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# PROSPECTIVE COMPARATIVE STUDY ON ETIOLOGIC DIAGNOSIS OF VENTILATOR-ASSOCIATED PNEUMONIA

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**ABSTRACT**

Ventilator-associated pneumonia (VAP) is a pulmonary infection highly prevalent and lethal. The clinical diagnostic is the most used, even though it is not specific, and the collection of the biological material, fundamental for the handling of these patients, does not have a definite gold standard method. The present study aimed to evaluate different methods, invasive or noninvasive, on VAP etiologic diagnosis. It is a prospective, observational study carried on in a tertiary hospital. Thirty (30) patients with a clinical VAP suspicion were submitted to the endotracheal aspirate (ETA), bronchoscopic bronchoalveolar lavage (BAL) and bronchoalveolar lavage non-bronchoscopic (BALNB), being patients its own controller. The measures of accuracy, sensibility, specificity, positive predictive value (PPV) and negative predicted value (NPV) for the ETA were 83%, 85%, 50%, 96% and 20% respectively. For the BALNB, the following were observed for accuracy, sensibility, specificity, PPV and PNV: 50%, 50%, 50%, 93% and 7% respectively. In relation to the BAL, the accuracy, sensibility, specificity, PPV and PNV found were 63%, 60%, 100%, 100% and 15%. The agreement between the isolated microorganisms in the BAL with the ones obtained with the BALNB and ETA was good, with kappa coefficient of 0.63 and 0.64 respectively, being the Gram negative bacteria the main etiological agents. Comparing invasive and non-invasive methods, we concluded that the ETA represents the best option to the etiologic confirmation of the VAP.



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## INTRODUCTION

Ventilator-associated pneumonia (VAP) can be defined as infection of the pulmonary parenchyma that occurs after 48 hours of tracheal intubation, classified as early, when it occurs until the fourth day (96 hours) of hospitalization, and as late when it occurs after this period (Torres *et al* 2017). It affects about 32-38% of patients admitted to intensive care units (Ferrer and Torres 2018, Véliz and Fica 2017) and is associated to increased mortality, more days of mechanical ventilation, length of stay and higher costs (Cornistein 2018).
The diagnosis of VAP remains controversial (Grgurich 2013). The collecting of biological material (tracheal and / or pulmonary secretion) and its quantitative analysis provide greater specificity, but there is no definition of the ideal method for laboratory confirmation (Roberts *et al*., 2017).

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As it's commonly caused by multidrug-resistant bacteria (Patro *et al* 2018), the precise identification of the causative agent and the knowledge of sensitivity profile allow the reduction of the initial scheme spectrum of antibiotic therapy and interruption in the event of negative cultures (Frantzeskaki 2018). The objectives of this study were to evaluate and compare the sensitivity and specificity of endotracheal aspirate (AET), bronchoalveolar lavage not bronchoscopic (LBANB) and bronchoalveolar lavage (BAL) in relation to the clinical diagnosis and to determinining the prevalence of the bacterias commonly isolated and their susceptibility profile.

## MATERIALS AND METHODS

**Study design and patient population:** This was a comparative prospective observational study of etiological definition methods in cases of VAP. The study was approved by the Ethics Committee of the Federal University of Uberlândia (CAAE 31607614.6.0000.5152) and duly

registered with the Clinical Trials under the number NCT 02363023. The study have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. Patients in the adult intensive care unit of Uberlândia's University Medical School, with the age above 18 and with suspicion of VAP were included with permission of parents or guardians who signed and agreed the free and informed consent. Patients with coagulation disorders, immunocompromised and when there was contraindication to disconnect the ventilator for sample collection were excluded. Thirty seven patients with clinical suspicion underwent collection of material by three methods. Seven were excluded due to contaminated culture in at least one of the methods. Twenty-eight had clinical diagnosis with radiological alteration and at least two other criteria. Patients were recruited by convenience, as suggestive VAP cases were identified and were followed for up to thirty days after this episode. The parameters analyzed were: age, gender, number of variables associated to the radiological criteria: leukocytosis/leukopenia, fever and purulent secretion, score the Clinical Pulmonary Infection Score (CPIS), ICU outcome (discharge or death), type of admission, the patient's origin, severity score Simplified Acute Physiology Score (SAPS III), presence of tracheostomy, length of hospital stay, use of prior and current antibiotic, radiological aspect of infiltration, hospitalization time until episode of VAP (early versus late VAP), PaO2 / FiO 2 and isolated bacteria and their susceptibility profile (Dalhoff *et al* 2018).

were submitted to the collection of ETA, BALNB and BAL, always in the same sequence by the same thoracic surgeon after suspection of VAP. The minimum values established for infection for endotracheal aspirate, bronchoalveolar lavage and bronchoalveolar lavage non bronchoscopic were respectively $10^5$, $10^4$ and $10^4$ CFU / mL. The ETA was obtained by suction catheter, without any solution previously was instilled into the airway before collection. BALNB was performed by inserting a catheter 14 French, with the tip pointing to the affected side blindly until he found resistance. At this time 100ml 0.9% saline, in 20ml aliquots, were instilled and aspirated with the same syringe. Fiberoptic bronchoscopy was carried out under sedation and after FiO2 ventilation with 100% for 5 minutes, 10 minutes after BALNB. No secretion was previously vacuumed to prevent device channel contamination. The same was impacted in the segment identified by radiologic examination, being injected 5 aliquots of 20 ml of saline and recovered with the same syringe and placed in a sterile container. Both lavages had washed the first rate vacuumed neglected. The material was immediately sent for microbiological studies using quantitative culture and the automated system used to perform the antibiotic sensitivity test was Vitek-2. The resistance criteria adopted were in accordance with standards of the Clinical and Laboratory Standards Institute (CLSI 2018): *Staphylococcus aureus* resistant to oxacillin; *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to carbapenems and Enterobacteriaceae (*Escherichia coli*, *Enterobacter* spp,

**Table 1. General sample characteristics**

| Variables | Results | |
|---|---|---|
| Age (years) | 44,97 (39 - 44)[1] | |
| Gender (%) | Female | 40 |
| | Male | 60 |
| SAPS [2] / Predicted Mortlity (%) | 59 (55 - 64)[1] / 39 (30 - 48)[1] | |
| Origin (%) | Emergency room | 70 |
| | Ward | 17 |
| | Other service | 13 |
| Mortality in ICU (%) | 33 | |
| Length of stay (days) | 47 (38 - 57)[1] | |
| $PaO_2/FiO_2$ start [3] (mmHg) | 272 (228 - 315)[1] | |
| Number of variables [4] | 1 | 7 |
| | 2 | 57 |
| | 3 | 36 |
| CPIS[5] (%) | < 6 | 13 |
| | ≥ 6 | 87 |
| Type of VAP[6] (%) | Early | 13 |
| | Late | 87 |
| Previous use of antibiotics (%) | 96 | |
| Antibiotics under use (%) | 76 | |
| Adequate antibiotics after cultures (%) | Yes | 50 |
| | No | 34 |
| | Unknown | 16 |
| Pattern of infiltrated pulmonary in the thorax r-ray (%) | Localized | 80 |
| | Difused | 20 |

[1] Confidence interval;
[2] Simplified Acute Physiology Score (SAPS III);
[3] Oxigen arterial pressure/fraction of oxygen inhaled en the day of clinical suspicion;
[4] Associated variable to the radiological criteria: leukocytosis/leukopenia, fever and purulent secretion;
[5] Clinical Pulmonary Infection Score;
[6] Ventilator associated pneumonia.

**Clinical diagnosis and Microbiological procedures:** Clinical diagnosis was confirmed based on the presence of novel or progressive pulmonary infiltrate in patients with mechanical ventilation for a time longer than or equal to 48h, and two or more of the following variables: fever (> 38 ° C); leukocytosis (> 12,000 cells / mm3) or leukopenia (< 4,000 cells / mm3); and purulent tracheal secretions. All participants of the study

*Klebsiella pneumoniae*) producing ESBL (Extended Spectrum Beta-lactamase). No intervention of the research team was done in the conduction of the case, and the decision to initiate and / or terminate the use of antibiotics was the attending physician responsability, as institutional protocol.

**Statistical analysis:** The variables were tested for normal distribution using the Shapiro-Wilk test. Quantitative variables

were compared using the Student's t test. For qualitative variables was performed the test of independence (or association) of chi-square, was performed with Monte Carlo simulations when appropriate, considering statistically significant results at p <0.05. Among the precision methodologies were estimated sensitivity proportions, specificity, accuracy, positive predictive and negative value and probability of false-positive and negative. In these assessors was also used the Kappa Cohen's membership measure for correlation analysis in relation to methods of isolated micro-organisms. A study of the relationship between the variables evaluated in relation LBANB, LBA and AET from the Spearman correlation was performed. Analyses were performed using the environment R: A Language and Environment for Statistical Computing (2013).

## RESULTS

Thirty seven (37) patients were evaluated with clinical suspicion of VAP between may 2015 and april 2016. Seven were excluded due to contamination of at least one of the samples.

clinical suspicion are shown in Table 2. It was observed a better accuracy and sensitivity in ETA, besides a specificity and PPV perfect for the LBA, with no method has managed a relevant VPN. In 54% of the cases there was agreement among the three methods, and in 16% were all negative and 38% positive all. In 84% of patients at least one method had an isolated bacteria. Yield of the methods were 50%, 56% and 83% respectively for BALNB, BAL and ETA.

The ETA obtained a Kappa index of 0.41 when compared to the BAL. Already the results of BALNB showed greater agreement with Kappa value: 0.47. The sensitivity, specificity, PPV, NPV, and accuracy in relation to the BAL are shown in Table 3. There was a predominance of Gram negative, with a higher prevalence of *Pseudomonas aeruginosa* (30%) and *Klebsiella pneumoniae* (20%). Bacteria isolated were considered multiresistant in 36% of positive cultures (Table 4). We noted Cohen's Kappa's coefficients of 0.64 and 0.63 for the relation in between microorganisms isolated from AET and LBANB respectively, when compared to the LBA.

**Table 2. Sensitivity, specificity, PPV, NPV and accuracy of the methods of sample collection in relation to the clinical suspicion**

| Method | Accuracy | Sensitivity | Specifity | PPV[(1)] | NPV[(2)] |
|---|---|---|---|---|---|
| BAL[(3)] | 63% | 60% | 100% | 100% | 15% |
| EAT[(4)] | 83% | 85% | 50% | 96% | 20% |
| BALNB[(5)] | 50% | 50% | 50% | 93% | 7% |

[(1)] PPV: positive predictive value;
[(2)] NPV: negative predictive value;
[(3)]BAL: bronchoalveolar lavage;
[(4)]EAT: endotracheal aspirate;
[(5)]BALNB: bronchoalveolar lavage non-bronchoscopic;

**Table 3. Sensitivity, specificity, PPV, NPV and accuracy of the EAT and BALNB collection in relation to BAL method**

| Method | Accuracy | Sensitivity | Specifity | PPV[(1)] | NPV[(2)] |
|---|---|---|---|---|---|
| EAT[(3)] | 73% | 100% | 38% | 68% | 100% |
| BALNB[(4)] | 73% | 70% | 76% | 80% | 66% |

[(1)] PPV: positive predictive value;
[(2)] NPV: negative predictive value;
[(3)] EAT: endotracheal aspirate;
[(4)] BALNB: bronchoalveolar lavage non-bronchoscopic;

**Table 4. Microbiological profile of cases of ventilator-associated pneumonia**

| Bacteria | General | | Resistant | |
|---|---|---|---|---|
| | n | % | n | % |
| *Pseudomonas aeruginosa*[a] | 9 | 30 | 2 | 22 |
| *Klebsiella pneumoniae*[b] | 6 | 20 | 2 | 33 |
| *Staphylococcus aureus*[c] | 5 | 16,6 | 3 | 60 |
| *Enterobacter spp*[b] | 2 | 6,6 | 2 | 100 |
| *Acinetobacter baumannii*[a] | 2 | 6,6 | 0 | --- |
| *Escherichia coli*[b] | 1 | 3,3 | 1 | 100 |
| *Candida sp* | 1 | 3,3 | 0 | --- |

[a] Resistant to carbapenems (Imipem e Meropenem);
[b] Producers of extended-spectrum beta-lactamase;
[c] Resistant to Oxacillin (multi-drug resistant *S. aureus*).

Two patients obtained only one clinical criteria associated with pulmonary infiltrated and, despite having reached CPIS score greater than 6, were considered without VAP. The vast majority of patients had already used or was in antibiotic use on the day of the diagnosis. On average there were mechanically ventilated 12.4 days at diagnosis. No deleterious effect as hypoxia, atelectasis or arrhythmia was observed. The general characteristics of the sample are identified in Table 1. Precision measures estimated for the methods in relation to

## DISCUSSION

In this comparative prospective study we found a disease with high incidence and mortality, with a considerable number of patients with inadequate empiric antibiotics and ETA was method with higher accuracy in the identification of the etiologic agent. The hospital mortality rate found (43%) was much higher than in the larger epidemiological survey (30.4%) of Rinaudo *et al*., 2015, probably due to the greater severity of

our cases and the high prior use of antibiotics. However mortality during ICU stay (33%) was much lower when compared to other Brazilian study (44.3%) (Guimarães 2006) and compatible with the predicted severity score. We observed 34% of inadequate initial antibiotic therapy, defined by bacteria resistant to the antibiotic prescribed empirically. Piskin *et al* (2012) found as predictor of inadequate initial antibiotic therapy the previous admission to a surgical ward, a fact associated with longer hospital stay and mechanical ventilation. These factors were also demonstrated by our sample, which obtained previous use of antimicrobials and trauma admission in 96% and 57% of the time, respectively. These patients however did not show different mortality from those in which the bacteria were sensitive. There is concern that the previous use of antibiotics, as well as the current use of it, can interfere with what is found in culture. However, a meta-analysis showed a reduction in accuracy of bronchial brushing, did not find the same change in the result of the LBA. (Gupta *et al* 2018). The clinical diagnosis of VAP aims prompt recognition and early treatment, since the delay in its start may worsen the prognosis (Iregui 2002). However the use of clinical criteria or prognostic scores is nonspecific methods (Koulenti *et al* 2009), although the literature has already shown good correlation with histopathology (Fabregas *et al*. 1999). Clinical scores have been used in an attempt to improve the accuracy of clinical method but studies are contradictory regarding their usefulness, sometimes showing benefits in guiding the appropriate antibiotic (Shan *et al*. 2011), other times showing poor results for diagnosis and therapeutic decision (Zilberberg and Shorr 2010).

The definition of the etiological agent of VAP aims the knowledge of the local flora, allowing the use of appropriate initial empiric antibiotic and the possibility of escalation (Safdar *et al* 2005). It is a fact that even different units in a hospital (Namias *et al*. 2000) and different locations (Ferrer and Torres 2018) have their own prevalences. The importance of prior knowledge of the most prevalent pathogens is due to the fact that it is common to find resistant bacteria to the initial antimicrobial (Kollef and Ward 1998) and that patients who have altered inadequate empirical antibiotic based on cultures has the same mortality of patients who remain with antibiotics not effectives (Souza-Oliveira *et al* 2016). Bronchoscopic invasive methods despite changing the handling of the antibiotic, do not alter the mortality compared to noninvasive methods such as the AET (Shorr *et al*. 2005). The BALNB has been accomplished to Levine probe (Minutoli *et al*. 1990) was modified and shown to be safe and good sensitivity (73%), and good correlation (r = 0.90) when compared with BAL (Leo *et al*. 2008). In a similar study Khilnani *et al* (2011) obtained a sensitivity and specificity of 83.3% and 71.5%, with good correlation with bronchial brush and BAL, suggesting a viable alternative to BAL. However, our study showed a yield of only 56%. Despite the good agreement with BAL, with Kappa 0.47, BALNB showed sensitivity and specificity values (70 and 76%) well below other studies (93 and 85% respectively) (Yildiz-Atikan *et al* 2015). Regarding the bacterial isolates there was 100% agreement regarding LBA when both were positive with Kappa 0.63.

Widely used for its ease and low cost, ETA has a sensitivity of 38-82% and specificity of 72-85%, with a negative predictive value of approximately 94% (American Thoracic Society 2005). The results demonstrate the usefulness of ETA to exclude the diagnosis when their culture is negative, reaching NPV of almost 100%. Studies using BAL showed that with resolution of the clinical picture (Kollef and Kollef 2005) or not (Raman *et al* 2013), negative cultures can be used to stop the use of antibiotics without worsening mortality. Khilnani *et al* (2011) showed a similar yield (52%) but with only 64% concordance (Kappa index 0.27) compared to BAL, well below the value obtained in this study (0.41 Kappa). Regarding the agreement between the isolated bacteria obtained Kappa index of 0.64. The BAL has a sensitivity and specificity of 42-93% and 45-100%, respectively in literature (Torres and El-Ebiary 2000) and our results are similar. The volume of saline solution used in the BAL was 100ml. However, it was not always identified the venue of the BALNB, which also used 100ml solution with total volume may have reached 200ml adding both washed. There is in the meta-analysis literature relating lower diagnostic accuracy when used less than 140ml. However this analysis included 26 studies, of which only 6 used more than 140ml (Michaud *et al* 2002). In relation to the dilution of the washed Baldesi *et al* (Baldesi *et al* 2009) showed no influence of the injected volume up to 150 ml, in the interpretation of the method. There is a predominance of Gram negative as demonstrated in other studies (Restrepo *et al*. 2013; Medell *et al*. 2012; Souza-Oliveira *et al*. 2016). In total, 36% of multiresistant bacterias were found, probably due to the high use of previous antibiotics. However it was not possible to find differences between early and late onset of VAP in relation to the sensitivity profile. Studies (Bouglé *et al* 2017; Bassi *et al* 2014) found a predominant prevalence of resistant bacteria in patients with late-onset VAP. Among the limitations of the study we can mention the few number of sujects and no tests to assess the quality of BAL as the search for ciliated cells or bronchial squamous epithelial cells. In addition, it was not a routine to request x-ray chest to prove the position of the catheter BALNB. This study is in line with the recommendations of the Infectious Disease Society of America (IDSA) in 2016 (Kalil *et al*. 2016), which does not recommend use of invasive methods in the etiologic diagnosis of VAP. We conclude that the ETA presents good accuracy, negative predictive value able to exclude the diagnosis of VAP with good agreement with the BAL. The BALNB do not showed satisfactory results to replace any of the other methods. There is a predominance of gram negative bacteria, with 36% of multiresistant.

**Ethical Approval:** All procedures were in accordance with ethical standards of the institutional committee and with the 1964 Helsinki declaration.

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