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YIELD OF PLEURAL FLUID COLLECTED FOR CULTURE IN TYPICAL/SPECIALIZED BLOOD CULTURE BOTTLES VS COLLECTED IN ORDINARY SYRINGES/BOTTLES

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Abstract

Background: *Pleural infections are highly morbid and lethal. Microbiologic diagnosis is critical in guiding proper antimicrobial therapy, but conventional pleural fluid culture on sterile bottles is of low sensitivity. New evidence suggests that inoculation of pleural fluid into blood culture bottles (BCBs) enhances diagnostic yield.*

Objective: *To compare the microbiological yield of pleural fluid cultures collected in blood culture bottles versus standard sterile containers and assess their diagnostic performance.*

Methods: *In the present prospective study at Pak Emirates Military Hospital Rawalpindi, 120 adult patients of suspected pleural infections were enrolled. Pleural fluid samples from each patient were inoculated in both aerobic/anaerobic BCBs and regular sterile containers simultaneously. All the samples were processed in accordance with microbiology laboratory protocols. The primary outcomes were culture positivity rate, distribution of pathogens, contamination rate, and time to positivity. Statistical analysis was done through Chi-square test and Kaplan-Meier analysis, and the level of $p < 0.05$ was taken as significant.*

Results: *Positive cultures from BCBs were obtained from 53.3% of 120 patients compared with 28.3% from standard containers ($p < 0.001$). Anaerobes were obtained only from the BCBs. The time to positivity was significantly reduced when using BCBs (18.6 hours vs 36.4 hours). Contamination rates were very low and comparable using both procedures. The most frequently isolated pathogens were Streptococcus pneumoniae, Staphylococcus aureus, and Escherichia coli.*

Conclusion: *Blood culture bottles significantly improve the microbiological yield of pleural fluid cultures, reduce time to detection, and improve recovery and identification of anaerobes that are not recovered by standard conventional methods. In practice, routine application of BCBs should be considered in clinics, and guidelines for standardized pleural fluid collection are needed.*

INTRODUCTION

Pleural infections, including complicated parapneumonic effusions and empyema, are major predictors of morbidity and mortality, especially in hospitalized and immunocompromised patients. Treatment is only successful if early and correct identification of the pathogen is performed to direct appropriate antimicrobial therapy. Nevertheless, the yield of conventional pleural effusion cultures is disappointingly low, ranging from 20% to 40% [1,2]. This diagnostic failure frequently triggers empirical broad-spectrum antibiotic therapy, which may result in suboptimal outcomes and enhanced antimicrobial resistance.

Routine practice is usually to aspirate pleural fluid into sterile syringes or tubes and submit it to the laboratory for inoculation onto media. The practice is at a disadvantage with some limitations: time delay in processing and transport, exposure to oxygen with risk of killing anaerobic bacteria, and limited volume of fluid inoculated for culture [3,4]. Also, absence of enriched media transport can lead to the death of fastidious bacteria during transport to the laboratory, further reducing the yield of diagnosis.

In the last few years, specialty blood culture bottles (BCBs), aerobic and anaerobic, have been proposed as a substitute for pleural fluid culture. BCBs contain nutrient media and growth stimulators that preserve bacterial viability and allow better detection of a broad spectrum of pathogens, including anaerobes and fastidious bacteria [5]. Direct pleural fluid inoculation at the bedside into BCBs decreases turnaround time and enhances microbial recovery. Studies have demonstrated that the use of BCBs greatly enhances culture positivity rates compared with the use of standard sterile containers [6–8].

In spite of increasing evidence to support this approach, clinical practice is still heterogenous, and most institutions continue to employ traditional collection methods. These guidelines, however, like the British Thoracic Society and American College of Chest Physicians, refer to BCBs only as an option and not routine practice because of the absence of sufficient large-scale prospective data [9,10]. Additional prospective studies to compare the relative diagnostic yield of the two methods need to be carried out.

Objectives of the Study

1. To compare the microbiologic yield of pleural fluid cultures collected using special aerobic and anaerobic blood culture bottles versus routine sterile syringes/containers.

2. To compare the diversity of organisms recovered from each collection method, including anaerobic and fastidious pathogens.

3. To determine whether bedside inoculation into blood culture bottles reduces time to positivity and improves diagnostic efficiency.

4. To compare the culture contamination or false positive rate in each method.

5. To produce evidence that can direct and standardize clinical protocols for the pleural fluid collection in suspected infections.

Materials and Methods

Study Design

This study was intended as a prospective, observational, comparative clinical study to evaluate the microbiological yield of pleural fluid cultures utilizing two distinct collecting methods: specialized blood culture bottles (BCBs) against regular sterile syringes or containers.

Setting

The research was performed in the Department of Pulmonology and Internal Medicine at Pak Emirates Military Hospital Rawalpindi, a tertiary care academic institution featuring a specialised microbiology laboratory. The study lasted between 6 to 12 months, from [insert dates].

Participants
All patients undergoing diagnostic or therapeutic thoracentesis for suspected pleural infections were considered eligible for inclusion in the study.

Inclusion and Exclusion Criteria
Eligible patients were adults aged 18 years or older who exhibited clinical and radiological indications of pleural infection, including fever, pleuritic chest pain, purulent pleural fluid, or signs of parapneumonic effusion or empyema, and from whom a minimum of 20 ml of pleural fluid was collected during diagnostic or therapeutic thoracentesis. Enrollment or Inclusion in the study was restricted to individuals who granted informed consent for participation. Patients were excluded if they had a confirmed malignant pleural effusion without clinical signs indicative of infection, if the volume of pleural fluid obtained was inadequate (<20 ml), or if they had undergone systemic antibiotic treatment for over 48 hours before sample collection. Further exclusion criteria encompassed hemorrhagic, chylous, or transudative effusions without clinical suspicion of infection, as well as instances where pleural fluid was not concurrently submitted using both specialized blood culture bottles and the standard sterile collection technique.

Protocol for Sample Collection
Aseptic thoracentesis yielded a total of 20–30 ml of pleural fluid. The harvested fluid was promptly partitioned into two segments:

Group A (Blood Culture Bottles):
10 ml of pleural fluid was immediately inoculated at the bedside into a set of aerobic and anaerobic blood culture bottles (e.g., BACTEC™, BacT/Alert™). Bottles were carefully flipped to facilitate mixing and promptly delivered to the microbiological laboratory (11).

Group B (Standard Collection Method):
An additional 10 ml of pleural fluid was put into a sterile plastic or glass container/syringe with a secure closure. The specimen was delivered to the laboratory within 30 minutes and processed utilizing standard culture techniques.

To mitigate sample bias, both specimens were procured during the same thoracentesis technique and submitted concurrently.

Transport and Culture Techniques Group A (BCBs):
Blood culture bottles (BCBs) were placed in automated blood culture systems, such BACTEC FX™ or BacT/ALERT 3D™, and incubated at 37°C, with continuous monitoring for microbial development over a period of 5 to 7 days.

Group B (Standard Method):
Samples were processed within two hours of collection, with pleural fluid introduced onto standard culture media, including blood agar, MacConkey agar, chocolate agar, and anaerobic media (12). The inoculation plates were incubated in both aerobic and anaerobic environments at 35–37°C and monitored for microbial growth for a duration of 5 days. Bacterial isolates were later identified utilizing established biochemical assays and automated techniques, including the VITEK® 2.
All pleural fluid samples underwent Gram staining, cell enumeration, and biochemical analysis, including pH, glucose, LDH, and protein assessment.

Outcome Measures
Primary Outcome
Culture Positivity Rate
The culture positivity rate was defined as the ratio of samples exhibiting clinically significant pathogen growth in blood culture bottles relative to standard containers.

Secondary Outcomes
The time of detection was quantified as the period from sample inoculation to the automated signal positivity in the blood culture bottle (BCB) group. The range or spectrum of pathogens was evaluated by analyzing the distribution of aerobic, anaerobic, and fastidious organisms identified by each culture technique. The contamination rate was assessed based on the presence of potentially contaminating organisms, such as skin flora, in both groups. The concordance with clinical diagnosis was assessed by matching culture results with the verified clinical diagnosis of pleural infection (13). The impact on

antibiotic management was measured by assessing if the culture result led to targeted antimicrobial therapy (14).

Statistical Analysis

Data were analyzed utilizing SPSS version 25.0 software package. Continuous variables, including patient age and time to culture positivity, was outlined using means and standard deviations or medians and interquartile ranges, as applicable.

Categorical variables such as gender, comorbidity presence, and culture positivity rates were represented as frequencies and percentages. The primary outcome—the disparity in culture positivity between pleural fluid samples obtained in blood culture bottles and those in normal sterile containers—was evaluated using the Chi-square test or Fisher's exact test, contingent upon anticipated frequencies. Time-to-positivity data were analyzed using Kaplan-Meier survival analysis and the log-rank test for comparison. The concordance between the two cultural methodologies was assessed utilizing Cohen's kappa coefficient. A p-value below 0.05 was deemed statistically significant for all comparisons. Multivariable logistic regression may be employed, if relevant, to account for potential confounding variables such as previous antibiotic usage, pleural fluid attributes (e.g., purulence, pH, LDH), and underlying comorbidities.

Ethical Considerations

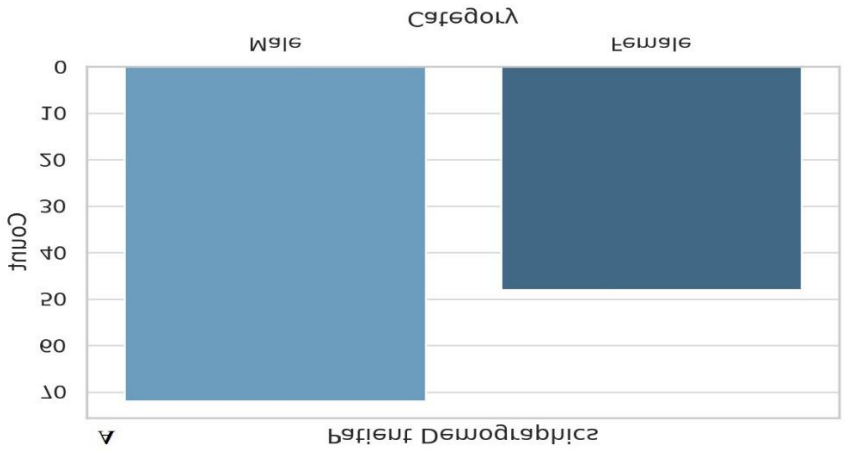
This study adhered to the ethical standards established in the Declaration of Helsinki and received approval from the Institutional Review Board (IRB) or Ethics Committee of **Pak Emirates Military Hospital Rawalpindi.** Before enrollment, all participants received comprehensive information about the study's objectives, procedures, potential hazards, and benefits, and written informed consent was acquired. Patient confidentiality and privacy were rigorously upheld during the study by anonymizing data and securely keeping all information. Participation in the study did not disrupt the standard of care, and patients were guaranteed that declining participation would not influence their medical treatment. Access to identifiable patient information was restricted to authorized members of the study team, and the data were utilized only for the objectives of this investigation.

Results

Patient Demographics and Clinical Characteristics

A total of 120 patients participated in the study, consisting of 72 males (60%) and
48 females (40%), with a mean age of 52.6 ± 14.3 years (range: 19–84 years) (Figure. 1A).

The predominant presenting symptoms were fever (89%), pleuritic chest discomfort (75%), and dyspnea (62%). Preexisting diseases comprised diabetes mellitus in 32%, chronic obstructive pulmonary disease (COPD) in 18%, and recent pneumonia in 46% of cases. All patients exhibited radiological evidence of pleural effusion, with 68% demonstrating multiloculated or complex effusions on ultrasonography or CT imaging. Purulent pleural effusion was observed in 41% of cases, whereas 27% exhibited a pleural fluid pH of less than 7.2 (Figure. 1B).



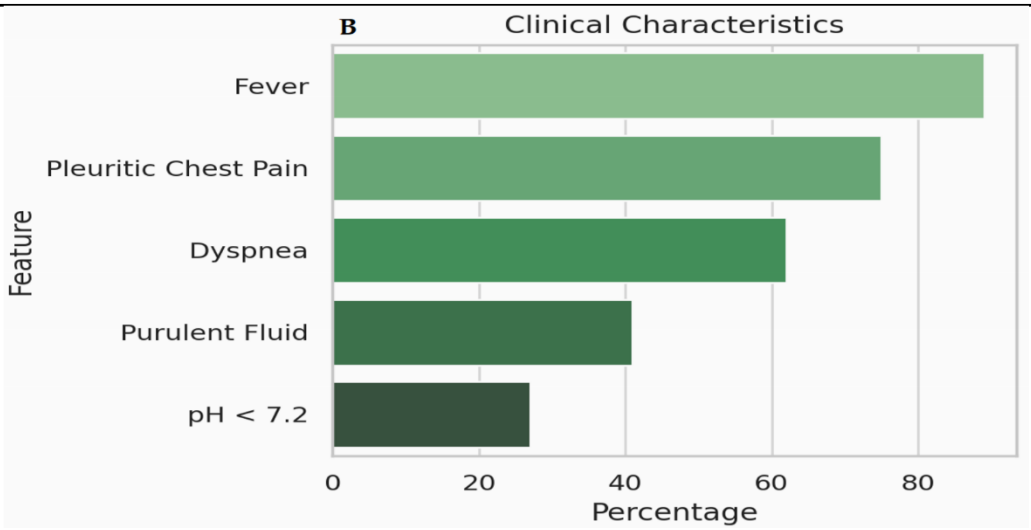


Figure. 1: Illustration of patients Demographics (A) and Clinical Characteristics (B).

Culture Positivity Comparison

Of the 120 pleural fluid samples obtained, aerobic and anaerobic blood culture bottles (BCBs) produced positive cultures in 64 cases (53.3%), while the usual sterile container method resulted in positive cultures in 34 cases (28.3%) (Table. 1, Figure. 2). The cultural positivity disparity between the two approaches was statistically significant ($p < 0.001$, χ^2 test), with a 25% absolute increase in detection rate (95% Confidence Interval [CI]: 13.2%–36.8%). Of these, 26 patients (21.6%) exhibited positive cultures using both methods, whereas 38 patients (31.6%) were positive alone in BCBs, and 8 patients (6.6%) were positive exclusively in standard cultures.

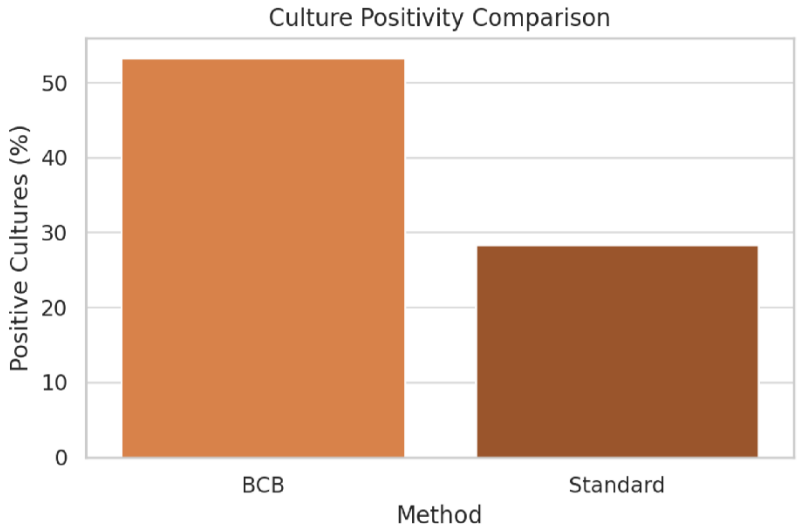


Figure. 2: Culture Positivity Comparison between BCB and Standard.

Table. 1: Diagnostic Yield and Clinical Impact of Pleural Fluid Cultures: BCBs vs Standard Method

Outcome	BCB Group (%)	Standard Method (%)	p-value	95% CI for Difference
Culture Positivity	53.3	28.3	<0.001	13.2 – 36.8

Anaerobe Detection	10.9	0	<0.01	–
Median Time to Positivity	18.6 hrs	36.4 hrs	<0.001	–
Contamination Rate	2.5	1.7	0.67	–

Pathogen Distribution

A wide range or spectrum of microorganisms was isolated (Figure. 3). The most common pathogens identified were,

- Streptococcus pneumoniae: 22 cases (34.4% of positive cultures)
- Staphylococcus aureus (including 4 MRSA): 14 cases (21.9%)
- Escherichia coli: 9 cases (14.1%)
- Klebsiella pneumoniae: 6 cases (9.4%)
- Anaerobic bacteria (e.g., *Peptostreptococcus*, *Bacteroides fragilis*): 7 cases (10.9%)
- Pseudomonas aeruginosa: 3 cases (4.7%)
- Others/rare pathogens: 3 cases (4.7%)

Notably, all anaerobic organisms were detected exclusively in the BCB group (Table. 1).

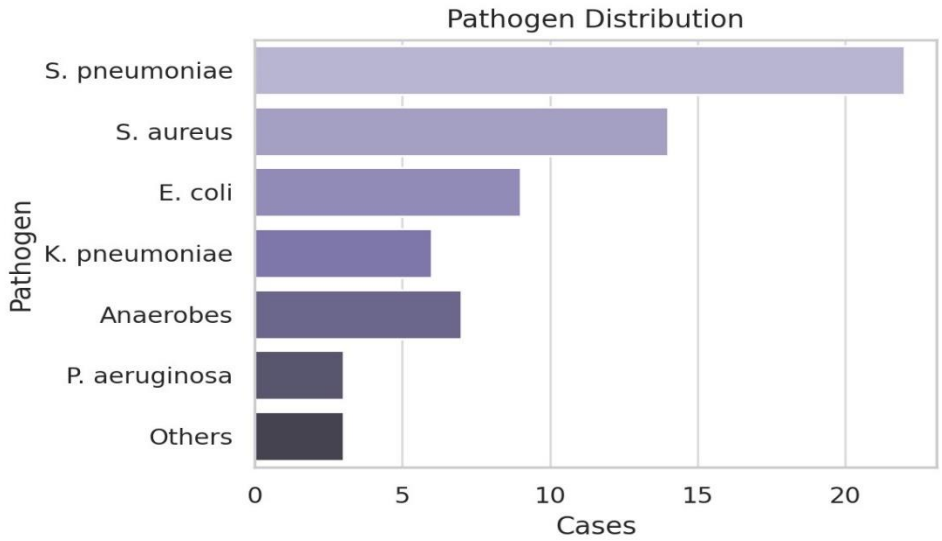


Figure. 3 Illustrate percentage distribution of different pathogens.

Contamination Rates

Presumptive contamination, characterized by the proliferation of typical skin flora or environmental organisms lacking clinical connection, was noted in 3 cases (2.5%) within the BCB group and 2 cases (1.7%) within the normal group (Table. 1, Figure. 4). The change was not statistically significant (p = 0.67, Fisher's exact test).

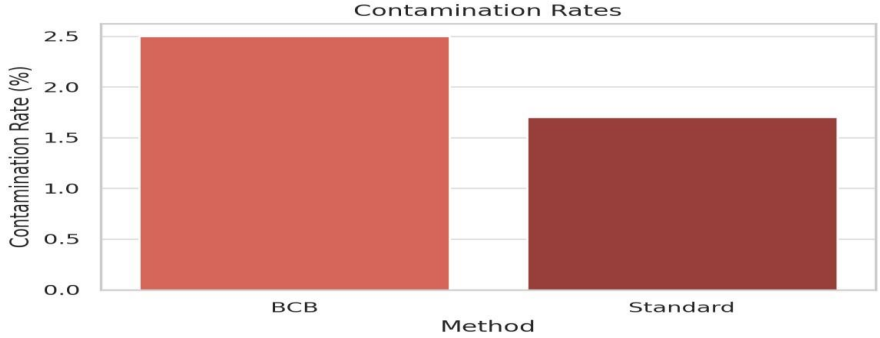


Figure. 4: Illustrate Contamination rate between BCB and Standard.

Time to Positivity

The median time to culture positive in the BCB group was 18.6 hours (Interquartile Range [IQR]: 12–32 hours), whereas in the standard technique group it was 36.4 hours (IQR: 24–60 hours). The difference was statistically significant ($p < 0.001$, log-rank test, Kaplan-Meier analysis), suggesting that BCBs enabled earlier detection of organisms (Table. 1, Figure. 5).

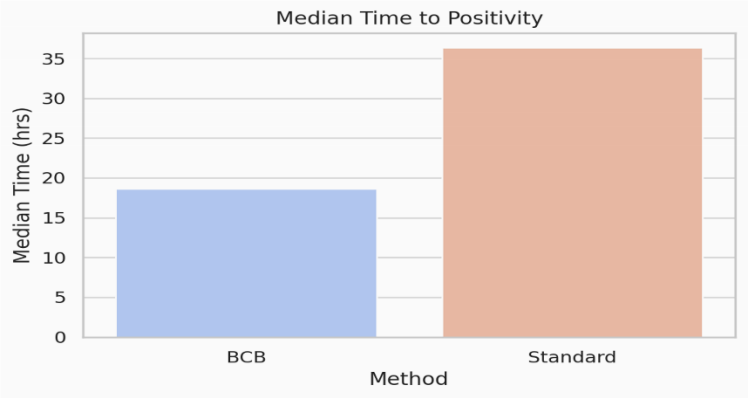


Figure. 5: Illustrate Median Time of culture Positivity between BCB and Standard.

The findings indicate that use blood culture bottles markedly enhance the diagnostic yield of pleural fluid cultures and decreases the time to detection. The improved recovery of anaerobic organisms and fastidious bacteria in the BCB group highlights its clinical significance in the treatment of pleural infections.

Discussion

This prospective investigation illustrates that inoculating pleural fluid into aerobic and anaerobic blood culture bottles (BCBs) markedly enhances microbiological yield relative to traditional sterile container techniques. The culture positivity rate was 53.3% in the BCB group compared to 28.3% in the standard group, reflecting a large absolute increase of 25%, with a statistically significant difference ($p < 0.001$). These findings are clinically significant, as precise and prompt pathogen identification is essential for customizing effective antibiotic therapy and enhancing outcomes in patients with pleural infections.

The increased yield noted in our study corresponds with earlier reported findings. Corcoran et al. in 2017 (15) in the PILOT trial and Maskell et al. in 2006 (16), both emphasized the enhanced detection rates of pathogens, particularly anaerobic bacteria, when pleural fluid was inoculated into blood culture bottles at the bedside. Our findings further corroborate this methodology, with anaerobes solely found in the BCB group, highlighting the significance of prompt bedside injection and suitable culture conditions. The median time to culture positivity significantly decreased in BCBs (18.6 hours compared to 36.4 hours), validating findings by Menzies et al. in 2005 (17), indicating that BCBs enhance early diagnosis and clinical decision-making.

The benefits of BCB use are multifaceted. In addition to increased sensitivity, BCBs create an ideal environment for the growth of fastidious and anaerobic organisms. This is particularly relevant in empyema and severe parapneumonic effusions, where anaerobes may assume a significant pathogenic role. Furthermore, BCBs are compatible with automated detection methods, hence minimizing labor and time in microbiology laboratories.

Nevertheless, practical considerations must be recognized. The utilization of BCBs necessitates staff training on appropriate bedside inoculation methods, and there may be apprehensions about availability and expense, especially in resource-limited settings. Notwithstanding these obstacles, the diagnostic benefits and potential influence on patient treatment warrant their regular application where feasible.

Our research possesses multiple limitations. The single-center design and limited sample size may restrict generalizability. Secondly, despite attempts to standardize collection techniques, operator variability may have impacted outcomes. Thirdly, we did not evaluate downstream clinical outcomes, such as

duration of hospitalization or adjustments to antibiotic therapy based on culture results, which could offer additional insight into the therapeutic efficacy of BCBs.

Based on our findings, we advise that pleural fluid specimens from patients with suspected empyema or complex effusions be consistently inoculated into both aerobic and anaerobic blood culture bottles at the bedside to improve diagnostic yield. Subsequent multicenter investigations with bigger cohorts should examine the influence of enhanced microbiological diagnosis on patient outcomes, antibiotic stewardship, and cost-effectiveness.

Summary and Conclusion

This prospective study demonstrates the diagnostic superiority of blood culture bottles (BCBs) compared to traditional sterile containers for the microbiological assessment of pleural fluid in patients with suspected pleural infections. The markedly elevated culture positivity rate, encompassing enhanced recovery of anaerobic organisms and diminished time to detection, emphasizes the importance of blood culture bottles (BCBs) in improving pathogen identification and enabling prompt, focused treatment.

From a clinical perspective, implementing BCBs for pleural fluid collection can significantly enhance diagnostic precision, facilitate antibiotic stewardship, and potentially diminish morbidity linked to delayed or insufficient treatment. These findings support the integration of BCB inoculation into standard diagnostic procedures, especially in cases of empyema or severe parapneumonic effusions.

Due to the ongoing diversity in pleural fluid culture practices, there is an urgent requirement for consensus-based, evidence-informed guidelines that encourage standardized collection and processing techniques. Further multicenter studies with bigger cohorts are necessary to validate these findings and evaluate their effects on clinical outcomes, resource utilization, and cost-effectiveness.

In conclusion, our research advocates for the incorporation of BCBs into the usual evaluation of pleural infections and urges the establishment of standardized protocols to enhance microbiological yield and patient care.

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