Comparison of endotracheal aspirate and non-bronchoscopic bronchoalveolar lavage in the diagnosis of ventilator-associated pneumonia in a pediatric intensive care unit

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Ventilator-associated pneumonia (VAP) is defined as pneumonia occuring in any period of mechanical ventilation. There is no optimal diagnostic method in current use and in this study we aimed to compare two non-invasive diagnostic methods used in diagnosis of VAP in children. This prospective study was conducted in 8 bedded Pediatric Intensive Care Unit at Ege University Children´s Hospital. Endotracheal aspiration (ETA) and non-bronchoscopic bronchoalveolar lavage (BAL) were performed in case of developing VIP after 48 hours of ventilation. Quantitative cultures were examined in Ege University Department of Diagnostic Microbiology, Bacteriology Laboratory. Fourty-one patients were enrolled in the study. The mean age of study subjects was 47.2±53.6 months. A total of 28 in 82 specimens taken with both methods were negative/negative; 28 had positive result with ETA and a negative result with non-bronchoscopic BAL and both results were negative in 26 specimens. There were no patients whose respiratory specimen culture was negative with ETA and positive with non-bronchoscopic BAL. These results imply that there is a significant difference between two diagnostic methods ($p<0.001$). Negative non-bronchoscopic BAL results are recognized as absence of VAP; therefore, ETA results were compared with this method. ETA's sensitivity, specificity, negative and positive predictive values were 100%, 50%, 100% and 48% respectively. The study revealed the ease of usability and the sensitivity of non-bronchoscopic BAL, in comparison with ETA.

***Key words:** endotracheal aspiration, non-bronchoscopic bronchoalveolar lavage, ventilator associated pneumonia, child.*

Ventilator-associated pneumonia (VAP) is defined as a nosocomial infection occurring in patients who are on mechanical ventilatory support, provided that infection is neither present nor in the incubation period at the time of intubation. It usually develops later than 48 hours after the initiation of the support. But there is no minimum period of time before which the ventilator must be in place in order for pneumonia to be considered ventilator associated[1]. VAP is the second most common nosocomial infection also in our unit, whereas bloodstream infections are reported as the most common in pediatric intensive care units[2]. Prolonged mechanical ventilation is an important factor associated with nosocomial pneumonia. Other risk factors are also similar to adults and there are several independent factors in pediatric VAP such as immunodeficiency, immunosuppression, neuromuscular blockade, burns, re-intubation, and transport from the Pediatric Intensive Care Unit (PICU) while intubated. Primary bloodstream infections are also strongly associated with VAP and total parenteral nutrition (TPN). Use of steroids and H2-blockers are other risk factors for developing the disease[3,4].

Although VAP causes high morbidity and

mortality rates, its diagnosis remains challenging despite many efforts in studies. Clinical and radiographic criteria including presence of fever, leukocyte counts, amount and character of tracheal secretions, and appearance of new or persistence of infiltrates in chest X-rays have been established by the Center for Disease Control and Prevention and are also commonly used. However these parameters have limited diagnostic value especially in the presence of Acute Respiratory Distress Syndrome (ARDS)[5]. Pugin et al.[6] combined the above mentioned parameters with oxygenation (PaO2/FiO2) and formed the Clinical Pulmonary Infection Score (CPIS) as a more standardized diagnostic tool for pneumonia . The CPIS has been used in pediatric patients and it is also found to be useful in patients on mechanical ventilation to diagnose VAP[7].

But use of diagnostic procedures is still a topic of debate because a gold standard for ventilator-associated pneumonia has never been clearly established. Isolation of causative agents by culture from biopsy of lung tissue specimens is difficult even though they are accepted as gold standard. It is also unusual to indicate the pneumonic pathogens at nonsterile sites such as blood or pleural fluid. Comparison of invasive and non-invasive procedures to obtain respiratory tract cultures has been the most common approach applied by research investigators.

Although there are many studies about usefulness and comparison of different techniques, such as endotracheal aspiration (ETA), blind bronchial sampling (BBS), and non-bronchoscopic bronchoalveolar lavage in adults, there are few studies conducted with children.

In this prospective study, we aimed to compare non-bronchoscopic BAL with ETA technique which is commonly used for diagnosis of VAP. We also tried to figure out the applicability and feasibility of non-bronchoscopic BAL in our unit.

Material and Methods

This prospective study was conducted in an 8-bedded pediatric intensive care unit of a tertiary care training Children's Hospital; University of Ege in Izmir from March 2012 to March 2013. Patients were daily evaluated for VAP. Pneumonia was suspected by the presence of a new or evolving infiltrate on chest X-ray, purulent or unsavoury airway secretions and the presence of systemic inflammatory response syndrome. Any increase in amount or changes to purulent and unsavoury character of secretions and worsening of X-ray findings accompanied with signs of inflammation in blood tests were listed as diagnostic criteria of VAP. ETA and non-bronchoscopic BAL specimens were collected respectively at the same session if VAP was suspected. Calculated CPISs higher than six points were suggested as the cut-off point for sampling [8]. Age, gender, day of mechanical ventilation, indication for hospitalization, type of bed head elevation (30° or 45°), type of secretions (purulent or serous), white blood cell count and antibiotics and sedatives used were recorded using a data collection form. Parental consent forms were signed by all children's parents and the study was approved by the Local Ethics Committee of Ege University.

Hemodynamic instable patients and patients with increased intracranial pressure, severe bronchospasm, severe hypoxemia, pneumothorax and pleural effusion were excluded from the study. Transient hypoxia ($SaO_2 < 90\%$) was defined as desaturation.

All samples were collected by the same personnel. A standard suction catheter was used for endotracheal aspiration (Bıçakçılar Tıbbi Cihazlar Sanayi ve Ticaret A.Ş.® , Türkiye). Endotracheal aspiration was obtained by using a sterile specimen tap after irrigating the trachea with 5 ml of sterile 0.9% NaCl solution and suction of a sample of sputum.

CombiCath® 60 cm x 2.7 mm lavage catheter (PRODIMED / Divison Plastimed, France) was used for non-bronchoscopic BAL. It is usually recommended to apply 20 ml of sterile saline for bronchoalveolar lavage in adults[9]. But there ise no consensus about the appropriate amount of lavage fluid in pediatric patients. Sachdev et al.[10] have used 3 ml of saline for babies weighing 5 kg, 5 ml of saline for children weighing between 5 kg and 10 kg, 7.5 ml of saline for those weighing 11 kg to 20 kg, and 10 ml of saline for patients weighing 20 kg[10]. In our study we used 5 ml for babies weighing less than 15 kg and 10 ml for children weighing more than 15 kg. Every patient was

monitored closely and 100% oxygenated to prevent or minimize desaturation. Hands were washed and dried throughly and the catheter package was opened in a sterile manner and laid on sterile field. A syringe was filled with sterile saline according to the patients weight. The catheter was attached to the endotracheal tube and advanced until resistance is met. Then the catheter was unlocked and the inner catheter was retracted. The saline solution in the syringe was infused and aspirated. It was feasible to obtain 2-3 ml of lavage fluid from all patients. The short duration of the procedure and no need for a bronchoscope are the major advantages of this non invasive method.

All samples were evaluated in the Ege University Hospital, Department of Medical Microbiology. They were vortexed and inoculated in blood agar, eosin methylene blue and chocolate agar plate. Presence of intracellular bacteria and Gram stain examinations were noted and leukocyte/epithelial cell ratio were evaluated according to Murray and Washington scale[11]. Positive samples were cultured for 24-48 hours and colony counts were reported. Samples were incubated for more than 48 hours (72 h) if the patient was under antibiotherapy. Identifications were made through automatized (VITEK 2 and VITEK MS, Biomerieux®, France) and conventional biochemical systems. Also sensitivity patterns were studied with disc diffusion, minimal inhibitory concentration methods and additionally with automatized systems (VITEK 2, Biomerieux®, France) according to Clinical and Laboratory Standards Institute (CLSI)[12] criteria.

Data were recorded on a sheet and transferred into SPSS for Windows (Version 16). Numeric data were reported as means ± SD. The chi-squared test was used to determine whether

Table I. Demographic and Clinical Characteristics of the Study Patients (n=41)

Age (months)	
Mean±SD	47.2 ± 53.6
Range	2-168
Cause of admission	
Respiratory	19 (46.3%)
Neuromuscular	5 (12.2%)
Central nervous system	5 (12.2%)
Cardiovascular	3 (7.3%)
Intoxication	3 (7.3%)
Hematological	3 (7.3%)
Trauma	2 (4.9%)
Vasculitis	1 (2.4%)
Time to procedure in PICU (days)	
Mean±SD	5.12 ± 3.6
Range	2-16
CPIS	
Mean±SD	6.32 ± 0.934
Range	5-8
Antibiotherapy	
Ceftriaxone	15 (36.6%)
Piperacillin-tazobactam + aminoglycosides or glycopeptide combination	15 (36.6%)
Carbapenemes	7 (17.1%)
Ceftazidime	3 (7.3%)
Clarithromycin	1 (2.4%)
Sedatives	
Benzodiazepines	35 (85.4)
Fentanyl	35 (85.4)

CPIS: Clinical Pulmonary Infection Score

Table II. Culture Results of Patients For Two Diagnostic Methods

Patient nr.	Endotracheal aspiration	Non-bronchoscopic BAL
1	negative	negative
2	negative	negative
3	negative	negative
4	negative	negative
5	negative	negative
6	negative	negative
7	negative	negative
8	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
9	negative	negative
10	negative	negative
11	Escherichia coli	negative
12	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
13	*Klebsiella pneumonia*	negative
14	Acinetobacter spp.	negative
15	*Staphylococcus aureus*	negative
16	*Klebsiella pneumonia*	*Klebsiella pneumonia*
17	*Staphylococcus aureus*	negative
18	*Stenotrophomonas maltophilia*	negative
19	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
20	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
21	*Staphylococcus aureus*	negative
22	negative	negative
23	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
24	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
25	*Branhamella catarrhalis*	*Branhamella catarrhalis*
26	*Streptococcus pneumonia*	negative
27	Acinetobacter spp	Acinetobacter spp
28	negative	negative
29	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
30	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
31	Acinetobacter spp.	negative
32	Escherichia coli	Escherichia coli
33	negative	negative
34	*Klebsiella pneumoniae*	negative
35	*Haemophilus influenzae B*	negative
36	*Haemophilus influenzae B*	negative
37	negative	negative
38	negative	negative
39	*Haemophilus influenzae B*	negative
40	*Haemophilus influenzae B*	negative
41	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*

BAL: Bronchoalveolar lavage

Table III. Comparison of ETA (endotracheal aspiration) and Non-Bronchoscopic BAL Culture Results

	ETA (+) non-bronchoscopic BAL (+)	ETA (+) non-bronchoscopic BAL (-)	ETA(-) non-bronchoscopic BAL (+)	ETA(-) non-bronchoscopic BAL (-)
Number of samples	26	28	-	28

BAL: Bronchoalveolar lavage

Table IV. Sensitivity, Specificity, and Positive/Negative Predictive Values of ETA as compared $\geq 10^4$ for non-bronchoscopic BAL

	Sensitivity	Specificity	NPV	PPV
ETA $\geq 10^5$ cfu/ml	% 100	% 50	% 100	% 48

ETA: Endotracheal aspiration, NPV: Negative Predictive Value, PPV: Positive Predictive Value

there is a significant difference between two diagnostic methods. Two-tailed test results were considered statistically significant at $p \leq 0.05$. Sensitivity, specificity, and positive/negative predictive values were calculated at a cut off point as 10^5 cfu/ml for ETA as compared 10^4 cfu/ml for non bronchoscopic BAL.

Results

This prospective study was conducted in a period of one year between March 2012 and 2013. The facility is a 8-bedded Pediatric Intensive Care Unit and has approximately 400 admissions per year. Ventilator utilization ratio was 0.61 and VAP rate was 21.5/1000 ventilator days in the unit during the study period. Utilization ratio was found to be at 50-75 % percentiles and VAP rate was at 75-90 % percentiles when compared with national surveillance reports[13].

Forty-one patients (27 boys and 14 girls) were enrolled in the study. The mean age of study subjects was 47.2±53.6 months. Days on the ventilator before the procedure ranged from 2-16, with a mean of 5.12 ± 3.6 days and a median of 3 days. Demographic and clinical characteristics of the study patients are summarized in Table I. Nineteen patients were intubated for underlying pulmonary diseases before they were suspected with VAP. Three of them had cystic fibrosis, six had bronchiolitis and nine was diagnosed with bronchopneumonia. One last patient had respiratory insufficiency due to foreign body aspiration. All patients were evaluated for VAP in the presence of any increase in amount or changes to purulent and unsavoury character of secretions and worsening of X-ray findings accompanied with signs of inflammation in blood tests and diagnosed with VAP. Two samples with both techniques were obtained. CPIS scores were calculated and noted for all patients with a mean value of 6.32 ± 0.934 but they were not used to decide to obtain specimens.

A total of 28 in 82 specimens taken with both methods were negative/negative when they were cultured; 28 had positive result with ETA and a negative result with non-bronchoscopic BAL and both results were positive/positive in 26 specimens. Culture results for two diagnostic methods for every patient were listed in Table II. There were no patients whose respiratory specimen culture was negative with ETA and positive with non-bronchoscopic BAL (Table III). These results imply that there is a significant difference between two diagnostic methods ($p<0.001$). Negative non- bronchoscopic BAL results are recognized as absence of VAP, therefore ETA results were compared with this method. ETA's sensitivity, specificity, negative and positive predictive value were 100%, 50%, 100% and 48% respectively (Table IV).

Pathogens isolated from ETA and non-bronchoscopic BAL specimen cultures were summarized in Table V. They were mostly consistent with nosocomial pathogenes which were described as ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter species*) and constitute more than 80% of ventilator-associated pneumonia (VAP) episodes[14].

There was no positive correlation between culture results and underlying diseases ($p=0.875$). Also no correlation was found between bed head elevation groups ($p=0.327$) and type of secretions ($p=0.777$).

Colony counts in positive cultures ranged between 10^3–10^5 cfu/ml for ETA and 10^2-10^5 cfu/ml for non-bronchoscopic BAL. Twenty-nine percent of specimen cultures drawn with ETA had a colony count of lower than threshold value of 10^5 cfu/ml. Most common complication noticed during procedures in 11 (27%) patients was transient desaturation which did not require additional support. Only in one patient, left upper lobe atelectasis developed and was assumed related to non-bronchoscopic BAL procedure or transposition of mucous secretions during lavage. But it was recovered in hours with positional change and increasing ventilatory support for a short interval.

Discussion

To date endotracheal aspiration is the most commonly used diagnostic procedure in our unit to show the pathogen microorganism responsible for VAP. ETA is easy to perform but it is usually been thought that diagnosis with ETA reads VAP rate in the unit higher than we expected. Especially in the absence of pulmonary symptoms, recurrent presentation of nosocomial pathogens in upper airways misguides and causes overtreatment. Therefore accurate diagnosis in VAP still continues to be a difficult problem. VAP can be diagnosed from lung tissue, pleural fluid, and blood culture besides tracheobronchial secretions. But lung biopsy in children is an extremely invasive procedure and even bronchoscopic BAL is recommended by Centers for Disease Control and Prevention (CDC) it is only performed in selected patients. However it is shown that non-bronchoscopic BAL; a noninvasive diagnostic method is comparable with invasive methods[9,15-17]. In this study we aimed to show the applicability of non-bronchoscopic BAL in children and compare the results of ETA with this method.

Patients were included to the study if VAP was suspected according to the CDC criteria. Selection was based on the decision of the clinician who was responsible for the patient. Although CPIS's were calculated for every patient and found mostly to be ≥ 6 points (n=32), there was no positive correlation between the groups. As there were no gold standard methods used in our study, the diagnostic value of CPIS could not be evaluated. There was also no positive correlation between underlying diseases and culture positivity or negativity with both techniques ($p=0.875$). But the presence of positive results with both methods in cystic fibrosis patients revealed that the lower airways can also be colonized with respiratory pathogens and it brings the idea that non-bronchoscopic BAL has limited diagnostic value in those patients. Also, even though there was no statistically significant difference between periods of time to diagnostic procedures in PICU, patients with positive results with both methods have prolonged hospitalization interval before intubation. This observation can also be explained with colonization of lower respiratory tract with

Table V. Pathogens Isolated From Endotracheal Aspiration and Non-Bronchoscopic BAL Specimen Cultures

Pathogen	Endotracheal aspiration	Non-bronchoscopic BAL
Escherichia coli	2	1
Pseudomonas aeruginosa	5	5
Klebsiella pneumoniae	3	1
Acinetobacter spp.	3	1
Staphylococcus aureus	3	-
Stenotrophomonas maltophilia	5	4
Streptococcus pneumoniae	1	-
Branhamella catarrhalis	1	1
Haemophilus influenza B	4	-

BAL: Bronchoalveolar lavage

nosocomial pathogens or immunosupression in prolonged hospitalization.

Pathogens isolated from ETA and non-bronchoscopic BAL specimen cultures were concordant if grown in cultures drawn with both methods. The most common causative agent was *Pseudomonas aeruginosa* in both groups. They were similar to reports of other studies which are called as ESKAPE group of pathogens[14].

All patients with possible and probable VAP were on empirical antibiotherapy according to recommendations of American Thoracic Society[18]. Third generation cephalosporins, extended spectrum antipseudomonal penicillines and aminoglycoside or glycopeptide combinations were most frequent choice of antibiotics. All therapies were modified and narrowed after culture results were obtained, but the information about these modifications was not enough to reveal a precise conclusion about the impact on antibiotic use ratios in the unit.

Although the non-bronchoscopic BAL procedure in children has no standardization like in adult population, we tried to use similar amounts of lavage fluid with previously studies to achieve the optimum aspiration volume. The amount of lavage fluid used to sample was usually reported as 25-30 ml in adults and the volume retrieval by aspiration ranged from at least 1 ml to 4-8 ml in different studies[19-21]. In pediatric population there is few data about the optimum amount of lavage fluid. Sachdev et al.[10] have reported that 3 ml of saline for babies weighing < 5 kg, 5 ml of saline for children weighing between 5 kg and 10 kg, 7.5 ml of saline for those weighing 11 kg to 20 kg, and 10 ml of saline for patients weighing > 20 kg was convenient for reproducibility of non-bronchoscopic BAL. In our study we devided the patients into two groups weighing <15 kg and > 15 kg. We used 5 and 10 ml respectively for non-bronchoscopic BAL. The aspiration volumes ranged between 1 to 4 ml for both groups which were adequate for specimen culture.

On the other hand establishing the applicability of non-bronchoscopic BAL was important as the reproducibility. The absence of any major complication during the study showed the safety of the technique which was an important outcome. Transient hypoxemia defined as desaturation ($SaO_2 \leq 90$ mmHg) was the most common reported side effect in similar studies[10,22,23] and seen in 11 of 41 of our patients. It was transient and did not require any additional support. Only atelactasis was seen in one patient shortly after non-bronchoscopic BAL procedure and it was assumed as related to the procedure or transposition of mucous secretions during lavage. It was recovered in hours with positional change and increasing ventilatory support for a short interval.

In this comparative study the most important result is the absence of any sample with positive non- bronchoscopic BAL culture result where ETA culture was found to be negative. This result can be interpreted as lower reproducibility of non-bronchoscopic BAL. But there are studies suggesting that non-bronchoscopic protected BAL is an easy, well tolerated and reproducible test in mechanically vantilated children[24]. It is more than likely that ETA samples are contaminated in case of negative non-bronchoscopic BAL and positive ETA results. In addition to these results the absence of a gold standard method and statistical nonsignificance of CPIS scores of the groups prevents the reach of a certain conclusion. But two diagnostic methods were found to be statistically different in chi-square tests when their positivity and negativity were compared ($p<0.001$).

The major limitation of this study is the absence of a gold standard method in use for diagnosis of VAP. Even though non-bronchoscopic BAL is not a gold standard method, it was only possible to compare these two methods based on the fact that non-bronchoscopic BAL has higher sensitivity and specificity when compared to bronchoscopic BAL. There are also studies which compare different diagnostic methods with each other. Sachdev et al.[10] have performed four diagnostic procedures (tracheal aspiration, blind bronchial sampling, non-bronchoscopic bronchoalveolar lavage, and bronchoscopic bronchoalveolar lavage) in the same sequence within 12 hours of clinical suspicion of ventilator-associated pneumonia in 30 patients. Sensitivity, specificity, positive and negative predictive values were compared with non-bronchoscopic BAL results with a

cut off 10^5 cfu/ml for ETA and 10^4 cfu/ml for non-bronchoscopic BAL. The bacterial density $>10^4$ cfu/ml in a bronchoscopic bronchoalveolar lavage sample was taken as reference standard. For non-bronchoscopic bronchial sampling at $>10^4$ cfu/ml cutoff, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 88%, 82%, 88%, 83%, and 87%, respectively . In the same study on calculating the operative indices for the ETA, the threshold value of 10^5 cfu/ml was found to be the most accurate, with a sensitivity of 84% and a specificity of 77%. On the other hand there are studies that compare ETA with other diagnostic procedures and reveal that use of ETA in treatment decisions would have led to needless antibiotic administration in 31% of VAP-negative patients at a cutoff of $>10^5$ cfu/ml and 42% at $>10^4$ cfu/ml. The use of ETA in VAP diagnosis is limited because of the rate of overdiagnosis[25]. Even though it was impossible to show statistically in this study that ETA causes overdiagnosis and overtreatment, the outcome that ETA has significant lower spesificity and positive predictive values as compared with non-bronchoscopic BAL can be speculated as the cause of higher rates of broad spectrum antibiotic use in the unit.

Another challenging result is that the twenty nine percent of specimen cultures had a colony count of lower than the threshold value of 10^5 cfu/ml. It can be hypothesized that this group of samples were contaminated because of lower colony counts. But even though the specimens were cultured for longer time periods (48-72 hours), antibiotic use seems to be effective on suppression of bacterial growth. In any case, culture results are guiding physicians to use or continue empirical antibiotherapy.

In conclusion, the study revealed that the ease of usability and the sensitivity of non-bronchoscopic BAL, in comparison with ETA.

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