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Role of semi-quantitative and quantitative cultures of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia

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RESEARCH

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Abstract

Background
Ventilator-associated pneumonia (VAP) is an important nosocomial infection among patients on mechanical ventilation. Modified clinical pulmonary infection score (CPIS) is used widely for diagnosis of VAP. There is a need for a non-invasive microbiological technique for diagnosis of VAP. Therefore, we performed a prospective study to determine the diagnostic value of semi-quantitative and quantitative cultures of endotracheal aspirates (EA) in VAP.

Method
During a period of 15 months from October 2006 to December 2007, a prospective observational cohort study was conducted at a tertiary care hospital. Semi-quantitative and quantitative cultures of EA were performed for patients with VAP.

Results
A total of 200 patients were prospectively evaluated, of whom 42 (21%) developed VAP. The semi-quantitative culture had 100% sensitivity, 58.2% specificity, 38.9% positive predictive value and 100% negative predictive value. Quantitative EA culture had 88.1% sensitivity, 84.2% specificity, 59.7% positive predictive value and 96.4% negative predictive value. The Receiver operating characteristic (ROC) curve of quantitative EA cultures showed an area under the curve of 0.861 ± 0.034.

Conclusion
The semi-quantitative EA culture had a good negative predictive value and therefore it may be useful for excluding VAP. The quantitative EA culture with a good sensitivity, specificity, positive and negative predictive values can be considered as an acceptable tool for diagnosis of VAP. In addition, the quantitative EA cultures can also guide the selection of appropriate antibiotics for treatment of VAP cases.

Key Words
Ventilator-associated pneumonia, quantitative culture, endotracheal aspirate, semi-quantitative culture, clinical pulmonary infection score.

Background
Ventilator-Associated Pneumonia (VAP) is defined as pneumonia that arises more than 48 hours after endotracheal intubation and initiation of mechanical ventilation (1). VAP is clinically suspected usually on the basis of the presence of fever, leukocytosis or leukopenia, purulent tracheal secretions and the presence of a new and/or persistent radiographic infiltrate. However, these clinical parameters individually have limited diagnostic value (2).

Pugin et al proposed the Clinical Pulmonary Infection Score (CPIS) for diagnosis of VAP as they found it to be very accurate (3). In a study carried out by Woske et al to evaluate three quantitative bronchoscopic methods for diagnosis of VAP, the CPIS was used to define the presence of VAP (4). Similarly, in another study, CPIS was used for early diagnosis of VAP in patients with severe brain injury receiving mechanical ventilation (5). The main advantage of CPIS over the culture based methods is the early diagnosis which helps timely administration of adequate empiric therapy (6).

Though VAP can be diagnosed clinically, quantitative culture of the lower respiratory tract secretions obtained

bronchoscopically is essential for knowing the antibiotic susceptibility of the etiological agent (7). However, bronchoscopy being a minimally invasive procedure cannot be performed in all patients suspected to have VAP. On the other hand endotracheal aspirates (EA) are easy to collect and may be useful for diagnosing VAP (8). Qualitative cultures of tracheal aspirate is not a specific diagnostic tool as it is associated with a high percentage of false-positives due to colonization of the lower respiratory tract (9).

Semi-quantitative and quantitative cultures of EA may be helpful in distinguishing active infection from colonization and may prove to be useful alternatives or adjuncts for clinical diagnosis of VAP (10). In a study from Japan, semi-quantitative cultures of endotracheal aspirate were observed to be poorly concordant with quantitative cultures obtained via non-bronchoscopic bronchoalveolar lavage (BAL) (11). But, in a randomized controlled trial conducted by Canadian Critical Care Trials Group, quantitative culture of the BAL and semi-quantitative culture of EA were associated with similar clinical outcomes and similar overall use of antibiotics (12). Mondi et al found that the use of quantitative EA in VAP diagnosis is limited because of the higher rate of over-diagnosis (13). However, a multicentric study from Spain concluded that the quantitative cultures of EA can be considered acceptable for the diagnosis of VAP (14). The results of these studies were conflicting.

In view of the above mentioned ambiguity in results, we performed a prospective study in a tertiary care hospital of India, to determine the diagnostic value of semi-quantitative and quantitative cultures of endotracheal aspirates in VAP in comparison to CPIS.

Method

Setting and Subjects

A prospective observational cohort study was conducted in the departments of Microbiology, Medicine and Anaesthesiology & Critical Care at Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER), a tertiary care hospital in Pondicherry, India. During a 15-month period from October 2006 to December 2007, all the adult patients on mechanical ventilation (MV) for > 48 hours in Medicine Intensive Care Unit (MICU) and Critical Care Unit (CCU) were included in this study. Patients with pneumonia prior to MV or within 48 h of MV were excluded. This study was approved by the institute's research and ethical committees and informed consent was obtained from the patient's next of kin.

Study Design and Data Collection

Demographic details and preliminary data were collected at ICU admission. All the patients included in this study were monitored at frequent intervals (every 3 days) for the development of VAP using CPIS till discharge or death (9). The CPIS was based on the assessment of 6 clinical features, each worth 0–2 points, and included: fever, leukocyte count, quantity and purulence of tracheal secretions, oxygenation, type of radiographic abnormality, and results of sputum culture and Gram stain. A CPIS > 6 was shown to be associated with 93% sensitivity and 100% specificity (7). We therefore considered patients with a CPIS > 6 to be suffering from VAP.

Microbiological processing

Endotracheal aspirates were collected from these patients by the respiratory therapist with strict aseptic precautions, following specimen collection guidelines. The specimens were taken immediately to the microbiology laboratory and processed.

Semi-quantitative culture

Semi-quantitative culture was performed based on the four-quadrant streak technique using a calibrated loop. EA cultures were read semi-quantitatively by observing the growth in the four quadrants, which suggested the approximate number of colony forming units per ml (CFU/ml) of the bacteria in the specimen (15). The cultures were graded as 1+, 2+, 3+ and 4+. Cultures showing a moderate to heavy growth with 3+ or 4+ grades were considered as positive.

Quantitative culture

EA was serially diluted in sterile normal saline as 1/10, 1/100, 1/1000 and 0.01 ml of 1/1000 dilution was inoculated on 5% sheep blood agar. After incubation at 37^0C in 5% CO_2 incubator for 24 h, colony count was done and expressed as CFU/ml. The number of CFU/ ml is equal to number of colonies on agar plate × dilution factor × inoculation factor. Therefore presence of even a single colony on the blood agar after inoculating 0.01 ml of 1/ 1000 times diluted EA was interpreted as more than 10^5 CFU/ ml (16). The organisms isolated from the clinical specimens were identified based on standard bacteriological procedures (17).

Statistical Analysis

The data were analysed with the statistical software SPSS 16.0 for Microsoft Windows (SPSS Inc., Chicago, IL, USA). Quantitative variables were expressed as mean ± SD. Means were compared using the Student *t* test or the Mann Whitney test. Qualitative variables were expressed as the frequency of distribution of each category. The Chi-square test or Fisher's exact test were used to compare patients in different groups. *P* values < 0.05 were considered statistically significant. Receiver operating characteristic (ROC) curves were generated for semi-quantitative and quantitative EA cultures and the area under the curve was calculated for evaluating their role in diagnosis of VAP. Percentage agreement and kappa factor were calculated to determine the agreement between semi-quantitative and quantitative EA cultures and CPIS. Sensitivity, specificity, positive predictive value and negative predictive value of different diagnostic criteria were determined using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA. Likelihood ratios were calculated according to Deeks and Altman (18).

Results

A total of 200 patients were prospectively evaluated during the study period, of whom 42 (21%) developed VAP during their ICU stay. The demographic details of the patients with and without VAP are summarised in Table 1.

Table 1. Demographic details of the VAP and non-VAP patients

Parameter	Non-VAP (n = 158)	VAP (n = 42)	*P* value (2-tailed)
Age (mean ± SD)	36.6 ± 16.4	41.5 ± 14.5	0.0797
Gender			
Male	93 (58.9%)	26 (61.9%)	0.8569
Female	65 (41.1%)	16 (38.1%)	
Primary diagnosis			
Poisoning[a]	54 (34.2%)	11 (26.2%)	0.4255
Neuromuscular disorders	21 (13.3%)	7 (16.7%)	0.7564
Intra-abdominal diseases	11 (7.0%)	5 (11.9%)	0.3362
Snake bite	11 (7.0%)	5 (11.9%)	0.3362
CNS infections	2 (1.3%)	5 (11.9%)	0.0050
Pregnancy-related disorders	17 (10.8%)	2 (4.8%)	0.3748
Trauma	12 (7.6%)	2 (4.8%)	0.7384
Cardiovascular diseases	10 (6.3%)	2 (4.8%)	1.0000
Respiratory diseases	2 (1.3%)	1 (2.4%)	0.5089
Intracranial haemorrhage/ thrombosis	7 (4.4%)	1 (2.4%)	1.0000
Miscellaneous[b]	11 (7.0%)	1 (2.4%)	0.4663

[a] It includes organophosphorous (insecticide), yellow oleander and atropine poisoning.

[b] Acute flaccid paralysis, frontotemporal intracranial space occupying lesion, cerebrovascular accident, multiple injury, hanging, sepsis, chondrosarcoma, renal cell carcinoma, chronic obstructive pulmonary disease with cardiac failure, CO_2 narcosis, diabetes mellitus with hypertension, diabetic nephropathy, neuroglycopenia, post hysterectomy, severe anaemia, chronic or acute renal failure.

No significant differences were found between the baseline variables of the patients with and without pneumonia. However, patients with CNS infections were noted to be significantly predisposed for the development of VAP. Most cases of VAP were caused by Gram-negative bacteria, which accounted for 81.3% of etiological agents. *Pseudomonas aeruginosa* (20.8%) and *Acinetobacter baumannii* (20.8%) were the most common Gram-negative bacteria associated with VAP and *Staphylococcus aureus* (14.6%) was the most common Gram-positive bacteria among patients with VAP.

Diagnostic values of semi-quantitative and quantitative cultures

A total of 465 EA specimens collected from 200 patients were studied. The mean number of EA specimens collected from each patient was 2.3 ± 1.5 (range, 1 to 7). The diagnostic values of semi-quantitative and quantitative EA cultures are summarised in Table 2. Semi-quantitative EA culture had 67% agreement with CPIS having a kappa (κ) factor of 0.37, while the quantitative EA culture had 85% agreement with CPIS and a kappa (κ) factor of 0.62.

Table 2. Diagnostic values of semi-quantitative and quantitative EA cultures

Parameter	Quantitative EA culture (95% confidence interval)	Semi-quantitative EA culture (95% confidence interval)	*P* value
Sensitivity	88.1 (74.3 – 96.0)	100 (91.6 – 100)	0.0551
Specificity	84.2 (77.6- 89.5)	58.2 (50.2 – 66.0)	< 0.0001
Positive predictive value	59.7 (46.5 – 71.9)	38.9 (29.6 – 48.7)	0.0140
Negative predictive value	96.4 (91.74 – 98.81)	100 (96.1 – 100)	0.1601
Positive likelihood ratio	5.57	2.39	-
Negative likelihood ratio	0.14	0	-

The ROC curves for semi-quantitative and quantitative EA cultures with the corresponding areas under the curve are shown in Figure 1 and Figure 2 respectively. The sensitivity/specificity relationship of quantitative EA culture is better compared to that of semi-quantitative EA culture.

In 5 out of the 42 VAP cases diagnosed on the basis of CPIS, the quantitative EA cultures were negative. However, in the remaining 37 cases, the mean ± SD number of days taken for diagnosis of VAP in patients on MV was 6.3 ± 5.0 and 5.8 ± 4.3 based on CPIS and quantitative EA culture respectively (*P* value 0.7698).

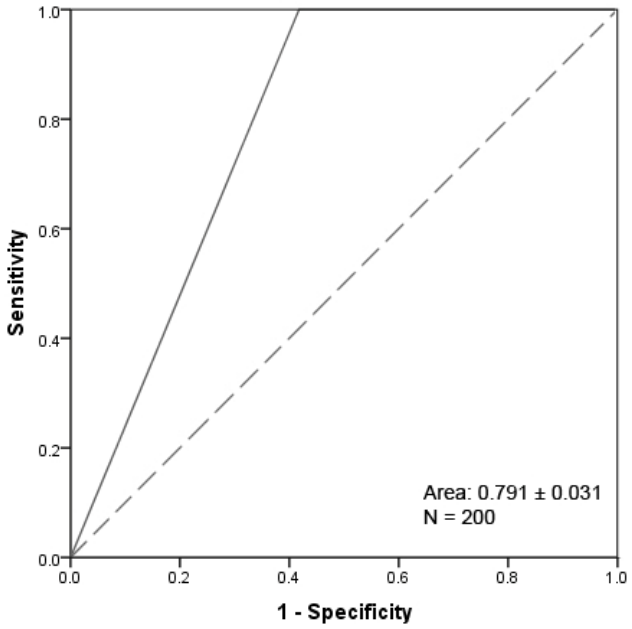

Figure 1. ROC curve of semi-quantitative EA cultures. Area under the curve: 0.791 ±0.031 (95% CI, 0.730 - 0.852).

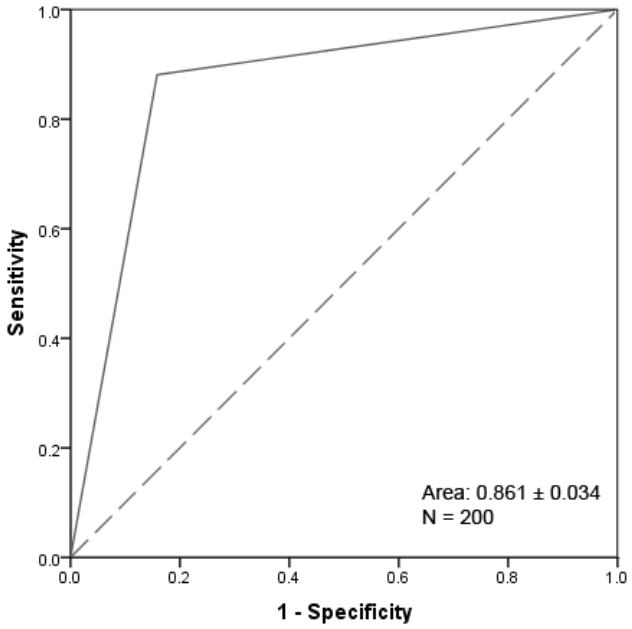

Figure 2. ROC curve of quantitative EA cultures. Area under the curve: 0.861 ±0.034 (95% CI, 0.796 - 0.927).

Discussion

VAP is an important nosocomial infection among ICU patients receiving MV. The clinical diagnosis of VAP can be made using the CPIS (1). Quantitative cultures of the lower respiratory secretions obtained by bronchoscopic techniques such as bonchoalveolar lavage (BAL) or protected specimen brushing (PSB), are essential for deciding appropriate therapy for the VAP patients. Although bronchoscopy has only a low inherent risk, it may rarely lead to cardiac arrhythmias, hypoxemia, or bronchospasm (19). In addition, bronchoscopy being a minimally invasive procedure is usually performed only in the later stages of VAP. But, any delay in the administration of appropriate antibiotic therapy is associated with higher morbidity and mortality (20). So, there is a need for a non-invasive technique which can be performed early in patients suspected to have developed VAP.

In this study we evaluated the semi-quantitative and quantitative cultures of endotracheal aspirates as diagnostic tools for VAP. We found that the sensitivity and negative predictive value of both the techniques were reasonably good and there was no statistically significant difference between the two. But the specificity and positive predictive value of semi-quantitative EA culture were too low. Moreover the semi-quantitative EA culture had a poor agreement with CPIS. Similarly, in a study by Fujitani et al the semi-quantitative EA culture was poorly concordant with non-bronchoscopic BAL culture and had unacceptable specificity and positive predictive value (11). Therefore, this technique cannot be used as an acceptable tool for diagnosis of VAP. Use of semi-quantitative EA cultures for guiding antibiotic therapy may also result in unnecessary antibiotic treatment of substantially more number of patients without VAP. In a study by Brun-Buisson *et al*, 18% patients were noted to had been unnecessarily treated with antibiotics based on semi-quantitative cultures of EA (15). The other inherent problem with semi-quantitative EA culture is its variability based on the diagnostic threshold employed. Conventionally, most studies have used moderate or heavy growth as the diagnostic threshold (11,21-23). But in a recent study, use of light growth as the diagnostic threshold was found to perform better than the moderate or heavy growth (11). These discordant results underscore the problems with optimizing a diagnostic threshold for semi-quantitative EA cultures. However, this technique may be useful for excluding VAP, as it had a good negative predictive value in our study.

In recent years, various investigators are recommending quantitative analysis of EA as a simple and useful tool for the diagnosis of VAP (7,14,24). Quantitative EA culture is a non-invasive and inexpensive technique. In our study the specificity and the positive predictive value of quantitative EA culture were significantly high compared to that of semi-quantitative EA culture. Quantitative EA culture also had very good positive and negative likelihood ratios, suggesting that it can be effectively used for diagnosis of VAP. Similarly, Liang et al also have showed that quantitative EA culture had acceptable sensitivity and specificity comparable to protected specimen brush (PSB) and bronchoalveolar lavage (24). In our study there was a relatively good agreement between the quantitative EA culture and CPIS with a kappa (κ) factor of 0.62. Similarly, various studies have reported that quantitative EA cultures showed a total agreement with BAL and PSB in patients with suspected VAP (24,25). We also observed that based on the ROC curves the quantitative EA culture is better than semi-quantitative EA culture for diagnosis of VAP. Therefore, the quantitative EA culture can be used as an acceptable tool for diagnosis of

VAP. In a study conducted to determine the reproducibility of quantitative cultures of EA, a mean persistence of 85%, 80%, 74% and 89% was observed for Enterobacteriaceae, *Pseudomonas* spp., *Staphylococcus* spp., and *Enterococcus* spp., respectively, at a threshold of ≥ 10^5 CFU/ml (26). They also found that the results of quantitative culture of ET are reproducible over a 2-day period (26). We observed that there was no statistically significant delay in diagnosis of VAP based on quantitative EA culture compared to CPIS. In addition, the quantitative EA cultures also provided the susceptibility pattern of the isolates, thereby guiding the selection of appropriate antibiotics for treatment of VAP cases.

Conclusion

The semi-quantitative EA culture had a good negative predictive value and therefore it may be useful for excluding VAP. The quantitative EA culture with a good sensitivity, specificity, positive and negative predictive values can be considered as an acceptable tool for diagnosis of VAP. In addition, the quantitative EA cultures can also guide the selection of appropriate antibiotics for treatment of VAP cases.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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