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Original Research Article

Bacteriological Spectrum and Antimicrobial Resistance Pattern of Culture-Positive Sputum Isolates in a Tertiary Care Hospital of Vidarbha, Maharashtra: A Five-Year Retrospective Analysis

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Abstract

Background: Lower respiratory tract infections are a significant cause of illness and death, especially in tertiary care hospitals where antimicrobial resistance is rising. Sputum culture remains an important tool for identifying pathogens and guiding appropriate treatment. This study analysed the bacterial profile and antimicrobial susceptibility pattern of culture-positive sputum samples.

Methods: A retrospective observational study was conducted in the Department of Microbiology, Government Medical College, Nagpur, from January 2021 to December 2025. Sputum samples received from outpatient departments, wards, and intensive care units were included. Only satisfactory samples based on Gram-stain Barlett's criteria were processed. Cultures were performed on blood agar and MacConkey agar, and organisms were identified using standard biochemical methods. Antimicrobial susceptibility testing was carried out by Kirby–Bauer disc diffusion method according to CLSI guidelines.

Results: A total of 2068 culture-positive sputum samples were analysed. Most isolates were from the respiratory-chest department (62.5%), followed by medicine (17.2%), surgery (8.9%), paediatrics (5.2%), obstetrics and gynaecology (5.0%), and orthopaedics (1.2%). Klebsiella pneumoniae was the most common isolate (71.4%), followed by Pseudomonas aeruginosa (18.0%), Acinetobacter spp. (5.6%), and Staphylococcus aureus (4.2%). Enterobacterales showed high resistance to beta-lactams and fluoroquinolones, with carbapenem resistance of 38–40%. Non-fermenters demonstrated variable resistance, with higher resistance in Acinetobacter spp. Colistin resistance was not detected.

Conclusion: Klebsiella pneumoniae predominated among sputum isolates with notable multidrug resistance. Rising carbapenem resistance among Enterobacterales and Acinetobacter spp. underscores the need for antimicrobial stewardship and regular surveillance to guide empirical therapy.

Keywords: Antimicrobial resistance, Antibiotic susceptibility test, Enterobacterales, Sputum culture.

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Introduction

Lower respiratory tract infections (LRTIs) continue to pose a significant burden on global health and are recognized as one of the major causes of morbidity and mortality in individuals of all age groups. [1] LRTIs comprise a spectrum of respiratory illnesses such as influenza, pneumonia, acute bronchitis, acute exacerbations of chronic obstructive pulmonary disease (COPD), and acute exacerbations of bronchiectasis. [2] The etiological agents responsible for these infections, along with their antimicrobial susceptibility profiles, vary according to geographical location and healthcare environment. [3] Frequently isolated bacterial pathogens in LRTIs include Klebsiella pneumoniae, Acinetobacter, Haemophilus influenzae, Moraxella catarrhalis, and Pseudomonas aeruginosa. [4] The emergence and spread of antibiotic-resistant bacteria have become a major therapeutic challenge, often resulting in severe disease, increased mortality, prolonged hospitalization, and a higher incidence of complications. [5] Among these resistant pathogens, Klebsiella pneumoniae producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases has become particularly concerning because of limited treatment options

and its potential for rapid dissemination within healthcare settings. [6] Microbiological examination of sputum is an essential diagnostic approach for determining the causative organisms in respiratory and pulmonary infections. [7] Periodic assessment of sputum culture isolates and their antibiotic susceptibility patterns provides valuable information for empirical treatment decisions and contributes to effective antimicrobial stewardship in hospitals. [8]

The present study was conducted at a 750-bedded tertiary care referral hospital serving the population of the Vidarbha region of Maharashtra. Surveillance of bacterial isolates from sputum samples and evaluation of their antimicrobial resistance trends over time are important for optimizing antimicrobial therapy and improving patient management. Therefore, this study was undertaken to analyse the bacterial profile and antimicrobial resistance patterns of culture-positive sputum samples received at a tertiary care government medical college and hospital in Maharashtra over a five-year duration from 2021 to 2025.

Aim: To determine the bacterial profile and antimicrobial resistance pattern of culture-positive sputum samples received at a tertiary care government medical college and hospital in the Vidarbha region of Maharashtra during a five-year period.

Objectives:

1. To analyse the department-wise distribution of culture-positive sputum isolates.
2. To study the organism-wise and gender-wise distribution of isolates.
3. To evaluate the year-wise trends of bacterial isolates.
4. To assess the antimicrobial susceptibility patterns of pathogenic organisms

Methodology

This retrospective observational study was performed in the Department of Microbiology of a tertiary care teaching hospital situated in the Vidarbha region of Maharashtra. The study covered a five-year period from January 2021 to December 2025. Sputum samples received in the microbiology laboratory from outpatient clinics, inpatient departments, and intensive care units during the study duration were reviewed and analysed.

Inclusion Criteria: Only sputum specimens fulfilling the acceptability criteria based on Gram stain evaluation using Bartlett's criteria [9] and showing bacterial growth on culture were included in the study. For every patient, only the initial isolate obtained during the defined study period with confirmed identification and antimicrobial susceptibility results was considered for analysis.

Exclusion Criteria:

- Duplicate isolates recovered from the same patient.
- Isolates showing unclear or intermediate antimicrobial susceptibility patterns.

All sputum specimens underwent preliminary Gram stain examination to determine specimen adequacy. Specimens had less than 10 epithelial cells per low-power field and at least 25 polymorph nuclear leukocytes, they were allowed for culture. Acceptable specimens were inoculated onto blood agar and MacConkey agar media and incubated aerobically at 37°C for 18–24 hours. After incubation, bacterial isolates were identified by assessing colony morphology, Gram staining features, and standard biochemical characteristics using conventional microbiological techniques.

Antimicrobial susceptibility testing of all isolates was carried out using the Kirby–Bauer disc diffusion technique on Mueller–Hinton agar, while colistin susceptibility was assessed by broth microdilution method. Interpretation of antimicrobial susceptibility results was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2024. [10] Quality control for identification and susceptibility testing was ensured using standard reference strains, namely Klebsiella pneumoniae ATCC 700603, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 25923. Data analysis and interpretation were performed using WHONET Antibiotic Susceptibility Surveillance Software.

The antimicrobial agents evaluated included beta-lactams, carbapenems, aminoglycosides, fluoroquinolones, and other commonly prescribed antibiotics. Colistin susceptibility, wherever applicable, was determined using minimum inhibitory concentration (MIC) testing methods. Laboratory data were further analyzed using WHONET software to assess the distribution of bacterial isolates according to department, organism type, gender, and study year. Resistance trends among Gram-positive and Gram-negative organisms were also studied.

Antibiotics were classified according to the World Health Organization (WHO) AWaRe classification 2023 [11] into Access, Watch, and Reserve categories to evaluate antimicrobial stewardship practices and resistance trends.

The Access group comprised first-line antibiotics with lower resistance potential, whereas the Watch group included broad-spectrum agents associated with increased risk of resistance development. The

Reserve category consisted of antibiotics reserved for the treatment of multidrug-resistant infections.

Organisms exhibiting intrinsic resistance to specific antibiotics were identified accordingly and excluded from susceptibility interpretation. Since the study was based solely on retrospective laboratory records without direct patient involvement or intervention, ethical committee approval was not considered necessary.

Results:

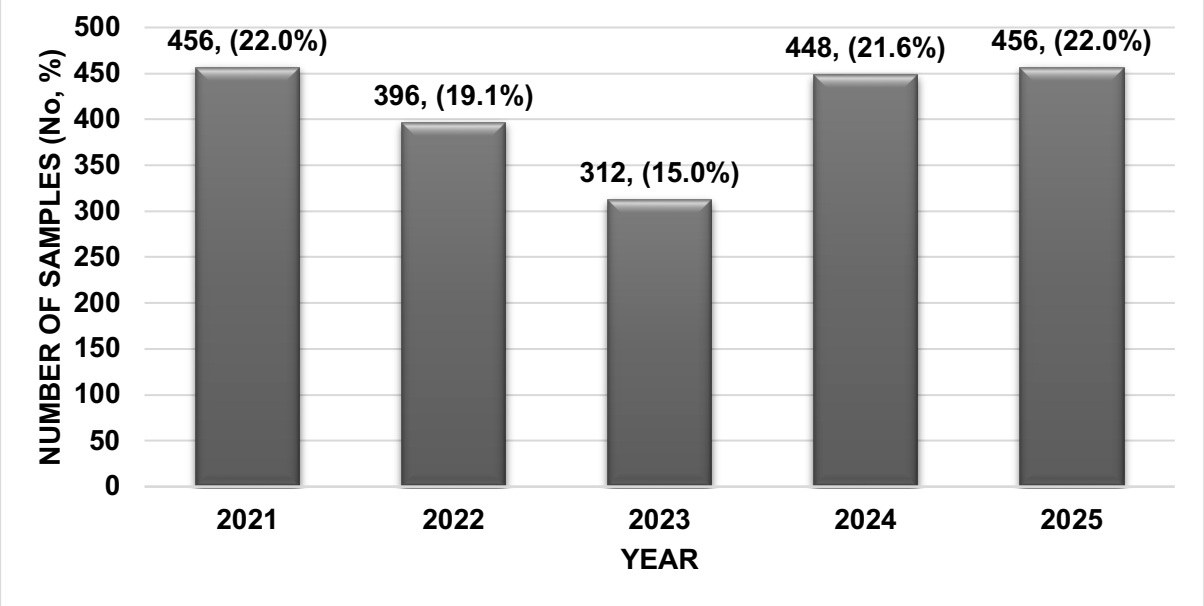

Fig 1: Year wise culture positive sputum samples. (N= 2068)

A total of 2068 culture-positive sputum samples were analysed over the study period.

The year-wise distribution showed that the highest number of isolates was observed in 2021 and 2025, each contributing 456 (22.0%) cases. This was followed by 2024 with 448 (21.6%) isolates. In 2022, 396 (19.1%) culture-positive samples were recorded. The lowest number of isolates was noted in 2023, with 312 (15.0%) cases.

Overall, a declining trend was observed from 2021 to 2023, followed by a subsequent rise in 2024 and 2025.

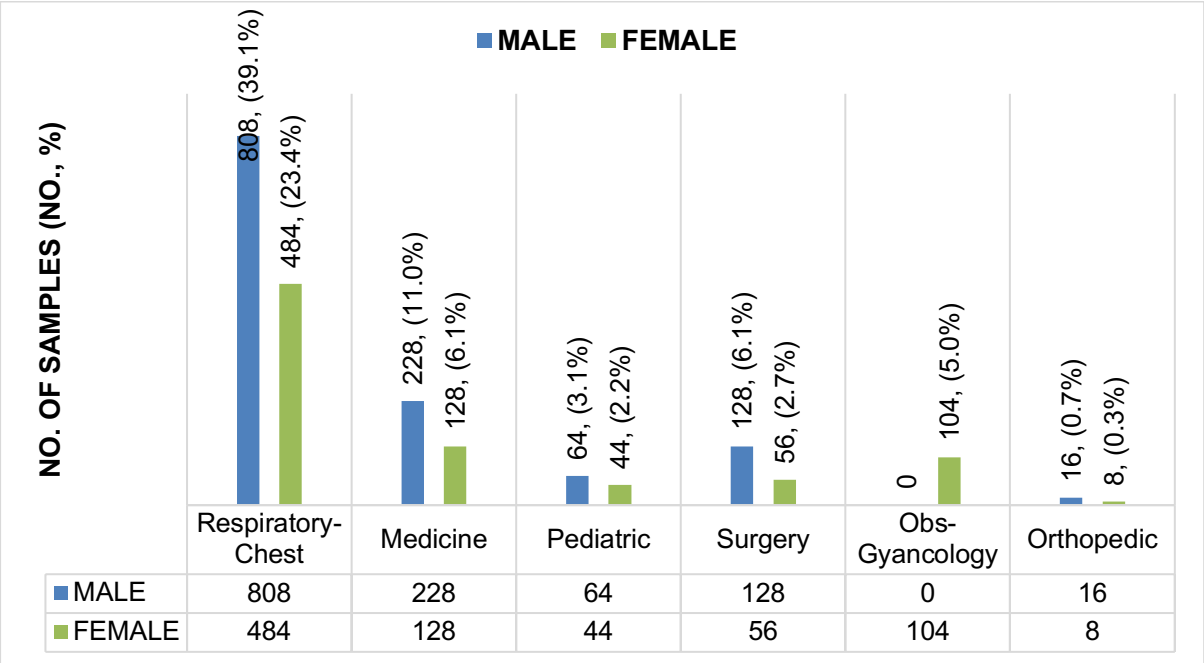

Fig 2: Gender-wise positive sample distribution from each department. (N= 2068)

Among 2068 culture-positive samples, males predominated across most departments.

The highest contribution was from the Respiratory-Chest department [808 (39.1%) males, 484 (23.6%) females], followed by Medicine [456 (11.1%) males, 128 (6.1%) females]. Paediatric and Surgery departments showed lower numbers. All 104 (5.0%) cases from Obstetrics and Gynaecology were females, while Orthopaedic contributed minimal cases.

Overall, male patients formed the majority except in Obstetrics and Gynaecology.

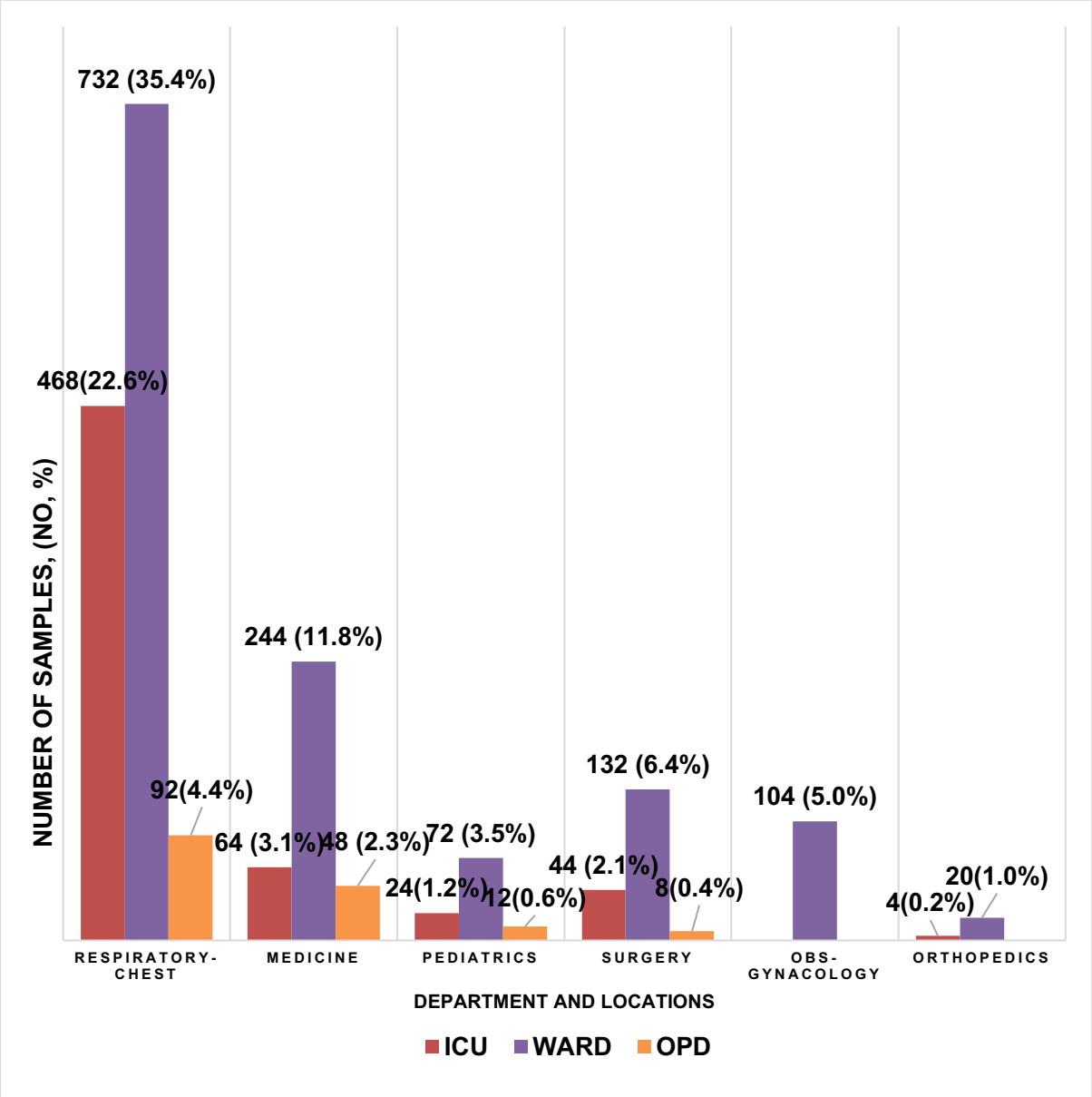

Fig 3: Location based culture positive sputum samples (N= 2068)

Most samples were received from the Respiratory-Chest department, mainly from ward patients (35.4%), followed by ICU (22.6%) and OPD (4.4%).

The Medicine department was the next major contributor, with ward samples accounting for 11.8%, while ICU and OPD contributions were lower.

Other departments such as Surgery, Paediatrics, Obstetrics & Gynaecology, and Orthopaedics contributed fewer samples overall, with wards being the main source across all departments.

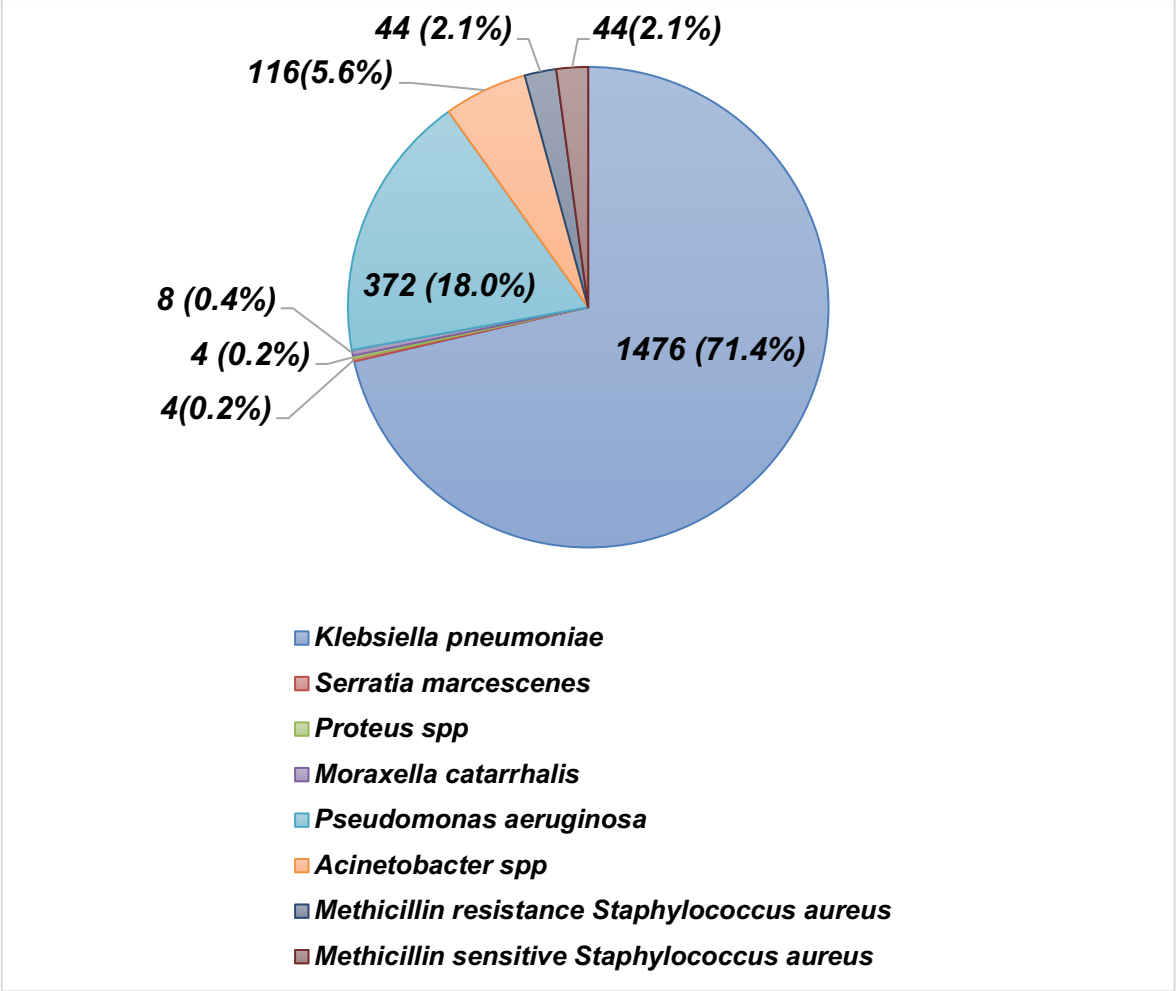

Fig 4: Organism wise culture positive sputum samples (N= 2068)

Among 2068 culture-positive sputum samples, Klebsiella pneumoniae was the leading isolate (71.4%), followed by Acinetobacter spp. (18.0%). Less frequent organisms included Moraxella catarrhalis and Proteus spp. (0.2% each). Overall, Gram-negative bacteria predominated.

Table 1: Antibiotic resistance pattern of gram negative organisms (n = 1980)

	Antibiotics	Entero bacterales (n = 1484) %	Pseudomonas aeruginosa (n = 372) %	Acinetobacter spp. (n= 116) %	Moraxella catarrhalis (n= 8) %
ACCESS	Amoxicillin-clavulanate	55.5	NR	NR	0
	Amikacin	34.8	4.3	51.7	NR
	Gentamicin	33.7	10.8	58.6	NR
	Doxycycline	72.2	NR	78.2	25
WATCH	Cefuroxime	71.4	IR	IR	37.5
	Ceftriaxone	59.3	NR	75.9	25.0
	Ceftazidime	57.1	46.2	75.9	25.0
	Cefepime	48.5	28.0	73.3	0
	Piperacillin-tazobactam	41.5	22.6	55.2	NR
	Ampicillin- sulbactam	59.4	NR	72.4	0
	Imipenem	38.5	7.5	44.8	NR
	Meropenem	40.4	7.5	41.4	NR
	Ciprofloxacin	55.3	15.1	72.4	12.5
	Levofloxacin	50.9	11.8	62.1	12.5
RESERVE	Aztreonam	33.7	18.3	NR	NR
	Colistin (MIC)	0	0	0	NR

IR: intrinsic resistance; NR: not recommended by CLSI 2024; MIC: only MIC is recommended for the isolate/ antibiotic.

Among the gram-negative bacteria, resistance to doxycycline was notably high in Enterobacterales (72.2%) and Acinetobacter spp. (78.2%). Acinetobacter spp. exhibited substantial resistance to third-generation cephalosporins, with ceftriaxone and ceftazidime resistance rates of 75.9% each, and also showed the highest resistance to carbapenems compared to other organisms studied. In contrast, Pseudomonas aeruginosa demonstrated relatively low resistance to imipenem and meropenem (7.5% each). No resistance to colistin was detected among the gram-negative isolates.

Table 2: Antibiotic resistance pattern of gram positive organisms (n = 88)

	Antibiotics	Staphylococcus aureus (n = 88) %
ACCESS	Penicillin	81.8
	Oxacillin	50
	Gentamicin	22.7
	Clindamycin	40.9
WATCH	Tetracycline	36.4
	Ciprofloxacin	54.5
	Levofloxacin	50.0
	Erythromycin	63.6
RESERVE	Linezolid	04.5
	Vancomycin	0

Among Staphylococcus aureus isolates, the highest resistance was observed against penicillin (81.8%) and erythromycin (63.6%). Resistance to fluoroquinolones was also considerable, with ciprofloxacin and levofloxacin resistance rates of 54.5% and 50.0%, respectively. Oxacillin resistance was detected in 50% of isolates, indicating a substantial prevalence of methicillin-resistant S. aureus (MRSA). In contrast, vancomycin resistance was not observed, while linezolid resistance remained low (4.5%).

Discussion:

Lower respiratory tract infections remain a significant cause of hospital admissions in tertiary care hospitals, with Gram-negative bacteria being the predominant pathogens.

In the present study, most culture-positive sputum isolates were obtained from the pulmonology department (62.5%), followed by medicine (17.2%) and surgery (8.9%). Comparable findings were reported by Ahmed SM et al. [12], where respiratory units contributed 75.5% of the samples. The predominance of Gram-negative bacilli observed in this study is also in accordance with the findings of Chandra A et al. [13], who highlighted the increasing burden of multidrug-resistant Gram-negative organisms in healthcare settings.

Klebsiella pneumoniae was identified as the predominant isolate, accounting for 71.4% of cases. Similar predominance have been reported by Yamer O et al., Singh J et al., and Gebre et al. [14–16], where Klebsiella pneumoniae was frequently isolated from respiratory infections, particularly among hospitalized patients. Enterobacterales in the present study showed high resistance to doxycycline (72.2%) and cefuroxime (71.4%). Comparable resistance levels of approximately 60–70% have also been documented by Mahale et al. [17] Carbapenem resistance among Enterobacterales ranged between 38% and 40%, indicating a concerning rise in resistance to last-line antibiotics.

Among non-fermenting Gram-negative bacilli, Acinetobacter spp. demonstrated greater multidrug resistance than Pseudomonas aeruginosa. The World Health Organization categorized carbapenem-resistant Acinetobacter baumannii as a critical priority pathogen in 2018. [18] Carbapenem resistance in Acinetobacter isolates exceeded 40% in the present study, whereas Sharma et al. [19] reported a resistance rate of 59%. In contrast, Pseudomonas aeruginosa showed relatively lower resistance to imipenem and meropenem (7.5% each). The elevated resistance among non-fermenters may be attributed to intrinsic resistance mechanisms, biofilm production, and acquisition of resistant genes.

Gram-positive organisms constituted a smaller proportion of isolates, with Staphylococcus aureus accounting for 4.2% of the total isolates while prevalence rates 24% and15.3% have been reported by Santella B et al. [20] and Regha IR et al. [21] respectively. No resistance to colistin was observed among Gram-negative isolates in the present study, which is similar to findings from recent surveillance studies. [20,22] The overall findings indicate an increasing prevalence of multidrug-resistant Gram-negative pathogens and reinforce the need for continuous antimicrobial surveillance, rational antibiotic use, and preparation of local antibiograms for effective empirical therapy.

Overall, the findings of this study highlight the increasing predominance of multidrug-resistant Gram-negative organisms in sputum samples.

Regular surveillance and development of local antibiograms are essential for guiding empirical therapy and reducing antimicrobial resistance.

Conclusion:

This five-year retrospective study highlights the high burden of respiratory tract infections and the increasing antimicrobial resistance among respiratory pathogens in tertiary care settings. Increasing resistance rates were observed among Enterobacterales, with notable carbapenem resistance in Enterobacterales and Acinetobacter spp., limiting therapeutic options. Among non-fermenting gram-negative bacilli, Acinetobacter spp. demonstrated greater multidrug resistance than Pseudomonas aeruginosa.

These findings stress the importance of continuous surveillance, hospital-specific antibiograms, antimicrobial stewardship, and strict infection control measures to support appropriate empirical therapy and reduce the spread of multidrug-resistant pathogens.

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