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Blood Culture Bottle and Standard Culture Bottle Methods for Detection of Bacterial Pathogens in Parapneumonic Pleural Effusion

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Background: Bacterial parapneumonic pleural effusions (PPEs) have high morbidity. The accurate identification of pathogens is vital for initiating the appropriate treatment. A previous study suggested that the use of blood culture bottles might improve the bacterial yield in PPEs.

Objectives: The aim of this study was to compare the culture positivity rate by the blood culture bottles and the standard culture bottles in bacterial PPEs.

Patients and Methods: Patients diagnosed with PPEs at the Khon Kaen Hospital, Khon Kaen, Thailand, which is an endemic area of melioidosis, were enrolled consecutively and prospectively. The study period was from June first, 2012 to December 31st, 2013. The inclusion criteria were adult patients aged > 18 years, with exudative, neutrophilic parapneumonic effusion. Of the pleural fluid samples, 5 mL from all the eligible patients were collected in both blood culture bottles and the standard culture bottles. Patient baseline characteristics, laboratory results, and culture results were collected and analyzed.

Results: During the study period, 129 patients met the study criteria. The bacteria-positive rate of pleural fluid culture using the standard culture bottle was 14.0%, whereas the positive rate using blood culture bottles was 24.0% ($P < 0.001$).

Conclusions: The blood culture bottle method is more effective than the standard culture bottle method for the detection of bacterial pathogens in PPE.

Keywords: Parapneumonic Pleural Effusion; Diagnosis; Culture; Efficacy

1. Background

Bacterial parapneumonic pleural effusion (PPE) accounts for 40% of community-acquired pneumonia cases and has high morbidity and mortality (1). A study in the UK showed that the mortality rate of bacterial PPE was 15% and 20% of patients needed to be hospitalized for several months (2). The most common causative pathogen was *Streptococcus* sp. (3). An early identification of the causative pathogen cultured from pleural samples can improve the treatment outcomes by indicating the appropriate antibiotics as well as treatment interventions such as surgical drainage (4, 5).

The use of blood culture bottles for ascites fluid or joint fluid gave a better yield of causative agents than did the standard culture bottle (6, 7). Similar results were obtained in a study to detect bacteria in pleural fluid in the UK. The blood culture bottle method increased the pathogen identification rate by 20.8% compared with sterile culture bottles in 53 bacterial PPEs (8). Pathogens of pneumonia, causes of PPE, may be different between Asian and the Western countries (9, 10). The northeastern parts of Thailand and Australia have a higher incidence of melioidosis or *Burkholderia pseudomallei* infection (11). Pulmonary melioidosis may have pleural effusions in 13.5% of patients (12). There are limited data of using blood culture bottles to yield the identification of bacterial pathogens in Asian patients with bacterial PPEs.

2. Objectives

The aim of this study was to compare the culture-positive rate by the blood culture bottles and the standard culture bottles in bacterial PPEs in different settings and larger study populations from the original study (8).

3. Patients and Methods

Patients diagnosed with bacterial PPE in Khon Kaen Hospital, Khon Kaen, Thailand, were enrolled consecutively and prospectively. The study period was from June first,

2012 to December 31st, 2013. The inclusion criteria were patients aged > 18 years and with exudative, neutrophilic PPE. The patients were excluded if causes other than bacterial pleural effusion were suspected including the presence of lymphocytes in the pleural fluid at levels of more than 50%, the presence of abnormal cells, lung abscesses, or bloody pleural effusions (13).

All the eligible patients signed informed consents prior to study participation. Pleural fluids of all the eligible patients were collected in blood culture bottles in addition to standard culture bottles to compare the organism identifications in both bottles. In this practice, a standard culture bottle is a sterile bottle without any media; 5 mL pleural fluid was put in both culture bottles. The bottles were transported together to the laboratory. Blood culture bottles were BacT/ALERT FA for aerobic organisms, containing soybean-casein digestive broth, sodium polyanethol sulfonate, pyridoxal HCl, menadione, hemin, activated charcoal, L-cysteine, carbohydrate, and amino acids (BioMe´rieux, Inc., Durham, N.C., USA) (14). Blood culture bottles were used for aerobic organisms because aerobic organisms are the common pathogens in bacterial PPE (8), and patients with pleural effusion from anaerobic pathogens were excluded. The blood culture bottles were incubated in BacT/Alert system at 35°C for seven days (15, 16), and the procedures for BacT/Alert system were followed (16), while pleural fluids in the standard culture bottles or sterile tubes were cultured using blood, MacConkey, and chocolate media. The organism identification procedures were similar in both groups of bottles.

Baseline patient characteristics, laboratory results, and culture results were collected and compared with the disease severity. The culture results using blood culture bottles and standard culture bottles were compared using descriptive statistics. The disease severity was classified into the most severe empyema thoracis, complicated PPE, and simple PPE as the least severity (17). In brief, empyema thoracis is diagnosed if the pleural fluid is purulent, while complicated PPE is warranted if one of the following features is met; positive pleural fluid smears for bacterial pathogens; pleural fluid glucose < 60 mg/dL, pleural fluid pH < 7.20, or loculated pleural effusion. Simple PPE is exudative, neutrophilic pleural effusion without evidence of complicated PPE and negative pleural fluid culture by bacterial culture in both types of culture bottles.

Following Menzies et al. (8), the proportions of the bacteria-positive pleural fluid cultures by blood culture bottles (58.5%) and standard culture bottles (37.7%) methods were used for sample size determination. The sample size was calculated by the differences of both mentioned proportions with a two-tailed, confidence level of 95%, power of 80%, and deviation of 15% by the McNamar's method. The estimated study population was 135 subjects. The main primary outcome of the study was the comparison of the culture-positive rates between the blood culture bottles and the standard culture bottles groups. Descriptive statistics were used to calculate means, standard deviation (SD), and proportions. Comparing the proportions between the groups was performed by chi-squared or Fisher's Exact test, when appropriate. Statistical analyses were executed by STATA 10.0 (College Station, Texas, USA).

4. Results

During the study period, 169 patients were suspected of bacterial PPE. Of those, 40 were excluded due to either lymphocytic pleural effusion (33 patients) or transudative pleural effusion (seven patients). In total, 129 patients (76.3%) met the study criteria; 80 (62.0%) with empyema thoracis; 19 (14.7%) with complicated PPE and 30 (23.3%) with simple PPE. Among these three levels of severity, baseline characteristics such as age, gender, and co-morbid diseases were comparable (Table 1). The pleural fluid features and peripheral blood analysis among these three groups were also similar (Table 2).

Table 1. Biographical Data of Patients With Bacterial Pleural Effusion Categorized by the Severity of Disease [a]

Clinical Features	All Patients (n = 129)	Empyema Thoracis (n = 80)	Complicated Parapneumonic Effusion (n = 19)	Simple Parapneumonic Effusion (n = 30)
Mean age, y	54 ± 14.2	53 ± 12.8	53 ± 15.4	45 ± 17.0
Males	106 (82.2)	69 (86.3)	19 (100)	18 (60.0)
Median duration, d	14 (7 - 21)	14 (7 - 30)	14 (7 - 21)	7 (4 - 14)
Previously received antibiotic	102 (79.1)	65 (81.3)	15 (79.0)	22 (73.3)
Median duration of previous antibiotic use, d	3 (1 - 7)	3 (1 - 7.5)	3 (1 - 9)	1 (0 - 3)
Co-morbid diseases	86 (64.3)	48 (60.0)	12 (63.2)	23 (76.7)
Diabetes mellitus	28 (21.7)	15 (18.8)	5 (26.3)	8 (26.7)
Alcohol consumption	14 (10.9)	12 (15.0)	1 (5.3)	1 (3.3)
Cancer	14 (10.9)	6 (7.5)	2 (10.5)	6 (20.0)
Cirrhosis	11 (8.5)	4 (5.0)	3 (15.8)	4 (13.3)
Previous tuberculosis	7 (5.4)	5 (6.3)	2 (10.5)	0
Community-acquired disease	104 (80.6)	65 (81.3)	16 (84.2)	23 (76.7)

[a] Data are presented as mean (SD), median (range), or number (percentage); alcohol consumption is more than 10 g/d.

Table 2. Laboratory Data of Patients With Bacterial Pleural Effusion Categorized by the Severity of Disease [a,b]

Features	All Patients (n = 129)	Empyema Thoracis Group (n = 80)	Complicated Parapneumonic Effusion (n = 19)	Simple Parapneumonic Effusion (n = 30)
Pleural Fluid				
Pus, No. (%)	80 (62.0)	80 (100)	0	0
pH, mean ± SD	7.1 (0.3)	6.9 (0.3)	7.0 (0.2)	7.4 (0.9)
Glucose, mg/dL	40 (2 - 111)	3 (1 - 38.5)	17 (2 - 58)	114 (83 - 138)
Protein, mg/dL	4.4 (3 - 5.3)	4.4 (2.6 - 5.4)	5.0 (3.8 - 5.5)	3.5 (2.7 - 4.6)
LDH, IU/L	1940 (687 - 6040)	4922 (2487 - 8666)	1785 (943 - 3999)	358 (229 - 716)
WBC, cells/mm^3 ($\times 10^3$)	4.0 (1.2 - 17.6)	9.4 (3.3-56.0)	3.4 (1.5 - 12.8)	2.0 (0.5 - 3.3)
Neutrophils, %	91 (76 - 96)	94 (88 - 97)	91 (79 - 97)	74 (61 - 783)
Lymphocytes, %	6 (3 - 21)	5 (3-9)	8 (2 - 17)	24 (8 - 33)
Blood				
WBC, cells/mm^3 ($\times 10^3$)	14.2 (10.0 - 20.8)	14.5 (9.4 - 21.7)	14.2 (12.0 - 21.7)	12.9 (9.9 - 17.8)
Neutrophils, %	82 (74 - 87)	82 (74 - 87)	80 (74 - 88)	84 (77 - 88)

[a] Abbreviations: LDH, lactate dehydrogenase; WBC, white blood cell.
[b] Data are presented as median (first-third quartile) unless indicated otherwise.

Table 3. Bacterial Detection Using Two Types of Culture Bottles

Culture Results	All Patients (n = 129)		Empyema Thoracis, (n = 80)		Complicated Parapneumonic Effusion (n = 19)	
	Standard Culture Bottle	Blood Culture Bottle	Standard Culture Bottle	Blood Culture Bottle	Standard Culture Bottle	Blood Culture Bottle
Culture-positive, %	18 (14.0) [a]	31 (24.0) [a]	16 (20.0) [a]	27 (33.8) [a]	2 (10.5)	4 (21.0)
Pathogens						
Streptococcus	6 (33.3)	15 (48.4)	5 (31.3)	14 (51.9)	1 (50.0)	1 (25.0)
Staphylococcus	2 (11.1)	1 (3.2)	1 (6.2)	1 (3.7)	1 (50.0)	0
Gram-negative	9 (50.0)	11 (35.5)	9 (56.3)	9 (33.3)	0	2 (50.0)
Mixed aerobic	1 (5.6)	1 (3.2)	1 (6.2)	1 (3.7)	0	0
Others	0	3 (9.7)	0	2 (7.4)	0	1 (25.0)

[a] There was a statistically significant difference between the bottle types, P value < 0.001 (P value for all other pairs > 0.05).

5. Discussion

The results of the previous study (8) wherein the blood culture bottle, specifically BacT/ALERT FA increased the bacterial identification rates in PPE, were confirmed. This study was different from the original study in terms of the study location (the UK vs an Asian tropical country), the sample size (53 vs 129), plus different possible organisms in the effusion. The overall bacterial identification rate in bacterial PPE by blood culture bottle in this study was 24.0% and even higher at 33.8% in the empyema group. The rates were lower than the original study (24.0% vs. 25.5%). This finding may be explained by the different types of blood culture bottles. The original study used the BACTEC PLUS bottle (8). Previous antibiotic use in this study population (79.1%), as shown in Table 1 (18), and delayed transport process to the culture lab (19) might also be considered as factors. The culture-positive rate by blood culture bottle in the study, however, was still higher than a previous report by Ferrer at 15.0% (20). Better bacterial culture yield for pleural effusion using blood culture bottles may be due to using charcoal-containing medium, while the standard culture bottles do not have such medium (14). Charcoal may increase the oxygenation, resulting in a higher rate of organism recovery.

In this study, the most common pathogen in the pleural fluid was *Streptococcus* sp., similar to the previous study in UK (5). *Burkholderia pseudomallei*, the causative agent of melioidosis, was found in five patients (31 patients, 16.1% in our study). Reechaipichitkul (12) reported pleural effusion in 12.2% and 15.3% of cases with acute and subacute/chronic pulmonary melioidosis, respectively. *S. pneumoniae* is usually the most common pathogen causing community-acquired pneumonia, but was not found in this study. This may also be explained by the high rate of previous antibiotic use (79.1%).

The main limitation of this study was that the size of the study population was somewhat lower than the calculated power sample size (126 vs. 135 subjects). The results, however, showed statistically significant advantages of the blood culture bottle method over the standard culture bottle method for the detection of bacteria in pleural effusion. The relatively low bacterial detection rate for the pleural fluid may be due to the high rate of previous antibiotic use. The results of this study strongly supported the previously report of Menzies et al. using a relatively larger sample size (8). Blood culture bottle method should be used in routine clinical practices for pathogen identification in pleural fluids of patients suspected of bacterial PPE. In conclusion, the blood culture bottle method was more effective than the standard culture bottle method for the detection of bacterial pathogens in PPE.

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Authors' Contributions

Study concept and design: Surapan Charoentunyarak and Sarassawan Kananuraks. Statistical analysis: Sarassawan Kananuraks. Data interpretation: all the authors. Drafting of the manuscript: Jarin Chindaprasirt; Panita Limpawattana; Kittisak Sawanyawisuth. Patient care: Surapan Charoentunyarak; Sarassawan Kananuraks. Critical revision of the manuscript for important intellectual content: Surapan Charoentunyarak and Kittisak Sawanyawisuth.

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