Comparison of Semi-Quantitative Endotracheal Aspirates to Quantitative Non-Bronchoscopic Bronchoalveolar Lavage in Diagnosing Ventilator-Associated Pneumonia

Shigeki Fujitani MD, Mark H Cohen-Melamed RRT, Raymond P Tuttle RRT, Edgar Delgado RRT, Yasuhiko Taira MD, and Joseph M Darby MD

BACKGROUND: Current strategies for diagnosing ventilator-associated pneumonia (VAP) favor the use of quantitative methods; however, semi-quantitative cultures of endotracheal aspirates are still commonly used. METHODS: The microbiological results of patients with suspected VAP who had both quantitative cultures with non-bronchoscopic bronchoalveolar lavage (BAL) and semi-quantitative cultures of endotracheal aspirate obtained within 24 hours of each other were retrospectively reviewed and compared, using a quantitative threshold of $\geq 10^4$ colony-forming units/mL as a reference standard. RESULTS: 256 patients with paired cultures were identified. Concordance between endotracheal aspirate (any growth of pathogens) and non-bronchoscopic BAL was complete in 58.2% and completely discordant in 23.8%. The sensitivity and specificity of endotracheal aspirate were 65.4% and 56.1%, which improved to 81.2% and 61.9% when antibiotic management decisions were considered in the analysis. Twenty-six patients had endotracheal aspirate cultures that were falsely negative for pathogens, with 61.5% of these patients demonstrating growth of non-fermenting Gram-negative rods or methicillin-resistant *Staphylococcus aureus* (MRSA) on non-bronchoscopic BAL. Overall, 45 patients (17.5%) among the entire cohort had false positive endotracheal aspirate cultures, with 19 of these patients (42.2%) demonstrating growth of non-fermenting Gram-negative rods or MRSA. CONCLUSIONS: Semi-quantitative cultures of endotracheal aspirate are poorly concordant with quantitative cultures obtained via non-bronchoscopic BAL. Although the performance of endotracheal aspirate improves when antibiotic treatment is considered, guiding therapy on the basis of semi-quantitative cultures may still result in failure to identify potentially multiple-drug-resistant pathogens, and would also tend to promote excessive antibiotic usage. Our data support the use of quantitative cultures in diagnosing VAP. *Key words: ventilator-associated pneumonia, endotracheal aspirate, non-bronchoscopic bronchoalveolar lavage, quantitative culture, semi-quantitative culture*. [Respir Care 2009;54(11):1453–1461.

Introduction

Inappropriate or inadequate antimicrobial therapy for ventilator-associated pneumonia (VAP) is associated with increased mortality[1-4] and the emergence of multiple-drug resistance,[5] which emphasizes the importance of a microbiologically based approach in the treatment of VAP. Current guidelines for the management of VAP emphasize the principles of early, epidemiologically based, broad-spec-

SEE THE RELATED EDITORIAL ON PAGE 1446

Shigeki Fujitani MD is affiliated with the Department of Critical Care, University of Pittsburgh, Pittsburgh, Pennsylvania, and with the Department of Emergency and Critical Care Medicine, St Marianna University School of Medicine, Kanagawa, Japan. Mark H Cohen-Melamed RRT, Raymond P Tuttle RRT, and Edgar Delgado RRT are affiliated with the Department of Respiratory Care, University of Pittsburgh Medical Center—Presbyterian University Hospital, Pittsburgh, Pennsylvania. Yasuhiko Taira MD is affiliated with the Department of Emergency and Critical Care Medicine, St Marianna University School of Medicine, Kawasaki City, Kanagawa, Japan. Joseph M Darby MD is affiliated with the Department of Critical Care, University of Pittsburgh, Pittsburgh, Pennsylvania.

trum antibiotics, microbiologically guided de-escalation of therapy, and the shortest possible course of effective antimicrobial treatment.[6] Adoption and widespread implementation of these guidelines should lead to improvements in clinical outcomes, antimicrobial utilization, and reductions in the frequency of multiple-drug-resistant (MDR) bacteria. Indeed, several studies have proved that this approach is effective.[7] The tailoring and de-escalation of antibiotic therapy to achieve these goals, however, is predicated in large part on cultures that accurately represent the causative pathogens in the lower respiratory tract, especially those that are MDR.

Despite the potential value of quantitative cultures of the lower respiratory tract in the management of VAP and the lack of validated diagnostic standards, qualitative or semi-quantitative cultures of endotracheal aspirates are still widely employed.[8-10] In the absence of anti-microbial therapy, endotracheal aspirate has good negative predictive value,[11] but it is commonly contaminated, and specificity is reduced because patients are frequently receiving antibiotics prior to suspected VAP.[9,10,12-14] Furthermore, endotracheal aspirate has poor concordance with cultures from open lung biopsy[15] and bronchoalveolar lavage (BAL),[16] and thus may add little more to the diagnostic assessment of VAP than a clinical diagnosis alone.[17]

Although a microbiological diagnosis is necessary, there is no consensus about the different microbiological tools for diagnosing VAP. Fagon et al reported in their multicenter randomized, uncontrolled trial ($n = 413$) that an invasive management strategy was associated with fewer deaths at 14 days, earlier attenuation of organ dysfunction, and less antibiotic use in patients suspected of having VAP.[18] Blinded, non-bronchoscopic BAL has been increasingly used in intensive care units (ICUs), and many studies have demonstrated that non-bronchoscopic BAL was an equivalent diagnostic method, compared to bronchoscopic BAL. Kollef et al studied the safety and efficacy of non-bronchoscopic BAL, and in this study non-bronchoscopic BAL was safely performed by respiratory therapists; good concordance of quantitative cultures of lower respiratory tract was seen between protected specimen brush and non-bronchoscopic BAL.[19] Other benefits of non-bronchoscopic BAL are decreased cost, time savings, and easy access, compared to bronchoscopic procedures.[19] Based on these evidences, non-bronchoscopic BAL has been the diagnostic tool of preference in our institution.

The authors have disclosed no conflicts of interest.

Correspondence: Shigeki Fujitani MD, Department of Emergency and Critical Care Medicine, St Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki City, Kanagawa, Japan 216-8511. E-mail: shigekifujitani@hotmail.com.

Because studies directly comparing the culture results of endotracheal aspirate to quantitative cultures of the lower respiratory tract are limited, and, in an ongoing effort to evaluate the safety and efficacy of non-bronchoscopic BAL as our preferred diagnostic tool, the aim of our investigation was to compare semi-quantitative cultures of endotracheal aspirate to quantitative cultures of the lower respiratory tract using non-bronchoscopic BAL in patients suspected of VAP. This investigation was reviewed and approved as a quality-improvement project. The institutional review board at the University of Pittsburgh Medical Center has determined that quality-improvement projects must be reviewed and approved by the University of Pittsburgh Medical Center's Total Quality Council. Projects so approved are, by our institution's definitions and processes, quality-improvement projects, outside of the institutional review board research process.

Methods

Data for this retrospective review were obtained from patients admitted to medical and surgical ICUs at the University of Pittsburgh Medical Center, a large tertiary referral center. Non-bronchoscopic BAL was performed using a commercially available kit (BAL-Cath, Kimberly-Clark Health Care, Roswell, Georgia).[20] All respiratory therapists at our institution were trained to perform the non-bronchoscopic procedure. Training occurred at the Winter Institute for Simulation Education and Research, a medical simulation and education center affiliated with our health system. The simulation center incorporates Web-based curriculum, simulation-based training, videotaped performance, data collection, and post-scenario debriefing at its core. Training focused on proper procedure and individual psychomotor skills, both of which can impact consistency in sample quality and patient safety.[21]

Sample Collection and Processing

The BAL-Cath telescoping catheter is passed through the endotracheal or tracheostomy tube, using an accompanying prepackaged access-port adapter. With the curved tip of the catheter directed toward the desired lung, the outer catheter is advanced into the left or right main bronchus. The inner catheter is then advanced into a "wedged" position, and sterile, physiologic saline solution is injected through the catheter and then re-aspirated. Saline lavage was performed with a typical volume of 100 mL, and the returned lavage fluid was collected in a sterile trap and sent immediately to the microbiology laboratory for quantitative culture. Endotracheal aspirate cultures were collected in a sterile sputum trap and then sent to the microbiology laboratory for semi-quantitative cultures. Endotracheal aspirate samples were streaked onto standard

Table 1. Semi-Quantitative Reporting of Microbial Growth in Cultures Perfomed in Petri Dishes

Report	Number of Colonies in Each Sector		
	1st	2nd	3rd
Rare	< 10	0	0
Light	≥ 10	< 5	0
Moderate	≥ 10	≥ 5	< 5
Heavy	≥ 10	≥ 5	≥ 5

culture media in 3 sectors consecutively. The microbiological growth of endotracheal aspirate was classified as rare, light, moderate, or heavy, based on the number of colonies in each of 3 sectors (Table 1).

Data Collection

Microbiological data were extracted from a search of the hospital's microbiology laboratory database. From April 1, 2002, to June 30, 2004, 2,000 lower-respiratory-tract samples obtained using non-bronchoscopic BAL were submitted from patients with suspected VAP. From these samples, patients with mechanical ventilation ≥ 48 hours were identified who had cultures of both endotracheal aspirate and non-bronchoscopic BAL obtained within 24 hours of each other. Data collected included demographics, duration of mechanical ventilation, ICU and hospital stay, Acute Physiology and Chronic Health Evaluation (APACHE III) score on the day that non-bronchoscopic BAL was performed, time differential between non-bronchoscopic BAL and endotracheal aspirate, antibiotic usage, and the bacterial isolates identified in culture.

Definitions

We defined our cases as having microbiological evidence of VAP if non-bronchoscopic BAL samples grew one or more respiratory pathogens at a diagnostic threshold of $\geq 10^4$ colony-forming units/mL. Paired samples were specifically evaluated by one of the authors (JMD) to determine whether antibiotic management would be altered if non-bronchoscopic BAL were used as the microbiological reference standard instead of endotracheal aspirate. In making these judgments it was assumed that broad-spectrum antimicrobial therapy sufficient to cover MDR pathogens was started empirically at the time endotracheal aspirate was obtained, and that subsequent modifications of that regimen would be made on the basis of those microbiological results. The performance of endotracheal aspirate as a diagnostic test was evaluated both microbiologically and clinically, as judged by the potential impact of endotracheal aspirate on antibiotic management, and then classified accordingly.

True Positive. Pathogens growing from endotracheal aspirate were identical to those obtained from non-bronchoscopic BAL, or pathogens isolated from endotracheal aspirate samples were disparate from non-bronchoscopic BAL, but the disparities were judged to have had no implications for antibiotic management.

True Negative. Paired samples with no growth from endotracheal aspirate or non-bronchoscopic BAL.

False Positive. Growth of pathogens was present from endotracheal aspirate, but the paired non-bronchoscopic BAL culture showed no growth at or above the defined growth threshold, or when endotracheal aspirate and non-bronchoscopic BAL both grew pathogens but the design of an antibiotic regimen based on the results of the endotracheal aspirate would result in excessive or overly broad antibiotic administration.

False Negative. Endotracheal aspirate cultures were those that showed either no growth in association with a positive non-bronchoscopic BAL, growth of some but not all pathogens identified on non-bronchoscopic BAL, or those that grew isolates that were completely disparate from those identified in non-bronchoscopic BAL. Endotracheal aspirate cultures were also considered to be false negative if the selection of an antibiotic regimen based on the results of the endotracheal aspirate would probably result in inadequate antibiotic coverage.

Endotracheal Aspirate. The threshold of rare/light/medium/heavy was defined as the endotracheal aspirate cultures that were either rare, light, medium, or heavy. The threshold of light/medium/heavy was defined as the endotracheal aspirate cultures that were either light, medium, or heavy. The threshold of medium/heavy was defined as the endotracheal aspirate cultures that were either medium or heavy. The threshold of heavy was defined that the endotracheal aspirate cultures were only heavy.

Analysis

Concordance between culture methods was expressed as a percentage and was determined by comparing the results of endotracheal aspirate to non-bronchoscopic BAL at a growth threshold of $\geq 10^4$ colony-forming units/mL. The results were considered to be completely concordant if both culture methods yielded either no growth or had identical growth of pathogens. The growth of pathogens by non-bronchoscopic BAL below the cutoff value of $\geq 10^4$ colony-forming units/mL was considered as no growth. The results were considered to be completely discordant when growth of pathogens occurred via one method and not by the other, or when pathogens grew via both meth-

Table 2. Clinical Characteristics of Study Population

Number of patients	256
Male (*n*, %)	173 (67.6)
Age (median, IQR)	57.5 (45.8–70)
Surgical ICU (*n*, %)	218 (85.2)
APACHE III at time of non-bronchoscopic BAL (median, IQR)	58 (43–76)
Antibiotics within 24 h of non-bronchoscopic BAL (*n*, %)	224 (87.5)
Interval from initiation of mechanical ventilation to non-bronchoscopic BAL (median, IQR d)	7 (4–17)
Interval between non-bronchoscopic BAL and endotracheal aspirate (median, IQR h)	12 (11–15)
Endotracheal aspirate prior to non-bronchoscopic BAL (*n*, %)	203 (79.3)
Microbiological criteria for VAP (*n*, %)	147 (57.4)
Proportion of late-onset VAP (≥ 5 d on mechanical ventilation) (*n*, %)	97 (66.0)
Duration of mechanical ventilation (median, IQR d)	20.5 (12–35.3)
ICU stay (median, IQR d)	17 (11–26)
ICU mortality (*n*, %)	41 (16.0)
Total hospital mortality (*n*, %)	90 (35.2)

IQR = interquartile range
ICU = intensive care unit
APACHE = Acute Physiology and Chronic Health Evaluation
BAL = bronchoalveolar lavage
VAP = ventilator-associated pneumonia

ods but the isolates identified were disparate. The microbiological results were considered to be incompletely concordant when endotracheal aspirate grew either more or fewer discrete pathogens than were isolated in non-bronchoscopic BAL. Statistical analysis was performed using a statistical software program (SPSS 10.0, SPSS. Chicago, Illinois). Continuous variables were expressed as median ± interquartile range unless otherwise indicated. The 95% confidence intervals were calculated for continuous variables. Agreement between the quantitative microbiological results obtained with non-bronchoscopic BAL and those obtained with endotracheal aspirate was assessed via the kappa statistic test.[22]

Results

The clinical characteristics of 256 patients with paired culture samples are shown in Table 2. The vast majority of the patients identified (85.2%) were admitted to a surgical ICU. A total of 224 (87.5%) patients received antibiotic therapy within the 24-hour period preceding sampling via non-bronchoscopic BAL, with 204 (91.1%) of these patients receiving broad-spectrum coverage. Non-bronchoscopic BAL cultures were obtained a median of 7 days (interquartile range 4–17 d) after the initiation of mechanical ventilation. Cultures obtained with endotracheal aspirate were obtained prior to non-bronchoscopic BAL in 203 (79.3%) patients. Of the population studied, 147 (57.4%) patients met the microbiological criteria for VAP.

Pathogens isolated via non-bronchoscopic BAL and endotracheal aspirate at each growth threshold are shown in Table 3. The majority of pathogens (64.2%) isolated via the reference standard of non-bronchoscopic BAL were Gram-negative rods. Non-fermenting Gram-negative rods (*Pseudomonas aeruginosa*, *Acinetobacter* species, and *Stenotrophomonas* species) accounted for 38.1% (56/147) of the Gram-negative isolates, with *Pseudomonas aeruginosa* the most frequent isolate among Gram-negative rods, at 21.8% (32/147). Of the Gram-positive isolates, 70.7% (58/82) were *Staphylococcus aureus*, with 32.8% (19/52) of these isolates identified as methicillin-resistant *Staphylococcus aureus* (MRSA). There was no statistical difference in the frequency of isolates between non-bronchoscopic BAL versus endotracheal aspirate at a threshold of rare/light/medium/heavy. The overall microbiological yield dropped substantially once the growth threshold was raised from rare/light/medium/heavy (100%) to light/medium/heavy (90.0%), from light/medium/heavy (90.0%) to medium/heavy (52.2%), and from medium/heavy (52.2%) to heavy (31.9%), respectively.

Microbiological concordance between non-bronchoscopic BAL and endotracheal aspirate at each of the growth thresholds is shown in Table 4. Complete concordance at the growth thresholds of rare/light/medium/heavy, light/medium/heavy, medium/heavy, and heavy were 58.2%, 61.7%, 60.9%, and 56.6%, with each 95% confidence interval overlapped. In contrast, complete discordance between endotracheal aspirate and non-bronchoscopic BAL increased when the threshold of rare/light/medium/heavy in endotracheal aspirate was compared with the threshold of heavy (23.8% vs 35.9%), and the threshold of light/medium/heavy was compared with the threshold of heavy (21.5% vs 35.9%), respectively.

The diagnostic test performance of endotracheal aspirate is shown in Table 5. Overall microbiological sensitivity and specificity of endotracheal aspirate at any growth level (rare, light, medium, or heavy) were 65.4% and 56.1%, respectively. When test performance was recalculated with antibiotic management decisions taken into consideration, sensitivity and specificity of endotracheal aspirate increased to 81.2% and 61.9%, respectively. Similarly, positive predictive value and negative predictive value increased from 61.7% and 60.0% to 71.3% and 73.7% when antibiotic management was considered. Among the 147 patients who met microbiological criteria for VAP, 26 patients (17.7%) had endotracheal aspirate cultures that were falsely negative for pathogens. Of those patients with false negative endotracheal aspirate cultures, 16 (61.5%) were falsely negative for MDR pathogens (non-fermenting Gram-neg-

Table 3. Bacterial Pathogens Isolated Based on Culture Method

Growth level	Endotracheal Aspirate				
	Non-bronchoscopic BAL ($\geq 10^4$ CFU/mL)	Rare, Light, Moderate, or Heavy	Light, Moderate, or Heavy	Moderate or Heavy	Heavy
Gram positive (*n*, %)	82 (35.8)	106 (39.3)	92 (37.9)	48 (34.0)	42 (48.8)
Methicillin sensitive *Staphylococcus aureus*	39 (17.0)	52 (19.3)	44 (18.1)	23 (16.3)	23 (26.7)
Methicillin resistant *Staphylococcus aureus*	19 (8.3)	32 (11.9)	27 (11.1)	12 (8.5)	10 (11.6)
Streptococcus pneumoniae	8 (3.5)	7 (2.6)	7 (2.9)	5 (3.5)	3 (3.5)
Streptococcus species	16 (7.0)	15 (5.6)	14 (5.8)	8 (5.7)	6 (7.0)
Gram negative (*n*, %)	147 (64.2)	164 (60.7)	151 (62.1)	93 (66.0)	44 (51.2)
Pseudomonas aeruginosa	32 (15.8)	37 (13.7)	36 (14.8)	22 (15.6)	14 (16.3)
Klebsiella species	21 (9.2)	29 (10.7)	25 (10.3)	13 (9.2)	5 (5.8)
Enterobacter species	20 (8.7)	24 (8.9)	22 (9.1)	14 (9.9)	7 (8.1)
Serratia species	18 (7.9)	14 (5.2)	14 (5.8)	8 (5.7)	1 (1.2)
Escherichia coli	15 (6.6)	20 (7.4)	18 (7.4)	10 (7.1)	2 (2.3)
Haemophilus species	13 (5.7)	12 (4.4)	11 (4.5)	9 (6.4)	7 (8.1)
Acinetobacter species	12 (5.2)	13 (4.8)	11 (4.5)	5 (3.5)	3 (3.5)
Stenotrophomonas species	12 (5.2)	10 (3.7)	10 (4.1)	9 (6.4)	4 (4.7)
Citrobacter species	2 (0.9)	1 (0.4)	1 (0.4)	1 (0.7)	0
Proteus species	2 (0.9)	4 (1.5)	3 (1.2)	2 (1.4)	1 (1.2)
Pathogens isolated (*n*, %)	229 (100)	270 (100)	243 (90)	141 (52.2)	86 (31.9)
95% confidence interval	NA	NA	86.5–93.5	46.2–58.2	25.6–36.8

BAL= bronchoalveolar lavage
NA = not applicable

Table 4. Concordance Between Endotracheal Aspirate and Non-bronchoscopic BAL in 256 Paired Samples

Concordance With Non-bronchoscopic BAL	Endotracheal Aspirate Growth in semi-quantitative culture (*n*, %, and 95% CI)			
	Rare, Light, Moderate, or Heavy	Light, Moderate, or Heavy	Moderate or Heavy	Heavy
Complete Concordance	149 (58.2) 52.2–64.2	158 (61.7) 56.7–67.7	156 (60.9) 54.9–66.9	145 (56.6) 50.5–62.7
Complete Discordance	61 (23.8) 18.6–29.0	55 (21.5) 16.5–26.5	74 (28.9) 23.3–34.5	92 (35.9) 30.0–41.8
Incomplete Concordance	46 (18.0) 13.3–22.7	43 (16.8) 12.2–21.0	26 (10.2) 6.5–13.9	19 (7.4) 4.2–10.6

BAL = bronchoalveolar lavage
CI = confidence interval

ative rods and MRSA). Among the entire cohort of 256 patients there were 45 patients (17.5%) who had false positive endotracheal aspirate cultures, with 19 of these patients (42.2%) being falsely positive for MDR pathogens. Semi-quantitative cultures threshold for endotracheal aspirate with the highest agreement with non-bronchoscopic BAL was light/medium/heavy (kappa = 0.28); however, thresholds did overlap with each other. When test performance of endotracheal aspirate was evaluated in the context of antibiotic management and compared to that based only on the microbiological data, there was better agreement of endotracheal aspirate (rare/light/medium/heavy-antibiotics) with non-bronchoscopic BAL (kappa = 0.44) than the best kappa using microbiological data alone (see Table 5).

Discussion

As the problems of excessive antibiotic use and antimicrobial resistance continue to grow, an accurate microbiological diagnosis of VAP is likely to be the key element in ensuring appropriate antibiotic coverage for MDR organisms, as well as for limiting the use and duration of empirically prescribed broad-spectrum antibiotics.[23] Within this context, this study probably examined the largest sample size to compare the traditional semi-quan-

Table 5. Performance of Endotracheal Aspirate in the Microbiological Diagnosis of VAP

Endotracheal Aspirate Growth Level	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Kappa (95% CI)
Rare, light, moderate, or heavy	65.4	56.1	61.7	60.0	0.22 (0.17–0.27)
Light, moderate, or heavy	63.2	65.0	67.2	60.9	0.28 (0.23–0.34)
Moderate or heavy	44.4	83.3	76.8	54.6	0.26 (0.21–0.32)
Heavy	30.4	94.4	88.2	49.8	0.22 (0.17–0.27)
Rare, light, moderate, or heavy (antibiotic decision)	81.2	61.9	71.3	73.7	0.44 (0.38–0.50)

VAP = ventilator-associated pneumonia
CI = confidence interval

titative cultures of endotracheal aspirate to a quantitative-cultures method such as that which we used (non-bronchoscopic BAL), and supports the growing body of evidence in favor of quantitative techniques in the diagnosis of VAP.

As far as qualitative or semi-quantitative cultures are concerned, only limited studies to evaluate semi-quantitative cultures of endotracheal aspirate for diagnosing VAP have been reported. Our study compared the concordance of semi-quantitative cultures of endotracheal aspirate to quantitative cultures of non-bronchoscopic BAL, based on identical pathogens. Cook et al summarized the efficacy of quantitative cultures of endotracheal aspirate for the diagnosis of VAP.[13] Similar to these results reported by Cook et al,[10,24-27] which showed that endotracheal aspirate qualitative and semi-quantitative cultures were poorly concordant with cultures that were obtained via invasive quantitative cultures methods, our study resulted in the lack of specificity in endotracheal aspirate semi-quantitative cultures for the diagnosis of VAP.

Endotracheal aspirate typically and consistently grows more organisms than do invasive quantitative cultures.[28,29] Although in practice, semi-quantitative methods similar to the method used in our study are commonly used, there are few studies to evaluate the efficacy of semi-quantitative cultures of endotracheal aspirate.[30,31] The sample size of these studies[30,31] was very small and there were no specific data to show the sensitivity and specificity in terms of pathogen basis.[30] In our study the specificity of endotracheal aspirate semi-quantitative cultures on the threshold of light/medium/heavy was 65% (see Table 5). This explains that there are risks of over-diagnosis of VAP using endotracheal aspirate semi-quantitative cultures, and inappropriate use of antibiotics. Moreover, as is often the case with analyzing the threshold of diagnosing VAP, the accuracy of endotracheal aspirate is dependent on the threshold used.

Concern about inaccuracy of the clinical approach and/or endotracheal aspirate to diagnose VAP has led many investigators to use the invasive quantitative approaches. Thus, once bacteria are detected in endotracheal aspirate, regardless of the threshold, many physicians have difficulty de-escalating antibiotics to the cases with possible colonization, especially with MDR colonization.[32]

Prior studies using semi-quantitative cultures and a diagnostic threshold showed that a diagnostic growth threshold of "moderate" or greater growth was employed for diagnosing VAP.[30,31,33,34] In our study the kappa score for the light/medium/heavy growth threshold was the highest of any threshold evaluated (kappa = 0.28), while raising the growth threshold to moderate or greater growth (medium/heavy) lowered sensitivity but increased specificity remarkably (see Table 5). Given the variability in sensitivity and specificity of semi-quantitative endotracheal aspirate and the absence of good scientific data that define a diagnostic threshold that optimizes outcomes, appropriate antibiotic selection based on these culture methods is problematic. In our study almost 18% of patients meeting microbiological criteria for VAP had endotracheal aspirate cultures that were falsely negative for pathogens, while a similar proportion of the total cohort of patients had falsely positive cultures. In our practice, when endotracheal aspirate semi-quantitative cultures have been used for diagnosing VAP, we have deemed that the rate of false negative should be very low. Against our expectation, our study showed that 26 patients had endotracheal aspirate cultures that were falsely negative for pathogens, with 61.5% of these patients demonstrating growth of non-fermenting Gram-negative rods or MRSA on non-bronchoscopic BAL.

Use of semi-quantitative cultures therefore has the potential to lead to inadequate or excessive antimicrobial therapy. Test performance hypothetically improves when endotracheal aspirate is used in concert with a clinical strategy that initially employs broad-spectrum antibiotics in cases of suspected VAP, followed by de-escalation based on culture results. Even with such an improvement, clinicians still may be reluctant to de-escalate treatment.[32] Of note, several studies reported that surveillance cultures were beneficial to detect MDR pathogens.[35-38] This means that surveillance cultures have high positive and negative predictive values. In contrast, our data showed a high rate of false negative endotracheal aspirate cultures, especially

MDR pathogens, which directly affect the low rate of negative predictive value. A high false negative rate of endotracheal aspirate cultures would place the patient at risk for inappropriate therapy. There could be several reasons for this discrepancy. This result could be explained as follows: (1) sampling technique of endotracheal aspirate was poor, (2) samples on endotracheal aspirate did not represent samples of lower respiratory tract (a prerequisite for good positive and negative predictive values might be related to a sampling frequency of at least several times[35]), or (3) antibiotics might influence samples from endotracheal aspirate and/or non-bronchoscopic BAL, as there was at most a 24-hour time difference between each sample collection.

Because of problems inherent in the microbiological diagnosis of VAP, attention has now been directed to the impact of diagnostic strategies on clinical outcomes such as mortality, antibiotic utilization, and de-escalation. Invasive strategies using bronchoscopy have been widely accepted on the basis of data suggesting improved mortality.[18] A subsequent meta-analysis of 4 studies[18,39-41] evaluating the effect of invasive approach for the diagnosis of VAP on antibiotic utilization and mortality showed that an invasive quantitative approach favorably affected antibiotic utilization without altering mortality.[42]

A recent multicenter randomized trial conducted by the Canadian Critical Care Trials Group compared the use of BAL with quantitative cultures to qualitative endotracheal aspirate in patients who had received mechanical ventilation for at least 4 days.[43] In this study there was no significant difference in 28-day mortality, the rates of targeted therapy, days alive without antibiotics, organ-systems dysfunction, or stay between the groups. This study was limited because approximately 12% of screened patients who were infected or colonized with pseudomonas and MRSA at the time of randomization were excluded from the study.[44,45] Approximately 40% of all screened patients were excluded because of being at high risk for potentially resistant bacteria. A recent prospective observational study comparing the de-escalation rate between patients undergoing quantitative cultures via BAL and undergoing qualitative endotracheal aspirate showed that the rate of de-escalation was significantly higher in the BAL group than in the endotracheal-aspirate group.[46] In our study, 42% (19/45) of patients were considered to have false positive cultures for MDR pathogens, which would have been a negative impact on any strategy that emphasizes de-escalation. It is precisely in this group of patients with suspected VAP that the greatest value in quantitative BAL is likely to be used in the context of a de-escalation strategy.[47,48]

Our study has several major limitations, including its retrospective nature, the absence of an accepted standard culture method, and the absence of affirmation of a clinical diagnosis of VAP. Other limitations of the study are the issue of time difference in regard to each sample collection and the fact that some endotracheal aspirate samples were obtained after non-bronchoscopic BAL, which might potentially affect the microbiological yield of endotracheal aspirate. Similar to other studies, the vast majority of samples of the lower respiratory tract were obtained from patients who had received previous or current antibiotics, potentially influencing the culture results. Therefore, non-bronchoscopic BAL should be performed when a new VAP without previous antibiotic therapy is suspected. This intervention is feasible when respiratory therapists are trained to perform non-bronchoscopic BAL. In Table 2 we mentioned that antibiotics within 24 hours of non-bronchoscopic BAL was 87.5%; however, we did not collect the data of antibiotic intervention between non-bronchoscopic BAL and endotracheal aspirate sample collection, which might have resulted in discordance between non-bronchoscopic BAL and endotracheal aspirate. Finally, potential bias was introduced when our data were analyzed in the context of a de-escalation strategy, but this bias, if anything, would tend to favor the use of endotracheal aspirate.

Conclusions

In comparing the microbiological yield of cultures of the lower respiratory tract using semi-quantitative methods to quantitative cultures using non-bronchoscopic BAL in a "real world" clinical context, substantial disagreement between these methods, in addition to growth-level-dependent variability in sensitivity and specificity of endotracheal aspirate, was observed. Our results further suggest that use of endotracheal aspirate may lead to errors in antibiotic management of VAP, with the potential for inadequate or excessive treatment. In consideration of current trends in the antibiotic management of VAP that favor a de-escalation strategy, we recommend that quantitative cultures of the lower respiratory tract should be employed to guide antibiotic therapy until better outcome data are available.

ACKNOWLEDGMENTS

We greatly appreciate the help with statistical analysis provided by Ha Hoang MPH, Department of Hospital Epidemiology, Cedars-Sinai Medical Center, Los Angeles, California.

REFERENCES

1. Alvarez-Lerma F. Modification of empiric antibiotic treatment in patients with pneumonia acquired in the intensive care unit. ICU-Acquired Pneumonia Study Group. Intensive Care Med 1996;22(5):387-394.
2. Dupont H, Mentec H, Sollet JP, Bleichner G. Impact of appropriateness of initial antibiotic therapy on the outcome of ventilator-associated pneumonia. Intensive Care Med 2001;27(2):355-362.

3. Kollef MH, Ward S. The influence of mini-BAL cultures on patient outcomes: implications for the antibiotic management of ventilator-associated pneumonia. Chest 1998;113(2):412-420.
4. Luna CM, Vujacich P, Niederman MS, Vay C, Gherardi C, Matera J, et al. Impact of BAL data on the therapy and outcome of ventilator-associated pneumonia. Chest 1997;111(3):676-685.
5. Kollef MH, Fraser VJ. Antibiotic resistance in the intensive care unit. Ann Intern Med 2001;134(4):298-314.
6. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171(4):388-416.
7. Niederman MS. De-escalation therapy in ventilator-associated pneumonia. Curr Opin Crit Care 2006;12(5):452-457.
8. Baughman RP. Diagnosis of ventilator-associated pneumonia. Microbes Infect 2005;7(2):262-267.
9. Camargo LF, De Marco FV, Barbas CS, Hoelz C, Bueno MA, Rodrigues M, Jr, et al. Ventilator-associated pneumonia: comparison between quantitative and qualitative cultures of tracheal aspirates. Crit Care 2004;8(6):R422-R430.
10. Cook D, Mandell L. Endotracheal aspiration in the diagnosis of ventilator-associated pneumonia. Chest 2000;117(4 Suppl 2):195S-197S.
11. Blot F, Raynard B, Chachaty E, Tancrede C, Antoun S, Nitenberg G. Value of gram stain examination of lower respiratory tract secretions for early diagnosis of nosocomial pneumonia. Am J Respir Crit Care Med 2000;162(5):1731-1737.
12. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165(7):867-903.
13. Mondi MM, Chang MC, Bowton DL, Kilgo PD, Meredith JW, Miller PR. Prospective comparison of bronchoalveolar lavage and quantitative deep tracheal aspirate in the diagnosis of ventilator-associated pneumonia. J Trauma 2005;59(4):891-896.
14. Torres A, Ewig S. Diagnosing ventilator-associated pneumonia. N Engl J Med 2004;350(5):433-435.
15. Hill JD, Ratliff JL, Parrott JC, Lamy M, Fallat RJ, Koeniger E, et al. Pulmonary pathology in acute respiratory insufficiency: lung biopsy as a diagnostic tool. J Thorac Cardiovasc Surg 1976;71(1):64-71.
16. Brun-Buisson C, Fartoukh M, Lechapt E, Honore S, Zahar JR, Cerf C, et al. Contribution of blinded, protected quantitative specimens to the diagnostic and therapeutic management of ventilator-associated pneumonia. Chest 2005;128(2):533-544.
17. Koenig SM, Truwit JD. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. Clin Microbiol Rev 2006;19(4):637-657.
18. Fagon JY, Chastre J, Wolff M, Gervais C, Parer-Aubas S, Stephan F, et al. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia. A randomized trial. Ann Intern Med 2000;132(8):621-630.
19. Kollef MH, Bock KR, Richards RD, Hearns ML. The safety and diagnostic accuracy of minibronchoalveolar lavage in patients with suspected ventilator-associated pneumonia. Ann Intern Med 1995; 122(10):743-748.
20. Levy H. Comparison of Ballard catheter bronchoalveolar lavage with bronchoscopic bronchoalveolar lavage. Chest 1994;106(6):1753-1756.
21. Tuttle RP, Cohen MH, Augustine AJ, Novotny DF, Delgado E, Dongilli TA, et al. Utilizing simulation technology for competency skills assessment and a comparison of traditional methods of training to simulation-based training. Respir Care 2007;52(3):263-270.
22. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33(1):159-174.
23. Yu VL, Singh N. Excessive antimicrobial usage causes measurable harm to patients with suspected ventilator-associated pneumonia. Intensive Care Med 2004;30(5):735-738.
24. Lambert RS, Vereen LE, George RB. Comparison of tracheal aspirates and protected brush catheter specimens for identifying pathogenic bacteria in mechanically ventilated patients. Am J Med Sci 1989;297(6):377-382.
25. Rumbak MJ, Bass RL. Tracheal aspirate correlates with protected specimen brush in long-term ventilated patients who have clinical pneumonia. Chest 1994;106(2):531-534.
26. Torres A, Puig de la Bellacasa J, Xaubet A, Gonzalez J, Rodriguez-Roisin R, Jimenez de Anta MT, et al. Diagnostic value of quantitative cultures of bronchoalveolar lavage and telescoping plugged catheters in mechanically ventilated patients with bacterial pneumonia. Am Rev Respir Dis 1989;140(2):306-310.
27. Villers D, Derriennic M, Raffi F, Germaud P, Baron D, Nicolas F, et al. Reliability of the bronchoscopic protected catheter brush in intubated and ventilated patients. Chest 1985;88(4):527-530.
28. Arora SC, Mudaliar YM, Lee C, Mitchell D, Iredell J, Lazarus R. Non-bronchoscopic bronchoalveolar lavage in the microbiological diagnosis of pneumonia in mechanically ventilated patients. Anaesth Intensive Care 2002;30(1):11-20.
29. Jourdain B, Novara A, Joly-Guillou ML, Dombret MC, Calvat S, Trouillet JL, et al. Role of quantitative cultures of endotracheal aspirates in the diagnosis of nosocomial pneumonia. Am J Respir Crit Care Med 1995;152(1):241-246.
30. Sauaia A, Moore FA, Moore EE, Haenel JB, Kaneer L, Read RA. Diagnosing pneumonia in mechanically ventilated trauma patients: endotracheal aspirate versus bronchoalveolar lavage. J Trauma 1993; 35(4):512-517.
31. Wood AY, Davit AJ 2nd, Ciraulo DL, Arp NW, Richart CM, Maxwell RA, et al. A prospective assessment of diagnostic efficacy of blind protective bronchial brushings compared to bronchoscope-assisted lavage, bronchoscope-directed brushings, and blind endotracheal aspirates in ventilator-associated pneumonia. J Trauma 2003; 55(5):825-834.
32. Heyland DK, Cook DJ, Marshall J, Heule M, Guslits B, Lang J, et al. The clinical utility of invasive diagnostic techniques in the setting of ventilator-associated pneumonia. Canadian Critical Care Trials Group. Chest 1999;115(4):1076-1084.
33. Pugin J. [The diagnosis of pneumonia in the ventilated patient]. Schweiz Med Wochenschr 1990;120(45):1672-1677. *Article in French.*
34. Singh N, Rogers P, Atwood CW, Wagener MM, Yu VL. Short-course empiric antibiotic therapy for patients with pulmonary infiltrates in the intensive care unit. A proposed solution for indiscriminate antibiotic prescription. Am J Respir Crit Care Med 2000;162(2 Pt 1):505-511.
35. Blot S, Depuydt P, Vogelaers D. Maximizing rates of empiric appropriate antibiotic therapy with minimized use of broad-spectrum agents: are surveillance cultures the key? Intensive Care Med 2008; 34(12):2130-2133.
36. Depuydt PO, Blot SI, Benoit DD, Claeys GW, Verschraegen GL, Vandewoude KH, et al. Antimicrobial resistance in nosocomial bloodstream infection associated with pneumonia and the value of systematic surveillance cultures in an adult intensive care unit. Crit Care Med 2006;34(3):653-659.
37. Depuydt P, Benoit D, Vogelaers D, Decruyenaere J, Vandijck D, Claeys G, et al. Systematic surveillance cultures as a tool to predict involvement of multidrug antibiotic resistant bacteria in ventilator-associated pneumonia. Intensive Care Med 2008;34(4):675-682.
38. Papadomichelakis E, Kontopidou F, Antoniadou A, Poulakou G, Koratzanis E, Kopterides P, et al. Screening for resistant gram-negative microorganisms to guide empiric therapy of subsequent infection. Intensive Care Med 2008;34(12):2169-2175.
39. Ruiz M, Torres A, Ewig S, Marcos MA, Alcon A, Lledo R, et al. Noninvasive versus invasive microbial investigation in ventilator-associated pneumonia: evaluation of outcome. Am J Respir Crit Care Med 2000;162(1):119-125.

40. Sanchez-Nieto JM, Torres A, Garcia-Cordoba F, El-Ebiary M, Carrillo A, Ruiz J, et al. Impact of invasive and noninvasive quantitative culture sampling on outcome of ventilator-associated pneumonia: a pilot study. Am J Respir Crit Care Med 1998;157(2):371-376.
41. Sole Violan J, Fernandez JA, Benitez AB, Cardenosa Cendrero JA, Rodriguez de Castro F. Impact of quantitative invasive diagnostic techniques in the management and outcome of mechanically ventilated patients with suspected pneumonia. Crit Care Med 2000;28(8):2737-2741.
42. Shorr AF, Sherner JH, Jackson WL, Kollef MH. Invasive approaches to the diagnosis of ventilator-associated pneumonia: a meta-analysis. Crit Care Med 2005;33(1):46-53.
43. Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. N Engl J Med 2006;355(25):2619-2630.
44. Fagon JY, Chastre J, Rouby JJ. Is bronchoalveolar lavage with quantitative cultures a useful tool for diagnosing ventilator-associated pneumonia? Crit Care 2007;11(2):123.
45. Kollef MH. Diagnosis of ventilator-associated pneumonia. N Engl J Med 2006;355(25):2691-2693.
46. Giantsou E, Liratzopoulos N, Efraimidou E, Panopoulou M, Alepopoulou E, Kartali-Ktenidou S, et al. De-escalation therapy rates are significantly higher by bronchoalveolar lavage than by tracheal aspirate. Intensive Care Med 2007;33(9):1533-1540.
47. Kollef MH. Inadequate antimicrobial treatment: an important determinant of outcome for hospitalized patients. Clin Infect Dis 2000;31(Suppl 4):S131-S138.
48. Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC, et al. Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. Am J Respir Crit Care Med 1998;157(2):531-539.

Petri dishes with cultured cells
(undated photograph by Roy Perry)
Courtesy National Library of Medicine