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DETECTION OF BACTERIA CAUSING VENTILATOR ASSOCIATED PNEUMONIA USING BRONCHOALVEOLAR LAVAGE CULTURE AND ENDOTRACHEAL ASPIRATE CULTURE

Asty H[1], Loesnihari R[2], Syarani F[3]

[1] Specialization Program of Clinical Pathology, Faculty of Medicine, University of North Sumatera, Medan, Indonesia. E-mail: dr.hairiahasty@yahoo.co.id
[2] Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera, Medan, Indonesia
[3] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of North Sumatera, Medan, Indonesia

ABSTRACT

Ventilator-Associated Pneumonia (VAP) is the most common hospital infection in the ICU. Proper and prompt diagnosis and treatment with adequate antibiotics can reduce the high mortality rate, prevent complications, and antibiotic resistance. One of invasive methods such as bronchoalveolar lavage can make a more accurate diagnosis and help with the choice of antibiotics, but it requires pulmonologists. Contrastingly, non-invasive methods such as endotracheal aspirate can be performed faster with fewer complications. The study aimed to determine the bacterial pattern and sensitivity of the bronchoalveolar lavage and endotracheal fluid. This was an observational study with cross-sectional approaches performed at the Intensive Care Unit of Adam Malik Hospital Medan, in August 2017-February 2018. The endotracheal aspirate and bronchoalveolar lavage of 23 patients who met the criteria and was suspected with VAP taken as sample. The samples obtained were then cultured and their sensitivity against antibiotics was tested using BD Phoenix. There was agreement of bacterial pattern but no significant difference of sensitivity and antibiotic resistance between bronchoalveolar cultures and endotracheal aspirate cultures. Endotracheal aspirate culture showed a sensitivity of 78.9% and a specificity of 75% to diagnose VAP. Information on the identification of bacteria and susceptibility test in patients with suspected VAP required appropriate sampling techniques. There were no significant differences between bronchoalveolar lavage culture and endotracheal aspirate culture to diagnose VAP. Endotracheal aspirate culture is a non-invasive diagnostic instruments that can be used as an alternative diagnostic instruments in patients with suspected VAP.

Key words: Ventilator-associated pneumonia, bronchoalveolar lavage, endotracheal aspirate, bacterial pattern, sensitivity

INTRODUCTION

Patients in the ICU have a risk of death not only from their critical illness but also from secondary processes such as hospital infections. Pneumonia that occurs in a hospital or hospital-acquired pneumonia is the second most common cause of infection in patients with critical illness. Among hospital-acquired pneumonia, 86% were associated with Ventilator-Associated Pneumonia (VAP) due to the installation of mechanical ventilation devices.[1]

Ventilator-associated pneumonia is pneumonia that occurs 48-72 hours after installation of mechanical ventilation devices.[2] The average mortality rate associated with VAP has been reported approximately 24% - 50% in hospital intensive care in Nigeria.[3] Proper and prompt diagnosis and treatment with adequate antibiotics can reduce the mortality rate from 33% to 71%.[1] In contrast, inappropriate antibiotic use can cause side effects and antibiotic resistance.[4]

Lower airway sampling can be carried out by non-invasive and invasive methods. The most common non-invasive method is aspiration of endotracheal fluid while the examples of invasive methods are bronchial stroke and Bronchoalveolar Lavage (BAL).[5] Invasive methods can make a more accurate diagnosis and determine the right antibiotics than non-invasive methods but invasive methods require pulmonologists.[6] Non-invasive methods can be performed faster, does not require pulmonary specialists, and causes smaller complications compared to invasive methods.[2]

Research by Shafi in 2015 showed that there were no significant differences between endotracheal aspirate culture and BAL culture results in 30 patients suspected of VAP there were with 26 bacterial growths observed in endotracheal aspirate cultures and 27 bacterial growths observed in bronchoalveolar lavage cultures. The most

commonly found microorganisms in these aquaculture were *Acinetobacter spp, Pseudomonas aeruginosa,* and *Klebsiella spp*. Endotracheal fluid culture showed good sensitivity (86%) but low specificity (63%) to diagnose VAP.[7]

Previous studies have shown that the need for appropriate and rapid antibiotics to reduce mortality caused by VAP requires information on the identification of bacteria, appropriate sample, and sensitivity tests in patients who use ventilators after 48 hours and suspected of having pneumonia. Based on this, the researchers wanted to conduct a study on the detection of bacteria that cause ventilator-associated pneumonia with bronchoalveolar lavage culture and endotracheal aspirate culture.

This study aimed to determine differences of the bacterial patterns and their sensitivity to antibiotics between bronchoalveolar lavage culture obtained using flexible fiberoptic bronchoscopy and aspirate endotracheal culture obtained using catheter hoses among patients with suspected ventilator-associated pneumonia.

METHODS

This study was an observational research with cross-sectional data collection method. Study was conducted at the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Adam Malik Hospital Medan in collaboration with the Department of Pulmonology and Faculty of Medicine, University of North Sumatera/Respiratory Medicine Adam Malik Hospital Medan during the period of August 2017-February 2018. The inclusion criteria of this study were all ventilator patients after 48 hours with suspected VAP in the Treatment Unit Intensive at the Adam Malik Hospital Medan, patients with suspected VAP based on the Clinical Pulmonary Infection Score (CPIS) criteria with a score of more than six.[8] The clinical pulmonary infection score can be seen in Table 1. Patients who were suffering from pneumonia before ventilator use (a score of more than six on CPIS criteria was indicator of pneumonia) and tuberculosis were excluded from this study.

Ethical Clearance was obtained from the Health Research Committee Faculty of Medicine, University of North Sumatra with a number of 305/TGL/KEPK FK USU-RSUP HAM/2017.

Endotracheal aspirate and BAL collection were performed by a pulmonologist. Endotracheal aspirate was collected using a catheter hose with aseptic technique. Then proceed with taking BAL with using flexible fiberoptic bronchoscopy. The samples obtained were placed into different sterile pots and immediately delivered to the Pathology Clinic laboratory of Adam Malik Hospital Medan.

The samples were then streaked in a solid medium of Blood Agar, Mc Conkey Media, and Chocolate Agar, and put in the incubator at 37^{0}C for 18-24 hours. The colony growth was observed, smeared and Gram stained. Examination was proceeded using BD Phoenix to determine the types of bacteria and pattern sensitivity.

The characteristics of the research subject were described in the form of tables. Chi-Square test was done to determine the difference between bronchoalveolar lavage culture and the endotracheal aspirate culture. Significance level of

Table 1. Clinical pulmonary infection score[8]

Component	Value	Score
Temperature (°C)	≥ 36.5 and ≤ 38.4	0
	≥ 38.5 and ≤ 38.9	1
	≥ 39.0 and ≤ 36.0	2
Leukocyte per mm^3	≥ 4000 and ≤ 11000	0
	< 4000 and > 11000	1
Tracheal secretions	Low	0
	Intermediate	1
	High	2
	Purulent	+1
Oxygenation		
PaO_2/FiO_2 (mmHg)	> 240 or there is ARDS	0
	≤ 240 and there is no ARDS	2
Thorax photo	There is no infiltrates	0
	Spotting or diffuse infiltrates	1
	Localized infiltrates	2

5% alpha was used, and sensitivity, specificity, positive predictive value and negative predictive value of endotracheal aspirate culture were determined using bronchoalveolar lavage culture as gold standard.[9]

RESULTS AND DISCUSSION

In this study, there were 23 research subjects who met the inclusion criteria. Their endotracheal aspirate was collected using a hose catheters and BAL were collected using a fiberoptic bronchoscopy device. The samples were cultured and sensitivity test was performed using BD Phoenix instruments.

In this study, there was no difference between males and females and underlying disease. The most common isolated bacterial spectrum and sensitivity testing for antibiotics in patients with suspected VAP at Intensive Care Unit Adam Malik Hospital Medan were determined.

Patients who entered the Intensive Care Unit Adam Malik Hospital generally have received antibiotics before the results of culture and sensitivity testing were obtained. The most commonly used antibiotic was Ceftriaxone among 11 subjects (47.8%). This was not different from the research conducted by Yagmurdur *et al.* in 2016, that patients have received antibiotics before they were given the bacterial culture and sensitivity results. Description of the subject characteristics was shown in Table 2.

Table 2. Basic characteristics of the study subjects

Demographic Characteristics	n = 23
Gender, n (%)	
Male	14 (60.9)
Female	9 (39.1)
Age, Year	
Mean	46.61
Median	47
Minimum	18
Maximum	85
Observation in 1 week, n (%)	
Death	8 (34.8)
Life	15 (65.2)
VAP, n (%)	
Early	8 (34.8)
Late	15 (65.2)
Antibiotics, n (%) *	
Ceftriaxone	11 (47.8)
Cefotaxime	1 (4.3)
Ceftazidime	1 (4.3)
Meropenem	8 (34.8)
Cefotaxime and Metronidazole	2 (8.7)

* Antibiotics used as empirical therapy

The results of BAL culture showed 19 samples (82.7%) with bacterial growth, 1 sample (4.3%) with no growth and 3 samples (13%) with not meaningful. Meanwhile, culture from endotracheal aspirate showed bacterial growth in 20 samples (87%) and no growth in 3 samples (13%). Of the 23 samples, there were 16 samples (69.6%) with the same results. Of 16 samples with the same results, there were 93.8% that showed similarities in the type of bacteria and 6.2% showed no bacterial growth (Table 3).

Table 3. Culture results of bronchoalveolar lavage and endotracheal aspirate

Culture Results	n = 23
Bronchoalveolar lavage	
Growth	19 (82.7)
No growth	1 (4.3)
Unconditional*	3 (13)
Endotracheal aspirate	
Growth	20 (87)
No growth	3 (13)
The same culture results	16 (69.6)
Types of bacteria	15 (93.8)
No growth	1 (6.2)

*Was not qualified in bronchoalveolar lavage culture, suggesting the growth of pure bacteria <104 CFU (Colony Forming Unit/mL)

The most common bacteria obtained from the results of BAL culture isolated using the most flexible fiberoptic bronchoscopy were *Pseudomonas aeruginosa* (21.7%, n=5), unconditional (13%, n=3) and no growth (4.3%, n=1). The bacterial culture from endotracheal aspirate using a catheter hose were *Acinetobacter baumanii* (21.7%, n=5) and no bacterial growth (13%, n=3). The detailed results of bacterial culture in the second group can be seen in Table 4.

Based on research by Shafi *et al*. in 2015, the results of bacterial culture obtained from endotracheal aspirate and bronchoalveolar lavage were nearly the same. 30% of *Acinetobacter baumanii* (n=9), 23.3% of *Pseudomonas aeruginosa* (n=7), and 13.3% of *Klebsiella pneumonie* (n=4) were found in bronchoalveolar lavage culture, while 30% of *Acinetobacter baumanii* (n=9), 20% of *Pseudomonas aeruginosa* (n=6) and 13.3% of *Klebsiella spp*. (n=4) were found in the culture of endotracheal fluid.

A total of 18 cultures (78.2%) showed similar results, 15 cultures (65.2) showed the same growth, 3 cultures (13%) showed no growth of bacteria, and 5 cultures (21.7%) showed contradictory results. After correction of the coincidence factor, interrater

Table 4. Types of organisms from bronchoalveolar lavage and endotracheal aspirate

Culture Results	Bronchoalveolar Lavage	Endotracheal Aspirate
No growth	1 (4.3)	3 (13)
Unconditional	3 (13)	0
Pseudomonas aeruginosa	5 (21.7)	4 (17.4)
Burkholderia cepacia complex	1 (4.3)	0
Klebsiella ozaeneae	4 (17.4)	3 (13)
Klebsiella pneumonia	2 (8.7)	4 (17.4)
Citrobacter diversus	1 (4.3)	1 (4.3)
Acinetobacter baumannii	4 (17.4)	5 (21.7)
Stenotropomonas maltophilia	1 (4.3)	1 (4.3)
Klebsiella oxytoca	1 (4.3)	1 (4.3)
Chromobacterium violaceum	0	1 (4.3)

Table 5. Agreement test between endotracheal aspirate culture and BAL

Endotracheal aspirate culture	Bronchoalveolar Lavage Culture		p
	Growth	No Growth	
Growth	15 (65.2)	1 (4.3)	0.033
No growth	4 (17.4)	3 (13)	

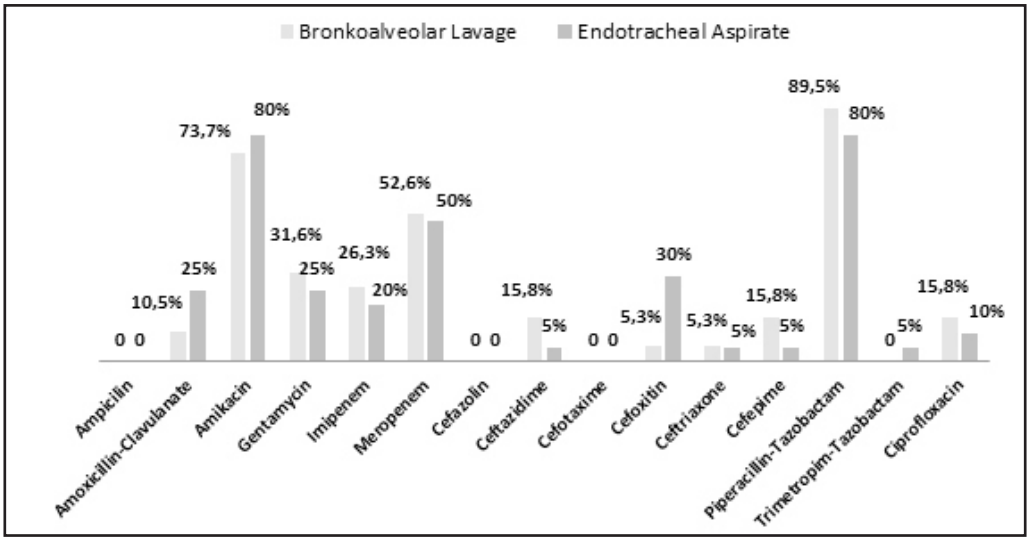

Figure 1. Antibiotic sensitivity profile of bacteria isolated from bronchoalveolar lavage culture using bronchoscopy fiber flexural optics and from endotracheal aspirate culture using catheter hose.

reliability (kappa value) of 0.416 was obtained. Based on the kappa test categorization, it was suggested that there was moderate agreement between endotracheal aspirate and bronchoalveolar lavage (Table 5).

In pneumonia, microorganisms usually enter the body by inhalation or aspiration. Generally, the microorganisms found in the upper respiratory tract are the same with the lower respiratory tract, but in some studies not found the same microorganism.[10]

The sensitivity of bacteria from bronchoalveolar lavage to Piperacillin-Tazobactam, Amikacin, and Meropenem was 89.5% (n=17), 73.7% (n=14) and 52.6% (n=10), respectively. A similar thing was found in the sensitivity test of bacteria from endotracheal aspirate culture showing the highest sensitivity to Piperacillin-Tazobactam. The bacterial sensitivity to Piperacillin-Tazobactam, Amikacin, and Meropenem was 80% (n=16), 80% (n=16) and 50% (n=10), respectively. These results can be seen in Figure 1.

The principle of VAP treatment is antibiotic de-escalation therapy which initially uses broad-spectrum antibiotics with high probability proceeded with the use of narrow-spectrum antibiotics based on microbiological results.

Table 6. Differences of antibiotic resistance and sensitivity patterns between bronchoalveolar lavage culture and endotracheal aspirate culture

Type of Antibiotics	–	Bronchoalveolar Lavage		Endotracheal Aspirate		p
		Diameter of inhibition zone (mm)				
		Resistance	Sensitivity	Resistance	Sensitivity	
Ampicillin		19 (100)	0	20 (100)	0	-
Ammoxicillin-Clavulanate		17 (89.5)	2 (10.5)	15 (75)	5 (25)	0.407[a]
Amikacin		5 (26.3)	14 (73.7)	4 (20)	16 (80)	0.716[a]
Gentamycin		13 (68.4)	6 (31.6)	15 (75)	5 (25)	0.648[b]
Imipenem		14 (73.7)	5 (263)	16 (80)	4 (20)	0.716[a]
Meropenem		9 (47.4)	10 (52.6)	10 (50)	10 (50)	0.869[b]
Cefazolin		19 (100)	0	20 (100)	0	-
Ceftazidime		16 (84.2)	3 (15.8)	19 (95)	1 (5)	0.342[a]
Cefotaxime		19 (100)	0	20 (100)	0	-
Cefoxitin		18 (94.7)	1 (5.3)	14 (70)	6 (30)	0.091[b]
Ceftriaxone		18 (94.7)	1 (5.3)	19 (95)	1 (5)	1.000[a]
Cefepime	1 (5.3)	15 (78.9)	3 (15.8)	19 (95)	1 (5)	0.294[a]
Piperacillin-Tazobactam		2 (10.5)	17 (89.5)	4 (20)	16 (80)	0.661[a]
Trimetropim-Tazobactam		19 (100)	0	19 (95)	1 (5)	1.000[a]
Ciprofloxacin		16 (84.2)	3 (15.8)	18 (90)	2 (10)	0.661[a]

[a,] Fischer's Exact, [b] Chi-Square

Table 7. Diagnostic test results of endotracheal aspirate culture and bronchoalveolar lavage culture to identify bacterial pathogen of associated ventilator pneumonia

Endotracheal Aspirate	Bronchoalveolar Lavage		Sen. (%)	Spe. (%)	PPV (%)	NPV (%)
	+	-				
+	15	1	78.9	75	93.8	42.9
-	4	3				

Empirical antibiotic therapy for early-onset VAP is beta-lactam/anti-beta-lactamase (amoxicillin-clavulanate), third-generation antipseudomonal cephalosporins (ceftriaxone, cefotaxime) or quinolone (levofloxacin). Whereas empirical antibiotic therapy for late-onset VAP is antipseudomonal cephalosporin (cefepime, ceftazidime), antipseudomonal carbapenem (meropenem, imipenem) or beta-lactam/anti beta-lactamase (piperacillin-tazobactam), supplemention with anti pseudomonal fluoroquinolone (ciprofloxacine or levofloxacine) or aminoglycosides (amikacine, gentamicine or tobramicine), supplemention with Linezolide or vancomicine (if suspected of MRSA).[9]

The results of the antibiotic sensitivity and resistance test of bronchoalveolar lavage culture and endotracheal aspirate culture showed no significant difference to all types of antibiotics (p> 0.05) (Table 6).

By using bronchoalveolar lavage culture as a gold standard, endotracheal aspirate culture showed sensitivity of 78.9% and specificity of 75%. Positive predictive value and negative predictive value were 93.8% and 42.9%, respectively (Table 7).

By using bronchoalveolar lavage culture as a gold standard, endotracheal aspirate culture showed a sensitivity of 78.9%, specificity of 75%, positive predictive value of 93.8% and negative predictive value of 42.9%. According to Shafi and colleagues, endotracheal aspirate culture showed good sensitivity (86%) but low specificity (63%) to diagnose VAP.

CONCLUSIONS AND SUGGESTIONS

There was moderate agreement of bacterial spectrum between cultures of BAL collected using fiber bronchoscopy optic bending and endotracheal aspirate collected using catheter hose. There was no significant difference of sensitivity and antibiotic resistance between BAL culture and endotracheal aspirate culture. Sensitivity and specificity of endotracheal aspirate culture were quite good at 78.90% and 75%, respectively. Both endotracheal aspirate cultures and bronchoalveolar lavage culture can be used to detect bacteria that cause VAP. Endotracheal aspirate can be used as an alternative to diagnose suspected VAP.

Based on the results of this study, the bacterial spectrum was not greatly different. Therefore, in areas with no pulmonologists and facility of flexible fiberoptic bronchoscopy, it was recommended to use a sterile hose catheter with aseptic techniques to collect endotracheal aspirate for bacterial culture and sensitivity tests to detect bacteria causing VAP.

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