DEBRE MARKOS UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL PROFILE, ANTIMICROBIAL SUSCEPTIBILITY PATTERN, AND ASSOCIATED FACTORS OF COMMUNITY-ACQUIRED PNEUMONIA AMONG ADULT PATIENTS AT DEBRE MARKOS COMPREHENSIVE SPECIALIZED HOSPITAL, NORTHWEST ETHIOPIA.

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DECLARATION

This thesis is entitled 'Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Factors of Community-Acquired Pneumonia Patients at Debremarkos Comprehensive Specialized Hospital, Northwest Ethiopia.'

This thesis was carried out by Abiyot Yitbarek under the supervision of Mr. Getachew Mengistu and Mr. Abebaw Admasu, College of Medicine and Health Sciences, for the award of the MSc Degree in Medical Microbiology.

I declare that this is original and has not been presented or published in this or other University or institution.

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ABBREVIATION AND ACRONYMS

APHI	Amhara Public Health Institute
ARTI	Acute Respiratory Tract Infection
AST	Antimicrobial Susceptibility Testing
CAP	Community-Acquired Pneumonia
CLSI	Clinical Laboratory Standard Institute
DMCSH	Debre Markos Comprehensive Specialized Hospital,
DRP	Drug-Resistant Pathogen
HTM	Haemophilus Test Medium
ICU	Intensive Care Unit
IRERC	Institutional Health Research and Ethics Review Committee
MDR	Multi-Drug Resistant
MRSA	Methicillin-resistant Staphylococcus *aureus*
OPD	Outpatient Diagnosis
PCR	Polymerase Chain Reaction
PNL	Polymorph Nuclear Leukocytes
SOP	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences

ABSTRACT

Background: Community-acquired pneumonia is a frequently encountered lower respiratory tract infection that leads to significant morbidity and mortality worldwide. Antibiotic resistance of frequently isolated bacterial pathogens from community-acquired pneumonia patients has complicated the selection of empirical therapy

Objective: To assess bacterial profile, antimicrobial susceptibility patterns, and associated factors of community-acquired pneumonia presumptive adult patients at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia.

Method: A hospital-based cross-sectional study was conducted among community-acquired pneumonia presumptive adult patients at Debre Markos Comprehensive Specialized Hospital from March 1, 2025, to May 30, 2025. Sputum samples were collected from 370 community-acquired pneumonia presumptive adult patients aseptically and inoculated onto MacConkey, Blood agar, and Chocolate agar plates. Antimicrobial susceptibility testing of the isolates was performed using the disk diffusion method on Mueller-Hinton agar. Data were analyzed using SPSS version 25 software. The statistical significance of the association was measured using bivariable and multivariable logistic regression analysis; a P-value < 0.05 was considered statistically significant.

Results: Of 370 cases, 33.2% (n = 123; 95% CI: 28.4%–37.8%) were found to have culture-confirmed community-acquired pneumonia. The most common isolate was *S. pneumoniae* (35.0%, n = 43). The Gram-positive bacteria were susceptible to erythromycin (86.4%) and clindamycin (66.7%), whereas the Gram-negative bacterial isolates were susceptible to meropenem (100%), followed by amikacin (93%), ceftazidime (84.2%), and cefepime (66.7%). urban residence ($p < 0.001$), cigarette smoking ($p = 0.008$), alcohol consumption ($p = 0.035$), a previous history of pneumonia ($p < 0.001$), comorbidity ($p = 0.014$), and malnutrition ($p = 0.043$) were significantly associated with culture-positive sputum.

Conclusion: This study found 33.2% prevalence of community-acquired pneumonia -causing bacteria. Alcohol use, cigarette smoking, a previous history of pneumonia, comorbidity, and urban residence, were significantly associated with community-acquired pneumonia. Therefore, culture-based bacterial identification and local antibiotic susceptibility testing should be performed regularly to ensure accurate diagnosis and effective treatment.

Keywords: Community-Acquired Pneumonia, Antimicrobial susceptibility profile, Bacterial pathogens

1. INTRODUCTION

1.1. Background

Pneumonia is a form of acute respiratory tract infection (ARTI) that affects the lungs. It is greatly associated with clinical and/or radiological evidence of consolidation of the lungs (1). Pneumonia develops when the defensive mechanisms of the lung are weakened and overwhelmed by a pathogen that has been either inhaled or aspirated (2). It can be caused by bacteria, viruses, or even fungi, but the most common type is bacterial pneumonia. Pneumonia is a common and potentially serious respiratory disease, mainly in children and the elderly (3).

Pneumonia is usually classified as community-acquired pneumonia, hospital-acquired pneumonia, or those occurring in the immunocompromised host or patient with underlying damaged lung, including suppurative and aspiration pneumonia (4). Community-acquired pneumonia (CAP) is an infection of the lung parenchyma that is not acquired in a hospital, long-term care facility, or other recent contact with the health care system (5). It is a frequently encountered lower respiratory tract infection that is reported as a major health problem, leading to significant morbidity and mortality worldwide (6). Constant exposure to contaminated air and frequent aspiration of nasopharyngeal flora makes the lung parenchyma susceptible to virulent microorganisms. Most microorganisms reach the lower respiratory tract by inhalation of contaminated microdroplets (7).

The diagnosis of CAP is a challenge to the evaluating physicians because the condition closely mimics the common cold or flu. Its diagnosis has relied on medical history and physical examination. However, the clinical characteristics of CAP cannot be reliably used to establish etiological diagnosis with adequate sensitivity and specificity. So, appropriate laboratory diagnosis using culture is very important in the diagnosis of CAP (8). Several risk factors are recognized for the development of CAP, including old age, smoking, alcoholism, immunosuppressive conditions, chronic obstructive pulmonary disease (COPD), asthma, cardiovascular disease, chronic liver or renal disease, diabetes mellitus, and recent viral upper respiratory infections (URTI) (9, 10). The bacterial etiology of CAP and drug-resistant patterns vary not only worldwide, but also within continents, regions, and smaller geographical areas (11). The reports also showed that *Streptococcus pneumoniae* is the most commonly isolated pathogen, identified in 20%-60% of cases, followed by *Haemophilus influenzae*, *Staphylococcus aureus*, *Chlamydia pneumoniae*,

Legionella species, and *Mycoplasma pneumonia*e (12, 13). Antibiotic treatment of CAP is usually empirical as the causative pathogen is rarely identified. Appropriate selection of empirical treatment depends on the common pathogens identified in previous etiological studies (14).
Community-acquired pneumonia is an issue of public health concern, being a leading cause of morbidity that often requires hospitalization, and a significant cause of mortality, especially in severe cases presenting with sepsis or requiring assisted ventilation (15). In CAP, immediate initiation of effective antibiotic therapy is crucial for a favorable outcome, and empirical choice of initial antibiotic therapy is the mainstay of treatment. However, the rapid emergence and widespread dissemination of microbial resistance have rendered most available antimicrobial agents ineffective. Evaluation of clinical response to initial therapy, ideally performed after 72 hours of starting treatment, is critical, as failure of such response carries a high risk of death (16).

According to the global burden of diseases, injuries, and risk factors, lower respiratory infections cause more than 2.3 million deaths globally, with a majority occurring in sub-Saharan Africa, including Ethiopia. Pneumonia can manifest in various ways, ranging from mild cases characterized by fever and cough to severe cases that result in sepsis and respiratory failure. Community-acquired pneumonia is a major contributor to global mortality and morbidity. Understanding the prevalence and common bacterial causes of CAP is crucial for clinicians to diagnose and improve patient outcomes and satisfaction accurately. (17). Community-acquired pneumonia is an acute illness that results from an infection of the lung parenchyma outside of a hospital setting. Understanding the prevalence of the bacterial etiology of CAP is very important for researchers, policymakers, and others to allocate resources for the proper management and prevention of this disease.

1.2. Statement of the problem

Globally, pneumonia is the third most common cause of death reported. It is a treatable and preventable disease described since ancient times, and continues to be a major cause of mortality all over the world (18). According to the 2021 Global Burden of Disease report, 2.1 million people died of pneumonia in that year (19). It was also reported that pneumonia occurs five times more frequently in developing than in the developed world, and is found to be the most common cause of hospital attendance in adults living in developing countries (20). In the elderly population, CAP remains the single most common cause of death, and the annual incidence was reported four times higher compared to that of younger populations.

In addition, older adults have higher rates of hospitalization and are more likely to die as a result of CAP (21). In adults, the incidence of CAP varies among countries, ranging from 1.6 to 11 per 1,000 adults, and 40%–60% of patients require hospitalization. High mortality of hospitalized CAP in adults >18 was observed in-hospital (18.5%), at 30 days (22.9%), and at one year (44.5%) after CAP onset. Mortality was more than double in older adults in comparison to younger patients (22).

In Africa, the mortality rate due to CAP among adult patients was reported to average between 6 to 15% (23). Community-acquired pneumonia is the fifth-largest killer in South Africa, accounting for 3.9% of all deaths in 2000 and a mortality of 20% for patients hospitalized with CAP (24).The frequency of bacterial CAP in Ethiopia varies between 38.7% and 45% (25, 26). An institution-based cross-sectional study was conducted among adult patients in Gondar, involving 312 cases. Of these, 39.4% were found to have culture-confirmed pneumonia. The high prevalence of bacteria-caused CAP and low susceptibility to ampicillin, tetracyclines, and amoxicillin-clavulanic acid indicate a significant burden of CAP in the community. Respiratory bacterial pathogens have a significant impact on public health, affecting both healthy individuals and immunocompromised hosts, as well as causing post-viral infections in community and hospital settings. In Ethiopia, CAP is treated empirically, and the diagnosis is not supported by confirmation of specific etiologic agents. This is due to the lack of culture and AST facilities in most health care facilities, as well as the time and cost required for laboratory procedures (1).

The antibiotic resistance of frequently isolated bacterial pathogens from CAP patients has complicated the selection of empirical therapy for CAP, including methicillin-resistant

Staphylococcus *aureus* (MRSA) and *Pseudomonas aeruginosa*. Research has been published attempting to identify Drug-Resistant Pathogen (DRP) in CAP through the implementation of probabilistic approaches in clinical practice. However, recent epidemiological data have shown that the incidence of DRP in CAP vary significantly according to local ecology, healthcare systems, and the countries where the studies were conducted (26). On average, older adults take 19 days to recover from severe community-acquired pneumonia. Recovery times are notably longer for individuals aged 75 years or older, those with comorbidities, and those with elevated white blood cell and creatinine levels. This condition results in various societal costs, including increased drug costs, additional health-service costs such as laboratory tests and hospitalizations, and greater drug resistance-related morbidity and mortality (27).

As stated previously, the bacterial etiology of CAP and antimicrobial susceptibility patterns vary among different geographical regions. Therefore, identifying the most common bacterial pathogen for CAP and its corresponding drug susceptibility profile is necessary to reduce complications and mortality associated with CAP. In Ethiopia, there are limited studies on the etiology of CAP and antimicrobial susceptibility patterns. However, similar studies were not conducted in this study area. As a result, this study was conducted in this study area to provide information regarding bacterial etiologies and drug susceptibility patterns among CAP presumptive adult patients at Debre Markos Comprehensive Specialized Hospital (DMCSH), Northwest Ethiopia.

1.3. Significance of the study

This study can provide information to physicians for empirical treatment in countries where routine antimicrobial susceptibility testing is not performed. As a result, a locally determined common etiology of CAP and antimicrobial susceptibility profile must be identified to indicate local empiric treatment.

Additionally, the study provides benefits to patients with CAP during the study period. It also has paramount importance in selecting the appropriate therapy against the right organism, which further reduces the rates of morbidity and mortality. It also will be used us an information for researchers, health sector administrators, and relevant bodies in planning and developing treatment guidelines.

2. LITERATURE REVIEW

2.1. Bacterial pathogens associated with community-acquired pneumonia

A study in a nationwide surveillance network system, upper and lower respiratory samples were assayed using a culture or polymerase chain reaction (PCR) from August 2018 to May 2020 in which two hundred sixty-four cases (25.8%) were isolated by culture, revealed that 131 cases were *S. aureus* (12.8%), 92 cases were *S. pneumoniae* (9%), and 20 were *H. infuenzae* (2%). The cultured, isolated bacteria may be a colonized pathogen. The proportion of codetection was 49.2% (13).

In a hospital-based prospective observational study, a total of 87 hospitalized patients diagnosed with CAP were enrolled consecutively from the medicine ward of Chittagong Medical College Hospital (CMCH). Sputum culture, blood culture, and PCR were positive in 60.9%, 1.1%, and 4.6% of the samples respectively. *K. pneumoniae* was identified in the sputum culture of the majority of the patients (39.1%), followed by *P. aeruginosa* (10.3%), *S. aureu*s and *Escherichia coli* (5.7%). The only sample that was positive in blood culture was *S. aureus*. *S. pneumoniae* was identified in all 4 PCR-positive cases. Gram-negative bacteria pre-dominate in the bacteriologic prole of CAP using conventional sputum and blood culture (28).

A study conducted at Beijing Shijitan Hospital from 2016 to 2017 found that out of 5,563 outpatients with fever, 391 were recruited for sputum cultures, and 391 had CAP. A total of 117 isolates of pathogenic bacteria were obtained from 176 cases of CAP. The main pathogenic bacteria were *K. pneumoniae* (27.4%), *E. coli* (17.9%), *S. aureus* (12.0%), *P. aeruginosa* (10.3%), and *S. pneumoniae* (9.4%) (29).

A prospective study was conducted in Kenya, with radiographically confirmed community-acquired pneumonia from May 2019 to March 2020. A potential etiologic agent(s) was identified in 57% including 43% viral, 5% combined viral and bacterial, 5% bacterial, and 4% Pneumocystis. The most common etiologic agent was Influenza A, which was associated with severe clinical disease. The most common underlying conditions were cardiovascular disease, diabetes, and lung disease, while HIV infection was identified in only 13% of patients. Critical care admission was required for 24%, and 31% had acute kidney injury, sometimes in combination with acute respiratory distress or sepsis (30).

A cross-sectional study was conducted in Bahir Dar among 414 participants with presumptive CAP diagnoses; bacterial pathogens were identified in 167 (40.3%) participants. Among these, multidrug resistance (MDR) accounted for 127(76%) of the isolates. The predominant isolates were *S. pneumoniae* at 60(35.9%) and *K. pneumoniae* at 30(18%) (25).

An institution-based cross-sectional study was conducted from April to June 2021 in Gondar, Northwest Ethiopia, involving 312 cases. Of these, 39.4% (n = 123; 95% CI: 34.1%–44.9%) were found to have culture-confirmed pneumonia. The most common isolates were *K. pneumoniae* (31.0%, n = 39), S. pneumoniae (26.2%, n = 33), and S. aureus (20.6%, n = 26) (1).

Astudy conducted in Arba Minch showed that out of 170 adult patients clinically diagnosed to have CAP, 73 (42.9%) were culture positive and the isolated bacteria were *S. pneumoniae* 20 (11.8%), *S. aureus* 18 (10.6%), *P. aeruginosa* 12(7.1%), *K. pneumoniae* 11 (6.5%), *E. coli* 5 (2.9%), *Proteus mirabilis* 2 (1.2%), *Proteus vulgaris* 1 (0.6%), and *H. influenzae* 4 (2.4%) (31). The other study conducted in Jimma found that out of 133 adult patients, 60 (45%) of the samples were positive for bacterial growth. *S. pneumoniae* 17 (12.8%), *S. aureus* 14 (10.5%), and *P. aeruginosa* 9 (6.8%) were the three common bacteria isolated in order of frequency (32).

Astudy conducted in Adama Hospital Medical College Of the 369 patients with CAP, bacterial pathogens were identified in 31.7% of cases. The most common isolates were *M. catarrhalis* (n = 15; 12.8%), *S. aureus* (n = 15; 12.8%), *K. pneumoniae* (n = 12; 10.3%), *E. coli* (n = 11; 9.4%), *P. aeruginosa* (n = 11; 9.4%), *Enterobacter* species (n = 11; 9.4%), and *Citrobacter* species (n = 11; 9.4%) (33).

2.2. Antimicrobial susceptibility pattern

At Chittagong Medical College Hospital, *K. pneumoniae* was identified in the sputum cultures of the majority of patients (39.1%), followed by P. aeruginosa (10.3%), Staphylococcus aureus, and E. *coli* (5.7%). The highly sensitive drugs were meropenem, levofloxacin, and amikacin. The mean (±SD) duration of hospital stay was 6.34±2.37. In-hospital mortality and 30-day mortality were 6.9% and 16.1% respectively (28).

A study conducted at Beijing Shijitan Hospital from 2016 to 2017 found that out of 5,563 outpatients with fever, 391 were recruited for sputum cultures, and 391 had CAP. A total of 117

isolates of pathogenic bacteria were obtained from 176 cases of CAP. The main pathogenic bacteria were *K. pneumoniae* (27.4%), *E.* coli (17.9%), *S. aureus* (12.0%), *P. aeruginosa* (10.3%), and *S. pneumoniae* (9.4%). A drug sensitivity test (DST) showed that *K. pneumoniae, E. coli*, and *P. aeruginosa* had good sensitivity to imipenem, cefoperazone/sulbactam, piperacillin/tazobactam, and amikacin. *S. aureus and S. pneumoniae* showed strong sensitivity to vancomycin, linezolid, and levofloxacin. Previous multiple antibiotic treatment was an independent risk factor for MDR-CAP (29).

In Dhaka, Bangladesh, of the 20 isolated *S. pneumoniae*, 19 (95%) were sensitive to amoxyclav, followed by 17 (85%) to ampicillin, 16 (80%) to ceftriaxone, 14 (70%) to levofloxacin, and 12 (60%) to doxycycline. The resistance rates to azithromycin and cefixime were 13 (65%) and 10 (50%), respectively. All (100%) the *S. aureus* were sensitive to amoxyclav, ampicillin, and doxycycline, and one (33.33%) was resistant to levofloxacin, azithromycin, ceftriaxone, and cefixime. All (100%) the Gram-negative bacteria were sensitive to meropenem, with variable sensitivity to different antimicrobial agents. Ceftriaxone was an effective drug against *K. pneumoniae* (92.85%) and *E. coli* (100%), but was not effective against *Acinetobacter baumannii.* Amikacin was found to be moderately effective against *P. aeruginosa* (83.34%) (34).

Study conducted by Wang B, et al, Of the 79 *S. pneumoniae* isolates in China, 55 (69.6%) were susceptible to penicillin, 7 (8.9%) were intermediate, and 6 (7.6%) were resistant to penicillin. The resistance rate to erythromycin was as high as 63.2% (43/69). In this study, no *S. pneumoniae* isolate was found to be resistant to amoxicillin or moxifloxacin. *H. influenzae* had a high susceptibility to antimicrobials; all isolates were susceptible to amoxicillin/clavulanic acid, ciprofloxacin, and ertapenem. Only three isolates were resistant to amoxicillin, and four isolates were resistant to clindamycin. *K. pneumoniae* also showed a high susceptibility to antimicrobial agents. Resistance rates to cefaclor and ceftriaxone were 15.4% and 7.7%, respectively. All isolates were susceptible to ertapenem (35).

A cross-sectional study was conducted in Adama Hospital Medical College from November 10, 2022, to November 30, 2023. Demographic, clinical data, and sputum samples were collected from patients with CAP (n = 369). Of the 369 patients with CAP, bacterial pathogens were identified. Among the identified isolates, resistance rates were high in Enterobacteriaceae, followed by Gram-

positive bacteria, and non-Enterobacteriaceae. Overall, 68 (58.1%) of the identified bacterial isolates were multidrug-resistant (MDR), with *K. pneumoniae* accounting for the highest proportion of multidrug-resistant isolates (91.7%), while *P. aeruginosa* accounted for the lowest proportion (9.1%) of MDR isolates. Those studies revealed a high prevalence (31.7%) of bacterial pathogens in CAP patients and a higher prevalence (58.1%) of MDR bacterial pathogens. Therefore, regular surveillance and monitoring systems are warranted for assessing predominant pathogens and antibiotic resistance patterns (33).

A cross-sectional study was conducted in Bahir Dar among 414 presumptively diagnosed study participants with CAP; bacterial pathogens were identified in 167 (40.3%) participants. Among these, multidrug resistance (MDR) accounted for 127(76%) of the isolates. The predominant isolates were *S. pneumoniae* (60, 35.9%) and *K. pneumoniae* (30, 18%). Overcrowded living conditions and alcohol use were found statistically associated with culture-positive sputum (25).

An institution-based cross-sectional study was conducted in Gondar, Northwest Ethiopia, involving 312 cases. The Gram-positive bacteria were susceptible to chloramphenicol (100%) and clindamycin (96.6%). Gram-negative bacteria were susceptible to gentamicin (87.5%), azithromycin (87.1%), ciprofloxacin (86.6%), and ceftriaxone (79.0%) but highly resistant to ampicillin (100%), followed by tetracycline (87.1%), doxycycline (86.4%), co-trimoxazole (80.6%), and amoxicillin-clavulanic acid (79.0%). Overall, 72.2% of the isolates were multidrug resistant to *K. pneumoniae* (94.9%, n = 37), E. coli (93.8%, n = 15), and *S. pneumoniae* (72.7%, n = 24). Only 7.7% of *S. aureus* isolates showed inducible clindamycin resistance (1).

In the study conducted at Arba Minch Hospital, *S. pneumoniae* isolates showed relatively high resistance (60%) to Oxacillin (a representative of the penicillin group), and all isolates were sensitive to trimethoprim-sulfamethoxazole. A high resistance rate of *S. aureus* was observed to Tetracycline (100%), Oxacillin (83.3%), Ampicillin (83.3%), Penicillin (83.3%), Trimethoprim-sulfamethoxazole (83.3%), Erythromycin (50%), and Doxycycline (50%). *P. aeruginosa* isolates showed that 50% resistant to Gentamycin. The antimicrobial testing of *K. pneumonia* and *H. influenza* isolates indicated that all isolates showed resistance (100%) to Tetracycline, Ampicillin, and Trimethoprim-sulfamethoxazole. *Proteus* and *E. coli* isolates showed resistance to

Tetracycline, Chloramphenicol, Doxycycline, Gentamycin, Ampicillin, and Trimethoprim-sulfamethoxazole. Multidrug resistance was also observed to several antimicrobial agents (31).

The study conducted in Jimma University Specialized Hospital, Jimma, stated that among 20 *S. pneumoniae* isolates, the overall resistance rate was high to oxacillin 11 (55%), and low resistance was observed for tetracycline 7 (35%), erythromycin 1 (5%), and chloramphenicol 1 (5%); while none of them were resistant to trimethoprim-sulfamethoxazole. Of 16 *S. aureus* isolates subjected to antimicrobial susceptibility testing, overall resistance rates were high to tetracycline 16(100%), oxacillin 13(81.3%), ampicillin 13(81.3%), penicillin 13(81.3%), trimethoprim-sulfamethoxazole 13(81.3%), erythromycin 12(75%), and doxycycline 8(50%). Low resistance was observed for ceftriaxone 5(31.3%), ciprofloxacin 5(31.3%), chloramphenicol 5(31.3%), and gentamycin 5 (31.5%). *P. aeruginosa* isolates showed relatively high resistance to gentamycin 5 (50%). Low resistance was observed for ceftriaxone 2 (20%) and ciprofloxacin 2 (20%). The antimicrobial testing of *K. pneumoniae, P. mirabilis, P. vulgaris*, *E. coli*, and *H. influenza* isolates showed that all (100%) isolates had high resistance to tetracycline, ampicillin, and trimethoprim-sulfamethoxazole. However, low or no resistance was observed to ciprofloxacin and ceftriaxone. This result showed that all isolates were resistant to two or more drugs except *S. pneumonia* and *H. influenza,* which showed that only 15% and 66.7% of the isolates were resistant to two or more drugs, respectively (32)

2.3. Factors associated with community-acquired pneumonia

Over the past two decades, different risk factors for community-acquired pneumonia have been recognized. Clinical conditions associated with an increased risk of community-acquired pneumonia include history of pneumonia (odds ratio [OR] <6·25); chronic cardiovascular diseases (OR <3·20); cerebrovascular disease, stroke, and dementia (OR <2·68); neurological or psychiatric conditions (OR <3·20); chronic respiratory diseases, including chronic obstructive pulmonary disorder (COPD) and Bronchitis (36).

In the study conducted in Europe, there was consistent evidence that smoking was associated with an increased risk of CAP as compared with non-smokers. The risk was also increased in individuals with higher consumption of alcohol. Higher levels of education were associated with a lower risk of CAP compared with individuals with a low level of education (37). A study conducted in Spain

found that the highest burden of CAP was associated with metabolic disease, cardiovascular disease, and diabetes. Lifestyle factors such as smoking, high alcohol intake, or being underweight have been associated with an increased risk of CAP (38).

A study conducted in Kenya found that a history of pneumonia was a significant risk factor for developing pneumonia. It was inversely associated with the number of years in education. Passive smoking and alcohol intake were not associated with pneumonia (39).

A cross-sectional study was conducted among 414 participants with presumptive CAP diagnoses; bacterial pathogens were identified in 167 (40.3%) participants. Overcrowded living conditions and alcohol use were found statistically associated with culture-positive sputum (25).

The other institution-based cross-sectional study was conducted from April to June 2021 in Gondar, Northwest Ethiopia, involving 312 cases. Aging (AOR: 3.248, 95% CI: 1.001– 10.545, p = 0.050), a history of pneumonia (AOR: 7.004, 95% CI: 3.591–13.658, p = 0.001), alcohol use (AOR: 6.614, 95% CI: 3.399–12.872, p < 0.001), and overcrowded living conditions (AOR: 4.348, 95% CI: 1.964–9.624, p = 0.001) were significantly associated with culture-positive sputum (1).

2.4. Conceptual framework

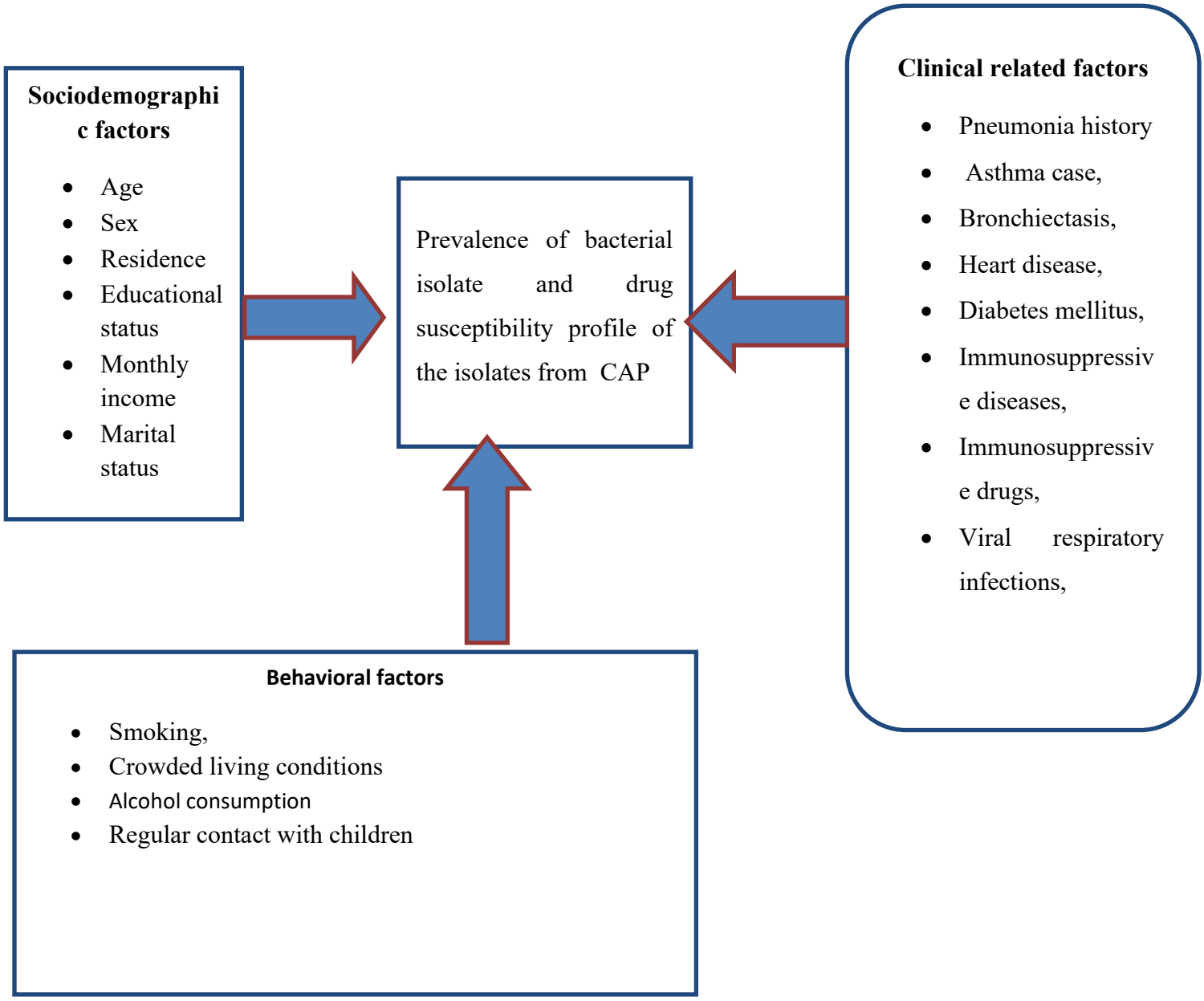


Figure 1. Conceptual framework

3. OBJECTIVES

3.1. General objective

To assess bacterial profile, antimicrobial susceptibility patterns, and associated factors of community-acquired pneumonia among pneumonia-suspected adult patients at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia

3.2. Specific objectives

- To determine bacterial profiles of CAP among adult pneumonia suspected patients at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia
- To determine the antimicrobial susceptibility pattern of common bacterial pathogens of CAP among pneumonia-suspected adult patients at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia
- To identify factors associated with CAP among pneumonia-suspected adult patients at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia

4. MATERIALS AND METHODS

4.1. Study area and setting

This study was conducted at Debre Markos Comprehensive Specialized Hospital, the only Comprehensive Specialized Hospital in the East Gojjam zone, located in Debre Markos. Debre Markos is a regio-politant town and the capital city of East Gojjam Zone. According to information obtained from the hospital's administrative office, it serves a population of over 3.5 million within its catchment area. The hospital provides services for adults aged 15 years and older in separate wards. In this hospital, adults with severe community-acquired pneumonia are admitted to the emergency ward, and further diagnosis and treatment are provided by internists, general practitioners, and nurses.

4.2. Study design and period

A hospital-based cross-sectional study was conducted from March 01, 2025, to May 30, 2025

4.3. Population

4.3.1. Source population

The source population consisted of all community-acquired pneumonia presumptive adult patients attending DMCSH.

4.3.2. Study population

The study population consisted of all adult patients suspected of having community-acquired pneumonia who attended DMCSH during the study period.

4.4. Sample size determination

The sample size was determined using a single population proportion formula as follows: $N = z^2 p(1-p)/ d^2$; where: N = The number of study subjects from which samples were taken; Z = Standard normal distribution value at 95% CI, which is 1.96; P= the prevalence of bacterial community-acquired pneumonia which is taken as 40.3% in previous studies from Bahir Dar city (25).

W= the margin of error taken as 5%.

Accordingly, the sample size was:

$$n = \frac{Z^2 pq}{W^2}$$

Where: n = Sample size, p = proportion 40.3%, q = Proportion of failure (1-p)

z = critical value 1.96, W = precision (marginal error) = 0.05 Thus the sample size is

$$n = \frac{(1.96)^2 \times 0.403 \times 0.597}{(0.05)^2} = \frac{0.9604}{0.0025} = 370$$

4.5. Sampling technique

A convenient consecutive sampling technique was employed to recruit 370 study participants who met the inclusion criteria.

4.6. Eligibility criteria

4.6.1. Inclusion criteria

All individuals aged 15 years or older who were clinically diagnosed with CAP and presented with typical symptoms of the disease, such as cough lasting less than 15 days, fever, and/or chest pain consistent with pneumonia, were considered for participation in the study and were included.

4.6.2. Exclusion criteria

Patients who were under antibiotic treatment for last 2 weeks and had a history of hospital admission for 14 days, were excluded from the study.

4.7. Study variables

4.7.1. Dependent variable

Prevalence of bacterial isolates

4.7.2. Independent variables

Age, sex, educational background, occupation, residence, smoking, alcoholism, crowded living conditions, previous pneumonia history, and presence of co-morbidities (asthma case, bronchiectasis, heart disease, diabetes mellitus, immunosuppressive diseases, and stroke), immunosuppressive drugs, viral respiratory infections, and regular contact with children.

4.8. Data collection and laboratory methods

4.8.1. Sociodemographic data collection

Socio-demographic and other related data were collected by trained nurses via face-to-face interviews using a structured questionnaire. The questionnaire was prepared in English and then

translated into Amharic, which was subsequently translated back into English to verify consistency

4.8.2. Sample collection and transportation

Sputum samples were collected from CAP presumptive adult patients at DMCSH. Deeply coughed-up sputum (5-10ml) was collected in a disposable, leakproof, sterile, and wide-mouthed container with a tight-fitting lid after giving proper instructions to the patient. After collection, the samples were transported to the DMCSH microbiology laboratory as soon as possible for further bacteriological processing. Sputum specimens were first inspected both macroscopically and microscopically after Gram staining, before culture. The specimens were accepted for further culturing if they contained at least 25 polymorph nuclear leukocytes (PNL) and less than 10 epithelial cells per low-power field (LPF) (41).

4.8.3. Isolation and identification of bacteria

The sputum specimen was inoculated on MacConkey (MAC), blood agar plate (BAP), and chocolate agar plate (CAP) using a sterile swab. Streak the inoculum in 4 quadrants on the plates to obtain single colonies. These inoculated MAC plates were incubated in an aerobic atmosphere at 35-37 °C for 18-24 hrs, and CAP and BAP were incubated at 35-37 °C for 18-24 hrs using a 5% CO_2 generating candle jar. All positive cultures were then characterized by a distinctive colony appearance on the respective media. Each grown bacterial colony was tested by Gram stain and confirmed by the pattern of biochemical reactions using standard methods. Biochemical tests like urease, indole, citrate, lysine decarboxylase (LDC), triple sugar iron agar (TSI), oxidase, Simon's citrate agar, Mannitol test, and Motility test were used to differentiate gram-negative bacteria. Catalase, coagulase, optochin, and bile solubility test results were used to distinguish between Gram-positive and Gram-negative bacteria. In addition, the X and V factors were used to enhance the growth of *H. influenzae* (42).

4.8.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on each bacterial isolate using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA). For *S. pneumoniae*, 5% sheep blood was added to MHA was used (43). Briefly, 3-5 pure colonies of each bacterium were picked and transferred to a tube containing 5 mL of sterile nutrient broth (Oxoid), and the suspension was

mixed thoroughly to ensure homogeneity. If the prepared suspension is less turbid, the suspension was adjusted to 0.5 McFarland turbidity standard by further addition of colony until the turbidity of the suspension is adjusted to a 0.5 McFarland turbidity standard (bacterial concentration of 1.5 × 10^8 colony forming unit/ml) or if the prepared suspension is over turbid, the suspension was adjusted by adding a sterile nutrient broth aseptically to standardize the size of the inoculums. A sterile swab was dipped in the suspension, and the entire surface of the MHA plate was uniformly flooded with the suspensions. The plate was then allowed to dry for approximately 30 minutes. The antimicrobial-impregnated disks were placed in the media using sterile forceps, ensuring that each disk was at least 24 mm away from the others to prevent overlapping zones of inhibition. After the disk is placed on the inoculated media, the plate is allowed to stand for 30 minutes, allowing the antibiotic to diffuse into the media. The plates were incubated at 35-37 °C for 24 hrs and observed for the zone of inhibition (44).

Grades of the susceptibility pattern were recognized as sensitive, intermediate, and resistant by comparing the zone of inhibition with current standards outlined in the CLSI guidelines. The following antibiotics discs selected based on the CLSI 2024 guidelinesline including Ampicilln (AMP, 10 µg) ftriaxone (CRO, 30µg), ceftazidime (CAZ, 30µg), and cefoxitin (FOX, 30µg)), tetracycline (TE, 30µg) and Meropenem (MEM, 30 µg), macrolides (azithromycin (AZM,15 µg) and erythromycin (EYR,15µg)), trimethoprim-sulfamethoxazole (SXT,1.25 + 23.75µg), gentamicin (GEN, 10µg)), clindamycin (CLD, 2 µg) and levofloxacillin (LEV, 30 µg) for Gram negative and (penicillin (P, 10µg), oxacillin (OX, 1µg), and piperacillin (TZP, 100µg)), tetracycline (TE, 30µg)), clindamycin (CLD, 2 µg) for Gram positive (45).

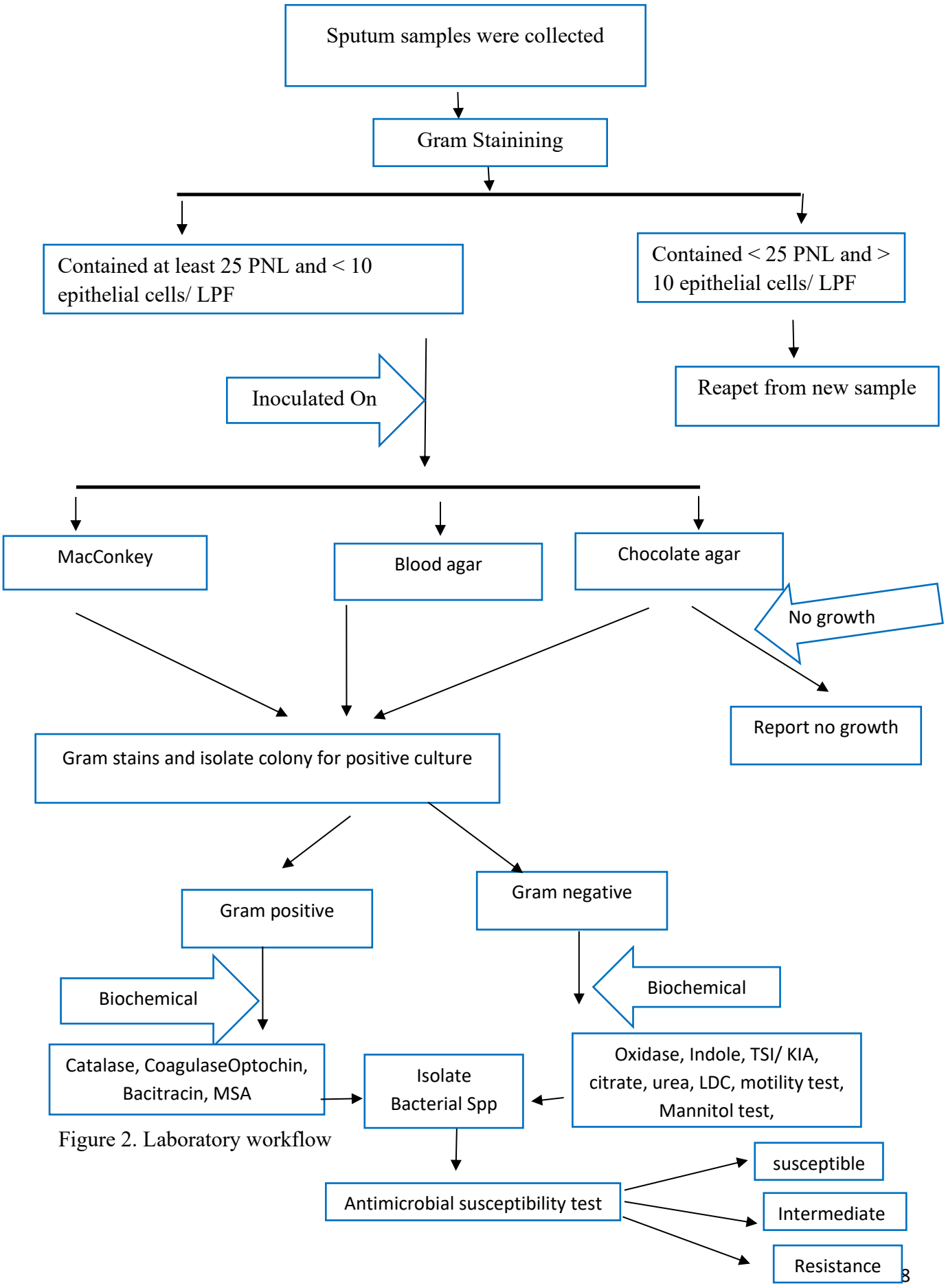


Figure 2. Laboratory workflow

4.9. Data quality assurance

The reliability of the study findings was guaranteed by implementing quality control (QC) measures throughout the entire laboratory work process. All materials, equipment, and procedures were adequately controlled. The pre-analytical, analytical, and post-analytical stages of quality assurance, as well as Standard Operating Procedures (SOPs), were strictly followed. A pre-tested questionnaire-guided interviews were used to collect data on socio-demographic characteristics. The sterility of culture media was checked by incubating 5% of the batch at 35-37 °C °C overnight and evaluating for possible contamination. The performance of all prepared media was checked by inoculating international standard strains such as *E. coli* (ATCC 25,922), *S. aureus* (ATCC 25923), *H. influenzae* (ATCC 49,247), and *S. pneumoniae* (ATCC 49,619), which were used to control the quality of the media. Moreover, senior laboratory professionals cross-checked the entire procedure and result interpretation, and the potency of the test antibiotics was monitored using control strains.

4.10. Data processing and analysis

Data were entered into Epi-Data version 3.1 and exported to SPSS version 25 software. The statistical significance of the association was measured by univariate and multivariate logistic regression analysis, odds ratio at 95% confidence interval, and a P value < 0.05 was considered statistically significant.

4.11. Ethical consideration

Ethical clearance was obtained from the Institutional Health Research and Ethics Review Committee (IRERC) of the College of Medicine and Health Sciences, Debre Markos University, and the Amhara Public Health Institute (APHI). An official letter of cooperation was provided to Debre Markos Comprehensive Specialized Hospital before data collection. A letter of permission was obtained from DMCSH. Written Informed consent and assent were obtained from study participants after explaining the purpose and objective of the study. Any patient who was unwilling to participate in the study was not required to participate. They were informed that all data and samples obtained from them were kept confidential by using codes instead of personal identifiers and were intended solely for the purpose of the study. The laboratory results from the study participant were communicated to their physicians for appropriate treatment management.

5. RESULT

5.1. Socio-demographic characteristics of participants

A total of 370 study participants were enrolled in the present study, with the majority being males 214(57.8%) and females 156 (42.2%), resulting in a sex ratio of 1:0.73. The ages of the study subjects ranged from 15 years to 82 years, with a mean age of 39.44 (± 14.007 standard deviation (SD)) years. Most of the study participants were in the 25-34 age group, comprising 110(29.7%). The majority of the study participants were urban dwellers, comprising 237 (64.1%), while 116 (31.4%) were college graduates or above. The majority of the study participants were farmers 112 (30.3%), followed by government employees 69 (18.6%), and merchants 66 (17.8%). Most of the study participants reported being married 227 (61.4%) and Orthodox 324 (87.6%), respectively (Table 1).

Table 1. Socio demographic characteristics of study participants

Characteristics		Frequency (%)
Sex	Male	214 (57.8)
	Female	156(42.2)
	15-24	46(12.4)
	25-34	110(29.7)
Age	35-40	62(16.8)
	41-50	80(21.6)
	51-60	41(11.1)
	>60	31(8.4)
	Single	97 (26.4)
Marriage	Married	227 (61.2)
	Divorced	17 (4.6)
	Widowed	29 (7.8)
Residence	Urban	237 (64.1)
	Rural	133 (35.9)
	Unable to read and write	93 (25.1)
Education	Primary school	69 (18.6)

	Secondary school	91 (24.6)
	Collage and above	117(31.7)
	Government employed	69 (18.6)
	Student	36 (9.7)
Occupation	Farmer	112 (30.3)
	Housewife	44 (11.9)
	Merchant	66 (17.8)
	Other	43 (11.6)

5.2. Clinical and behavioral characteristics of participants

Besides, nearly one-third 128(34.6%) of the study participants were alcohol users; 27 (7.3%) had a previous history of pneumonia; and 112 (30.3%) study participants shared utensils. Alternatively, of all study participants, only 47 (12.7%) were asthmatic cases, and 30 (8.1%) were malnourished (Table 2).

Table 2. Clinical and other relevant data of study participants

Characteristics		Frequency (%)
Alcohol consumption	Yes	128 (34.6)
	No	242 (65.4)
Cigarette smoking	Yes	17 (4.6)
	No	353 (95.4)
Contact with pneumonia patient	Yes	56 (15.1)
	No	314(84.9)
Utensils sharing	Yes	112 (30.3)
	No	258 (69.7)
Asthema	Yes	47 (12.7)
	No	323 (87.3)
Pre-morbid illness	Yes	91 (24.5)
	No	279 (75.4)
Previous pneumonia history	Yes	27 (7.3)
	No	343 (92.7)

Nutritional status	Mulnutrited	30 (8.1)
	Normal	340 (91.9)
Immunosuppressive diseases	Yes	6 (1.6)
	No	364 (98.4)

5.3. The distribution of bacterial isolates among adult CAP patients

In this study, the overall culture-positive sputum for bacterial isolates from clinically diagnosed CAP patients was 33.2% (123/370; 95% CI: 28.4%–37.8%). Both gram-positive and gram-negative bacterial isolates were recovered with a 53.7% (n = 123) and a 46.3% (n = 123) prevalence, respectively. In this study, *S. pneumoniae* (35%, n = 43) was the most frequently isolated bacterium, followed by *K. pneumoniae* (22.8%, n = 28), *S. aureus* (18.7%, n = 23), and *E. coli* (8.9%, n = 11) (Fig. 1).

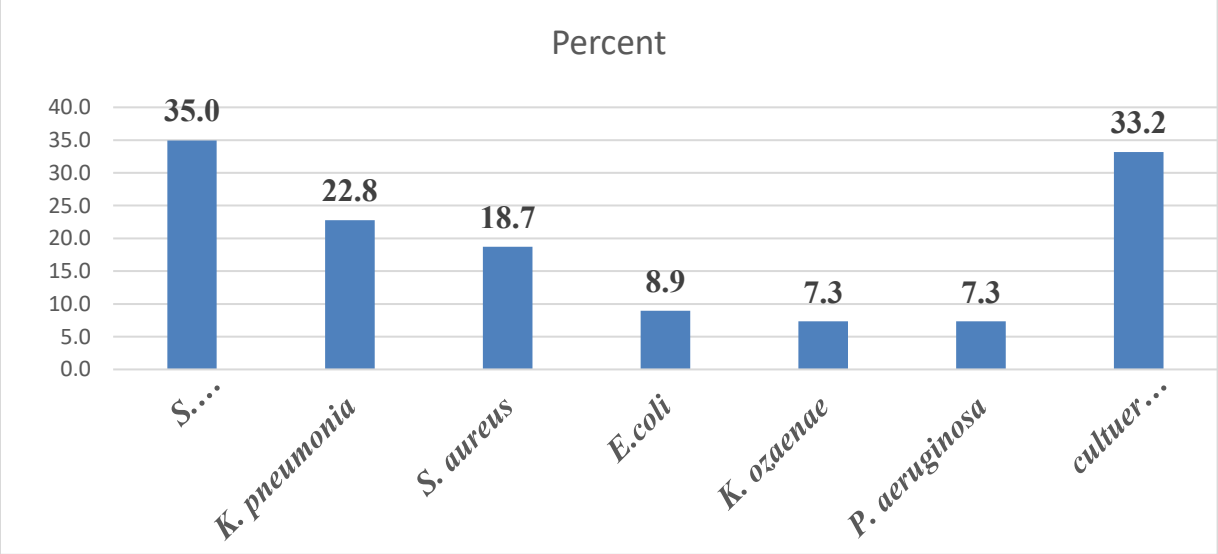


Figure 3. The distribution of bacterial isolates from sputum specimens of cap adult patients.

5.4. Antimicrobial susceptibility patterns of the bacterial isolates

In this study, Gram-positive isolates were found to be sensitive to Erytheromycin (86.4%) and clindamycin (66.7%). *S. pneumoniae* isolates were sensitive to Erytheromycin (95.3%) but resistant to Trimethoprim-sulfamethoxazole (93%), tetracycline (53.5%), and azithromycin

(46.5%). Antimicrobial susceptibility of *S. aureus* isolates to levofloxacin, clindamycin, erythromycin, and cefoxitin was 95.7%, 69.6%, 60.9%, and 56.5%, respectively. *S. aureus* isolates were penicillin-resistant (80.8%), and 43.5% were methicillin-resistant *S. aureus* (MRSA) (Table 3).

The Gram-negative bacterial isolates showed a high level of susceptibility to some of the tested antimicrobial agents. For instance, 100% of them were sensitive to Meropenem, followed by Amikacin (93%), Ceftazidime (84.2%), and Cefepime (66.7%). However, they showed a high level of resistance to trimethoprim-sulfamethoxazole (78.3%), levofloxacin (77.2%), and tetracycline (71.4%). Most of the isolated *K. pneumoniae* showed higher resistance to levofloxacin, trimethoprim-sulfamethoxazole, and tetracycline, with resistance rates of 82.1%, 82.1%, and 71.4%, respectively. But *K. pneumoniae* isolates were susceptible to meropenem (100%), followed by amikacin (92.9%), Ceftazidime (85.7%), Cefepime (71.4%), and ceftriaxone (64.3%). *E. coli* isolates also showed sensitivity 100 % to meropenem, 90.9% to amikacin, and 81.8% to Ceftazidime. A high level of resistance to Cefazolin (80.9%) and ciprofloxacin (77.2%) was detected in *E. coli* isolates. *P. aeruginosa* was sensitive to Meropenem (100 %) and Ceftazidime (77.8%), but resistant to Levofloxacillin (88.9%) and piperacillin and Cefepime (66.7% each) (Table 4).

Table 3. Antimicrobial susceptibility pattern of Gram positive bacteria isolates among CAP patients

Antibiotics (%)	Bacterial isolates %

	Pattern	*S. pneumoniae* (n = 43)	*S. aureus* (n = 23)	Total (n = 66)
Erytheromycin	S	40 (93.0)	16 (60.9)	56(84.8)
	I	1 (2.3)	0	1 (1.5)
	R	2(4.7)	7 (39.1)	9 (13.6)
Tetracycline	S	18 (41.9)	12 (52.2)	30 (45.5)
	I	2 (4.7)	0	2 (3.0)
	R	23 (53.5)	11(47.8)	34 (51.5)
Cefoxitine	S	N/A	12(52.2)	12 (18.2)
	I	N/A	1(4.3)	1 (1.5)
	R	N/A	10 (43.5)	10 (15.2)
Levofloxacin	S	N/A	22 (95.7)	22 (95.7)
	I	N/A	1 (4.3)	1 (1.5)
	R	N/A	1(4.3)	1(4.3)
Tremthoprim	S	3 (7%)	5 (21.7)	8 (12)
sulphamethoxazole	I	0	0	0
	R	40 (93%)	18 (78.3)	58 (88)

Note. N/A: Not applicable, S: sensitive and R: resistant

Table 4. Antimicrobial susceptibility pattern of Gram-nagative bacterial isolates among CAP patients

Antibiotics	Bacterial isolates

(%)	Pattern	*K. pneumoniae*(n = 28)	*E. coli (*n=11)	*P.aeruginosa (n=9)*	K. azaenae (n=9)	Total (n = 57)
Ceftazidime	S	22 (78.6)	8(72.7)	7 (77.8)	7(77.8)	44 (77.2)
	I	2 (7.1)	1 (9.1)	0	1 (11.1)	4 (7.0)
	R	4 (14.3)	2 (18.2)	2 (22.2)	1 (11.1)	9 (15.8)
Levofloxacillin	S	5(17.9)	5(45.5)	1 (11.1)	2 (22.2)	13 (22.8)
	I	0	0	0	0	0
	R	23 (82.1)	6 (54.5)	8 (88.9)	7 (77.8)	44 (77.2)
Meropenem	S	28 (100)	11(100)	9 (100)	9 (100)	57(100)
	I	0	0	0	0	0
	R	0	0	0	0	0
Cefepime	S	19 (67.9)	8 (72.7)	3 (33.3)	7 (77.8)	38 (66.7)
	I	1 (3.6)	0	0	0	1 (1.8)
	R	8 (28.6)	3 (27.3)	6 (66.7)	2 (22.2)	19 (33.3)
Tremthoprim sulphamethoxazole	S	5 (17.9)	N/A	3 (33.3)	2 (22.2)	10 (21.7)
	I	0	0	0	0	0
	R	22(78.6)	N/A	6 (66.7)	7 (77.8)	36 (78.3)

Note. N/A: Not Applicable, S: Sensitivend R: Resistance

5.5. Multi-drug resistance patterns of bacterial isolates

Overall, 66.7% (n = 82) of bacterial isolates were multidrug resistant (MDR; resistance to at least one antibiotic from three or more classes) . Among the total MDRs (53.6%, n = 15), *K. pneumoniae*, (72.1%, n = 31) was *S. pneumoniae*, (56.5%, n = 13) was *S. aureus*, (81.8%, n = 9) was *E. coli*, (88.9%, n = 8) was *P. aeruginosa*, and (66.7%, n = 6) was *K. azaenae* (Table 5).

Table 5. Multi drug resistance pattern of bacterial isolates among CAP patients

Antimicrobial Clasese	Bacterial Isolates

No of class	Antimicrobial risistance	S. *pneumoniae (n=43)*	K. *pneumoniae (n=28)*	*S. aureus (n=23)*	*E. coli (n=11)*	*P. aeruginosa (n=9)*	K. azaenae (n=9)	Total% (n= 123)
R0	Non	2	0	0	0	0	0	2
R1(For one)	Amp	4	5	7	0	0	0	16
	TZP	0	0	0	0	1	0	1
R2(For Two)	AMP, SXT	4	5	2	0	0	1	12
	AMP, TE	2	2	1	1	0	2	8
	AMC, AZM	0	1	0	1	0	0	2
R3(For three)	AMP, TE, AZM	3	0	0	0	0	0	3
	AMP, TE, SXT	4	1	0	0	0	0	5
	AMP, CAZ, SXT	0	0	0	0	0	1	1
	TZP, CAZ, LEV	0	2	0	0	3	0	5
R4(For three)	AMP, GEN, TE, SXT	0	0	6	0	0	0	6
	TZP, LEV, SXT, CAZ	0	3	0	1	3	0	7
	AMP, CRO, SXT, TE	0	4	0	0	0	0	4
R5(For five)	AMP, CAZ, SXT, CRO, LEV	0	0	0	0	0	1	1
	TZP, CAZ, SXT, FEP, LEV	0	0	2	0	2	1	5
	AMP, CLD, SXT, TE, LEV	5	0	0	3	0	0	8
	AMP, SXT, AZM, CLD, TE	5	0	0	3	0	0	8
R6(For six)	AMP, GEN, SXT, TE, FOX, LEV	0	0	3	0	0	0	3
	AMP, GEN, SXT, LZD, CLD, TE	0	0	2	0	0	0	2
	AMP, SXT, VAN, AZM, CLD, TE	3	0	0	0	0	0	3
	AMP, CRO, CAZ, TE, SXT, LEV	0	0	0	2	0	2	4
	OX, TE, EYR, CLD, SXT, AZM	5	0	0	0	0	0	5
	AMP, CLD, AZM, OX, SXT, TE,	6	0	0	0	0	0	6
R7(For seven)	AMP, TZP, CRO, CAZ, SXT, LEV, TE	0	5	0	0	0	1	6

	Total	43(100)	28(100)	23(100)	11(100)	9(100)	9(100)	123(100)
	MDR/ ≥ 3drug	31(72.09)	15(53.6)	13(56.5)	9(81.8)	8(88.9)	6(66.7)	82(66.7)

Key: R0=Sensitive, R1=Resistant for one antibiotic,R2= Resistant for two antibiotic,R3= Resistant for three antibiotic,R4= Resistant for four antibiotic,R5 = Resistant for five antibiotic.R6= Resistant for six antibiotic,R7= Resistant for seven antibiotic, MDR=Multidrug resistance,AMP= Ampicillin, CRO= Ceftriaxone, CAZ= Ceftazidime,FOX= Cefoxitin,TE= Tetracycline,MEM= Meropenem,AZM= Azithromycin, EYR= Erythromycin, SXT=Trimethoprim-sulfamethoxazole, GEN= Gentamicin,CLD=Clindamycin,LEV= Levofloxacillin,TZP=Piperacillin, VAN=Vancomycin, OX=Oxacillin, LZD=Linazollid

5.6. Factors associated with culture-positive sputum among CAP patients

Different socio-demographic characteristics of study participants were assessed for possible association with bacterial culture positive. Generally, there was no statistically significant association between the prevalence of bacterium isolate and socio-demographic factors by binary logistic analysis. Different clinical factors assessed for possible association with CAP infection shows a high percentage of bacterium isolate were found to have in crowded living condition 67(54.5%) compare to not crowded, but association was not statistically significant.

After bivariate analysis, multivariate logistic regression, were performed to avoid the confounding effect, the culture positivity of CAP had a significant association with the residence urban (AOR 8.214, 95% CI: 3.632-18.576, p <0.001), cigarete smoking(AOR: 6.478, 95% CI: 1.643-25.547, p = 0.008), alcohol consumption (AOR: 1.98, 95% CI: 1.05-3.735, p = 0.035) previous history of pneumonia (AOR: 6.956, 95% CI: 2.932-20.217, p = <0.001), commorbidity (AOR: 2.647, 95% CI: 1.215-5.767, p = 0.014), and malnutrited (AOR: 0.358, 95% CI: 0.132-0.968, p =0.043) (Table 6).

Characteristics	Culture	Bivariate Analysis	Multivariate Analysis	P-value

		Positive (%)	Negative (%)	COR (95% CI)	AOR (95% CI)	
Sex	Male	70 (56.9)	144(58.3)	0.945(0.610-1.463)	0.705(0.380-1.307)	0.267
	Female	53(43.1)	103 (41.7)	1		
Residence	Urban	100(81.3)	158(64.0)	2.449(1.453-4.129)	8.214(3.632-18.576)	<0.001
	Rural	23(18.7)	89(36.0)	1		
Cigarette smoking	Yes	10(8.1)	7(2.8)	3.034(1.126-8.177)	6.478(1.643-25.547)	0.008
	No	113(91.9)	240 (97.2)	1		
Alcohol consumption	Yes	58(47.1)	70(28.3)	2.256(1.440-3.536)	1.98(1.05-3.735)	0.035
	No	65(52.8)	177(71.7)	1		
History of pneumonia	Yes	17(13.8)	10(4.0)	3.801(1.684-8.578)	6.956(2.932-20.217)	<0.001
	No	106(86.2)	237(96.0)	1		
Comorbidity	Yes	27(22.0)	25(10.1)	2.497(1.378-4.525)	2.647 (1.215-5.767)	0.014
	No	96(78.0)	222(89.9)	1		
Nutritional status	Malnourished	14(11.4)	16(6.5)	0.539(0. 254-1.145)	0.358(0.132-0.968)	0.043
	Normal	109(88.6)	231(93.5)	1		
Immunosuppressive diseases	Yes	4(3.3)	2(0.81)	4.12(0.74-22.799)	0.993(0.125-7.914)	0.995
	No	119(96.7)	245(99.2)	1		
Asthmatic case	Yes	20(16.3)	27(10.9)	1.582(0.848-2.952)		0.149
	No	103(83.7)	220(89.1)	1		

Table 6. Bivariable and multivariable analysis of factors associated with culture-positive sputum among CAP patients

6. DISCUSSION

In this study, the overall prevalence of culture-positive sputum of bacterial CAP among adult patients was 33.2% (123/370; 95% CI: 28.4%–37.8%). This finding is comparable with previous Ethiopian studies in Dessie (38.7%) (46). However, our finding is lower than other findings reported in Bahir Dar (40.3%) (47), Arba Minch (40.0%) (48), Sudan (42.0%) (49), Jimma, Ethiopia (45.0%) (26), India (48.0%) (50), Tripura, North-eastern India (58.8%) (51), Egypt (50.4%) (52), China (55.1%) (29), and Saudi Arabia (46.6%) (53). There was some documented evidence in Bangladesh (27.61%) (54) and Tanzania (20.4%) (55), which was lower than our finding. This disparity could be attributed to differences in geographic location, study period, and sampling methods used for the study population (46).

Sixty-six (53.7%) of the isolates were gram-positive bacteria, while fifty-seven (46.3%) were gram-negative bacteria, indicating the predominance of gram-positive bacteria in causing CAP. This is due to the presence of various virulence factors that facilitate colonization and invasion of the respiratory airways, as well as the prior use of ineffective antibiotics by individuals outside the hospital, which results in the selection of gram-positive bacteria and an increased transmission of resistance genes. Findings documented by other studies in Ethiopia show that gram-negative bacteria were dominant (1).

In this study, the most frequent etiologic agent isolated from adult patients with CAP was *S. pneumoniae* (35.0%). Similarly, *S. pneumoniae* was the most commonly isolated bacterium from previous studies in Saudi Arabia (34.0%) (53) and Bahir Dar (35.9%) (47). However, our finding is higher than studies in India (13.3%) (50), Bangladesh (19.1%) (54), Dessie (24.8%) (46), Jimma (28.3%) (26), and Arba Minch (11.8%) (48). *K. pneumoniae* was the second most frequently isolated bacterium, accounting for 22.8% of the isolates. This result is comparable to studies conducted in Northeastern India (20.4%) (50) and Bahir Dar (18.0%) (47) . However, our finding is significantly higher compared to studies reported in Bangladesh (13.3%) (54), Saudi Arabia (12.0%) (53), Jimma (11.7%) (26), and Arba Minch (4.7%) (48). But it is lower than a study finding in India (29.1%) (50), China (27.4%) (29), Tanzania (29.9%) (55), and Dessie (28.0%) (46). This is due to their high prevalence in hospital settings, which can spread to the community through contaminated hands that can be found in the environment and on human mucosa, their capsular nature, the establishment of hypermucoviscous variants, and the acquisition of antibiotic

resistance genes. Additionally, a high intake of alcohol in the study participants that promotes alcohol-mediated dysbiosis also contributes to *K. pneumoniae* infection (56).

S. aureus accounted for 18.7%, which is comparable with other studies, Dessie (18.5%) (46) but higher than studies reported in Saudi Arabia (13%) (53) and Bangladesh (2.86%) (54). In our study, *E. coli* accounted for 8.9%. This finding is comparable with the studies conducted in Tanzania (9.1%) (55) . But it is higher than the studies in Bahir Dar (5.9%) (47), Saudi Arabia(5%) (53), Jimma, (3.8%) (26), Sudan (2.4%) (49) and lower than a study finding in China (17.9%) (29). The varying proportion of bacterial isolates among studies in different areas may be attributed to variations in geographic distribution of bacterial isolates, associated risk factors, sample size, type of specimen used, specimen collection and processing methods, and the methods used in each investigation.

In this study, the antimicrobial susceptibility patterns of the recovered bacterial isolates were assessed, and the predominant isolate, *S. pneumoniae*, was found to be 93% resistant to trimethoprim-sulfamethoxazole and 53.5% Resistant to Tetracycline. However, (95.3%) of the *S. pneumoniae* were sensitive to Erythromycin. This finding is comparable with local studies conducted in Bahir Dar (96.7%) (47), Jimma (95%) (26), South Ethiopia (95%) (57). Moreover, our study showed that (82.1%) of the *K. pneumoniae* isolates were resistant to Levofloxacillin and Sulphamethoxazole. Further, (71.4%) of the *K. pneumoniae* isolates were found to be resistant to Tetracycline. In addition, the present study noted a high level of antimicrobial resistance to Tetracycline, Levofloxacin, and Trimethoprim-sulfamethoxazole. This might be related to a high rate of prescription and self-medication of these drugs in the study area. On the other hand, most of the isolates in this study were susceptible to Meropenem (100%), Erythromycin (95.3%), and Amikacin (93%) (47, 57).

The overall prevalence of MDR was at 66.7% which is comparable to the studies in Gonder (72.2%) (1) and in Jimma, the prevalence was at 62.7% (26). However, slightly higher than the studies conducted in Arbaminch, Ethiopia, the prevalence was 60.3% (57). MDR in resource-constrained settings is highly contributed to by the widespread misuse of antimicrobials by patients due to a lack of access to appropriate treatment and the use of drugs due to inadequate dosing or incomplete treatment courses. A high level of MDR was observed among *P.aeruginosa* (88.9%), followed by *E. coli* (81.8%) and *S. pneumoniae* (72.1%). Comparable findings were reported in

Bahir Dar (47). High prevalence of MDR might be related to different factors, such as misuse and overuse of antimicrobials, poor adherence to treatment, poor infection control in health care and community settings, and poor hygiene and sanitation (58). Our study measured factors associated with culture positive sputum among patients suspected of having CAP. In our study, risk factors such as alcohol use ($p = 0.035$), cigarette smoking ($p = 0.008$), a previous history of pneumonia ($p < 0.001$), comorbidity ($p = 0.014$), and urban residence ($p < 0.001$) were significantly associated with CAP. In different studies, determinants like alcohol consumption (37, 46, 47), previous history of pneumonia (39, 59) cigarette smoking (46, 60, 61), comorbidity (62, 63), were reported as possible risk factors for CAP.

7. LIMITATIONS

Study limitations are the cross-sectional and single-center design, which limit generalizability and causal inference. Additionally, molecular studies to identify resistance genes were not performed, which would have been more revealing about mechanisms of antimicrobial resistance.

8. CONCLUSION AND RECOMMENDATIONS

In this study, a high prevalence of culture-confirmed CAP was observed among adults, with frequently isolated pathogens including *S. pneumoniae*, *K. pneumoniae*, and *S. aureus*. The most effective antibiotics against Gram-positive bacteria were Erythromycin and Levofloxacin, whereas Meropenem, Ceftazidime, Amikacin, Ceftriaxone, and Cefepime were effective against Gram-negative bacteria. Gram-negative bacteria were highly levofloxacin-resistant, followed by trimethoprim-sulfamethoxazole and tetracyclines. The predominant isolate, *S. pneumoniae,* showed a higher level of MDR, and it was resistant to Tetracycline, Azithromycin, and Sulfamethoxazole. In our study, risk factors such as alcohol use cigarette smoking, a previous history of pneumonia, comorbidity, and urban residence, were significantly associated with CAP. These high figures indicate to need of further multicenter and longitudinal studies through molecular characterization of resistance genes to comprehend and control the spread of AMR more effectively. Regular culture and local susceptibility testing, as well as special attention to factors such as vaccine coverage, are essential for better management of CAP in the study area. Periodical monitor the antibiotic resistance patterns to aid physicians to choose empirical treatments for better management of CAP.

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10. ANNEXES

ANNEX I: Information Sheet and Consent Form

Study Title: Bacteriology of Pathogens Associated with Community Acquired Pneumonia among Presumptive Adult Patients at Debremarkos Comprehensive Specialized Hospital, North West Ethiopia

Name of the Organization: Debremarkos University, College of Medicine and Health Sciences, Department of Laboratory Sciences.

1. Purpose of the Research Project: The aim of this study is to assess bacterial pathogens and antimicrobial susceptibility profile among CAP presumptive adults at Debremarkos Comprehensive Specialized Hospital, North West Ethiopia. In line with the findings, it was provide base line information for health sector administrators and concerned bodies in planning and developing treatment guide lines.

2. Procedure: In order to perform the indicated study at Debremarkos Comprehensive Specialized Hospital you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. The required sputum sample was collected currently at the hospital Laboratory. Then, you were requested to give your consent to the sample collector. Socio- demographic information was collected from the study participants using structured questionnaires via interview technique; since you fulfill the criteria you are kindly requested to give the required sample and genuine answers to the prepared questionnaire.

3. Risk associated with the study: They were not be at any physical or psychological risk and no damage resulting from the research procedures or during collection of the sputum ample.

4. Benefits of the study: Based on the diagnosis result they were treated accordingly. 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 for empirical treatment of community acquired pneumonia.

5. Compensation for participation: They were not receive any payment for your participation in this research study.

6. Confidentiality of your information- All information gathered from the study participant were remain confidential. Their participation in this study is strictly anonymous. Personal information

was be treated confidentially and under no circumstances was it be transmitted to any person or organization. The results of this study was evaluated and summarized, and a feedback of the results to the study participants were given by principal investigator.

7. Right to Refusal or Withdraw: Their participation in the study is absolutely voluntary; they have full right to refuse from participating in this research. They can refuse to give sample and not to respond any or all the questionnaires and this were not affect you on using any kind of services from the hospital.

8. Person to Contacts: This research project was reviewed and approved by Ethical review board of College of Medicine and Health Sciences, Debremarkos University. If you want to know more information, you can contact the following individuals and you may ask at any time.

1. Miss. Abyot Yitbarek, principal investigator

   Mobile: +251912725809/ e-mail: yitbarekabyot@gmail.com

2. Mr. Getachew Mengistu (Ass. professor) (advisor), Debremarkos University

   Mobile+251953590840/ e-mail: gechomlt@gmail.com

3. Mr. Abebaw Admasu (Msc) (advisor), Debremarkos University

   Mobile+251913214879// e-mail: woldkiros15@gmail.com

Consent form

Serial No----------Name of health institution----------Card No---------- Date----------

I the undersigned study participant have been well informed about the objective of the study entitled "Bacteriology of Pathogens Associated with Community Acquired Pneumonia Among Presumptive Adult Patients at Debremarkos Comprehensive specialized Hospital, North West Ethiopia

I am also told that all the information obtained at any course of the study is to be kept confidential. Moreover, I was also been well informed of my right to keep hold of, decline to cooperate and drop out of the study if I want and none of my actions were have been any bearing at all on my overall health care and hospital access.

I agreed voluntarily to provide the requested samples from me.

Name and signature of study participant _____ Date_____

Name and signature of investigator _____ Date_____

Annex II: Amharic Version Study Participant Information and Consent Form

የመረጃና የስምምነት ዉል ቅጽ

የጥናቱ ርእስ; በማህበረሰቡ ዉስጥ በጎልማሳ የዕድሜ ክልል ላይ በሚገኙ ሰዎች ላይ የሚከሰተዉን የሳንባ ምች በሽታ የሚያመጡ የባክቴሪያ ዝርያዎችን በደብረማርቆስ ሆስፒታል በሚታከሙ ሕመምተኞች በአክታ ናሙና ውስጥ መኖራቸውን መለየት; በምን ያህል መጠን እንዳሉ ማወቅ፤ እና በምን መድሃኒት ሊድኑ እንደሚችሉ ማወቅ ለበሽታው አጋላጭ የሆኑ ሁኔታዎችን ማወቅ፡፡

የድርጅቱ ስም- በደብረማርቆስ ዩኒቨርሲቲ ህክምና ና ጤናሳይንስ ኮሌጅ የላቦራቶሪ ሳይንስ ትምህርት ክፍል፡፡

1.የጥናቱዓላማ; በማህበረሰቡ ዉስጥ በጎልማሳ የዕድሜ ክልል ላይ በሚገኙ ሰዎች ላይ የሚከሰተዉን የሳንባ ምች በሽታ የሚያመጡ የባክቴሪያ ዝርያዎችን በደብረማርቆስ ሆስፒታል በሚታከሙ ሕመምተኞች በአክታ ናሙና ውስጥ መኖራቸውን መለየት፤በምን ያህል መጠን እንዳሉ ማወቅ፤እና በምን መድሃኒት ሊድኑ እንደሚችሉ ማወቅ ለበሽታው አጋላጭ የሆኑ ሁኔታዎችን ማወቅ አያይዞም ከጥናቱ የሚገኘው ውጤት ለጤና ተቋም አስተዳዳሪዎች ለሚመለከታቸው አካላት እና ለሕሙማን ለራሳቸው በዚህ ባክቴርያ የሚከሰተውን ሕመምና ሞት ለመከላከል መሠረታዊ መረጃዎችን ይሰጣል፡፡

2.የአሰራርሂድት፡ይህን ጥናት በደብረማርቆስ ሆስፒታል ለመስራት የጥናቱ ተሳታፊ እንዲሆኑ ተጋበዘዋል፡፡ለመሳተፍ ፍቃደኛ ከሆኑ የጥናቱን ዓላማ መረዳትና ፍቃደኝነትዎን መግለፅ ይጠበቃል፡፡ለዚህ ጥናት የሚያስፈልገው ናሙና የሚሰበሰበው በሆስፒታሉ ላቦራቶሪ ዉስጥ ይሆናል፡፡እንዲሁም በተጨማሪ ማህበራዊ ነክ ሁኔታን የሚያሳዩ ጥያቀዎች ከያንዳንዱ የጥናቱ ተሳታፊዎች በመጠይቁ መሰረት ይሰበሰባል፡፡ እርስዎም መስፈርቱን እስካሟሉ ድረስ የሚያስፈልገዉን ናሙና ለመስጠት እና ለተዘጋጀው ጥያቄ ትክክለኛ ምላሽ እንዲሰጡ በትህትና ይጠየቃሉ፡፡

3.ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት፡በዚህ ጥናት ዝርዝር አሰራር ሂደት እና ናሙናዉ በሚወሰድበት ጊዜ አካላዊ ወይም አእምሮአዊ ጉዳት አይኖርም፡፡

4.ጥቅሞች፤በምርመራ ውጤትዎ መሠረት ሕክምና ያገኛሉ በተጨማሪም ይህጥናት የላቦራቶሪ አገልግሎት በሌለበት ጤና ተቋም ትክክለኛ መድሃኒት ለመስጠት እንደአመላካች ሆኖ ያገለግላል፡፡

5. ለተሳትፎ የሚሰጥ ማካካሻ- ምንም አይነት የካሳትፎ ክፍያ የለዉም፡፡

6. የጥናቱ መረጃ ሚስጥራዊነት-ሁሉም ከተሳታፊዎች የሚሰበሰቡ መረጃዎች በሚስጢር የሚያዙ እና የሚጠበቁ ይሆናሉ፡፡ በማንኛውም ምክንያት ተሳታፊዎች እነማን መሆናቸውን የሚያሳይ በመጠይቁ ይሁን በሌላ ነገር አይኖርም፡፡ የተሰበሰቡ መረጃዎች ለሶስተኛ ወገን ተላልፎ አይሰጥም፡፡በተጨማሪም ውጤቱ የሚለካዉ ይሁን ተሰብስቦ የሚያዘው በዋና አጥኚ ነው፡፡

7.የመዉጣት (የማቋረጥ) መብት በዚህ ጥናት ላይ መሳተፍዎ በሙሉ ፍቃደኝነት ላይ የተመሰረተ ነው:: ጥናቱን የማቋረጥ ሙሉ መብት አለዎት፡፡ ናሙናም ሆነ ለመጠይቁ መልስ ያለመስጠት ከሆስፒታሉ የሚያገኙትን ማንኛውንም አገልግሎት አይገድብም ::

8.የሚያገኙዋቸው ሰዎች ይህ ጥናት በደብረማርቆስ ዩኒቨርሲቲ የስነምግባር ምርምር ኮሚቴ ናሕክምናና ጤና ሳይነንስ ኮሌጅ የላቦራቶሪ ትምህርት ክፍል ተልኮ የሚጸድቅ ይሆናል፡፡ ጥያቄ ካለዎት ተጨማሪ መረጃ ከፈለጉ በማንኛዉም ጊዜ ከዚህ በታች የተጠቀሰዉን አድራሻ መጠቀም ይችላሉ፡፡

የስምምነት ዉል

ተራ ቁጥር----------የሚታከምበት ጤና ተቋም----------የካርድቁጥር----------

ቀን----------

እኔ ከዚህ በታች ስሜ የተጠቀሰዉና የፈረምኩት የጥናቱ ተሳታፊ በማህበረሰቡ ዉስጥ በጎልማሳ የዕድሜ ክልል ላይ በሚገኙ ሰዎች ላይ የሚከሰተዉን የሳንባ ምች በሽታ የሚያመጡ የባክቴሪያ ዝርያዎችን በደብረማርቆስ ሆስፒታል በሚታከሙ ሕመምተኞች በአክታ ናሙና ውስጥ መኖራቸውን መለየት፤ በምን ያህል መጠን እንዳሉማ ወቅ፤ እና በምን መድሃኒት ሊድኑ እንደሚችሉ ማወቅ ለበሽታው አጋላጭ የሆኑ ሁኔታዎችን ለማወቅ የሚደረገውን ጥናት አላማና ጥቅም በሚገባ ተረድቻለሁ፡፡ ጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍ በራሴ ፍቃድ የሚወሰን መሆኑም ተገልጾልኛል፡፡ በተጨማሪም ከጥናቱ ባልሳተፍም ሆነ አቋርጬ ብወጣ ከሆስፒታሉ በማገኘዉ የህክምና አገልግሎት ምንም አይነት ችግር እንደማይደርስብኝ ተነግሮኛል፡፡በመሆኑም ከእኔ ናሙና መዉሰድ አስፈላጊ መሆኑን ስለተስማማሁበት ለመስጠት ሙሉ ፈቃደኛ መሆኔን በፊርማዬ እገልጻለሁ፡፡

የተሳታፊ ስምና ፊርማ _____ ቀን _____

የተመራማሪ ስምና ፊርማ _____ቀን _____

Annex III: Questionnaire & Laboratory Data Collection Form

Bacteriology of Pathogens Associated with Community Acquired Pneumonia Among Presumptive Adult Patients at Debremarkos Comprehensive specialized Hospital, North West Ethiopia

AnnexIIIa: Questionnaire:

Code No	

Hospital Card No	
Date of sample collection	
Health Organization	

I. Socio demographic information:

S. No	Question	Code	Response
Q 11	Sex	1 = Male, 2 = Female	
Q12	Age	---------- Years	
Q13	Residence	1 = Rural 2 = Urban	
Q14	Occupation	1. Governmentemployee/Civil servant 2. Farmer 3. Merchant 4. House wife 5. Daily labor 6. Other specify	
Q15	Education status	1 = Illiterate, 2 = Primary schools(1-8) 3 = Secondary schools(9-12) 4 = Higher education(diploma and above)	

II. Factors associated with CAP

Q21	Do you drink alcohol	1=Yes 2=No	
Q22	Do you smoke cigarette?	1=Yes 2=No	
Q23	Do you have asthma?	1=Yes 2=No	
Q24	Do you have diabetes?	1=Yes 2=No	

Q25	Do you live in crowded room condition?	1=Yes 2=No	
Q26	Do you have previous pneumonia history?	1=Yes 2=No	
Q27	If you say yes for no 26 did you take antibiotic?	1=Yes 2= No	
Q28	Do you have heart disease?	1=Yes 2=No	
Q29	Do you have immunosuppressive diseases?	1=Yes 2=No	
Q30	If you say yes for no 29 did you take immunosuppressive drugs?	1=Yes 2=No	
Q31	Do you know your HIV/AIDS status?	1=Yes 2=No	
Q32	If you say yes for no 31 Have you a positive result?	1=Yes 2=No	

You have finished the interview Thank you!

Annex III b: Laboratory data collection form:

Code No	
Hospital Card No	
Date of sample collection	
Health Organization	

1. Antibiotic susceptibility pattern:

Antibiotic	Sensitivity Results:					
	Content	S	I	R	Reading (mm)	Interpretation
Ceftriaxone	30µg					

Ciprofloxacin	5µg					
Tetracycline	30µg					
Chloramphenicol	30µg					
Erythromycin	15µg					
Doxycycline	30µg					
Penicillin	10µg					
Gentamycin	10µg					
Trimethoprimsulfamethoxazole	1.25+23.75 µg					
Ampicillin	10µg					
Oxacillin	1µg					

Annex IV: Amharic Version of Questionnaire

የአማርኛ መጠይቅ

በማህበረሰቡ-ዐስጥበጎልማሳየዕድሜክልልላይበሚገኙሰዎችላይየሚከሰተዉንየሳንባምችበሽታየሚያመጡየባክቴሪያዝርያዎችን በደብረማርቆስ ሆስፒታል በሚታከሙ ሕመምተኞች በአከታ ናሙና ውስጥ መኖራቸውን መለየት፤ በምን ያህል መጠን እንዳሉ ማወቅ፤እናበምንመድሃኒትሊድኑእንደሚችሉማወቅ ለበሽታዉ አጋላጭ ሁኔታዎችን ለመለየት የተዘጋጀ መጠይቅ፡፡

የኮድቁጥር	
የመታከሚያካርድቁጥር	
ናሙናዉየተወሰደበትቀን	
የጤናተቋሙ ስም	

ሀ. ማህበራዊ እና ኢኮኖሚያዊ መረጃ

ተ.ቁ	ጥያቄ	መለያ ምልክት	ምላሽ
1	ጾታ	1. ወንድ 2. ሴት	
2	እድሜ	---------አመት	
3	መኖሪያ ቦታ	1. ገጠር 2. ከተማ	
4	ስራ	1. የመንግስትሰራተኛ 5. የቀን ስራ 2. ገበሬ 6. ሌላ ከሆነይግለጹ---------- 3. ነጋዴ 4.የቤትእመቤት	
5	የትምህርትደረጃ	1 ያልተማረ 2 የመጀመሪያ ደረጃ (1-8) 3 ሁለተኛ ደረጃ (9-12) 4 ከፍተኛ ትምህርት(ዲፕሎማ እና ከዛ በላይ)	

ለ. ለበሽታዉ መባባስ አጋላጭሁኔታዎች

ተ.ቁ	ጥያቄ	መለያምልክት	ምላሽ
1	የአልኮል መጠጥ ይጠጣሉ?	1.አዎ 2.አልጠጣም	

2	ሲጋራ ያጨሳሉ	1.አዎ 2.አላጨስም	
3	የአስም በሽታ አለብዎት	1.አዎ 2.የለብኝም	
4	የስኳር ህመም አለብዎት	1.አዎ 2. የለብኝም	
5	ቤት ዉስጥ በተጨናነቀ ክፍል ነዉ የሚኖሩት	1.አዎ 2. አይደለም	
6	ባለፉት ወራቶች የሳንባ ምች ታመዉ ያዉቃሉ	1.አዎ 2.የለም	
7	ለተራቁጥር 6 መልስዎ አዎ ከሆነ መድሃኒት ወስደዉ ነበር	1.አዎ 2.የለም	

መጠይቁን ጨርሰዋል አመሰግናለሁ

Annex V: Laboratory Procedures

A. Sputum Specimen collection

1. Instruct the patient to clean or rinse his mouth to reduce the number of contaminating oropharyngeal bacteria.

2. Give the patients a sterile screw-cap sputum cup and tell them to produce expectorated sputum resulting from deep cough which is about 2ml.

3. To prevent contamination of the outside of the container, the patient should be

Instructed to press the rim of the container under the lower lip to catch the entire expectorated cough sample

4. Tightly screw on the cap of the container. Wipe off any spilled material on its outside with a tissue moistened with disinfectant, but take care not to let any disinfectant enter the container

5. Label the sputum cup with patient identifying information, and Deliver the specimen to the laboratory as quickly as possible. A delay in examining the specimen may cause a false result.

6. Wash your hands and instruct the patient to wash his hand as well(44).

B. Gram stain procedures

1. Select a purulent portion of the specimen and prepare a smear

2. Fix the dried smear

3. Cover the fixed smear with crystal violet stain for 30–60 seconds.

4. Rapidly wash off the stain with clean water. *Note*: When the tap water is not clean, use filtered water or clean boiled rainwater.

5. Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds.

6. Wash off the iodine with clean water.

7. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water. Caution: Acetone–alcohol is highly flammable; therefore use it well away from an open flame.

8. Cover the smear with neutral red stain for 2 minutes.

9. Wash off the stain with clean water.

10. Wipe the back of the slide clean, and place it in a draining rack for the smear to air dry.

11. Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

12.Record the results and assess suitability for culture(44)

C. Culture media preparation

1. Read the manufacturer's instructions on the bottle of dehydrated agar media of interest.

2. Measure the appropriate amount of dehydrated powder media and distilled water using an electronic balance on clean dry paper and measuring cylinder respectively.

3. Drop a measured amount of distilled water in a flask and add the measured amount of powder.

4. Close the flask with cotton foiled with aluminum foil

5. Heat the flask on a hot plate to dissolve the mixture until it vapors

6. When the agar mixture is completely dissolved, remove the flask from the hot plate

7. Autoclave the agar mixture for 15 min at 120^{o}c. Do not autoclave the preparation if indicated in the manufacturer's instruction.

8. Remove the flask of sterilized agar from autoclave and allow it to cool to about 50°C.

9. Quickly pour the melted, sterile agar into a series of petri dishes until about one-third of the dish is filled and wait until it solidifies by partially covering the dishes.

10. Invert the petri dishes if the agar is solidified to avoid the moisture from accumulating on the agar surfaces that has been condensed from the vapor.

11. Incubate one petridish from each batch of preparation in the incubator for at least 24 hours to check sterility of preparation before use.(44).

D. Biochemical testing procedures

Identification of Gram negative bacteria will be based on their test result with a series of biochemical tests.

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, mannitol, urea agar and motility medium.
3. Incubate at 35-37 ^{O}c for 18-24 hours.
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the biochemical tests and their motility characteristics by comparing with the identification chart.

E. Antimicrobial Susceptibility Testing

1. Prepare a suspension of the test organism by emulsifying several colonies of the organism in a small volume of nutrient broth until it matches with a turbidity of 0.5 McFarland standard
2. Take a sample from the suspension with a sterile swab (squeeze the swab against the side of the test tube to remove the excess fluid).
3. Spread the inoculums evenly over Muller-Hinton (MHA) agar plate with the swab (add 5% sheep blood on MHA for fastidious organisms like *S.pneumoniae* and incubated in HTM media 5% CO2 at 35–37°C for 16–18 h use instead of MHA for *H .influenzae*)
4. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
5. Incubate the plate aerobically at 35-37ºC for 18-24 hours
6. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
7. Interpret the reaction of the test organism to each antibiotic used as sensitive, intermediate, or resistance as per the standard

Sensitive– zone of inhibition is wider or equal to the CLSI recommended zone size for each isolated strain

Intermediate– If the zone of inhibition is within the intermediate range of CLSI guideline

Resistance– If the zone of inhibition is below the stated zone size on CLSI guideline.

Annex VI: Dummy Tables

Table 1: Socio-Demographic Characteristics of CAP presumptive adult Patients at Debremarkos Comprehensive Specialized Hospital, 2025

Variables	Frequency % N=	Bacterial isolates		Others
		Positive	Negative	
Age in year. ➢ 18-30 ➢ 31-40 ➢ 41-50 ➢ ≥51 ➢ Total				
Sex ➢ Male ➢ Female ➢ Total				
Education ➢ Primary ➢ Secondary ➢ No education ➢ University ➢ Total				

Residence ➢ Urban ➢ Rural ➢ Total				
Smoking ➢ Yes ➢ No ➢ Total				
Alcohol consumption ➢ Yes ➢ No ➢ total				
Asthma ➢ yes ➢ no ➢ total				

Table 2-List of bacteria isolated from sputum of CAP presumptive adult patients at Debremarkos Comprehensive Specialized Hospital

Bacteria type	Bacteria	Number(N)	N%
Gram positive			
Gram negative			

Table 3-Antimicrobial susceptibility pattern of isolated bacteria among CAP presumptive adult patients at Debremarkos Comprehensive Specialized Hospital

Drugs name	Gram positives			Gram negatives		
	S	I	R	S	I	R
Ceftriaxone						
Ciprofloxacin						
Tetracycline						
Chloramphenicol						
Erythromycin						
Doxycycline						
Penicillin						
Gentamycin						
Trimethoprimsulfamethoxazole						
Ampicilin						
Oxacilin						

Table4-List of MDR pattern for each bacterial isolate

Bacterial isolates	Total No	AST pattern			
		R_0	R_1	R_2	R_3

Declaration

I, the undersigned, declare that this thesis is my own original work, has not been presented for a degree in any other University, and that all sources of materials used for the thesis have been duly acknowledged.

Abyot Yitbark Sign----------Date----------

Approved by Advisors

1. Mr Getachew Mengistu Sign----------Date ----------

2. Mr. Abebaw Admasu Sign----------Date ----------