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Original Research Article

A Study of Microbiological Spectrum and Antimicrobial Sensitivity in Pleural Infections: Empyema Thoracis and Parapneumonic Effusions

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Abstract:

Background: Pleural infections, including empyema thoracis and parapneumonic effusions, remain a significant cause of morbidity and mortality despite advances in antimicrobial therapy and drainage techniques. The evolving bacteriological profile and rising antimicrobial resistance warrant periodic local surveillance to guide empirical therapy.

Aim: To determine the microbiological spectrum and antimicrobial sensitivity patterns of organisms isolated from pleural infections and to compare the yield of conventional culture with enrichment (BHI broth) methods.

Methods: A prospective study was conducted on 100 patients with pleural infections aged >15 years at a tertiary care hospital. Pleural fluid samples were subjected to direct microscopy, standard aerobic culture, and inoculation in Brain Heart Infusion (BHI) broth. Isolates were identified by standard biochemical tests, and antibiotic susceptibility was determined using the Kirby–Bauer disc diffusion method as per CLSI 2015 guidelines.

Results: The majority of patients were males (72%), aged 26–45 years. Fever (95%) and cough (92%) were the most common symptoms. Standard culture yielded 25% positivity, while BHI broth improved detection to 40% (p=0.02). Pseudomonas aeruginosa (45%) was the predominant isolate, followed by Klebsiella pneumoniae (20%) and Staphylococcus aureus (15%). Methicillin-resistant S. aureus (MRSA) accounted for 50% of staphylococcal isolates, while 35.2% of Gram-negative bacilli produced extended-spectrum β-lactamases (ESBLs). Imipenem and Vancomycin showed 100% sensitivity against Gram-negative and Gram-positive isolates, respectively.

Conclusion: Gram-negative organisms, particularly Pseudomonas aeruginosa, predominate in pleural infections. BHI broth enhances culture yield, and rising MRSA and ESBL prevalence underscore the need for rational antibiotic use and continuous resistance monitoring.

Keywords: Empyema Thoracis, Parapneumonic Effusion, Pseudomonas Aeruginosa, MRSA, ESBL, Antimicrobial Resistance, BHI Broth, Pleural Infection.

Introduction

Pleural infection encompassing complicated parapneumonic effusions (CPPE) and empyema thoracis remains a substantial cause of hospitalisation, procedure-related morbidity, and death worldwide despite advances in diagnostics and drainage techniques [1,2]. Adult incidence appears to be rising in several regions, with contemporary cohorts reporting persistent mortality and prolonged length of stay, underscoring the need for context-specific microbiological and antimicrobial data to guide empirical therapy [1,3,4].

The bacteriology of pleural infection differs meaningfully from that of pneumonia. While classic community-acquired pathogens (e.g., Streptococcus pneumoniae) are encountered, modern adult series highlight frequent isolation of the Streptococcus anginosus (milleri) group and Staphylococcus aureus, including methicillin-resistant strains; anaerobes are under-recognised unless specifically targeted by culture methods [2,5,6]. In healthcare-associated disease, Gram-negative bacilli (including Pseudomonas aeruginosa and Enterobacterales) and MRSA are comparatively more prevalent, shaping initial antimicrobial choices [2,7]. The growing contribution of antimicrobial resistance (AMR) further complicates management and may prolong illness or necessitate surgical intervention when initial therapy is discordant [3,8–10].

Accurate pathogen identification is pivotal but challenging. Pleural fluid culture sensitivity is limited—especially after antibiotics—prompting guideline-endorsed strategies that include obtaining

pre-antibiotic samples when feasible, using blood culture bottles for pleural fluid inoculation, and considering molecular assays (e.g., broad-range 16S rRNA PCR) to enhance yield [1,6,11–13]. Such approaches not only refine definitive therapy but also inform local antibiograms that can calibrate empiric regimens for community- versus hospital-acquired infection.

Timely, appropriate antimicrobials alongside effective pleural drainage remain the cornerstone of care [1,2,7]. Current European and British guidance advocate broad empiric coverage tailored by acquisition setting (community vs healthcare-associated), with de-escalation once microbiology is available and early review for source control failure [1,7]. However, practice must be anchored in up-to-date local susceptibility data. This study therefore aims to delineate the current microbiological spectrum and antimicrobial sensitivity patterns in pleural infections (empyema thoracis and parapneumonic effusions) at our centre, providing evidence to optimise empiric therapy and stewardship.

Materials and Method

The present prospective study was conducted in the Upgraded Department of Microbiology, Government General and Chest Hospital, Hyderabad, over a period of six months from March 2016 to August 2016. The study protocol was reviewed and approved by the Institutional Ethics Committee prior to commencement.

A total of 100 patients aged above 15 years of either sex presenting with empyema thoracis or pleural effusion were included. Patients were recruited from both outpatient and inpatient departments of the hospital.

Inclusion Criteria

- Clinically suspected cases of pneumonia with pleural effusion.
- Patients with pneumonia in association with COPD or diabetes mellitus.
- Empyema secondary to lung abscess or bronchiectasis.
- Patients above 15 years of age.
- Pleural fluid samples with pH <7.2, glucose <40 mg/dL, and protein <5.4 g/dL.

Exclusion Criteria

- Patients unwilling to provide informed consent.
- Patients with persisting fever and cough for more than 3 weeks.
- Patients with tuberculous empyema.
- Patients showing infiltrates or consolidation in the upper lobe on chest X-ray.

Thoracic empyema was defined as pleural effusion fulfilling at least one of the following criteria:

1. Presence of frank pus on pleural aspiration.
2. Positive Gram stain for bacteria in pleural fluid.
3. Positive bacterial culture from pleural fluid.

Method

After obtaining informed consent, detailed history and clinical examination were performed. Data included epidemiological characteristics (age, sex, comorbidities such as smoking, diabetes, alcoholism, previous pleural effusions), clinical features (fever, cough, chest pain, dyspnea, weight loss), and laboratory parameters (chest X-ray, pleural fluid pH, glucose, and protein levels).

Sample Collection and Processing

Pleural fluid was collected aseptically by thoracocentesis or during intercostal tube (ICT) drainage. Approximately 2–3 mL of pleural fluid was distributed into three sterile containers:

- **Specimen 1:** Biochemical analysis (pH, glucose, protein)
- **Specimen 2:** Microbiological analysis (microscopy and aerobic culture)
- **Specimen 3:** Inoculation into blood culture broth (Brain Heart Infusion, BHI).

Rejection Criteria

Specimens were rejected if:

- Patient identifiers were incomplete or mismatched.
- Samples were unlabelled or improperly collected.

Microbiological Methods

- **Direct Microscopy**

Samples were centrifuged at 3000 rpm for 10 minutes; smears from the sediment were Gram-stained and examined under oil immersion for pus cells and bacteria. Controls used were Staphylococcus aureus ATCC 25923 (Gram-positive) and Escherichia coli ATCC 25922 (Gram-negative).

- **Culture and Incubation**

Pleural fluid was inoculated on Blood Agar (BA), MacConkey Agar (MA), and Chocolate Agar (CA) plates and incubated at 37°C for 24 hours. CA plates were incubated in 5% CO_2 at 35–37°C for 18 hours. Plates with no growth were reincubated up to 48 hours before reporting as sterile. Additionally, 3 mL of pleural fluid was inoculated into BHI broth and observed for turbidity for up to 7 days.

- **Identification of Isolates**

Colonies were identified using Gram staining and standard biochemical tests, including:

- **For Gram-positive cocci:** Catalase and Coagulase (slide and tube) tests.
- **For Gram-negative bacilli:** Catalase, oxidase, motility, indole, methyl red, Voges–Proskauer, citrate, urease, triple sugar iron (TSI), nitrate reduction, sugar fermentation, and decarboxylase tests.

- **Antimicrobial Susceptibility Testing (AST)**

AST was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar following CLSI 2015 guidelines. Inoculum turbidity was adjusted to 0.5 McFarland standard. Plates were incubated at 35°C for 18–24 hours, and zones of inhibition were measured in millimeters. Results were interpreted as sensitive, intermediate, or resistant.

Antibiotics Tested

For Gram Positive Isolates

Ampicillin, Amoxycillin–Clavulanic acid, Azithromycin, Clindamycin, Co-trimoxazole, Linezolid, Cefoxitin, Vancomycin, Ciprofloxacin, Gentamicin, Ceftazidime, Amikacin.

For Gram Negative Isolates

Ampicillin, Ceftazidime, Gentamicin, Amikacin, Amoxycillin–Clavulanic acid, Piperacillin–Tazobactam, Cotrimoxazole, Aztreonam, Imipenem, Ciprofloxacin, Cefoperazone, Cefotaxime.

Detection of Resistant Strains

- Methicillin-Resistant Staphylococcus aureus (MRSA): Detected using 30 µg Cefoxitin disc. Isolates with zone diameter < 22 mm were labeled as MRSA.
- Extended Spectrum β-Lactamase (ESBL) Production: Identified using Ceftazidime (30 µg) and Ceftazidime–Clavulanic acid (30/10 µg) combined disc test; an increase of > 5 mm in zone diameter indicated ESBL production.

Statistical Analysis

All data were entered into Microsoft Excel 2016 and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic and clinical characteristics.

- Continuous variables (e.g., age, biochemical parameters) were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) as appropriate.
- Categorical variables (e.g., culture positivity, type of organism, antibiotic resistance pattern) were expressed as frequency and percentage.
- Comparisons between groups were performed using the Chi-square test or Fisher's exact test for categorical variables, and the Student's t-test for continuous variables.
- A p-value <0.05 was considered statistically significant.

Observation and Results

Table 1: Distribution of baseline profile among study population

Parameter	Frequency	Percentage
Age (Years)		
15-25	17	17%
26-35	35	35%
36-45	20	20%
46-55	14	14%
56-65	10	10%
66-75	4	4%
Gender		
Male	72	72%
Female	28	28%
Risk Factors		
Diabetes Mellitus	22	22%
Smoking	52	52%
COPD	8	8%
Alcoholism	18	18%
Clinical Symptoms		
Cough	92	92%
Dyspnoea	89	89%
Fever	95	95%
Chest pain	83	83%
Constitutional symptoms	48	48%

This table summarizes the demographic and clinical characteristics of the 100 patients included in the study. The age distribution reveals that the majority of patients (35%) belonged to the 26–35-year age group, followed by 20% in the 36–45-year range, indicating that pleural infections were more prevalent among young to middle-aged adults. A smaller proportion (4%) were elderly (66–75 years). Males constituted a larger proportion (72%) compared to females (28%), showing a clear male predominance—possibly due to higher exposure to risk factors such as smoking and occupational hazards. Among comorbid conditions, smoking was the most common (52%), followed by diabetes mellitus (22%) and alcoholism (18%), while COPD accounted for 8% of cases. Regarding clinical presentation, fever (95%) was the most frequent symptom, followed closely by cough (92%) and dyspnea (89%). Chest pain was reported in 83% of patients, while constitutional symptoms such as malaise and weight loss were seen in 48%. These findings reflect that fever and respiratory symptoms dominate the clinical profile of pleural infections.

Table 2: Comparison of culture methods

	Standard culture	BHI Broth	p-value
Culture Positivity	25	40	0.02
Culture Negativity	75	60	
Total	100	100	

This table compares the culture positivity between the standard culture method and Brain Heart Infusion (BHI) broth enrichment. Out of 100 samples, the standard culture yielded growth in 25 cases (25%), whereas BHI broth showed positivity in 40 cases (40%). The difference was statistically significant ($p = 0.02$), suggesting that BHI broth enrichment substantially improved the detection rate of pathogens. This indicates that BHI broth provides a more favorable environment for the growth of fastidious or low-count organisms often missed by conventional culture. Hence, incorporating enrichment media enhances microbiological yield in pleural infection diagnosis.

Table 3: Comparison of isolates in pleural fluid culture and BHI broth

Organism	No. of isolates Pleural fluid n=25	No. of isolates BHI broth n=40
Pseudomonas aeruginosa	12(48%)	18(45%)
Klebsiella pneumoniae	4(16%)	8(20%)
Proteus species	3(12%)	5(12.5%)
Escherichia coli	2(8%)	3(7.50%)
Staphylococus aureus	4(16%)	6(15%)
Total isolates	25(100%)	40(100%)

This table details the bacterial isolates recovered using the two culture techniques. Pseudomonas aeruginosa was the most frequently isolated organism in both pleural fluid (48%) and BHI broth (45%), reflecting its predominant role in pleural infections, especially in hospital-acquired or post-interventional cases. Klebsiella pneumoniae was the second most common isolate (16% vs. 20%), followed by Proteus species (12% vs. 12.5%), Escherichia coli (8% vs. 7.5%), and Staphylococcus aureus (16% vs. 15%). The slight increase in total isolates with BHI broth (40 vs. 25) reinforces that enrichment culture enhances microbial recovery. The similar relative distribution across both methods also indicates consistency in the microbial spectrum, dominated by gram-negative organisms.

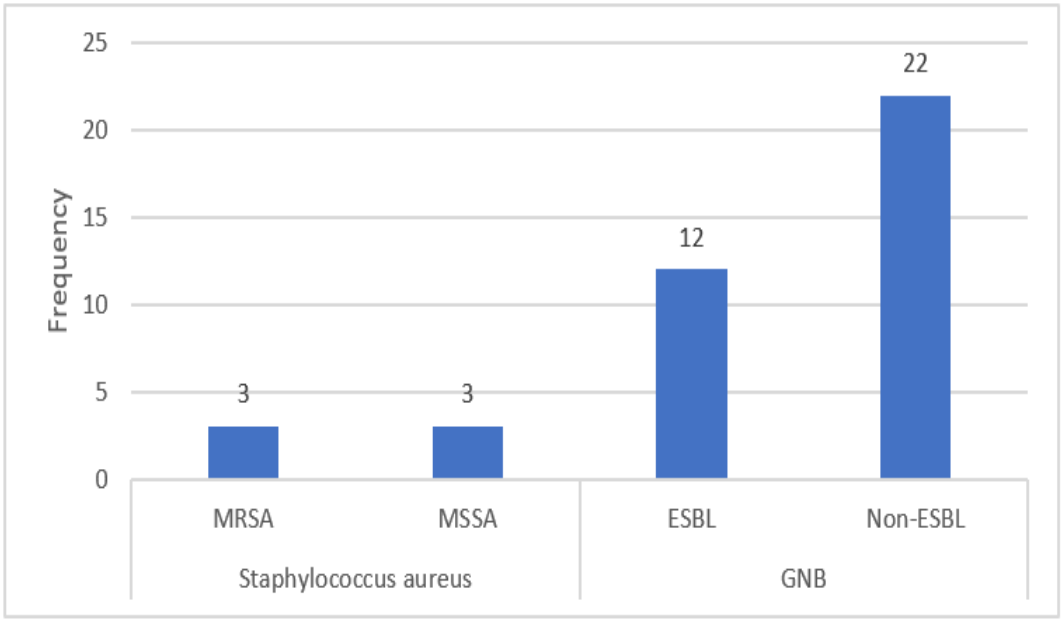

Figure 1:

This figure illustrates the distribution of multidrug-resistant pathogens among the isolates. Methicillin-resistant Staphylococcus aureus (MRSA) represented a significant proportion of S. aureus isolates, indicating the rising trend of resistance among gram-positive cocci in pleural infections. Similarly, Extended Spectrum Beta-Lactamase (ESBL) production was noted among gram-negative isolates, particularly Klebsiella pneumoniae and Escherichia coli. The overall figure emphasizes that antimicrobial resistance is a major concern in pleural infections, necessitating continuous surveillance and judicious antibiotic use.

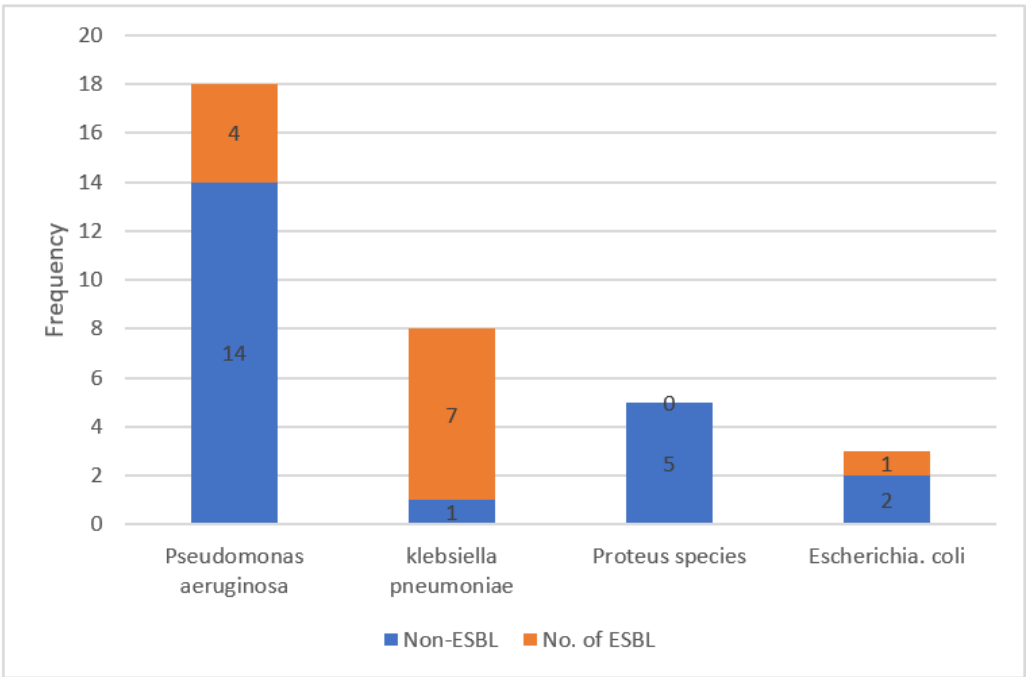

Figure 2: ESBLs among different organisms

This figure depicts the organism-wise distribution of ESBL producers. Klebsiella pneumoniae demonstrated the highest proportion of ESBL production, followed by Escherichia coli and Pseudomonas aeruginosa. The data suggest that ESBL-mediated resistance is particularly common among Enterobacteriaceae, which complicates empirical therapy and necessitates reliance on carbapenems or other higher-line antibiotics. The figure underscores the importance of antibiotic

stewardship and the need for periodic local antibiogram updates to guide clinicians in selecting appropriate antimicrobial regimens.

Discussion

Empyema thoracis continues to be a significant clinical problem, despite the advent of potent antibiotics, due to the rise of antibiotic-resistant organisms, nosocomial infections, and immunocompromised hosts. The present study of 100 clinically diagnosed cases of pleural infections found that community-acquired pneumonia remains the predominant cause, followed by chronic obstructive pulmonary disease and bronchiectasis, aligning with prior studies by Malhotra et al. [14] and Mandapakala et al. [15]. Historically, tuberculosis accounted for most empyema cases in India, but with effective anti-tubercular therapy, bacterial pneumonia now predominates.

The age distribution revealed that the most affected group was 26–45 years, which corresponds to findings by Saxena et al. [16], Acharya et al. [17], and Vardhan et al. [18]. Males were more commonly affected (72%), giving a male-to-female ratio of 2.57:1, similar to Acharya et al. [17] and Saxena et al. [16]. This male predominance may relate to higher exposure to risk factors such as smoking and occupational stress. Smoking (52%) was the leading risk factor, followed by diabetes mellitus (22%) and alcoholism (18%), which is consistent with Kamat et al. [19] and Acharya et al. [17]. Fever (95%) and cough (92%) were the most common presenting symptoms, in line with the findings of Girish et al. [20] and Kundu et al. [21]. This pattern highlights that acute febrile respiratory symptoms predominate in empyema presentations.

In standard culture, only 25% of pleural fluid samples were positive, while the BHI (Brain Heart Infusion) broth technique increased yield to 40%. This 15% improvement ($p = 0.02$) demonstrates the superiority of enrichment methods, agreeing with studies by Menzies et al. [22] and Charoentunyarak et al. [23]. The use of BHI broth improves detection in low-bacterial-load specimens, underscoring its value in diagnostic microbiology.

The majority of isolates were Gram-negative bacilli (GNB) (85%), compared with only 15% Gram-positive cocci (GPC). Pseudomonas aeruginosa was the most common isolate (45%), followed by Klebsiella pneumoniae, Proteus spp., and Staphylococcus aureus. These findings correspond with Indian data from Gupta et al. [24], Mohanty et al. [25], and Goel et al. [26], who also reported predominance of GNB. The increasing role of Pseudomonas reflects hospital-acquired infections and prior antibiotic exposure. Comparatively, earlier studies (Acharya et al. [17], Kundu et al. [21]) observed Staphylococcus aureus and Streptococcus pneumoniae as frequent isolates, indicating a shift in the microbial pattern over time.

Among S. aureus isolates, 50% were Methicillin-resistant (MRSA), which aligns with findings by Jain et al. [21] and Sowmya et al. [22], indicating high MRSA prevalence in community-acquired pneumonia. Extended-spectrum beta-lactamase (ESBL) production was detected in 35.2% of GNB isolates, particularly Klebsiella pneumoniae, Pseudomonas aeruginosa, and E. coli. These results are consistent with Jain et al. [27] and Sowmya et al. [28], who reported ESBL prevalence between 40–70%, underscoring the growing threat of beta-lactam resistance.

Vancomycin remained universally effective against GPCs, consistent with reports by Jain et al. [27] and Saxena et al. [16], whereas Linezolid and Azithromycin showed 83.3% sensitivity. Among GNBs, Imipenem was 100% sensitive, followed by Ciprofloxacin, Aztreonam, and Ceftazidime (88.8%). These results corroborate findings of Goel et al. [26] and Gagneja et al. [29], who emphasized high sensitivity to carbapenems and declining efficacy of third-generation cephalosporins due to overuse.

Conclusion

Pleural infections remain a major clinical concern, predominantly affecting males aged 26–45 years, with fever and cough as the most common symptoms. The study revealed that Gram-negative bacteria, particularly Pseudomonas aeruginosa, are the leading pathogens. Use of BHI broth significantly improved culture yield compared to standard methods, emphasizing its diagnostic advantage. The emergence of MRSA (50%) and ESBL-producing Gram-negative bacilli (35.2%) highlights growing antimicrobial resistance. Imipenem and Vancomycin were the most effective antibiotics, underscoring the need for rational antibiotic use, regular microbial surveillance, and culture-guided therapy to improve patient outcomes.

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