BACTERIAL ISOLATES AND THEIR ANTIBIOTIC RESISTANCE PATTERN AMONG ADULT HOSPITALIZED PATIENTS WITH LOWER RESPIRATORY TRACT INFECTIONS AT JIMMA UNIVERSITY MEDICAL CENTER, SOUTHWEST ETHIOPIA.

BY:-

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Abstract

Introduction: *Lower respiratory tract infections (LRTIs) are major cause of morbidity and mortality throughout the world. Moreover, in recent years, there has been dramatic rise in prevalence of multidrug-resistant bacteria among respiratory pathogens in both hospital and community setting. Updated knowledge on etiology and antibiotic susceptibility pattern of lower respiratory tract infections is essential for optimal management of patients.*

Objective: *The objective of this study was to determine bacterial isolates and their antibiotic resistance pattern among adult hospitalized patients with lower respiratory tract infections at Jimma University Medical Center, Southwest Ethiopia, 2019*

Materials and Methods: *A cross sectional study was conducted from April to October 2019. Demographic and related clinical data were collected from 189 study participants using a structured questionnaire. Respiratory sample from adults suspected with lower respiratory tract infection was collected and processed to identify potentially pathogenic bacteria using the conventional culture according to standard bacteriological techniques. Antibiotic susceptibility testing was performed by using Kirby Bauer disk diffusion method. Data was analyzed using SPSS version 23. Chi-square and odds ratios were calculated and P values <0.05 was considered as statistically significant.*

Results: *Out of the 189 suspected study participants with lower respiratory infections, 102(54.0%) of participants were a culture positive. The predominant bacterial isolates were Klebsiella species, Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter species, Escherichia coli and Streptococcus pneumonia. Overall, 92(82.1%) of the isolates were multidrug resistance (MDR); 71.9% of gram-negative isolates were ESBL positive. Having two or more exacerbations in previous one year [AOR 6.59 (95%CI: 1.06-38.73)] was found independent associated risk factor for positive sputum culture from acute exacerbation of chronic obstructive pulmonary (AECOPD) patients & being age ≥65 years [AOR 4.21 (95%CI: 1.12-14.64) and chronic respiratory disease [AOR 3.68 (95%CI: 1.29-10.50)] from community acquired pneumonia(CAP) were found independent associated risk factor for positive sputum culture.*

CONCLUSIONS: *High prevalence of multidrug resistance in bacterial isolates of lower respiratory tract infections are clear characteristics of this study. 71.9% of Gram-negative isolates were ESBL (*Extended spectrum of betalactamase*) positive. Management of bacterial LRTI better to guide by culture isolation and antimicrobial susceptibility testing to decrease morbidity and mortality. Empiric antibiotic therapy should consider the prevalence of antibiotic resistant pathogens in our community and certain risk factors that may increase the occurrence of MDR bacterial pathogens.*

Keywords: - ***Lower Respiratory Tract Infections, Hospitalized patients, Potentially Bacterial Pathogens, Multi Drug Resistance***

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List of abbreviations

AECOPD	Acute Exacerbation of Chronic Obstructive Pulmonary Disease
AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
CAP	Community Acquired Pneumonia
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
ESBL	Extended Spectrum of Betalactamase
FEV_1	Forced Expiratory Volume in first second
FVC	Forced Vital Capacity
GNB	Gram Negative Bacilli
GOLD	Global Initiative for Chronic Obstructive Lung Disease
JUMC	Jimma University Medical Center
LPF	Lower Power Field
LRTI	Lower Respiratory Tract Infection
MDR	Multidrug Resistance
MHA	Muller Hinton Agar
MRSA	Methicilin Resistance Staphylococcus aureus
HCAI	Healthcare associated pneumonia
PMN	Polymorphomonuclear cells
PPB	Potentially pathogenic bacteria
SECs	Squamous epithelial cells

1. Introduction

1.1. Background

Lower respiratory tract infection (LRTI) is a general description of a collection of disease (affecting trachea, bronchi and lung parenchyma). It encompassing different clinical presentations and etiologies. LRTIs in adults include pneumonia (either clinical suspected or radiological confirmed), influenza, acute bronchitis and exacerbation of chronic airways diseases (1, 2).

Lower respiratory tract infections are a leading cause of morbidity and mortality throughout the world, the sixth overall cause of death, and the second overall cause of disability-adjusted life years (DALYs). However, most of these conditions are largely preventable causes of disease and death. There have been changes in the epidemiology of LRTIs and burden had decreased in children younger than 5 years of age in the past decades but increase in the burden in older individuals > 70 years of age(3).

Several studies showed that older age, smoking, alcoholism, pulmonary disease, cardiovascular disease, chronic liver or renal disease, neurological disease, diabetes mellitus, previous respiratory tract infection and immunosuppressive therapy are contributing factors in the susceptibility of individuals to lower respiratory tract infections (4-6). Several bacteria such as *S. pneumoniae, H. influenzae, Pseudomonas spp., Acinetobacter spp., Klebsiella spp., S. aureus, M. catarrhalis* and *atypical bacteria* have been most commonly identified bacterial etiologies from LRTIs(3, 7-10). In Ethiopia, *S. pneumoniae* was the most frequent isolated bacteria followed by *S. aureus* or *K. pneumoniae* from CAP patients (11-13).

Although early initiation of antibiotic therapy is the cornerstone for treating for lower respiratory bacterial infection, exposure to antibiotics leads to selection pressure with a risk of emergence of bacterial resistance leading to difficulty in patient care (14). In addition, etiology of LRTIs have differences in bacterial profiles and antibiotic susceptibility patterns were identified geographically and time depending upon the antibiotic pressure on that certain locality(15, 16). Therefore, proper selection of empirical treatment depends on the common pathogens identified in etiological studies. However, there is limited information about causes and antimicrobial resistance situation of lower respiratory tract infections in Ethiopia.

1.2. Statement of the problem

Lower respiratory infections are is a major public health problem and a leading cause of morbidity and mortality around the world. According to Global Burden of Disease Study in 195 countries of LRTIs and indicated that, for 2016, LRTIs were the leading infectious disease cause of death and the sixth leading cause of death overall. They estimated that LRTIs caused 2.38 million deaths, which account for 4.4% of all deaths in people of all ages and 91.8 million disability-adjusted life years (DALYs) worldwide. Nearly 1.1 million deaths occur in adults older than 70 years. While the burden had decreased in children younger than 5 years of age, it remain increased in many regions for individuals older than 70 years. In 2016, about 336.46 million episodes of LRTIs and nearly 66 million hospital admissions due to lower respiratory infections among all ages. In Sub-Saharan Africa, LRTIs remain the third after HIV/AIDS and malaria in terms of causes of mortality (3). In Ethiopia, the top five leading causes of age standardized premature mortality and death rates in 2015 were lower respiratory infections, tuberculosis, diarrheal disease, ischemic heart disease, and HIV/AIDS (17).

AECOPD is most common associated with the overgrowth of pathogenic bacteria, especially *S. pneumoniae*, *H. influenzae*, *P. aeruginosa* and *Moraxella catarrhalis* (9, 10). On the other hand, *S. pneumonia*, *S. aureus* and Gram-negative bacteria such as *H. influenzae*, *Pseudomonas* spp, *Acinetobacter* spp., *Klebsiella* spp. and other gram negatives have been recovered from pneumonia (8, 12, 18). Because culture results are not available immediately, clinicians prescribe antibiotics empirically, commonly based on the medical history and physical examinations of the suspected individuals. On the other hand, inappropriate use of antibiotics both in and out of hospital has resulted in an exponential increase in antibiotic resistance that have been associated with excessive morbidity, mortality, and healthcare costs(14). In addition, in the past few decades, the emergency of multidrug-resistant organisms (MDROs) also become a major problem for clinicians when deciding antimicrobial therapy empirically(18). In addition, resistance for these β-lactam antibiotics due to the production of β-lactamases are becoming a worldwide problem (19).

Data on bacterial etiologies of LRTIs in Ethiopia are very limited. To the best of our knowledge, there is no data about bacterial profile of LRTIs among adult hospitalized patients in Ethiopia. Therefore, this study was conducted to give updated information on pattern of bacterial etiological agent of LRTIs and its antibiotic resistance pattern in the current study setting.

1.3. Significance of the study

Lower respiratory tract infections (LRTIs) are the leading infectious disease causes of morbidity and mortality throughout the world, given the expanding rate of multidrug-resistant pathogens and the emergence of new pathogens. Although early initiation of antibiotic therapy is the basis for management LRTIs, increasing resistance to antibiotics by respiratory pathogens has complicated the use of empirical treatment with traditional agents and a definitive bacteriological diagnosis and susceptibility testing would be required for effective management of LRTIs (14). In addition, etiology of LRTIs have differences in bacterial profiles and antibiotic susceptibility patterns from place to place and time depending upon the antibiotic pressure on certain locality(16). Improved knowledge of likely pathogens and the likely susceptibility of bacterial pathogens could help guide antibiotic prescribing decisions and thus help to avoid unnecessary antibiotic use and to decrease antimicrobial resistance.

Since there is limited information on etiologies of LRTIs in Ethiopia before, this study will add updated information on bacterial profile and its antibiotic susceptibility pattern among study participants in current study area. Knowledge of the local bacteriological profile & antibiogram help us to decrease the number of failure cases recorded with empirical treatment during LRTIs. Moreover, it is necessary for finding out the resistant pathogens so treatment can be planned according to organisms, which may decrease the mortality and morbidity. This is also essential as to allow for effective and cost saving management strategy and reducing the emergence of drug resistance. Generally, it will give information on causative agent, sensitivity pattern especially for those health personnel involved in either planning or management of patient, and it will be used as a base line for future study.

2 Literature review

Lower respiratory tract infections are the leading infectious disease that cause for huge morbidity and mortality throughout the world. LRTIs is not a single disease but a group of specific infection with different epidemiology, pathogeneses, clinical presentations, and outcomes (2, 20). From an epidemiological point of view, LRTIs in adults include Pneumonia (either clinical suspected or radiological confirmed), influenza, acute bronchitis and exacerbation of chronic obstructive airways disease The etiology and symptom of lower respiratory diseases vary with age, season, the type of population at risk, geographical location and other factors(1, 2).

A number of recent studies, mainly from the US and Europe, have investigated the burden and incidence of LRTIs in adults. According to Global Burden of Disease Study, in 2016, LRTIs caused 2.38 million deaths which account for 4·4% of all deaths in people of all ages and 91.8 million disability-adjusted life years (DALYs). Nearly 1.1 million deaths occur in adults older than 70 years. There was a total of 336.46 million Episodes of lower respiratory infection and nearly 66 million hospital admissions due to lower respiratory infections among all ages in 2016 (3). GDB 2018 report suggested that nearly three-quarters of lower respiratory infection deaths occurred in older children and adults, with a particularly high burden in adults older than 70 years.

In retrospective study of hospitalized patient due to pneumonia from National Inpatient Sample (NIS), database of hospitalizations in the United States of America (US), there were an estimated 20,361,181 pneumonia-associated hospitalizations in 2001-2014, representing an pneumonia-associated hospitalization rate of 464.8 per 100,000 population (95% CI 462.5-467.1), with 7.4% (SE 0.03) in-hospital deaths(21). A literature review from Europe, regardless of showing wide variations in the different regions, indicated that the burden of pneumococcal CAP in adults remained high, especially in elderly patients with comorbidities and despite use of the 23-valent pneumococcal polysaccharide vaccine. Overall, high rates of hospitalization and length of hospital stay were documented, particularly in older patients (22). LRTI are the most common bacterial infections among patients in intensive care units (ICUs), occurring in 10-25% of all ICU patients and resulting in high overall mortality, which may range from 22% to 71%(2).

On the other hand, Similarly, the Global Burden of Disease Study reports a prevalence of 251 million cases of COPD globally in 2016(3). AECOPD have an enormous impact, both personal and economic, on patients and their families. A population-based survey of patients with COPD

performed in nine countries in the Asia-Pacific region found that 46% of patients had at least one exacerbation in the previous year and 19% required hospitalization(23).

The etiological agent of LRTIs is not the same across different countries even varies within the same country, with time due to differences in the frequency of use of antibiotics, environmental pollution, co-morbidities, awareness of the disease and life expectancy (15, 16). According to Global Burden of Disease Study analysis of LRTIs (2016), more than 50% of lower respiratory tract infection deaths in 2016 were attributable to bacterial etiologies, which indicated that the pneumococcus was the most common cause of LRTIs among all ages despite the change in epidemiology regarding frequency and etiology in different part of the world(3). Furthermore, the pneumococcus has been reported to be the most common cause of LRTIs in several studies (7, 24).

Study from Cameroon on bacterial etiologies of Lower Respiratory Tract Infections among Adults tested by using conventional bacterial culture and a commercial reverse-transcriptase real-time polymerase chain reaction (RT-PCR) assay. One hundred forty-one adult patients with LRTIs were enrolled in the study. Among the participants, 46.8% were positive for at least one bacterium. *S. pneumoniae* and *Haemophilus influenzae* were the most detected bacteria with 14.2% (20/141) followed by *K. pneumoniae*, 9.2% (13/141), Staphylococcus aureus,7.1% (10/141), and Moraxella catarrhalis, 4.3% (6/141). Bacterial coinfection accounted for 23% (14/61) with *Haemophilus influenzae* being implicated in 19.7% (12/61). The diagnostic performance of RT-PCR for bacteria detection (43.3%) was significantly different from that of culture (17.7%) ($P< 0.001$)(7).

A cross-sectional hospital-based study conducted in Egypt, two hundred and seventy CAP patients were included. Bacteria represented 50.4% of them. *K. pneumoniae* was the most prevalent bacterium (10.37%) followed by *S. pneumoniae* and *P. aeruginosa* (7.78% each). Overall, 76.2% of isolates showed a multidrug resistant phenotype: 82.61% (19/23) *S. pneumoniae*, 89.66 % (26/29) *K. pneumoniae*, 65.22% (15/23) *Pseudomonas aeruginosa*, 87.50% (7/8) Escherichia coli and 81.25 % (13/16) Staphylococcus aureus. Broad spectrum β-lactams, especially carbapenems, and moxifloxacin showed in vitro efficacy on most of the tested isolates. Forty-three cases (15.9%) were nonresponders, 37 (86%) of them showed bacterial etiology. The highest rate of nonresponsiveness (30.43%) was observed in cases receiving antipseudomonal/antipneumococcal β-lactam plus a fluoroquinolone for suspected *P. aeruginosa* infection(8).

Another a prospective hospital-based study on adult CAP was conducted in Leyte, Central Philippines from May 2010 to May 2012. Blood, sputum, and nasopharyngeal samples obtained from patients were used to identify pathogens using standard microbiological culture methods and PCR. Results: More than half of the patients had an underlying disease, including pulmonary tuberculosis (22%). The detection rate was higher for bacteria (40%) than viruses (13%). *Haemophilus influenzae* (12%) was the most commonly detected bacterium and influenza virus (5%) was the most commonly detected virus. The proportion of CAP patients with Mycobacterium tuberculosis infection was higher in the younger age group than in the older age group. Among CAP patients, 14% died during hospitalization, and drowsiness on admission and SpO2 <90% were independent risk factors for mortality(25).

Similar study was conducted to identify the bacterial etiology of LRTIs among patients who attended the Central Chest Clinic in city of Colombo, Sri Lanka. Sputum samples were collected from 1,372 patients over the age of 18 years with suspected LRTIs during the year 2015. Out of all sputum cultures processed, 404 (29.4%) resulted positive for pathogenic bacterial organisms. Coliforms (n = 176, 43.6%), and *Pseudomonas aeruginosa* (n = 117, 29%) were the most common isolated bacteria, followed by Moraxella (n = 47, 11.6%), *Haemophilus influenzae* (n = 23, 5.7%), and *S. pneumoniae* (n = 18, 4.4%). The two most common bacteria isolated showed a high sensitivity for co-amoxyclav, quinolones, 3rd generation cephalosporins, carbapenems and aminoglycosides, while coliforms were highly resistant (98%) to ampicillin. *S. pneumoniae* showed a high resistance for penicillin (67%) and erythromycin (61%), while *Haemophilus influenza* showed a good sensitivity to co-amoxyclav (96%)(26).

A cross-sectional, observational study conducted in Bangladesh, a total of 120 hospitalized diabetic patients diagnosed with CAP were involved. Sputum for culture showed that out of 120 (100%) patients, *K. pneumoniae* was detected in 53 (44.2%) patients, *S.aureus* in 18 (15.0%), *Pseudomonas* species in 16 (13.3%) patients, *Acinetobacter* spp. in 10 (8.3%), Escherichia coli in 9 (7.5%) patients and 14 (11.7%) patients had growth of other organisms. Sensitivity pattern of different bacterial growth in sputum to commonly used antibiotics like ceftriaxone, ciprofloxacin, amikacin and imipenem were as follows Klebsiella *spp.* (19%, 47%, 74%, 96% respectively), *S. aureus* (11%, 33%, 78%, 67% respectively), *Pseudomonas* (19%, 75%, 81%, 88% respectively),

Acinetobacter (0%, 0%, 20%, 50% respectively), *E. coli* (22%, 22%, 100%, 100% respectively). All (100%) of the *Pseudomonas* and *Acinetobacter* were susceptible to colistin(27).

Hospital based cross sectional study was conducted in India among 100 patients with CAP was conducted in Southern India. Sputum culture showed that out of 100 patients 39 had an identifiable etiology with 12 patients having evidence of mixed infection. Micro-organisms isolated in sputum culture were *S. pneumoniae* (31%) followed by, *Pseudomonas* pyogens (15%), *K. pneumoniae* (13%). AFB smear was found to be positive in 6 patients. Organisms were found to be susceptible for piperacillin plus tazobactum (41%), aminoglycocides (amikacin-46%, gentamicin-31%), third generation cephalosporins (Cefotaxim-36%, Ceftriaxone-18%) and macrolides (Erythromicin-31%, Azithromycin-18%). Sensitivity to chloramphenicol was observed in 31% sputum culture positive patients. Ciprofloxacin sensitivity was seen among 49%(28)

A surveillance study for VAP incidence was conducted in 73 hospitals in Asia, *Acinetobacter* spp. (33.5%) was the most commonly isolated pathogen from VAP patients, followed by *P. aeruginosa* (23.9%), *K. pneumoniae* (15.4%), and S. aureus (13.8%)(29). On the other hand, other study in Egypt showed that *Klebsiella Spp.* was the most common followed by *P. aeruginosa* were the most prevalent in HAP (30). An article review in America on the results of the SENTRY Antimicrobial Surveillance Program (1997–2008), reported that a consistent 6 organisms*: S. aureus* (28.0%), *P. aeruginosa* (21.8%), *Klebsiella* species (9.8%), Escherichia coli (6.9%), *Acinetobacter* species (6.8%), and *Enterobacter* species (6.3%) as causative organisms of about 80% of episodes of HAP and VAP(31). From literatures review on last decades have documented GNB prevalence higher than 50% in patients with healthcare associated pneumonia, prevalence varies depending on geographic areas, with a consistently higher GNB prevalence in the Asian region in patients with pneumonia (both CAP and VAP), *K. pneumonia* and *P. aeruginosa* were the most common GNB isolated(32).

In Ethiopia, study conducted in Arbaminch Hospital indicated as prevalence of bacterial pathogens of CAP was 42.9%. Majority of tested bacterial isolates (> 86%) were susceptible to Ceftriaxone and Ciprofloxacin. Multidrug resistances (MDR) were also observed to most (60.3%) bacterial isolates(13). Similarly, study done at Jimma specialized Hospital showed that *S. pneumoniae* and *S.aureus*(11). Another recent study in Bahirdar among CAP on bacteriology of community acquired pneumonia in adult patients, Northwest Ethiopia. From total of 414 cases, bacterial

pathogens were identified from 167 (40.3%) participants. Among these, multidrug resistance (MDR) accounted for 127(76%) of the isolates. The predominant isolates were *S. pneumoniae* at 35.9% and *Klebsella pneumoniae* at 18%. Overcrowded living conditions [AOR 1.579 (95%CI: 1.015–2.456)] and alcohol use [AOR 4.043 (95% CI, 2.537–6.443)] were found that statistically associated with culture positive sputum(12).

In recent years, there has been dramatic rise in antibiotic resistance among pathogens of lower respiratory tract. Hence, selection of empiric antibiotic therapy for patients with LRTIs should consider the prevalence of antibiotic resistant pathogens in the hospital and community setting to decrease morbidity and mortality. There is very limited information on etiology and antibiotics susceptibility pattern of Lower respiratory tract pathogens among adult hospitalized patients in Ethiopia. Therefore, this study was undertaken with the objective of determining the current etiological agents of lower respiratory tract infections and antibiotics resistance pattern of bacterial isolates among hospitalized patients at Jimma University Medical Center.

3 Objective of the study

3.1 General objective

- To determine bacterial isolates and their antibiotic resistance pattern among adult hospitalized patients with lower respiratory tract infections at Jimma University Medical Center, Southwest Ethiopia, 2019

3.2 Specific objectives

- To determine bacterial isolates and their antibiotic resistance pattern in patients admitted with clinical diagnosis of LRTIs at JUMC.
- To describe antibiotic resistance pattern of bacterial isolates in patients admitted with clinical diagnosis of LRTIs at JUMC.
- To assess factors associated with culture positivity in patients admitted with clinical diagnosis of LRTIs at JUMC.

4 Materials and methods

4.1 Study area

The study was conducted at Jimma University Medical Center in Jimma town, Jimma zone in Oromia regional state south west Ethiopia. Jimma, the capital town of the Jimma zone, is located 354kms southwest of Addis Ababa. The total projected population of the town from 2007 central statistical agency (CSA) census report is 130, 254. Total area of the town is estimated about 4,623 hectare & its temperature is ranges from 20-30^{0}C and the average rainfall is 800-2500mm^3. It is located at an altitude of 1750-2000m above sea level.

Jimma University Medical Center, located in Jimma town, is one of the oldest public hospitals in the country. It was established in 1938 during the Italian occupation for the service of their soldiers. Jimma University Medical Center is the only teaching and referral hospital in the Southwestern part of the country. It provides specialized health services through its different clinical and diagnostic departments for about 15 million people from the South West part of the country. It has a bed capacity of 800 and more than 1448 staff, including both supportive and professional staff.

4.2 Study design and period

A cross sectional study was conducted at Jimma University Medical Center from April 2019 to October 2019, to determine bacterial isolates and their antibiotic resistance pattern among adult hospitalized patients with lower respiratory tract infections.

4.3 Study Variables

- ✓ **Dependent Variable**

  Culture result, Type of bacteria & Antibiotics susceptibility

- ✓ **Independent Variables**

Age	Sex
Occupation	Alcohol consumption
Residence	Smoking status
Severity of COPD	Nature of ventilation
Comorbidities	History of steroid/antibiotic use
No. of exacerbation's in previous one year	Nature of specimens
Length of hospital stay	

4.4 Study participants

4.4.1.1 Source population

All adult patients admitted to medial wards of JUMC during the study period.

4.4.2 Study participants

All adult patients who were hospitalized due to lower respiratory infection at JUMC and, who fulfill inclusion criteria, voluntary to participate and gave written informed consent to take part in the study.

4.5 Eligibility criteria

4.5.1 Inclusion criteria

Individuals, age ≥18 years and clinically diagnosed with LRTIs by the concerned physicians depending upon the presence of cardinal respiratory symptoms (with cough as the main, with at least one other symptom like sputum production, dyspnea, fever, wheeze or chest discomfort/pain) and who have adequate sputum sample.

4.5.2 Exclusion criteria

All participants with known cases of tuberculosis and patient who unable/unwilling to participate were excluded from the study.

4.6 Sample size and sampling technique

Sample size

The sample size was determined using single population proportion formula considering the 95% confidence level taking the proportion as 81.5% from previous study conducted in Nigeria(33) and tolerable error of 0.05, the formula used to calculate the sample size was as follow:

$$n = \frac{(Z\ \alpha/2)^2\ p\ (1-p)}{d^2}$$

Where n= the minimum sample size

$\frac{z\alpha}{2}$ =1.96 (95% confidence level)

p= 81.5%, taken from study conducted in Nigeria

d= margin of error (5%)

Therefore, the value of n is calculated as follows:

$$n = \frac{(1.96)^2 \times 0.815\,(1-0.185)}{(0.05)^2} \longrightarrow n = 232$$

The approximate monthly average hospitalization rate of patient with lower respiratory tract infection at the JMUC was 76, and we used correction formula by taking N, the maximum population hospitalization rate in the same study period was 532, since N (532) < 10,000.

$$nf = \frac{n}{1+\frac{n}{N}} = 232/\ 1+232/532 = 162$$

Finally, by considering 15% non-response rate, 189 hospitalized patients suspected with LRTI were included.

Sampling Technique

Non-probability consecutive sampling technique was applied.

4.7 Data collection

4.7.1 Sociodemographic and related clinical data

Principal demographic data and other important clinical data from each participants such as age, sex, residence, smoking status, comorbidities, history of antibiotic use and physical characteristics of sputum were collected by trained data collectors using self-administer structured questionnaire (APPENDIX II).

4.7.2 Laboratory data

Specimen Collection and processing

Expectorated sputum specimens were collected in sterile sputum cup (falcon tube) by standard collection procedures after ordering them to rinse their mouth with water. Patients were instructed to take a deep breath, hold it shortly, and then cough vigorously to produce quality sputum. The sputum sample was transported to department of Medical Microbiology laboratory where the bacteriological analysis was performed. Quality of expectorated sputum samples were assessed by Bartlett's scoring method(34) (Table 1). In addition, sputa were screened for non-prescreened cases (94 cases) for pulmonary tuberculosis by using the Ziehl-Neelsen (ZN) staining technique and three of them were smear positive for mycobacterium tuberculosis.

Nurses perform tracheal aspiration by passing a new suction catheter into the endotracheal tube. If initially unable to suction adequate secretions, 3 ml of normal saline was injected into the endotracheal tube, and a repeat attempt was made.

Gram stain

The specimens were subjected to macroscopic examination for physical appearance of the specimens and microscopic examination to determine the acceptability of the specimen for culture. Gram-stained smears were prepared from most visually purulent portions of sputum specimen. The quality of sputum was assessed by determining the numbers of squamous epithelial cells (SECs) and polymorphomonuclear cells (PMN) in gram-stained smear of the specimen through microscopic examination within the following categories: <10, 10-25, or >25 cells per representative (100x) low power fields (LPF). The presence of PMN was graded as 0, +1 and +2, whereas SECs were graded as -2, -1 and 0 after observing a minimum of 20 LPF. The scores were added and the specimen with 1 or greater than 1 score was considered as acceptable specimen while sputum specimen with zero or less scores were classified as being of non-acceptable, then the good quality sputum specimens were further processed. While endotracheal aspirate was cultured without prescreening, as it usually contain material directly from alveolar spaces with very little contamination from upper respiratory secretions(34).

Table 1: Bartlett Grading System for screening sputum quality(34)

Number of polymorphonuclear cells(PMN) per LPF (x10 objective)	Grade
< 10	0
10 – 25	+1
>25	+2
Presence of mucus	+1
Number of epithelial cells per LPF (x10 objective)	Grade
< 10	0
10 – 25	-1
>25	-2
Total Score	

LPF- Lower power field

Washing Technique

Accepted samples were mixed with physiological saline (1:10 vol), vortexed and centrifuged for 10 minutes at 1500 rpm. An equal volume of N- acetyl- L cysteine was added to the pellet for homogenization and the mixture was incubated at 37°C for about 15 minutes(35).

Specimen culture

Samples accepted after microscopic evaluation and washing procedures, sputum was inoculated directly onto blood agar base (Accumix, Tulib diagnostics Ltd, India), MacConkey agar (Oxoid Ltd, England) and chocolate agar (Accumix, Tulib diagnostics Ltd, India) plates for bacterial isolation. Washed samples as described above was further diluted 1: 10 - 1:1000 in sterile saline and 10 µl aliquot from 1: 100 and 1:1000 dilution was inoculated on sheep blood agar, MacConkey agar and chocolate agar plates for quantitative evaluation(36). The chocolate agar plate was incubated in an incubator (5-10% CO_2) at 37 ºC for 24- 48 hours while blood agar and MacConkey agar were incubated in an aerobic atmosphere at 37 ºC for 24 hours. Suspicious colony was sub-cultured on suitable solid culture media for purification, there after further procedures were processed or preserved on appropriate media, and stored in a refrigerator (4 º C) for subsequent analysis as necessary. Isolated micro-organisms were considered as significant and accepted as causative pathogens only if they reached a count of 10^6 CFU/mL, except for *S. pneumoniae* where 10^5CFU/mL was estimated to be sufficient(37)

Identification of isolated organisms

The identification of isolates was accomplished using the standard microbiological techniques, which involved morphological colony studies, gram staining, and a battery of biochemical tests like Catalase test, Oxidase test, Indole, Citrate utilization test, Urease production, Hydrogen sulfide production, Sugar fermentation test, Coagulase test and Optochin sensitivity tests were done(38).

Antimicrobial Susceptibility Test

The antimicrobial of the test strains against different antibiotics were performed using the Kirby Bauer disk diffusion method on Mueller Hinton agar (Oxoid Ltd, England) as per the Clinical Laboratory Standards Institute guidelines(39). Based on the CLSI guideline, we used

Penicillin(P:10µg), Oxacillin(OX:1µg), Ciprofloxacin(CIP:5µg), Chloramphenicol(CAF:30µg), Erythromycin(ERT:15µg), Azithromycin(AZE:15µg), Doxycycline(DOX:30µg), Trimethoprim-sulfamethoxazole(SXT:1.25/3.75µg), Clindamycin (CLD:2µg), Vancomycin(VCM:30µg), Gentamycin(GTM:10µg), Tobramycin(TOB:10µg) & Cefoxitine (FOX:30µg) for gram positive isolates. While, for gram negatives, we used Ampicillin(AMP:10µg), Gentamycin(GTM:10µg), Amikacin(AMK:30µg), Tobramycine(TOB:10µg), Amoxicillin Clavulanicacid(AMC:20/10µg), Trimethoprim-sulfamethoxazole(SXT:1.25+23.75µg), Ceftriaxone(CRO:30µg), Ceftazidime (CAZ:30µg), Cefepime(CFP:30µg), Cefuroxime(CXM:30µg), Doxycycline(DO:30µg), Ciprofloxacin(CIP:5µg), Chloramphenicol(C:30µg), Cefoxitine(FOX:30µg) and Meropenem (Mer:10µg) according to CLSI guidelines(39).

Briefly, one to two isolated colonies from freshly streaked plates were suspended in 1ml saline, adjusted to a 0.5 McFarland standard. A sterile swab was dipped in the suspension and excess suspension was removed by pressing the swab against the wall of the tube. The entire surface of MHA plate was uniformly flooded with suspensions and allowed to dry for about 5 minutes.

The antimicrobial impregnated disks were placed by using sterile forceps at least 24 mm away from each other to avoid the overlapping zone of inhibition. After the surface of the agar plate dried for about 5 minutes, , antibiotic disks were applied onto the inoculated plate and the disks were placed on agar plates and allowed to stand for about 15 minutes to dissolve the antibiotics in the media, then the plates were inverted and incubated for about 16 to 24 hours at 37°C, inhibition zone were measured. Müeller Hinton agar, supplemented with 5% sheep blood was used for testing *S. pneumonia* and for *H. influenzae*, MHA chocolate agar was used. Interpretation of zone of inhibition diameter was interpreted as susceptible, intermediate and resistant by comparison of zone of inhibition as indicated in CLSI guidelines(39).

Extended-Spectrum Beta-Lactamases (ESBL), AmpC and Carbapenemase detection

Isolates resistant to third generation cephalosporins and/or cefepime were tested for ESBL and/or AmpC production by disc diffusion confirmation test with MAST^{TM} D68C combination disc sets. The test was performed and interpreted in accordance to the manufacturer's instructions (MASTDISCS™ID, UK). A 0.5 McFarland suspension of the test isolate was inoculated uniformly on MHA, then each of four discs (A–D) were placed onto the agar in accordance with the manufacturer's instructions (Mast Group). Disc A contained cefpodoxime (10 mg), disc B contained cefpodoxime (10 mg) and an unidentified ESBL inhibitor, disc C contained cefpodoxime (10 mg) and an unidentified AmpC inhibitor, and disc D contained cefpodoxime (10 mg) and both the AmpC and ESBL inhibitors. On the other hand, isolates resistant to meropenem were tested for carbapenemase production by disc diffusion confirmation test with MAST^{TM} D73C combination disc sets. A 0.5 McFarland suspension of the test isolate was inoculated uniformly on MHA, then each of five discs (A–E) were placed onto the MHA in accordance with the manufacturer's instructions (Mast Group). Disc A contained Penem discs (10 mg), disc B contained Penem (10 mg) and an unidentified MBL inhibitor, disc C contained Penem (10 mg) and an unidentified KPC inhibitor, and disc D contained Temocillin and unidentified MBL (10 mg) inhibitors. After overnight incubation in room air at 35-37 ^{0}C, zone diameters were read using caliper and recorded on excel sheet. Zone diameters from Microsoft Excel were transported to Mast group ESBL/AmpC and CARBA plus calculator spreadsheet (Mast group, UK) and interpreted as ESBL or/and AmpC and carbapenemase positive or negative accordingly.

4.8 Quality Control

The quality of data was controlled starting from the time of questionnaires preparations. The Questionnaires were developed by reviewing relevant literatures on the subject to ensure reliability. All specimens were collected according to the standard operating procedure of specimen collection and the quality of the specimens were checked based on Bartlett's acceptance and rejection criteria. Furthermore, in order to decrease a heavy growth of commensal organisms from sputum culture, sputum washing technique and quantitative culture of sputum were used(35, 36). The sterility of culture Medias were ensured by incubating 5% of each batch of the prepared media at 37^{o}c for 24 hours. Quality control tests were done for check testing the performance of media & antibiotics using the following standard American Type Culture Collection (ATCC) control strains.

Pseudomonas aeruginosa-ATCC 27853, *E. coli* ATCC 25922 *and S. aureus* ATCC 25923 control strains were used as controls accordingly(39). ESBL positive ATCC 700603 *Klebsiella pneumoniae* and ESBL negative *E. coli* ATCC 25922 control strains were used as positive and negative control respectively. To standardize the inoculums density of bacterial suspension for the susceptibility test, 0.5 MacFarland standard was used. The data was entered and cleaned in EpiData then exported to SPSS Software version 23 and excel then analyzed by them.

4.9 Statistical Analysis

After checking the data for completeness, missing values, and coding of the questionnaires, raw data was entered in EpiData version 3.1 and exported to SPSS version 23 Software and excel. Frequency and percentages distribution were generated using SPSS software and excel to describe the relative proportions of relevant variables. Results were presented in texts, tables and graphs. Chi-square or Fisher-exact test and odds ratios with corresponding confidence interval were calculated when appropriate to ascertain association between patients' demographic, clinical characteristics and the outcome. A *P-value* at a level of 0.05 considered as statistically significant.

4.10 Ethical considerations

Before the commencement of the study, ethical approval was obtained from Jimma University, Institute of Health Sciences Ethical Review Committee and after that, a formal letter was given to Jimma University Medical Center Medical ward with copy of this project proposal objectives of the study to explain to them. The ethical clearance certificate reference number was IHRPGD/565/2018. Written informed consent was obtained from the study participants or their guardian. Confidentiality of individual patients' information was maintained during data collection, analysis and interpretation. The laboratory preliminary results of the study participant were reported to their physicians. In addition, culture result was also reported to the responsible physicians in charge for possible intervention.

Plan for dissemination

Final report will be submitted to the School of Medical Laboratory Sciences, Jimma University and the findings would be presented to the academics of the University. The summary of the report will be also submitted to the hospital administration. Moreover, the paper will be published on either national or at International Journal to communicate to the scientific community.

4.11 Operational Definitions

In agreement with previous literatures, the following terms are defined (1, 40, 41)

COPD acute exacerbation: an acute sustained worsening of the COPD patient's condition, from the stable state (in the patient's baseline dyspnea and cough or sputum, or both and beyond normal day to day variation that result in additional therapy.

Lower respiratory tract infection:-An acute illness of lower respiratory tract, usually with cough as the main symptom, with at least one other lower respiratory tract symptom (sputum production, dyspnea, fever, wheeze or chest discomfort/pain)

Community-acquired pneumonia (CAP):- pneumonia that is acquired outside the health care facility setting or from the community.

Healthcare associated pneumonia (HCAP): - pneumonia that is not incubating at the time of hospital admission and occurring 48 h or more after admission

Multi drug resistance (MDR): - Multidrug resistance is an acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

5 RESULTS

General clinical description of study participants

Over a period of six months from 1 April to 18 October 2019, a total of 189 study participants were enrolled on the study of determining bacterial profile and antibiotics susceptibility patterns of isolates from lower respiratory infections (LRTIs). From these study participants, 39/189(20.6%) were chronic obstructive pulmonary disease patients with acute exacerbation (AECOPD), 109189(57.7%) were community acquired pneumonia patients and 41/189(21.7) were patients with healthcare associated pneumonia. Of these, 54% (102/189) of the participants were males, while 46% (87/189) were female patients.

5.1 Demographic and clinical characteristics of study participants

In this study, from 189 study participants, 54% (102/189) of the participants were males, while 46% (87/189) were female patients, resulting in an overall male to female ratio of 1.2:1 with median age of patients was 45 year (interquartile range 25). In this study, history of exposure to tobacco smoke was presented in 28(71.8%), 14(12.8%) and 6(14.6%) of AECOPD, CAP and HCAI patients respectively. From the participants, 92.3%, 67% and 92.7% of the COPD-exacerbation, CAP and HCAI patients had taken antibiotics within the three months before enrolled in the current study respectivelly. In addition, (25)64.2% and (17)43.6% of AECOPD patients were inhalant steroid and systemic steroid users before data collection respectively. Sociodemographic and clinical characteristics of the patients are summarized in Table 3.

Table 2: Demographic and clinical characteristics of study participants with lower respiratory infections (n = 189) at JUMC, south west, Ethiopia, 2019

	Clinical diagnosis					
	AECOPD		CAP		HCAI	
Variables	Culture negative cases, 12(30.8%)	Culture positive cases, 27(69.2%)	Culture negative cases, 61(66%)	Culture positive cases, 48(44%)	Culture negative cases, 14(34.2%)	Culture positive cases, 27(65.8%)
Age, year(mean)	65.13±10.92	66.52± 10.61	41.94±15.91	45.58±17.43	40.79±12.44	41.22±14.39
Sex Female	1(12.5)	7(87.5)	34(59.6)	23(40.4)	7(31.8)	15(68.2)
Male	11(38.7)	20(61.3)	27(51.9)	25(48.1)	7(36.8)	12(63.2)
Residence						
Urban	5(35.7)	9(64.3)	28(43.7)	20(56.2)	5(31.2)	11(68.8)
Rural	7(28)	18(62)	33(67.2)	28(32.8)	9(36)	16(64)
Tobacco smoking status						
No	3(27.3)	8(72.7)	55(57.9)	40(42.1)	14(40)	21(60)
Yes	9(32.1)	19(67.9)	6(42.8)	8(57.1	0	6(100)
Alcohol consumption						
No	11(33.3)	22(66.7)	56(58.3)	40(41.7)	13(35.1)	24(64.9)
Yes	1(16.7)	5(83.3)	5(38.5)	8(61.5)	`1(25)	3(75)
No. of exacerbations in previous one year ≤1	3(14.3)	18(85.7)	NA		NA	
≥2	10(55.6)	8(44.4)				

Inhalant steroid use						
No	6(42.9)	8(57.1)	ND			
Yes	6(24)	19(76)				
Systemic steroid use						
No	10(45.4)	12 (55.6)	ND			
Yes	2(11.7)	15(88.2)				
History of antibiotics usage in past 3 months						
No	1(33.3)	2(66.7)	24(66.7)	12(33.3)	3(100)	0
Yes	11(30.6)	25(69.4)	73(50.7)	36(49.3)	11(28.9)	27(71.1)
Renal disease	3(37.5)	5(62.5)	4(33.3)	8(66.7)	2(22.2)	7(77.8)
Diabetes	0	2(100)	5(62.5)	3(37.5)	1(16.7)	5(83.3)
CHF	3(50)	3(50)	2(18.2)	9(81.8)	1(12.5)	7(87.5)
Chronic respiratory disease	12(30.8)	27(69.2)	8(33.3)	16(66.6)	1(20)	4(80)
HIV infection	0	1(100)	3(27.3)	8(72.7)	1(25)	3(75)
Others [a,b,c]	3(37.5)	5(62.5)	6(54.6)	5(44.4)	1(20)	4(80)

AECOPD: acute exacerbation of chronic obstructive pulmonary disease, CAP: community acquired pneumonia, HCAI: healthcare associated pneumonia, CHF: cardiac heart failure, RVI: retroviral infection, chronic respiratory disease: bronchial asthma plus bronchiectasis plus COPD plus post TB fibrosis, NA: not applicable, ND: not data collected, **[a]:** AECOPD**;** malignancy pus dyspepsia**, [b]:** CAP**;** malignancy pus dyspepsia plus goiters swelling, **[c]: HCAI;** malignancy pus dyspepsia plus urinary tract infection plus goiters swelling

5.2 Bacterial profile of Lower respiratory tract infections.

In this study, an overall prevalence of potentially pathogenic bacterial isolates (PPB) was 54% (95% CI; 47–61). A total 112 bacterial isolates were identified which containing 12 different bacterial types in 102 participates, from which 89(79.5%) were gram-negatives whereas 23(20.5%) were gram-positives. The distribution of the isolates is summarized in Fig. 1. The most frequently identified bacteria was *Klebsiella spp.* 25(22.3%), *Pseudomonas aeruginosa* 24(21.4%), *S.aureus 14*(12.5%), *Acinetobacter spp.* 11(9.8%), *Escherichia coli* 10(8.9%) and *S. pneumoniae* 9(8.0%). Separately, *Pseudomonas aeruginosa* was the most frequently isolated organism from both AECOPD and healthcare associated pneumonia patients where as *Klebsiella spp.* was most frequently isolated organisms from CAP patients. More than one bacterial isolates (polybacteria) were revealed from 10(5.3%) of study participants. The most frequent coinfection occurred by *K. pneumoniae* and *P. aeruginosa*

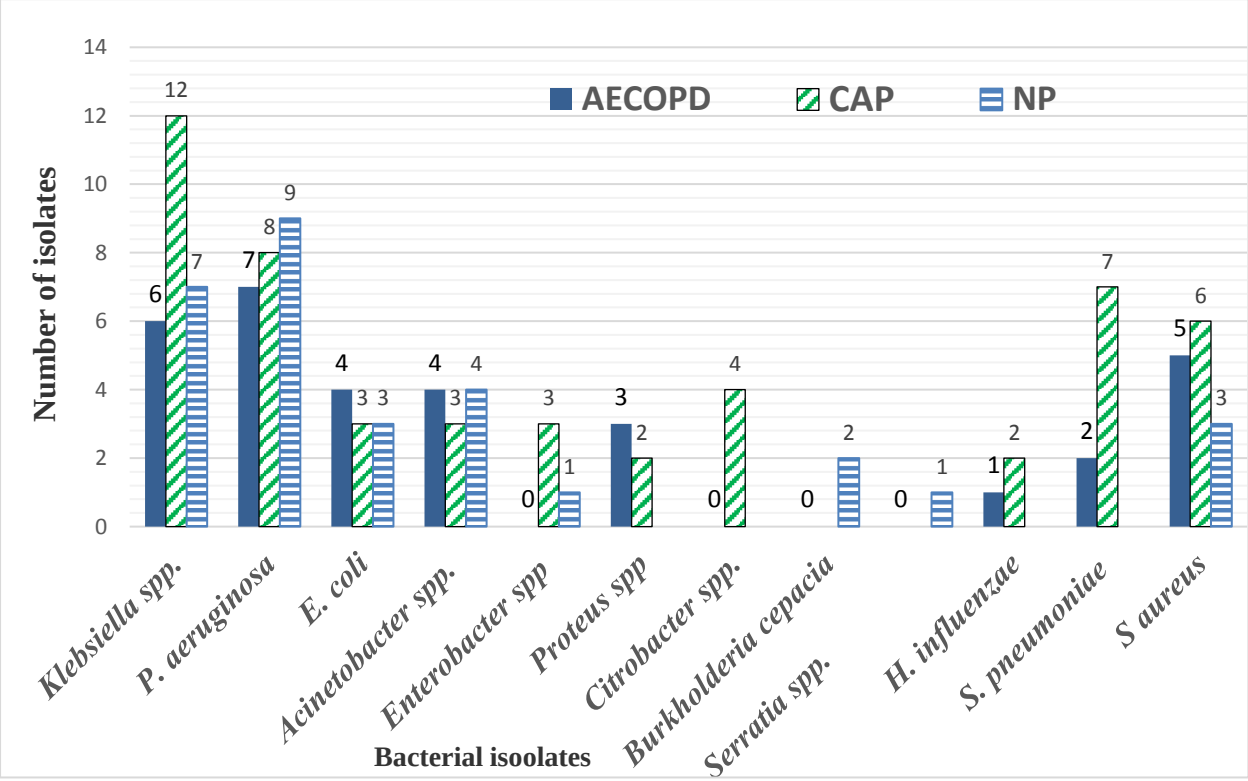

Figure 1: Bacterial profile of lower respiratory tract infections at JUMC, southwest, Ethiopia, 2019, AECOPD: acute exacerbation of chronic obstructive pulmonary disease, CAP: community acquired pneumonia, HCAI: healthcare associated pneumonia

5.3 Antibiotic Susceptibility Patterns of Bacterial Isolates from lower respiratory tract infection

All antibiotics used in the antibiotic susceptibility assay were selected based on the CLSI guidelines 2017. Table 4 shows the resistance rates of isolated bacterial pathogens of lower respiratory tract infection against tested antibiotics. In the present study *Klebsiella spp.* showed high rate of resistance at 100% to ampicillin, 80% to cefuroxime and trimethoprim sulfamethoxazole, 76.0% to doxycycline, 64.0% to chloramphenicol and 52% to Amoxicillin-clavulanate. In addition, in the range (56.0-72.0%) of *Klebsiella* spp found resistant to third and fourth generation cephalosporins. Whereas *Klebsiella spp.* found to be susceptible at 88% to meropenem, 84% to Amikacin, 80% to gentamycin and 60% susceptible to ciprofloxacin. Similarly, 91.7% of *P. aeruginosa* found be resistant to ceftazidime, cefepime and Amoxicillin-clavulanate. Moreover, 41.9% of *P. aeruginosa* were found to be resistant ciprofloxacin. While, (79.2%) and (75%) of these isolates were found susceptible to meropenem and amikacin respectively. Clinical isolates of *Acinetobacter spp* also exhibited high resistance rates to third- and fourth-generation cephalosporins (81.8-100%), Ciprofloxacin (63.6%), doxycycline (81.8%), Trimethoprim-Sulfamethoxazole (100%) and meropenem (45.5%)

On the other hand, *S. aureus* showed high resistance 100% to penicillin, 78.6% to Trimethoprim-sulfamethoxazole, 71.4% to doxycycline, 64.3 to azithromycin, and chloramphenicol. While *S. aureus* showed relatively good sensitivity 71.4 % to gentamicin and ciprofloxacin from tested antibiotics. In addition, 85.7% of isolated *S. aureus* were Methicillin resistant (Table 5). *S. pneumoniae* also exhibited high resistance to Trimethoprim-sulfamethoxazole (77.8%), Oxacillin (66.7%) and azithromycin (55.6%). However, 88.9% and 77.8% of *S. pneumoniae* were susceptible to vancomycin and clindamycin (Table 5).

Table 3: Antibiotic Susceptibility Patterns of Gram negative Bacterial Isolates from lower respiratory tract infections at JUMC, southwest, Ethiopia, 2019

Isolates	No		Antibiotic resistance pattern (R %)													
			TOB	CN	AMK	CAZ	CRO	FEP	AMC	CAF	SXT	CXM	AMP	CIP	MRP	DOXY
Klebsiella spp.	25	R	20	20	16	68	72	56	52	64	80	80	100	40	12	76
E.coli	10	R	20	20	20	70	70	70	70	60	90	100	100	60	20	90
Citrobacter spp.	4	R	25	25	0	100	100	75	50	75	100	100	100	50	0	75
Proteus spp.	5	R	40	40	20	80	80	80	60	80	100	100	100	60	20	100
Enterobacter sp	4	R	50	50	0	75	100	75	75	50	75	100	NT	50	0	100
Serratia spp.	1	R	0	0	0	100	100	100	100	100	100	100	100	100	0	100
P.aeruginosa	24	R	33.3	25	25	91.7	100	91.7	91.7	87.5	NT	NT	NT	41.9	20.8	NT
Acinetobacter sp.	11	R	18.2	18.2	37.5	100	100	81.8	81.8	81.8	100	100	NT	63.6	45.5	81.8
B.cepacia	2	R	0	0	50	100	100	100	100	100	100	100	NT	100	50	100
H.influenzae	3	R	NT	NT	NT	NT	33.3	0	0	0	33.3	33.3	66.7	0	0	66.7
Total	89	R	23.3	20.9	23.3	82.6	82.0	75.3	68.5	68.5	87.6	92.1	99.4	48.3	19.1	84.3

Key, TOB-Tobramycin, CN - Gentamycin, CAZ-Ceftazidime, FEP-Cefepime, AMC Amoxicillin-clavulanate, CRO - Ceftriaxone, CIP - Ciprofloxacin, FOX - Cefoxitin, MRP – Meropenem, CAF-Chloramphenicol, CXM-Cefuroxime, AMP- Ampicillin, SXT-Trimethoprim-sulfamethoxazole, Doxy-Doxycycline, and AMK- Amikacin, NT- not test

Table 4: Antibiotic usceptibility patterns of gram-positive bacterial isolates from lower respiratory tract infections at JUMC, southwest, Ethiopia, 2019

Isolate		Antibiotic resistance pattern (%)											
	No	P	OX	ERY	CLD	VAN	SXT	CAF	CIP	DOX	CN	AZM	TOB
S.aureus	14	100	85.7	64.3	50	NT	78.6	64.3	28.6	71.4	28.6	64.3	28.6
S.pneumoniae	9	NT	77.8	44.4	22.2	11.1	66.7	55.5	NT	44.4	NT	55.6	NT
Total	23			60.9	34.9		78.3	43.5		60.9		65.2	

Key, P- Penicillin, OX –Oxacillin, TOB-Tobramycin, CN - Gentamycin, CIP - Ciprofloxacin, FOX - Cefoxitin, SXT-Trimethoprim-sulfamethoxazole, Doxy-Doxycycline, ERY-Erythromycin, AZE- Azithromycin, CLD-Clindamycin, VAN-Vancomycin and CAF Chloramphenicol, NT- not tested

5.4 Distribution of multi-drug resistant (MDR) isolates of lower respiratory tract infection

Overall, multidrug resistance was observed in 82.1% of isolates. Of this 93.75%, 90% and 70% of MDR bacterial isolates were isolated from AECOPD, HCAI and CAP patients respectively (Table 6). The MDR level among frequent isolates was as follows, *S. aureus* 14(100%), *Acinetobacter spp.* 11(100%), *P. aeruginosa 23*(95.8%), *E. coli* (80%), *Klebsiella spp*.16 (64%) and *S. pneumoniae* 5(55.5%) (Table 7). This study finding showed that there is significant differences in multidrug resistance profiles of isolates from acute exacerbation of COPD patients and those isolates from patients with healthcare associated pneumonia and community-acquired pneumonia patients ($p< 0.010$) (Table 6).

Table 5: Frequency of multidrug resistant bacterial isolates among lower respiratory tract infections at JUMC, southwest, Ethiopia, 2019

Variables	NMDR(%)	MDR(%)	*P value*
CAP	15(30)	35(70)	P = 0.010*
HCAI	3(10)	27(90)	(Df = 2, X^2 = 9.23)
AECOPD	2(6.3)	30(93.8)	

* Statistically significant

Table 6: Destributions of antibiotics resistant bacterial isolates among tested antibiotics classes

Isolates	NO	Penicillins		3rd and 4th generation cephalosporin			aminoglycoside			Phenicols	Doxycycline	Folate pathway	amoxicillin + clavulates	Fluoroquinolone	cephamycins	carbapenems	Lincosamides	macrolides
		P	Amp	CRO	CAZ	FEP	TOB	CN	AMK	CAF	DOX	SXT	AMC	CIP	FOX	MRO	CLD	ER
Klebsiella spp.	25	-	25	18	17	14	5	5	4	16	19	20	13	10	13	3	-	-
E.coli	10	-	10	7	7	7	2	2	2	6	9	9	7	6	5	2	-	-
Proteus spp.	5	-	5	4	4	4	4	4	1	4	5	5	3	3	3	1	-	-
Citrobacter spp.	4	-	4	4	4	3	1	1	0	3	3	5	2	2	2	0	-	-
Enterobacter spp.	4	-	4	4	3	3	2	2	0	3	3	4	3	2	2	0	-	-
Serratia spp.	1	-	1	1	1	1	0	0	0	1	1	1	1	1	1	0		
P. aeruginosa	24	-	-	24	22	22	8	6	6	21	-	-	22	10	20	5	-	-
Acinetobacter spp.	11	-	-	11	11	9	2	2	3	9	9	11	9	7	10	5	-	-
B.cepacia	2		-	2	2	2	0	0	1	2	2	2	2	2	2	1		
H. influenza	3	-	2	2	-	0	-	-	-	0	2	1	0	0	-	0	-	-
S.aureus	14	14	-	-	-	-	5	4	-	9	10	11	-	4	12	-	7	9
S.pneumoniae	9	-	-	-	-	-	-	-	-	5	4	6	-	-	7	-	2	4

Key, CAZ-Ceftazidime, FEP-Cefepime, AMC Amoxicillin-clavulanate, CRO - Ceftriaxone, MRP – Meropenem, CAF-Chloramphenicol, AMP-Ampicillin, P- Penicillin, TOB-Tobramycin, CN - Gentamycin, CIP - Ciprofloxacin, FOX - Cefoxitin, SXT-Trimethoprim-sulfamethoxazole, Dox-Doxycycline, ERY-Erythromycin, CLD-Clindamycin and AMK- Amikacin,

5.5 Prevalence of ESBLs, AmpC β-Lactamases and carbapenemases in gram-negative isolates

Of the 89 GNB isolates, 45(50.6%) was found ESBL producers only, 8(9.0%) were AmpC β-lactamase producers only, and 19(21.3%) was found both ESBL and AmpC β-lactamase producers. In addition, 19.1% of GNB were carbapenemase producers. ESBL producers were commonly isolated from the AECOPD patients 18(72%), followed by patients with healthcare associated pneumonia 15(55.6%) and CAP patients 12(32.4%) (fig.2). Moreover, the prevalence of the ESBL-producing *Klebsiella spp*., *P. aeruginosa, E.coli* and *Acinetobacter spp.* was 36%, 62.5%, 62.5% and 54.5% respectively (table 8).

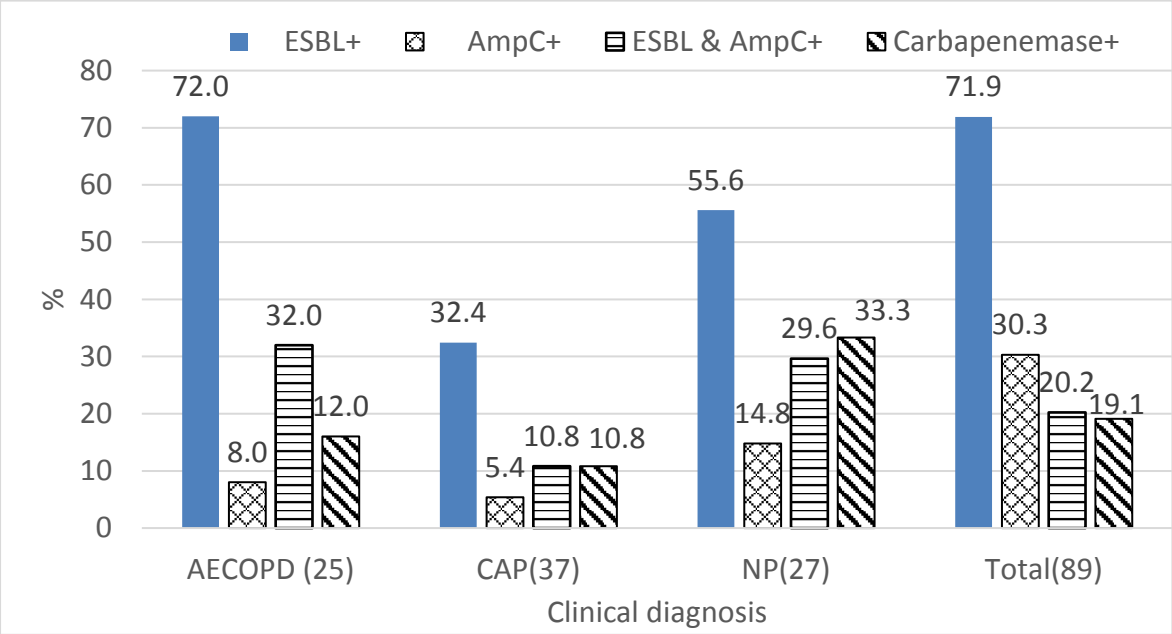

Figure 2: Distribution of ESBLs, AmpC β-Lactamases and carbapenemases producing bacterial isolates among lower respiratory tract infections at JUMC, southwest, Ethiopia, 2019, MDR: multidrug resistance, NMDR: non multidrug resistance

Table 7: Frequency of ESBL and AmpC beta lactamase producing clinical isolates of lower respiratory tract infections at JUMC, southwest, Ethiopia, 2019

Isolates	MDR	ESBL+	AmpC+	ESBL& AmpC+
Klebsiella spp.(25)	16	6(24)	2(8)	4(16)
P. aeruginosa.(24)	23	10(41.7)	2(4.2)	5(20.8)
E. coli.(10)	8	3(30)	1(10)	2(20)
Acinetobacter spp.(11)	11	2(18.8)	1(9.1)	4(36.4)
Enterobacter spp.(4)	4	2(50)	1(25)	0
Proteus spp.(5)	4	2(40)	1(20)	1(20)
Citrobacter spp.(4)	3	1(25)	0	1(25)
B. cepacia.(2)	2	0	0	1(50)
Serratia spp.(1)	1	0	0	1
H. influenzae.(3)	1	0	0	0

MDR: multidrug resistance, ESBL: extended beta lactamase

5.6 Factor associated with sputum culture positivity in patient admitted with lower respiratory tract infections

In this study, bivariate and multivariable analysis was conducted for selected variables that are adequate for logistic regression analysis. For acute exacerbation of COPD relative significant association (P<0.25) was found between being age ≥ 65 years, [COR 4.8 (95%CI: 1.04-22.10)], p ≤ 0.044], having two or more exacerbations in previous one year [COR 7.27 (95%CI: 1.33-39.86)], p ≤ 0.022] and systemic steroid use, [COR 6.25 (95%CI: 0.14-34.12)], p ≤ 0.034] and positive sputum culture (table 8). In multivariable analysis, the only variable, having two or more exacerbations in previous one year [AOR 6.59 (95%CI: 1.06-38.73*)]*, p ≤ 0.037] was found to have statistically significant association with positive sputum culture for PPB. The wider confidence interval indicated that we used smaller sample size for the analysis. On the other hand, being age older than 65 years and systemic steroid use were failed to show a significant association with the prediction of sputum culture positivity for PPB in the multivariable analysis.

Similarly, for CAP, bivariate analysis was conducted and variables with significant association(P<0.25) was found between being age (36-49)years [COR 2.5 (95%CI: 0.85-6.34)], p ≤ 0.102], between (50-64)years [COR 2.27 (95%CI: 0.77-6.74), p ≤ 0.139], older than 65 years [COR 5.42 (95%CI: 1.64-17.56), p ≤ 0.005], alcohol consumption [COR 2.24 (95%CI: 0.68-7.35), *p* ≤ 0.184], renal disease[COR 2.85 (95%CI: 0.80–10.11), p ≤ 0.105], Chronic respiratory disease [COR 3.31 (95%CI: 1.27-8.61), p ≤ 0.014], previous use of antibiotic [COR 1.95 (95%CI: 0.85-4.47), p ≤ 0.116] and culture positivity respectively (table 8).

All variables with a significance value (P<0.25) in the bivariate analysis were entered in to binary logistic regression model and in multivariable analysis, the variables being age older than 65 years [AOR 4.21 (95%CI: 1.12-14.64*)]*, P ≤ 0.024) and chronic respiratory disease [AOR 3.68 (95%CI: 1.29-10.50*)]*, P ≤ 0.015) were found to have statistically significant association with sputum culture positivity (Table 8). In the multivariable analysis, being age < 65year, alcohol consumption, renal disease and previous use of antibiotics are failed to show a significant association with the sputum culture positivity (table 8).

Table 8: Bivariate and multivariable analysis of factors associated with sputum culture positivity for PPB among suspected patients with lower respiratory tract infection at JUMC, southwest, Ethiopia, 2019

	Clinical diagnosis											
	AECOPD						CAP					
Variables	Culture positive		COR (95% CI)	P-value	AOR (95% CI)	P-value	Culture positive		COR (95% CI)	P-value	AOR (95% CI)	P-value
	Yes n (%)	No n (%)					Yes n (%)	No n (%)				
Age, year												
18-35							12(28.6)	30(71.4)				
36-49							13(48.1)	14(51.9)	2.5(0.85-6.34)	0.102	2.54(0.86-7.48)	0.024
50-64	10(52.6)	9(47.4)	4.8(1.04-22.10)			0.134	10(47.6)	11(52.4)	2.27(0.766-6.74)	0.139	3.1(0.962-9.98)	*
>65	16(84.2)	3(15.6)		0.044	3.66(0.67- 19.99)		13(68.4)	6(31.6)	5.42(1.67-17.56)	0.005	4.21(1.12-14.64)	
Sex												
Female	7(87.5)	1(12.5)					23(40.4)	34(59.6)	1.37(0.64-2.92)	0.421		
male	20(64.5)	11(35.5)					25(48.1)	27(51.9)				
Residence												
Urban	9(64.3)	5(35.7)					20(40.4)	28(59.6)	1.19 (0.55-2.55)	0.658		
Rural	18(72)	7(28)	1.43(0.35-5.79)	0.617			28(48.1)	33(54.1)				
Tobacco smoking status												
No	8(72.3)	3(27.3)					40(42.1)	55(57.9)	1.83 (0.59-6.67)	2.29		
Yes	19(32.1)	9(67.9)					8(57.1)	6(42.9)				
Alcohol consumption												
No	22(66.7)	11(33.3)					40(41.7)	56(58.3)	2.24(0.68-7.35)	0.184	1.3(0.32-5.30)	0.710
Yes	5(83.3)	1(16.7)					8(7.3)	5(4.6)				

Inhalant steroid use												
No												
Yes	8(57.1)	6(42.9)	2 (0.50-7.99)	0.333			ND					
	19(76)	6(24)										
Systemic steroid use												
	12(5.6)	10(45.4)	6.25(1.14-	0.034	4.56(0.75-27.69)	0.14	ND					
No	15(88.2)	2 (11.7)	34.12)			2						
Yes												
History of Antibiotic use												
No	2(66.7)	1(33.3)					12	24(22)	1.95(0.85-	0.116	1.69(0.67-4.30)	0.266
Yes	25(30.6)	11(69.4)					36(33)	37(33.9)	4.47)			
Exacerbation in previous one year												
≤ 1	11(40.7)	10(83.3)	7.27(1.33-	0.022	6.59(1.06-38.73)	0.03	NA					
	16(59.3)	2(16.7)	39.86)			7						
≥2												
Renal disease												
No	22(71.0)	9(29.0)					40(36.7)	57(52.3)	2.85(0.80-	0.105	2.93(0.76-11.49)	0.117
Yes	5(62.5)	3(37.5)					8(7.3)	4 (3.7)	10.11)			
CHF												
No	24(72.7)	9(27.3)					39(39.8)	59(60.2)				
Yes	3(50)	3(50)					9(81.8)	2(18.2)				
Chronic respiratory disease												
	0	0					32(29.4)	53(48.6)	3.31(1.27-	0.014	3.68(1.29-10.50)	0.015
No	27(69.2)	12(30.8)					16(14.7)	8 (7.3)	8.61)			*
Yes												

AECOPD: acute exacerbation of chronic obstructive pulmonary disease, CAP: community acquired pneumonia, HCAI: healthcare associated pneumonia, CHF: cardiac heart failure, RVI: retroviral infection, chronic respiratory disease: bronchial asthma plus bronchiectasis plus COPD plus post TB fibrosis, NA: not applicable, ND: not data collected, COR=crude odds ratio; CI=confidence interval; AOR=adjusted odds ratio; ∗ statically significant

5.7 Inpatient mortality

In this study, inpatient mortality of AECOPD, healthcare associated pneumonia and community acquired pneumonia patients was 8(20.5%), 10(24.4%) and 2(1.8%) respectively with overall mortality of 10.6%. Of these deceased cases, 8(42.1%) and 5(26.3%) case mortality was attributed to multidrug resistant *P. aeruginosa* and *Acinetobacter* spp. induced infections respectively, even though statistically insignificant association between MDR and case fatality as inpatient in the present study ($p < 0.166$) (Table 9)

Table 9: Association between antimicrobial resistance and treatment outcomes of study participants at JUMC, southwest, Ethiopia, 2019

Variables	NMDR	MDR	*P*
Previous antibiotics therapy [a]			
No	6(40.0)	9(60.0)	P = 0.005
Yes	10(11.5)	77(88.5)	(df =1, X^2 = 7.86)
Hospital mortality			
No	15(18.1)	68(81.9)	P = 0.166
Yes	1(5.3)	18(94.7)	(df = 1, X^2 = 1.92)
Hospital stay(day)[b]			
<15	1(14.3)	16(85.7)	P = 0.693
≥15	1(5)	9(95)	(df = 1, X^2 = 0.156)

[a] treated with antibiotics in the past three months before enrolled in the study,

[b] for patient with healthcare associated pneumonia, MDR: multidrug resistance, NMDR: non multidrug resistance.

6 Discussions

In this study, an overall culture positivity was 54% (95% CI; 47–61). This is in comparable with studies reported in Cameroon 46.8 %(7) and Egypt 50.4%(8). While lower rate was reported from in previous studies in Ethiopia range from 40.3-45.0%(11-13), Indian 42.9%(42) and Philippines 40.0% (25). In addition, higher rate was reported from Nigeria 81.5% (33) and India 73.6% (9). In the present study, 89(79.5%) and 23(20.5%) of bacterial isolates were account for gram-negatives and gram-positives respectively. This finding was comparable with study finding reported from India 77.6%(16) and Sri Lanka 93.1%(26) which account for gram-negative bacteria. On the other hand, study from Nigeria73.3% (33) and Egypt 80.0%(43) of isolates were gram-positives.

In this study, the most frequently isolated bacteria was *Klebsiella spp.* at 22.3% and *P. aeruginosa at* 21.4%. This finding is comparable with studies reported from Nigeria *P. aeruginosa* at 35.3% and *K. pneumoniae* at 17.2%(16) and Egypt *K. pneumoniae at* 10.37% followed by *P. aeruginosa* at 7.78%(8). On the other hand, *S.aureus at* 12.5% and *S. pneumoniae* at 8.0% the third and the sixth predominant organism in our study. This is lower compared to other studies conducted in Ethiopia and in different geographical regions of the world that *S. pneumoniae* followed by *H. influenzae* and/or *S. aureus* then other gram-negatives were predominantly responsible for lower respiratory tract infections (7, 12, 24, 25).

The inconsistency in prevalence and distribution of etiologies of LRTIs in different studies might be explained by variation in study design and geographical area, difference in specific methodologies, prior use of antibiotics, and presence of comorbidities. In addition LRTIs by gram-negative bacteria was more associated with hospitalization whereas predominance of *S. pneumoniae* and *H. influenzae* was more common in community-based outpatients (44, 45). Furthermore, difference in culture positivity and distribution of microorganisms could be the result of different methods of sample collection, transportation time and the number of organism present in the sample and different diagnostic approaches. The relative lower prevalence rate of bacterial isolates in our study finding may be explained by the most of study participants (77.2%) was engaged for antibiotics before enrolled in the study.

According to our study findings, gram-negative bacteria (GNB) had high role in LRTIs especially among CAP in contrary to previous study report in our country. Although different bacteria and respiratory viruses can be responsible for LRTIs, *S. pneumoniae*, *H. influenzae* and atypical pathogens have been known the most common causative pathogens. GNB, such

as *P. aeruginosa*, *K. pneumonia*, *E. coli*, *Acinetobacter baumannii* and *Stenotrophomona maltophilia* have emerged as causes of CAP in the past few decades with an estimated prevalence rate ranging from 2% to 30%(32, 46). Our finding also comparable with these statistics, where GNB were attributed for 32% of our CAP Cases.

In addition, in this study, GNB have great role on LRTIs this may explained by first, more than half of study participants were with comorbidities like chronic respiratory disease, cardiac diseases, HIV and others. Several prospective observational studies identified different comorbidities as independent risk factor for pneumonia due to GNB and/or bacterial CAP in general (47-51). In the presence of comorbid conditions like bronchiectasis and other chronic respiratory disease, usually there is persistent mucus production with mechanical change that result impaired host immunity, and the presence of potential pathogenic bacteria in the airways that result in microbiome imbalances result in increased airway inflammation and which may be promotes colonization and leading to infection (6, 51). Second, although we failed to find significant association between prior use of antibiotics and pneumonia due to GNB or bacterial pneumonia in general, prior use of antibiotics among majority of study participants may explain the reason for predominance of gram-negative bacteria. Other studies, identified prior use of antibiotics as an independent risk factor for pneumonia due to GNB(47, 51). The explanation could be earlier symptom onset in patients with respiratory disease with subsequent earlier treatment, selection of patients with GNB and seeking medical care due to treatment failure(6, 51). Last, increasing the number of aging population who are usually harboring colonizers of GNB result in aspiration due to inefficient coughing may be another reason for significant role GNB on LRTIs specially with severe illness requiring hospital admission(52). On the other hand, low rates of *S. pneumoniae* and *H. influenzae* in our study might be due to lower sensitivity of the used conventional identification method.

In this study, High antibiotics resistance rate was observed against most groups of tested antibiotics (Table 4 & 5). Different guidelines recommend macrolides as prophylaxis for acute exacerbation of COPD(53). Similarly, combination therapy with amoxicillin/clavulanate or cephalosporin and macrolide or doxycycline or macrolide with respiratory fluoroquinolone for initial empirical treatment for hospitalized patients with LRTIs without risk factor for MRSA or *pseudomonas* induced infection (1, 53, 54). In this study, about 85.7%, 73.9%, 68.5%, 65.2% and 75.3-82.6% of bacterial isolates were resistant to trimethoprim-sulfamethoxazole, doxycycline, amoxicillin clavulanic acid, macrolides and cephalosporins respectively. This result is in comparable with study finding reported in Egypt (8) and India(16). This may be

due to the regular use of these antibiotics that have been prescribed frequently, which contribute to the development of drug resistance. In addition, most of study subjects were exposed to antibiotics before the current admission.

Resistance rate of *Klebsiella spp.* was 52.0% to Amoxicillin-clavulanate, third and fourth generation cephalosporins range from 72.0% to 56.0% and doxycycline 76.0%, While *Klebsiella spp.* showed good susceptibility to ciprofloxacin 60.0%, amikacin 84.0% and meropenem 88.0%. This finding was in agreement with other studies reported from Nigeria (33), Egypt (8) and Bangladesh(27). On the other hand, ceftriaxone resistance rate in this study was higher than previous studies in Ethiopian ranging from (13.2-19.4%). This difference may be due to our study subjects were hospitalized patients commonly with underlying diseases. Similarly, *P. aeruginosa*, showed very high resistance toward cephalosporines and Amoxicillin-clavulanate with 91.7% and whereas *P. aeruginosa* showed good sensitivity to meropenem 79.1%, amikacin 75.0%, and ciprofloxacin 58.1%, which is in line with other studies (16, 55).

We also noticed that 85.7% of isolated *S. aureus* were MRSA. This is comparable with previous studies from Ethiopia and elsewhere in the globe reported that MRSA was account for 81.3%, Jimma (11), 83.3% Arba Minch, (13) and, 75% Bahirdar (12) and other studies reported from other countries (8, 9, 28). Furthermore, *S. pneumoniae* was found 77.8% resistance to Oxacillin and trimethoprim sulfamethoxazole, 55.6% azithromycin and 44.4% doxycycline. On the other hand, 88.9% and 77.8% of these isolates were found to be susceptible to vancomycin and clindamycin respectively. This result is almost similar with findings reported from different studies (8, 16, 26). However, previous studies conducted in Ethiopia, Jimma, Oxacillin 55% and tetracyclin 35.0% and SXT 0% (11) and Bahirdar, Oxacillin 56.7% tetracyclin 45% and SXT 8.3% (12) reported lower resistance rate for *S. pneumoniae* in opposite to our study.

The emergence and rapid spread of MDR bacteria is a worldwide health problem that put additional burden on low-income countries. This makes the clinical management of infectious disease including LRTIs by prescribing effective empirical antibiotics has been challenging for physicians(46). In addition, the emergence of MDR pathogens, including MRSA, *Pseudomonas aeruginosa* and Enterobacteriaceae can cause CAP including organisms producing extended spectrum b-lactamase further complicate the choice of appropriate antibiotic treatment in CAP, that requires updated treatment guideline when the risk of each of

these pathogens is elevated(54). There for this scenario support current study finding. In the present study 93.3%, 90% and 70% of isolates from acute exacerbation of COPD, healthcare associated pneumonia and community, acquired patients were multidrug resistant respectively with an overall MDR rate of 82.1% including MRSA. This is comparable with study reported from other studies (8, 12) accordingly. Similarly recent reported study by title of emergence of high drug resistant bacterial isolates from patients with health care associated infections at Jimma University Medical Center also underline a dramatic increasing of antibiotics resistance in the current study setting (56).

The most commonly used empirical antibiotics in the study participants against LRTIs caused by such MDR bacteria was β-lactam antibiotics. However, resistance for these β-lactam antibiotics mainly due to the production of β-lactamases are becoming a worldwide problem. These can be developed when bacterial gene mutate continuously in response to overuse or misuse of β-lactam antibiotics(19, 57). In the present study, the prevalence of ESBL-producing GNB was 71.9%, with 72.0% attributed to isolates from acute exacerbation of COPD; this could be due to repeated exposure to such beta lactams drugs during frequent exacerbations. Present ESBL finding was lower than study reported in Gondar Ethiopia ESBL+ at 85.8%(58) and Ababa Ethiopia ESBL+ at 78.6%(59). However, the magnitude of ESBLs production among GNB in our study was higher than studies reported in Jimma Ethiopia 40.4% (56), Addis Ababa Ethiopia 57.7%(60), Nigeria 58.0%(61) and Nepal 28.1% (55). Present study reported high prevalence of the ESBL among *P. aeruginosa (*62.5%), *E. coli (*62.5%), *Acinetobacter* spp. (54.5%), and *Klebsiella* spp. (36.0%)**.** This finding is comparable with study done in Wolaita Ethiopia, 62.5% of *P. aeruginosa* & 55.8% of *A. baumannii*(62), in Jimma Ethiopia 46.7% of *Klebsiella spp. &* 51.6% of *E. coli*(56), and in Nepal 26.7% of *K.pneumoniae (55)* isolates were ESBL producers. However, our finding is higher than study reported from endotracheal aspirate in India, 42.30% of *P.aeruginosa* and 17.95% of *A.baumannii*(63) and in Nepal(11.1%) of *A.baumannii* (55) were ESBL producers . On the other hand, in the present study ESBL production in 36.0% *Klebsiella* spp. and 62.5% *E. coli* which is comparable with study done in clinical sample in Jimma Ethiopia 46.7% *K.pneumoniae &* 51.6% *E. coli* (56) and Nepal *K.pneumoniae* (26.7%)(55). While, highest ESBLs production was observed in *K. pneumoniae* (85.4%) in Addis Ababa Ethiopia (60). This variation might be due to differences in study population, type of specimen used, sample size, the extent of antibiotic use, geographic variations and detection methods used. This observation suggested that the ESBLs that were

generally widespread among members of Enterobacteriaceae are also found in *P. aeruginosa* and *Acinetobacter* spp.

Concerning to carbapenems, which is last choice of antibiotics against ESBL producers, 19.1% of gram-negative isolates were found meropenemase producers in the current study. Of which 20.8% and 45.5% were attributed by *P.aeruginosa* and *Acinetobacter* spp. respectively. Comparable with study reported in Gondar Ethiopia 16.2%(58), Jimma Ethiopia (25%) (56). This, 19.1% resistance rate was lower than study reported in India, 85% of LRTI isolates were carbapenem resistant (28). Variation in resistance to carbapenem may be due to difference in availability and owing to little use of them because of these antibiotics are expensive. On the other hand, 33.3% of carbepenem resistance were isolates recovered from patient with healthcare associated pneumonia. This higr resistance might be due to inappropriate use of this antibiotics for hospital acquired infections.

High resistance to these commonly recommended antibiotics might be due to irrational use of antibiotics and self-medication of these drugs in community and hospital settings. Moreover, this result also highlight a lack of antimicrobial stewardship and effective infection control practices in the study area in particular. In Ethiopia, there are indications of the misuse of antibiotics in and out of hospital settings. These, together with rapid spread of resistant bacteria and poor surveillance system, contributed to the problem of AMR(64). In addition recent systematic review report on bacterial infection in Ethiopia indicated that majority of the bacterial isolates were resistant to commonly used antibiotics(65). Hence, antibiotic resistance is an important clinical concern in our country as a big problem, which demands tireless efforts to rationalize antibiotic use in and out of hospital settings.

An important finding of this study was identifying some factors associated with sputum culture positivity from both AECOPD and CAP suspected cases. Experience of two or more exacerbation in the previous one year [AOR 6.6 (95%CI: 1.06-38.73)], ($p \leq 0.037$) was found an independent factor associated with culture positivity among AECOOP cases. AECOPD patient with two or more exacerbation in the last one year were 6.59 times more likely to have sputum culture positive. This is inline other studies which identified frequent exacerbation as independent factor associated with culture positivity(44, 66). Frequent exacerbations are associated with faster decline in lung function. This decline in lung function favors colonization and/ or infection with bacterial pathogen by failure in host immunity, such as epithelial cell

damage, mucous hypersecretion and inflammatory cell infiltrates which makes them susceptible for frequent exacerbation (67).

On the other hand, being age ≥ 65 year [AOR 4.2 (95%CI: 1.12-14.64), and chronic respiratory disease [AOR 3.7 (95%CI: 1.29-10.50)], (p≤0.037) were identified as factors associated with culture positivity from CAP in this study. This finding was consistent with several international studies, the incidence of CAP increased with age and with the presence of comorbidities (5, 51, 68). In the current study, Being Age ≥ 65 were 4.2 times more likely to have positive sputum clture compared to the age group 18-35 years. A Systematic Review of observational studies suggested that age is a well-known risk factor for CAP(5). In this review elderly age was associated with an increased risk of CAP compared with the age <65, the risk of CAP was increased in ≥ 65 years old; adjusted AORs: 1.07 (1.01 – 1.19) per year of increase. Study report from Spain, determined age ≥65 as independent predictor for bacterial CAP AOR, 1.82(1.08-3.05)(50). In addition, study reported from Taiwan determined age ≥65 as independent predictor for CAP AHR: 2.81 (2.40 - 3.29)(48).

There are satisfactory evidences that advanced age is important risk factor that directly related with physiological changes associated with aging and a greater presence of chronic disease. Impairment of mucociliary clearance, impairment of alveolar defense, ineffective cough, and swallowing disorders are altered lung mechanisms of defense, which increase susceptibility of the persons in elderly age to CAP (68, 69).

The other key identified factor associated with culture positivity from CAP by this study was chronic respiratory disease. CAP patient with chronic respiratory disease were 3.7 times more likely to have bacterial pneumonia. This finding is in consistent with studies reported from Germany, chronic air way disease is an independent risk factor of culture-positive CAP patients due to PA; AOR: 4.78 (1.89–12.07)(47), another observational prospective study in Spain, the presence of Chronic respiratory disease(OR, 2.20; 95% CI, 1.36-3.57; *P*=0.001) independent risk factor of culture-positive bacterial CAP(50). In addition case control study in USA demonstrated that, the presence of Bronchiectasis(OR, 8.3; 95% CI, 1.7–46.6; *P*=0.01 were independent Risk factors for pneumonia due to GBN(49).

Chronic respiratory disease may be more susceptible to develop pneumonia due to persistent mucus production with some physiological change that result impaired host immunity, and the presence of potential pathogenic bacteria in the airways that result in microbioma imbalances result in increased airway inflammation and which may be promotes colonization and infection.

Another explanation could be earlier symptom onset of milder CAP in patients with airway disease with subsequent earlier treatment and a potential effect of co-treatments like inhaled steroids (6, 51).

In this study, inpatient mortality of AECOPD, healthcare associated pneumonia and community acquired pneumonia patients was 20.5%, 24.4% and 1.8% respectively, an overall rate of 10.6% with 65% *P. aeruginosa* and *Acinetobacter* species induced infections. In a study in Egypt, the inpatient mortality among VAP patients was 31.8% with 25% *P. aeruginosa* induced infections (70). Other study in Japan reported CFR due to CAP was 3.2% (71). Other the other hand, mortality rate among COPD exacerbation patients was reported from Egypt(50%)(72) and Serbia(6.2%) with 100% *P. aeruginosa* induced infection (73). The this study, mortality was lower than reported CFR from Egypt and Japan, and higher than CRF reported from Serbia. The reason could be difference in severity of the disease, sample size difference and better treatment option.

Limitation of the study

This study have several limitations, first, due to resource limitation, some common etiologies of LRTIs was not explored. Antibiotic treatment prior to the diagnosis could also be biases for real pathogen frequencies identified in this study. Moreover, sputum samples and tracheal aspirate were the specimen used in this study, which may be contaminated. To overcome this problem, we have used sputum validity criteria proposed by different researchers. Although combinations of aminoglycosides and carbapenems were tested, other beta-lactams, beta-lactamase inhibitors and newer fluoroquinolones, such as tigacycline, colistin, piperacillin/tazobactam and levofloxacin, were not tested. The other limitation was the limited sample regarding culture positive for PPB in some clinical form of lower respiratory tract infection (healthcare associated pneumonia) did not permit us to perform a multivariable analysis for the identification of exclusive factor associated with culture positivity. In the last, the study site was limited to JUMC, where the data obtained might not be representative of the entire Ethiopian hospitalized adult population.

7 Conclusions

In this study, sputum culture positivity was 54.0% with large proportion caused by *Klebsiella species, P. aeruginosa* and *S. aureus*. High rate of antibiotic resistance of these bacteria to commonly prescribed antibiotics was observed in this study. Moreover, isolated bacterial strain also showed high rate of MDR pattern, ESBL and AmpC. However, Most of the isolates were found susceptible to carbapenems, aminoglycosides and fluoroquinolones. Having two or more exacerbation in previous one year was found independent factor associated with sputum culture positivity in acute exacerbation of COPD whereas being age ≥65 years and chronic respiratory disease were found independent factor associated with sputum culture positivity in community acquired pneumonia. Mortality rate was more common among patients with *P. aeruginosa* and *Acinetobacter* species.

8 Recommendations

Based on the research finding, the following recommendations are forwarded:

- This study provides evidence that majority of the bacterial isolates from LRTIs were multi drug resistant strains. To address this problem, it need coordination of multidisciplinary efforts (physicians, infection control group, and microbiologists) and recommends continued surveillance on type of etiology and their antibiotic resistance, effective infection control practices and strict adherence to national and international treatment guideline to reduce prevalence and spreading of AMR.
- Establishment of antimicrobial stewardship (to improve appropriate use of antibiotics and reduce unnecessary use).
- Management of bacterial lower respiratory tract infection better to be guide by culture isolation and antimicrobial susceptibility testing that may aid to decrease morbidity and mortality.
- Empiric antibiotic therapy should consider the prevalence of antibiotic resistant pathogens in our community and certain risk factors that may increase probability of MDR bacterial pathogens.
- Furthermore, Large-scale national level research is required for further validate the etiology and drug susceptibility patterns of pathogens by considering atypical bacteria, viral, fungal and all other potential bacteria that can involve in lower respiratory tract infections.
- Ministry of health should work to improve laboratory capacity to improve diagnostic capacity of the lower respiratory tract infections.

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APPENDICES

APPENDIX I: INFORMED CONSENT

PART 1: Information Sheet

Title: Bacteriology of Lower Respiratory Tract Infection and Antibiotics Susceptibility Pattern of Isolate in Jimma University Medical Center, southwestern Ethiopia, 2019 GC.

Name of Investigator: Abdulhakim Mussema

Name of the Organization: Jimma University

Introduction: You are invited to participate as a study participant in this study voluntarily. It is very important that you read and understand the following information. The information given is to describe the purpose, procedures, benefits and risks of the research study. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me about them.

Purpose: - The main objective of the study is to determine bacterial profile of lower respiratory tract infection and their antibiotic susceptibility patterns.

Study protocol and procedures

The study procedures and protocol will be carefully explained to you, and if you need more information, you can freely ask about them whenever you would like. If you are willing to participate, you need to understand the purpose of the study and you are requested to give your consent. No procedure will be initiated before you offer your consent to participate in the study.

1. Questionnaire

You will be asked few questions. The questions will take approximately 3-5 minutes to be completed.

2. Specimen culture

Trained (selected) data collectors (nurses and medical laboratory professionals) will collect the required clinical sample. Before specimen collection, you will be wash your mouth with water to decrease mouth bacterial contamination and dilution by saliva and you will breathe deeply and then cough vigorously into a cup; these are standard procedures for sputum collection for

this type study purpose. Your sputum will be tested in the laboratory in order to know the cause of infection.

Potential risks and discomfort

1. **Questionnaires:** You do not have to answer any question that you do not feel comfortable with. Any information that you provide to the researcher is completely confidential and you will not be identified.
2. **Symptom:** Before, you cough vigorously into a cup, you will be asked to wash your mouth and to breathe deeply. However if you feel discomfort, you should breath as you would normally do.

Potential benefits

There is no direct benefits to you from participating in this study. However, based on the diagnosed result you will be treat accordingly. Moreover, this study will have a great value on preventive measures in our health institutes and in the community. The results of this study have importance to treat the patients and to use as a baseline for effective treatment in the absences of laboratory investigation.

Compensation for participation: You will not receive any payment for your participation in this research study.

Confidentiality of your information- All information gathered from the study participant will remain confidential. Your participation in this study is strictly anonymous. Personal information will be treated confidentially and under no circumstances will it be transmitted to any person or organization. It will not be shared with or given to anyone except your physician upon your request.

Voluntary participation - Your participation in the study is voluntary; no one is obliged to take part. Refusal to participate will involve no penalty. Each study participant is free to withdraw consent and discontinue participation in this study at any time. As aware of study objective, this study is not associated with any governmental or non-governmental bodies rather our interest is purely academic. Your willingness to participation is very important for this study success.

Would you be willing to? A. Yes ☐ B. No ☐

Whom to Contact

If you have any questions, you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact.

Mr. Abdulhakim Mussema Telephone: 0910828546

Email: abdulhakimmusseema5@gmail.com

PART 2: Written Consent Certificate

I. Written Consent Certificate(English Version)

I confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my idea. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. I_____ do here by give consent to Dr. /Mr. /Mrs. /Miss _____to include me in the proposed research.

Participant code _____

Participant (signature) _____date_____

Name of the data collector_____

Data collector (signature) _____date_____

II. **Written Consent Certificate(Afaan Oromoo Version)**

Qorannicha irratti hirmaachuuf waliigaluun koo kaayyoo fi haala qorannichaa haala ifaa ta'een hubachuu fi yaada kiyya yoon jijjiire mirga addaan kutuu qabaachuu koo hubachuu nan mirkaneessa, odeeffannoo waa'ee qorannichaa ilaalchisee naaf kennamee jira. Yeroon barbaade adabbii fi tajaajila dhabuu tokko malee qorannicha keessaa bahuu akkan dandahu naaf himameera, qorannichi afaanin ani hubadhuun naaf ibsame. Ani _____ Dr. /Mr. /Mrs. /Miss _____qorannoo yaadame keessatti hirmaachuf waliigaleera.

Koodii hirmaataa _____

Mallatoo hirmaataa _____guyyaa_____

Maqaa nama raga funaanuu_____

Mallatoo nama raga funaanuu _____guyyaa_____

III. **Written Consent Certificate (Amharic Version)**

የስምምነት ውል

በዚህ በተዘጋጀው የመረጃ ቅፅ መሠረት የጥናቱን ዓላማና ጥቅም በግልጽ ተረድቻለሁ:: ጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍም በራሴ ፍቃድ የሚወሰን መሆኑንም ተገልጾልኛል። በተጨማሪም ከጥናቱ ባልሳተፍም ሆነ አቋርጬ ብወጣ ከጤና ተቋሟቱ በማገኘው የህክምና አገልገሎት ምንም አይነት ችግር ወይም እንግልት እንደማይደርስብኝ ተነግሮኛል። በዚህ መሠረት ያለጥናቱ ቡድን አባላት ተፅዕኖ በሙሉ ፈቃደኝነት በዚህ ጥናት ውስጥ በመሣተፍ የሚጠበቅብኝን አስተዋፅዖ ለማበርከት በፊርማዬ አረጋግጣለሁ።

የተሳታፊው የሚስጥር ቁጥር ----------

የተሳታፊው ፊርማ ----------, ቀን ----------

የመረጃ ሰብሳቢው ስም ----------

የመረጃ ሰብሳቢው ፊርማ ----------, ቀን ----------

APPENDIX II: QUESTIONNAIRE

Jimma University, Institute Of Health, Faculty of Health Sciences, School of Medical Laboratory Science.

APPENDIX II: QUESTIONNAIRE

Questionnaires to determine Bacterial profile of Lower Respiratory Tract Infection and Antibiotic Susceptibility patterns of isolates at Jimma University Medical Center, South West Ethiopia, 2019.

Participant serial number _____, Identification code_____

Participant address_____, Phone number _____

Data collector name _____ Date _____, Signature_____

Instruction: Read/ listen the questions and select the appropriate response; for question 1, enter age in years.

SN	QUESTIONS	CODING CATEGORIES
1.	Age(in years) _____	
2.	Sex	1. Male 2. Female
3.	Residence	1. Urban 2. Rural
4.	Occupation	1. Civil servant 2. Farmer 3.Merchant 4.House wife 5. Daily labor 6. Other (specify)_____
5.	Clinical diagnosis	1. AECOPD 2. CAP 3.HCAI
6.	Tobacco smoking status (current or former)	1. Yes 2. No
7.	Alcohol consumption	1. Yes 2. No
8.	If patient with healthcare associated pneumonia, length of hospital stay(day)	
9.	If ICU admitted patient	1. Ventilated 2. Non ventilated
10.	If AECOPD case, number of acute exacerbation in previous year_____	
11.	If AECOPD case, GOLD stage (severity)	1. Mild 2. Moderate 3. Severe 4. Very severe
12.	Comorbidities/Underlying diseases	1. Diabetes mellitus 2. Chronic renal failure 3. Congestive heart failure 4. Malignancy 6. Other (specify)_____

13.	If diagnosis is AECPD, steroid usage Systemic Inhalant	 1. Yes 2. No 1. Yes 2. No
14.	Have been on antibiotic treatment for the last three months	1. Yes 2. No
15.	If yes in Q14 above, what type of Antibiotics have been used_____	
16.	Discharge outcomes	1. Death as Inpatient 2. survived to hospital discharge
17.	Nature of specimens(sputum)	mucoid Mucopurulent Purulent

THANK YOU!

APPENDIX III: Media and Reagents

A. Media

MacConkey Agar

50g of MacConkey agar base in 1litre distilled water

Autoclave at 121^0C for 15 minutes.

Blood Agar

37.5g in 1000ml of distilled water.

Autoclave at 121^0C for 15 minutes.

Cooled to 50-55^0C and added 5-7% sterile sheep blood.

Mixed well before pouring.

Chocolate Blood Agar

37.5g in 1000ml of distilled water.

Autoclave at 121˚C for 15 minutes.

Added 5-7% sterile blood the temperature is until 75-80^0C.

Mueller Hinton Agar

38.0g in 1000ml of distilled water
Autoclave at 121˚C for 15 minutes.

Mueller Hinton Agar with 5% sheep blood

38.0g in 1000ml of distilled water

Autoclave at 121˚C for 15 minutes.

Cooled to 50-550C and added 5-7% sterile sheep blood.

Kliger Iron Sugar (KIA) Agar (REMEL, UK)

59.5g in 1000ml of distilled water

Autoclave at 121˚C for 15 minutes.

Simmons Citrate Agar (HiMedia Laboratories Pvt. Ltd. India)

24.2g in 1000ml distilled water

Autoclave at 121˚C for 15 minutes.

Urease Agar (HiMedia Laboratories Pvt. Ltd. India)

24.01g of urea base in 950ml distilled water

Autoclave at 115˚C for 20 minutes.

Cool to 50˚C and add 50ml of sterile 40% urea

Lysine Iron Agar (HiMedia Laboratories Pvt. Ltd. India)

34.56g in 1000ml distilled water
Autoclave at 121˚C for 15 minutes.

Sulfide Indole Motility (REMEL, UK)

30g in 1000ml distilled water.

Autoclave at 121˚C for 15 minutes

Skim milk, tryptone, glucose, glycerol storage medium (STGG)

Mix 2 g Skim milk powder, 3 gm Tryptone soya broth, 0.5 g Glucose, 10 ml Glycerol in 100 ml distilled water

Dispense in 1.0 ml amounts in screw-capped 1.5-ml vials.

Autoclave at 121˚C for 15 minutes

Store STGG frozen at -20°C or refrigerate until use. Use STGG medium within 6 months of preparation

B. **Reagents**

Gram staining

Crystal violet (2%) l0g crystal violet in 100ml

Absolute alcohol in 1 litter of distilled water (primary stain)

Grams Iodine l0g Iodine in 20g KI (fixative)

Acetone Decolorizing agent

Carbol fuchsine 1% Secondary stain

Normal Saline

0.89% Sodium Chloride

0.89g Sodium chloride in 100ml distilled water

Autoclave at 115˚C for 15 minutes.

McFarland standard 0.5

Barium chloride (1%) 0.05ml

Sulphuric acid (1%) 9.95ml

APPENDIX IV: Laboratory methods

Sputum collection procedure

1. Assemble the necessary materials required: Clean, sterile, single use, plastic, disposable containers.
2. Ask patient to rinse his/her mouth with water to reduce specimen contamination
3. Instruct the patient to sit on a chair or at the edge of the bed.
4. Take a deep breath, 3-4 times.
5. Hold the breath for 3-5 sec after each inhalation before exhaling.
6. Cough after the last inhalation.
7. Empty sputum into the container.

8. Carefully and tightly, replace the cap.
9. Label the specimen with the patient's name, doctor's name, specimen type, and the date and time collected.
10. Note on specimen type that sputum was coughed.
11. Send the specimen to the laboratory immediately.

Gram staining Procedure and Examination:

1. Assemble the necessary materials reagents.Gram's Iodine, Acetone-Alcohol, Safranin
2. Prepare the smear from the culture or from the specimen.
3. Allow the smear to air-dry completely.
4. Rapidly pass the slide (smear upper most) three times through the flame.
5. Cover the fixed smear with crystal violet for 1 minute and wash with distilled water.
6. Tip off the water and cover the smear with gram's iodine for 1 minute.
7. Wash off the iodine with clean water.
8. Decolorize rapidly with acetone-alcohol for 30 seconds.
9. Wash off the acetone-alcohol with clean water.
10. Cover the smear with safranin for 1 minute.
11. Wash off the stain wipe the back of the slide. Let the smear to air-dry.
12. Examine smear with the area of maximal purulence for polymorph leukocytes (PMNL) and squamous epithelial cells (SEC) by using low power field (LPF) magnification.
13. Sputum specimens containing <10 epithelial cells and >25 leukocytes/ low power field (LPF), fulfill criteria for culture.

Washing sputum specimens

1. Mix the specimens with physiological saline (1:10 vol)
2. Vortex and centrifuge for 10 minutes at 1500 rpm.
3. Add an equal volume of N- acetyl- L cysteine to the pellet for homogenization and
4. Incubate the mixture at 37°C for 15 minutes.

Sputum specimen culture

1. Further dilute washed sputum samples 1: 10, 1:100 and 1:1000 in sterile normal saline.
2. Inoculate 10 ul aliquots of 1:100 and 1:1000 on sheep blood agar, Mac Conkey agar and chocolate agar plates for quantitative evaluation.
3. Incubate chocolate agar plates in an incubator (5- 10% CO2) at 37 ºC for 24- 48 hours while incubate blood agar and MacConkey agar in an aerobic atmosphere at 37 ºC for 24 hours.
4. If isolated bacteria reach a count of 10^6 CFU/mL, consider as significant and accept as

causative pathogens except for *Streptococcus pneumoniae* where 10^5CFU/mL estimated to be sufficient.

- ✓ CFU/ml=number of colonies X dilution factor

Biochemical Tests

Kligler Iron Agar Test

This test was performed on colonies which were observed to be Gram negative rods under the microscope and suspected to belong to the Enterobacteriaceae family. The test was performed by picking part of the colony and inoculating on Kligler agar using a straight sterile loop. The setup was incubated overnight. After overnight incubation, the tubes were examined for the following reaction; a red slant, yellow butt, Hydrogen sulphide production and a crack in the medium. The various Gram negative rods reacted differently in this medium and their reaction helped in their identification.

Indole Test

The indole test was done on colonies which were Gram negative rods. To perform the test, loopful of colonies was inoculated into a sterile peptone broth and incubated overnight. The following day, a few drops of Kovacs'reagent were added to the culture broth, using a Pasteur pipette. Appearance of a red layer indicated that the test was positive whereas yellow layer indicated that the test was negative. Escherichia coli was suspected if the colonies were lactose fermenting, non mucoid colonies and were Gram negative rods and positive to the indole test.

Citrate Utilization Test

This test was done to determine the ability of an organism to utilize citrate. This test was done by stabbing citrate agar in a test tube with organisms which were Gram negative using a sterile straight loop. After overnight incubation, a change in colour of the agar from green to blue implies a positive test and no change in colour implies a negative test. Among the Gram negative rods, Klebsiella, Proteus and Pseudomonas species were suspected because these bacteria are able to utilize citrate. The composition, preparation and mode of action of citrate agar are presented in appendix.

Urease Test

This test was used to identify Gram negative rods which have the ability to produce urease. Colonies of the suspected bacteria were stabbed into urea agar in a test tube using a sterile straight loop. After overnight incubation, a change in colour from yellow to pink of the urea agar confirmed the ability of the organisms to produce urease. Proteus species was thus suspected, if the colonies were lactose non fermenters on MacConkey and produced swarming on blood agar

Oxidase Test

This was done on growths which were non lactose fermenters on MacConkey and identified as Gram negative rods on Gram staining. A slide was used to pick colony from an agar plate. The colony was smeared on a filter paper soaked with the oxidase reagent (Appendix). The filter paper was examined for a colour change after a few seconds. The appearance of a purple / bluish colouration indicated the presence of Pseudomonas species.

Catalase Test

This test was done to differentiate bacteria that produce the enzyme catalase, such as Staphylococcus from non-catalase producing bacteria such as Streptococci. This was done by using a sterile bacteriological loop to pick a colony of the test organism from the blood agar plate and immersing into a test tube containing 3% Hydrogen peroxide. Within one minute, gas bubbles were seen produced and rising from the Hydrogen peroxide indicating that the organism is catalase positive. The test is positive for Staphylococcus species and negative for Streptococcus species.

Coagulase Test

This test was performed on organisms which were Gram positive cocci in clusters after Gram's stain. It was used to differentiate between coagulase positive Staphylococcus and coagulase negative Staphylococcus. This was done by emulsifying the suspected colonies in a few drops of saline placed on a clean slide. A few drops of plasma were added to the emulsified colonies. The appearance of clumps on the slide after mixing indicated the presence of coagulase positive Staphylococcus which is Staphylococcus aureus. S.aureus gives a coagulase positive result which other Staphylococcus species are negative for.

Optochin Test

The Optochin test done on colonies which produced alpha hemolysis on blood agar and chocolate agar but did not grow on MacConkey agar. Again it was performed on colonies which were Gram positive cocci in chains to differentiate between Streptococcus pneumoniae and Streptococcus viridans. Colonies of the test organism were streaked or cross hatched on blood agar using a sterile loop. An optochin disc was placed directly on the cross hatched surface of the agar plate. This was incubated in a Carbon dioxide jar. After overnight incubation, the plates were examined for zones of clearance around the optochin discs. Streptococcus pneumoniae is optochin positive; therefore, forms zones of inhibition around the optochin disc whilst Streptococcus viridans is optochin negative, hence, no zone of inhibition forms around the optochin disc.