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Original Article

Bacteriological Correlation of Sputum and Bronchoalveolar Lavage Cultures in Suspected Cases of Lower Respiratory Tract Infections

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ABSTRACT

Introduction: Lower respiratory tract infections (LRTIs) are a leading cause of global morbidity and mortality, necessitating accurate microbiological diagnosis for effective antimicrobial stewardship. Sputum is the primary non-invasive diagnostic specimen, but its reliability is often limited by oropharyngeal contamination, leading to frequent false-negative results. Bronchoalveolar lavage (BAL) provides a more representative sample of the lower airway but is invasive. This study aimed to evaluate the bacteriological correlation between sputum and BAL cultures and describe associated antimicrobial susceptibility patterns in patients with suspected LRTIs.

Materials and Methods: A descriptive cross-sectional study was conducted on 140 adult patients (≥18 years) with suspected LRTIs in the Department of Respiratory Medicine at the Pacific Institute of Medical Sciences (Udaipur, India) between July 2023 and December 2024. Paired expectorated sputum and BAL samples were collected simultaneously and processed for aerobic culture and antimicrobial susceptibility testing (Kirby–Bauer disk diffusion method) following CLSI-2019 guidelines. The quality of sputum samples was microscopically assessed before culture.

Results: The mean age of participants was 54.98 pm 13.53 years, with a pronounced male predominance (90.71%). Chronic lung disease (47.86%) and smoking (64.29%) were the most common comorbidity and risk factor, respectively. BAL demonstrated significantly higher culture positivity (80.71%) compared to sputum (25.00%). Pseudomonas aeruginosa was the most common isolate in both samples, but its detection rate was significantly higher in BAL (27.86%) than in sputum (7.14%) ($p<0.001$). Similar highly significant differences were noted for Acinetobacter baumannii (BAL: 15.00%, Sputum: 2.14%; $p<0.001$) and Escherichia coli (BAL: 12.14%, Sputum: 1.43%; $p<0.001$). Isolates from both samples showed substantial antimicrobial resistance, particularly Gram-negative organisms. In BAL, Acinetobacter baumannii showed resistance to macrolides in all detected cases, while E. coli was most sensitive to beta-lactams.

Conclusion: Sputum culture exhibits a high false-negative rate (75%) compared to BAL in diagnosing LRTIs, severely underestimating the prevalence of Gram-negative pathogens like P. aeruginosa and A. baumannii. BAL is superior for definitive microbiological diagnosis, particularly in a population with high prevalence of chronic lung disease and exposure risk factors. These findings reinforce the need for targeted antibiotic policies guided by lower airway sampling, especially in cases of severe illness or where sputum results are inconclusive.

Keywords: *Lower respiratory tract infections (LRTIs); Sputum culture; Bronchoalveolar lavage (BAL); Antimicrobial susceptibility; Gram-negative pathogens.*

INTRODUCTION

Lower respiratory tract infections (LRTIs), including pneumonia, bronchitis, and infective exacerbations of chronic lung disease, remain a critical public health challenge globally [1, 2]. Data from the Global Burden of Disease (GBD) 2019 study indicated that lower respiratory infections accounted for approximately 2.49 million deaths in 2019, consistently ranking among the deadliest communicable diseases worldwide [2, 3]. This persistent burden is exacerbated by delayed diagnosis, limited access to specialized microbiology, and the escalating crisis of antimicrobial resistance (AMR) [1, 4].

Accurate microbiological confirmation is paramount for guiding rational, pathogen-directed antimicrobial therapy [4]. LRTIs are caused by a wide spectrum of organisms, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and increasingly, multidrug-resistant (MDR) Gram-negative bacilli [3, 5]. The rise of MDR organisms in both community and hospital settings complicates empirical treatment and increases the urgency for precise bacteriological identification [4].

The microbiological diagnosis of LRTIs is inherently difficult, as clinical and radiological findings are often non-specific [6]. Expectorated sputum is the traditional, non-invasive first-line specimen, undergoing routine work-up via Gram stain and culture [6, 7]. However, the utility of sputum culture is severely limited by common contamination from oropharyngeal flora, which can obscure true lower airway pathogens, resulting in misleading results and potentially inappropriate antibiotic prescribing [8, 9, 10]. To address this, many guidelines recommend microscopic screening and rejection of poor-quality samples [9]. Even with quality control, sputum often fails to identify a pathogen, especially in patients who have received prior antibiotics [10].

These limitations motivate the use of bronchoscopy-based techniques, particularly bronchoalveolar lavage (BAL), in selected, often critically ill, patients [11]. BAL involves instilling and re-aspirating sterile saline into the distal airways, yielding a sample that more accurately reflects the microbial milieu of the infection site while minimizing upper airway contamination [11]. Numerous studies support the superior diagnostic yield of BAL compared to sputum, particularly in severe or complex conditions like pulmonary tuberculosis and cystic fibrosis, where BAL has demonstrated superior sensitivity [12, 13, 14].

However, BAL is invasive, requires specialized resources, and carries inherent procedural risks, limiting its use in routine practice [11]. Clarifying the bacteriological concordance between sputum and BAL is crucial: high concordance would support the reliance on non-invasive sputum, while significant discordance, especially concerning high-risk or MDR pathogens, would justify a lower threshold for performing BAL [15, 16]. Local data comparing pathogen profiles and resistance patterns in these two specimens are essential for developing evidence-based diagnostic algorithms and institutional antibiotic policies [16, 17].

This study aims to evaluate the bacteriological correlation between sputum and BAL cultures in patients with suspected lower respiratory tract infection (LRTI) and describe the associated antimicrobial susceptibility patterns. By quantifying the agreement between these two commonly used specimens, the findings are intended to inform practical decisions regarding the necessity of BAL for optimal diagnosis and treatment of LRTIs in a resource-constrained setting.

MATERIALS AND METHODS

Study Design and Setting

This was a descriptive cross-sectional study conducted over 18 months, from July 2023 to December 2024, at the Department of Respiratory Medicine, Pacific Institute of Medical Sciences (PIMS), Udaipur, Rajasthan, India. The study included patients from both outpatient and inpatient services.

Study Population and Enrollment

A total of 140 adult patients (age >18 years) clinically suspected of having an LRTI were enrolled. Enrollment was contingent upon obtaining informed written consent and physician confirmation of the patient's suitability for bronchoscopic evaluation.

Inclusion Criteria:

- Age >18 years.
- Symptoms suggestive of acute respiratory infection (e.g., cough, fever, dyspnoea, or radiographic evidence of consolidation/infiltrates).
- Patients advised bronchoscopy with consent for the procedure.

Exclusion Criteria:

- Patients refusing consent for bronchoscopy.
- Individuals with unstable cardiac or systemic disease (e.g., recent myocardial infarction, stroke).
- Confirmed pulmonary tuberculosis (positive CBNAAT/AFB in sputum or BAL).

- Pregnant women and patients deemed unfit for bronchoscopy by the attending physician.

Data Collection and Sample Processing
Demographic (age, sex, occupation) and clinical data (symptoms, comorbidities, risk factors, radiological findings, and clinical diagnosis) were collected using a standardized questionnaire. Sputum and BAL samples were collected simultaneously from each participant and transported to the microbiology laboratory within 30 minutes for immediate processing.

Sample Collection
1. **Sputum Collection:** Expectorated sputum was obtained by deep cough. Sample quality was microscopically assessed for squamous epithelial cells and polymorphonuclear leukocytes (PMNs) before further analysis.

2. **Bronchoalveolar Lavage (BAL) Collection:** BAL fluid was collected during flexible bronchoscopy under local anesthesia and conscious sedation. A sterile saline aliquot (20–50 mL) was instilled into the targeted segment and re-aspirated for analysis.

Laboratory Procedures
1. **Microscopic Examination:** Smears were prepared from the purulent portion of both samples for Gram staining and Ziehl-Neelsen (ZN) staining.

2. **Aerobic Culture:** Samples were inoculated onto Blood Agar (BA) and MacConkey Agar (MA) and incubated aerobically at 37^0 C for 24 hours. Isolates were identified using standard microbiological procedures, including colony characteristics, Gram staining, and a panel of biochemical reactions based on Bergey's Manual of Systematic Bacteriology.

3. **Antimicrobial Susceptibility Testing (AST):** AST was performed on Mueller–Hinton Agar (MHA) using the Kirby–Bauer disk diffusion method, interpreted according to Clinical and Laboratory Standards Institute (CLSI)-2019 guidelines [17].

Ethical Approval
Institutional Ethical Committee clearance was obtained prior to commencement, and informed written consent was secured from all participants.

RESULTS
Demographic and Clinical Characteristics
The study included 140 patients. The mean age was 54.98 pm 13.53 years, with the majority belonging to the 46–60 years (45.00%) and 61–75 years (37.14%) age groups (Table 1). There was a significant male predominance (90.71%).

Table 1. Age and Gender Distribution of Study Participants (N=140**)**

Characteristic	**Number of Patients**	**Percentage (%)**
Age Group (years)		
18–30	12	8.57
31–45	13	9.29
46–60	63	45.00
61–75	52	37.14
Mean Age ± SD	*54.98 ±13.53*	
Gender		
Male	127	90.71
Female	13	9.29

Occupational analysis showed that laborers (28.57%) and farmers (22.14%) constituted the largest proportion of cases. The most common presenting symptoms were cough (98.57%), shortness of breath (78.57%), and expectoration (76.43%).

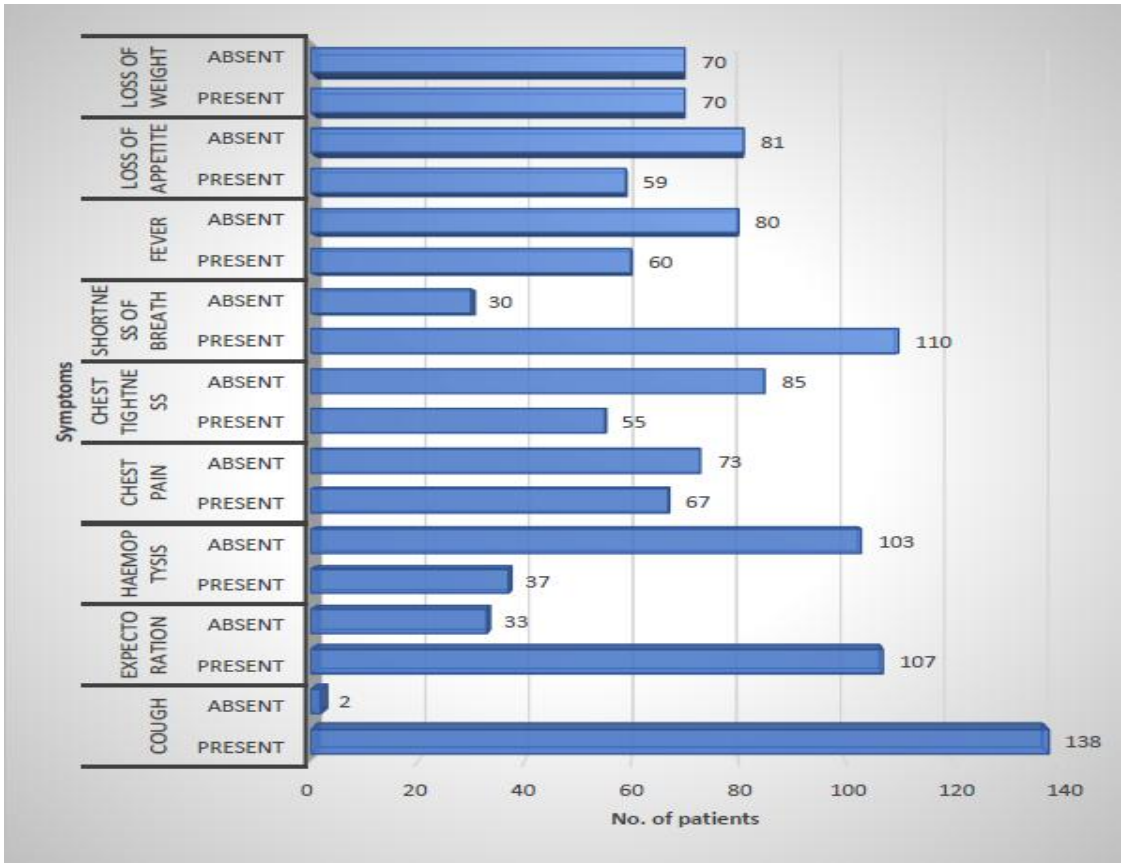

Fig 1: **Distribution of cases according to symptoms**

Comorbidities and Risk Factors: Chronic Lung Disease (CLD) was the most common comorbidity (47.86%), followed by Diabetes Mellitus (13.57%) and Hypertension (8.57%). Smoking (current smokers: 44.29%; ex-smokers: 20.00%) was the dominant risk factor (64.29% total), followed by alcohol consumption (25.00%).

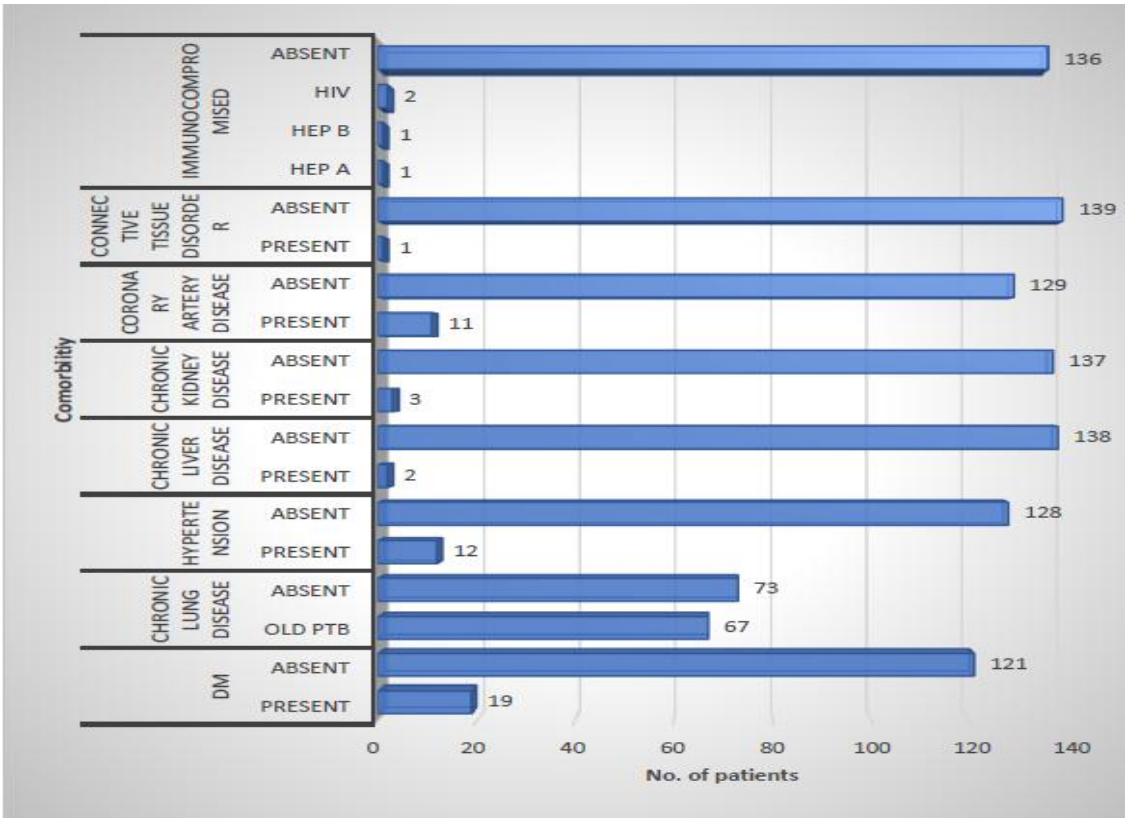

Fig 1: Distribution of cases according to comorbidity

Microbiological Findings and Correlation

A pronounced difference in culture positivity was observed, with BAL cultures showing positive growth in 80.71% of samples, significantly higher than the 25.00% positivity rate seen in sputum cultures.

Table 2. Comparison of Culture Results (Sputum vs BAL) (N=140**)**

Organism	**Sputum No. (%)**	**BAL No. (%)**	***p*-value**
Pseudomonas aeruginosa	10 (7.14)	39 (27.86)	<0.001
Acinetobacter baumannii	3 (2.14)	21 (15.00)	<0.001
Escherichia coli	2 (1.43)	17 (12.14)	<0.001
Klebsiella pneumoniae	5 (3.57)	11 (7.86)	<0.001
Citrobacter koseri	2 (1.43)	7 (5.00)	0.05
CoNS	3 (2.14)	2 (1.43)	0.65
Enterobacter cloacae	2 (1.43)	2 (1.43)	0.90
Staphylococcus aureus	5 (3.57)	4 (2.86)	0.90
Streptococcus pneumoniae	3 (2.14)	3 (2.14)	0.90
Citrobacter freundii	—	4 (2.86)	—
Pseudomonas fluorescens	—	3 (2.14)	—
Total Positive Cultures	**35 (25.00)**	**113 (80.71)**	
Total Negative Cultures	**105 (75.00)**	**27 (19.29)**	

Highly significant differences (p<0.001) in detection rates in favor of BAL were noted for *P. aeruginosa*, *A. baumannii*, *E. coli*, and *K. pneumoniae*. Organisms such as *C. freundii* and *P. fluorescens* were detected exclusively in BAL cultures.

Antimicrobial Susceptibility Patterns

Table 3. Antibiotic Resistant Pattern in Different Aerobic Organisms in Sputum

Organism	**Total Isolates (N)**	**Beta-Lactams Resist. (%)**	**Aminoglycosides Resist. (%)**	**Macrolides Resist. (%)**	**Quinolones Resist. (%)**
Pseudomonas aeruginosa	10	4 (21.0)	3 (27.27)	3 (25.0)	0 (0.00)
Klebsiella pneumoniae	5	3 (15.79)	2 (18.18)	5 (41.67)	5 (35.71)
Staphylococcus aureus	5	3 (15.79)	1 (9.09)	2 (16.67)	3 (21.43)
Acinetobacter baumannii	3	2 (10.53)	1 (9.09)	1 (8.33)	2 (14.29)
Streptococcus pneumoniae	3	2 (10.53)	1 (9.09)	1 (8.33)	2 (14.29)
CoNS	3	1 (5.26)	0 (0.00)	0 (0.00)	1 (7.14)
Escherichia coli	2	2 (10.53)	2 (18.18)	0 (0.00)	0 (0.00)
Citrobacter koseri	2	1 (5.26)	1 (9.09)	0 (0.00)	0 (0.00)
Enterobacter cloacae	2	1 (5.26)	1 (9.09)	0 (0.00)	1 (7.14)
Negative (No growth)	105	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	**140**	**19 (100)**	**11 (100)**	**12 (100)**	**14 (100)**

Table 4. Antibiotic Resistant Pattern in Different Aerobic Organisms in BAL

Organism	**Total Isolates (N)**	**Beta-Lactams Resist. (%)**	**Aminoglycosides Resist. (%)**	**Macrolides Resist. (%)**	**Quinolones Resist. (%)**
Pseudomonas aeruginosa	39	9 (36.00)	15 (28.30)	35 (35.71)	10 (47.62)
Acinetobacter baumannii	21	0 (0.00)	21 (39.62)	0 (0.00)	0 (0.00)
Escherichia coli	17	1 (4.00)	5 (9.43)	6 (42.86)	5 (23.81)
Klebsiella pneumoniae	11	3 (12.00)	6 (11.32)	0 (0.00)	2 (9.52)
Citrobacter koseri	7	7 (28.00)	0 (0.00)	0 (0.00)	0 (0.00)

Staphylococcus aureus	4	1 (4.00)	1 (1.89)	1 (7.14)	1 (4.76)
Citrobacter freundii	4	0 (0.00)	4 (7.55)	0 (0.00)	0 (0.00)
Pseudomonas fluorescens	3	1 (4.00)	1 (1.89)	0 (0.00)	1 (4.76)
Streptococcus pneumoniae	3	1 (4.00)	1 (1.89)	1 (7.14)	0 (0.00)
CoNS	2	1 (4.00)	0 (0.00)	0 (0.00)	1 (4.76)
Enterobacter cloacae	2	1 (4.00)	0 (0.00)	0 (0.00)	1 (4.76)
Total	**113**	**25 (100)**	**53 (100)**	**14 (100)**	**21 (100)**

The percentage values for the antibiotic classes refer to the proportion of resistance among all positive isolates tested for that class in the respective sample type.

In BAL isolates, *Acinetobacter baumannii* showed complete sensitivity to macrolides and quinolones (0.00% resistance), indicating these as potentially effective empirical options. Conversely, it demonstrated high resistance to aminoglycosides (39.62% of all aminoglycoside-resistant isolates were *A. baumannii*). *P. aeruginosa* showed high resistance across all tested classes.

DISCUSSION

This study evaluated the diagnostic correlation between non-invasive sputum culture and invasive BAL culture in 140 adult patients with suspected LRTIs, revealing a stark contrast in diagnostic yield and pathogen detection.

The mean age of the cohort (54.98 ± 13.53 years) and the high prevalence of cases in the 46–75 age bracket align with epidemiological data suggesting heightened vulnerability in middle-aged and older adults due to cumulative environmental exposures, smoking, and underlying chronic diseases [18, 19]. The pronounced male predominance (90.71%) is consistent with many regional and national studies, attributed largely to higher rates of smoking and occupational exposure [18, 20]. The dominance of chronic lung disease (47.86%) and smoking (64.29%) underscores the role of pre-existing pulmonary impairment in predisposing individuals to bacterial colonization and subsequent infection [21].

The most critical finding is the substantial difference in culture positivity: 75.00% of sputum samples were negative, compared to only 19.29% of BAL samples. This 55-point difference highlights the **superior diagnostic yield of BAL** and confirms the well-recognized limitation of sputum sampling, which is highly susceptible to oropharyngeal contamination, leading to high false-negative rates [8, 11]. This finding strongly supports the use of BAL for definitive diagnosis in patients with moderate-to-severe illness, non-resolving pneumonia, or prior antibiotic exposure.

The comparison of specific pathogens is even more revealing. While *P. aeruginosa* was the most frequent isolate, its prevalence was four times higher in BAL (27.86%) than in sputum (7.14%) ($p<0.001$). Similar highly significant discrepancies were found for *Acinetobacter baumannii* and *E. coli*—all organisms frequently associated with hospital-acquired infections, prior antibiotic exposure, and chronic respiratory disease [22]. Sputum, in these cases, critically *underestimated* the true pathogen load and incidence of these Gram-negative organisms in the lower airway. The detection of pathogens exclusively in BAL (e.g., *C. freundii*, *P. fluorescens*) further validates BAL's indispensable role in identifying high-risk pathogens that might be entirely missed by non-invasive methods, leading to inappropriate empirical therapy [23].

Conversely, organisms such as *Staphylococcus aureus* and *Streptococcus pneumoniae* showed no significant difference between sputum and BAL ($p>0.05$). This suggests that these organisms may colonize both upper and lower airways or represent true infections detectable by both methods, potentially making sputum a more reliable initial screen for these specific typical pathogens.

The antibiotic sensitivity analysis revealed an alarming degree of antimicrobial resistance, particularly among the prevalent Gram-negative isolates detected by BAL. For instance, *Acinetobacter baumannii* showed complete resistance to macrolides in BAL. The observed resistance patterns, such as the high rates in *P. aeruginosa*, reflect evolving local AMR trends. Given the magnitude of discordance, relying solely on sputum to determine the infection's true susceptibility profile is highly risky and likely contributes to the overuse of broad-spectrum antibiotics.

In conclusion, while non-invasive sputum remains the practical first-line sample, its inherent diagnostic limitations, particularly the high false-negative rate for critical Gram-negative pathogens, are a major concern. The findings strongly advocate for **a lower threshold for performing BAL** in selected patient groups—especially those with chronic lung disease, history of smoking/alcohol use, prior antibiotic exposure, or severe illness—to secure a precise microbiological

diagnosis and enable effective, pathogen-directed therapy, thereby improving patient outcomes and reinforcing antimicrobial stewardship [24].

CONCLUSION

This study establishes a statistically significant bacteriological discordance between sputum and bronchoalveolar lavage (BAL) cultures in adult patients with suspected LRTIs. Sputum yielded a high false-negative rate (75.00%) and severely underestimated the prevalence of clinically relevant Gram-negative pathogens, specifically *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *E. coli*, all of which were detected significantly more frequently in BAL ($p<0.001$). The antimicrobial susceptibility data from BAL highlight a substantial burden of resistance, necessitating accurate culture results for targeted therapy. While sputum is a convenient first-line test, BAL is demonstrably superior and is essential for definitive microbiological diagnosis, particularly in a cohort with high comorbidity burden. We recommend incorporating BAL when sputum is inconclusive, to optimize patient management, enhance antimicrobial stewardship, and limit the spread of multidrug-resistant organisms.

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