Quantitative Culture of Endotracheal Aspirates in the Diagnosis of Ventilator-Associated Pneumonia in Patients With Treatment Failure*

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Study objective: To study the correlation of bacteriology between quantitative cultures of protected specimen brush (PSB), BAL, and quantitative endotracheal aspirate (QEA) in ventilator-associated pneumonia (VAP) patients with treatment failure.

Design: Prospective observational clinical study.

Setting: A 15-bed medical ICU of tertiary medical center.

Patients: Forty-eight patients receiving mechanical ventilation with clinical suspected VAP who had been treated with antibiotics for at least 72 h without improvement.

Intervention: QEA, PSB, and BAL were performed with patients receiving antibiotics. The diagnostic thresholds for QEA, PSB, and BAL were 10^5, 10^3, and 10^4 cfu/mL, respectively.

Measurements and results: Microbial culture findings were positive in 24 BAL samples (50%), in 23 PSB samples (48%), and in 28 QEA samples (58%). The correlations between of QEA vs PSB and QEA vs BAL were significant (\( r = 0.567 \) and \( r = 0.620, p < 0.01 \), respectively). The most commonly isolated microorganisms were Acinetobacter baumannii (27%), Staphylococcus aureus (24%), Stenotrophomonas maltophilia (15%), and Pseudomonas aeruginosa (10%). Using the predetermined criteria, bacterial pneumonia was diagnosed in 28 of 48 suspected VAP episodes based on PSB and/or BAL results. The diagnostic efficiency of QEA at threshold of 10^5 cfu/mL had a sensitivity of 92.8% and a specificity of 80%.

Conclusions: QEA correlated with PSB and BAL in patients with suspected VAP who responded poorly to the existent antibiotic treatment. QEA missed only two cases of bacterial pneumonia diagnosed by invasive PSB and/or BAL with acceptable sensitivity and specificity. More importantly, QEA is noninvasive and easily repeatable. Early use of QEA is helpful to clinical physicians in decision making with regard to antibiotics use.

CHEST 2002; 122:662–668

Key words: BAL; diagnosis; protected specimen brush; quantitative endotracheal aspirate; treatment failure; ventilator-associated pneumonia

Abbreviations: PSB = protected specimen brush; QEA = quantitative endotracheal aspirate; VAP = ventilator-associated pneumonia

The etiologic diagnosis of ventilator-associated pneumonia (VAP) is critical to the appropriate antibiotic therapy. Over the past 2 decades, the most extensively investigated diagnostic techniques have been quantitative cultures of bronchosopic protected specimen brush (PSB) and BAL.1–3 The diagnostic specificity of these invasive techniques has been shown to be 80 to 90%, compared with direct culture of lung tissue4,5; however, the value of invasive methods to improve outcome of patients with VAP is still controversial. Heyland et al6 and Fagon et al7 showed that the invasive bronchoscopic techniques led to better outcome and antibiotic consumption compared with noninvasive strategy. By contrast, Luna et al8 reported a high mortality rate of 60 to 91% in patients with clinical suspicion of VAP regardless of whether BAL cultures guided antibiotic treatment. Ruiz et al9 also reported that...
the outcome of VAP was not influenced by the techniques used for microbial investigation. It was only the adequate initial empiric antibiotics that affected patient outcome. Several studies demonstrated similar results.

Because of the controversies regarding bronchoscopic quantitative cultures as well as its inherent cost and risk, there has been a reappraisal of noninvasive cultures of quantitative endotracheal aspirate (QEA) in the management of VAP. Given its low cost and ease to perform, QEA seems attractive. However, it has limitations. The diagnostic threshold of recovered bacteria and the influence of antibiotic therapy on its diagnostic yield have not been established. Earlier studies produced conflicting conclusions. This is at least in part because they only compared QEA with PSB, since this would have made QEA less specific. Besides, different diagnostic thresholds had been used. In the absence of direct culture of lung tissue, which is usually not feasible clinically, we believed that combined PSB and BAL cultures would be more accurate in diagnosing VAP than either technique alone, and comparing QEA with both cultures would be more appropriate. The aim of our study, therefore, was to compare noninvasive QEA with both PSB and BAL in patients receiving mechanical ventilation who had unsuccessful treatment with systemic antibiotics. We assessed the agreement of microbial cultures between these two diagnostic methods, and tried to reevaluate the diagnostic value of QEA in this group of patients.

Materials and Methods

This study was conducted between December 1997 and June 1999 in the medical ICU of Mackay Memorial Hospital, a 15-bed ICU for adult patients from multiple disciplines. During the study period, a total of 596 patients were admitted to the medical ICU. Patients who had received mechanical ventilation for > 72 h and received antibiotic therapy for > 72 h for either pulmonary or extrapulmonary conditions before development of a new and/or progressive radiographic infiltrates were included. Patients also had to have the following: temperature > 38°C or < 35°C, leukocyte count of > 12,000/μL or < 4,000/μL or band form > 10%, and purulent tracheobronchial secretions. Patients with AIDS or receiving cancer chemotherapy were excluded. No modification or discontinuation of antibiotics was attempted before the study until completion of specimen collection. The study was approved by the hospital research committee, and informed consent was obtained from the patients’ next of kin.

Study Protocol

The following protocol was performed in the same sequence in all cases. First, endotracheal aspiration was performed with sterile technique using a 22-inch, 12F suction catheter with a mucus collection tube (Mucus Extractor; Syphon Chemical; Tamsui, Taiwan). The catheter was introduced through the endotracheal tube for at least 30 cm. Gentle aspiration was performed without instilling saline solution. The first aspirate was discarded, and the second aspirate was collected for evaluation. Chest vibration or percussion for 10 min was used to increase the retrieved volume (≥ 1 mL) in case that the patient produced very little secretions. Patients with tracheal aspirates < 1 mL were excluded from the study. Then, after adequate sedation with IV midazolam and/or curarization with pancuronium, and adjusting ventilator settings to a fraction of inspired oxygen of 100% with proper rate and tidal volume, a bronchoscope (Olympus BF30; Olympus; Tokyo, Japan) was passed through the endotracheal tube via a specific adaptor without topical anesthesia. No endobronchial suction was attempted during the advance of the bronchoscope. PSB (Microbiology Brush, product No.130; Mill-Rose Laboratories; Mentor, OH) sampling was obtained from the orifice of a lung segment with the most radiographic abnormality or new infiltrates. Following PSB sampling, the bronchoscope was then introduced again and wedged into the same segmental bronchial orifice where PSB was conducted. Seven aliquots of 20 mL of sterile saline solution were instilled and aspirated gently. The first two aliquots were discarded, and the last five aliquots were pooled for analysis. All the retrieved specimens (QEA, PSB, and BAL) were sent to the microbiology laboratory immediately after collection.

Microbiological Processing

Endotracheal aspirate and BAL samples were mechanically liquefied and homogenized by vortexing for 1 min with glass beads, followed by centrifuging at 3,000 revolutions per minute for 10 min. PSB samples were aseptically cut and placed in a sterile tube containing 1 mL of 0.9% saline solution and vortexed for 1 min. All three types of specimens were serially diluted in sterile 0.9% saline solution with final concentrations of 10⁴, 10³, and 10² for QEA; 10⁵, 10⁴, and 10³ for PSB; and 10⁶, 10⁵, and 10⁴ for BAL. The specimens were then plated on sheep blood agar, chocolate, and MacConkey agar. All plates were incubated overnight in a 5% carbon dioxide incubator at 37°C. Isolates were characterized by colony morphology and Gram stain.

Statistical Analysis

The results were expressed as mean ± SD. Correlations between quantitative cultures were calculated by linear regression analysis and tested by Spearman test using software (SPSS version 8.0 for Windows; SPSS, Chicago, IL). The diagnostic thresholds for PSB and BAL were 10⁵ cfu/mL and 10⁶ cfu/mL, respectively. Thus, pneumonia was established by positive culture finding of either PSB or BAL. For QEA, the diagnostic thresholds were studied at 10⁵ cfu/mL. Sensitivity, specificity, predictive values, and diagnostic accuracy of QEA were calculated based on individual cases.

Results

From December 1997 to June 1999, 48 patients were prospectively studied. The study group consisted of 26 men and 22 women with a mean age of 65.3 ± 15.2 years (range, 33 to 82 years) and mean admission APACHE (acute physiology and chronic health evaluation) score of 21 ± 6 (range, 16 to 30). The primary underlying diseases for admission to the ICU were COPD with acute exacerbation (n = 6,
12%), cardiogenic emergency (n = 5, 10%), sepsis with acute respiratory failure (n = 10, 21%), pneumonia (n = 13, 27%), renal failure (n = 5, 10%), drug overdose (n = 3, 6%), neurologic emergency (n = 3, 6%), and other (n = 3, 6%). Mean duration of mechanical support before enrollment was 5.1 ± 3.7 days (range, 3 to 10 days). All of the patients received antibiotic therapy before the investigation. The mean duration of antibiotic therapy was 6.5 ± 1.2 days (range, 3 to 9 days; Table 1). The administration of antibiotics was based on the judgment of the attending physicians, and modification of antibiotics was not attempted until diagnostic evaluation.

Results of Quantitative Cultures

A total of 41 microorganisms were isolated at concentrations above the diagnostic thresholds by either of these techniques. The most frequently isolated bacteria were Acinetobacter baumannii (n = 11, 27%), Staphylococcus aureus (n = 10, 24%), Stenotrophomonas maltophilia (n = 6, 15%), and Pseudomonas aeruginosa (n = 4, 10%). The isolated microorganisms from these techniques were listed in Table 2. Comparisons of isolated microorganisms between QEA and BAL and between QEA and PSB are shown in Figure 1 and Figure 2, respectively. There was a significant correlation between the quantitative cultures of microorganisms obtained from endotracheal aspirate and PSB (p = 0.567, p < 0.01), as well as from endotracheal aspirate and BAL (p = 0.620, p < 0.01).

Diagnostic Value of QEA

In this study, pneumonia was diagnosed based on PSB culture findings ≥ 10^4 cfu/mL and/or BAL culture findings ≥ 10^4 cfu/mL. According to the diagnostic criteria, pneumonia was present in 26 of 48 cases (54.2%). There was a total agreement of bacterial cultures between PSB and BAL in 43 cases (21 patients with positive results of both cultures and 22 patients with both negative culture findings). Five cases had discrepancy, which included three BAL-positive/PSB-negative cases and two BAL-negative/PSB-positive cases. QEA coincided with both PSB and BAL in 37 cases (19 positive and 18 negative culture findings). Partial agreement was noted in five cases (QEA positive with either PSB or BAL positive) and disagreement in six cases (QEA positive with negative PSB/BAL in four cases, and QEA negative with positive PSB/BAL in two cases).

We then analyzed diagnostic efficiency of QEA at the threshold of 10^5 cfu/mL by various diagnostic criteria of pneumonia (Table 3). If only PSB was taken as reference culture for VAP, the sensitivity and specificity of QEA were 91.3% and 72%, respectively. When BAL was the reference culture, the values were 91.7% and 75%, respectively. If pneumonia was defined by either of positive PSB and BAL findings, the sensitivity was 92.8%, specificity was 80%, positive predictive value was 86.7%, and negative predictive value was 88.9%. For QEA threshold at 10^6 cfu/mL, the specificity was increased to 95.4%, but the sensitivity markedly decreased to 44.4%.

Discussion

The results of this study showed that the quantitative cultures of endotracheal aspirates were comparable to those using invasive bronchoscopic methods in patients already receiving antibiotics. There was a total agreement in bacteriology between QEA and combined PSB/BAL on 37 patients (19 patients with positive culture findings and 18 patients with

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>QEA</th>
<th>BAL</th>
<th>PSB</th>
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<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>11</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S aureus</td>
<td>9</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>P aeruginosa</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chryseobacterium species</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>2</td>
<td>0</td>
<td>1</td>
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</table>

*Data are presented as mean ± SD (range) or No. (%) unless otherwise indicated.
negative culture findings). QEA at the cutoff point of $10^5$ cfu/mL can detect pneumonia with a sensitivity of 92.8%, a specificity of 80%, and a negative predictive value of 88.9%. When only PSB was taken as reference, the specificity of QEA was 72%. The specificity of QEA increased to 80% when combined PSB and BAL were used as diagnostic criteria. The high negative predictive value would have allowed clinicians to withhold antibiotics more confidently and shifted attention to possible extrapulmonary origin of infection. However, QEA in this study had a specificity of 80%, suggesting that if antibiotics were to be selected based on only QEA result, approximately 10% of the patients would have been unnecessarily exposed to more antibiotics. On further analysis of these four cases of positive QEA findings with negative PSB/BAL findings, we found that one case had PSB at $10^2$ to $10^3$ cfu/mL and three cases had BAL at $10^3$ to $10^4$ cfu/mL. We cannot exclude the possibility of existing lung infection, because no lung tissue cultures were performed in this study. However, given that more and more resistant microbials emerge due to inappropriate use of antibiotics and its association with increasing mortality, the potential risk of overtreatment based on only QEA culture cannot be overemphasized, although this overtreatment with antibiotics appeared relatively low in this study. In this study, we also found the same bacteria isolated by these three techniques showed a similar drug-sensitivity pattern. This further strengthened the usefulness of QEA in term of antibiotic selection.

El-Ebiary et al. first reported that QEA using $10^5$ cfu/mL as a threshold was a relatively sensitive (70%) and relatively specific (72%) method to diagnose VAP. Although QEA was less specific than PSB and BAL, its negative predictive value was higher than PSB (72% vs 34%, respectively). In a later study, Jourdain et al. found that with a cutoff threshold of $10^6$ cfu/mL, QEA had a diagnostic sensitivity of 68% and a specificity of 84% for VAP compared with PSB, but there was only a 40% agreement between the isolated microorganisms by the two techniques. Marquette and colleagues also reported that using a cutoff point of $10^6$ cfu/mL, QEA had a high level of agreement (84%) with PSB. Sanchez-Nieto and colleagues found a 71% agreement between QEA, PSB, and BAL culture techniques. If the PSB and BAL were combined together, the agreement between invasive and noninvasive methods was increased to as high as 92%. Their findings were very similar to those of this study. The discrepancies in

![Figure 1. Correlation of quantitative cultures between QEA and BAL specimens ($\rho = 0.620, p < 0.01$). Each point represents a single microorganism ($n = 58$).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21981/)
these published reports are not really elucidated. The possible explanations might be different patient populations, different cut-off thresholds used, the portion of patients receiving antibiotics, less standardized performing techniques of QEA culture and absence of “gold standard” reference for VAP.

The diagnostic threshold for PSB and BAL have been well studied by comparison with quantitative culture of lung tissue.2,19 The threshold, however, has not been standardized for QEA. Jourdain et al18 demonstrated that different cutoff diagnostic levels of QEA had different sensitivity and specificity results. The sensitivity and specificity were 90% and 26%, respectively, for the threshold of $10^3$ cfu/mL; and 21% and 92%, respectively, for $10^7$ cfu/mL. They found the threshold of $10^6$ cfu/mL gave the greatest accuracy in diagnosis with a sensitivity of 68%, but the specificity decreased to 66%. However, a review of past studies13,17,18 showed investigations were performed using different cut-off points ($10^5$ cfu/mL or $10^6$ cfu/mL), and even the same group changed the diagnostic threshold in their studies13,20 at different times. Our study showed QEA at $10^5$ cfu/mL gave a sensitivity of 92.8% and a specificity of 80%. One major difference between our study and those of Jourdain et al18 and Marquette et al13 was that both PSB and BAL were used in our study, while Jourdain et al18 used only PSB culture for comparison. It had been shown that negative PSB culture findings with positive BAL culture findings and vice versa could occur.4 Actually, five cases of disagreement between PSB and BAL were noted in our study. The causes of false-negative findings could be incorrect brush placement, inadequate brushing volume, poor BAL fluid return, and prior antibiotic use. PSB and BAL may be supplemental to each other in diagnosing VAP. Therefore, combined PSB with BAL as reference will undoubtedly increase the diagnostic specificity of QEA. This was confirmed in this study.

Another important point to discuss is that only

![Figure 2](image_url)

**Figure 2.** Correlation of quantitative cultures between QEA and PSB specimens ($\rho = 0.507$, $p < 0.01$). Each point represents a single microorganism ($n = 58$).

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### Table 3—Diagnostic Accuracy of QEA According to Different Diagnosis Criteria

<table>
<thead>
<tr>
<th>Diagnostic Reference</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSB only</td>
<td>91.3</td>
<td>72.0</td>
<td>75.0</td>
<td>90.0</td>
</tr>
<tr>
<td>BAL only</td>
<td>91.7</td>
<td>75.0</td>
<td>78.6</td>
<td>90.0</td>
</tr>
<tr>
<td>PSB and/or BAL</td>
<td>92.8</td>
<td>80.0</td>
<td>86.7</td>
<td>88.9</td>
</tr>
</tbody>
</table>
patients receiving no antibiotics or withholding antibiotics were included in studies of Jourdain et al\textsuperscript{18} and Marquette et al,\textsuperscript{13} while our investigation as well as that of El-Ebiary et al\textsuperscript{17} studied patients who received prior antibiotics at the time of investigation. Prior antibiotic therapy has been an issue of debate in quantitative culture of lower airway samples.\textsuperscript{21–24} It has been shown that following antibiotic use, cultures of lower airway samplings are less sensitive than they are in the absence of antibiotics; therefore, lowering threshold values may be more appropriate in patients receiving antibiotic therapy who are suspected of having VAP.\textsuperscript{21,22} Some investigators\textsuperscript{25} recommended withholding antibiotics for > 48 h before performing quantitative cultures because of concern for potential false-negativity related to antibiotic use. Thus, this difference of using different cutoff points (10\textsuperscript{5} cfu/mL vs 10\textsuperscript{6} cfu/mL) between our study and that of Jourdain et al\textsuperscript{18} and Marquette et al\textsuperscript{13} was probably explained, at least in part, by the presence of antibiotic therapy. In fact, we were unable to prove the impact of antibiotic therapy on the diagnostic yield of quantitative cultures in our study. When infection develops as a superinfection or drug resistance, lower airway cultures are able to detect causative microorganisms responsible for VAP.\textsuperscript{19,21} Given that our patients all had worsening or unresolved lung infiltrates despite current antibiotic therapy, it suggested infection with resistant microorganisms, if present. This could account for the high agreement between QEA and PSB/BAL results in our study. Besides, withholding antibiotics in critically ill patients with clinically suspected VAP may expose them to risk with unfavorable consequences. This, however, cannot be extrapolated to suggest that antibiotic modification does not influence the sensitivity of quantitative cultures, since we always modified antibiotics only after the quantitative culture procedures. We are still concerned that the newly added antibiotics might affect the growth of microorganisms if they happened to be sensitive to these drugs.

There are several limitations in our study that merit consideration. First, we did not use lung tissue cultures, either needle lung aspirate or postmortem lung culture, as reference cultures. Therefore, we cannot definitely exclude that PSB and BAL might miss microorganisms present in the lung that caused worsening or progressive lung infiltrates in our patients. This may lead to incorrect results in regard to the diagnostic sensitivity and specificity of QEA. However, the aim of our study was to investigate the correlation between QEA and PSB/BAL. Our findings showed a high level of agreement between these two techniques. Second, a small sample size, 48 patients, was studied. Although our patients represent the population of a medical ICU, a further study of larger sample size is warranted. Third, all the patients had been receiving systemic antibiotic therapy; this could have influenced the sensitivity and specificity of the cultures. We chose not to withhold antibiotics in our study because of concern over the potential risk associated with discontinuing antibiotic treatment in these critically ill patients. Our findings have shown that QEA still has comparable results with invasive PSB and BAL even in such circumstances.

In conclusion, our study demonstrates that QEA at a cutoff point of 10\textsuperscript{5} cfu/mL is a practical diagnostic method in clinical suspected VAP. It can be easily and repeatedly performed to help clinicians in decision making regarding antibiotic use. Given its high negative predictive value, early use of QEA is warranted. Physicians may focus their attention to extrapulmonary origin of infection in the presence of negative QEA findings. Invasive diagnostic methods of PSB and BAL might follow QEA if the latter is positive to decrease the risk of unnecessary antibiotic therapy. Such a stepwise approach could be more cost-effective.

\textbf{References}

1. Chastre J, Fagon JY. Invasive diagnostic testing should be routinely used to manage ventilated patients with suspected pneumonia. Am J Respir Crit Care Med 1994; 150:570–574