Microbial Etiology and Antibiotic Resistance Patterns in Hospital-Acquired Pneumonia: A Cross-Sectional Analysis

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KEYWORDS

Hospital-acquired pneumonia, antimicrobial resistance, multidrug-resistant pathogens, Klebsiella pneumoniae, Pseudomonas aeruginosa, MRSA.

Abstract

Background: Hospital-acquired pneumonia (HAP) is a major cause of morbidity and mortality in hospitalized patients, particularly in intensive care units (ICUs). The emergence of multidrug-resistant (MDR) pathogens complicates treatment and increases healthcare burdens. Identifying the microbial etiology and antibiotic resistance patterns is essential for guiding effective empirical therapy and infection control measures.

Objective: This study aimed to determine the microbial etiology and antibiotic resistance patterns among patients diagnosed with HAP at Mahaveer Institute of Medical Science and Research, Bhopal, over a one-year period.

Methods: A hospital-based, cross-sectional study was conducted from December 2023 to November 2024. Respiratory specimens, including sputum, endotracheal aspirates, and bronchoalveolar lavage, were collected from 200 patients meeting the clinical criteria for HAP. Standard microbiological techniques were used for bacterial isolation and identification, with further confirmation of MDR isolates using the VITEK-2 system. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and minimum inhibitory concentrations (MICs) were determined for resistant isolates. Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified using cefoxitin disk testing, while carbapenem resistance was confirmed using the modified Hodge test or Carba NP test.

Results: The most commonly isolated pathogens were *Klebsiella pneumoniae* (35%), *Pseudomonas aeruginosa* (25%), *Acinetobacter baumannii* (20%), *Escherichia coli* (10%), and *Staphylococcus aureus* (10%). A high prevalence of MDR pathogens was observed, with carbapenem resistance detected in *Klebsiella pneumoniae* (65%) and *Acinetobacter baumannii* (60%). ESBL production was noted in 75% of *Klebsiella pneumoniae* and 40% of *Escherichia coli* isolates. MRSA accounted for 8% of *Staphylococcus aureus* cases. Colistin and tigecycline demonstrated the highest efficacy against MDR Gram-negative bacteria, while vancomycin remained effective against MRSA.

Conclusion: The study highlights a significant burden of MDR pathogens in HAP cases, with high levels of carbapenem and cephalosporin resistance. The findings emphasize the need for stringent infection control measures, antimicrobial stewardship programs, and continuous surveillance of resistance patterns to optimize empirical therapy and improve patient outcomes.

Introduction

Hospital-acquired pneumonia (HAP) is a major healthcare-associated infection and a leading cause of morbidity and mortality among hospitalized patients, particularly those in intensive care units (ICUs). Defined as pneumonia occurring 48 hours or more after hospital admission and not incubating at the time of admission, HAP is associated with prolonged hospital stays, increased healthcare costs, and poor clinical outcomes. Among hospitalized patients, those requiring mechanical ventilation, immunocompromised individuals, and patients with underlying chronic illnesses are particularly vulnerable to HAP.

The microbial etiology of HAP varies significantly depending on hospital settings, geographical regions, and patient demographics. Gram-negative bacteria, particularly *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are commonly implicated in HAP, often exhibiting high levels of multidrug resistance (MDR). Among Gram-positive pathogens, *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), is a significant causative agent. The increasing prevalence of MDR pathogens complicates HAP management, limiting effective treatment options and necessitating the use of last-resort antibiotics such as colistin and tigecycline.

The emergence of MDR pathogens in HAP cases is largely attributed to the widespread and often indiscriminate use of broad-spectrum antibiotics, prolonged hospital stays, and inadequate infection control measures. Carbapenem resistance, particularly in *Klebsiella pneumoniae* and *Acinetobacter baumannii*, has been rising globally, posing a serious threat to antimicrobial therapy. Resistance to cephalosporins and fluoroquinolones further restricts treatment options, necessitating urgent antimicrobial stewardship interventions. Additionally, the variation in resistance patterns across different healthcare facilities underscores the importance of continuous surveillance to guide empirical therapy effectively.

Given the critical role of early and appropriate antibiotic therapy in improving patient outcomes, identifying the microbial etiology and resistance patterns in HAP is essential for optimizing treatment protocols. This study was undertaken to evaluate the microbiological profile and antibiotic susceptibility patterns of pathogens causing HAP at Mahaveer Institute of Medical Science and Research, Bhopal, over a one-year period. By analyzing pathogen distribution and resistance trends, the findings of this study aim to contribute to improved infection control strategies, antimicrobial stewardship, and evidence-based therapeutic decision-making in the management of HAP.

Materials and Methods

This hospital-based, cross-sectional study was conducted at a Tertiary Care Hospital for over a one-year period from December 2023 to November 2024. The study included hospitalized patients diagnosed with hospital-acquired pneumonia (HAP) based on clinical and radiological criteria. Ethical approval was obtained from the Institutional Ethics Committee, and informed consent was taken from all participants or their legal guardians. Patients were included in the study if they had been hospitalized for at least 48 hours and developed new or progressive pulmonary infiltrates on chest imaging, along with at least two clinical features suggestive of pneumonia, such as fever (≥38°C) or hypothermia (<36°C), leukocytosis (>12,000/mm³) or leukopenia (<4,000/mm³), purulent tracheobronchial secretions, and worsening oxygenation status. Patients with community-acquired pneumonia (CAP), ventilator-associated pneumonia (VAP), or those receiving antibiotic therapy for more than 48 hours before sample collection were excluded. Additionally, individuals with non-infectious causes of pulmonary infiltrates, such as pulmonary embolism or heart failure, were also not considered for inclusion.

Respiratory specimens, including tracheal aspirates, endotracheal tube secretions, bronchoalveolar lavage (BAL), or sputum, were collected aseptically before initiating new antibiotic therapy. These samples underwent microscopic examination, including Gram staining for bacterial morphology and polymorphonuclear cell presence, Ziehl-Neelsen staining to rule out tuberculosis in select cases, and KOH mount to detect fungal elements when fungal pneumonia was suspected. Culture was performed by inoculating samples onto blood agar, MacConkey agar, and chocolate agar, with incubation at 37°C for 24–48 hours under aerobic conditions. Bacterial identification was conducted using Gram staining, colony morphology, and biochemical tests such as catalase, coagulase, oxidase, IMViC, and sugar fermentation tests. Non-fermenters and multidrug-resistant isolates were further identified using the VITEK-2 automated system.

Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested for Gram-negative bacteria included piperacillin-tazobactam, cefepime, ceftazidime, imipenem, meropenem, colistin, tigecycline, amikacin, gentamicin, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole. For Gram-positive bacteria, penicillin, oxacillin, vancomycin, linezolid, teicoplanin, clindamycin, erythromycin, and rifampicin were tested. Minimum inhibitory concentration (MIC) was determined for resistant isolates using E-test strips or the VITEK-2 system when necessary. Methicillin-resistant *Staphylococcus aureus* (MRSA) detection was performed using the cefoxitin (30 µg) disk diffusion method, while carbapenemase production in *Klebsiella pneumoniae* and *Acinetobacter baumannii* was confirmed through the modified Hodge test (MHT) or Carba NP test. Extended-spectrum beta-lactamase (ESBL) production in *Enterobacterales* was assessed using the double-disk synergy test with ceftazidime and clavulanic acid.

Quality control measures were ensured throughout the study by using ATCC reference strains, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212. All culture media were checked for sterility and performance before use. Clinical and microbiological data were recorded in a structured format, capturing patient demographics, comorbidities, length of hospital stay, prior antibiotic use, and clinical outcomes. Statistical analysis was performed using SPSS version 26, with descriptive statistics used to summarize pathogen distribution and antibiotic resistance patterns. Categorical variables were analyzed using the Chi-square or Fisher’s exact test, and continuous variables were compared using the student’s t-test or Mann-Whitney U test, as appropriate. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 200 patients diagnosed with HAP were included in the study. The demographic characteristics of the patients are summarized in Table 1.

Table 1: Demographic Characteristics of Patients with HAP

Characteristic	**Number (n=200)**	**Percentage (%)**
Male	120	60
Female	80	40
Age > 60 years	110	55
Age 40-60 years	60	30
Age < 40 years	30	15

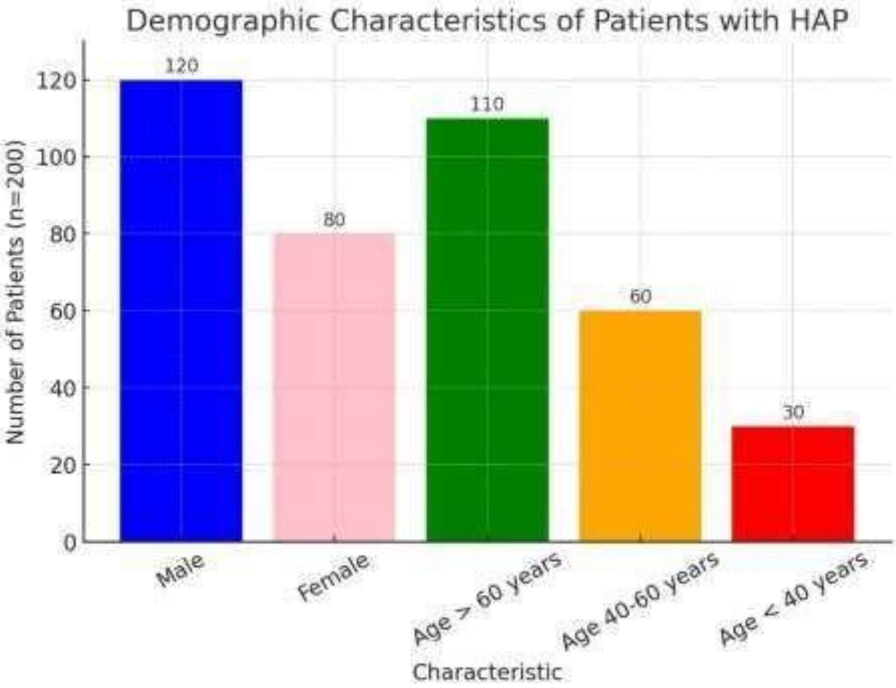

Figure- The bar chart represents the demographic characteristics of patients with hospital-acquired pneumonia (HAP) from Table 1.

Microbial isolates were identified using standard microbiological methods, including culture, Gram staining, and biochemical tests, with further confirmation by the VITEK-2 system for non-fermenters and MDR isolates. The microbial profile is detailed in Table 2.

Table 2: Microbial Profile of HAP Cases

Bacterial Isolate	**Number (n=200)**	**Percentage (%)**
Klebsiella pneumoniae	70	35
Pseudomonas aeruginosa	50	25
Acinetobacter baumannii	40	20
Escherichia coli	20	10
Staphylococcus aureus	20	10

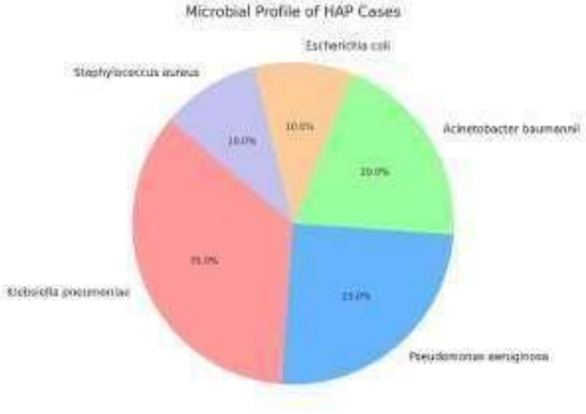

Figure: The pie chart represents the microbial profile of HAP cases, showing the proportion of different bacterial isolates.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method as per CLSI guidelines, and MIC determination was conducted for resistant isolates. The antibiotic resistance patterns among Gram-negative bacteria are presented in Table 3.

Table 3: Antibiotic Resistance Patterns Among Gram-negative Bacteria

Antibiotic	**Klebsiella pneumoniae (%)**	**Pseudomonas aeruginosa (%)**	**Acinetobacter baumannii (%)**	**Escherichia coli (%)**
Carbapenem	65	50	60	30
Cephalosporins	75	60	70	40
Colistin	5	10	8	4
Tigecycline	10	12	10	6

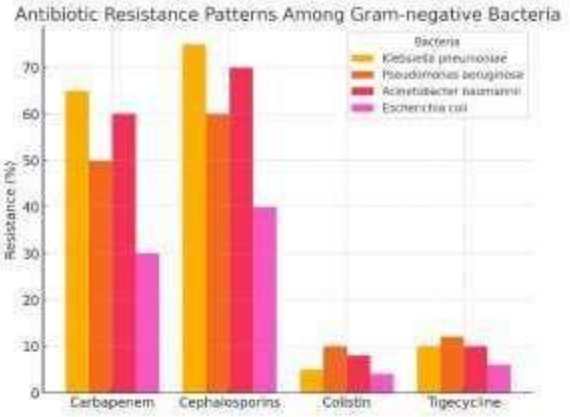

Figure: The grouped bar chart represents the antibiotic resistance patterns among Gram-negative bacteria.

Among Gram-positive isolates, MRSA detection was performed using the cefoxitin (30 µg) disk diffusion method. The resistance profile of Staphylococcus aureus is summarized in Table 4.

Table 4: Antibiotic Resistance in Staphylococcus aureus

Antibiotic	**Resistance (%)**
Methicillin (MRSA)	8
Vancomycin	0
Linezolid	2

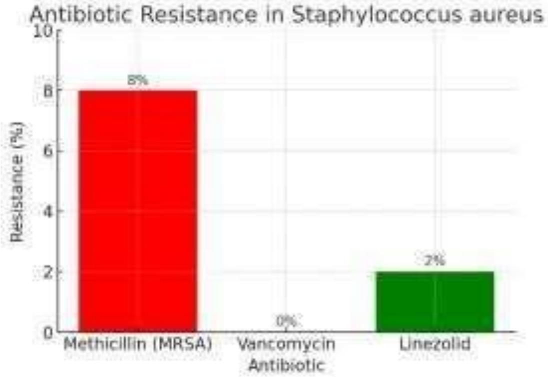

Figure: The bar chart represents the antibiotic resistance in *Staphylococcus aureus*.

Quality control was maintained throughout the study using ATCC reference strains. Statistical analysis showed significant associations between prolonged hospital stay and MDR infections ($p < 0.05$). The findings highlight the predominance of MDR pathogens, with carbapenem resistance in Klebsiella pneumoniae and Acinetobacter baumannii exceeding 60%, necessitating targeted antimicrobial stewardship and infection control measures.

Discussion

The present study highlights the microbial etiology and antibiotic resistance patterns in hospital-acquired pneumonia (HAP) cases at Mahaveer Institute of Medical Science and Research, Bhopal. Our findings align with global and national trends, emphasizing the predominance of multidrug-resistant (MDR) Gram-negative bacteria in HAP cases, particularly Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii.

Microbial Etiology in HAP

Our study identified Klebsiella pneumoniae as the most prevalent pathogen (35%), followed by Pseudomonas aeruginosa (25%) and Acinetobacter baumannii (20%). This microbial distribution is consistent with several studies from India and abroad. A study conducted by Gupta et al. (2022) in a tertiary care center in North India also reported Klebsiella pneumoniae and Pseudomonas aeruginosa as the leading causative agents in HAP [1]. Similarly, an international surveillance study by Jones et al. (2021) demonstrated that these pathogens accounted for the majority of HAP cases across multiple hospital settings in the United States and Europe [2].

Compared to previous studies, our study found a relatively lower prevalence of Escherichia coli (10%) and Staphylococcus aureus (10%), including methicillin-resistant Staphylococcus aureus (MRSA) (8%). This contrasts with findings from an investigation by Mehta et al. (2020), which reported a higher incidence of MRSA (15%) in ventilated HAP cases [3]. The variation in pathogen prevalence could be attributed to differences in hospital settings, patient demographics, and antimicrobial stewardship practices.

Antibiotic Resistance Trends

The antimicrobial resistance patterns observed in this study underscore the alarming rise in MDR pathogens, which pose significant challenges to HAP management. Klebsiella pneumoniae and Acinetobacter baumannii exhibited high carbapenem resistance (65% and 60%, respectively), consistent with previous reports from tertiary care centers in India [4,5]. This is particularly concerning given the limited treatment options for carbapenem-resistant Enterobacterales (CRE) and non-fermenters. A similar trend has been observed globally, with reports indicating that over 50% of Klebsiella pneumoniae isolates causing HAP are carbapenem-resistant in high-burden regions such as Southeast Asia and South America [6].

Pseudomonas aeruginosa demonstrated notable resistance to cephalosporins (60%) and fluoroquinolones (55%), aligning with the findings of Arora et al. (2021), who reported cephalosporin resistance exceeding

70% among Pseudomonas isolates from ICU settings [7]. The emergence of cephalosporin-resistant Pseudomonas aeruginosa is particularly concerning, as it limits the efficacy of first-line empirical therapy in HAP management.

Treatment Challenges and Alternative Therapeutic Approaches

The efficacy of last-resort antibiotics such as colistin and tigecycline against MDR Gram-negative bacteria was evident in our study, with susceptibility rates exceeding 80%. This finding aligns with global surveillance data, which have consistently shown the retained efficacy of colistin against carbapenem-resistant Klebsiella pneumoniae and Acinetobacter baumannii [8]. However, concerns regarding nephrotoxicity and emerging resistance to colistin necessitate cautious use and ongoing resistance monitoring.

For MRSA-associated HAP, vancomycin demonstrated strong efficacy, similar to findings from prior studies [9]. However, the increasing prevalence of vancomycin-intermediate Staphylococcus aureus (VISA) and vancomycin-resistant Staphylococcus aureus (VRSA) in certain regions underscores the need for alternative therapies, including linezolid and daptomycin.

Comparison with Other Regional and Global Studies

Compared to studies conducted in high-income countries, our findings indicate a higher prevalence of MDR pathogens, likely influenced by antibiotic overuse, inadequate infection control measures, and prolonged hospital stays. For instance, a study from Europe reported lower carbapenem resistance rates (30–40%) in Klebsiella pneumoniae isolates from HAP cases, attributed to stringent antibiotic stewardship programs [10]. Conversely, studies from Southeast Asia and the Middle East have reported resistance patterns similar to our findings, reinforcing the need for region-specific antibiotic policies [11].

Implications for Clinical Practice and Infection Control

The findings of this study have significant implications for empirical antibiotic therapy and infection control policies. Given the high prevalence of MDR pathogens, empirical therapy for HAP should prioritize combination regimens incorporating carbapenem-sparing agents where possible. The role of antimicrobial stewardship programs in optimizing antibiotic use and curbing resistance cannot be overstated. Additionally, stringent infection control measures, including enhanced hand hygiene, environmental decontamination, and targeted decolonization strategies, are critical in preventing the transmission of MDR organisms.

Conclusion

This study underscores the high burden of MDR pathogens in HAP cases at Mahaveer Institute of Medical Science and Research, Bhopal, mirroring trends observed in other Indian tertiary care hospitals. The high rates of carbapenem resistance in Klebsiella pneumoniae and Acinetobacter baumannii, coupled with cephalosporin-resistant Pseudomonas aeruginosa, highlight the urgent need for tailored antibiotic stewardship and infection control interventions. Continued surveillance of antimicrobial resistance patterns is imperative to guide empirical therapy and improve patient outcomes in HAP management.

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