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Comparative Study of Pleural Fluid Culture in Standard Culture Bottle and Blood Culture Bottle for the Evaluation of Empyema

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Abstract

Background: *Bacterial pleural infection has been a substantial clinical challenge since ancient times. Identification of the infecting bacteria by culture of pleural fluid is of great importance for clinical care. Inoculating pleural fluid into blood culture bottles at the bedside can potentially improve the yield of pleural fluid culture.*

This study was done to assess whether addition of blood culture bottle sample to the standard bottle sample increases the rate of detection in pleural fluid culture for the evaluation of empyema.

Materials and Methods: *A cross sectional observational study was conducted in patients with clinical presentation compatible with empyema with purulent pleural fluid drainage during thoracocentesis for a period of 1 year from 1st January 2013 to 31st December. Those having sputum positive tuberculosis were excluded. Two samples were collected in sterile bottle for standard culture and one sample in blood culture bottle from every patient enrolled into the study. The second standard culture was taken to assess whether the increase in yield was due to repetition of culture. All samples sent to microbiology laboratory of same institute. The standard bottle samples were subjected to Gram staining and inoculated to blood, MacConkey and chocolate agar. Blood culture bottle containing pleural fluid was incubated at 37^0 c for upto 7 days. A Gram stain was performed on any positive bottle and subculture was done to identify the organism. Identification of cultures were done by standard biochemical tests in microbiology laboratory. Further evaluation and management of patients done according to the department protocol*

Results: *A total of 60 patients were enrolled in the study.The standard laboratory culture of pleural fluid was positive in 19 (31.6%) cases. A second standard culture did not produce an increase in bacterial yield. Pleural fluid culture in blood culture bottle was positive in 24 (40%) cases. Addition of blood culture bottle culture to standard laboratory culture increased culture positivity by 8.4 (chi square value 41.7 with p <0.001). All cases which are culture positive in standard bottle were also positive in blood culture bottle. Klebsiella pneumoniae was the most common bacterium identified in pleural fluid culture. Blood culture bottle culture identified two extra cases of Pseudomonas aeruginosa, one case of Escherichia coli and Klebsiella pneumoniae, and a mixed infection case*

Conclusion: *Inoculating pleural fluid into blood culture bottles at the bedside increases the rate of bacterial pathogen identification in empyema, when compared to standard laboratory culture.*

Keywords: *Empyema, Blood culture bottle, Standard laboratory culture.*

Background

Bacterial pleural infection has been a substantial clinical challenge since ancient times. It is a major cause of morbidity and mortality, and its incidence continues to rise both in adults and children[1,2,3]. About 40% of all patients with pneumonia will have an associated pleural effusion, although a minority will require an intervention for a complicated parapneumonic effusion or empyema[4,5]. Delay in diagnosis, failure to institute appropriate antimicrobial therapy, and inadequate drainage of pleural space contribute to increased morbidity and mortality in these patients[3].

Prompt evaluation and therapeutic intervention appears to reduce morbidity and mortality as well as healthcare costs associated with pleural infection[3]. Pleural fluid characteristics remain the most reliable diagnostic test to guide management[1,6.] Identification of the infecting bacteria by culture of pleural fluid is of great importance for clinical care. Pleural fluid culture also provides the sensitivity profile of the isolated microorganism to various antibiotics. This will help the clinician for proper antibiotic selection. Conventional pleural fluid cultures, especially in the event of the prior use of antibiotics, exhibit a low sensitivity. About 40% of cases has a negative culture results[2]. These patients are treated with empirical antibiotics that cover the spectrum of likely pathogens, resulting in polypharmacy and its associated disadvantages. Anaerobic antibiotic treatment is frequently given empirically, as anaerobes are often implicated in empyema although their pick-up rates by standard laboratory cultures are poor[7]. Peripheral blood culture can increase the identification rate of the causative organism, while sputum cultures are positive less often than pleural fluid cultures[8,9]. Inoculating pleural fluid into blood culture bottles at the bedside can probably improve the yield of pleural fluid culture[10,11]. Several studies of this approach suggest clinically significant higher bacterial isolate rates[12-16]. In other clinical settings, inoculation of various other fluids like peritoneal dialysate[14,17], peritoneal fluid [18] and synovial fluid[14,19] into blood culture bottles has been shown to be clinically useful.

This study assesses whether inoculating pleural fluid into blood culture bottle identifies more bacteria than standard laboratory culture in the evaluation of empyema.

Materials and Methods

After getting approval from the Institutional Ethical Committee, a cross sectional observational study was conducted in the Department of Pulmonary Medicine, Government Medical College, Thrissur, Kerala. Patients with clinical presentation compatible with empyema with purulent pleural fluid drainage during thoracocentesis for a period of 1 year from 1st January 2013 to 31st December 2013 were included in the study. Those having sputum positive tuberculosis were excluded. The baseline characteristics of patients were noted. Two samples were collected in sterile bottle (universal container for culture sample) for standard culture and one sample in blood culture bottle from every patient enrolled into the study. The second standard culture was taken to assess whether the increase in yield was due to repetition of culture. Venous blood culture and sputum culture samples were also taken. All samples sent to microbiology laboratory of same institute. The standard bottle samples were subjected to Gram staining and inoculated to blood, MacConkey and chocolate agar. Blood culture bottle containing pleural fluid was incubated at 37^0 c for upto 7 days. A Gram stain was performed on any positive bottle and subculture was done to identify the organism. Identification of cultures were done by standard biochemical tests in microbiology laboratory. Further evaluation and management of patients done according to the department protocol.

Ethics

Ethical clearance was obtained from Institutional Ethical Committee Government Medical College, Thrissur, Kerala

Results

A total of 60 patients were enrolled in the study. Among them 56 (93.3%) were males and 4 (6.7%) were females. 38(63.3%) patients had history of antibiotic use before enrolment in the study. Also 15(25%) patients had undergone thoracocentesis previously from other centres. Diabetes Mellitus was the most frequent comorbidity, found in 27(45%) patients. 8(13.3%) patients had hypertension, 9(15%) patients had history of chronic lung disease and 23(38.3%) patients had Gastroesophageal reflux disease. Dental infection was present in 36(60%) patients. Chest x-ray showed mild pleural effusion in 5(8.3%), moderate in 47 (78.3%) and massive in 26 (43.3%) patients. 26 (43.3%) patients had encystment of pleural effusion. Sputum culture was positive in 5 (8.3%) patients and blood culture in 2 (3.3%) patients. The standard laboratory culture of pleural fluid was positive in 19 (31.6%) cases. A second standard culture did not produce an increase in bacterial yield. Pleural fluid culture in blood culture bottle was positive in 24 (40%) cases (Table 1). There was high agreement between the two methods of pleural fluid culture (kappa value=0.82). Addition of blood culture bottle culture to the standard laboratory culture increased culture positivity by 8.4 (chi square value 41.7 with p <0.001). All cases which are culture positive in standard bottle were also positive in blood culture bottle. *Klebsiella pneumoniae* was the most common bacterium identified in pleural fluid culture. Blood culture bottle culture identified two extra cases of *Pseudomonas aeruginosa*, one case of *Escherichia coli*, one case of *Klebsiella pneumoniae* and one mixed infection case (Table 2 and Figure 1). Additional yield in blood culture bottle was more for gram negative organism. A history of antibiotic use before diagnostic thoracocentesis had decreased pleural fluid culture positivity. In patients with a history of antibiotic use before diagnostic thoracocentesis culture positivity was 13/38(34%) whereas that in patients without a prior history of antibiotic use was 12/22(54%).

Table 1. Microbial culture results

Patient characteristics	Culture positivity, n (%)
Pleural fluid culture	
Standard culture bottle 1	19 (31.6)
Standard culture bottle 2	19 (31.6)
Blood culture bottle	24 (40)
Sputum culture	5 (8.3)
Venous blood culture	2 (3.3)

Table 2. Pleural fluid culture results for each culture method

pathogen	Sterile bottle, n (%)	Blood culture bottle, n (%)
No pathogen	41 (68.3)	36 (60)
Streptococcus pneumoniae	4 (6.67)	4 (6.67)
Streptococcus pyogenes	1 (1.7)	1(1.7)
Staphylococcus aureus	2 (3.3)	2 (3.38)
Escherichia coli	2 (3.3)	3 (5)
Klebsiella pneumoniae	6 (10)	7 (11.7)
Pseudomonas aeruginosa	3 (5)	5 (8.3)
Mixed infection	1 (1.7)	2 (3.3)

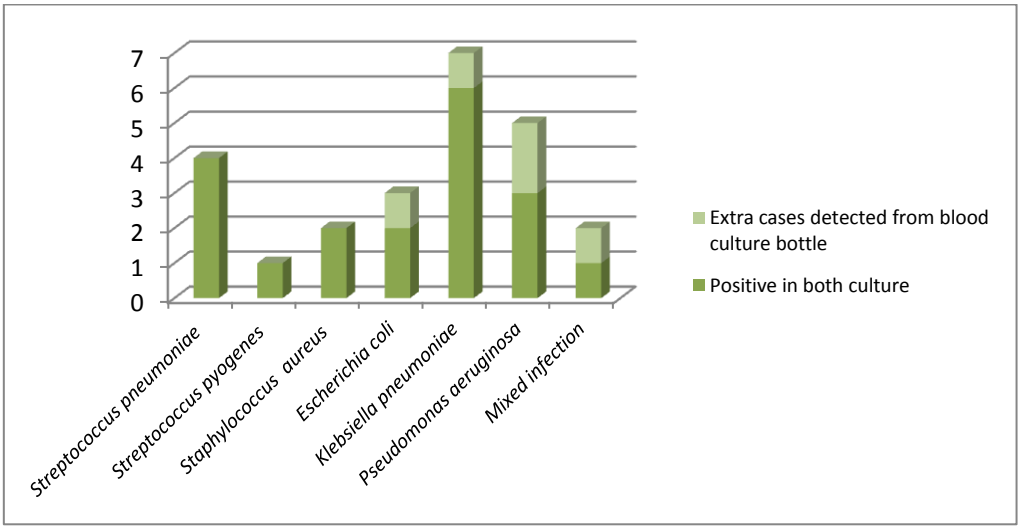

Figure 1 The value of adding blood culture bottle culture methods to standard culture in detection of pathogen

Discussion

This study demonstrates that inoculation of pleural fluid into blood culture bottles increases diagnostic yield of culture. Pleural fluid culture in blood culture bottle increases the bacterial yield by 8.4% (from 31.6% to 40%) when compared to standard culture. All cases which are positive in standard culture were also positive blood culture bottle culture.

Several other studies of this approach have shown similar results. A prospective study by Menzies SM et al in 62 patients with suspected pleural infection from four centres in the UK showed the addition of blood culture bottle culture increased the yield of culture by 20.8 %[20]. Another study in 1999 by Ferrer A et al also found that pleural fluid culture positivity increased significantly by additionally analysing samples in blood culture bottles[12]. Together with these two studies the current study results show that blood culture bottle culture is a valuable adjunct to standard laboratory plated culture in empyema and should be part of standard care. It is very interesting that this finding is similar to the results in other 'non-blood' uses of blood culture bottle culture. In bacterial peritonitis, peritoneal fluid culture in blood culture bottle increased the bacterial isolation rate by 29-49%[18], from a positivity rate on standard culture of 42-54%. In synovial fluid culture, blood culture bottle culture increased the bacterial isolation rate by about 20% over the 10-20% culture positivity with standard culture[14,19].

This study used repeated standard laboratory culture as a control intervention to test whether the improved yield of organisms using blood culture bottles was simply due to repeating the culture process. But second standard culture didn't produce any increase in bacterial yield. Hence blood culture bottle culture is superior to simple repetition of standard culture.

Klebsiella pneumoniae was the most frequent bacterium identified in pleural fluid culture by both culture techniques. There are many other studies showing similar results. A study by Lin et al[21] in 2007 found that most frequent bacteria isolated were aerobic gram negative group. Among them *Klebsiella pneumoniae* was the most frequent isolate. An Indian study by Ramana et al[22] in 2012 have also concluded that most common bacteria identified in pleural fluid culture were *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

The additional yield obtained in blood culture bottle was predominantly gram negative organism and few mixed infection. This results suggest that inoculation of pleural fluid into blood culture bottles increases diagnostic yield especially in empyema caused by gram negative organism. This study demonstrated a differential rates of bacterial identification between subjects with or without a history of antibiotic use before diagnostic thoracocentesis. In patients with a history of antibiotic use before diagnostic thoracocentesis, culture positivity was 13/38(34%) whereas that in patients without a prior antibiotic use was 12/22(54%). This may suggest that all patients should be subjected to diagnostic thoracocentesis before starting antibiotics as far as possible.

Limitations of the Study

There were several potential limitations to this study. Firstly, this was not a blinded study, which could have lead to several bias in identifying bacteria during culture. In addition, anaerobic culture was not done. Different bacteria may have preferential growth in different media, and the spectrum of bacterial infection differs worldwide. Hence this study should be repeated in other regions to ascertain the exact magnitude of benefit with blood culture bottle culture. A larger study may also be needed to address whether bottle culture is particularly beneficial in subgroups of clinical settings, for example taking into consideration antibiotic treatment prior to sampling, the size of the effusion, the likely bacterial load or degree of sepsis.

Conclusion

Inoculating pleural fluid into blood culture bottles at the bedside increases the rate of bacterial

pathogen identification in empyema, when compared to standard laboratory culture. This increased yield appears to be specific to the use of blood culture bottles, and not due to repetition of culture process.

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