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UNRAVELLING THE BIOCHEMICAL WEB: INSIGHTS INTO PATHOGENS AND RESISTANCE IN LOWER RESPIRATORY INFECTIONS

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ABSTRACT

Background: Lower respiratory tract infections (LRTIs) are a major cause of morbidity and mortality, particularly in intensive care unit (ICU) patients. Identifying the causative pathogens and understanding antimicrobial resistance (AMR) patterns is critical for optimising treatment and improving patient outcomes.

Objective: This study used bronchoalveolar lavage (BAL) samples in ICU settings to investigate the prevalence of infectious pathogens and antimicrobial resistance in patients with LRTIs.

Methods: BAL samples were collected from ICU patients with LRTIs and analysed for bacterial, viral, and fungal pathogens. Biochemical tests and molecular techniques were employed to identify pathogens and detect antimicrobial resistance genes, including CTX-M, OXA-48-like, and NDM.

Results: The study revealed that Klebsiella pneumoniae, Escherichia coli, and Human Rhinovirus/Enterovirus were the significant causative microorganisms in LRTIs. Fungal pathogens were infrequent in the analysed samples. A high prevalence of antimicrobial resistance genes, including CTX-M, OXA-48-like, and NDM, was detected in most samples, indicating significant multidrug resistance among the bacterial pathogens.

Conclusion: The findings highlight the critical need for antimicrobial stewardship, the use of rapid diagnostic techniques, and the development of targeted antimicrobial therapies to manage LRTIs effectively in ICU patients. Addressing the high prevalence of AMR genes is essential for improving infection control strategies and patient outcomes at the local level.

Keywords: Bronchoalveolar lavage, Bacterial pathogens, Antimicrobial resistance, Viral infections, Respiratory tract infections.

INTRODUCTION

LRTIs are a significant cause of morbidity and mortality in hospitalized patients, leading to a substantial economic burden. It causes nearly 2.4 million deaths globally among people of all ages. South Asia, along with sub-Saharan Africa and Southeast Asia, have a high fatality rate among these [1]. There has been a consistent rise in hospitalisation (including ICU) rates in recent years among the elderly population for community-acquired pneumonia (CAP)[2]. The increasing prevalence of AMR has become a primary global health concern. It complicates the management of respiratory infections.

Bacteria and viruses are the primary cause of LRTI, but clinically, it's tough to distinguish because of the similarity of their symptoms. Some rapid tests are available for specific pathogens like Streptococcus pneumoniae and RSV, along with markers like procalcitonin to differentiate between bacterial and viral infections. However, these tests alone often aren't enough to diagnose and treat LRTI effectively [3].

The BAL procedure, coupled with rapid microbiological testing, is an essential diagnostic tool that samples lower respiratory tract secretions. It facilitates the identification of pathogens and provides valuable knowledge of the microbial landscape of the LRTI within a few hours. It also enables clinicians to effectively adjust broad-spectrum antibiotics early in the management. Quickly figuring out what's causing a respiratory infection and spotting signs of antibiotic resistance can help avoid the overuse of broad-spectrum antibiotics.

This study aims to investigate the biochemical characteristics of BAL samples using FilmArray Pneumonia Plus Panel (PNplus panel). It focuses on identifying the prevalence of different pathogens and AMR genes. This research seeks to inform more effective treatment protocols by understanding the microbiological profile and resistance patterns at a local level. The findings from this study will contribute to the growing body of knowledge on respiratory infections. It will offer insights into AMR that could improve patient outcomes and infection control practices.

METHODOLOGY

Study Design and Setting

This observational study was conducted at Bahria International Hospital, Lahore, from April 2022 to August 2024. Adult patients aged 18 years and older, admitted to the Intensive Care Unit (ICU) with a clinical suspicion of lower respiratory tract infections (LRTI), were included in the study. Patients were excluded if they had received antibiotic treatment within 48 hours before sample collection or were under 18. Ethical approval was obtained from the hospital's ethics committee, and informed consent was secured from all participants or legal guardians.

Sample Collection and Processing

Bronchoalveolar lavage (BAL) samples were collected using a standardised bronchoscopy procedure under strict aseptic conditions. The procedure was performed by trained healthcare professionals using sterile suction catheters. Collected aspirates were immediately transported to the microbiology laboratory in sterile containers for further processing.

Upon receipt, the BAL samples were processed using the FilmArray Pneumonia Plus Panel (PNplus panel) technique to identify bacterial, viral, and fungal pathogens. The panel also screened for antimicrobial resistance (AMR) genes. All laboratory procedures followed standard protocols to ensure sample integrity and reliability of results.

Data Analysis

Data on the frequency and distribution of pathogens and AMR genes were recorded. Prevalence rates were calculated, and statistical analyses were performed using Microsoft Excel. Descriptive statistics, including means, standard deviations, and percentages, were used to summarise the data. The final results were presented in tabular and graphical formats to highlight the key findings of pathogen distribution and AMR gene prevalence.

RESULTS

Thirty-nine patients participated in this study, with a mean age of 61.13 years, ranging from 32 to 81 years. Among these, 22 males and 17 females presented with symptoms indicative of LRTI and were admitted to the ICU for further management. The gender distribution is shown in Figure 1.

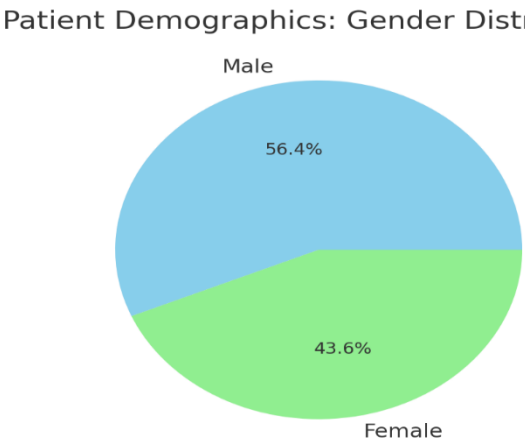

figure 1: Gender Distribution

The analysis of BAL samples showed a significant presence of bacterial pathogens. Klebsiella pneumoniae was the predominant bacteria, found in 23 patients (59%), followed by *Escherichia coli* in 18 patients (46%) and *Pseudomonas aeruginosa* in 16 patients (41%). Other bacterial isolates included *Acinetobacter calcoaceticus-baumannii complex* in 13 patients (33%), *Staphylococcus aureus* in 9 patients (23%), and both *Streptococcus pneumoniae* and *Haemophilus influenzae*, each detected in 5 patients (13%). Less common bacteria such as *Klebsiella oxytoca*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Stenotrophomonas maltophilia*, *Serratia marcescens*, *Streptococcus agalactiae*, and *Proteus spp* were each identified in a single patient. In three patients, no bacterial pathogens were detected. This is illustrated in Figure 2**.**

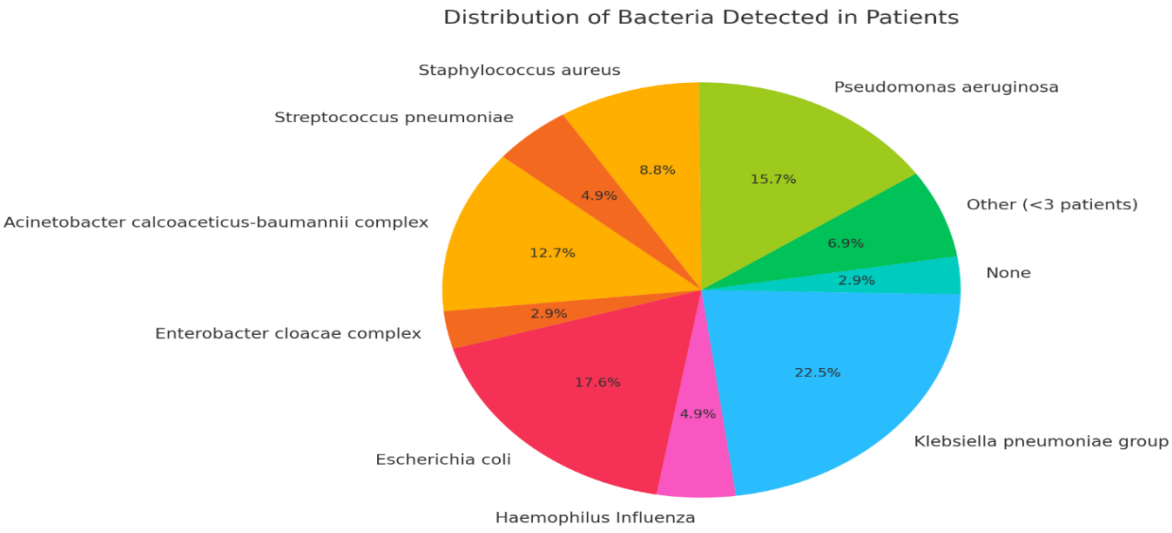

Figure 2: Bacterial Pathogen Distribution

Viral pathogens were also identified in a subset of the patients. The most frequently detected virus was Human Rhinovirus/Enterovirus, present in 8 patients (21%). Influenza A and Respiratory Syncytial Virus (RSV) were each identified in 2 patients (5%). However, most patients, 27 (69%), did not show any viral pathogens in their samples**.** (Figure 3).

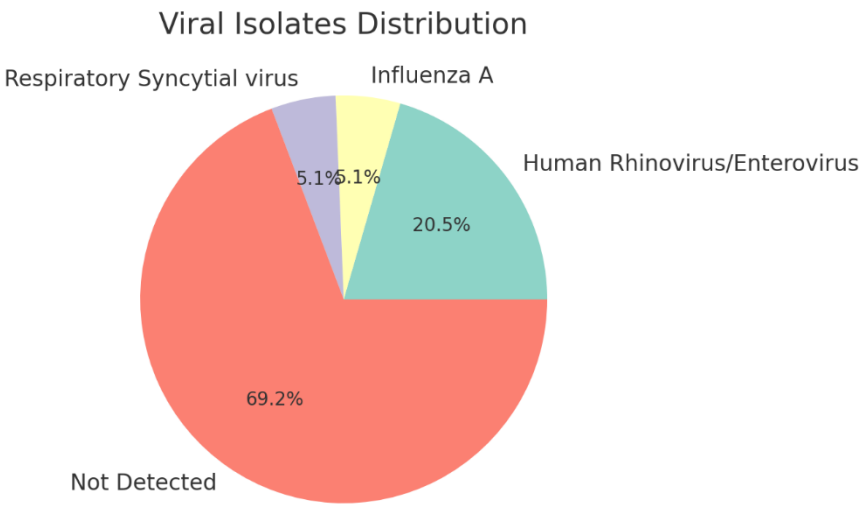

Figure 3: Viral Pathogen Distribution

Fungal pathogens were detected in only 2 cases: *Candida albicans* in one patient and *Aspergillus fumigatus* in another, accounting for 3% each of the total patient population. The remaining 37 patients (95%) showed no fungal growth. (Figure 4)

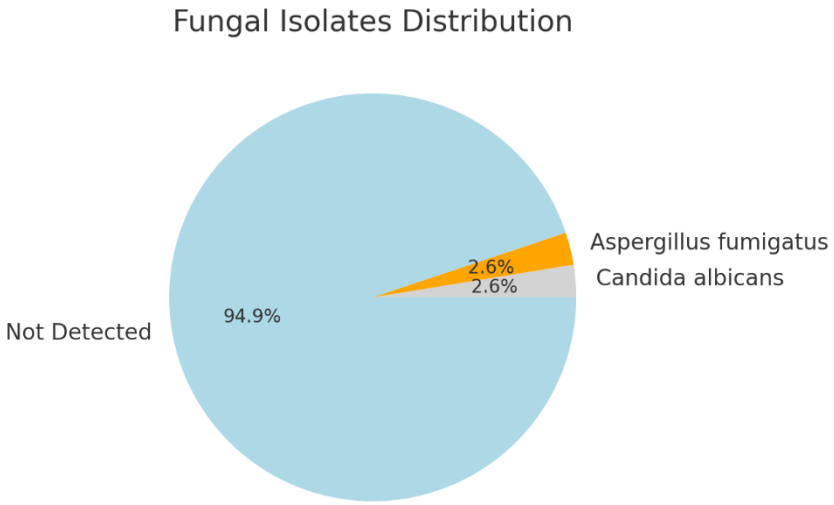

Figure 4: Fungal Pathogen Distribution

Bacterial isolates were tested for AMR genes, and a high prevalence of resistance genes was observed. CTX-M gene, associated with extended-spectrum beta-lactamase (ESBL) production, was the most common, detected in 28 patients (72%). The OXA-48-like gene, which encodes β-lactamase, was found in 25 patients (64%), and the NDM gene, associated with New Delhi metallo-beta-lactamase, was present in 27 patients (69%). The Verona integron-encoded metallo-beta-lactamase (VIM) gene was detected in 14 patients (36%). Methicillin resistance genes mecA/C and MREJ were identified in 4 patients (10%), whereas the IMP gene, linked to imipenemase metallo-beta-lactamase, was found in 3 patients (8%). Eight patients (21%) did not exhibit any screened resistance genes. (Figure 5).

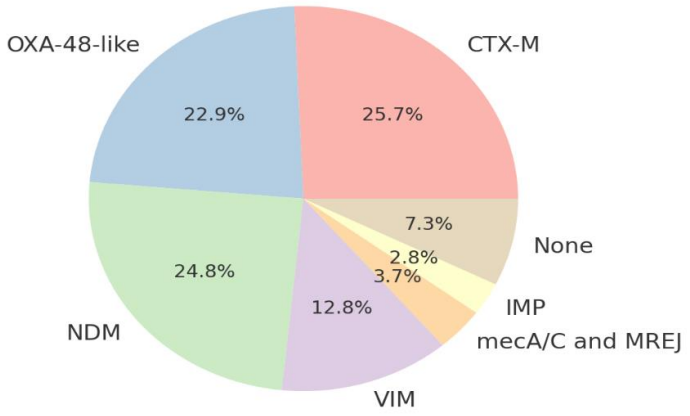

Figure 5: AMR Gene Distribution

The study included the detection of Mycobacterium tuberculosis (MTB) genes. Trace levels of MTB genes were found in 6 patients (15%), and high levels were detected in 1 patient (3%). No MTB genes were detected in 7 patients (18%), while the analysis was not applicable to 25 patients (64%).

DISCUSSION

The most predominant pathogens identified in BAL samples in this study were *Klebsiella pneumoniae* (59%), *Escherichia coli* (46%), *Pseudomonas aeruginosa* (41%) and *Acinetobacter baumannii* (33%). This type of microbiological pattern has traditionally been associated with hospital-acquired infections [4]. Their emergence in community-acquired cases signals a concerning shift in the epidemiology of respiratory diseases in Pakistan. A retrospective study by Uluç et al. (2024) reported Klebsiella pneumonia (24.8%) and *Pseudomonas aeruginosa* (14.4%) to be significant pathogens in BAL samples among the ICU population. They also found *Acinetobacter baumannii* (11.7%), consistent with our findings regarding prevalence but contrasts in detection percentage compared to total patients. The detection of non-albicans *Candida* (28.9%) in Uluç et al.'s aspiration samples suggests the importance of fungal pathogens in these clinical scenarios. However, our study only found one patient with *Candida albicans* [5]. Our study contrasts with a study done by Catia et al. (2011), in which the most common aetiologies were Streptococcus pneumoniae (42%) in ICU patients. Blood and urine samples were also used for testing in this study, along with BAL samples conducted in Spain[6]. This discrepancy with our results may be due to regional differences in pathogen prevalence and site of sampling.

Our study observed many resistance genes, with the CTX-M gene being the most common, detected in 28 patients (72%). CTX-M encodes ESBL, a common cause of community-acquired infections in Asia, South America, and Africa. These infections are mostly due to ESBL-E. coli[7]. The NDM-1 gene encodes a metallo-β-lactamase (MBL) that inactivates all β-lactams except aztreonam. It is identified mostly in Escherichia coli and K. pneumoniae and, to a lesser extent, in other enterobacterial species. It is implicated in pulmonary infection, UTI and other infections. It has been reported extensively in the Indian subcontinent [8.] It is consistent with our study (from Pakistan), which shows a high prevalence of the NDM gene (69%). The blaOXA-48 gene encodes the OXA-48 enzyme, which catalyses imipenem. K. pneumoniae is in plasmid and has been involved in outbreaks, such as one in Istanbul, from 2006 to 2007. It has also been identified in E. coli and Citrobacter freundii in countries like Turkey, Lebanon, and Belgium**.** Detection may be challenging due to low resistance levels, and its spread could be underestimated [9]. Our study, the OXA-48-like gene was found in 25 patients (64%), a significantly higher prevalence than earlier localised reports. This highlights the broader dissemination of OXA-48-like genes in our patient population, reinforcing concerns that its spread

may be more extensive. With around 150 variants, OXA enzymes show wide genetic diversity and varying β-lactam hydrolysis spectra, contributing to increasing reports of resistance in Gram-negative bacteria. The presence of VIM genes in 36% of patients reflects global trends in the spread of MBL, as noted by study Logan & Weinstein (2017), particularly in carbapenemase-producing *Klebsiella pneumoniae*. This is linked to multidrug resistance, reducing the effectiveness of carbapenems, often the last line of defence against severe infections [10]. The high prevalence of VIM genes in this ICU population indicates ongoing transmission of resistant organisms. It highlights the need for stringent infection control and new treatment options to combat MBL-producing bacteria. Our study finds methicillin resistance genes mecA and MREJ in 10% of patients and the IMP gene in 8%. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a key example of a disease that acquires resistance through horizontal gene transfer. It does this via mobile elements like the SCCmec cassette that carries the mecA gene. This has led to widespread, hard-to-treat hospital and community infections[11; due] to these AMRs, the WHO has placed carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and resistant *Enterobacteriaceae on* its priority list of pathogens[12].

Our study found that *Human Rhinovirus/Enterovirus* was the most frequently detected virus (21%), followed by Influenza A (5%) and RSV (5%). These findings are consistent with those reported by Altay-Kocak et al. (2022). It showed rhinovirus as the most prevalent virus (25.2%) and influenza A as the second most prevalent at 12.1%. Altay-Kocak, et al.'s study, included samples from patients across all age groups at a university hospital[13]. Tchatchouang et al.(2023) reported in a cross-sectional study conducted in Cameroon a viral prevalence of 22.1% among hospitalised LRTI patients with *Human rhinovirus* in 13% of patients as a predominant viral pathogen. However, the Cameroonian study also identified coronavirus and influenza A virus as significant contributors to LRTIs, with 7.8% of patients experiencing concomitant viral and bacterial co-infections. The viral detection rate was 20% in BAL (7/35) and 23.8% in sputum (10/42). Additionally, the Cameroonian study's context in the pre-COVID-19 era provides valuable information into the viral landscape before the global pandemic, highlighting the consistent role of common respiratory viruses across different geographic regions [14].

Our study found fungal pathogens in a few cases, with *Candida albicans* and *Aspergillus fumigatus* detected in only one patient (3%). This is lower than the 28.75% prevalence of fungal infections, predominantly *Candida albicans*, found in a cross-sectional study in India [15]. The discrepancy may stem from regional environmental differences and sample types, highlighting the need for tailored approaches to diagnosing and managing fungal infections in LRTIs. Meersseman et al. (2009) similarly noted the rarity of invasive fungal infections in the ICU, finding *Candida* in respiratory samples of 57% of critically ill patients but no cases of histologically proven *Candida* pneumonia. It suggested colonisation rather than actual infection [16]. Chakraborti et al. (2023) reported a higher fungal presence (5%), with *Candida albicans* as the dominant coloniser and 17% of patients showing invasive fungal disease. Their higher incidence likely reflects the high-risk population of long-term mechanically ventilated patients, who face increased risk due to factors like indwelling catheters and immunosuppressive therapies [17].

Our study's detection of MTB genes indicates a low prevalence of MTB-related infections among ICU patients with LRTIs. Trace levels of MTB genes were found in 15% of patients, suggesting either latent infection or minimal bacterial load, likely not contributing to the acute symptoms. However, one patient (3%) had high levels of MTB genes, which pointed to an active or significant infection. Studies like Lawn & Zumla (2011) stress the need for timely TB detection in high-risk groups to prevent worsened outcomes [18]. Interestingly, 18% of patients showed no MTB genes, indicating that not all ICU respiratory infections are linked to tuberculosis. In 64% of cases, MTB testing was not applicable, either due to clinical presentations that did not suggest TB or prior screenings ruling it out. The prevalence of resistance genes in our study likely resulted from the extensive use of broad-spectrum antibiotics. However, our study's relatively small sample size is a limitation because it may not fully represent the broader patient population. Additionally, generalizability is further limited by single-centre findings. More significant, multi-center studies with large sample populations are required to validate these results. Besides these limitations, our study's findings carry important

implications for clinical practice. The high prevalence of multidrug-resistant pathogens necessitates the urgent need for robust antimicrobial stewardship programs. It also highlights the importance of rapid diagnostic tests to identify resistance genes that could help clinicians initiate appropriate therapies more swiftly.

CONCLUSION

This study shows that *Klebsiella pneumoniae* and *Escherichia coli* are significant players in LRTI in ICU patients, with high antimicrobial resistance due to genes like CTX-M, OXA-48-like, and NDM. It highlights the complexity of these infections and the importance of thorough testing by finding viral and bacterial pathogens and fungal and tuberculosis genes.

REFERENCES

1. GBD 2016 Lower Respiratory Infections Collaborators (2018). Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet. Infectious diseases, 18(11), 1191–1210. https://doi.org/10.1016/S1473-3099(18)30310-4
2. Ramirez, J. A., Wiemken, T. L., Peyrani, P., Arnold, F. W., Kelley, R., Mattingly, W. A., Nakamatsu, R., Pena, S., Guinn, B. E., Furmanek, S. P., Persaud, A. K., Raghuram, A., Fernandez, F., Beavin, L., Bosson, R., Fernandez-Botran, R., Cavallazzi, R., Bordon, J., Valdivieso, C., Schulte, J., … University of Louisville Pneumonia Study Group (2017). Adults Hospitalized With Pneumonia in the United States: Incidence, Epidemiology, and Mortality. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 65(11), 1806–1812. https://doi.org/10.1093/cid/cix647
3. Murphy, C. N., Fowler, R., Balada-Llasat, J. M., Carroll, A., Stone, H., Akerele, O., Buchan, B., Windham, S., Hopp, A., Ronen, S., Relich, R. F., Buckner, R., Warren, D. A., Humphries, R., Campeau, S., Huse, H., Chandrasekaran, S., Leber, A., Everhart, K., Harrington, A., … Bourzac, K. M. (2020). Multicenter Evaluation of the BioFire FilmArray Pneumonia/Pneumonia Plus Panel for Detection and Quantification of Lower Respiratory Tract Infection Agents. Journal of clinical microbiology, 58(7), e00128-20. https://doi.org/10.1128/JCM.00128-20
4. Shebl E, Gulick PG. Nosocomial Pneumonia. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535441/
5. Uluç, K., Akkütük Öngel, E., Köylü İlkaya, N., Devran, Ö., Çolakoğlu, Ş. M., & Kutbay Özçelik, H. (2024). Analysis of 332 fiberoptic bronchoscopies performed in a respiratory intensive care unit: a retrospective study. European review for medical and pharmacological sciences, 28(4), 1433–1438. https://doi.org/10.26355/eurrev_202402_35465
6. Cillóniz, C., Ewig, S., Polverino, E., Marcos, M. A., Esquinas, C., Gabarrús, A., Mensa, J., & Torres, A. (2011). Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax, 66(4), 340–346. https://doi.org/10.1136/thx.2010.143982
7. Bush, K., & Bradford, P. A. (2020). Epidemiology of β-Lactamase-Producing Pathogens. Clinical microbiology reviews, 33(2), e00047-19. https://doi.org/10.1128/CMR.00047-19
8. Nordmann, P., Poirel, L., Toleman, M. A., & Walsh, T. R. (2011). Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria?. The Journal of antimicrobial chemotherapy, 66(4), 689–692. https://doi.org/10.1093/jac/dkq520
9. Poirel, L., Naas, T., & Nordmann, P. (2010). Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrobial agents and chemotherapy, 54(1), 24–38. https://doi.org/10.1128/AAC.01512-08
10. Logan, L. K., & Weinstein, R. A. (2017). The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. The Journal of infectious diseases, 215(suppl_1), S28–S36. https://doi.org/10.1093/infdis/jiw282

11. Chambers, H. F., & Deleo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature reviews. Microbiology, 7(9), 629–641. https://doi.org/10.1038/nrmicro2200
12. Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., Theuretzbacher, U., … WHO Pathogens Priority List Working Group (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet. Infectious diseases, 18(3), 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3
13. Altay-Kocak, A., Sarzhanova, S., Tapisiz, A., Dizbay, M., Basustaoglu, A., & Bozdayi, G. (2022). Retrospective evaluation of viral respiratory tract infections in a university hospital in Ankara, Turkey (2016-2019). Journal of infection in developing countries, 16(5), 857–863. https://doi.org/10.3855/jidc.14427
14. Tchatchouang, S., Kenmoe, S., Nzouankeu, A., Njankouo-Ripa, M., Penlap, V., Donkeng, V., Pefura-Yone, E. W., Fonkoua, M. C., Eyangoh, S., & Njouom, R. (2023). Viral etiology of lower respiratory tract infections in adults in the pre-COVID-19 pandemic era: A cross-sectional study in a single center experience from Cameroon. Health science reports, 6(5), e1234. https://doi.org/10.1002/hsr2.1234
15. Chiti, B., Seth, R. J., & Madoriya, K. (2023). Prevalence of lower respiratory tract fungal infection at a tertiary care hospital in Central Madhya Pradesh, India: A cross-sectional study. National Journal of Laboratory Medicine, 12(4), MO09-MO12. https://doi.org/10.7860/NJLM/2023/64013.2784
16. Shamim, Shelley; Agarwal, Abinash1; Ghosh, Bijan Kumar; Mitra, Mrinmoy. Fungal pneumonia in intensive care unit: When to suspect and decision to treatment. The Journal of Association of Chest Physicians 3(2):p 41-47, Jul–Dec 2015. | DOI: 10.4103/2320-8775.158837
17. Chakraborti, A., Jaiswal, A., Verma, P. K., & Singhal, R. (2018). A Prospective Study of Fungal Colonization and Invasive Fungal Disease in Long-Term Mechanically Ventilated Patients in a Respiratory Intensive Care Unit. Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine, 22(8), 597–601. https://doi.org/10.4103/ijccm.IJCCM_181_18
18. Lawn, S. D., & Zumla, A. I. (2011). Tuberculosis. Lancet (London, England), 378(9785), 57–72. https://doi.org/10.1016/S0140-6736(10)62173-3