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Effects of antibiotics on protected specimen brush sampling in ventilator-associated pneumonia

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Effects of antibiotics on protected specimen brush sampling in ventilator-associated pneumonia. E. Prats, J. Dorca, M. Pujol, L. Garcia, B. Barreiro, R. Verdaguer, F. Gudiol, F. Manresa. ©ERS Journals Ltd 2002.

ABSTRACT: The effects of antibiotic treatment on the results of protected specimen brushing (PSB) in ventilator-associated pneumonia were prospectively assessed by performing this procedure before antibiotic treatment, and 12, 24, 48 and 72 h after initiation of antibiotic treatment, in 35 ventilated patients who developed pneumonia during mechanical ventilation.

The number of micro-organisms isolated, their concentration (colony-forming units (cfu)·mL^{-1}), and the number of cases with a positive PSB ($\geq 10^3$ cfu·mL^{-1}) were evaluated.

Within 12 h of the initiation of effective antibiotic treatment a rapid, significant decrease in the numbers of organisms isolated, their individual concentrations and the percentage of positive PSB results were observed. Certain bacterial species (*Streptococcus pneumoniae*, *Haemophilus influenzee*) appeared to be more vulnerable to antibiotics than others (Staphylococcus aureus, *Pseudomonas aeruginosa*, *Acinetobacter baumanni*).

This data confirms that prior antibiotic treatment, even after only a few hours of activity, significantly decreases the sensitivity of protected brush specimen; this effect appears to be particularly marked among the species involved in early ventilator associated pneumonia.

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During the last two decades many studies have analysed the potential usefulness of distal bronchial sampling for microbiological quantitative culture in order to guide antibiotic treatment in patients suffering from ventilator-associated pneumonia (VAP). The conclusions of these reports differ widely and, consequently, the debate on the diagnostic efficacy of the technique, its therapeutic value and, in particular, its influence on outcome, is still open [1–3].

The fact that prior antibiotic treatment may produce false-negative or false-positive results in bacterial cultures is well recognized [4, 5]. In spite of its great importance, this clinical issue has not been appropriately assessed. Most of the major series published to date include large numbers of patients already receiving antibiotics when the procedures were performed, and details concerning the nature and duration of these treatments are generally scarce. It seems logical to argue that "prior antibiotics" does not represent a homogeneous situation since it may include, for instance, an antibiotic regimen initiated before the development of pneumonia for the treatment of a different infection, or the use of antibiotic treatment immediately after the diagnosis of pneumonia but several hours before bronchial sampling is performed.

The possible influence of prior antibiotics on quantitative bacterial cultures of distal bronchial samples has been analysed retrospectively in several studies [5–7], as well as in occasional prospective series including small numbers of cases [8, 9]. According to the data available, antibiotics seem to decrease bacterial numbers in bronchial samples, producing false-negative results.

Nevertheless, the precise influence of antibiotics on quantative bacterial cultures has not been adequately reported previously. This is, without doubt, a critical issue because the major advantage of any diagnostic procedure, in terms of therapeutic management, is to rule out disease and thus prevent unnecessary treatment. In the context of VAP, if prior antibiotics significantly increase the number of false-negative results, their therapeutic value would be questionable. Furthermore, the epidemiological data reported in series, which included large proportions of patients on antibiotics may be biased because these studies may have underestimated the more vulnerable bacteria.

The aim of this study was to prospective analyse the influence of effective antibiotic treatment on bronchoscopic protected specimen brush (PSB) quantitative cultures by serial bronchial sampling after administration of antibiotics had been initiated.

Methods

Inclusion criteria

During a 24-month period a prospective study of mechanically-ventilated patients with a clinical suspicion of pneumonia was carried out in a 30-bed general intensive care unit (ICU) at a 1,000-bed teaching hospital. Patients were considered for inclusion in the study if they fulfilled all the following criteria: 1) mechanical ventilation for at least 48 h before the development of pneumonia; 2) presence of macroscopically purulent bronchial secretions; 3) appearance of a new infiltrate on chest radiography; 4) no change in antibiotic strategy during the 3 days preceding the diagnostic procedure; 5) no selective digestive decontamination or endotracheal antibiotics administered during hospitalization. Subjects were included in the study definitively after at least one bacterial species was cultured in concentrations $\geqslant 1\times10^4$ colony forming units (cfu)·mL^{-1} in PSB samples, and the effectiveness of the empirical antibiotic treatment was confirmed by sensitivity testing (fig. 1). For the purpose of the study a limit of 1×10^4 cfu·mL^{-1} was used instead of the conventional 1×10^3 cfu·mL^{-1} only as an inclusion criterion, in order to avoid borderline episodes as well as to allow a certain margin in the assessment of the change in the concentration. Informed consent was obtained from relatives of the patients and the study protocol was approved by the authors institution's ethical committee for clinical research.

Study protocol

Bacteriological follow-up of the cases was carried out by repeated PSB during the first 72 h of antibiotic therapy. The first bronchoscopy (PSB1) was performed before a new and empirical antibiotic treatment was started in all cases. Patients were premedicated intravenously with midazolam and a short-acting paralytic agent (pancuronium bromide) 10 min before the procedure, but topical anaesthesia was not used. Then, without stopping mechanical ventilation a fibreoptic bronchoscope (Olympus BF1T30D; Olympus, New Hyde Park, NY, USA) was introduced through a special adapter and advanced without suction to the bronchial orifice of a lung segment identified radiologically as the one containing the new infiltrate. The PSB (Microbiology Brush; Mill-Rose Lab Inn, OH, USA) was then advanced to a subsegmental, peripheral position. After dislodging the distal plug to obtain lower airway secretions for microbial cultures, the brush was retracted into the inner cannula, and the whole unit was removed from the bronchoscope. Finally a sample of bronchial secretions was collected for Gram-staining by direct suction through the inner channel of the bronchoscope. PSB brush was cut aseptically into a sterile tube containing 1 mL of Ringer's solution and vortexed for 1 min. Specimens were immediately sent to the laboratory for quantitative cultures. Two calibrated loops of 0.01 and 0.001 mL were used to plate the samples onto blood agar, chocolate agar medium, McConkey agar, and Sabouraud medium. Bacterial growth was expressed as cfu·mL^{-1}. Successive PSBs were performed by the same operator in the same subsegment of the lung at 12 (PSB2), 24 (PSB3), 48 (PSB4) and 72 h (PSB5) after the beginning of antimicrobial treatment.

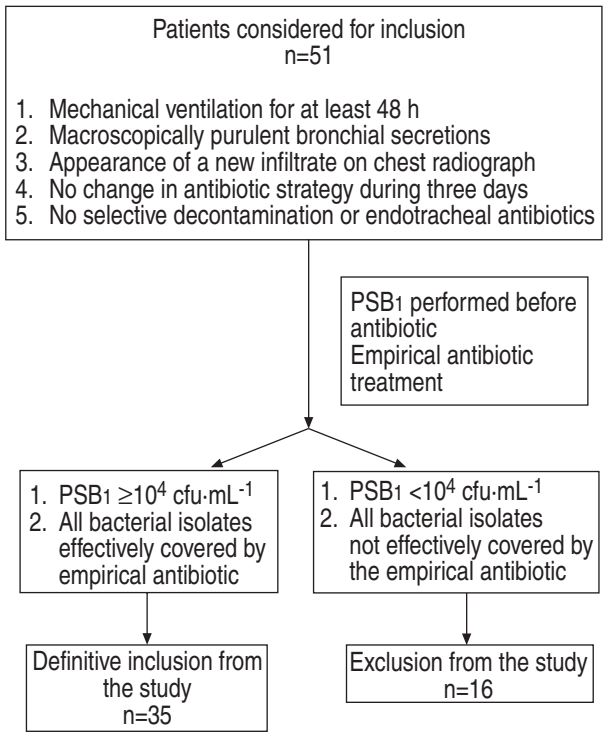

Fig. 1. – Trial profile. PSB: protected specimen brush.

Empirical treatment of patients

Immediately after the first bronchoscopy was performed, empirical antibiotic treatment was started. The choice of antibiotics was left to the discretion of each attending physician in the context of current recommendations for the management of nosocomial pneumonia at the authors' institution. Antibiotic treatment for early-onset pneumonia was amoycillin-clavulanate or a second-generation cephalosporin plus cloxacillin and for late-onset pneumonia the regimen included an antipseudomonal betalactam plus tobramycin. This treatment was occasionally modified on the basis of initial microbiological data such as Gram-staining of bronchial secretions.

Data collection

The following clinical variables were recorded: age, sex, severity of underlying medical condition stratified according to the criteria of McCabe and Jackson [10], Acute Physiological and Chronic Health Evaluation (APACHE) II score on admission to the ICU, indications for ventilatory support, prior antimicrobial therapy at the time of examination, duration of mechanical ventilation temperature, white blood cell count ($1\times10\cdot L^{-1}$), oxygen tension in arterial blood/inspiratory oxygen fraction ratio, a radiological

score determined by modification of the technique described by WEINBERG *et al.* [11] and antibiotic prescribed in the current episode of pneumonia. To evaluate the bacteriological evolution the following parameters in each PSB sample were analysed: number of micro-organisms isolated, number of cfu·mL^{-1} and number of cases with a positive PSB culture ($\geq 1\times10^3$ cfu·mL^{-1}).

Statistical analysis

Descriptive analysis was performed and results were expressed as mean±SD or as a fraction of total. Means were compared using Wilcoxon's rank-sum test. McNemar's test was used to determine the statistical significance of differences for binomial paired samples and the Chi-squared test was used for categorical variables. Differences between groups were considered to be significant at $p<0.05$.

Results

Population studied

Thirty-five patients (29 males and 6 females) were included definitively (fig. 1). Mean age was 46±18 yrs (range, 16–79) and the average number of ventilator days was 4.6±4 (range, 2–19). In 26 (72%) cases, pneumonia developed during the first 4 days of mechanical ventilation. Other demographic and clinical characteristics of the patients are shown in table 1. The primary indication for ventilatory support was closed head injury in 48% of cases, other neurological emergencies (20%), postoperative respiratory failure (14%), multiple trauma (9%), cardiac failure (6%) and exacerbation of chronic obstructive pulmonary disease (3%).

Twenty-eight patients (77%) had not been receiving antimicrobial therapy prior to the study. The other seven patients were receiving antibiotic therapy for a previous infection for at least 3 days. The antibiotics prescribed were: cephalosporin (n=4), penicillin (n=1), imipenem (n=1) and amoxycillin-clavulanate (n=1). The empirical antibiotic treatment prescribed in the cases studied is summarized in table 2. A single antibiotic was used in 22 cases and a combination of two drugs in the others. The overall hospital mortality rate for the study group was 38% (14/35 patients). Seventy-one per cent of all deaths were due to causes other than VAP; 11 suffered early VAP and most of them died from neurological causes (closed-head injury, neurological emergency). The mean time from diagnosis of VAP to death was 8.3±5 days for the 14 patients, for those dying with early-VAP the time was 5.8±2 days.

Five of the 35 patients included could not complete the study. In three cases the patients died before the study was finished and the other two were excluded due to contraindications to bronchoscopy during the follow-up (severe hypoxaemia or haemodynamic deterioration). However, in all cases the results of at least the first 24 h of antibiotic treatment were recorded and therefore they were taken into consideration. Although, 5 serial PSB were performed in each patient, side-effects were unusual and only in five cases was limited bronchial bleeding reported.

Table 1. – Clinical characteristics of the patients

Patient characteristics	
Age yrs	46±17 (16–79)
APACHE score	13.1±4.5 (5–23)
Severity of underlying disease	
Fatal or ultimately fatal within 5 yrs	8
Nonfatal	27
Duration of prior ventilation days	4.6±4 (2–19)
Temperature °C	38.4±0.4 (38–39.3)
WBC $10^9\cdot L^{-1}$	12.1±3.4 (5.6–18.6)
$P_{a,O_2}/F_{i,O_2}$	217±76 (87–382)
Radiological score	3.89±1.3 (2–7)

Data are presented as mean±SD with range in parentheses unless otherwise stated. APACHE: Acute Physiological and Chronic Health Evaluation; WBC: white blood cell; P_{a,O_2}: oxygen tension in arterial blood; F_{i,O_2}: inspiratory oxygen fraction.

Table 2. – Antibiotics prescribed in the studied episodes of ventilator-associated pneumonia (VAP)

	Early-onset pneumonia	Late-onset pneumonia
Amoxycillin-clavulanate	18	1
Imipenem		3
Imipenem+Tobramycin#	1	3
Cefuroxime+Cloxacillin	4	
Ceftazidime+Tobramycin#	1	2
Piperacillin-tazobactam+ Tobramycin#	2	

#: VAP presented on the fourth day of mechanical ventilation and the attending physician considered that combined antibiotherapy usually prescribed in late-onset pneumonia was required.

Initial protected specimen brush culture results

A total of 66 micro-organisms were isolated in concentrations $\geq 1\times10^4$ cfu·mL^{-1} among PSB1 samples (table 3). Twenty-eight were Gram-positive bacteria and 38 were Gram-negative. More than one isolate was recovered in 24 (66%) of the patients. The most commonly isolated species was *Haemophilus influenzae* (n=17), followed by *Streptococcus pneumoniae* (n=12) and *Staphylococcus aureus* (n=12), most of them in polymicrobial isolates. All the pathogens recovered from the initial PSB in the seven patients who had received antibiotics before the study were resistant to these antibiotics (table 4). These organisms were: *Acinetobacter baumannii* (five cases), *Pseudomonas aeruginosa* (two cases), and *Proteus mirabilis* (one case).

Results of serial protected specimen brush cultures

In the analysis of serial PSB and according to standard recommendations [2, 5], bacterial species

Table 3. – Bacterial species recovered from the first protected specimen brush (PSB) samples at numerically significant concentrations

Organism	Total	Monomicrobial isolates	Polymicrobial isolates
Gram-positive bacteria	28		
Streptococcus pneumoniae	12	1	11
Other *Streptococci*	3		3
Staphylococcus aureus	12	1	11
Corynebacterium equii	1		1
Gram-negative bacteria	38		
Haemophilus influenzae	17	3	14
Acinetobacter baumannii	7	5	2
Pseudomonas aeruginosa	5	2	3
Escherichia coli	4	1	3
Proteus mirabilis	2		2
Citrobacter spp.	1		1
Klebsiella oxytoca	1		1
Moraxella catarrhalis	1		1

Numerically significant concentrations in PSB samples were $\geqslant 1\times10^3$ cfu·mL^{-1}.

Table 4. – Details of patients on antibiotics before protected specimen brush (PSB) sampling

Indication	Antibiotic treatment	Duration of prior antibiotic days	Aetiological micro-organisms of VAP	Initial PSB1 concentrations cfu·mL^{-1}
Meningitis	Penicillin	5	*Pseudomonas aeruginosa*	1×10^5
Abdominal infection	Imipenem	5	*Proteus mirabilis*+	1×10^5
			Acinetobacter baumannii	1×10^5
Postoperative prophylaxis	Cefuroxime	4	*Acinetobacter baumannii*	7×10^4
Bronchial superinfection	Amoxicllin-clavulanate	4	*Acinetobacter baumannii*	1×10^5
Postoperative prophylaxis	Cefuroxime	4	*Pseudomonas aeruginosa*	1×10^5
Postoperative prophylaxis	Cefuroxime	5	*Acinetobacter baumannii*	8×10^4
Bronchial superinfection	Cefuroxime	3	*Acinetobacter baumannii*	1×10^5

VAP: ventilator-associated pneumonia.

cultured in a concentration of $\geqslant 1\times10^3$ cfu·mL^{-1} were accepted as significant bacterial isolate and consequently a positive PSB result. From the initial 66 (100%) micro-organisms isolated in PSB1 samples, 34 (50%) were recovered by PSB2 cultures, 23 (34%) from PSB3, 9 (13%) from PSB4 and finally only 2 (4%) of the initial pathogens persisted at high concentrations in the PSB5 culture (fig. 2). Accordingly, the number of positive PSBs steeply declined from the initial 35 positive to 25 at 12 h, 19 at 24 h, 7 at 48 h and only 2 at 72 h (fig. 3).

Parameters reflecting the bacterial load, such as number of colony counts of each individual micro-organism also decreased significantly after the introduction of antibiotic therapy. The mean number of colony counts decreased from 80,300±35,200 cfu·mL^{-1}

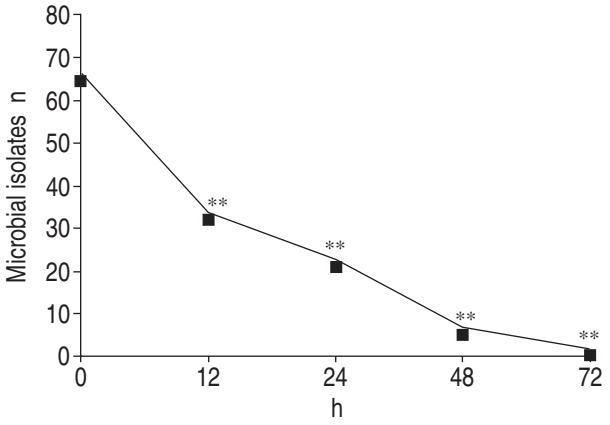

Fig. 2. – Evolution of microbial isolates grown in significant concentrations during the first 72 h of antibiotic treatment. **: p<0.01 (McNemar's test).

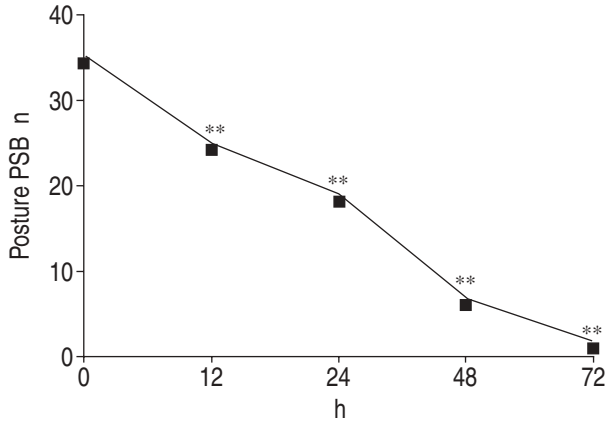

Fig. 3. – Evolution of positive protected specimen brush (PSB) samples during the first 72 h of antibiotic treatment. **: p<0.01 (McNemar's test).

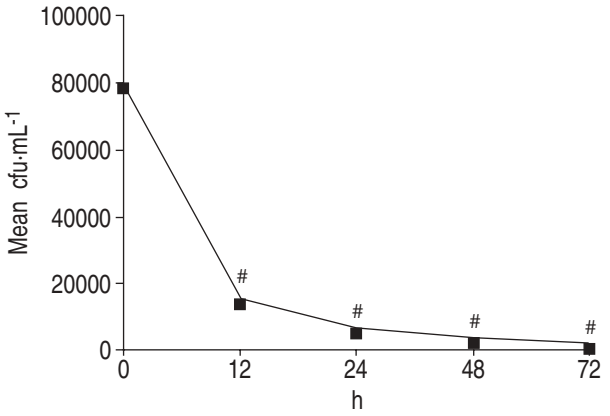

Fig. 4. – Evolution of number of colony forming units (cfu)·mL^{-1} of individual bacteria during the first 72 h of antibiotic treatment. #: p<0.00001 (Wilcoxon test).

in the PSB1 to 15,500±27,130 cfu·mL^{-1} in the PSB2 samples (p<0.0001). In general, bacterial counts decreased sharply during the first 12–24 h of the study (fig. 4).

The trend of the change observed in PSB results seems to be related to the nature of the microbiological isolates. *H. influenzae* and *S. pneumoniae* isolates decreased rapidly after the administration of effective antibiotic therapy. These two pathogens accounted for 42% of all the micro-organisms isolated. During the first 12 h of antibiotic treatment, the number of isolates halved compared with the initial cultures, and completely disappeared after 48 h. However, *S. aureus* and certain Gram-negative bacilli such as *A. baumannli* and *P. aeruginosa* showed a slower rate of decrease during the study period (p<0.05). After 48–72 h of the antibiotic therapy some of these pathogens persisted at numerically significant concentrations (1×10^3 cfu·mL^{-1}). The evolution of the most frequently isolated organisms is shown in figure 5.

Finally, in four cases (11 %) follow-up PSB cultures showed the appearance of five new species, four at numerically significant concentrations and one at low concentration $<1\times10^3$ cfu·mL^{-1} (table 5). These new emerging species were: *S. aureus* methicillin resistant (n=1), *A. baumannli* (n=2) *Enterobacter aerogenes* (n=1), *Serratia marcescens* (n=1) and *P. aeruginosa* (n=1). Resistance to the initial antimicrobial agents was documented in all cases.

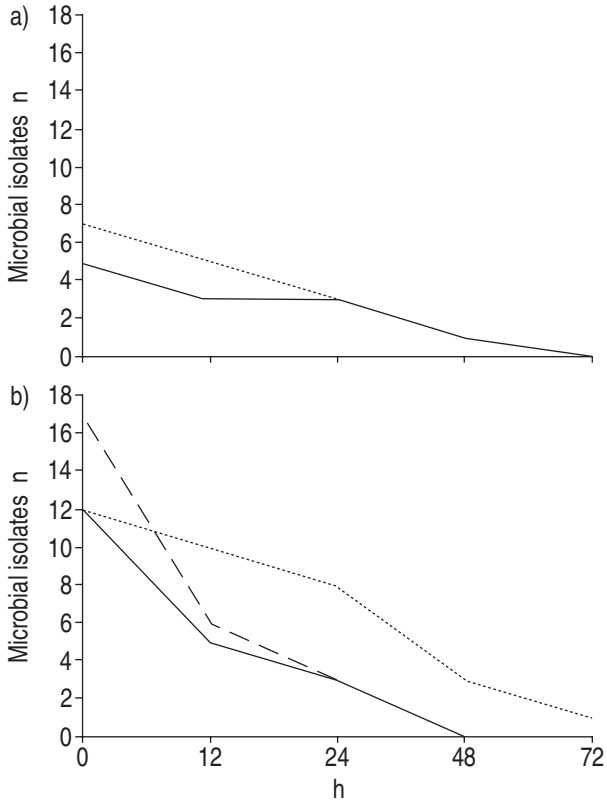

Fig. 5. – Evolution of the most commonly isolated species. Showing in a) *Acinetobacter baumannii* (·····) and *Pseudomonas aeruginosa* (——) and in b) *Haemophilus influenza* (– – –), *Staphylococcus aureus* (·····) and *Streptococcus pneumoniae* (——).

Discussion

This study confirms that antibiotics can have a marked effect on PSB results, even after a short period of time. According to this data, after only 12 h of effective antibiotic treatment, PSB missed 28% of VAP episodes. In addition, among the remaining positive cases, the procedure missed 50% of the species involved, and as predicted this trend increased in later samples. The potential implications of these findings for the management of VAP are obvious.

To date, only a few reports in the literature have specifically analysed the effect of an adequate antibiotic therapy on the results of PSB, and most of these studies have demonstrated a decrease in its

Table 5. – New pathogens recovered during follow-up

Aetiological micro-organisms of VAP	Antibiotic treatment of VAP	Aetiologies of superinfection	Time of superinfection h	PSB cfu·mL^{-1}
Haemophilus influenzae	Amoxycillin clavulanate	MRSA	48 (PSB4)	7×10^4
Moraxella catarrhalis	Amoxycillin clavulanate	*Enterobacter Aerogenes*	72 (PSB5)	3×10^4
Staphylococcus aureus		*Acinetobacter baumannii*		7×10^3
Streptococcus pneumoniae	Amoxycillin clavulanate	*Acinetobacter baumannii*	24 (PSB3)	8×10^3
		Pseudomonas aeruginosa		1×10^3
Haemophilus influenzae	Amoxycillin clavulanate	*Serratia marcescans*	48 (PSB4)	3×10^2

VAP: ventilator-associated pneumonia; PSB: protected specimen brush; MRSA: methicillin-resistant *Staphylococcus aureus*.

efficacy, with a fall in sensitivity to <60% in patients already receiving antibiotics [1]. These data have been also confirmed by results obtained in quantitative cultures of samples of pulmonary tissue recovered immediately *post mortem* [5, 6]. These studies have shown that in the context of adequate antibiotic therapy, the culture of bronchopulmonary samples obtained by PSB or bronchoalveolar lavage, as well as that of lung tissue of most patients with histological evidence of VAP gives negative results or very low quantitative cultures. The period of time required to eradicate a sensitive micro-organism from the lung is not known, but according to the results of MONTRAVERS *et al*. [8], 3 days of antibiotic therapy can eliminate most of the micro-organisms present at the beginning of the infection. GARRAD and A'COURT. [12] and BLAVIA *et al.* [9] obtained similar results using serial cultures of different respiratory samples from patients with pulmonary infections. In support of the authors' previous data [13], the present study showed that once adequate empirical antibiotic therapy had been introduced, bacterial concentrations in respiratory samples rapidly decreased. Thus, after just 12 h of antibiotic treatment, 28% of all the cultures obtained by PSB in patients with pneumonia became negative, and after 24 h this figure reached 46%. These data point to the need to obtain respiratory samples well before the introduction of the antibiotic; otherwise, the threshold figure of 1,000 cfu·mL^{-1} loses its discriminatory value. Some authors [7, 14] have suggested lowering this cut-off point, *i.e.* the diagnostic threshold of the bronchoscopic techniques, in order to maintain the diagnostic accuracy of these endoscopic procedures. According to SOUWEINE *et al.* [7], in patients who have received empirically appropriate antibiotic therapy the reduction of the threshold figure to 100 cfu·mL^{-1} would increase the sensitivity of the procedures without modifying their specificity. In contrast, in a case in which a patient with pneumonia being treated with antibiotics suffers a pulmonary superinfection, the micro-organisms responsible for this second infection will be resistant to the first antibiotic and hence the PSB cultures will be positive [8, 15].

This study could be criticized for lacking a definitive diagnostic technique such as a histological sample of lung tissue. According to the guidelines of the American College of Chest Physicians [16], all the cases in this study should be considered as "probable pneumonia". However, in the absence of previous antibiotic treatment a very good correlation has been observed between the criteria for the histological diagnosis of pneumonia and positive quantitative cultures of samples of lung tissue, as well as those obtained by PSB [7, 15]. In this study, the patients were not receiving antibiotics before they suffered pneumonia, or if they were, pneumonia appeared several days after an empirical antibiotic regimen had been started for other reasons, and PSB was carried out before antibiotics were modified. Therefore, in these cases the threshold figure of 1,000 cfu·mL^{-1} can be accepted as a valid indicator of the presence of infection. Similarly, in all the cases clinical follow-up allowed us to evaluate the clinical and radiological evolution of the pulmonary infection and rule out alternative causes for fever and pulmonary infiltrates.

The aetiological spectrum of pneumonia in these patients may differ from other reports which analysed the efficacy of PSB in the aetiological diagnosis of pneumonia [17–20]. According to the present authors' experience as well as that of other groups, the timing of the presentation of the pulmonary infection and the absence of previous antibiotics modulate the involvement of different causative micro-organisms in the pneumonia. In 64% of cases described here the pneumonia appeared during the first 4 days of mechanical ventilation, and were therefore early pneumonias [21]. In these episodes, the infection is the consequence of aspiration or introduction of micro-organisms present in the oropharynx during the endotracheal intubation. The most frequently observed micro-organisms in early pneumonia are *S. pneumoniae*, *S. aureus*, and *H. influenzae*, present in 62% of the isolates in this study. As a consequence, the pathogenesis, and therefore the aetiological spectrum, must be different from those in late pneumonia [22]. As far as the pneumococcus is concerned, these results appear to be in accordance with those of other series dealing with severe community acquired pneumonia [23, 24], where the authors emphasize that *S. pneumoniae* was seldom isolated in patients already receiving antibiotics. Conversely, late-onset pneumonia that occurs after 4 days of mechanical ventilation is more commonly caused by *P. aeruginosa* and multiresistant Gram-negative bacilli. It is well known that previous broad-spectrum antibiotic therapy facilitates the presentation of highly resistant organisms [25], as was observed in 30% of patients in this study.

In this series, the impact of antibiotics on the results of the cultures of the PSB samples was not homogeneous for all species. Most organisms responsible for early pneumonia, such as *S. pneumoniae* and *H. influenzae* disappear rapidly from the serial cultures of PSB samples. In contrast, other organisms such as *S. aureus*, *P. aeruginosa* or *A. baumannii* are more difficult to eradicate and therefore can still be cultivated from samples even after 48 h of adequate antibiotic treatment. These results corroborate the data of GARRAD and A'COURT [12] who demonstrated the persistence of *P. aeruginosa* in respiratory secretions several days after adequate treatment, suggesting that the behaviour of this organism may be different from other species. Similar results were obtained by SMITH *et al.* [26] studying an animal model of polymicrobial pneumonia caused by *S. aureus* and *S. pneumoniae*. Although amoxycillin-clavulanate was effective against these micro-organisms, SMITH *et al.* [26] observed that the number of colonies of *S. aureus* cultured from the pulmonary tissue decreased more slowly than in the case of the pneumococcus. The causes and the clinical implications of the persistence of the organisms hours or even days after the introduction of an empirically appropriate antibiotic are not known.

Today, distal bronchial sampling methods such as PSB are no longer considered mandatory for the

microbiological diagnosis of VAP, considering that quantitative cultures of proximal secretions have demonstrated an acceptable degree of correlation with these more sophisticated procedures [27, 28]. Nevertheless, the present authors' experience strongly suggests that this simpler approach may also be influenced by antibiotics.

According to the data obtained in this study, many of the figures on diagnostic efficacy of distal bronchial sampling procedures reported in the literature may be considered as inaccurate. Protected specimen brush has a poor diagnostic value in ventilator-associated pneumonia patients already receiving antibiotics, even for a few hours, when the samples are collected. This is particularly true in cases of early pneumonia, because the most prevalent organisms in this context, *Streptococcus pneumoniae* and *Haemophilus influenzae*, are extremely vulnerable to the effect of antibiotics.

References

1. Niederman MS, Torres A, Summer A. Invasive diagnostic testing is not needed routinely to manage suspected ventilator-associated pneumonia. *Am J Respir Crit Care Med* 1994; 150: 565–569.
2. 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.
3. Sanchez-Nieto JM, Torres A, Garcia-Cordoba F, *et al.* Impact of invasive and noninvasive quantitative culture sampling on outcome of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 1998; 157: 371–376.
4. Chastre J, Viau F, Brun P, *et al.* Prospective evaluation of the protected specimen brush for the diagnosis of pulmonary infections in ventilated patients. *Am Rev Respir Dis* 1984; 130: 924–929.
5. Torres A, El-Ebiary M, Padro L, *et al.* Validation of different techniques for the diagnosis of ventilator associated pneumonia. *Am J Respir Crit Care Med* 1994; 149: 324–331.
6. Chastre J, Fagon JY, Bornet-Lecso M, *et al.* Evaluation of bronchoscopic techniques for the diagnosis of nosocomial pneumonia. *Am J Respir Crit Care Med* 1995; 152: 231–240.
7. Souweine B, Veber B, Bedos JP, *et al.* Diagnostic accuracy of protected specimen brush and bronchoalveolar ravage in nosocomial pneumonia: Impact of previous antimicrobial treatments. *Crit Care Med* 1998; 39: 236–244.
8. Montravers P, Fagon PJ, Chastre J, *et al.* Follow-up protected spedmen brushes to assess treatment in nosocomial pneumonia. *Am Rev Respir Dis* 1993; 147: 33–34.
9. Blavia R, Dorca J, Verdaguer R, Carratala J, Gudiol F, Manresa F. Bacteriological follow-up of nosocomial pneumonia by successive protected specimen brush. *Eur Respir J* 1991; 4: 407S.
10. McCabe WR, Jackson CG. Gram-negative bacteriemia: etiology and ecology. *Arch Intern Med* 1962; 110: 847–855.
11. Weinberg PF, Matthay MA, Webster RO, Roskov KV, Golstein IM, Murray JF. Biologically active products of complement and actute lung injury in patients with the sepsis syndrome. *Am Rev Respir Dis* 1984; 130: 791–796.
12. Garrad CS, A′Court CD. The diagnosis of pneumonia in the critically ill. *Chest* 1995; 108: Suppl. 2, 17S–25S.
13. Prats E, Dorca J, Irigaray R, *et al.* Bacteriological follow-up of ventilator-associated pneumonia during the first 24 hours of antibiotic treatment. *Am J Respir Crit Care Med* 1995; 151: A791.
14. Tirnsit JF, Misset B, Goldstein FW, Vaury P, Carlet J. Reappraisal of distal diagnostic testing in the diagnosis of ICU-acquired pneumonia. *Chest* 1995; 108: 1632–1639.
15. Johanson WG Jr, Seidenfeld JJ, Gornez P, de los Santos R, Coalson JJ. Bacteriologic diagnosis of nosocomial pneumonia following prolonged mechanical ventilation. *Am Rev Respir Dis* 1988; 137: 259–264.
16. Wunderink R, Mayhall G, Gibert C. Methodology for clinical investigation of ventilator-associated pneumonia. *Chest* 1992; 102: 580S–588S.
17. Rouby JJ, Martin de Lasale E, Poete P, *et al.* Nosocomial bronchopneumonia in the critically ill. Histologic and bacteriologic aspects. *Am Rev Respir Dis* 1992; 146: 1059–1066.
18. Fagon JY, Chastre J, Hance AJ, *et al.* Detection of nosocomial lung infection in ventilated patients. Use of a protected specimen brush and quantitative culture techniques in 147 patients. *Am Rev Respir Dis* 1988; 138: 110–116.
19. Fagon JY, Chastre J, Domart Y, *et al.* Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of protected specimen brush and quantitative culture techniques. *Am Rev Respir Dis* 1989; 139: 877–888.
20. De Castro FR, Violan JS, Capuz BL, Caminero J, Gonzalez B, Manzano JL. Reliability of the bronchoscopic protected catheter brush in the diagnosis of pneumonia in mechanically ventilated patients. *Crit Care Med* 1991; 19: 171–175.
21. American Thoracic Society. Hospital-acquired pneumonia in adults: diagnosis, assessment of severity, initial antimicrobial therapy, and preventive strategies. *Am J Respir Crit Care Med* 1996; 153: 1711–1725.
22. Langer M, Cigada M, Mandelli M, Mosconi P, Tognoni G and ICUGIC. Early onset pneumonia: a multicentric study in intensive care units. *Intensive Care Med* 1987; 13: 342–346.
23. Jimenez P, Saldias F, Meneses M, Silva ME, Wilson MG, Otth L. Diagnotic fibreoptic bronchoscopy in patients with community-aquired pneumonia. Compatison between bronchalveolar lavage and telescoping plugged atheter cultures. *Chest* 1993; 103: 1023–1027.
24. Moine P, Vercken JB, Chevert S, Chastang C, Gajdos P. Severe community-aquired pneumonia. Etiology, epidemiology and prognosis factors. French Study Group for Community-Acquired Pneumonia in the Intensive Care Unit. *Chest* 1994; 105: 1487–1495.
25. Neu HC. The crisis in antibiotic resistance. *Science.* 1992; 257: 1064–1073.
26. Smith GM, Boon RJ, Beale AS. Influence of Clavulanic Acid on the activity of amoxicillin against

an experimental *Streptococcus pneumoniae Staphylococcus aureus* mixed respiratory infection. *Antimicrob Agents Chemother* 1990; 34: 210–214.
27. Marquette CH, Georges H, Wallet F, *et al.* Diagnostic efficiency of endotracheal aspirates with quantitative bacterial cultures in intubated patients with suspected pneumonia. *Am Rev Respir Dis* 1993; 148: 138–144.
28. EI-Ebiary M, Torres A, Gonzalez J, *et al.* Quantitative cultures of endotracheal aspirates for the diagnosing of ventilator assodated pneumonia. *Am Rev Respir Dis* 1993; 148: 1552–1557.