of the hundred cases 51 were female and 49 were males.
- The incidence of chronic respiratory diseases was more among male cases.
- Chronic bronchitis 30% cases were observed more in this study.
- Lower respiratory tract infections were common among COPD cases.
- Incidence of bacterial infection was 25 % and fungal infection was 10 %.
- Negative for bacterial culture was 75 %.
- Negative for Fungal culture was 90 %.
- Negative culture for both bacteria and fungus were 65 %.

- The percentage of pathogens causing lower respiratory infection in Chronic respiratory diseases patient was 43 % gram-negative bacteria,28 % gram positive bacteria,14 % of yeast and 15 % of Dimorphic fungi.
- Among the gram negative bacteria, the predominant pathogen was Klebsiella pneumoniae 46%, Acinetobacter baumanii 33%, Escherichia coli 13% and Pseudomonas aeuroginosa 6%.
- Among the gram positive bacteria, the predominant pathogen was Staphylococcus aureus 50%, Streptococcus pneumonia 30% and Enterococcus faecalis 20%.
- Among the fungus candida was 50% and Aspergillus was 50%.
- High antimicrobial resistance was noted in Acenitobacter baumanii and the least antimicrobial resistance was noted in pseudomonas aeruginosa.
- 7 samples of Klebsiella pneumoniae isolated were send for conventional PCR and test positive for hemolysin gene and confirmed as Klebsiella pneumoniae by molecular method.

CONCLUSION

It is important to diagnose chronic respiratory diseases much early before clinically evident stage to prevent and reduce infection related morbidity and mortality.

Results from BAL fluid analysis have to be evaluated with regard to the underlying disease and the whole clinical picture in chronic respiratory diseases patients.

It is evident that the colonizing microbial flora of the respiratory tract are responsible for infections in Chronic Respiratory diseases and frequent cause of exacerbations.

Phenotypic tests are economical and so can be used for screening. PCR molecular characterization of the isolates are costlier. This may be utilized to identify drug resistance pattern in the isolates which can influence the choice of antibiotic therapy.

Gram negative bacterial infections are on the rise with high prevalence of drug resistance. Prompt treatment with appropriate antibiotics with improve the outcome of the disease and decrease the number of episodes of exacerbation and severity of the disease.

Inappropriate choice of antibiotic and inadequate treatment poses a risk of Multidrug resistant pathogens which are difficult to treat and needs higher antibiotics which are expensive and not afford to many in our setting.

Institutional antibiograms are an important tool for detecting and monitoring antimicrobial resistance patterns among microbial pathogens. This can help in guiding the policy making decisions for the ethical use of antibiotics in treatment setting.

BIBLIOGRAPHY

1. WHO. Global surveillance, prevention and control of CHRONIC RESPIRATORY DISEASES.A comprehensive approach;2007:5-14.

2. Australian Institute of Health and Welfare. chronic respiratory conditions including asthma and COPD [internet].http:/www.aihw.gov.au/chronicrespirator conditions/.last accessed on.

3. Koul PA.Chronic obstructive pulmonary disease: Indian guidelines and the road ahead. Lung India. 2013 Jul;30(3):175-7. doi: 10.4103/0970-2113.116233.

4. Meduri GU, Beals DH, Maijub AG, Baselski V. Protected bronchoalveolar lavage. A new bronchoscopic technique to retrieve uncontaminated distal airway secretions. Am Rev Respir Dis. 1991 Apr;143(4 Pt 1):855-64.

5. Meduri GU, Beals DH, Maijub AG, Baselski V. Protected bronchoalveolar lavage. A new bronchoscopic technique to retrieve uncontaminated distal airway secretions. Am Rev Respir Dis. 1991 Apr;143(4 Pt 1):855-64.

6. Joao Carlos Pereira Gomes, Wilson L Pedreira Jr, Evangelina M P, Araujo A, Francisco G Soriano, Elnara M Negri et al.Impact of BAL in the Management of Pneumonia With Treatment failure-Positivity of BAL Culture Under Antibiotic Therapy. CHEST 2000;118:1739–46.

7. Mary K. York,Peter Gilligan, and Deirdre L Church. Processing and Interpretation of Lower respiratory tract specimens.In:Lynne S Garcia,Henry D Isenberg(eds). Clinical Microbiology Procedure Handbook.2nd ed.ASM PRESS,Washington DC 2007;1:3.11.2.

8. Initial processing, inoculation and incubation of aerobic bacteriology specimens. In:Henry D Isenberg, editor. Clinical Microbiology Procedure Handbook.1st Ed.ASM PRESS,Washington DC.1992;1:1.4.12.

9. Kahn Frederick and Jones J Jefrey.Analysis of Bronchoalveolar lavage specimen from immunocompromised patients with protocol applicable in microbiology Laboratory.J of Clin Microbiology 1988 june;1150-1155.

10. Rasmussen TR, Korsgaard J, Møller JK, Sommer T, Kilian M. Quantitative culture of bronchoalveolar lavage fluid in community-acquired lower respiratory tract infections. Respir Med. 2001 Nov;95(11):885-90.

11. Knox KS, Meinke L. Role of bronchoalveolar lavage diagnostics in fungal infections. Clin Chest Med. 2009 Jun;30(2):355-65, viii. doi: 10.1016/j.ccm.2009.02.010.

12. Müllerová H, Shukla A, Hawkins A, Quint J. Risk factors for acute exacerbations of COPD in a primary care population: a retrospective observational cohort study. BMJ Open. 2014 Dec 18;4(12):e006171. doi:10.1136/bmjopen-2014-006171.

13. Merino-Sánchez M, Alfageme-Michavila I, Reyes- Núñez N, Lima-Alvarez J. [Prognosis in patients with pneumonia and chronic obstructive pulmonary disease]. Arch Bronconeumol. 2005 Nov;41(11):607-11.

14. Vélez L1, Correa LT, Maya MA, Mejía P, Ortega J, Bedoya V, Ortega H. Diagnostic accuracy of bronchoalveolar lavage samples in immunosuppressed patients with suspected pneumonia: analysis of a protocol.. Respir Med. 2007 Oct;101(10):2160-7.

15. Kottmann RM, Kelly J, Lyda E, Gurell M, Stalica J, Ormsby W, Moon K, Trawick D, Sime PJ. Bronchoscopy with bronchoalveolar lavage: determinants of yield and impact on management in immunosuppressed patients. Thorax. 2011 Sep;66(9):823. doi: 10.1136/thx.2010.145540.

16. Kottmann RM, Kelly J, Lyda E, Gurell M, Stalica J, Ormsby W, Moon K, Trawick D, Sime PJ. Bronchoscopy with bronchoalveolar lavage: determinants of yield and impact on management in immunosuppressed patients. Thorax. 2011

Sep;66(9):823. doi: 10.1136/thx.2010.145540. Epub 2010 Sep 13.

17. Lin SH, Kuo PH, Hsueh PR, Yang PC, Kuo SH. Sputum bacteriology in hospitalized patients with acute exacerbation of chronicobstructive pulmonary diseasein Taiwan with an emphasis on Klebsiella pneumonia and Pseudomonas aeruginosa. Respirology. 2007 Jan;12(1):81-7.

18. Singh AK, Sen MR, Anupurba S, Bhattacharya P. Antibiotic sensitivity pattern of the bacteria isolated from nosocomial infections in ICU. J Commun Dis. 2002 Dec;34(4):257-63.

19. Torres A, El-Ebiary M. Invasive diagnostic techniques for pneumonia: protected specimen brush, bronchoalveolar lavage, and lung biopsy methods. Infect Dis Clin North Am 1998;12: 701–22.

20. Bari MR, Hiron MM, Zaman SM, Rahman MM, Ganguly KC. Microbes responsible for acute exacerbation of COPD. Mymensingh Med J. 2010 Oct;19(4):576-85.

21. Bonten MJ, Weinstein RA. The role of colonization in the pathogenesis of nosocomial infections. Infect Control Hosp Epidemiol. 1996 Mar;17(3):193-200.

22. Hohenadel IA, Kiworr M, Genitsariotis R, Zeidler D, Lorenz J. Role of bronchoalveolar lavage in immunocompromised patients with pneumonia treated with a broadspectrum antibiotic and antifungal regimen. Thorax. 2001 Feb;56(2):115-20.

23. Biswas D, Agarwal S, Sindhwani G, Rawat J. Fungal colonization in patients with chronic respiratory diseases from Himalayan region of India. Ann Clin Microbiol Antimicrob. 2010 Sep 20;9:28. doi: 10.1186/1476-0711-9-28.

24. Phukan AC, Sarmabordoloi JN, Mahanta J. Bronchopulmonary candidiasis in a tertiary referral hospital of Assam, India. Indian J Med Sci. 2000 Nov;54(11):491-4.

25. Cruz C H R, Vieille O P, Fuentes H D, Ponce E E, Piontelli L E. Frequency of pulmonary mycoses determined by analyzing lung secretion samples. Rev Med Chil. 2012 May;140(5):595-601. doi: 10.4067/S0034-98872012000500006.

26. Tarrand JJ, Lichterfeld M, Warraich I, Luna M, Han XY, May GS, Kontoyiannis DP. Diagnosis of invasive septate mold infections.A correlation of microbiological culture and histologic orcytologic examination. Am J Clin Pathol. 2003 Jun;119(6):854-8.

27. Changdeo, S.A. Species distribution, virulence factors and antifungal susceptibility profile of Candida isolated from Oropharyngeal lesions of HIV infected patients. Int J Curr Microbiol App Sci., 3(1): 453-460 (2014)

28. Snelders E, van der Lee HA, Kuijpers J, Rijs AJ, Varga J, Samson RA, Mellado E, Donders AR, Melchers WJ, Verweij PE. Emergence of azole resistance in Aspergillus fumigatus and spread of a single resistance mechanism. PLoS Med. 2008 Nov 11;5(11):e219. doi: 10.1371/journal.pmed.0050219.

29. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, Haas A, Ruhnke M, Lode H. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin Infect Dis. 2002 Mar 1;34(5):563-71. Epub 2002 Jan 22.

30. Vivek KU, Nutan Kumar DM Microbiological profile of bronchoalveolar lavage fluid in patients with chronic respiratory diseases: a tertiary care hospital study. Int J Med Res Rev 2016;4(3):330-337. doi: 10.17511/ijmrr.2016.i03.08.

31. Washinton C. Winn Jr., et al. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed:68-80

32. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed M100S, Wayen, PA, USA, 2016

33. Collee. JG, Fraser AG et al, Mackie and McCartney Practical Medical Microbiology, 14th ed 152

34. Bartlett, J. G., J. Alexander, J. Mayhew, N. Sullivan-Sigler, and S. L. Gorbach. 1976. Should fiberoptic bronchoscopy aspirates be cultured? Am. Rev. Respir. Dis. 114:73-78.
35. Wimberly, N., L. J. Faling, and J. G. Bartlett. 1979. A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture. Am. Rev. Respir. Dis. 119:337-343.
36. Linder, J., and S. I. Rennard. 1988. Development and application of bronchoalveolar lavage, p. 1-16. In Bronchoalveolar lavage. ASCP Press, Chicago.
37. Meduri, G. U., and J. Chastre. 1992. The standardization of bronchoscopic techniques for ventilator-associated pneumonia. Chest 102:557s-564s.
38. Papin, T. A., J. P. Lynch III, and J. G. Weg. 1985. Transbronchial biopsy in the thrombocytopenic patient. Chest 88:549-552.
39. Steinberg, K. P., D. R Mitchell, R J. Maunder, J. A. Milberg, M. E. Whitcomb, and L. D. Hudson. 1993. Safety of bronchoalveolar lavage in patients with adult respiratory distress syndrome. Am. Rev. Respir. Dis. 148:556-561.
40. Chalfin, D. B., M. S. Niederman, and A. M. Fein. 1992. Protected specimen brush (PSB) or empiric therapy (ET) for the diagnosis and treatment of nosocomial pneumonia (NP)? A decision analysis. Chest 102:96S.
41. Fein, A. M., S. H. Feinsilver, and M. S. Niederman. 1993. Nonresolving and slowly resolving pneumonia. Diagnosis and management in the elderly patient. Clin. Chest Med. 14:555-569.
42. McCabe, R E., and J. S. Remington. 1991. Open lung biopsy, p. 105-117. In J. Shelhamer, P. A. Pizzo, J. E. Parrillo, and H. Masur (ed.), Respiratory disease in the immunosuppressed host. J. B. Lippincott Co., Philadelphia.

43. Baselski, V. S., M. Eltorky, J. J. Coalson, and J. P. Griffin. 1992. The standardization of criteria for processing and interpreting laboratory specimens with suspected ventilator-associated pneumonia. Chest 102:571S-579S.
44. Cockerill, F. R, W. R Wilson, and H. A. Carpenter. 1985. Open lung biopsy in immunocompromised patients. Arch. Intern. Med. 145:1398-1404.
45. Davis, G. S., M. S. Giancola, M. C. Costanza, and R B. Low. 1982. Analyses of sequential bronchoalveolar lavage samples from healthy human volunteers. Am. Rev. Respir. Dis. 126:611-616.
46. Goldstein, R. A., and P. K. Rohatgi. 1990. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. Am. Rev. Respir. Dis. 142:481-486.
47. Hadley, W. K., and V. L. Ng. 1989. Organization of microbiology laboratory services for the diagnosis of pulmonary infections in patients with human immunodeficiency virus infection. Semin. Respir. Infect. 4:85-92.
48. Linder, J., and S. Rennard. 1988. Processing and analysis of bronchoalveolar lavage specimens, p. 17-44. In Bronchoalveolar lavage. ASCP Press, Chicago.
49. Baselski, V. S., M. Eltorky, J. J. Coalson, and J. P. Griffin. 1992. The standardization of criteria for processing and interpreting laboratory specimens with suspected ventilator-associated pneumonia. Chest 102:571S-579S.
50. Bordelon, J. Y., P. Legrand, W. C. Gewin, and C. V. Sanders. 1983. The telescoping plugged catheter in suspected anaerobic infections. A controlled series. Am. Rev. Respir. Dis. 128:465-468.
51. Higuchi, J. H., J. J. Coalson, and W. G. Johanson, Jr. 1982. Bacteriologic diagnosis of nosocomial pneumonia in primates. Usefulness of the protected specimen brush. Am. Rev. Respir. Dis. 125:53-57.
52. Kahn, F. W., and J. M. Jones. 1986. Bronchoalveolar lavage in the rapid diagnosis of lung disease. Lab. Manage. 24:31-35.

53. Kahn, F. W., and J. M. Jones. 1988. Analysis of bronchoalveolar lavage specimens from immunocompromised patients with a protocol applicable in the microbiology laboratory. J. Clin. Microbiol. 26:1150-1155.

54. Rouby, J.-J., M.-D. Rossignon, M.-H. Nicolas, E. M. deLassale, S. Cristin, J. Grosset, and P. Viars. 1989. A prospective study of protected bronchoalveolar lavage in the diagnosis of nosocomial pneumonia. Anesthesiology 71:679-685.

55. Baselski, V. S., M. K. Robison, L. W. Pifer, and D. R Woods. 1990. Rapid detection of Pneumocystis carinii in bronchoalveolar lavage samples by using cellufluor staining. J. Clin. Microbiol. 28:393-394.

56. Chastre, J., J.-Y. Fagon, P. Soler, M. Bornet, Y. Domart, J.-L. Trovillet, C. Gibert, and A. J. Hance. 1988. Diagnosis of nosocomial bacterial pneumonia in intubated patients undergoing ventilation: comparison of the usefulness of bronchoalveolar lavage and the protected specimen brush. Am. J. Med. 85:499-506.

57. Chastre, J., J.-Y. Fagon, P. Soler, Y. Domart, J. Pierre, M. C. Dombret, C. Gibert, and A. J. Hance. 1989. Quantification of BAL cells containing intracellular bacteria rapidly identifies ventilated patients with nosocomial pneumonia. Chest 95:190S- 192S.

58. Baselski, V. 1993. Microbiologic diagnosis of ventilator-associated pneumonia. Infect. Dis. Clin. N. Am. 7:331-357.

59. Levine, S. J. 1992. An approach to the diagnosis of pulmonary infections inimmunosuppressed patients. Semin. Respir. Infect. 7:81-95.

60. Skootsky, S. A., and R K. Dye. 1993. The changing relationship between clinicians and the laboratory medicine specialist in the managed care era. Am. J. Clin. Pathol. 99:S7-S11.

61. Farr, B. M., D. L. Kaiser, B. D. W. Harrison, and C. K. Connolly. 1989. Prediction of microbial etiology at admission to the hospital for pneumonia from the presenting clinical features. Thorax 44:1031-1035.
62. Ferrer, M. A., A. Torres, A. Xaubert, J. P. dela Bellacasa, C. Agusti, J. Gonzalez, R. deCelis, and M. T. J. deAnta. 1992. Diagnostic value of telescoping plugged catheters in HIV-infected patients with pulmonary infiltrates. Chest 102:76-83.
63. Meduri, G. U. 1990. Ventilator-associated pneumonia in patients with respiratory failure: a diagnostic approach. Chest 97:1208-1219.
64. Violan, J. S. F. R. deCastro, J. C. Luna, A. B. Benitez, and J. L. M. Alonso. 1993. Comparative efficacy of bronchoalveolar lavage and telescoping plugged catheter in the diagnosis of pneumonia in mechanically ventilated patients. Chest 103:386-390.
65. Haley, L. D., J. Trandel, and M. B. Coyle. 1980. Cumitech 11, Practical methods for culture and identification of fungi in the clinical microbiology laboratory. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
66. Linder, J., and S. Rennard. 1988. Processing and analysis of bronchoalveolar lavage specimens, p. 17-44. In Bronchoalveolar lavage. ASCP Press, Chicago.
67. Baselski, V. 1993. Microbiologic diagnosis of ventilator-associated pneumonia. Infect. Dis. Clin. N. Am. 7:331-357.
68. Chauncey, J. B., J. P. Lynch, R C. Hyzy, and G. B. Toews. 1990. Invasive techniques in the diagnosis of bacterial pneumonia in the intensive care unit. Semin. Respir. Infect. 5:215-225.
69. Crone, P. B. 1948. The counting of surface colonies of bacteria. J. Hyg. 46:426-430.

70. Pingleton, S. K., J.-Y. Fagon, and K. V. Leeper. 1992. Patient selection for clinical investigation of ventilator-associated pneumonia. Criteria for evaluating diagnostic techniques. Chest 102: 553S-556S.

71. Fang, G.-D., M. Fine, J. Orloff, D. Arisumi, V. Yu, W. Kapoor, J. T. Grayston, S. P. Wang, R. Kohler, R. Muder, Y. C. Yee, J. D. Rihs, and R. M. Vickers. 1990. New and emerging etiologies for community-acquired pneumonia with implications for therapy. A prospective multicenter study of 359 cases. Medicine (Baltimore) 69:307-316.

72. Bartlett, J. G. 1977. Diagnostic accuracy of transtracheal aspiration bacteriologic studies. Am. Rev. Respir. Dis. 115:777-782.

73. Bartlett, J. G. 1993. Anaerobic bacterial infections of the lung and pleural space. Clin. Infect. Dis. 16:S248-S255.

74. Korvick, J. A., V. L. Yu, and G. Fang. 1987. Legionella species as hospital-acquired respiratory pathogens. Semin. Respir. Infect. 2:34-47.

75. Rello, J., E. Quintana, V. Ausina, A. Net, and G. Prats. 1993. A three year study of severe community-acquired pneumonia with emphasis on outcome. Chest 103:232-235.

76. Arduino, R. C., P. C. Johnson, and A. G. Miranda. 1993. Nocardiosis in renal transplant recipients undergoing immunosuppression with cyclosporine. Clin. Infect. Dis. 16:505-512.

77. Chapman, S. W., and J. P. Wilson. 1990. Nocardiosis in transplant patients. Semin. Respir. Infect. 5:74-79.

78. Kenny, G. E., G. G. Kaiser, M. K. Cooney, and H. M. Foy. 1990. Diagnosis of Mycoplasma pneumoniae pneumonia: sensitivities and specificities of serology with lipid antigen and isolation of the organism on soy peptone medium for identification of infections. J. Clin. Microbiol. 28:2087-2093.

79. Marmion, B. P., J. Williamson, D. A. Worswock, T.-W. Kok, and R J. Harris. 1993. Experience with newer techniques for the laboratory detection of Mycoplasma pneumoniae infection: Adelaide, 1978-1992. Clin. Infect. Dis. 17:S90-S99.

80. Grayston, J. T., L. A. Campbell, C.-C. Ku, C. H. Mordhorst, P. Saikku, D. H. Thom, and S.-P. Wang. 1990. A new respiratory tract pathogen: Chlamydia pneumoniae strain TWAR. J. Infect. Dis. 161:618-625.

81. Baughman, R. P., M. N. Dohn, R. G. Loudon, and P. T. Frame. 1991. Bronchoscopy with bronchoalveolar lavage in tuberculosis and fungal infections. Chest 99:92-97.

82. Burns, D. N., R. J. Wallace, Jr., M. E. Schultz, Y. Zhang, S. Q. Zubairi, Y. Pang, C. L. Gibert, B. A. Borwn, E. S. Noel, and F. M. Gordin. 1991. Nosocomial outbreak of respiratory tract colonization with Mycobacterium fortuitum: demonstration of the usefulness of pulsed field gel electrophoresis in a epidemiologic investigation. Am. Rev. Respir. Dis. 144:1153-1159.

83. Bigby, T. D., D. Margoiskee, J. L. Curtis, P. F. Michael, D. Sheppard, W. K. Hadley, and P. C. Hopewell. 1986. The usefulness of induced sputum in the diagnosis of Pneumocystis cannii pneumonia in patients with the acquired immunodeficiency syndrome. Am. Rev. Respir. Dis. 133:515-518.

84. Englund, J. A., C. J. Sullivan, M. C. Jordan, L P. Dehner, G. M. Vercelloti, and H. H. Balfour, Jr. 1988. Respiratory syncytial virus infection in immunocompromised adults. Ann. Intern. Med. 109: 203-208.

85. Anderson, D. J., and M. C. Jordan. 1990. Viral pneumonia in recipients of solid organ transplants. Semin. Respir. Infect. 5:38-49.

86. Bottone, E. J. 1991. Diagnosis of acute pulmonary toxoplasmosis by visualization of invasive and intracellular tachyzoites in Giemsa- stained smears of bronchoalveolar lavage fluid. J. Clin. Microbiol. 29:2626-2627.

87. Current, W. L, and L. S. Garcia. 1991. Cryptosporidiosis. Clin. Microbiol. Rev. 4:325-358.

88. Genta, R. M., P. Miles, and K. Fields. 1989. Opportunistic Strongyloides stercoralis infection in lymphoma patients. Report of a case and review of the literature. Cancer 63:1407-1411.

89. Yee, B., J.-I. Hsu, C. B. Favour, and E. Lohne. 1992. Pulmonary paragonimiasis in southeast Asians living in the central San Joaquin Valley. West. J. Med. 156:423-425.

90. Cameron, M. L., J. A. Bartlett, H. A. Gallis, and H. A. Waskin. 1991. Manifestations of pulmonary cryptococcosis in patients with acquired immunodeficiency syndrome. Rev. Infect. Dis. 13:64-67.

91. George, R. B., S. G. Jenkinson, and R. W. Light. 1978. Fiberoptic bronchoscopy in the diagnosis of pulmonary fungal and nocardial infections. Chest 73:33-36.

92. Prechter, G. C., and V. B. S. Prakash. 1989. Bronchoscopy in the diagnosis of pulmonary histoplasmosis. Chest 95:1033-1036.

93. Kahn, F. W., J. M. Jones, and D. M. England. 1986. The role of bronchoalveolar lavage in the diagnosis of invasive pulmonary aspergillosis. Am. J. Clin. Pathol. 86:518-523.

94. Sapira, J. D., and J. Cochran. 1993. Community-acquired versus hospital-acquired pneumonia: What are we talking about? A skeptic's review. Infect. Dis. Newsl. 12:57-64.

PROFORMA

NAME:

AGE/SEX:

ADDRESS:

OCCUPATION:

DIAGNOSIS:

IP No/OP No:

WARD:

CENTRAL LAB No:

MICRO No:

DATE OF SAMPLE COLLECTION:

CHIEF COMPLAINTS

FEVER:

COUGH:

BREATHLESSNESS:

OTHERS:

PAST HISTORY

TREATMENT HISTORY:

DIABETES:

HYPERTENSION:

PULMONARY TB:

ASTHMA/ ALLEGRY:

PERSONAL HISTORY

SMOKER:

ALCOHOLIC:

OCCUPATION:

CLINICAL DIAGNOSIS:

WORK SHEET

SPECIMEN: **BRONCHOALVEOLAR LAVAGE**

METHOD OF COLLECTION: **POST BRONCHOSCOPY**

MACROSCOPIC EXAMINATION:

MICROSCOPIC EXAMINATION:

1. 10% KOH WET MOUNT:

CULTURE:

1. NUTRIENT AGAR:
2. MACCONKEY AGAR:
3. BLOOD AGAR:
4. SDA:

MOTILITY:

STAINING

1. GRAM STAIN:
2. LPCB
3. ACID FAST STAIN:
4. KINYOUN'S STAIN:
5. INDIA INK STAIN:

BIOCHEMICAL REACTIONS:

1. CATALASE:
2. OXIDASE
3. SUGAR FERMENTATION TESTS:`
4. IMViC:
5. UREASE:
6. TSI:
7. LAO:
8. COAGULASE:

MICRO ORGANISM ISOLATED:

SPECIAL TESTS IF ANY:

ANTI MICROBIAL SUSCEPTIBILITY TEST: **ANTIBIOGRAM ON MHA BY KIRBY BAUER METHOD**

BACTERIA

S. No	DRUG	ZONE	SENSITIVE	RESISTANT	REMARK
1					
2					
3					
4					
5					
6					
7					
8					
9					

CONSENT FORM

I _____ hereby give consent to participate in the study conducted by **DR.BASHEER AHAMED.M,** Post Graduate in Department of Microbiology , Thanjavur Medical College, Thanjavur – 613004 and to use my personal clinical data and result of investigation for the purpose of analysis and to study the nature of disease. I also give consent for further investigations.

Place :

Date : Signature of participant

INFORMATION SHEET

- We are conducting a prospective and analytical study on

  "STUDY OF BACTERIAL AND FUNGAL PROFILE IN BRONCHOALVEOLAR LAVAGE FROM CHRONIC RESPIRATORY DISEASES PATIENTS ATTENDING A TERTIARY CARE CENTRE IN THANJAVUR AND MOLECULAR CHARACTERISATION FOR THE COMMONEST ORGANISM ISOLATED." in the department of Microbiology, Thanjavur Medical college, Thanjavur.
- At the time of announcing the results and suggestions, name and identity of the patients will be confidential.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator **Signature of participant**

Date:

MASTER CHART

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
1	31	F	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
2	40	M	COPD	Staphylococcus aureus	S	R	No	S	S	S	No	No	S	__
3	23	F	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
4	75	F	Emphysema	Acinetobacter baumanii	R	R	No	R	R	R	No	No	R	__
5	65	F	Bronchietasis	No Growth	__	__	__	__	__	__	__	__	__	__
6	55	M	Bronchitis	Staphylococcus aureus	S	R	No	S	S	S	No	No	S	__
7	54	M	Chronic Pleural disease	No Growth	__	__	__	__	__	__	__	__	__	__
8	55	F	Bronchietasis	No Growth	__	__	__	__	__	__	__	__	__	__
9	70	F	COPD	Enterococcus faecalis	S	R	S	S	R	No	S	S	No	__
10	60	M	Emphysema	Acinetobacter baumanii	S	R	No	R	R	R	No	No	S	__
11	33	M	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
12	65	M	COPD	Streptococcus pneumoniae	S	R	R	R	R	No	S	S	No	__
13	45	M	Bronchitis	No Growth	__	__	__	__	__	__	__	__	__	__

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
14	55	M	Bronchiectasis	No Growth	__	__	__	__	__	__	__	__	__	__
15	50	M	Bronchitis	No Growth	__	__	__	__	__	__	__	__	__	__
16	50	F	Asthma	Staphylococcus aureus	S	R	No	S	S	S	No	No	S	__
17	41	F	Pulmonary fibrosis	No Growth	__	__	__	__	__	__	__	__	__	__
18	52	M	Emphysema	No Growth	__	__	__	__	__	__	__	__	__	__
19	70	F	COPD	Streptococcus pneumoniae	S	S	S	S	S	No	S	S	No	__
20	30	F	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
21	47	M	Bronchitis	No Growth	__	__	__	__	__	__	__	__	__	__
22	55	M	Chronic Pleural disease	No Growth	__	__	__	__	__	__	__	__	__	__
23	65	F	Emphysema	Escherichia coli	R	R	No	No	R	S	No	No	S	__
24	44	M	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
25	23	F	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
26	35	M	Bronchitis	No Growth	__	__	__	__	__	__	__	__	__	__

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
27	47	F	Pulmonary fibrosis	No Growth	—	—	—	—	—	—	—	—	—	—
28	70	F	Emphysema	Staphylococcus aureus	S	S	No	S	S	S	No	No	S	—
29	48	F	Bronchitis	No Growth	—	—	—	—	—	—	—	—	—	—
30	35	F	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
31	44	M	Bronchitis	No Growth	—	—	—	—	—	—	—	—	—	—
32	82	M	COPD	Streptococcus pneumoniae	No	S	S	S	S	No	S	S	No	
33	47	F	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
34	52	F	Chronic Pleural disease	Enterococcus faecalis	No	R	S	S	S	No	S	S	No	
35	30	M	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
36	40	M	Bronchitis	No Growth	—	—	—	—	—	—	—	—	—	—
37	75	M	COPD	Klebsiella pneumoniae	S	R	No	R	R	S	No	No	S	P
38	23	F	Cystic fibrosis	No Growth	—	—	—	—	—	—	—	—	—	—
39	45	F	Bronchiectasis	No Growth	—	—	—	—	—	—	—	—	—	—

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
40	65	F	Emphysema	Pseudomonas aeuroginosa	S	S	No	No	S	S	No	No	S	__
41	70	F	COPD	Candida albicans	__	__	__	__	__	__	__	__	__	__
42	55	F	Bronchiectasis	No Growth	__	__	__	__	__	__	__	__	__	__
43	50	M	Bronchitis	No Growth	__	__	__	__	__	__	__	__	__	__
44	45	F	Chronic Pleural disease	No Growth	__	__	__	__	__	__	__	__	__	__
45	65	F	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
46	65	F	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
47	54	M	Bronchiectasis	Staphylococcus aureus	S	S	No	S	S	S	No	No	S	
48	57	M	Emphysema	No Growth	__	__	__	__	__	__	__	__	__	__
49	58	M	Bronchiectasis	No Growth	__	__	__	__	__	__	__	__	__	__
50	36	M	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
51	45	M	Bronchitis	Escherichia coli	S	R	No	R	S	S	No	No	S	__
52	58	M	Pulmonary fibrosis	No Growth	__	__	__	__	__	__	__	__	__	__

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
53	76	F	COPD	Aspergillus flavus	—	—	—	—	—	—	—	—	—	—
54	30	F	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
55	18	M	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
56	55	F	Bronchiectasis	No Growth	—	—	—	—	—	—	—	—	—	—
57	50	F	Emphysema	No Growth	—	—	—	—	—	—	—	—	—	—
58	50	M	Pulmonary fibrosis	No Growth	—	—	—	—	—	—	—	—	—	—
59	45	F	Bronchiectasis	No Growth	—	—	—	—	—	—	—	—	—	—
60	60	F	COPD	Acinectobacter baumanii	S	R	No	R	R	S	No	No	S	—
61	50	F	Chronic Pleural disease	No Growth	—	—	—	—	—	—	—	—	—	—
62	50	M	Bronchitis	No Growth	—	—	—	—	—	—	—	—	—	—
63	56	F	Bronchiectasis	Aspergillus flavus	—	—	—	—	—	—	—	—	—	—
64	31	F	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
65	68	M	COPD	No Growth	—	—	—	—	—	—	—	—	—	—

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
66	28	M	Chronic Pleural disease	No Growth	—	—	—	—	—	—	—	—	—	—
67	43	M	Bronchiectasis	No Growth	—	—	—	—	—	—	—	—	—	—
68	40	F	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
69	67	M	COPD	Aspergillus niger	—	—	—	—	—	—	—	—	—	—
70	53	M	Emphysema	No Growth	—	—	—	—	—	—	—	—	—	—
71	60	F	COPD	No Growth	—	—	—	—	—	—	—	—	—	—
72	38	F	Bronchiectasis	No Growth	—	—	—	—	—	—	—	—	—	—
73	43	M	Bronchitis	No Growth	—	—	—	—	—	—	—	—	—	—
74	60	F	COPD	Klebsellia pneumoniae	S	R	No	S	S	S	No	No	S	P
75	45	F	Pulmonary fibrosis	Candida albicans	—	—	—	—	—	—	—	—	—	—
76	30	F	Asthma	No Growth	—	—	—	—	—	—	—	—	—	—
77	58	M	Bronchiectasis	Klebsiella pneumoniae	S	R	No	S	S	S	No	No	S	P
78	70	M	COPD	No Growth	—	—	—	—	—	—	—	—	—	—

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
79	35	M	Asthma	No Growth	__	__	__	__	__	__	__	__	__	__
80	65	M	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
81	74	M	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
82	66	F	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
83	70	M	COPD	Klebsiella pneumoniae	S	S	No	S	S	S	No	No	S	P
84	35	M	Asthma	Acinetobacter baumanni	S	S	No	S	S	S	No	No	S	__
85	42	M	Bronchitis	No Growth	__	__	__	__	__	__	__	__	__	__
86	60	F	COPD	Klebsiella pneumoniae	S	S	No	S	S	S	No	No	S	P
87	55	F	Bronchiectasis	No Growth	__	__	__	__	__	__	__	__	__	__
88	70	F	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
89	75	M	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
90	37	M	Asthma	Candida albicans	__	__	__	__	__	__	__	__	__	__
91	37	F	Bronchiectasis	No Growth	__	__	__	__	__	__	__	__	__	__

SNO	AGE	SEX	DIAGNOSIS	ORGANISM	AK 30µg	AU 20/10 µg	CY 2 µg	CF 75 µg	CPM 30µg	I 10µg	LZ 30µg	VA 30 µg	PT 100/10 µg	PCR
92	42	M	Chronic Pleural disease	No Growth	__	__	__	__	__	__	__	__	__	__
93	60	F	COPD	No Growth	__	__	__	__	__	__	__	__	__	__
94	36	F	Bronchietasis	Klebsiella pneumoniae	S	S	No	S	S	S	No	No	S	P
95	42	M	Bronchitis	Candida albicans	__	__	__	__	__	__	__	__	__	__
96	60	M	COPD	Klebsiella pneumoniae	S	S	No	S	S	S	No	No	S	P
97	48	F	Bronchitis	Acinetobacter baumanni	S	S	No	S	S	S	No	No	S	__
98	25	M	Asthma	Aspergillus niger	__	__	__	__	__	__	__	__	__	__
99	47	F	Asthma	Aspergillus fumigates	__	__	__	__	__	__	__	__	__	__
100	24	M	Asthma	Candida albicans	__	__	__	__	__	__	__	__	__	__

AK – AMIKACIN; AU – AMOXICILLIN/CLAVULANIC ACID; CY – CLINDAMYCIN; CF – CEFOPERAZONE;

CPM – CEFEPIME;

I – IMEPENAM; LZ – LINEZOLIDE; VA – VANCOMYCIN; PT – PIPERACILLIN/TAZOBACTAM;

S – SENSITIVE; R – RESISTANT; NO – NO DISC RECOMMENDED

P – PCR POSITIVE

LIST OF TABLES

LIST OF PICTORIAL DIAGRAMS

APPENDIX 1

Potassium Hydroxide Mount

Principle

The KOH clears out the background scales or cell membranes that may be confused with fungal hyphal elements in microscopy of clinical specimens. Gentle heating also accelerates clearing of artifacts.

Procedure

1. Emulsify the specimen in a drop of 10% KOH on a microscopic slide with the help of a loop.
2. Apply gentle heat by passing the slide over a Bunsen flame for 3-4 times.
3. Cover the smear with the cover slip.
4. Leave it for 5-10 minutes.
5. Examine the slide under low (10X) and high power (40X) magnifications.
6. Examine the slide for 15-20 minutes for demonstration of shining fungal elements.

APPENDIX 2

Gram's Staining

Procedure

1. Cover the smear with methyl violet (basic dye). Allow it to stand for one minute.
2. Rinse the smear gently under tap water.
3. Cover the smear with Gram's iodine (mordant) and allow it to stand for one minute.
4. Rinse the smear again gently under tap water.
5. Decolourise the smear with 95% alcohol.
6. Rinse the smear again gently under tap water.
7. Cover the smear with dilute carbol fuchsin (counter stain) for 30 seconds to 1 minute.
8. Rinse the smear again gently under tap waterand air dry it.
9. Observe the smear first under low power (10X) objective and then under oil immersion (100X) objective.
10. Record the observation.

APPENDIX 3

Acid-Fast Staining (Ziehl-Neelsen)

Procedure

1. Heat fix the smear by passing the slide 2-3 times gently over the flame with the smear side up. Allow the smear to be air dried.
2. Put the smears on a slide rack and cover the smears with strong carbol fuchsin. Allow it to stain for 5 minutes.
3. Heat the slide intermittently.
4. Rinse the smear gently under tap water.
5. Cover the smear with 20% sulphuric acid for at least 10 minutes for decolourisation.
6. Wash the slides thoroughly with water to remove all traces of acid.
7. Cover the smear with Loeffler's methylene blue for 15-20 seconds.
8. Rinse the smear again under tap water and air dry.
9. Observe the smear first under low power (10X) objective and then under oil immersion (100X) objective.
10. Record the observation.

APPENDIX 4

Modified Acid-Fast (Kinyoun's) stain

To stain Nocardia by Kinyoun Acid fast staining method.

Procedure

1. Prepare smear and fix with heat.
2. Added strong carbol fuchsin over the smear. Allow it to act for 5 minutes. Stain need to be heated.
3. Decolourize with 1% sulphuric acid and wash.
4. Counter stain with methylene blue for 1 minute and wash.
5. Dry the slide in air and examine under oil immersion and record the findings.

APPENDIX 5

May-Grunwald Giemsa Stain

Procedure

1. Fix the smear with methanol for 5 to 10 minutes.
2. Stain in diluted May-Grunwald solution for 10 minutes.
3. Without washing transfer the slide to working Giemsa stain.
4. Stain in Giemsa solution for 15 minutes.
5. Wash and differentiate by keeping the water on slides for 1-2 minutes until desired colour balance is achieved.
6. Air dry, mount and examine under the microscope.

APPENDIX 6

Nutrient agar

Prepared from ready to used dehydrated powder.

Contents

Peptone

Lab-Lemco powder

Yeast extract

Sodium chloride

Agar

Nutrient agar is used at a concentration of 2.8 g in 100 ml of distilled water.

1. Sterilize by autoclaving at 121˚C for 15 minutes.
2. Dispense aseptically the requied amount of nutrient agar.
3. Store in cool dark place.

APPENDIX 7

Blood agar

Contents

Nutrient agar 500 ml

Sterile defibrinated blood 25 ml

1. Sterilize the medium by autoclaving at 121˚C for 15 minutes. Transfer to a 50 ml water bath.
2. When the agar is colled to 50˚C, add aseptically the sterile blood and mix gently but well. Avoid air bubble formation.
3. Dispense aspectically in 15 ml amounts in sterile petri dishes.
4. Store the plates at 2-8˚C in plastic bags to prevent loss of moisture.

APPENDIX 8

CHOCOLATE AGAR

When blood agar is heated, red cells are lysed and the medium becomes brown in color.

It supplies the growth factors required for the growth of fastidious organisms.

1. Heat the medium in a 70˚C water bath until it becomes brown in colour. This takes about 10-15 minutes. During this time the media should be mixed gently several times.
2. Allow the medium to cool to about 45˚C, remix and dispense in sterile petri dishes.
3. Store the plates at 2-8˚C in plastic bags.

APPENDIX 9

MacConkey agar

Prepared from ready to use dehydrated powder.

Content:

Peptone.

Lactose.

Bile salts.

Sodium chloride.

Neutral red.

Agar.

The medium is used at a concentration 5.2 g in 100 ml distilled water.

1. Sterilize the media by autoclaving at 121˚C for 15 mins.
2. When the medium has cooled to 50-55˚C, mix well and dispense aseptically in sterile petri dishes.
3. Store the plates at 2-8˚C in plastic bags to prevent loss of moisture.

APPENDIX 10

Sabouraud Dextrose Agar

Basal media used to isolate common fungal pathogens.

Ingredients

Peptone	10 gm
Dextrose	40 gm
Agar	20 gm
Distilled water	1000 ml

Preparation

1. Autoclave the above ingredients at 121˚C for 15 minutes and adjust final pH at 5.6.
2. Dispense in tubes and allow to cool in slanted position.

APPENDIX 11

Mueller-Hinton Agar

Beef extract	2 gms
Acidicase Peptone	7.5 gms
Starch	1.5 gms
Agar	17 gms
Distilled water	1000 ml

Dissolve the ingredients in one liter of distilled water. Mix thoroughly. Heat with frequent agitation and boil for one minute. Adjust pH to 7.4 ± 0.2. Sterilize by autoclaving.

Use

Standard medium for antimicrobial susceptibility testing.