Prevalence of bacterial pathogens, their antimicrobial susceptibility patterns and associated factors among patients suspected of bacterial pneumonia attending dessie referral hospital, northeast Ethiopia

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Abstract

Background: Pneumonia is the most common cause of morbidity and mortality in developing countries, mostly caused by different species of bacterial pathogens. Hence, patient management needs awareness of the pathogens and Antimicrobial Susceptibility Testing (AST). This study was aimed to assess the type of bacterial isolates and their antimicrobial susceptibility patterns among pneumonia suspected patients at Dessie Referral Hospital, Amhara region, Dessie, Northeast Ethiopia. Materials and Methods: A hospital-based cross-sectional study design was employed among pneumonia suspected patients from February to April 2020. Socio-demographic characteristics and associated risk factors were collected using pretested questionnaire and clinical data was extracted by reviewing medical records. Sputum specimens were collected and inoculated into chocolate agar, blood agar, Manitol salt agar and MacConkey agar which then incubated at 35 OC or 37 OC for 24-48 hours. Bacterial species were identified based on Gram stain, colony characteristics and biochemical techniques. Antimicrobial susceptibility testing was done using Kirby–Bauer disc diffusion technique. The data was entered in to Epi-Info version 7.1.5 and analyzed with SPSS software version 20.P-Value<0.05 at 95% CI was considered as statistically significant. Results: A total of 406 sputum specimens were collected and cultured among which157 (38.7%) were positive for different bacterial pathogens. The predominant pathogens were Klebsiella pneumoniae with 28.0% (n=44), Streptococcus pneumoniae with 24.8% (n=39), Staphylococcus aureus with 18.5% (n=29) and Pseudomonas aeruginosa with 14.0% (n=22). Majority of the isolates exhibited resistance to Ampicillin with 81.5% (n=53) followed by penicillin with 75.9% (n=22) and amoxicillin-clavulanate with 61.2% (n=43). Multivariable logistic regression showed significant association of culture positivity with older age(AOR = 2.43, CI: 1.12-5.28, p-value = 0.025), cigarette smoking (AOR=4.67, CI: 2.39-9.20, p-value<0.001) and alcohol use (AOR=5.58, CI: 3.14-9.92, p-value<0.001). Resistance to Ampicillin and penicillin was associated with repeated prescription and use. Conclusions: This study found high prevalence of bacterial pneumonia in the study area and high rate of bacterial resistance was observed in Ampicillin, penicillin and amoxicillin –clavunalate. Repeated prescriptions and use of antimicrobials were significantly independent factors of bacterial resistance. Therefore, expanding routine bacterial culture and identification with antimicrobial susceptibility testing and strengthening regular surveillance systems are essential for appropriate patient care.

Keywords: Antimicrobial susceptibility test; Bacterial pneumonia; Dessie Referral Hospital

Accepted on 02 August, 2021

Introduction

Pneumonia causes health crisis in terms of morbidity, mortality, health expenses and days of work lost (1), most commonly caused by bacteria [2]. High incidence, admission rate and mortality rate of bacterial pneumonia was identified as a contributing factor for both health crisis and economic burdens by several studies, although the unique treatment protocol applied by each country complicated to easily compare the treatment costs. The increase in the burden of bacterial pneumonia with age needs more attention to the disease in the future.

The annual incidence rate of Community-Acquired Pneumonia (CAP) in the age group of 18-39 and > 75 years was 6/1000 and 34/1000 respectively. Among all cases, 20-40% of patients need admission from which 5-10% of them are admitted to intensive care units due to severe complications such as septic shock, extra-pulmonary organ dysfunction and acute respiratory distress syndrome (ARDS). Moreover, the overall mortality among adults from CAP is 6–15%, which magnifies the importance of identifying and treating patients with this disease [3].

Pneumonia-causing bacteria can be transmitted by different ways; inhalation of droplets (e.g. C. pneumoniae and M. pneumoniae), environmentally (L. pneumophila) and micro-aspiration of a potential pathogen after colonization of the nasopharynx (e.g. S. pneumoniae, H. influenzae or S. aureus). Conjugate vaccines has reduced the incidence of pneumonia

caused by S. pneumoniae and H. influenzae type B (Hib). However, these bacteria are still the leading causes of the disease; as only 30% of children receive the correct treatment [4].

The main risk factors are demographic and socioeconomic factors (such as age, sex marital status, occupational status), lifestyle factors (such as cigarette smoking, alcohol drinking) and environmental factors (such as dust, gasoline, contact with animals, painting) and viral infections of the respiratory tract, as it compromises the respiratory tract and results in bacterial colonization and infection [5].

In developing countries, bacterial pneumonia is treated usually empirically; by medical history and physical examinations in which the etiologic agent is rarely identified and results in high prevalence of MultiDrug-Resistant (MDR) pathogens. Hence, identifying the most common bacterial pathogens and their antimicrobial susceptibility patterns is a key to reduce morbidity and mortality due to bacterial pneumonia. Therefore, this study aimed to assess the prevalence of bacterial pathogens, their antimicrobial susceptibility patterns and associated risk factors among patients suspected for bacterial pneumonia attending Dessie Referral Hospital, Northeast Ethiopia.

Materials and methods

Study design and setting

A facility based cross-sectional study was conducted from February to April 2020 in Dessie Referral Hospital, Amhara regional state, Dessie, Northeast Ethiopia. The hospital had around 500 beds with annual ambulatory cases of around 14,400; hence, it was purposefully selected to conduct this study. All pneumonia suspected patients visiting the hospital were used as the source population and all patients aged > 5 years who were clinically suspected for bacterial pneumonia were included in the study. Patients who were under antimicrobial treatment within the last 14 days during data collection were excluded from the study [6].

Sample size and sampling technique

A single population proportion formula was used to determine the sample size; based on the previous study with 40.3% pneumonia prevalence (20).By considering 5% margin of error, 95% confidence level and 10% non-response rate, a total of 406 participants were proposed and systematically recruited.

Data collection

Data related to socio-demography and risk factors for bacterial pneumonia was collected by pre-tested and structured questionnaire through face-to-face interview. Gene Xpert results, initial diagnosis and nutritional status of study participants were collected by reviewing medical records. Sputum specimens were collected in a sterile, disposable, leak proof and wide-mouthed container with tight-fitting lid. To reduce the number of commensals, the purulent parts of the sputum specimens were washed in about 5 ml of sterile physiological saline. To keep pathogens (such as S. pneumonia and H. influenzae) alive, the washed sputum specimens were inserted to a cotton-wool swab which then inserted in containers of Amies transport medium, then all specimens were transported with cold box to Amhara Public Health Institute (APHI) Dessie branch reference laboratory for culture and antimicrobial susceptibility testing.

Bacterial isolation and identification

All specimens received for culture were evaluated macroscopically followed by microscopic inspection by Gram stain before culture analysis began. Thus, sputum specimens with at least 25 polymorph-nuclear leukocytes and<10 epithelial cells per low power field and >10 bacteria per high-powered field were processed for culture.

The sputum specimens appropriate for culture were inoculated into Blood agar plate (BAP), MacConkey Agar plate (MAC), Manitol Salt Agar (MSA) and Chocolate agar plate (CHO). Subsequently, BAP, MAC and MSA were incubated at 37°C for 18-24 hours while CHO (in humid, 5% CO2 atmosphere) were incubated for 18–24 hours at 35°C-37°C. All the plates were examined for growth after 24 hours; the plates without growth were further incubated for up to 48 hrs. The colonies were sub-cultured on BAP and MAC for further identification. Bacterial species were identified based on Gram stain, colony characteristics (such as size, shape, pigmentation, color) zones of hemolysis and other biochemical characteristics. *S. pneumonia* were identified by catalase and optochin (5 µg) sensitivity tests. S. aureus was confirmed by catalase, Coagulase and the Manitol fermentation tests. Chocolate agar, enriched with factor V (NAD) and factor X (hemi) was used to enhance the growth of *H. influenzae*. Gram-negative isolates of bacteria were inoculated onto different biochemical tests such as motility, Indole, urea, Lysine decarboxylase, triple sugar iron agar and citrate utilization tests for identification.

Antimicrobial susceptibility testing

Antimicrobial Susceptibility Testing (AST) for bacterial isolates was performed according to Clinical and Laboratory Standards Institute (CLSI) recommendations. The applied discs were Tetracycline (TE_30 µg), Erythromycin (E_15 µg), Penicillin (P_ 10 µg), Ceftriaxone (CRO_30 µg), Doxycycline (DA_30 µg), Chloramphenicol (C_30 µg), Trimethoprim-Sulphametoxazole (TMP-SMX_ 1.25+23.75µg), Ciprofloxacin (CIP_5 µg), Gentamicin (CN_10 µg), Ampicillin (AMP_ 10 µg), imipenem (IMP_ 10 µg), cefepime (PEP_30 µg), amoxicillin/ clavulonic acid (AMC_20/10 µg), piperacillin/ tazobactam (TZP_100/10 µg), amikacin (AK_30 µg), Ceforoxime (CXM_30 µg), ceftazidime (CAZ_30 µg), Chloramphenicol (CAF_30µg), meropenem (MER_10 µg), Aztreonam (AZT_30µg), oxacillin (OXA_1 µg) and Cefoxitin (CXT_ 30µg).

A young culture growth of bacterial suspensions was prepared by picking parts of similar colonies with a sterile wire loop in which these suspensions were adjusted to McFarland 0.5

turbidity standard. The bacterial suspensions in a sterile broth were incubated up to 2 hours to allow the bacteria to reach their log-phase in growth. Then, inoculums were swabbed on to Muller- Hinton agar. After drying the agar for 3-5 minutes, the antimicrobial impregnated disks were placed with sterile forceps on the agar surface in such a way that each disk was placed at least 24 mm away from each other to avoid the overlapping zone of inhibition. After placing the discs, the plates were allowed to stand for 30 minutes to help the antimicrobial to be dissolved in the media. Following inverting and incubating for 24 hours at 37o C, the plates were read for the diameter of zone of inhibition. The susceptibility patterns were graded as sensitive, intermediate and resistant. Muller-Hinton Agar (MHA) supplied with 5% sheep blood was used for *S. pneumoniae* while Muller-Hinton Agar containing 1.0% hemoglobin and 1.0 % IsoVitaleX supplement (CHOC-MHA) was used for *H. influenzae*.

Laboratory quality control

Manufacturer instructions and bacteriological standard procedures were followed strictly throughout the whole technical processes including culture media preparation, inoculation and AST testing. The sterility of culture media was checked by incubating 5% of the batch at 35-37OC overnight and was evaluated for possible contamination. The standard reference bacterial strains such as S. aureus (ATCC®25923), *H. influenza* (ATCC® 49247), E. coli (ATCC® 35218) and *S. pneumonia* (ATCC® 49619) were used as a quality control (25).

Data quality control

Training was given for the data collector about data collection procedures and interview techniques. To assure the quality of the data, pre-tested, structured questionnaire was used for data collection. The questionnaire was objective-based and logically sequenced. It was checked daily by the principal investigator for its completeness.

Data analysis

Data was checked for completeness, cleaned, coded and entered in to Epi Info version 7.1 software and then exported to SPSS version 20 for analysis. Frequency, proportions and summary statistics were used to describe study participants in relation to relevant variables. Bivariate and multivariate logistic regression analysis was carried out to identify association between bacterial pathogens, antimicrobial resistance and possible risk factors. Odds ratio and p value were used to assess the presence and degree of association. P-value<0.05 at 95% CI was considered as statistically significant.

Ethical approval and consent to participate

Ethical clearance was obtained from University of Gondar, College of Medicine and Health Sciences Institutional Review Board. Informed written consent was obtained from the study participants after explaining the purpose and objective of the study. The laboratory results from the study participants were communicated to their physicians for appropriate patient management. Data from all patients were kept confidential.

Limitations of the study

This study did not consider the “atypical” intracellular bacteria such as *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella pneumophila* since they cannot be cultured by routine culture methods. This study did not also consider the anaerobic bacteria (Prevotella spp., Fusobacterium spp. and Clostridium spp.) as routine culture methods are mostly aerobic. Hence, it underestimates the actual prevalence in the study area. This study did not include serotyping techniques to *Homophilus influenzae* and *Streptococcus pneumoniae*. Characterization of methicillin resistant *Staphylococcus aureus* (MRSA) was not done.

RESULTS

Socio-demographic and clinical data characteristics

This study was conducted among 406 pneumonia suspected patients of which 221 (54.4%) were males and 249 (61.3%) were urban dwellers with 158 (38.9%) participants unable to read and write (Table-1). The median age of study participants was 45.0 with a range of 10-95 years. Among study participants, 131 (32.3%) were smokers and 158 (38.9%) were alcohol consumers.

Table1. *Socio-demographic characteristics of pneumonia suspected patients in Dessie referral hospital, Dessie.*

Characteristics	Frequency	Percent (%)
Sex		
Male	221	54.4
Female	185	45.6
Age (in years)		
5-14	30	7.4
15-24	57	14
25-44	96	23.6
45-64	67	27.6
>64	156	38.4
Residence		
Urban	249	61.3
Rural	157	38.7
Educational level		
Unable to read & write	158	38.9
Read & write only	48	11.8
Primary education	56	13.8
Secondary education	67	16.5
College and above	77	19

Among all participants, 43 (10.6%) were HIV positives and 39(9.6%) had active TB cases at the time of data collection. As indicated in Table 2, asthma, diabetes and hypertension comorbidities accounted 6%, 5% and 3% respectively .

The nutritional status of children (n= 30) was assessed by mid upper arm circumferences (MUAC) and body mass index (BMI) all of which (n=30, 100%) showed normal nutritional status.

Table 2. *Clinical characteristics of pneumonia suspected patients in Dessie referral hospital, Dessie*

Characteristics	Frequency	Percent
HIV		
Positive	43	10.6
Negative	363	89.4
TB		
Positive	39	9.6
Negative	385	90.4
Asthma		
Yes	23	6
No	383	94
Diabetes		
Yes	20	5
No	386	95

Prevalence of bacterial pathogens

Bacterial isolates were identified from 157 (38.7 %) participants of which 155 (98.7%) were identified as community-acquired pneumonia (CAP) while 2 (1.3%) were hospital-acquired pneumonia (HAP). Gram-negative isolates accounted 89 (56.7%) while the rest 68 (43.3%) were Gram-positive.

The frequently isolated pathogens were Klebsiella pneumonia with 44(28.0%), Streptococcus pneumonia with 39 (24.8%), Staphylococcus aureus with 29(18.5%), Pseudomonas aeruginosa with 22 (14.0%), Haemophilus influenzae with 11(7.0 %), Escherichia coli with 7(4.5%), Acinetobacter baumannii with 3(1.9%) and Klebsiella oxytoca with 2 (1.3%), (Table 3).

Additionally, Out of all study participants (406) screened for TB, Mycobacterium tuberculosis was detected in 39(9.6%) of them, from which rifampicin-resistant was detected in 5(12.8%) of the cases.

Table 3. *The prevalence of bacterial isolates identified from pneumonia suspected patients in Dessie referral hospital.*

Bacterial isolates	Frequency	Percent
Gram-positive (n = 68)		
S. pneumonia	39	24.8
S.aureus	29	18.5
Gram-negative (n = 89)		
K. pneumonia	44	28
P. aeruginosa	22	14
H. influenza	11	7
E. coli	7	4.5
A. baumannii	3	1.9
K. oxytoca	2	1.3
Overall	157	38.7

Antimicrobial susceptibility patterns of bacterial isolates

Among gram negative isolates, resistance to tetracycline, ampicillin, amoxicillin-clavulanate, co-trimoxazol and chloramphenicol for *Klebsiella pneumoniae*, which was the most frequently isolated species, were 95.5%, 93.2%, 88.6%, 88.6%, and 79.5% respectively.

Whereas, low resistance to ciprofloxacin (2.3%), Ceforoxime (4.5%), piperacillin-tazobactam (4.5%), ceftazidime (6.8%) and amikacin (9.1%) was observed for *Klebsiella pneumoniae* isolates. Pseudomonas aeruginosa isolates showed more resistance to ceftazidime (63.6%) and gentamycin (54.5%), while Haemophilus influenzae isolates were more resistant to tetracycline (90.9%) and ampicillin (54.5%),

Among gram positive isolates, *Streptococcus pneumonia*, the second most frequent isolate of all species, showed higher resistance to oxacillin (56.4%), penicillin (56.4%) and erythromycin (48.7%)and lower resistance to Clindamycin (10.3%), co-trimoxazol (12.5%) and ciprofloxacin (17.9%), while Staphylococcus aureus resist more to tetracycline (86.2%) and co-trimoxazol (72.4%), (Table 4).

Moreover, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be 34.5% (n=10). Multi Drug Resistance (MDR), resistance to 3 or more antimicrobials, was observed in 99 (63.1%) of the isolates and high level of MDR was observed among *Klebsiella pneumoniae* (97.7%) and *Staphylococcus aureus* (89.7%), followed by Pseudomonas aeruginosa (45.5%) and *Haemophilus influenzae* (36.4%).

DISCUSSION

In this study, the overall prevalence of bacterial isolates was 38.7%. This was consistent with the studies reported from Ethiopia, Bahir Dar (40.3%), Ethiopia, Mekelle (43.7%), Ethiopia, Arbaminich (40.0% 42.9%) and Nigeria (42.9%). However, the finding in this study was higher than the findings from Brazil (22.7%), China (23.6%), and USA (30.0%) (32) while it was lower than the findings in Ethiopia, Jimma (45.0% and 47.7%), China (45.3%), Cameroon (46.8%), Iceland (52.0%), Bangladesh (52.4%) and Pakistan (64.2%). The inconsistency might be due to sample size variation, type of specimen used, geographical variation and immune status of the study participants. For example, a study conducted in

Gambia used lung aspiration and sputum specimens for culture and molecular techniques while a study conducted in Iran (39) used pleural fluid in addition to sputum specimens for culture and molecular tests. Additionally, as indicated by a report from Japan, sputum culture (with a total prevalence of 48.0%) blood culture (with a total prevalence of 2.9), polymerase chain reaction (PCR) (with a total prevalence of 50.0%) and urinary antigen tests for S. pneumoniae (with a total prevalence of 13%) were used to detect bacterial pathogens responsible for pneumonia.

The predominant bacterial isolates, in this study, were *Klebsiella pneumoniae* (28.0%) and Streptococcus pneumoniae (24.8 %). It was consistent with studies conducted in Ethiopia and Nepal while the rank of these two isolates was reciprocal in other studies. Moreover, Staphylococcus aureus (18.5%) and *Pseudomonas aeruginosa* (14.0%) were the third and fourth most dominant isolates with consistency in a study conducted in China with a prevalence of (12.0%) and (10.3%) respectively. However, Streptococcus pneumoniae was reported as the most predominant species in many studies such as those conducted in Jimma, Ethiopia (12.8% and 57.6%), Bahir Dar, Ethiopia (35.9%), Gambia (91%), Spain (44%), Bangladesh (19.5%), Iceland (20%), Iran (24.4%) and China (32.6%). Additionally, a large, international study conducted in USA reported Pseudomonas aeruginosa as the most dominant species with a prevalence of 9.0% (44) while another study conducted in Japan reported *Haemophilus influenzae* as the most dominant pathogen with a prevalence of 9.0%.

In this study, *Streptococcus pneumoniae* (28.8%) and *Klebsiella pneumoniae* (24.0%) were the predominant isolates from Gram-positive and Gram-negative accordingly. These two pathogens were reported as predominant in different studies. However, it is poorly understood, although there are suggestions that it may possibly due to their capsular nature and the emergence of strains from both species that can acquire additional genetic traits. The prevalence of *Staphylococcus aureus* was 18.5% (n =29), with the prevalence of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) being 34.5% (n=10). This MRSA prevalence was lower than a study reported from Ethiopia (44.4%). The present study showed that MRSA was a causative pathogen of pneumonia which is coincided with other studies with varied prevalence. The general prevalence of Gram-positive and Gram-negative isolates was 43.3% (n=68) and 56.7% (n=89) respectively. This general prevalence was consistent with studies conducted in Ethiopia; Gram-positive isolates=(50.3%), Gram-negative isolates=(49.7%) (20) and Gram- positive isolates=(52.1%), Gram-negative isolates=(47.9%). It was also consistent with a study conducted in Ghana (40% and 58% for Gram-positive and negative isolates respectively).

In this study, there was an increased prevalence of drug-resistant isolates that could possess a significant health risk. *Klebsiella pneumoniae* was resistant to tetracycline in 95.5% which is comparable to the study conducted in Bahir Dar, Ethiopia (100%) and higher than the study conducted in Jimma Ethiopia (8.0%) (33). This organism was resistant to Ampicillin in 93.2%; higher than studies conducted in Ethiopia (8.0%) (33) China (71.9%) and lower than studies conducted in Ethiopia (100%) and Brazil (100%) (30). However, it was sensitive to ciprofloxacin in 97.7%; comparable to studies conducted in Ethiopia (96.7%) and China (96.9%). But it was higher than studies conducted in Brazil (85.7%) and Bangladesh (57.1%) while it was lower than the study conducted in Ethiopia (100%).

Streptococcus pneumoniae was resistant to oxacillin in 56.4%; comparable with studies conducted in Bahir Dar, Ethiopia (56.7%) and Jimma, Ethiopia (55.0%). This organism was less resistant to Clindamycin (10.3%) which is comparable with a study conducted in Ethiopia (8.3%). *Staphylococcus* aureus was resistant to penicillin in 75.9% which is comparable with studies in Bahir Dar, Ethiopia (75%) (20), Mekelle, Ethiopia (77.8%) and Brazil (75.0%). *Pseudomonas aeruginosa* was resistant to Gentamicin (59.1%); comparable with studies conducted in Ethiopia (57.9%) and Bangladesh (66.7%) while it was higher than studies conducted in Ethiopia (5.0%), China (14.7%) and Brazil (3.4%).

The overall prevalence of Multi-Drug Resistant (MDR) isolates in this study was 63.1% (n= 99/157). This finding was comparable with studies conducted in Jimma and Arbaminich, Ethiopia with the prevalence of 56.7% and 54.8% respectively. But it was lower than a study conducted in Ethiopia, Bahir Dar (76.0%) and higher than studies conducted in Ethiopia, Mekelle (17.9%) (26) and China (24.5%). Generally, the differences in antibiotic resistance patterns could be due to different reasons poor quality of antibiotics; antibiotic prescribing differences in different areas; misuse and/ or incomplete treatment courses of antibiotics due to lack of awareness and lack of access to appropriate treatment; empirical treatment due to the absence of well-organized bacteriology laboratories; and variations in buying and using antibiotics from private pharmacies without prescription which is common in Ethiopia.

From a total of 157 culture positive study participants, in general, 155 (99%) of them were due to community-acquired pneumonia (CAP) and two (1%) of them were due to hospital-acquired pneumonia (HAP). The two cases of HAP developed symptoms at 3rd and 5th days after initially admitted due to accident and inability to urinate respectively.

In the present study, age group of >64 years was 2.4 times more likely to have bacterial pneumonia compared to the age group of 5-15 years. Several studies showed aging as a risk factor for bacterial pneumonia such as those conducted in Spain, Pakistan, Japan and USA. The decline of the immune status in the older age may be the possible reason. However, there were studies conducted in Mekelle, Ethiopia and Ghana that indicated young age as the risk factor for the disease.

In this study, smoking increases the risk of bacterial pneumonia 4.7 times compared to those who were non-smokers. There were other studies which indicated cigarette smoking as the risk factor for this disease such as those conducted in Kenya and Spain. This may be due to the fact that smoking decreases the number and, at the same time, the action of cilia facilitating the entry of microorganisms to the respiratory tract.

Alcohol consumers (although this study did not identify the level of consumption) were 5.6 times more likely to have bacterial pneumonia compared to those who were non-consumers. Several studies conducted in Ethiopia, China, Spain, England and Europe showed that alcohol consumption increased the risk of bacterial pneumonia. This may be due to the fact that the sedative properties of alcohol minimize oropharyngeal tone that results in a high risk of aspiration of pathogens from the upper respiratory tract. Additionally, high levels of alcohol consumption can alter the alveolar macrophage function, hence withdrawing pulmonary defense against infection. Alcohol depresses cough, decreases endothelial adherence, lowers chemotaxis and suppresses B and T-cell spreading out which contributes to reduced clearance mechanism of lung cells. Although not statistically significant, bacterial pneumonia was higher among study participants with chronic co-morbid diseases, as the immunity gets weakened.

Conclusion

The overall prevalence of bacterial isolates in this study was 38.7%, which is high with Klebsiella *pneumonia*, 28.0%, and *Streptococcus pneumonia*, 24.8%, being the predominant isolates. High prevalence of multi-drug resistant (MDR), 63.1%, and methicillin-resistant Staphylococcus aureus (MRSA), 34.5%, were also observed. The predominant isolate *Klebsiella pneumonia* was highly resistant to tetracycline, 95.5%, followed by Ampicillin, penicillin, amoxicillin/ clavulonic acid, co-trimoxazole, erythromycin and tetracycline in 81.5%, 75.9%, 61.2%, 56.4%, 48.5% and 40.4% of the isolates respectively. Older age, cigarette smoking and alcohol use were factors associated with culture positivity and repeated prescription and use of antimicrobials were significantly independent factors of bacterial resistance. Therefore, expanding routine bacterial culture and identification with antimicrobial susceptibility testing and strengthening regular surveillance systems are essential for appropriate patient care and focus should be given to life style factors to minimize the risk of the disease.

Data Availability

The dataset used to support the findings of this study are available from the corresponding author upon request E.mail:tedydes21@gmail.com

Conflicts of interests

The authors declare that they have no competing interests.

Authors' Contributions

TD conceived the research idea. TD has also involved in the data collection and interpretation of the results. MT and MJ have involved in interpretation of the result and evaluating the scientific content of the study. MM has involved in data analysis and rationalizing the method section and manuscript preparation. All authors read and approved the final manuscript for submission.

Acknowledgments

We would like to thank University of Gondar, Amhara Public Health Institute-Dessie Branch and Dessie Referral Hospital for their support. We also thank Mr. Abdu Hussein for his contribution in the data collection process. Finally, our acknowledgment goes to the study participants.

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