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TECHNIQUE

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Rapid identification of *Staphylococcus aureus* in bronchoalveolar lavage fluid using a DNA probe (Accuprobe)

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Abstract *Objective: Staphylococcus aureus* is one of the prominent causative agents of ventilator-associated pneumonia (VAP). Gram staining of bronchoalveolar lavage (BAL) fluid is not always reliable. A nonisotopid probe (Accuprobe) has been developed by Gen-Probe for the specific identification of *S. aureus* isolated from cultures. This study was undertaken to assess the reliability of this probe for the early diagnosis of *S. aureus* VAP.
Design: A prospective study in 120 consecutive patients.
Setting: Department of intensive care medicine at a university hospital.
Patients: 120 ventilated patients (70 males and 50 females; mean age 52±12 years; mean simplified acute physiologic score = 13±4) were studied.
Interventions: 164 bronchoalveolar lavages were performed (none of the patients received prior antibiotic therapy).
Measurements and results: S. aureus was identified 29 times at significant concentrations ($\geq 10^4$ cfu/ml) and 7 times at $<10^4$ cfu/ml. The sensitivity and specificity of the Accuprobe system were 100 and 96%, respectively. We found agreement between quantitative cultures and probes in 96.3% of cases.
Conclusions: We conclude that this probe provides a rapid (≤7 h) and accurate diagnosis of *S. aureus* pulmonary infection.

Key words *Staphylococcus aureus* · Rapid identification · Nosocomial pneumonia · Bronchoalveolar lavage · Protected specimen brush · DNA probe

Introduction

Mechanically ventilated patients in intensive care units (ICU) are commonly afflicted by nosocomial pulmonary infections, which have been acquired either prior to admission or as a complication of mechanical ventilation itself. The exact incidence of the latter has yet to be established, although reports have indicated a range of 9–60% [1, 2]. Unfortunately, the establishment of such a diagnosis continues to be troublesome for various reasons: common clinical signs and symptoms of pneumonia may not be completely reliable (a 30% rate of false positives and false negatives has been reported [3], intubated patients suffer from colonization of the upper airways by gram-negative bacilli and micro-organisms, which lead to contamination during simple methods such as endotracheal aspiration or fiberoptic bronchoaspiration [3], and the specificity and sensitivity of the different diagnostic methods are often reduced by prior antibiotic treatment [4]. Most cases of nosocomial pneumonia are caused by aspiration of small amounts of oropharyngeal and/or gastric secretions that contain high concentrations of aerobic bacteria [4]. Large numbers of patients who do not have bacterial pneumonia are exposed to expensive and ineffective antibiotics that increase the risk of colonization with the potential emergence of multiresistant micro-organisms. In fact, prior antimicrobial therapy increases

the rate of pneumonia due to multiresistant organisms such as *Pseudomonas aeruginosa* or methicillin-resistant staphylococci [1]. Because appropriate antibotic treatment may significantly affect the survival of ventilated patients with pneumonia [2, 5], rapid identification of ICU patients requiring antimicrobial therapy and the selection of appropriate antibiotics are important clinical goals. Nosocomial lower respiratory tract infection can be caused by a large variety of micro-organisms, making it difficult to design empirical antimicrobial therapy. Microscopic analysis of bronchoalveolar lavage (BAL), however, is useful for initial prediction of significant bacterial growth and for directing antibiotic therapy [6].

Staphylococcus aureus is the most common gram-positive pathogen associated with nosocomial pneumonia. A non-isotopic nucleic probe (Accuprobe), has been developed recently by Gen-Probe (Gen-Probe, San Diego, Calif., USA) for the direct, specific 1-h identification of *Staph. aureus* isolated from culture [7].

The present study was undertaken to assess prospective the reliability of the Accuprobe for the early diagnosis of *S. aureus* pulmonary infection. To our knowledge, this is the first evaluation of a DNA probe specific for *S. aureus* on BAL samples.

Patients and methods

All patients had at least 48 h of mechanical ventilation before being included in the study. Inclusion criteria were: fever ($>38.3\,^{\circ}C$) and a new or progressive infiltrate on chest roentgenogram with either leukocytosis ($\geq 10000/mm^3$) or a macroscopically purulent tracheal aspirate.

A total of 120 ventilated patients (70 males and 50 females; mean age 52 ± 12 years; mean simplified acute physiologic score $= 13\pm4$) were studied (7 ± 2 days of prior mechanical ventilation). Between March and September 1992; 164 BALs were performed in a 30-bed medical/surgical intensive care unit of the Lyon University Hospital (none of the patients received antibiotic therapy before bronchoscopy). The fiberoptic bronchoscope (FOB) was inserted through the endotracheal tube via a sterile connector (Double swivel cobb adapter, Peters, Bobigny, France). Suction and injection of lidocaine were avoided. The FOB was positioned next to the orifice of the sampling area to visualize the entrance to the desired bronchial subsegment. The sampling area was selected according to the location of the new or progressive infiltrate on the chest roentgenogram. Hypoxemia and patient ventilator asynchrony were minimized by delivering high fractional inspired oxyen (100%) and providing adequate sedation. BAL was performed by injecting two aliquots of 50 ml sterile normal saline. The fluid was then withdrawn by hand suction into the syringes used for infusion. The BAL fluid was filtered, pooled, and divided into two samples for bacteriologic and cytologic analysis. The samples were transported to the laboratories within 30 min of collection. A 5-ml sample BAL fluid was taken for the processing of the Accuprobe system. The BAL fluid amount of recovered was 28 ± 6 ml.

Cytology

One aliquot of BAL fluid was cytocentrifuged for 5 min at 600 rpm. The air-dried slides were stained using May-Grünwald-Giemsa stain. Differential cell counts were performed on 400 cells. BAL samples were considered technically invalid and excluded when more than 5% of the cells counted were ciliated bronchial cells.

Quantitative cultures

BAL fluid was homogenized using repeared aspirations with a Pasteur pipette. Serial dilutions (1/100, 1/1000, 1/10000, 1/100000) of each sample were prepated in saline. Diluted specimens were placed on culture media 30 min after collection; 0.1 ml of each sample dilution was inoculated into the following agar culture media: 5% sheep's blood, chocolate, McConkey, Buffer Charcoal Yeast Extract Agar, and Sabouraud-dextrose. Plates were evaluated for growth at 48 h and discarded after 5 days, except Sabouraud, which was evaluated at 4 weeks. Gram and Ziehl-Neelsen staining was performed using undiluted samples. All microorganisms isolated were identified by standard laboratory methods [8]. There was no screening for *Mycoplasma* or viruses. Cultures were classified as positive ($\geq 10^4$ cfu/ml) or negative. Growth below 10^4 cfu/ml was called insignificant or negative [9]. The gram stain was defined as negative if no organisms were seen. The gram stain was considered positive only if the identified organism correlated with the morphology of the organism grown in significant concentrations in BAL.

Probe processing

A 1-ml sample of BAL fluid was cultured in 4 ml of enrichment brain-heart broth for 6 h at 37 °C with agitation. The culture was centrifuged for 15 min at 4000 *g*. The resulting supernatant was discarded and a 0.01-ml loopful of pellet was tested according to the Gen-Probe procedure.

The Accuprobe system (Gen-probe, San Diego, Calif., USA, Cat No. 2875) uses a chemiluminescent labeled, single stranded DNA probe, which is complementary to the ribosomal RNA of the target organism. After release of the ribosomal RNA from the organism, the labelled DNA probe combines with the ribosomal RNA of the target organism to from a stable DNA : RNA hybrid. The selection reagent allows for the differentiation of nonhybridized and hybridized probes. The labeled DNA : RNA hybrids are measured in a luminometer. A positive results is a luminometer reading equal to or greater than the cut-off (50000 related light units). A value less than the cut-off is considered negative.

Diagnosis of bacterial pneumonia

Pneumonia was diagnosed when there were new persistent infiltrates and purulent tracheal secretions with a positive quantitative culture of BAL fluid and an improvement in clinical status with appropriate antibiotic therapy.

The absence of pneumonia was indicated by the lack of significant growth on BAL fluid and resolution of clinical and radiographic infiltrates without antibiotic therapy or when an alternative diagnosis was established. *S. aureus* nosocomial pneumonia was diagnosed when this pathogen was isolated, and showed significant growth in culture, from a patient with clinical features of pneumonia.

Statistical analysis

The results are expressed as mean$\pm$standard deviation. The sensitivity, specificity, and predictive values were calculated according to standard formulas [10].

Results

Patients

The indications for ICU admission were medical ($n = 38$), trauma ($n = 60$), and postoperative care ($n = 22$). Seventy-eight cases of bacterial pneumonia were identified.

Cytologic data

In all cases bronchial contamination was ruled out by the inclusion criteria of $\leq 5\%$ ciliated cells. In our study, the BAL fluid of all patients contained less than 1% ciliated bronchial cells.

Bacteriologic data

A total of 86 samples were considered sterile or insignificant. Seventy-eight bacteria were isolated in numerically significant concentrations form the BAL samples ($\geq 10^4$ cfu/ml) as follows: 30 cases with a gram-negative bacilli $\geq 10^4$ cfu/ml; 38 cases with a gram-positive bacteria $\geq 10^4$ cfu/ml; 10 cases with a mixed sample (gram-positive bacteria + gram-negative bacilli); *S. aureus* was identified 29 times in numerically significant concentrations. We found seven cases with *S. aureus* $< 10^4$ cfu/ml.

Reliability of the Accuprobe system

In all of the cases with *S. aureus* $\geq 10^4$ cfu/ml, Accuprobe was positive (Table 1). The probe was negative in all the other cases, even when *S. aureus* was present at $< 10^4$ cfu/ml.

Table 1 Reliability of the Accuprobe system

	Probe+	Probe−
S. aureus $\geq 10^4$ cfu/ml	29 (17.7%)	0 (0%)
No *S. aureus* $\geq 10^4$ cfu/ml	**6 (3.7%)**	129 (78.6%)

In six cases we noted (3.7%) discrepancies probe+, culture−) (Table 2) between culture and probe results: in two cases, the fluid was hemorrhagic, in one case *S. aureus* grew in the BAL sample 21 days before bronchoscopy and again 21 days after; in the last two instances the discrepancy (in the same patient) was still "unexplained".

The overall sensitivity was 100% and the specificity, 96%. The positive and negative predictive values were, respectively, 83 and 100%. Agreement with cultures was observed in 96.3% of all cases.

Reliability of the gram stain in identifying *S. aureus*

Gram stain was positive in only 55% of samples with *S. aureus* in a significant concentration.

Discussion

Nosocomial pneumonia is associated with a very high mortality in patients receiving mechanical ventilation, and prolongs the length of stay in the ICU, thus imposing a significant economic burden. This excess mortality is independent of the underlying disease and of the initial severity of the illness.

Table 2 The six cases presenting discrepancies between cultures and probe results

Patient no.	Gram stain	Cytology	Culture ($\geq 10^4$ cfu/ml)	Probe cutoff = 50000
1	Gram negative bacilli	Hemorrhage Polymorphonuclear leukocytes + + +	*P. aeruginosa*, *Serratia marcescens*	167452
2	Gram negative bacilli	Hemorrhage	*S. marcescens*	181149
3	No germs	Few cells Polymorphonuclear leukocytes +	*Haemophilus influenzae* *Candida albicans*	129259 (21 days before *S. aureus* pneumonia)
4.1	No germs	Polymorphonuclear leukocytes + + +	Sterile	289682
4.2	No germs	Polymorphonuclear leukocytes + + +	Sterile	297302
5	Gram positive cocci	Polymorphonuclear leukocytes + + +	Sterile	484163 (21 days after *S. aureus* pneumonia)

Clinical features, radiographic finding, and analysis of tracheal aspirate are of little value in distinguishing ventilated patients with pneumonia [6]. Secretions obtained using BAL evaluated by quantitative cultures are useful in distinguishing patients with and without pneumonia [6, 9]. However, results are not available immediately, and a significant delay may affect the care of the critically ill patient. In fact, in patients with pneumonia, recognition of the causative organism is important in order to institute rational antimicrobial therapy. Two studies [2, 5] have show, using multivariate analysis, that appropriate antibiotic treatment may significantly improve the survival of ventilated patients with pneumonia. In these two studies, combined mortality rates of patients with appropriate and inappropriate treatment were 28 and 64%, respectively. Moreover, the use of empirical broad-spectrum antimicrobial therapy in patients without infection is potentially harmful, because it facilitates colonization and superinfections with more virulent organisms [1]. Combination antibiotic regimens, including betalactams and aminoglycosides, are considered as standard therapy.

Gram-positive cocci account for 20–30% of the pathogens of which *S. aureus* in the organism most frequently found [11]. In 1992, *S. aureus* represented 25% of the strains isolated in pulmonary samples in our ICU. It was the second most frequently detected organism after *P. aeruginosa*.

Many investigators have reported a close correlation between the findings of the Gram stain of the BAL sample and the results of quantitative cultures [12, 13]. However, for other investigators, the reliability of the Gram stain is debatable. In the study by Rello et al. [14], Gram staining indicated the final bacteriological diagnosis in only 46% of the cases. Our results – 55% – are comparable.

S. aureus nosocomial pneumonia has become an important infection not only because of an apparently increasing incidence, but also because of its high mortality [15]. Since 1988, we have observed a high incidence of *S. aureus* as a cause of nosocomial pneumonia in our ICU. Fagon et al. [1] observed that the mortality caused by *P. aeruginosa, Acinetobacter,* and *S. aureus* was greater than that of all other types of ventilator-associated pneumonia.

Laboratory diagnosis of *S. aureus* infection is usually performed by microscopic examination and cultures of clinical specimens. The DNA probes have, in recent years, found applications in routine microbiology [16]. Rapid identification of *S. aureus* can be performed by using DNA probe assay. The Accuprobe (Gen-Probe, San Diego, Calif.) *S. aureus* culture indentification is a chemiluminescent acridinium ester labeled DNA probe which utilized the technique of nucleic acid hybridization for the rapid identification of *S. aureus*. Accuprobe detects *S. aureus* in BAL samples much earlier (< 7 h) than conventional cultures. Reagents used to perform an Accuprobe assay cost approximately $7. In BAL, a colony count of 10^4 cfu/ml represents $10^5 - 10^6$ bacteria/ml in the original specimen. Studies in nonventilated patients have shown $10^4 - 10^5$ cfu/ml to be an appropriate cut-off to differentiate contamination from infection. The sensitivity of this probe in a liquid medium is about 10^6 cfu/ml. This level is not sufficient to detect *S. aureus* in BAL. A 6-h preculture was performed to allow detection at the expected level. The agreement between quantitative cultures and the threshold of the probe detection was previously assessed.

After comparing the probe results with the results of quantitative cultures, we obtained 96.3% agreement. We found six cases with discrepancies between positive probes and negative or insignificant quantitative cultures of BAL samples. Two BAL samples were hemorrhagic. Hemorrhage is an occasional complication of bronchoscopy, but the effects of hemorrhage on culture results have not been reported. However, the presence of blood in BAL could affect the reliability of this probe. In two other cases, *S. aureus* was isolated in the lung, 21 days before and 21 days after BAL. These two cases underscore the problem of the reliability of bacteriological findings. In the same patient, we found discrepancies between probe and culture results. This may be explained by hybridization with other substances (case 4.1 and 4.2 in Table 2).

Our findings suggest that the Accuprobe allows a rapid and accurate diagnosis of *S. aureus* ventilator-associated pneumonia. The Accuprobe *S. aureus* test offers a rapid, objective method for the definitive identification of *S. aureus* based on the detection of specific ribosomal RNA sequences that are unique to *S. aureus*.

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