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Reproducibility of Quantitative Cultures of Endotracheal Aspirates from Mechanically Ventilated Patients

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Ventilator-associated pneumonia is frequently diagnosed with quantitative cultures of samples obtained by bronchoscopic techniques, a method associated with high costs and potential adverse effects. Quantitative cultures of endotracheal aspirates are easier and cheaper to obtain, and good correlations between the results of this method and those of bronchoscopic methods have been reported. However, the reproducibility of quantitative cultures of endotracheal aspirates has never been determined. We studied the quantitative analysis of endotracheal aspirates from 21 mechanically ventilated patients taken during two study days with 2- and 6-h intervals between samplings. In all, 140 endotracheal aspirates were obtained. For mechanically ventilated patients, the median variation of quantitative culture results was 12.3% (range, 0 to 63%), corresponding to 0.7 log CFU/ml. Furthermore, variation was independent of the interval of time between samplings. Persistence of significant numbers of pathogens in quantitative culture results ($\geq 10^5$ CFU/ml) of the consecutive endotracheal aspirates occurred in 82% of samples. We conclude that results of quantitative cultures from endotracheal aspirates are reproducible and may be useful in diagnosing ventilator-associated pneumonia.

The diagnosis of ventilator-associated pneumonia is based upon a combination of clinical, bacteriological, and radiographic criteria. Quantitative cultures of samples obtained by bronchoscopic techniques, such as protected specimen brush sampling and bronchoalveolar lavage, have been introduced to increase specificity (10, 12). Although the use of bronchoscopy has been advocated by some (1), objections to its use include high costs and the possibility of adverse effects like hypoxemia, hemorrhage, and fever (7). Recently, good correlations between the results of quantitative cultures of protected specimen brush or bronchoalveolar lavage samples and those of quantitative cultures of endotracheal aspirates have been reported (2, 5, 8, 9, 11). In these studies, endotracheal aspirates were obtained by deep suctioning from the bronchi through the endotracheal tube. However, the reproducibility of quantitative culture results of endotracheal aspirates taken during the day is unknown. Therefore, we analyzed variations in quantitative culture results of samples obtained by endotracheal aspiration of mechanically ventilated patients.

The study was performed in the general intensive care unit (ICU) of the University Hospital Maastricht, Maastricht, The Netherlands. Mechanically ventilated patients who needed ≥5 endotracheal suctionings a day because of increased production of respiratory secretions were included. Endotracheal aspirates were obtained from study patients on day 1 at 8:00 a.m., 10:00 a.m., 12:00 noon, 2:00 p.m., and 4:00 p.m. and on day 2 at 8:00 a.m. and 2:00 p.m.

Endotracheal aspirates were collected by the nursing staff, who were instructed about the study before it started. After donning of sterile gloves, sampling was performed with a sterile catheter with a specimen trap kit (Lukens specimen container; Sherwood Medical, Tullamore, Ireland). For blind sampling, the catheter was introduced via the endotracheal tube for at least 30 cm without suctioning. After positioning of the catheter, aspirates were suctioned and directly collected into sterile containers. The samples were transported within 15 min to the Laboratory of Medical Microbiology, where they were Gram stained and cultured semiquantitatively and quantitatively.

The presence of polymorphonuclear neutrophils (PMN) was determined in Gram stain and graded per high-power field (HPF) under oil immersion (magnification, ×1,000) as follows: no PMN, 1+ (1 to 2 PMN/HPF), 2+ (2 to 5 PMN/HPF), 3+ (5 to 10 PMN/HPF), and 4+ (>10 PMN/HPF).

For semiquantitative analysis, purulent aspirate was washed three times in 0.9% sterile saline before inoculation on blood (CM 271; OXOID) cysteine lactose electrolyte-deficient agar (CM 423; OXOID), *Haemophilus* selective agar, and streptococcus and staphylococcus selective agar. The number of CFU was determined by the four-quadrant method and classified as follows: no growth, 1+, 2+, 3+, or 4+ (3).

For quantitative analysis, 1 ml of aspirate was mixed with 2 ml of sputolysin (11474; Merck) and directly after being vortexed thoroughly, 100 μl was added to 9.9 ml of 0.9% sterile saline and serial dilutions were made. The lower detection threshold of quantitative cultures was 10^4 CFU/ml. If the result of the quantitative culture was below the detection threshold and the semiquantitative culture yielded bacterial growth, the quantitative culture result was assumed to be 10^2 CFU/ml. If two or more species were cultured in one endotracheal aspirate, the quantity of each species was determined and the mean quantitative growth result of all isolated species for that endotracheal aspirate was calculated.

The coefficient of variation, which is the standard deviation (SD) expressed as a percentage of the mean, of the results from consecutive quantitative cultures of endotracheal aspirates was calculated for each patient (4). The coefficient of

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TABLE 1. Quantitative culture results of all cultured isolates

Isolates	No. of positive patients	Quantitative culture results		
		No. of positive samples	Median log CFU/ml	Range (log CFU/ml)
Nosocomial pathogens				
Enterobacteriaceae	15	53	5.9	4.3–7.5
Pseudomonas spp.	15	83	6.6	4.5–8.8
Enterococcus spp.	8	19	5.3	4.5–6.2
Yeasts	7	22	5.5	4.5–8.6
Oropharyngeal floras				
Staphylococcus spp.	9	35	5.4	4.5–7.4
Streptococcus spp.	2	5	5.7	5.3–6.3
Neisseria spp.	1	4	5.3	4.6–6.3
Haemophilus spp.	2	5	6.2	5.5–7.3
Moraxella spp.	1	4	6.5	5.1–7.2
Other	8	33	6.0	4.8–7.4

variation was calculated for each species per patient separately. Analysis of variance (ANOVA) and the Friedman two-way ANOVA were used to determine the influences of time of sampling and patient variation on total variance. In all statistical analyses the logarithm of the quantitative culture result was used. Furthermore, the persistence of significant quantities of isolated species in the consecutive samples of a patient was determined by dividing the number of samples with significant quantities of species (counted after the first culture with significant growth) by the total number of samples. Persistence was determined for thresholds of significance of $\geq 10^5$ and $\geq 10^6$ CFU/ml.

Twenty-one patients (18 male and 3 female) were studied. Their median age was 70 years (range, 18 to 87 years), and the mean admission acute physiology and chronic health evaluation (APACHE II) score was 21 (SD = 8). Three patients had a tracheostomy. The median length of stay in the ICU before the start of the study was 6 days (range, 1 to 63 days). On entrance into the study, 16 patients received antibiotics for a median of 2 days (range, 0 to 6 days). Antibiotic therapy was unaltered during the 2-day study period.

In all, 140 endotracheal aspirates were obtained and microorganisms were isolated from 127 samples. *Enterobacteriaceae*, *Pseudomonas* spp., *Staphylococcus* spp., *Enterococcus* spp., and yeasts were cultured most frequently. No large differences existed among the quantitative culture results (expressed as median log CFU per milliliter) analyzed per species (Table 1).

The median coefficient of variation for quantitative culture results, as calculated for each patient over the 2-day study period, was 12.3% (range, 0 to 63%), which means a variation of approximately 0.7 log CFU/ml. On study day 1 the median coefficient of variation was 11% (range, 0 to 60%), and on study day 2 the median coefficient of variation was 8% (range, 0 to 63%). When all culture results of all patients were analyzed (total variation), patient variation determined a significant part of the total variation ($P < 0.0001$ by ANOVA) whereas the time of sampling did not ($P = 0.35$). Categorization of the quantitative culture results in interval groups ($<10^4$, $\geq 10^4$ to $<10^5$, $\geq 10^5$ to $<10^6$, $\geq 10^6$ to $<10^7$, $\geq 10^7$ to $<10^8$, and $\geq 10^8$ CFU/ml) did not reveal a significant effect of time of sampling on total variation either ($P = 0.40$, by the Friedman two-way ANOVA).

The mean persistence of significant quantities of species in consecutive quantitative culture samples was 82% (SD, 22%) at a threshold of $\geq 10^5$ CFU/ml and 72% (SD, 31%) at a threshold of $\geq 10^6$ CFU/ml. Separate analyses for the most prevalent pathogens yielded a mean persistence of 85, 80, 74, and 89% for *Enterobacteriaceae*, *Pseudomonas* spp., *Staphylococcus* spp., and *Enterococcus* spp., respectively, at a threshold of $\geq 10^5$ CFU/ml. At a threshold of $\geq 10^6$ CFU/ml, persistence was 79% for *Enterobacteriaceae*, 66% for *Pseudomonas* spp., and 48% for *Staphylococcus* spp. Persistence of *Enterococcus* spp. at this threshold could not be determined since only one case remained.

When semiquantitative culture results were compared, we found an excellent correlation with quantitative culture results of endotracheal aspirates ($r = 0.86$; data not shown); all semiquantitative culture results with 4+ growth had quantitative culture results of $\geq 10^6$ CFU/ml. In contrast, a weak correlation was found between quantitative culture results and the number of PMN in Gram-stained endotracheal aspirates ($r = 0.30$; data not shown), which confirms observations made by Morris et al., who recovered similar numbers of organisms from samples with different numbers of PMN (6).

The results of this study demonstrate that quantitative cultures of endotracheal aspirates have a median variation of 12.3%. The variation in culture results was independent not only of the interval between culturing (2 or 6 h) but also of the time of sampling. The mean persistence of quantities of species of $\geq 10^5$ CFU/ml in the consecutive samples was 82%. The variations observed as well as the persistence rates after a first culture with a growth of $\geq 10^5$ CFU/ml were similar for the most prevalent pathogens. Importantly, antibiotic therapy was unaltered during the study period. Moreover, although the nursing staff was instructed how to obtain endotracheal aspirates before the study, there was no strict monitoring during the study, thereby imitating real situations. Therefore, we conclude that results of quantitative culturing of endotracheal aspirates are reproducible over a 2-day period. If further studies demonstrate good correlation between the results of this technique and those of quantitative cultures from bronchoscopic techniques, analysis of endotracheal aspirates may be an easy, inexpensive, and safe alternative for the diagnosis of ventilator-associated pneumonia that can be performed by a nursing staff or respiratory therapists.

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