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Enhanced Diagnostic Yield of Pleural Infection: The Role of Pleural Fluid Inoculation into Blood Culture Bottles

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ABSTRACT

Background: Pleural infection is a major complication of pneumonia and other thoracic diseases associated with high morbidity and mortality. Conventional pleural fluid culture has limited sensitivity, usually resulting in a negative culture because the patient has received some antibiotics or because the organism is a fastidious organism. When pleural fluid is inoculated directly into blood culture bottles, there may be higher diagnostic yield, lower time to pathogen detection, and better clinical decision-making.

Objective: To determine if inoculation of pleural fluid into blood culture bottles would result in improved pathogen yield and detection time compared to conventional culture, and perform a spectrum and antibiotic susceptibility analysis of isolates to help guide empirical therapy.

Methodology: A prospective cross-sectional study was conducted at Saidu Medical College and Teaching Hospital in Swat, Pakistan, from January 2023 to December 2023. 190 patients with pleural infection were eligible for the study. Pleural fluid samples were split to send for direct conventional culture and for inoculation of blood culture bottles. Data was analyzed with SPSS version 26. A P-value less than 0.05 was considered statistically significant.

Results: For direct conventional culture, 58 (30.5%) pleural fluid samples had bacterial growth but for samples sent for blood culture bottles 108 (56.8%) were positive which is a relative increase of 26.3% detection rate depending on sample processing using blood culture bottles (P<0.001). The most common pathogens were Staphylococcus aureus (20%), Klebsiella pneumoniae (9.5%) and Acinetobacter spp (6.3%). Gram-positive isolates in the sample were 100% sensitive to vancomycin and linezolid. Gram-negative isolates had the highest sensitivity to colistin (76%) and significant resistance to carbapenems (61% resistant) and high resistance to cephalosporins (>70% resistant).

Conclusion: Inoculation of pleural fluid into blood culture bottles enhances pathogen recovery and time to detection when compared with conventional culture methods.

Keywords: Pleural Infection; Empyema; Blood Culture Bottle; Diagnostic Yield; Antibiotic Susceptibility; Pakistan

Introduction

Pleural infection - including complicated parapneumonic effusions and empyema - remains a significant global burden, with variability in morbidities and mortalities. Mortalities, especially for managing empyema, can range from 5% to 15%, with increased incidence seen in recent years.[1] Timely microbiological diagnosis of a pleural infection is important for establishing the correct treatment plan; however, conventional culture methods only recover pathogens in about 40% of investigations.[2]

Routine pleural fluid aspiration is generally performed using sterile containers, but sensitivity may be poor after prior antibiotic use along with the potential isolation of fastidious organisms and delayed processing to the laboratory. Therefore, some clinicians have implemented bedside inoculation of pleural fluid in to blood culture bottles, which has shown an increase in recovery of bacteria/microorganisms along with decreased time to detection.[3] The liquid in blood culture bottles is nutritionally -deep, and for some models, has bacteria neutralizing resins, which boosts viability and growth comapared with standard culture methods.[4]

Many studies have reported this benefit across different contexts. Menzies et al., in a prospective cohort of 62 patients, found that adding bottle culture improved pathogen yield from 37.7% to 58.5% ($P < 0.001$).[5] In a similar prospective cohort study based on data from Thailand found improved positivity rates from 14.0% with and without standard culture to 24.0% with use of blood culture bottles as well ($P < 0.001$).[6] In Pakistan and neighboring regions, similar benefits have been observed: a study from Rawalpindi found yields of 53.3% with use of blood culture bottles vs. 28.3% with routine containers, therefore improving diagnostic sensitivity in their region and also reporting anaerobic pathogen recovery that was otherwise not detected.[7]

Additionally, blood culture bottle inoculation usually cuts the time to detection. In one study, time to positivity decreased nearly in half—from 36.4 hours for standard containers to 18.6 hours for blood culture bottles—offering more opportunities for earlier directed therapy.[7]

Besides improved yield and faster turnaround time, these approaches may also affect the patterns of pathogens isolated. While traditional organisms such as Streptococcus pneumoniae, Staphylococcus aureus, and Gram-negative bacilli abound, the inclusion of enrichment media in culture may allow recovery of polymicrobial or anaerobic organisms not detectable on standard culture-- thus helping extend empirical antibiotic coverage.[7,8]

Regional differences in the distribution of bacteria and their susceptibility to antimicrobials provide further justification for local data. A recent report from Egypt and adjacent areas has observed more S. aureus and Klebsiella pneumoniae in pleural infections and inconsistent resistance patterns, especially among Gram-negative isolates, emphasizing the necessity of using local susceptibility patterns when implementing empiric antibiotic regimens.[9]

Despite the clear benefits, we have limited high quality studies directly relevant to tertiary-care settings in Pakistan. As antimicrobial resistance to first-line treatments increases and resources become limited, the use of cost-effective diagnostic strategies, which provide a higher yield, is crucial to achieve better clinical outcomes.

With the increasing evidence that blood culture bottle inoculation of pleural fluid is superior to conventional culture methods, and given the lack of data from our region, this study at Saidu teaching Hospital aims to evaluate whether blood culture bottle inoculation of pleural fluid improves diagnostic yield, decrease time to detection, and better guide the choice of empirical antibiotics than routine culture methods when assessing pleural infections in a tertiary-care hospital in Pakistan.

Objective

To determine whether inoculation of pleural fluid into blood culture bottles enhances the diagnostic yield of pathogens compared to conventional culture techniques in patients with pleural infection. Secondary objectives include comparing the time to pathogen detection between the two methods, characterizing the spectrum of isolated microorganisms, evaluating their antibiotic susceptibility patterns, and identifying the most effective empirical antibiotic options in our setting.

Methodology

This was a prospective cross-sectional analytical study conducted at the Department of Pulmonology and Department of Pathology, Saidu Medical College and Teaching Hospital, Swat, Pakistan, over a period of 12 months from January 2023 to December 2023. The study was approved by the Institutional Review Board (IRB) of Saidu Medical College (Ref. No. 02-12/SMC/22)), and written informed consent was obtained from all participants or their legal guardians. The study adhered to the principles of the Declaration of Helsinki (2013 revision).

In total, there were 190 patients admitted into the study based on their clinical and radiological suspicion for pleural infection. The inclusion criteria indicated participants needed to be 15 years of age or older, had pleural effusion meeting at least one of the clinical criteria which included; aspirate fluid which was purulent, positive Gram stain or culture of pleural fluid, pleural fluid pH of less than 7.20, or pleural fluid glucose <40mg/dL with normal glucose in blood. Patients were excluded from the study if they were found to have a transudative pleural effusion based on Light's criteria, had a confirmed tuberculous pleural effusion (i.e., positive GeneXpert test,

AFB smear or culture, or ADA > 40 U/L), metastasis via cytology or biopsy, or broad-spectrum antibiotics for greater than seven days prior to their presentation.

All enrolled patients underwent a full clinical assessment. This included a detailed history, complete with a full physical examination, laboratory studies were also done as a standard procedure, including a complete blood count (CBC), an erythrocyte sedimentation rate (ESR), a C-reactive protein (CRP) level, renal function tests, and liver function tests. Then, to radiologically confirm the presence of pleural effusion, all patients underwent a chest radiograph or CT scan of the chest. Lastly, the pleural fluid aspiration was performed under ultrasound guidance, and the aspiration was performed under strict aseptic technique for analysis of the effusion.

Approximately 20 mL of pleural fluid was aspirated from each patient and processed by two separate methods. The sample was split, with 10 mL submitted for routine microbiological culture in a sterile container. A separate 5-10 mL aliquot was inoculated at the bedside directly into aerobic blood culture bottles (BacT/ALERT®, bioMérieux, France) before being taken to the microbiology laboratory. With the routine culture method, the pleural fluid was centrifuged and the deposit was used to perform a Gram stain followed by inoculation of Blood agar, MacConkey agar, and Chocolate agar plates (Oxoid, UK). The plates were incubated under appropriate atmospheric conditions for 37 °C: aerobically for Blood agar and MacConkey agar; Chocolate agar with 5% CO_2. Inspection for growth was performed daily for 48 to 72 hours. A culture was reported to be negative if there was no growth after 72 hours. The inoculated blood culture bottles were also incubated and monitored for up to 5 days in the automated BacT/ALERT® system. When a bottle tested positive on the BacT/ALERT® system, aliquots were extracted for Gram staining, and subcultured to same agars used for the conventional culture method. All organisms isolated as part of both methods were identified using standard biochemical methods.

Susceptibility testing for all bacterial isolates was performed using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar, and the results were interpreted by following the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. The antibiotic panels were determined by the isolate's Gram reaction. For Gram-positive bacteria, the panels included antibiotics like penicillin, erythromycin, clindamycin, linezolid, vancomycin, and teicoplanin. For Gram-negative isolates, the panels included drugs like carbapenems, cephalosporins, fluoroquinolones, aminoglycosides, and colistin.

The primary outcome of the study was to evaluate the increase in pathogen detection rate by inoculating pleural fluid into blood culture bottles. The primary outcomes were (1) the time to detection of causative pathogens for each method, (2) the total spectrum of organisms isolated, and (3) the antibiotic susceptibility profile of the isolates recovered.

The data that was collected were all entered and analyzed using SPSS version 26.0. Continuous variables were reported as mean ± standard deviation (SD), while categorical variables were reported as frequency and percentage. The Chi-square test was used to compare the proportion of cultures positive by the two methods. Paired continuous data, such as time to detection, was analyzed using the Wilcoxon signed-rank test. A P value of < 0.05 was considered statistically significant for all analyses.

Results

The study was carried out at Saidu Teaching Hospital, Pakistan and involved 190 patients with a diagnosis of pleural infection. Of the patients, 114 (60%) were male and 76 (40%) were female. The mean age of the patients was 48.6 ± 15.2 years old (range: 15–82 years). Comorbidities were evident in 78 patients (41.1%), diabetes mellitus in 34 (17.9%), hypertension in 22 (11.6%) and obstructive pulmonary disease (COPD) in 22 (11.6%). Empyema was clinically diagnosed in 52 (27.4%) patients and 138 (72.6%) had a parapneumonic effusion. Right-sided effusions were common (106, 55.8%) compared to left-sided effusions (84, 44.2%) (Table 1).

Of the 190 pleural fluid samples, routine direct culture found bacteria growth in 58 (30.5%) and blood culture bottle inoculation was positive in 108 (56.8%). There was an overall 26.3% increase in detection rate ($P < 0.001$), further supporting a higher yield for the blood culture bottle (Table 2).

Of the 190 samples collected, bacterial growth was detected by direct method in 30.5% of samples and by blood culture bottle inoculation in 56.8% of samples. Blood culture bottle positivity increased by 26.3% compared with the direct culture method, and this difference was statistically significant ($P < 0.001$). These results demonstrate that blood culture bottle inoculation is far more sensitive in detecting causative pathogens in pleural (Figure 1).

The duration of time it took to identify a pathogen was significantly different for the diagnostic methods. A direct Gram stain gave the quickest identification result, finding pathogens in 34 samples (17.9%) in 1 to 2 hours. As for culture-based lab identification, blood culture bottle had a median time to positive result at 21 hours (range 10–48) compared to the conventional, which yielded positive results at 48 to 72 hours. This difference was statistically significant ($P < 0.001$). Looking at the antibiotic susceptibility patterns, for Gram-positive isolates 100% sensitivity was observed for vancomycin and linezolid while teicoplanin displayed 94% sensitivity. The same isolates showed considerable resistance to penicillin (72%) and erythromycin (58%). Resistance trends were more pronounced with Gram-negative isolates with only

Table 1. Demographic and clinical characteristics of patients (N=190)

Variable	Frequency	Percentage (%)
Sex		
Male	114	60.0
Female	76	40.0
Age		
<50 years	100 (52.6)	52.6
≥50 years	90 (47.4)	47.4
Mean ± SD	48.6 ± 15.2	
Comorbidities		
Diabetes mellitus	34	17.9
Hypertension	22	11.6
COPD	22	11.6
Type of effusion		
Empyema	52	27.4
Parapneumonic effusion	138	72.6
Side of effusion		
Right	106	55.8
Left	84	44.2

slight susceptibility across most classes tested, but 76% sensitivity to colistin and only 39% sensitivity to carbapenems; with cephalosporins and fluoroquinolones having less than 30% sensitivity. To summarize, these results highlight the crucial role of glycopeptides and oxazolidinones as the only likely effective therapies for Gram-positive pleural infections in this clinical context (Figure 2).

Colistin demonstrated the highest susceptibility (76%) followed by carbapenems (39%). In contrast, there was low sensitivity to cephalosporins (28%) and fluoroquinolones (25%), indicating significant resistance trends among Gram-negative knowledge pathogens. These data indicate limited therapeutic options, and provide caution about the use of last-line antibiotics in pleural infections (Figure 3).

Discussion

The present study at Saidu Teaching Hospital assessed the effectiveness of inoculating pleural fluid into blood culture bottles as compared to standard culture methods in patients with pleural infection. In addition to describing the cohort and the patient characteristics, we assessed the diagnostic yield, time to pathogen, bacteria type, and antibiotic susceptibility. In this section, we discuss the implications of each major finding in light of current literature and how they can inform clinical practice.

We noted a substantially higher positivity (56.8%) using blood culture bottles than 30.5% with traditional methods (difference 26.3%, $P < 0.001$). Javaid et al. also reported similar findings in Peshawar. Blood culture bottles detected pathogens in 59%, compared to sterile

Table 2. Pathogens detected by direct culture vs. blood culture inoculation

Pathogen	Direct culture n (%)	Blood culture bottle n (%)
No growth	132 (69.5)	82 (43.2)
Staphylococcus aureus	22 (11.6)	38 (20.0)
Coagulase-negative staph	3 (1.6)	14 (7.4)
Klebsiella pneumoniae	10 (5.3)	18 (9.5)
Acinetobacter spp.	6 (3.2)	12 (6.3)
Pseudomonas aeruginosa	7 (3.7)	9 (4.7)
Enterococcus spp.	2 (1.1)	3 (1.6)
Streptococcus mitis	0	4 (2.1)
Candida spp.	0	2 (1.1)

syringes, which detected pathogens in 30% of the cases, where we obtained similar results.[10] Cholasseri et al. in India also reported an 8.4% difference when measuring using a dedicated blood culture bottle compared to standard containers.[11] In addition, multitasking independently, and in a multi-center study, Ferrer et al., also observed a yield improvement from 44% to 64% with blood culture bottle methods.[8] These consistent observations reinforce the greater diagnostic sensitivity for this method.

With blood culture bottles, we found a median detection time of 21 hours, distinctly shorter than the 48-72 hours for standard culture. Direct comparisons of detection times are limited, but Javaid et al. suggested shorter recovery times when using blood culture bottles.[10] In pneumonia, Heo et al. definitively showed that using

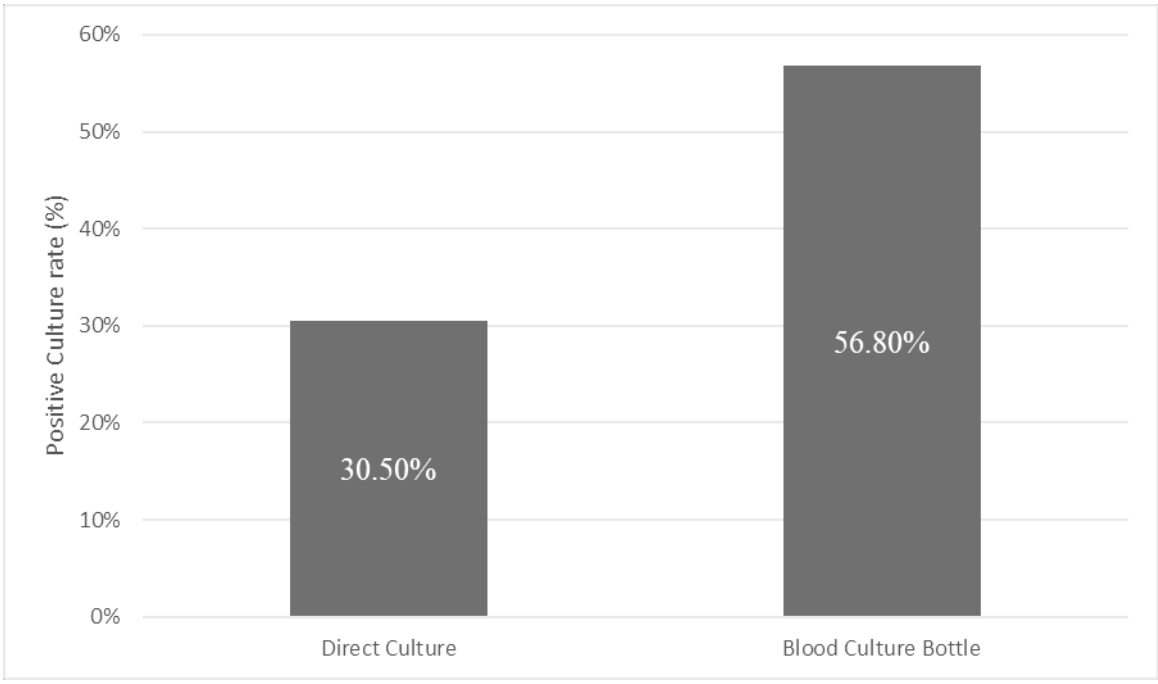

Figure 1. Comparison of detection rates between direct culture and blood culture bottle

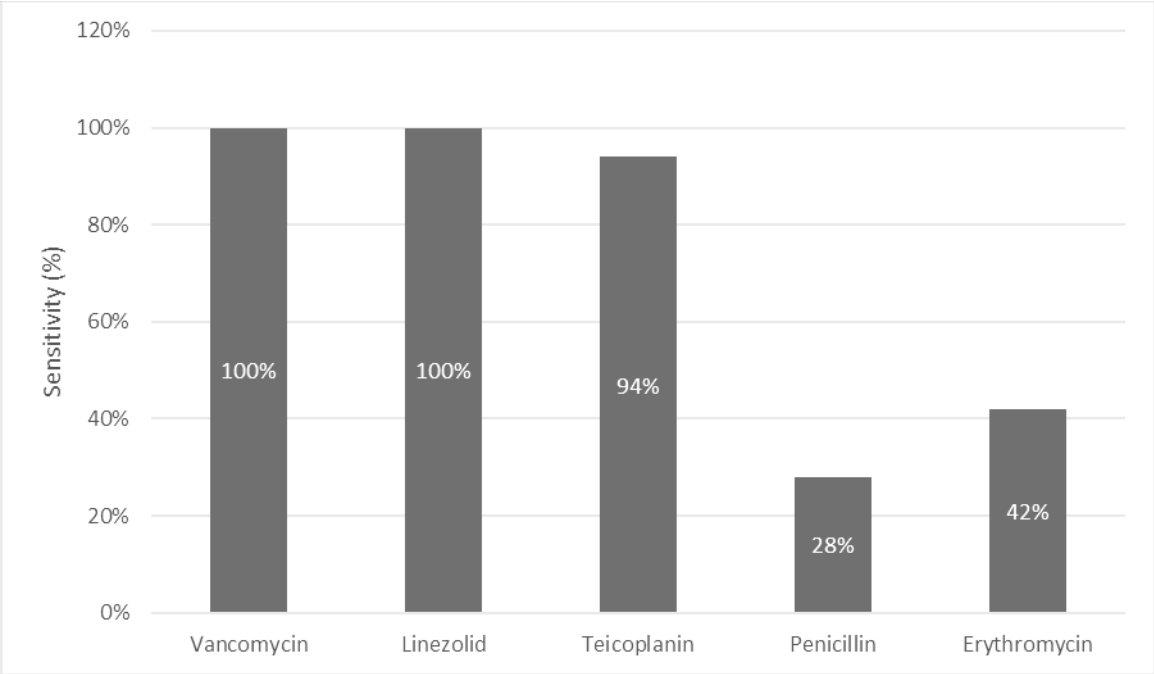

Figure 2. Susceptibility profile of Gram-positive isolates

blood culture bottles in conjunction with bronchoalveolar lavage fluid decreased detection time,[12] and the advantage of shorter recovery time may be applicable to pleural infections. Timely detection may allow more early targeted therapy, which is important in managing pleural infections.

The predominant organism in our cohort was Staphylococcus aureus followed by Klebsiella pneumoniae, Acinetobacter, and Pseudomonas, along with the added detections of Streptococcus mitis and Candida using the blood culture bottle method. Atif et al. reported a high prevalence of Pseudomonas (18.1%) and Klebsiella (10%) in their isolates of empyema in Pakistan which is also in agreement with our distribution of GNB.[13] Sharma & Agarwal conducted a similar study in India and also reported the same selection in pleural fluid cultures with S. aureus being the predominant cultured pathogen and the Gram negative bacilli being the dominant bacteria culture as well.[14] Habibie & Hamdani completed an empirical study in Indonesia that found a broader variety of pathogens in empyema cases, which consistently reflects geographic variability but similarities in the type of pathogen.[15]

Our results for Gram-positive isolates of 100% sensitivity to vancomycin and linezolid and 94% sensitive to teicoplanin (with resistance to penicillin and erythromycin) reflect results of similar Egyptian cohorts, where its similarly observed glycopeptides performed well in action.[9] As for the Gram-negative isolates, results were not promising, with only 39% sensitivity to carbapenems and 76% to colistin, as Atif et al. reported high resistance to standard empiric agents and similarly advocated for last-line agents.[13] Sharma & Agarwal again reported low susceptibility of Gram-negatives compared to cephalosporins and fluoroquinolones again, respectively.[14]

Collectively, our results demonstrating heightened diagnostic yield, rapid detection, and comprehensive antimicrobial susceptibility data strongly advocate adopting pleural fluid inoculation into blood culture bottles in routine practice. This supports earlier initiation of effective treatment and improves pathogen identification precision.

Some of the strengths of our study were its prospective design, large patient population (N=190), and careful comparison of the diagnostic methods. However, limited to a single-center study, our results cannot necessarily be generalized across other health care settings. Additionally, our laboratory did not utilize dedicated anaerobic culture methods that were not simply standard media, possibly underestimating the yield of anaerobic pathogens.

Conclusion

This study shows that inoculating pleural fluid into blood culture bottles substantially increases the diagnostic yield of pathogens, as compared to standard culture methods, in addition to reducing the time to detection of pathogens. The organism spectrum showed that Staphylococcus aureus and Gram-negative organisms such as Klebsiella pneumoniae and Acinetobacter spp. predominated, and had significant resistance to standard antibiotics.

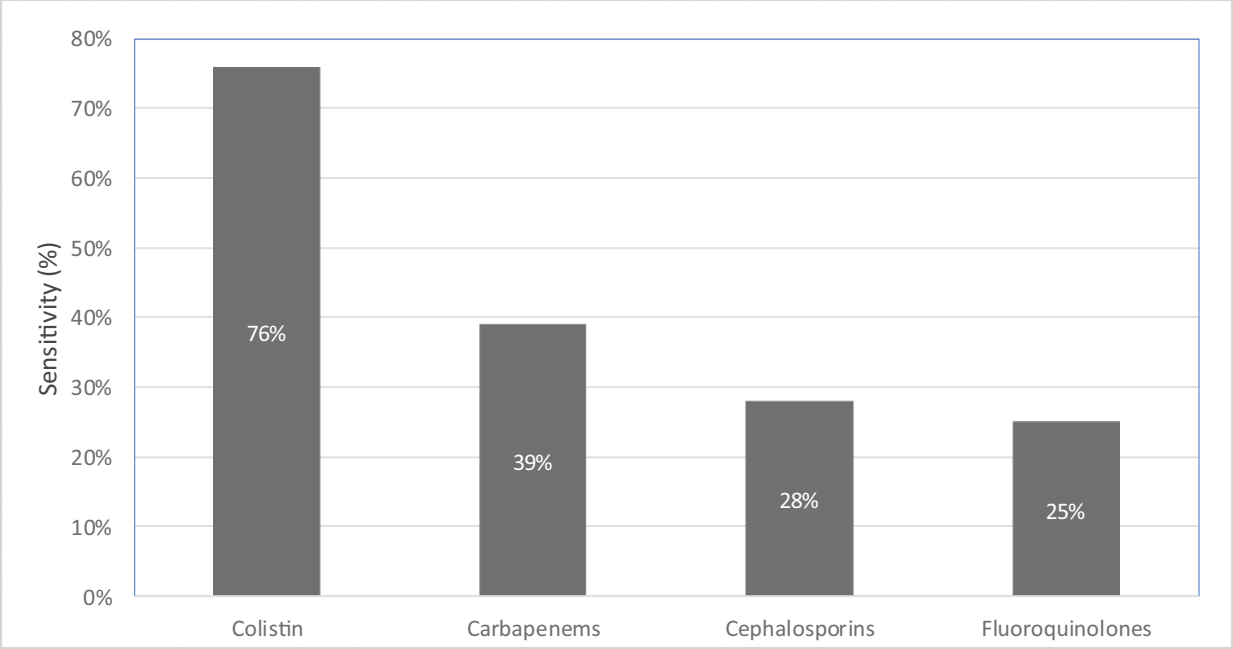

Figure 3. Susceptibility profile of Gram-negative isolates

Vancomycin, linezolid, and colistin were effective against Gram-positive and Gram-negative pathogens, respectively.

Overall, these results highlight the value of routine blood culture bottle inoculation in the diagnosis of pleural infections, especially in low-resource settings, where timely and accurate diagnosis can maximize the use of effective early empirical therapy, and ultimately patient outcomes. Adopting blood culture bottle inoculation into national diagnostic protocols at tertiary care centers could improve microbiological yield, judicious use of antibiotics, and ultimately the management of pleural infections.

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