

Isolation & identification of gram-negative bacilli isolated from various respiratory samples and their antibiotic susceptibility pattern

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ABSTRACT

Background: Globally, respiratory infections are responsible for a large proportion of illness and fatal outcomes. The importance of gram-negative rod organisms as causative agents in respiratory illnesses is becoming more widely acknowledged and are often associated with multidrug resistance, making treatment challenging.

Objective: To determine which Gram-negative Bacilli are frequently isolated from respiratory samples and to ascertain the isolated Gram-negative Bacilli's patterns of antibiotic susceptibility.

Procedure: The Department of Microbiology carried out a cross-sectional investigation between October 2024 and March 2025. Standard microbiological procedures were used to process 245 of the 650 samples from suspected patients that were obtained from IPD and OPD patients. Bacterial isolates were determined using culture characteristics, biochemical profiling, and gram reaction. Antimicrobial response was tested using the agar disk diffusion method in compliance with CLSI 2024 standards.

Results: The majority of the organisms included gram-negative bacteria, notably Klebsiella species (43.26%) occurring most frequently common isolation, with Pseudomonas ranking second species (26.53%), E. coli (24.48%), Acinetobacter species (4.48%) and Proteus species (1.22%). Biochemical testing showed distinct profiles: E. coli was indole and Methyl red (MR) positive; K. pneumoniae was citrate, urease, and Voges proskauer (VP) positive; Pseudomonas species were oxidase and catalase positive; Proteus species was catalase positive and oxidase negative; while Acinetobacter species were oxidase and VP negative but urease and MR positive. Among Klebsiella isolates the highest sensitivity was seen for Colistin (100%) followed by Tigecycline (98.11%), Meropenem (90.13%) and Doxycycline (70%). Among Escherichia coli the highest sensitivity was seen in colistin (100%) followed by Doxycycline (83.33%) and Amoxicillin (80.3%). In case of Pseudomonas species the highest sensitivity was seen in Colistin (95.31%) followed by Netilmicin (93.21%) and Piperacillin (81.54%). In Acinetobacter species the highest sensitivity was seen in Colistin (100%) followed by Tigecycline (100%) and Doxycycline (62.5%).

Conclusion: E. coli, K. pneumoniae, and P. aeruginosa were commonly obtained from respiratory samples. The majority of isolates remained susceptible to tigecycline and colistin, but there were multiple types of antibiotic resistance, notably reduced susceptibility to amoxicillin and third-generation cephalosporins. Effective antibiotic treatment and resistance monitoring rely on routine susceptibility testing and culture.

KEYWORDS: Gram-negative bacilli, Kirby-Bauer method, Respiratory samples, Respiratory tract infections.

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INTRODUCTION

Among the highest prevalent infectious diseases in the world, respiratory tract infections (RTIs) significantly increase morbidity and age-independent mortality.¹ Respiratory tract diseases are one of the most common illnesses that affect humans and the leading cause of infectious disease-related death across the world.² Acute illnesses lasting 21 days or less are classified as lower respiratory tract infections. Coughing is usually the main symptom of these infections, with associated symptoms include wheezing, dyspnea, sputum production, and chest pain or discomfort. LRTI is a broad name for a group of illnesses that include pulmonary disease, acute inflammation of the bronchi, and flare-ups among individuals with chronic lung

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conditions.³ Nosocomial lung infection, community-acquired pneumonia (CAP), acute and chronic inflammatory bronchial conditions in individuals among chronic airflow obstruction patients and bronchial dilation constitute prevalent respiratory illnesses.⁴ Acinetobacter baumannii, Escherichia coli, Klebsiella

pneumoniae, *Pseudomonas aeruginosa*, Enterobacteriaceae species, including *Stenotrophomonas maltophilia* are the gram-negative bacteria that have been found to be resistant in respiratory conditions.⁵ Treatment failure is a common occurrence for patients containing *P. aeruginosa* infections owing to the development of tolerance. Because of its ability to promote gene changes, it produces mutants resistant to clinical achievable concentrations of all anti-infective agents medications utilized in treatment approach.⁶ serious nosocomial infections, more commonly in ICUs are caused by *P. aeruginosa*. However, it has also been seen in community-dwelling pneumonia patients, and it is well acknowledged as a primary contributor to persistent respiratory infections in bronchiectasis and COPD patients.⁷ *Escherichia coli*, *Klebsiella pneumoniae*, along with other Enterobacteriaceae are associated with a variety of infection of the respiratory tract seen with healthcare facilities; however, numerous research has as well shown that these GNB are present in community acquired infectious diseases.⁸ They display a propensity to become resistant to numerous antibiotics, which renders treatment ineffective. They can also secrete ESBLs, like carbapenem- hydrolyzing enzymes, which is capable of hydrolyze the majority of β - lactam agents, including carbapenems.⁹ an opportunistic bacteria called *Acinetobacter baumannii* is often linked to infection outbreaks, which primarily affect intensive care units. Patients with severe sickness have reported having respiratory infections after being hospitalized due to *A. baumannii*. Multi drug resistant strains of *A. baumannii* have been documented more often in recent years, especially with regard to carbapenem class antimicrobial agents, have been associated with increased morbidity and mortality.¹⁰ Bacteria, viruses, fungi, and protozoa are examples of infectious agents that can cause infection. *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Candida albicans* are just a few of the many organisms that fall under this category. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common organisms.¹¹

All things considered, this study provides significant insights regarding the distribution and patterns of antibiotic susceptibility of gram-negative bacilli isolated from respiratory samples. The results could help with early detection of antimicrobial resistance, appropriate antibiotic selection, accurate pathogen identification, and effective treatment of respiratory infections.

MATERIAL AND METHODS

The Department of Microbiology was the site of this cross-sectional observational investigation, Era University, Lucknow, over a six-month period from October 2024 to March 2025. The primary objective of this study was to determine the common Gram-negative rod shaped bacteria obtained from respiratory samples and to ascertain the patterns of antibiotic susceptibility of the isolated Bacilli. Patients visiting both the inpatient (IPD) and outpatient (OPD) departments provided a total of 650 clinically suspected respiratory samples. The sample size was

determined using the standard prevalence-based method $n = Z_{\alpha/2} \times p \times q / I^2$, where p was the power of the study, q was $100 - p$, and I was the acceptable error. In order to guaranty sufficient representation and statistical reliability for resistance pattern analysis, the computed sample size was modified to 245. Patients of all ages and genders with clinically verified respiratory illnesses were included in the study. Sputum, endotracheal aspirate, and Bronchoalveolar lavage sample bacterial isolates were analyzed. To maintain analytical consistency and prevent confusing interpretation of antimicrobial susceptibility patterns, organisms that are typical respiratory tract flora and mixed infection growth following gram staining were eliminated. Standard microbiological procedures were followed in the aseptic collection of specimens. Sputum samples were collected in sterile, disposable, wide-necked, plastic sample cup with a screw cover, making sure the cap was securely fastened after collection. A tissue dampened with a disinfectant was used to clean up any item spilled on the containers outside.

The sample was obtained from an early morning deep cough, preferably immediately after waking up, and before any antibiotic therapy had been started. While Bronchoalveolar Lavage Samples were collected during the procedure of bronchoscopy and Endotracheal Aspirate (ETA) Samples were collected by sterile suction catheter in a sterile container. In order to preserve pathogen viability, all samples were quickly transferred in sterile, appropriately labeled containers to the microbiology lab within one to two hours.

Upon receipt in the laboratory, all samples were checked for quality, quantity and integrity. For sputum samples Barletts scoring was performed to rule out specimens containing saliva. After direct Gram staining, all samples were cultured aerobically on Blood enriched agar, MacConkey selective medium, and Nutrient culture substrate with the use of the usual loop approach. For eighteen to twenty-four hours, petri dishes were maintained at 37°C. Colony morphology, Gram reaction, and other culture features were used to identify the bacterial isolates. Enhanced by traditional biochemical tests.

The CLSI 2024 advice were followed when conducting antimicrobial sensitivity testing on MHA medium using the agar diffusion assay. β -lactams, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, glycopeptides, and last-resort medications like colistin and tigecycline were all part of the antibiotic panel. Based on CLSI breakpoints, zones of inhibition were assessed and classified as sensitive, moderate, or resistant.

Patterns of antibiotic sensitivity have been regarded as possible stand-ins for underlying mechanisms like carbapenemase activity and extended-spectrum β -lactamase generation. Descriptive statistics were used for statistical analysis, and the findings were presented as percentages and frequencies. Finding the typical Gram-Negative bacillary organisms that were recovered from respiratory specimens and figuring out their patterns of antibiotic susceptibility were the main goals.

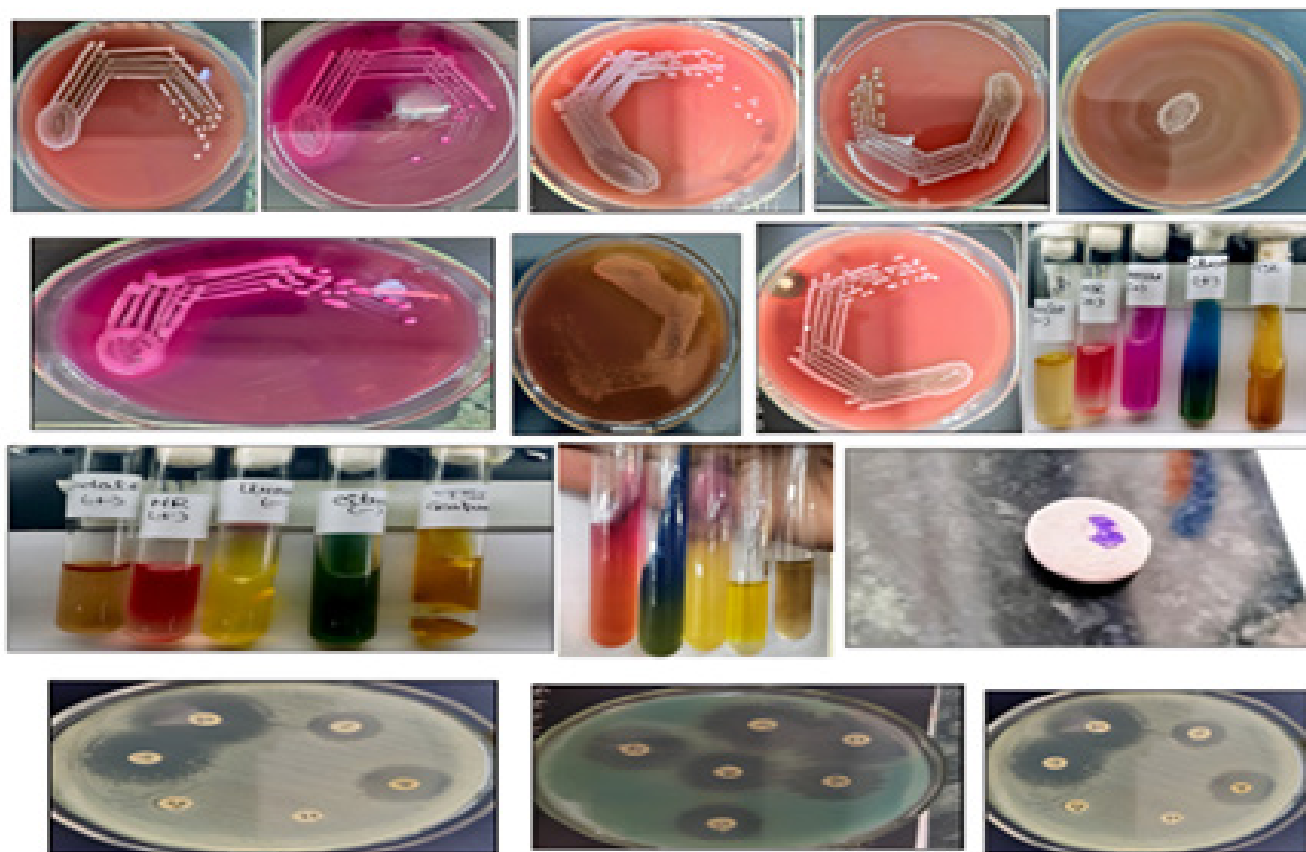


Figure1: showing 1. Culture plate identification for 1.1 *Escherichia coli* on blood agar, 1.2 *Escherichia coli* on MacConkey agar, 1.3 *Klebsiella* species on blood agar, 1.4 *Klebsiella* species on MacConkey agar, 1.5 *Pseudomonas aeruginosa* on blood agar, 1.6 *Pseudomonas aeruginosa* characteristics on MacConkey agar, 1.7 *Proteus* on Blood agar plates & 1.8 *Acinetobacter* species on Blood agar plates while the biochemical tests are showing for 2. *Klebsiella pneumoniae*, 2.1 *Escherichia coli*, *Pseudomonas* species 3. Oxidase (+) for *Pseudomonas* species, 4. AST pattern of *Klebsiella* species, *Escherichia coli*, *Pseudomonas aeruginosa*.

RESULTS

The current investigation was carried out at Era's university, unit of Microbiology. Overall, 650 samples (sputum, BAL, and ETA) were received and systematically processed between October 2024 and March 2025.

Out of the total of 650 respiratory samples received 245 samples showed significant growth of Gram-negative bacilli. Of these 170 (69.39%) samples were obtained from IPD and 75 (30.61%) from OPD.

Demographic and Host Risk Stratification

The corresponding age category of 51–90 aged years constituted the bulk of infections (38.78%), followed by 26–50 years old (36.73%), indicating a higher infection prevalence among the economically active population. Male patients constituted 67.35% of cases, reflecting possible increased occupational exposure.

From a variety of respiratory samples, 245 different

organisms were identified. The majority of isolates were obtained from sputum samples, accounting for 155 (63.26%) of the total isolates. Endotracheal aspirate samples contributed 75 (30.61%) isolates, while bronchoalveolar lavage samples yielded 15 (6.12%) isolates. Thus, sputum samples constituted the primary source of organism isolation, followed by endotracheal aspirates and bronchoalveolar lavage.

Among all 245 isolates, *Klebsiella* species were the most frequently isolated bacteria (106; 43.26%), followed by *Pseudomonas* (65; 26.53%) and *E. coli* (60; 24.48%). *Acinetobacter* (11; 4.48%) and *Proteus* (3; 1.22%) were less frequently isolated.

Thus, *Klebsiella* predominated in sputum and ET aspirates, whereas *Pseudomonas* mostly found in BAL samples.

Biochemical studies showed various reactions: *E. coli* was indole and MR positive, *K. pneumoniae* was citrate, urease, and VP positive, *Pseudomonas* species demonstrated positive oxidase and catalase reactions, *Proteus* species were

catalase positive and oxidase negative, but *Acinetobacter* species were both demonstrated negative oxidase and VP reactions along with positive urease and MR reactions.

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Table 1: Organisms isolated from various respiratory samples

Samples	No of isolates	Percentage
Sputum	155	63.26%
Endotracheal Aspirate	75	30.61%
Bronchoalveolar lavage	15	6.12%
TOTAL	245	100%

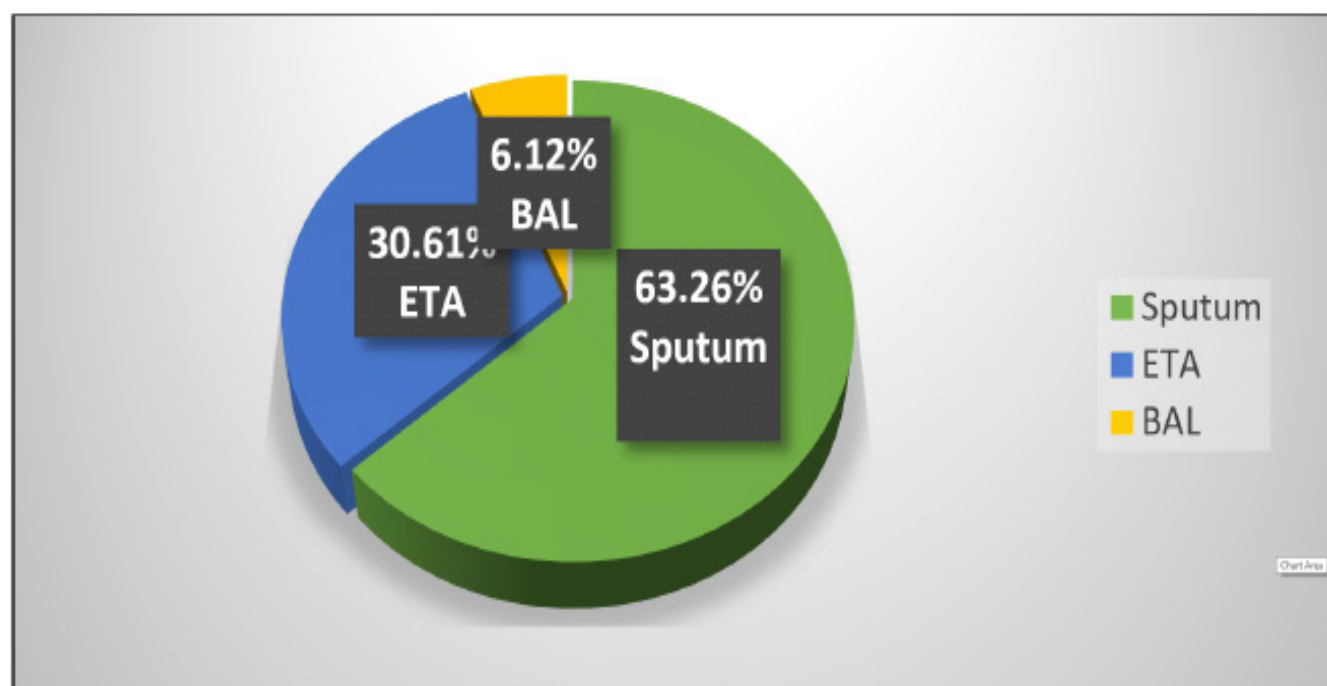


Figure 2: Pie chart showing percentage of different respiratory samples were used to isolate bacteria

Table-2: Distribution of bacterial isolates in various respiratory samples (n=245)

Bacterial isolates	Sputum (n=155)	Endotracheal aspirate (ETA) (n=75)	Bronchoalveolar lavage (BAL) (n=15)	Numbers
<i>Klebsiella species</i>	60 (38.70%)	44 (58.66%)	2 (13.33%)	106
<i>Pseudomonas species</i>	48 (30.97%)	8 (10.66%)	9 (60.0%)	65
<i>E. coli</i>	38 (24.51%)	19 (25.33%)	3 (20.0%)	60
<i>Acinetobacter species</i>	7 (4.51%)	3 (4.0%)	1 (6.66%)	11
<i>Proteus species</i>	2 (1.29%)	1 (1.33%)	0 (0%)	03
TOTAL	155 (100%)	75 (100%)	15 (100%)	245

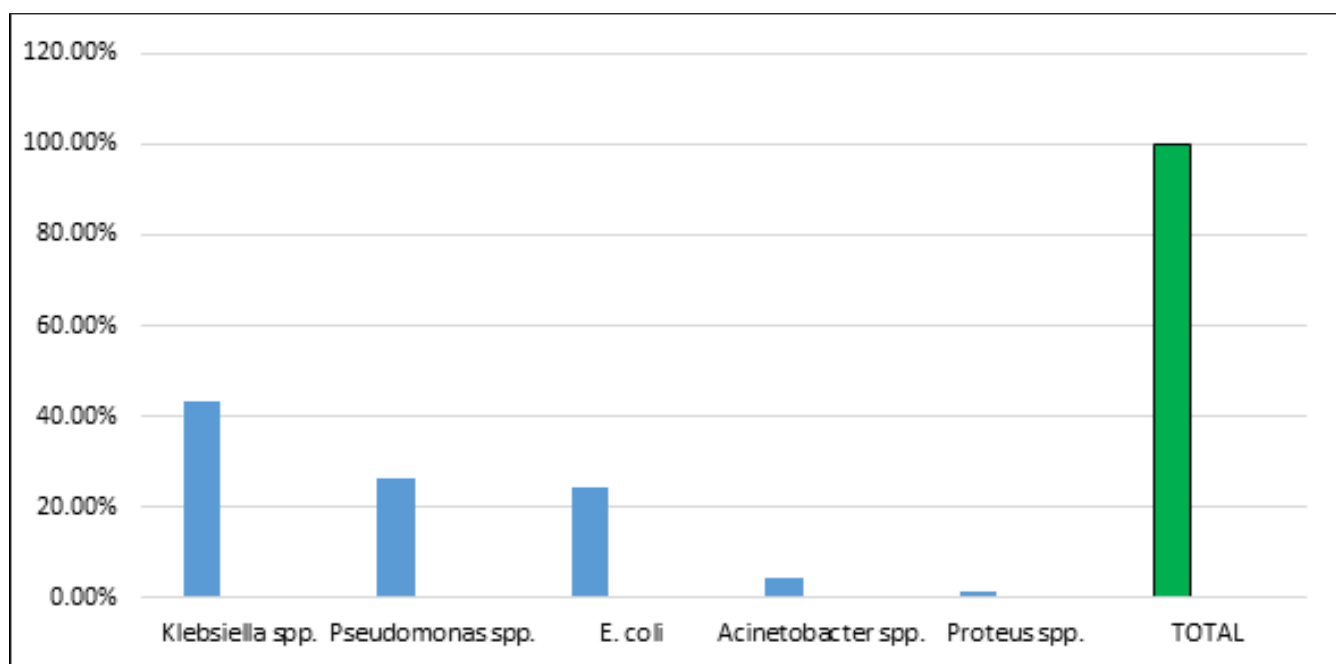


Figure 3: Bar graph showing the percentage wise distribution of gram-negative bacteria

Table 3: Klebsiella, E. coli, Pseudomonas, and Acinetobacter species' patterns of antibiotic sensitivity

S.NO	ANTIBIOTICS	KLEBSIELLA SPECIES (n=106)	E. COLI (n=60)	PSEUDOMONAS SPECIES (n=65)	ACINETOBACTER SPECIES (n=11)
1	DOXYCYCLINE	70%	83.33%	-	62.5%
2	TIGECYCLINE	98.11%	75.6%	-	100%
3	COLISTIN	100%	100%	95.31 %	100%
4	LEVOFLOXACIN	33.3%	76.6%	60.2%	-
5	AMOXICILLIN	1.89%	80.3%	-	-
6	AMIKACIN	46.23%	66.6%	69.5%	-
7	CEFTRIAXONE	7.3%	16.6%	-	-
8	GENTAMICIN	33.02%	53.3%		-
9	PIPERACILLIN+ TAZOBACTAM	33.02%	65%	81.54%	-
10	NETILMYCIN	34.91%	50%	93.21%	-
11	MEROPENEM/ IMIPENEM	90.13%	52.8%	55.38%	-
12	CEFOPERAZONE+ SULBACTAM	46.23%	60.3%	61.54%	-
13	CEFTAZIDIME	-	-	30%	-
14	CEFEPIME	-	-	52.31%	-
15	TOBRAMYCIN SULPHATE	-	-	67.69%	-
16	CIPROFLOXACIN	-	-	60%	-

DISCUSSION

The aim of the present study was to evaluate the antibiotic susceptibility patterns along with bacteriological profile of Gram-negative rods isolated from different respiratory specimens at tertiary care teaching Hospital.

Out of 650 respiratory samples processed, 245 (37%) were culture-positive for Gram-negative bacilli. The higher positivity rate in in-patients (69.39%) as compared to outpatients (30.61%) highlights the increased burden of hospital-acquired infections.¹² A male predominance (67.35%) and higher prevalence in the 51–90 years age group (38.78%) was noted, likely due to increased hospitalization and comorbidities in elderly males, which aligns with previously reported data.¹³

Sputum was the most common sample type (63.26%), followed by endotracheal aspirate (30.61%) and bronchoalveolar lavage (6.12%). This is similar to how LRTIs are typically diagnosed, particularly in critical care environments. *Klebsiella*, *Pseudomonas*, and *E. coli* are the most common Gram-negative bacteria that cause respiratory infections. Multidrug resistance (MDR) is a well-known characteristic of these pathogens, especially in hospital settings.^{14–16}

The isolates of *Klebsiella* were highly sensitive to meropenem/imipenem (90.13%), tigecycline (98.11%), and colistin (100%). The low effectiveness of earlier β -lactams and aminoglycosides in monotherapy is shown by the significant resistance to amoxicillin (1.89%), ceftriaxone (7.3%), and gentamicin (33%). Despite the fact that carbapenem resistance has been rising worldwide, these results highlight the significance of colistin and carbapenems as last-line treatments.

While *E. coli* isolates were 100% sensitive to colistin, they were more sensitive to doxycycline (83.33%) and amoxicillin (80.3%) than *Klebsiella*. *E. coli* bacteria were less sensitive to gentamicin (53.3%) and ceftriaxone (16.6%). Although this may alter depending on the situation, the greater susceptibility to doxycycline is noteworthy and may suggest a lower resistant selection pressure in this area.

Acinetobacter isolates were 100% sensitive to colistin and tigecycline but showed lower susceptibility to other antibiotics. For this infection, which is often linked to antibiotic resistance in intensive care units, this trend is typical. The utility of colistin and tigecycline as the last-resort options for *Acinetobacter baumannii* is consistent with findings from previous literature.¹⁴

Our study highlights prevalence of Gram-negative respiratory infections, particularly in hospitalized and elderly populations. The antibiotic sensitivity profiles observed support the continued use of carbapenems, aminoglycosides, and colistin in serious infections, while underlining the reduced effectiveness of cephalosporins and fluoroquinolones in many isolates.

CONCLUSION

Of the 650 respiratory samples, 245 (37.69%) contained gram-negative bacteria. *Klebsiella* species were the most common, followed by *Pseudomonas* species and *E. coli*. Those between the ages of 51 and 90 were most affected (38.78%), whereas men were more likely to be positive (67.35%). *Klebsiella* species demonstrated the maximum sensitivity to tigecycline (98.11%), Meropenem (90.13%), and colistin (100%), but *E. coli* was most sensitive to colistin (100%). The effectiveness of tigecycline and colistin against *Acinetobacter* species was 100%. Regular antibiograms and local antibiotic laws are crucial because most isolates were multidrug resistant.

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