

MICROBIOLOGICAL PROFILE AND ANTIBIOTIC RESISTANCE PATTERN OF RESPIRATORY PATHOGENS ISOLATED FROM SPUTUM SAMPLES: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Introduction: Respiratory tract infections are common reasons for sputum culture and empirical antibiotic therapy. Increasing antimicrobial resistance among Gram-negative respiratory isolates has reduced the dependability of routinely used beta-lactam antibiotics. Local antibiogram data are therefore necessary for rational treatment selection and antimicrobial stewardship.

Aim: To determine the microbiological profile of organisms isolated from sputum samples and to assess the antibiotic resistance pattern of major bacterial respiratory pathogens.

Materials and Methods: This prospective observational study included 1500 non-duplicate sputum samples collected from clinically suspected respiratory tract infection cases. Among the study samples, 600 (40.0%) were from males and 900 (60.0%) were from females. Adults aged 18-64 years constituted the largest group 910 (60.7%), followed by elderly patients aged ≥ 65 years 510 (34.0%), adolescents aged 13-17 years 50 (3.3%) and children aged 1-12 years 30 (2.0%). All samples were cultured on Blood agar and MacConkey agar and incubated aerobically at 37°C for 18-24 hours. Isolates were identified using colony characteristics, Gram staining, standard biochemical reactions and VITEK-2 when required. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method and interpreted according to Clinical and Laboratory Standards Institute recommendations. Data were analysed using descriptive statistics, and frequencies and percentages were calculated for culture outcome, demographic distribution, isolate profile and antibiotic resistance pattern.

Results: Pathogenic growth was obtained in 660 of 1500 samples (44.0%). Females contributed 900 samples (60.0%) and adults aged 18-64 years formed the largest group (910, 60.7%). Among pathogenic isolates, *Klebsiella pneumoniae* was most frequent (290, 43.9%), followed by *Pseudomonas aeruginosa* (130, 19.7%), *Candida albicans* (110, 16.7%) and *Escherichia coli* (90, 13.6%). Resistance was high to ceftriaxone, cefuroxime and amoxiclav, while comparatively lower resistance was observed with imipenem and meropenem.

Conclusions: Gram-negative bacteria predominated among sputum isolates, with substantial resistance to commonly used antibiotics. Routine culture, periodic antibiogram review and culture-guided de-escalation are important for effective respiratory infection management.

KEYWORDS: Bacterial culture; Drug susceptibility; Gram-negative bacilli; *Klebsiella* infections; *Pseudomonas* infections; Stewardship.

INTRODUCTION

Respiratory tract infections remain a major cause of clinical consultation, diagnostic testing, antibiotic prescription and hospital admission. Lower respiratory tract infections are particularly important because delayed recognition and inappropriate empirical therapy can increase complications in susceptible patients. Sputum culture continues to assist clinicians when a bacterial cause is suspected, especially in patients with persistent symptoms, comorbid illness, advanced age or healthcare exposure [1]. At the same time, antimicrobial resistance has become a major threat to the success of empirical therapy and has increased the need for local susceptibility data [2,3].

The clinical consequences of resistance extend beyond laboratory reporting. Resistant pathogens can delay clinical response, increase the need for second-line drugs, prolong hospital stay and raise treatment cost. These effects are most evident when initial therapy does not cover the causative organism or when broad-spectrum agents are used repeatedly without microbiological confirmation [4,5]. Therefore, empirical prescribing for respiratory infections should be supported by updated institutional or regional antibiograms rather than by older assumptions about common pathogens.

Gram-negative bacilli have emerged as frequent respiratory isolates in many hospital and diagnostic laboratory settings. Their resistance is driven by several mechanisms, including beta-lactamase production, altered

membrane permeability, efflux pump activity, plasmid-mediated resistance transfer and biofilm formation [6-8]. These mechanisms allow organisms to withstand multiple drug classes and complicate therapy in patients who have received antibiotics before submitting respiratory specimens.

Klebsiella pneumoniae and *Pseudomonas aeruginosa* are clinically important because they are associated with virulence, environmental persistence and multidrug resistance. *Klebsiella pneumoniae* has a prominent capsule and several pathogenicity factors that support survival in the host. *Pseudomonas aeruginosa* has intrinsic resistance, adapts well to moist healthcare environments and forms biofilms, making eradication difficult in chronic or severe respiratory disease [9,10]. *Escherichia coli* is less classically considered a respiratory pathogen, but it can be recovered from sputum in debilitated or healthcare-exposed individuals and should be interpreted with clinical correlation. Extended-spectrum beta-lactamase-producing Enterobacterales further restrict available therapeutic choices [11].

Reliable laboratory processing is essential for meaningful interpretation. Good-quality sputum collection, culture on suitable media, Gram staining, biochemical identification and susceptibility testing are standard elements of bacteriological evaluation [12]. The Kirby-Bauer disc diffusion method remains widely used because it is economical, reproducible and suitable for routine diagnostic laboratories when standard procedures are followed [13,14]. Antibiotic susceptibility results also require careful clinical interpretation because a sputum isolate can represent infection, colonisation or contamination, depending on the patient and sample quality [15].

Automated systems such as VITEK-2 can improve turnaround time and standardisation, particularly when laboratories process a large number of specimens or encounter complex resistance patterns [16,17]. However, the practical value of both manual and automated approaches depends on regular quality control and timely communication with clinicians. Periodic local analysis of sputum isolates can guide empirical therapy, encourage de-escalation and support antimicrobial stewardship. The present study was conducted to describe the microbiological profile of sputum culture isolates and assess the antibiotic resistance pattern of major bacterial respiratory pathogens in a dual microbiology study setting in Hyderabad, India.

Aim

To analyse the distribution of organisms isolated from sputum culture samples and to determine the antibiotic resistance pattern of major bacterial respiratory pathogens. The secondary objective was to describe the distribution of culture-positive isolates according to age group and gender.

MATERIALS AND METHODS

Study design and duration: This was a prospective observational study conducted over three months from February 2023 to April 2023.

Study setting: The study was conducted at the Indian Immunologicals Limited, Gachibowli, Hyderabad 500032, Telangana, India.

Study population: The study included sputum samples received from patients clinically suspected to have respiratory tract infection. Samples were accepted irrespective of age and gender when they were properly collected, transported in sterile leak-proof containers and suitable for routine microbiological processing.

Inclusion criteria: All properly labelled, non-duplicate sputum samples received during the study period from clinically suspected respiratory tract infection cases were included.

Exclusion criteria: Inadequate sputum samples, grossly contaminated specimens, improperly labelled samples, repeat samples from the same illness episode and samples with insufficient quantity or unsuitable quality for processing were excluded.

Sample size: A complete enumeration method was used. All eligible samples received during the study period were analysed, giving a final sample size of 1500 sputum samples. A separate formal sample size calculation was not applied because the study objective was descriptive laboratory surveillance rather than hypothesis testing.

Sample collection and processing: Sputum samples were collected in sterile containers after routine instructions and transported promptly to the laboratory. Macroscopic assessment and microscopic screening were performed where applicable. Samples were inoculated on Blood agar and MacConkey agar and incubated aerobically at 37°C for 18-24 hours. Colony morphology, haemolysis, lactose fermentation, pigmentation and growth characteristics were recorded. Gram staining and standard biochemical tests, including indole, citrate, urease, oxidase and triple sugar iron reactions, were used for organism identification. Selected isolates were processed further using VITEK-2 for automated identification [12,16,17].

Antibiotic susceptibility testing: Antibiotic susceptibility testing was performed by the Kirby-Bauer disc diffusion method on appropriate media. Zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute recommendations. VITEK-2 susceptibility results were used for selected isolates when required [13,14]. Resistance percentages were calculated for major bacterial isolates tested against selected antimicrobial agents.

Study variables: The main variables were culture outcome, isolated organism, age group, gender and percentage resistance to selected antibiotics among major bacterial isolates. *Candida albicans* was reported separately as a fungal isolate and interpreted cautiously because sputum recovery frequently reflects airway or oropharyngeal colonisation.

Measures to minimise bias: Duplicate specimens from the same clinical episode were excluded to avoid over-

representation. Uniform culture media, standard identification procedures and CLSI-based susceptibility interpretation were used to improve reproducibility. De-identified laboratory data were analysed to reduce observer influence from patient identifiers.

Statistical analysis: Data were entered in Microsoft Excel and analysed using descriptive statistics. Categorical variables were summarised as frequencies and percentages. Inferential statistical testing was not performed because the study was designed to describe isolate distribution and resistance pattern.

Ethical considerations: The analysis used de-identified diagnostic laboratory data and did not include direct patient contact or personal identifiers.

RESULTS

Culture outcome and demographic distribution

A total of 1500 sputum samples were processed during the study period. Pathogenic growth was observed in 660 samples (44.0%), while 840 samples (56.0%) showed non-pathogenic growth, normal flora or no significant growth. Females contributed a larger proportion of samples than males. Adults aged 18-64 years formed the largest age category, followed by patients aged 65 years and above [Table/Fig-1].

[Table-1]: Culture outcome and demographic distribution of sputum samples (n=1500).

Variable	Category	Number	Percentage (%)
Culture outcome	Pathogenic growth	660	44.0
Culture outcome	Non-pathogenic/no significant growth	840	56.0
Gender	Male	600	40.0
Gender	Female	900	60.0
Age group	1-12 years	30	2.0
Age group	13-17 years	50	3.3
Age group	18-64 years	910	60.7
Age group	>=65 years	510	34.0

Distribution of pathogenic isolates

Gram-negative bacteria constituted the major group among culture-positive samples. *Klebsiella pneumoniae* was the predominant isolate, followed by *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli*. *Proteus mirabilis*, *Citrobacter freundii*, *Enterobacter* species and *Pseudomonas putida* were recovered in smaller proportions [Table/Fig-2].

[Table-2]: Frequency of pathogenic isolates in sputum samples (n=660).

Organism	Number of isolates	Percentage (%)
<i>Klebsiella pneumoniae</i>	290	43.9
<i>Pseudomonas aeruginosa</i>	130	19.7
<i>Candida albicans</i>	110	16.7
<i>Escherichia coli</i>	90	13.6
<i>Proteus mirabilis</i>	10	1.5
<i>Citrobacter freundii</i>	10	1.5
<i>Enterobacter</i> species	10	1.5
<i>Pseudomonas putida</i>	10	1.5
Total	660	100.0

Pathogenic isolates by age group and gender

Among the 660 pathogenic isolates, most were recovered from adults aged 18-64 years, followed by elderly patients aged 65 years and above. Female patients accounted for 380 isolates and male patients for 280 isolates. *Klebsiella pneumoniae* remained the leading isolate in both adult and elderly groups [Table/Fig-3].

[Table-3]: Distribution of pathogenic isolates by age group and gender (n=660).

Organism	1-12 y	13-17 y	18-64 y	>=65 y	Male	Female	Total
<i>Klebsiella pneumoniae</i>	0	10	180	100	120	170	290
<i>Pseudomonas aeruginosa</i>	0	10	80	40	50	80	130
<i>Escherichia coli</i>	0	10	60	20	40	50	90
<i>Candida albicans</i>	10	10	60	30	50	60	110
Others	0	10	30	0	20	20	40
Total	10	50	410	190	280	380	660

Morphological and biochemical identification

Major Gram-negative bacterial isolates showed characteristic cultural and biochemical patterns. *Klebsiella*

pneumoniae produced mucoid lactose-fermenting colonies with citrate and urease positivity. *Escherichia coli* showed lactose-fermenting colonies with indole positivity. *Pseudomonas aeruginosa* showed non-lactose-fermenting colonies, pigment production and oxidase positivity [Table/Fig-4].

[Table-4]: Key morphological and biochemical features used for identification of selected bacterial isolates.

Organism	Gram stain and colony morphology	Key biochemical reactions
<i>Klebsiella pneumoniae</i>	Gram-negative bacilli; mucoid lactose-fermenting colonies	Indole negative, citrate positive, urease positive, TSI A/A with gas, oxidase negative
<i>Escherichia coli</i>	Gram-negative bacilli; smooth lactose-fermenting colonies	Indole positive, citrate negative, urease negative, TSI A/A with gas, oxidase negative
<i>Pseudomonas aeruginosa</i>	Gram-negative bacilli; non-lactose fermenter with greenish pigment	Indole negative, citrate positive, urease negative, TSI K/K, oxidase positive
<i>Proteus mirabilis</i>	Gram-negative bacilli; non-lactose fermenter with swarming	Indole negative, citrate negative, urease positive, TSI K/A with H ₂ S, oxidase negative
<i>Citrobacter freundii</i>	Gram-negative bacilli; non-lactose-fermenting colonies	Indole negative, citrate positive, urease negative, TSI A/A with H ₂ S, oxidase negative
<i>Enterobacter</i> species	Gram-negative bacilli; lactose-fermenting colonies	Indole negative, citrate positive, urease negative, TSI A/A, oxidase negative

Antibiotic resistance pattern

Resistance varied across the major bacterial isolates. Ceftriaxone, cefuroxime and amoxiclav showed high resistance among *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Resistance to imipenem and meropenem was comparatively lower than resistance to cephalosporins and amoxiclav, although carbapenem resistance was not absent [Table/Fig-5].

[Table-5]: Antibiotic resistance profile of major bacterial respiratory pathogens.

Antibiotic	<i>Klebsiella pneumoniae</i> resistance (%)	<i>Escherichia coli</i> resistance (%)	<i>Pseudomonas aeruginosa</i> resistance (%)
Ceftriaxone	80	70	85
Cefuroxime	75	65	85
Amoxiclav	70	60	80
Ciprofloxacin	50	45	55
Gentamicin	40	35	45
Imipenem	10	5	15
Meropenem	15	10	20

Manual and automated susceptibility testing

The VITEK-2 automated system supported faster reporting and standardised interpretation for selected isolates, while Kirby-Bauer disc diffusion remained a practical and low-cost method for routine susceptibility testing. The main operational differences are summarised in [Table/Fig-6].

[Table/Fig-6]: Comparison of automated and manual antibiotic susceptibility testing methods.

Parameter	VITEK-2 automated method	Kirby-Bauer manual method
Time required	Rapid; approximately 6-12 hours	Longer; approximately 18-24 hours
Interpretation	Automated and standardised	Manual and operator-dependent
Reproducibility	High when quality control is maintained	Variable if technique is inconsistent
Human error	Lower during reading and interpretation	Possible during measurement or interpretation
Support for selected resistance patterns	Supports recognition of selected resistance patterns	Depends on disc selection and interpretive accuracy
Cost	Higher	Lower
Suitability	Advanced or high-volume laboratories	Basic and routine diagnostic laboratories

DISCUSSION

The present study evaluated 1500 sputum samples and found pathogenic growth in 44.0% of specimens. This culture positivity indicates that sputum culture retains practical value in suspected respiratory infection, but it also shows that more than half of the submitted samples did not yield significant pathogenic growth. Non-significant culture can occur due to viral illness, prior antibiotic exposure, poor specimen quality, low bacterial load or colonisation by commensal flora. These observations support culture-based decision-making before unnecessary escalation of empirical antibiotics, because inadequate or poorly targeted therapy is associated with unfavourable

clinical outcomes [1,4,18].

The isolate profile showed predominance of Gram-negative organisms, with *Klebsiella pneumoniae* as the commonest isolate, followed by *Pseudomonas aeruginosa* and *Escherichia coli*. This pattern is relevant because Gram-negative bacilli are common in patients with healthcare exposure, structural lung disease, chronic illness and repeated antibiotic use. Similar findings have been reported in studies from other settings, although the dominant pathogen varies according to patient population, specimen quality, hospital referral pattern and local antibiotic pressure [19,20].

Adults and elderly patients accounted for most pathogenic isolates. This distribution probably reflects the higher burden of chronic respiratory disease, comorbid illness and healthcare contact in these age groups. Female predominance in both sample submission and culture-positive isolates should be interpreted with caution because the present study used laboratory records and did not assess population-level disease incidence. Therefore, this observation is better understood as a pattern of sample receipt and isolate recovery rather than proof of gender-related susceptibility.

The most important clinical finding was the high resistance to ceftriaxone, cefuroxime and amoxiclav among major bacterial isolates. This resistance profile suggests that these commonly prescribed antibiotics have limited reliability for empirical use in similar laboratory catchment settings. Resistance in Gram-negative bacilli is driven by extended-spectrum beta-lactamases, AmpC beta-lactamases, carbapenemases, porin changes, efflux pumps and horizontal transfer of resistance determinants [21-23]. Gentamicin and ciprofloxacin also showed notable resistance, indicating that susceptibility confirmation is important before their use whenever culture results are available.

Carbapenems showed comparatively better activity than cephalosporins and amoxiclav. Nevertheless, resistance to imipenem and meropenem was present, and this finding has stewardship importance. Carbapenems are reserve agents for severe infection and multidrug-resistant Gram-negative disease; unrestricted use can accelerate the emergence of carbapenem-resistant Enterobacterales and resistant non-fermenters. A policy of early culture collection, susceptibility-guided therapy and prompt de-escalation can help preserve these agents [24,25].

Candida albicans was isolated in a considerable proportion of culture-positive samples. In sputum specimens, *Candida* isolation commonly reflects airway or oropharyngeal colonisation rather than invasive fungal pneumonia. Antifungal therapy should not be started solely on the basis of sputum isolation. Clinical status, host immune profile, radiological findings and supportive microbiological evidence should guide interpretation.

The study also highlights the complementary roles of manual and automated methods. Kirby-Bauer disc diffusion is affordable and suitable for routine diagnostic laboratories when quality control is maintained. VITEK-2 can shorten reporting time and improve standardisation, especially in high-volume laboratories or when resistance patterns are complex. However, both methods require careful correlation with sample quality, clinical diagnosis and prior antibiotic exposure. The conversion of routine laboratory results into periodic antibiograms can strengthen empirical prescribing and support antimicrobial stewardship in respiratory infection management.

Limitations

This was a dual-setting prospective observational study conducted over a short three-month period; therefore, seasonal variation and wider regional trends were not fully assessed. Detailed clinical data, inpatient or outpatient status, prior antibiotic use, radiological diagnosis, comorbidities and treatment outcomes were unavailable for all samples. Molecular detection of resistance genes was not performed. Sputum culture interpretation can be influenced by specimen quality and upper airway colonisation, especially for *Candida albicans*.

CONCLUSION

The study showed predominance of Gram-negative organisms among sputum culture isolates, with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as the leading bacterial pathogens. High resistance to ceftriaxone, cefuroxime and amoxiclav indicates that empirical therapy should be guided by local culture and susceptibility data. Carbapenems retained comparatively better activity, but their use requires stewardship oversight. Regular antibiogram review, appropriate specimen processing, timely reporting and culture-guided de-escalation are essential for improving respiratory infection management and limiting antimicrobial resistance.

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