Comparative Study of Etiological Diagnosis of Nosocomial Pneumonia

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Nosocomial pneumonia is a common complication in patients on mechanical ventilation and results in significant mortality. Diagnosis of pneumonia in patients who are intubated and under mechanical ventilation is difficult, even with the aid of clinical, laboratorial, and endoscopic tests. The objective of this study was to compare three methods of tracheal sputum collection in patients with a clinical and radiological diagnosis of pneumonia. Twenty-two patients with a clinical diagnosis of liver disease were enrolled, 18 years of age or older, 13 males and nine females, who had been mechanically ventilated over an intubation period of 5.86 ± 4.62 days. These patients were being treated in intensive care unit (ICU) of the Liver Transplantdepartment. Secretion collection was carried out according to a protocol with three distinct methods: endotracheal aspiration with a closed aspiration system, Bal cath and bronchoalveolar lavage. Of the 22 patients analyzed, 21 (95.4%) showed one or more infectious agent when the closed aspiration system was used. With the Bal cathâ collection, 19 patients (86.3%) had one or more infectious agents; in the collection by bronchoalveolar lavage, 10 patients (45.4%) presented one or more infectious agent. According to the laboratorial analysis, 14 different microorganisms were isolated, the most frequent of which were *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* We concluded that aspiration with the closed system produced the most effective results in comparison with those of bronchoalveolar lavage and the Bal cath®, and may be an acceptable method for diagnosing hospital-acquired pneumonia when no fiberoptic technique is available.

Key-Words: Pneumonia, microorganisms, bronchoalveolar lavage, tracheal aspiration, closed aspiration system.

The greatest rates of hospital-acquired pneumonia occur in patients on mechanical ventilation with an endotracheal tube or tracheostomy, and are associated with more than one agent. Bacterial agents are the most important microorganisms responsible [1,2].

The incidence of hospital-acquired pneumonia has increased over the last two decades [3]. We know that nosocomial infections of the lower respiratory tract represent a significant challenge to intensive therapy specialists because of their high prevalence and significant morbidity and mortality.

Specialists in infectious diseases and intensive therapy face great difficulties in the diagnosis and treatment of lower respiratory tract infections, and recent studies have proved that hospital-acquired pneumonia contributes with 60% of deaths by hospital-acquired infections [3].

Craven et al., 1986 [1] and Meduri et al., 1992 [4], reported that, despite great technological and antimicrobial therapy advances in seriously ill patients, the mortality rate in patients with hospital-acquired pneumonia (HAP) continues high.

The etiological diagnosis of pneumonia is not yet established, and treatment is based on epidemiological, clinical, radiographic, and laboratorial characteristics. The most commonly used criteria for the diagnosis of hospital-acquired pneumonias are the appearance of a new infiltrate, its progression on chest X-rays, fever, leucocytosis, and purulent tracheobronchial secretion. The presence of three of these criteria allows the diagnosis to be made [3,5].

Advances in diagnostic methods for pneumonia, molecular epidemiology techniques, nosocomial prevention strategies, invasive treatments, adequate use of antibiotics and prophylaxis, and in immunotherapy are challenges for our present decade. Bronchoscopy with bronchoalveolar lavage (BAL) or protected specimen brush (PSB) has shown advantages in the diagnosis of pneumonia in patients on mechanical ventilation [6].

In light of the difficulties to define the etiological agent by hemoculture, several researchers have tried to establish criteria for this diagnosis by means of BAL and the best method for lavage collection [7]. In 1972, Johanson et al. were the first to describe the use of a fiberoptic bronchoscope for BAL in the diagnosis of pneumonia associated with mechanical ventilation. This method facilitates sputum collection in specific areas of the lungs [8]. Other authors have tried to quantify the number of colonies present and the best method for sputum collection, but none has been conclusive [8-12]. Quantitative culture with a protected catheter is important in order to guarantee non-contamination of the upper portion by the respiratory system. This occurs because the catheter tip is enclosed and is only exposed once the catheter has reached the region in which the lavage will be done. [13,14].

Schwartz et al. in 1998 criticized the identification of contaminating agents by simple tracheal aspirate (EA) [15]. On the other hand, in 1991, Papazian et al. and Wu et al., 2000, carried out a comparative study of secretions with a quantitative culture by simple tracheal aspirate and

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bronchoscopy, and demonstrated a good correlation between the two procedures in identifying the agent [16,17].

Aware of the possible complications that bronchoscopy could cause in the patient, Ballardâ developed a protected catheter, the Bal cathâ, for bronchoalveolar lavage. The procedure can be done in both the right and the left lung, according to positioning of the catheter tip [18]. Bronchoscopy with BAL and the Bal cathâ were compared, and proved similar for diagnosis of the causal agent of the pneumonia [18].

The objective of this study was to compare, by means of quantitative and qualitative cultures, three methods for collecting lung secretions in patients on mechanical ventilation: closed system endotracheal aspirate (ETA/CS), aspirate with the Bal cath®, and bronchoscopy with BAL.

Material and Methods

Twenty-two liver disease patients with clinical and radiographic diagnoses of pulmonary infection were prospectively studied. They were maintained on mechanical ventilation and empirical antibiotic treatment prescribed by the medical team, as necessary, in intensive care unit (ICU) of the Liver Transplant and Surgery Clinic of the University of São Paulo Medical School Clinics Hospital. The trial was approved by the Ethics Committee for Analysis of Research Projects of the HCFMUSP (CAPPesq) and all those responsible for the patients signed an informed consent form that had been previously approved by the CAPPesq. The document was also signed by the bronchoscopist responsible for the study.

The trial included both male and female patients 18 years of age or older with a diagnosis of pneumonia, submitted to orotracheal intubation and under mechanical ventilation. Patients with any contraindication for bronchoscopy such as hemodynamic instability not drug-reversible, a final positive expiratory pressure (PEEP) over 15 cm H_2O, and an inspired oxygen fraction (FiO_2) over 70% were excluded.

All patients analyzed were receiving antibiotics because of the severity of their disease, except for patient number 2. Antibiotics were used empirically in patients with a declining clinical condition, and the severe clinical state of many patients was an obstacle to collecting specimens without the use of antibiotics. For the diagnosis of pneumonia, criteria used were those of the Centers for Disease Control and Prevention (CDC) [19].

Subjects were enrolled in the study after a diagnosis of pneumonia made by the medical team. Bronchoscopy was used as the gold standard for the study. Patients were randomized by a drawing of sealed envelopes for the sequence of sputum collection methods, with intervals of approximately two hours between procedures.

Tracheal Secretion Spevimen Collection

For collection of lung secretions, endotracheal aspiration was performed with (ETA/CS) using Trach Care® from Ballard Medical Products® (USA), a closed aspiration system with 4.6 mm in diameter (14 French – Fr) and 54 cm in length, and two connecting pieces – one for the cannula and the other for the heat and moisture exchange (HME) and the ventilator circuit. The aspiration tube is protected by a plastic sleeve along the full length. The HME is a Gibeck Humid-Ventâ Filter, a combination of a heat and humidity exchanger associated to a bacterial/viral filter. This system is routinely used in ICUs and was designed for single use to be substituted every 24 hours or when necessary.

Specimen collection in the compromised lobe was performed by means of the BAL Cath® system, a catheter for bronchoalveolar lavage without bronchoscopy made by Ballard® Medical Products (USA). It is comprised of an internal 12 French (12 Fr) catheter protected by a 16 French (16 Fr) catheter with a two-port access proximal end, one for the syringe with saline solution and the other for the vacuum, which is the access route for aspiration. There is an entrance port for oxygen, when necessary. This method of collection is similar to the closed system of tracheal aspiration, but with the Bal cathâ, we can direct the catheter tip to the right or left bronchus after selecting the compromised lobe by chest X-ray.

Collection of Bronchoscopic Specimens

The bronchoscopy with BAL was performed by the bronchoscopist on call of the Endoscopy Department of the Hospital who had been designated by the bronchoscopist responsible for the study.

According to Howard et al., 1994, BAL functions as a non-invasive "liquid biopsy" or a "mirror" of what goes on in the pulmonary parenchyma, both for cytological analysis of substances and particles, and microbiological analysis. BAL is fundamentally a fibrobronchoscopy procedure [18].

Lavage Site

The affected site was identified by a radiographic study, tomography, gallium scanning, etc. The technique required placement of the bronchoscope in a subsegmental bronchus for posterior aspiration.

Lavage Liquid Total Volume Used

A volume of at least 100 mL divided into 20-50 mL quotas was necessary in order to obtain an adequate sample of alveolar surface related to the subsegmental bronchus to be explored. Magnetic resonance studies have also demonstrated that there is no advantage in using large volumes (greater than 150 mL) for an adequate sampling of this alveolar space. Additionally, larger volumes are related to a worsening in lung function. BAL is performed with saline solution and at room temperature.

Aspiration Technique

There are studies that show that the aspiration pressure should be between 20 and 80 mmHg, around 60 mmHg. Greater pressures usually lead to the most common failures (bleeding

and distal collapse, as well as trauma to cells). To attain this ideal pressure, gentle syringe aspiration, gravity drainage, and aspiration with constant pressure can be used. This care with aspiration allows a 40% minimum retrieval of the instilled volume, which is a good quality marker of the lavage.

In our study, the bronchoalveolar lavage was performed by introduction of the fiberoptic bronchoscope into a distal airway, wedged in a segmental bronchus of the abnormal lobe. Suction was avoided before the specimen removal. Non-bacteriostatic saline was then infused in 20 mL quotas to a final volume of 80-120 mL for lavage of the pulmonary segment and rapidly aspirated with a sterile syringe. The aspirated fluid was pooled in a sterile flask and sent to the microbiology laboratory soon after collection. This procedure was carried out with the patient under sedation, ventilated with 100% FiO_2.

The electrocardiogram, pulse oximetry, and arterial blood pressure were monitored during the procedures. Collection of pulmonary secretion material with the closed tracheal aspiration system and the Bal cathâ was performed by physiotherapists of the intensive therapy unit of the Liver Transplant and Surgery Clinic.

In collecting sputum by these two methods, antisepsis and instillation of 20 mL saline solution were used, and for the bronchoalveolar lavage, 120 mL of saline solution were instilled. The average volume of retrieved instilled fluid was 20% using BAL, 47% with the Bal cath®, and 55% with the closed aspiration system of endotracheal aspirate.

The microbiological processing of the specimens was performed according to the Essential Procedures for Clinical Microbiology of the American Society for Microbiology, 1998 [20].

Microbiological Analysis

The three specimens were immediately transferred to the microbiology laboratory for Gram staining and culture. A 0.01-mL calibrated loop was placed into the respective specimens and then onto the center of three media plates (blood agar, chocolate agar, and MacConkey agar). The media plates were then streaked using the pin-wheel streak method and incubated in CO_2 at 35°C. Microbiology analysis was performed using CLSI recommendations. An automatic method (Vitek) was used for initial identification and sensibility profile. Bacterial culture growth was quantified according to the number of colonies observed per plate: fewer than 10 colonies per plate represented less than 10^3 colony-forming units (CFU)/mL; 10 to 100 colonies per plate represented 10^3 to 10^4 CFU/mL; 100 to 1,000 colonies per plate represented 10^4 to 10^5 CFU/mL; and more than 1,000 colonies per plate represented greater than 10^5 CFU/mL. The results of cultures of EAT/ES, Bal cathâ and BAL were obtained within 24 to 48 hours. We used a previously established and validated quantitative threshold ($\geq 10^3$ CFU/mL) for all sampling methods to support the diagnosis of ventilator-associated pneumonia [20].

Statistical Analysis

The statistical method used was descriptive, expressed in percentages. The number of organisms of each bacterial species and the total number of bacteria obtained from specimen cultures were expressed in CFU/mL of fluid. Other results were expressed as means ± standard deviations (SD), as fractions of the total number of patients and as percentages of the total values [22].

Results

Of the 22 patients analyzed, in the ETA/CS collection 21 patients (95.4%) had one or more infectious agent. With the Bal cath®, 18 patients (86.3%) had one or more agent. With BAL, 8 patients (36.3%) had the presence confirmed of one or more infectious agent. The results were expressed in CFU/mL. A culture grown from one single pathogen was reported as being 10^3, 10^4, 10^5 CFU/mL.

In all, fourteen microorganisms were detected in the cultures obtained from the BAL samples. Counts were $^3 10^5$ CFU/mL for five microorganisms, $3x10^4$ CFU/mL for one microorganism, $2x10^4$ CFU/mL for two microorganisms, $5x10^4$ CFU/mL for one microorganism, and 10^5 CFU/mL for six microorganisms. Twelve patients had negative cultures. In two patients, a microorganism was identified but quantification was not performed.

With the Bal cath®, 28 microorganisms were identified in the samples. Counts were $\geq 10^5$ CFU/mL for ten microorganisms, 10^5 CFU/mL for ten microorganisms, $13x10^3$ CFU/mL for one microorganism, $8x10^3$ CFU/mL for one microorganism, $14x10^3$ CFU/mL for one microorganism, $3x10^4$ CFU/mL for one microorganism, $2x10^4$ CFU/mL for one microorganism, $37x10^3$ CFU/mL for one microorganism, $5x10^4$ CFU/mL for one microorganism, and 10^4 CFU/mL for one microorganism. Four patients had negative cultures.

The ETA/CS method also detected 28 different microorganisms in cultures obtained from the samples, and the counts were $\geq 10^5$ CFU/mL for ten microorganisms, $3x10^4$ CFU/mL for one microorganism, $3x10^3$ CFU/mL for one microorganism, $8x10^4$ CFU/mL for one microorganism, 10^5 CFU/mL for 12 microorganisms, 10^3 CFU/mL for two microorganisms, and 10^4 CFU/mL for one microorganism. Only one patient had a negative culture (Tables 1 and 2).

Prior antibiotic treatment was used empirically as needed. Only one patient did not receive antibiotics because the conclusion of tests was awaited in order to identify the appropriate drug (Table 2).

According to the laboratorial analysis, 11 microorganisms were isolated, and the most frequent were *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* (Table 2).

Patient characteristics and retrieve volume obtained using each method of collection and the sequential order of sputum collection of the procedures can be seen in Table 3.

Discussion

Nosocomial pneumonia is an infectious process of the lower respiratory tract involving pulmonary parenchyma that

Table 1. Microorganisms obtained by culture of respiratory secretions using three different methods of collection in 22 patients with nosocomial pneumonia

Agent	ETA/CS	Bal cath®	BAL
Escherichia coli	1	1	1
Klebsiella pneumoniae	4	4	3
Pseudomonas aeruginosa	4	4	3
Providencia stuartii	1	1	0
Coagulase-negative *Staphylococcus*	4	5	2
Staphylococcus aureus	7	3	1
Pseudomonas maltophilia	1	1	0
Corynebacterium sp	2	2	1
Candida albicans	1	1	2
Staphylococcus epidermidis	1	1	1
Acinetobacter baumannii	1	1	1
Stenotrophomonas maltophylia	3	3	1
Corynebacterium xerosis	1	1	0
Aspergillus fumigatus	0	0	1

ETA/CS: endotracheal aspirate/closed system; Bal cath®: catheter for bronchoalveolar lavage without bronchoscopy, BAL: bronchoalveolar lavage.

is acquired after a hospital stay for more than 48 hours and that was not present on admission [1,4,5].

Despite being a topic that generates intense discussion and clinical interest, the ideal technique for diagnosis and etiology in critically ill patients under treatment in the ICU is still uncertain, especially in the subgroup of patients that need mechanical ventilation [10,12].

Intubation is known as an important factor in promoting airway colonization with potential pathogens and can contribute towards the presence of a large number of microorganisms in cultures of lavage fluid in patients without pneumonia [10]. There are many studies on this theme, but no absolute consensus exists yet for defining a positive result. The diagnosis of hospital-acquired pneumonia is difficult because clinical, biological, and radiological signs are neither sensitive nor specific [4]. Papazian, 1995, and Baughman, 2005, showed that clinical criteria alone are not sufficient for the diagnosis of nosocomial pneumonia [16,21].

Several invasive techniques for specimen collection were described as potentially useful in improving efficacy of the diagnosis of hospital pneumonia. Blot et al., in 2000 [9], described that invasive diagnostic techniques can improve clinical administration, reduce the use of antibiotics, and possibly improve the prognosis of mechanically ventilated patients with suspected nosocomial pneumonia [8].

In this study, some patients received empirical antibiotic treatment. Although this is not the ideal procedure as suggested by some authors [17,23], these patients were in a severe clinical condition preceding liver transplantation. This prior use of antibiotics may have altered the precision of the pulmonary samples. We opted for randomly drawing the sequence of the three procedures in order to avoid doubt in contamination of events. According to the laboratorial analysis, 11 distinct microorganisms were isolated, and the most frequent were *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* In 2005, a study by Shaw also identified the first two agents besides *Acinetobacter baumannii* as the most common microorganisms, and discussed the empirical use of antibiotics and digestive tract colonization [5].

Sauaia et al., 1993, described that antibiotic treatment is frequently initiated before a specific pathogen is identified by culture and that changes according to the predominant pathogen [23].

Blot et al., 2002, reported that prior antibiotic treatment, prescribed to manage a former septic episode not related to the suspected pneumonia, did not affect the diagnosis of respiratory samples [9]. For this reason, when there is a suspicion of nosocomial pneumonia, it is important to collect respiratory sample before any change in antibiotic therapy. In most episodes of suspected pneumonia, this approach may allow an earlier and more appropriate administration of antibiotic treatment when needed, avoiding unnecessary treatments [17]. This approach may lead to an improvement in patient results, a lower risk of emergency use of antibiotics, and a more appropriate utilization of resources [24].

To Bello 1996, the routine of bronchoscopic technique use, such as BAL and PSB (protected specimen brush), for example, is limited by various time-consuming and relatively expensive factors and because of the costs of the sampling brush and the need for a fiberoptic guide. Additionally, fiberoptic bronchoscopy may not be available 24 hours-a-day in many ICUs. Therefore, research for easier and alternative techniques for the diagnosis of pneumonia is justified [6]. In conclusion, our findings suggest that the "blind" protected specimen brush has a similar diagnostic yield and specificity to bronchoscopic techniques. Sampling with the "blind" protected specimen brush (Accu-Cath) is a simple and reliable alternative. This could be interesting in those circumstances where standard bronchoscopic techniques are not available.

Table 2. Microorganisms obtained by quantitative culture of respiratory secretions collected by three different methods from 22 patients with nosocomial pneumonia

Pt	agent	ETA/CS (CFU/mL)	Bal cath® (CFU/mL)	BAL (CFU/mL)	Antibiotics
1	*E. coli*	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	Vancomycin and ganciclovir
	K. pneumoniae	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	
2	*P.aeruginosa*	$\geq 10^5$	$\geq 10^5$	ABF	No antibiotic
	P.stuartti	$\geq 10^5$	$\geq 10^5$		
	Coagulase-negative S.	$\geq 10^5$	$\geq 10^5$		
3	*S. aureus*	-	ABF	ABF	Cefepime, vancomycin, amphotericin
4	*Pseudomonas maltophilia*	$\geq 10^5$	$\geq 10^5$	ABF	Amphotericin, vancomycin, imipenem
5	*P. aeruginosa*	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	Vancomycin, imipenem, amphotericin,
	Coryneobacterium sp	$3x10^4$	$\geq 10^5$	$3x10^4$	ganciclovir
6	*C. albicans*	$8x10^4$	13×10^3	10^5	Vancomycin and cefepime
7	*S. aureus*	$\geq 10^5$	$\geq 10^5$	ABF	Vancomycin and cefepime
	Coagulase-negative S.	10^5	$\geq 10^5$		
8	*S. epidermidis*	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	Vancomycin, imipenem, amphotericin,
	C. albicans	ABF	ABF	present, but w/o CFU	ciprofloxacin
9		ABF	ABF	ABF	Clindamycin, cefotaxime
10	*K. pneumoniae*	$\geq 10^5$	$8x10^3$	$\geq 10^5$	Vancomycin, cefepime, amphotericin, metronidazole
11	*Acinetobacter baumannii*	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	Vancomycin, amphotericin, imipenem
12	*S. aureus*	10^3	-	ABF	Ceftriaxone, clindamycin
	Coagulase- negative S.	-	10^5		
13	*P. aeruginosa*	10^5	$14x10^3$	10^5	Ampicillin, cefotaxime, norfloxacin
	Coagulase-negative S.	10^5	$3x10^4$	$2x10^4$	
	Aspergillus fumigatus	ABF	ABF	present but w/o CFU	
14	*K. pneumoniae*	10^3	10^5	ABF	Vancomycin, amphotericin, Ceftazidime
15	*S. aureus*	10^5	10^5	ABF	Vancomycin, amphotericin, imipenem, acyclovir
16	*S. aureus*	10^4	$2x10^4$	$2x10^4$	Vancomycin, imipenem, amphotericin
	K. pneumoniae	$3x10^3$	10^5	10^5	
	Stenotrophomonas maltophilia	10^5	10^4	$\geq 10^5$	
17	*Stenotrophomonas maltophilia*	$\geq 10^5$ $^3 10^5$	$37x10^3$	ABF	Vancomycin, imipenem
18	*S. aureus*	10^5	10^5	ABF	Vancomycin, imipenem, amphotericin
19	*P. aeruginosa*	10^5	10^5	10^5	Clindamycin, cefotaxime
20	*Corynebacterium xerosis*	10^5	10^5	ABF	Clindamycin, ceftriaxone
21	*S. aureus*	10^5	ABF	ABF	Cotrimaxazole
22	*P. aeruginosa*	10^5	10^5	10^5	Vancomycin, ganciclovir, imipenem,
	E. coli	ABF	$5x10^4$	$5x10^4$	cotrimaxazole, amphotericin

ETA/CS: endotracheal aspirate/closed system; BAL cath: catheter for bronchoalveolar lavage without bronchoscopy, BAL: bronchoalveolar lavage; w/o: without; PT:patient(s).

In their study, Wu et al., 2002 [17], compared protected catheter, BAL, and endotracheal aspirate and described the diagnostic efficacy of endotracheal aspirate using a 10^5 number. It was obtained a 92.8% sensitivity and an 80% specificity. They concluded that since endotracheal aspirate is a non-invasive technique, it is easily reproducible. The results of this study confirm recent discoveries, in contrast to results from some former studies [17].

Blot et al., 2000, suggest that the combination of Gram stain examination of paired plugged telescoping catheter (PTC) and endotracheal aspirate (EA) may contribute to the early diagnosis of HAP in about two-thirds of mechanically ventilated patients and to guide the empiric therapy when needed [9].

The study conducted by Zedtwitz-Liebenstein et al., 2005, reported that a number lower than or equal to 10 units/mL of

Table 3. Patients characteristics and retrieved volume using three different methods of respiratory secretion collection for quantitative cultures and sequential order of collection in 22 patients with nosocomial pneumonia

PT	Gender	Age (years)	OTI (days)	Antibiotic (days)	Ret. Vol. BAL (mL)	Ret. Vol. Bal cath® (mL)	Ret. Vol. ETA/CS(mL)	Collection sequence
1	F	71	2		11	10	10	1 2 3
2	M	63			58	5	12	2 3 1
3	F	66		2	50	10	10	3 1 2
4	F	43	13		0	5	0	1 2 3
5	F	52	11		30	15	15	1 3 2
6	F	68	9	8	30	0	0	3 1 2
7	F	78	2		40	15	10	1 2 3
8	M	50	15	22	15	15	23	1 3 2
9	F	46	2	2	25	4,5	4	1 2 3
10	M	59	1		28	15	15	2 1 3
11	F	66	1		20	10	15	3 1 2
12	M	67	3		20	10	15	1 3 2
13	M	57	5		16	8	10	3 2 1
14	M	56			12	8	10	3 1 2
15	M	46	7		13	10	15	1 3 2
16	M	58			20	10	15	3 1 2
17	F	60	2		48	10	10	1 3 2
18	M	53	9		45	14	10	1 3 2
19	F	49	3	3	21	9	5	2 1 3
20	F	62	7		20	13		2 1 3
21	F	66	14		30	8	12	3 1 2
22	F	71	16		20	10	15	1 2 3
N= 22 13F/ 9M								
Mean		59.41	6.78	7.40	26.00	9.75	11.00	
SD		9.35	5.21	8.53	14.45	3.88	5.44	
% of retrieved volume					20%	47%	55%	

OTI (days): orotracheal intubation duration; Ret. Vol. BAL (mL): retrieved volume of bronchoalveolar lavage in milliliters; Ret. Vol. ETA/CS (mL): retrieved volume of endotracheal aspirate in milliliters; Ret. Vol. Bal cath® (mL): retrieved volume by Bal cath® (mL); F: female; M: male. Sequence: 1: Endotracheal aspirate with closed aspiration system (ETA/CS),2: Bal cathâ, 3: Bronchoalveolar lavage (BAL).

colonies requires discussions about treatment, since it questions the great variation in dilution during the BAL procedure, which could influence quantitative results. They inform that using urea to determine the dilution quotient of the sample might make the results more reliable [24].

According to Chastre et al., 1988, several considerations suggest that BAL may be useful in establishing the diagnosis of pneumonia. Lavage is a practical and safe method for obtaining cells and secretions of the lower respiratory tract. The technique shows a relatively large area of the lung, the recovered cells and liquid can be microscopically examined immediately after the procedure, and it is convenient for cultures using quantitative techniques [25]. Preliminary studies indicated that when the lavage fluid from patients with pneumonia is cultivated by quantitative techniques, large numbers of organisms are recovered in essentially all cases [1,26].

The use of bronchoscopic techniques allows etiologic diagnosis of infection and leads to directed antimicrobial therapy in opposition to empirical treatment [27].

Therefore, larger numbers of organisms can grow in cultures of patients without pneumonia presenting COPD (chronic obstructive pulmonary disease) or intense colonization of airways, a problem that can limit the usefulness of lavage. In these cases the quantitative culture may be essential. Sauaia et al, 1993, reported that in a former study carried out by their group in a series of patients with ventilation and clinically inclined to develop nosocomial pneumonia, it was suggested that quantitative culture of BAL fluid is not very useful in identifying patients with or without pneumonia because of its low specificity [23].

The technique of quantitative cultures of endotracheal aspirates, used initially in the 1960s for a more precise diagnosis of pneumonia in non-intubated patients, has recently been used for mechanically ventilated patients. BAL with quantitative cultures has been promoted to contamination detour of upper airways [26]. We observe that sample collection by ETA/CS (95.4%) was the method with the greatest number of infectious agents, and BAL was the method that presented the least number of agents (45.4%). The work

of Michel at al., 2005, shows that the routine of cultures by means of ETA/CS may facilitate prescription of adequate antibiotics in 95% of patients, while the results of BAL are awaited [11].

Non-bronchoscopic techniques are used in mechanically ventilated patients essentially because the endotracheal tube, which bypasses the proximal airways and allows easy access to the lower airways. Disadvantages include the potential sampling errors inherent to a blind technique and the lack of airway visualization [28,29].

The ideal "shortcut" for considering a quantitative BAL culture as positive is controversial, but an initial point of 10^4 CFU/mL seems to furnish the greatest specificity without losing sensitivity. Sauaia et al., 1993, however, observed that BAL samples of patients with histologically confirmed pneumonia who were being treated with antibiotics show an "insignificant" bacterial growth, and suggested that in these cases, the conventional "shortcuts" may not be appropriate [23].

In this same study, the authors reported that according to their experiences and a review of available literature, the quantitative culture of BAL can not be recommended for routine use in mechanically ventilated patients. They also comment that in patients with a probable diagnosis of pneumonia, data suggest that quantitative cultures of endotracheal aspirates may be an alternative to quantitative cultures of BAL, although this observation needs additional studies [7,30]. Nevertheless, they concluded that quantitative cultures of endotracheal aspirates have a significant correlation with quantitative cultures of BAL, and therefore, the endotracheal aspirate may be a valid alternative to BAL, even though critiques of Lorenti at al., in 2005, reveal that this system does not reduce the incidence of pneumonia related to the respirator [31].

Papazian et al., 1995, commented that the adverse effects of BAL procedures included hypoxemia and a syndrome similar to sepsis involving fever and a drop in arterial blood pressure and could imitate the indication for BAL, especially in patients with hypoxia and a $PO_2 < 60$ mmHg [16].

Comparing the qualitative and quantitative recovery of bacteria using four different techniques, the authors concluded that these four methods provide reasonably similar qualitative and quantitative recovery of bacteria from the lower airways of intubated mechanically ventilated patients. In addition, routine Gram stain and semi-quantitative aerobic culture of endotracheal aspirate may provide useful information in patients with suspected ventilator-associated pneumonia [32].

The fact that we did not collect these data to ascertain that these complications may arise and thus justify the facts more precisely may have been a drawback in our study.

Conclusion

We conclude that, by means of a closed system of endotracheal aspirate, the results obtained are more effective in comparison to BAL and Bal cath®. In most of the patients analyzed, positive cultures were detected in endotracheal aspirate with a closed aspiration system, while a large part of the sampling collected during BAL had an absent bacterial flora, which coincided with recent studies described in literature.

Endotracheal aspirate quantitative cultures may be an acceptable tool for diagnosing nosocomial pneumonia when no fiberoptic technique is available. Endotracheal aspirate with a closed system has advantages such as being an economical technique, it is quick and easy to use, and it is easier on the patient.

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