Anto P V1/ Afr.J.Bio.Sc. 6(5) (2024). 707-715 **ISSN: 2663-2187**

https://doi.org/ 10.33472/AFJBS.6.5.2024. 707-715

African Journal of Biological Sciences

Unveiling the Microbial Mysteries: Exploring the Multi Drug resistant Bacterial Trends in Sputum from Individuals Suspected for Tuberculosis in a Tertiary care hospital, Chennai

Anto P V[1], Vidyaa Nayaki Suppiah[2], Sukumar Bavadharani[3]

1. Tutor, Department of Microbiology, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University)
2. Assistant Professor, Department of Microbiology, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University)
3. Assistant Professor, Department of Microbiology, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University)

Corresponding Author - Anto P V

Article History
Volume 6, Issue 5, Apr 2024
Received: 16 Apr 2024
Accepted: 23 Apr 2024
doi: ***10.33472/AFJBS.6.5.2024. 707-715***

Abstract

Introduction: Respiratory tract infections, especially lower respiratory infection (LRTI) is the most common infection, accounting for over 90% cases, with over 7 million deaths annually. India, being a developing country, accounts for 25% of tuberculosis worldwide. Diagnosing and differentiating Tuberculosis from other LRTI pose a severe challenge. There are many microorganisms which can lead to LRTI, commonest being Streptococcus pneumoniae, Klebsiella species etc. Emergence of antibiotic resistance is a major concern as it leads to significant increase in the mortality and morbidity.

Aim: the aim of this study is to identify the bacterial pathogens isolated from sputum samples suspected of tuberculosis. Materials and

Methods: A descriptive cross-sectional study was carried out for 6 months. ZN staining was done to rule out tuberculosis, the samples were further inoculated into appropriate culture media and appropriate biochemical test was perform to identify the isolate along with AST according to CLSI 2022 guidelines. Statistical analysis of the data was done using SPSS version 22. Results: Of 453 samples, 197 samples had bacterial growth, the commonest being Streptococcus pneumonia (31%), followed by Klebsiella pneumoniae (29%) and Staphylococcus aureus. Our study also showed it has highest MDR isolates among Klebsiella, Pseudomonas aeruginosa, etc, which was similar to other studies. This raises the alarm of increasing antibiotic resistance among microorganisms, thereby significantly affecting the quality of life among patients.

Conclusion: There is a increasing threat to the community due to emergence of multi drug strains. In order to efficiently cut down the emergence and spread of multi drug strain, a structured outline of investigations has to be followed to find out the isolates and antimicrobial susceptibility test as per guidelines has to be followed to avoid use of unnecessary antibiotics.

Key words: Lower Respiratory Tract Infections (LRTI), Tuberculosis, Pneumoniae, Klebsiella pneumonia, Streptococcus pneumonia, MDR

Introduction

Respiratory tract infections (RTIs) are the most common type of infections in humans, with lower respiratory tract infections (LRTIs) accounting for over 90% of cases [1]. Viruses are the main cause of Upper Respiratory Tract Infections (URTIs), while bacteria are more commonly responsible for LRTIs compared to fungi and viruses. These infections result in over 7 million deaths annually [2]. Differentiating tuberculosis (TB) from other lower respiratory tract infections, like bacterial pneumonia, can be challenging, especially in less developed countries. This difficulty can lead to more severe consequences, including a higher mortality rate. [3].

India is among the 22 countries that have the highest incidence of tuberculosis infections and is estimated to account for 25% of all tuberculosis cases worldwide. Pulmonary tuberculosis is the most common form of tuberculosis [4]. Numerous global studies have highlighted *Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Moraxella catarrhalis, Escherichia coli, Acinetobacter Spp.,* and *Streptococcus pyogenes* as the most powerful pathogens responsible for causing respiratory tract infections (RTIs) [5]. Respiratory bacterial pathogens can have a significant impact on public health by causing infections in individuals with weakened immune systems as well as those who are generally healthy. These pathogens, such as *S. pneumoniae, K. pneumoniae* and *H. influenzae*, are capable of causing post-viral infections in various settings, including hospitals and communities. This is due to their capsules, which provide them with antiphagocytic properties that allow them to thrive in the airways and lead to pneumonia. [6].

The issue of antibiotic resistance is a significant concern in developing countries, primarily due to frequent exposure to infectious diseases, excessive use of antibiotics, poor quality of antibiotics, and limited financial resources [7]. Globally, there has been a rise in multi-drug resistance (MDR), posing a serious threat to public health. Recent studies have shown the emergence of MDR bacterial strains from diverse sources like humans, birds, cattle, and fish. This underscores the importance of regular antimicrobial susceptibility testing to determine the most effective antibiotic treatment and to detect any emerging MDR strains. [8].

As far as we know, there have been no previous efforts made to investigate the bacterial prevalence of sputum samples from patients suspected of having tuberculosis in India. Therefore, this research was conducted to analyse the range of bacteria present in the sputum of these patients and to assess the susceptibility of these isolates to antibiotics especially to focus on MDR organisms.

Materials and Methods

This wasa Descriptive cross-sectional study conducted in a tertiary care hospital, Chennai. Over a period of 6 months, starting from January to June 2023. The study included 453 individuals suspected of having TB who visited the Hospital during this time.

Upon arrival, each patient was instructed to provide an early morning sputum sample in a dry, clean and leak-proof container. These samples were then carefully transported to the laboratory for further analysis. At the laboratory, the sputum samples were examined under a microscope using the Ziehl-Neelsen staining method to detect the presence of AFB bacilli.Subsequently, the samples were involved in bacteriological testing.

Following a similar protocol outlined in a prior study, uncentrifuged samples were streaked onto various agar plates including Chocolate agar, Blood agar and MacConkey agar under sterile conditions. The agar plates were then incubated at 37°temperature to facilitate bacterial growth.Identification of the bacterial isolates was carried out based on their unique morphological and biochemical characteristics [9]. This comprehensive approach allowed for a thorough analysis of the samples to determine the presence and nature of TB in the suspected patients.

The Antibiotic Susceptibility Test was performed using a modified Kirby-Bauer disc diffusion method in compliance with CLSI guidelines (CLSI 2022). A total of 16 commonly used antibiotics sourced from Hi-Media, based in India, were tested during the process.

Every set of media and chemicals underwent rigorous testing to ensure both sterility and effectiveness. To maintain quality control during the study, *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) are used.

Statistical analysis: The data analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 23.0. The frequency and percentage of each value were reported.

Results

The research involved 453 Sputum samples taken from individuals suspected for Tuberculosis, out of which 197(43%) had bacterial growth other than Mycobacteriumand 256(57%) werenormal commensals (Figure 1). Out of this 197, 64% (126) were male and 36% (71) were female. The age range of the patients varied from 1 to 96 years old. The majority (39.5%) were from 40 to 60 years old. More than 76% of them were Rural residents and 27.4% were immunocompromised patients who had various other health issues such as Diabetes mellitus, HIV etc,Also, only 3(1.5%) were confirmed for TB positive (Table-1).

Among these 197 bacterial isolates, 79 (40%) were gram positive cocci(GPC) and 118 (60%) were gram negative bacilli. Out of these, 31% were *Streptococcus pneumoniae* and 29% were *Klebsiella pneumoniae*. Other than these, *Staphylococcus aureus, Acinetobacter baumannii, Citrobacter koseri, Enterobacter aerogens,* and *Pseudomonas aeruginosa* are the other organisms that are isolated(Figure 2).

The antimicrobial sensitivity of the organisms that are isolated has been shown in Tables 2(for GPC)& 3 (for GNB). The antimicrobial sensitivity results of these isolates showed that most of the organisms, that is more than 60% were Multi drug resistant (MDR) organisms.

Characteristics	Frequency n=197 (%)

Sex	Male	126 (64)
	Female	71 (36)
Age	1-10	2 (1.01)
	11-20	18 (9.1)
	20-40	47 (23.8)
	40-60	78 (39.5)
	≥61	52 (26.3)
Residence	Urban	46 (23.3)
	Rural	151 (76.6)
TB infection	Yes	3 (1.5)
	No	194 (98.5)
Immunocompromised	Yes	54(27.4)
	No	143 (72.5)
Cigarette smoking	Yes	124(62.95)
	No	58 (29.44)
	In past	15 (7.6)
Alcohol consumption	Yes	87 (44.1)
	No	98 (49.7)
	In Past	12 (6.09)

Table 1 Socio-demographic characteristics, Clinical and other relevant data of the study participants

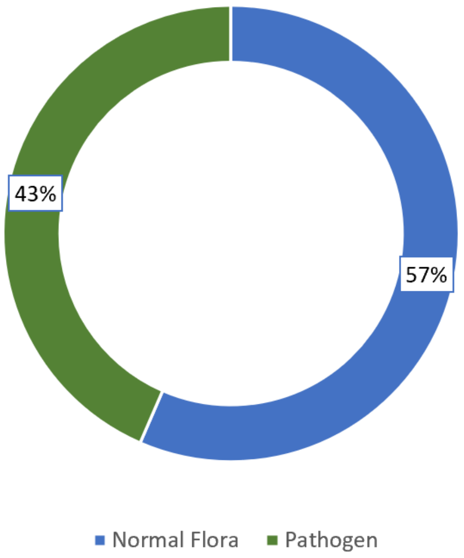

Figure 1 Total number of organisms isolated from samples

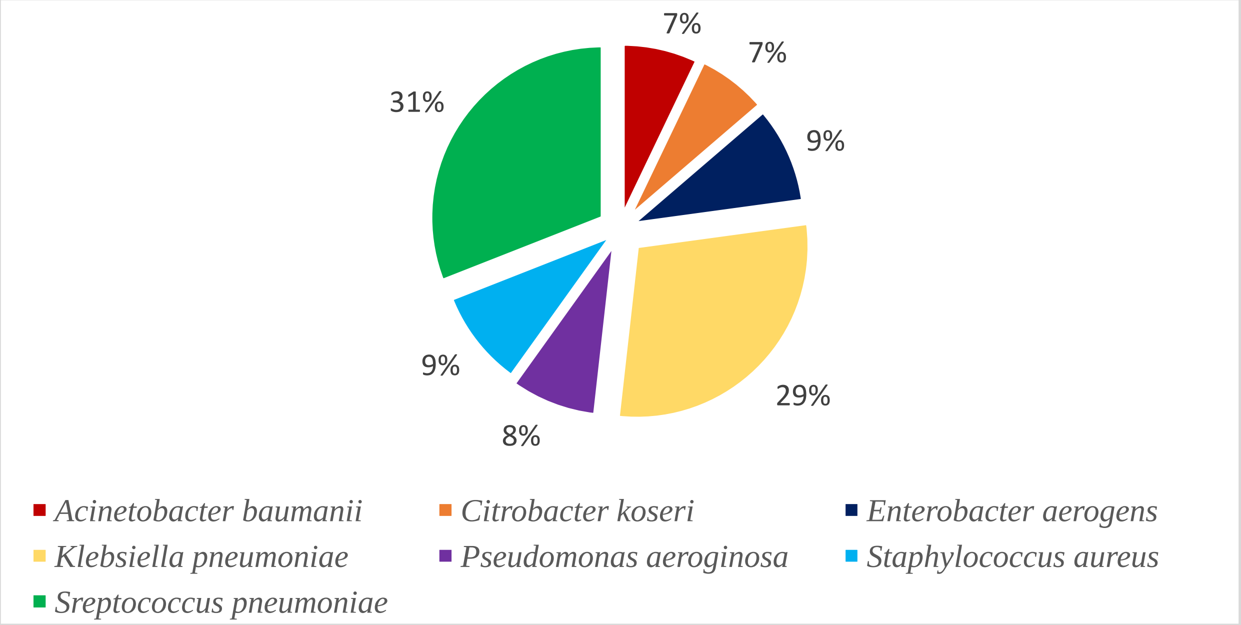

Figure 2 Bacterial Distribution

GPC Organisms	Penicillin	Co-trimoxazole	Cefotaxime	Erythromycin	Clindamycin	Tetracycline	Vancomycin	Linezolid
Staphylococcus aureus (n=18)	3(16)	6 (33)	6(33)	7 (39)	7 (39)	8 (44)	15 (83)	18 (100)
Streptococcus pneumoniae (n=61)	7 (11)	11(18)	13(21)	21(34)	21(34)	24(39)	53(86)	57(93)

Table 2 Antibiotic Sensitivity Pattern of Gram-Positive Cocci

GNB Organisms	Ceftazidime	Ciprofloxacin	Amikacin	Meropenem	Piperacillin and Tazobactam	Gentamicin	Polymyxin B	Levofloxacin
Acinetobacter baumanii(n=14)	2(14)	2 (14)	4 (28)	13(93)	8(56)	7 (50)	14(100)	13(93)
Citrobacter koseri (n=13)	2 (15)	2(15)	3(23)	7(54)	11(84)	7(54)	13(100)	13(100)
Enterobacter aerogenes (n=18)	1 (5)	3 (16)	6 (32)	15 (83)	17 (94)	9 (50)	18 (100)	18 (100)
Klebsiella pneumoniae (n=57)	1 (1.7)	4 (7)	11 (19)	47 (82)	46 (80)	36 (63)	57 (100)	54 (94)
Pseudomonas aeruginosa (n=16)	11 (68)	2 (12.5)	9 (56)	11 (68)	9(56)	7 (43)	16 (100)	16 (100)

Table 3 Antibiotic Sensitivity Pattern of Gram-Negative Bacilli

Discussion

Lower respiratory tract infections caused by multidrug-resistant organisms are a serious global threat, particularly in critically ill patients, leading to increased healthcare costs and potentially life-threatening consequences. Understanding the local infection rates, bacteriological profiles, and antibiotic resistance patterns is essential to address this challenge. The focus of this study is on the prevalence of Multi Drug resistant Bacterial pathogens in Sputum samples obtained from individuals suspected of having Tuberculosis at a leading tertiary care hospital in Chennai, India. A striking revelation from this research is the alarming levels of antibiotic resistance observed among the identified pathogens. Surprisingly, a majority of the bacterial strains exhibited extremely high resistance to various classes of antibiotics. This trend underscores the urgent need for effective strategies to combat antibiotic resistance, as these drugs are crucial for managing infections.

Our research revealed that male patients were the most affected by Lower Respiratory Tract Infections (LRTIs), comprising 64% of the cases (126 patients). This aligns with a recent study conducted in Nepal, which also showed a higher prevalence of LRTIs among males at 54.44%. The increased incidence of LRTIs among men in our study could be attributed to lifestyle factors such as smoking and alcohol consumption. Furthermore, the majority of participants in our study were in the 40 – 60 years age group, accounting for 39.5% of the total number (n=78). This finding is consistent with previous studies conducted by Attia et al 2019 and Ramesh Sharma Regmi et al., 2020, where a prevalence of 53% and 55.76% in this age group was reported, respectively [9]. Individuals in the 40–60 age group are more vulnerable to infections due to their weakened immune system and increased exposure to environmental factors.

This study accounted 62.95% bacterial growth from the smokers. This rate is in par with other study carried out by Ngekeng*et al.,* where it was 61.10% [5]. Also, in another study by V Sindhanai*et al.,* about 67 (48.55%) patients who were smokers, had predominant growth of bacterial isolates [10].

The rate of bacterial growth among alcohol consumers was 44.1% in this study, which was in concordance with a study carried out in Nigeria with a prevalence of 53.9% [5]. Higher prevalence of bacterial growth in those who were habitual to smoking and alcoholism may be because smoking and alcoholism usually increase risk to lower respiratory tract infections by diminishing mucosal immunity [11].

In this research, it was observed that bacterial growth was detected in the sputum of 43% (197 out of 453) individuals suspected of having tuberculosis. This finding aligns closely with a similar study conducted by Ramesh Sharma Regmi et al., 2020, which reported a bacterial growth rate of 47.33% (71 out of 150) among individuals suspected of having tuberculosis [9]. Conversely, a study from Nigeria reported a higher growth rate of approximately 61.37% [5]. The discrepancies in these findings could be attributed to the diverse collection and processing methods utilized across different locations.

This study was carried out to find out the most common isolate in sputum sample among tuberculosis suspected patients. The predominant isolate in our study was *Streptococcus pneumoniae*, followed by *Klebsiella pneumoniae* and *Staphylococcus aureus* and

Enterobacter aerogens. The results were slightly different to studies conducted by V Sindhanai*et al*, Muluneh Assefa *et al*, Ramesh Sharma Regmi [8,9,10]. Study conducted by V sindhanai*et al* showed*Klebsiella pneumonia* is the predominate isolate, followed by *Pseudomonas aeruginosa* [10], whereas in a study by Muluneh Assefa *et alKlebsiella pneumonia* was the predominant isolate followed by *Streptococcus pneumoniae* [8]. In a study by Ramesh Sharma Regmi, Pseudomonas was the predominant isolate This shows the geographical diversity in distribution of the microbial pathogens [9].

The most commonly isolated gram-negative bacillus in our study was *Klebsiella pneumoniae*, accounting for 29% of all isolates. This represents a significant increase compared to a study conducted nearly ten years ago, which reported a prevalence of only 10.8% for *K. pneumoniae* [12]. A more recent study by Miriti *et al.*, 2023, found that *Klebsiella species* had an isolation rate of 19.8% [13]. The shifting prevalence of *K. pneumoniae* is alarming, particularly for patients at a higher risk of lower respiratory tract infections.

Furthermore, we have identified *P. aeruginosa* at a rate of 8%, which aligns with the results reported by Ramesh Sharma Regmi et al., 2020 who observed isolation rates of approximately 9.33% and 11.96% respectively [9, . The high prevalence of *P. aeruginosa* in sputum samples may be attributed to its versatile nature in colonizing various ecological habitats, including air pollutants, animals, and humans.

Out of 18 *Staphylococcus aureus* isolates, 16 isolates were Methicillin resistant isolates. 15 isolates were sensitive to vancomycin whereas all isolates were sensitive to Linezolid, which was similar to study by V. Sindhanai*et al* [9]. In our study most of the isolates of Staphyloccoccus aureus were resistant to Penicillin, cotrimaxozole, erythromycin and clindamycin, whereas all isolates were sensitive to Linezolid.

Of 61 isolate *Streptococcus pneumoniae*, only around 35-40% of the isolates were sensitive to Clindamycin, Erythromycin and Tetracyclin , similar to study by Assefa M*et al* where maximum percentage of the isolates showed resistance to these drugs [8]. In our study nearly 85% of the isolates were sensitive to Vancomycin.

Of 14 isolates of *Acinetobacter baumanii* , most of the isolates were resistant to ciprofloxacin, amikacin, gentamicin and ceftazidime , whereas 93% of the isolates were sensitive to meropenem and Levoflaxacin. This was similar to a study by BirasenBehra*et al* where Acinetobacter isolates were resistant to most classes of drugs [14].

The present study documented highest MDR isolates in the following order *Klebsiella pneumoniae*, *Acinetobacter* sp, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Citrobacter* sp. Among the antibiotics that were tested, most of the gram negative organisms were found to be resistant to ceftazidime, ciprofloxacin and amikacin. *Klebsiella pneumoniae* (98.3%, 93%, 81%) was found to be the predominant isolate resistant to all the three aforementioned drugs respectively. Similar to our findings, RegmiRS *et al.*, also reported *K. pneumoniae* as the top contributor among the Gram-negative isolates [9]. Being the second most common MDR isolate, *Acinetobacter* sp was found to be resistant to Levofloxacin (7%)

and meropenem (7%) group of drugs. Whereas, ceftazidime and ciprofloxacin had a resistance percentage of 96% for *Acinetobacter* sp. However, *Pseudomonas aeruginosa, Enterobacter aerogenes* and *Citrobacter* sp. was found to be resistant to all the other drugs in varying order.

In a previous study done by Pokhrel *et al.*, the authors have reported 55.56% MDR isolates which is higher than a study carried out in Kathmandu (47.57%) [15]. Another study in the same city also showed similar prevalence of MDR isolates (57.50%) [16]. Streptococcus pneumoniae was found to account for a higher resistance rate when compared with S*taphylococcus aureus.* This was in par with another study conducted byScott J *et al.*, and Nambafu *J et al.,* where Streptococcus pneumoniae was reported as the frequent contributor [17,18].

CONCLUSION

Our study clearly emphasizes the increase in respiratory infections caused by multidrug-resistant bacteria in patients suspected of having Tuberculosis. It is necessary to develop guidelines for reducing the use of antibiotics like carbapenems and colistin. It is evident that utilizing antibiotics more selectively, establishing surveillance programs to monitor antimicrobial resistance and implementing effective infection control measures are important in combating the rise of Multi drug-resistant bacteria. Further research is necessary to gain a deeper understanding of the patterns of antimicrobial resistance, as this information will be vital in shaping recommendations for empirical antibiotic treatment of common infections by national and regional regulatory authorities.

Reference

1. Thomas M, Bomar P, Koutsothanasis GA. Upper respiratory tract infection. Treasure Island: StatPearls Publishing; 2019. https ://www.ncbi.nlm.nih.gov/books /NBK53 2961/.
2. Ozyilmaz E, Akan OA, Gulhan M, Ahmed K, Nagatake T. Major bacteria of community-acquired respiratory tract infections in Turkey. Japanese journal of infectious diseases. 2005 Feb 28;58(1):50-2.
3. Attia EF, Pho Y, Nhem S, Sok C, By B, Phann D, Nob H, Thann S, Yin S, Noce R, Kim C. Tuberculosis and other bacterial co-infection in Cambodia: a single center retrospective cross-sectional study. BMC pulmonary medicine. 2019 Dec;19:1-7.
4. Bindu KH, Sainath E, Kondaveti S. A PROSPECTIVE STUDY OF DRUG RELATED PROBLEMS IN TUBERCULOSIS PATIENTS UNDERGOING TREATMENT IN A TERTIARY CARE HOSPITAL. Int J Acad Med Pharm. 2024;6(1):385-91.
5. Ngekeng S, Pokam BT, Meriki HD, Njunda AL, Assob JC, Ane-Anyangwe I. High prevalence of bacterial pathogens in sputum of tuberculosis suspected patients in Buea. British Microbiology Research Journal. 2015 Nov 21;11(5):1-8.
6. Feldman C, Anderson R. Pneumococcal virulence factors in community-acquired pneumonia. Current opinion in pulmonary medicine. 2020 May 1;26(3):222-31.
7. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrobial Resistance & Infection Control. 2017 Dec;6(1):1-8.
8. Assefa M, Tigabu A, Belachew T, Tessema B. Bacterial profile, antimicrobial susceptibility patterns, and associated factors of community-acquired pneumonia among adult patients in Gondar, Northwest Ethiopia: A cross-sectional study. PloS one. 2022 Feb 1;17(2):e0262956.
9. Regmi RS, Khadka S, Sapkota S, Adhikari S, Dhakal KK, Dhakal B, Lamsal B, Kafle SC. Bacterial etiology of sputum from tuberculosis suspected patients and antibiogram of the isolates. BMC research notes. 2020 Dec;13:1-6.

10. Sindhanai V, Jayavarthinni M, Abarna V, Sethumathavan K. Prevalence of bacterial and fungal agents in lower respiratory tract infections regarding chronic obstructive pulmonary disease in a tertiary care hospital. Int J Acad Med Pharm. 2023;5(1):447-51.
11. Akingbade OA, Ogiogwa JI, Okerentugba PO, Innocent-Adiele H, Nwanze J, Okonko I, et al. Prevalence and antibiotic susceptibility pattern of bacterial agents involved in lower respiratory tract infections in Abeokuta, Ogun State, Nigeria. Rep Opin. 2012;4:25–30.
12. Hasan AZ, Al-sulami A, Al-taee A. Gram positive and gram negative bacteria from sputum of clinically tuberculosis suspected patients. Int J Curr Res. 2015;7:14289–91.
13. Miriti DM, Muthini JM, Nyamache AK. Study of bacterial respiratory infections and antimicrobial susceptibility profile among antibiotics naive outpatients visiting Meru teaching and referral hospital, Meru County, Kenya in 2018. BMC microbiology. 2023 Jun 29;23(1):172.
14. Behera B, Sahu KK, Bhoi P, Mohanty JN. Prevalence and antimicrobial susceptibility patterns of bacteria in ICU patients with lower respiratory tract infection: A cross-sectional study. Journal of Acute Disease. 2020 Jul 1;9(4):157-60.
15. Pokhrel BM, Koirala J, Mishra SK, Dahal RK, Khadga P, Tuladhar NR. Multidrug resistance and extended spectrum betalactamase producing strains causing lower respiratory tract and urinary tract infection. J Inst Med. 2006;28:19–27.
16. Thapa P, Bhandari D, Shrestha D, Parajuli H, Chaudhary P, Amatya J, et al. A hospital based surveillance of metallo-beta-lactamase producing gram negative bacteria in Nepal by imipenem-EDTA disk method. BMC Res Notes. 2017;10:1–6.
17. Scott J, Hall A, Muyodi C, Lowe B, Ross M, Chohan B, Mandaliya K, et al. Etiology, outcome and risk factors for mortality among adults with acute pneumonia in Kenya. Lancet. 2000;355(9211):1225–30.
18. Nambafu J, Achakolong M, Mwendwa F, Bwika J, Riunga F, Gitau S, et al. A prospective observational study of community acquired pneumonia in Kenya: the role of viral pathogens. BMC Infect Dis. 2021;21(703):1–9.