Accuracy of Tracheal Aspirate Gram Stain to predict Staphylococcus aureus in Ventilator-Associated Pneumonia

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Abstract

**Introduction:** Gram stain can be used to direct initial empiric antimicrobial therapy while culture is not available. This conduct could reduce the incidence of inappropriate initial therapy and adverse outcomes. However, several studies have attempted to determine the value of the Gram stain in the diagnosis and therapy in different populations of patients with VAP, with conflicting results.

**Methods:** This prospective single-center open cohort study enrolled 399 patients from December 2005 to December 2010 to evaluate the accuracy of the Gram stain to predict the existence of *Staphylococcus aureus* in culture in patients suspected of VAP. The inclusion criteria was being suspected VAP by ATS / IDSA criteria. Respiratory secretion samples were collected by tracheal aspirate for standard bacterioscopic analysis of Gram stain and culture.

**Results:** Samples of respiratory secretions collected by tracheal aspirates of 392 patients were analyzed by Gram stain and culture. When Gram-positive cocci were arranged in clusters, the sensitivity was 68.4%, specificity 97.8%, positive predictive value 88.1% and negative predictive value 92.8% for predicting the presence of *Staphylococcus aureus* in culture (*p* < 0.001).

**Conclusions:** Gram stain of tracheal aspirate could discard the presence of *Staphylococcus aureus* in patients with clinical diagnosis of VAP with a 92.8% Negative Predictive Value. Therefore, 7.2% of patients with *Staphylococcus aureus* would be not protected by an empiric treatment that limits antimicrobial coverage to *Staphylococcus aureus* only when Gram positive cocci in clusters are identified.
Keywords: *Staphylococcus aureus*, Gram stain, tracheal aspirate, ventilator-associated pneumonia.

**Introduction**

Ventilator-associated pneumonia (VAP) is a nosocomial pneumonia that arises 48-72 hours after endotracheal intubation [1, 2] and is associated with increased morbidity, mortality and costs in critically ill patients [3, 4]. VAP is difficult to diagnose being suspected using a combination of clinical, radiological and microbiological criteria[5]. The presence of a new or progressive radiographic infiltrate added to two of three clinical criteria (greater than 38.0 °C, leukocytosis or leukopenia, and purulent respiratory secretions fever) represent the most accurate combination of criteria for starting empirical antibiotic treatment [6]. The initiation of broad-spectrum antibiotics followed by reduction of antimicrobial spectrum after identification of the etiologic germ is the strategy used by the majority of intensive care units (ICU). The choice of broad-spectrum antibiotics is based on many factors, including the time of ICU admission, prior use of antibiotics and sensitivity profile of the local flora[4, 7]. The etiological cause of pneumonia is defined by culture of tracheal aspirate or sputum initial microscopic evaluation [8, 9]. This assessment is performed by Gram stain and can be used to direct initial empiric antimicrobial therapy while culture is not available, conduct that causes low incidence of inappropriate initial therapy and no adverse outcomes [7, 10, 11]. However, several studies have attempted to determine the value of the Gram stain in the diagnosis and therapy in different populations of patients with VAP, with conflicting results [4, 12-15]. In our hospital, aiming at restricting antibiotics overuse and prevent multidrug-resistant organisms induction and associated costs, a protocol created by the local
Hospital Infection Control Committee precludes the use of vancomycin for initial empiric treatment of VAP in the absence of gram-positive cocci on gram stain of tracheal aspirate. To validate this protocol, we developed the present study to evaluate the accuracy of the Gram stain to predict the existence of *Staphylococcus aureus* in culture in patients suspected of VAP.

**Methods**

This prospective single-center open cohort study enrolled 399 patients from December 2005 to December 2010. The inclusion criteria for the study were being suspected VAP by ATS / IDSA criteria [1, 5] and have respiratory secretion sample collected by tracheal aspirate for standard bacterioscopic analysis of Gram stain and culture. Patients who did not achieve criteria for VAP by Clinical Pulmonary Infection Score (CPIS) [2, 4] were excluded. All patients were admitted to the ICU of the university teaching hospital. Data were prospectively collected by researchers of the Infection Control Committee and registered in a database. Respiratory secretions were collected by tracheal aspirate. All samples were Gram stained by the microbiology technicians and read by hospital microbiologists. Samples with more than 10 epithelial cells per field with 100x magnification were discarded and new material was collected for analysis. After evaluating smear by Gram stain, cultures were plated on chocolate agar and depending on the results also on blood agar, azide blood agar and McConkey agar. Analysis of the colonies were performed in order to quantify, identify the germ and its antimicrobial susceptibility profile. A culture was considered positive when identified more than $10^5$ CFU/ml.

Statistical analysis: Data were analyzed using SPSS ® 19 software (IBM, 2010). Relationships between culture result and Gram stain were tested with the chi-square test. Statistical significance was accepted at $P < 0.05$. 
The present study was approved by the Hospital de Clínicas de Porto Alegre Research Ethics Committee, which, considering the nature of the study, waived requirements for informed consent.

**Results**

The study design is shown in figure 1.

Samples of respiratory secretions collected by tracheal aspirates of 392 patients were analyzed by Gram stain and culture.

Gram positive cocci, including in clusters, chains and diplococcus, were seen on Gram stain of 148 (37.7 %) samples.

Gram positive cocci in clusters were identified in 59 (15.1%) samples, 52 (88.1%) of these isolated *Staphylococcus aureus* in the culture.

Crosstabulation of Gram stain results and *Staphylococcus aureus* in tracheal aspirate cultures is shown in Table 1.

Accuracy of Gram stain to predict *Staphylococcus aureus* in tracheal aspirate is shown in Table 2.

When Gram-positive cocci were arranged in clusters, the sensitivity was 68.4%, specificity 97.8%, positive predictive value 88.1% and negative predictive value 92.8% for predicting the presence of *Staphylococcus aureus* in culture \((p < 0.001)\).

Twenty eight (7.1%) samples read Gram positive cocci in chain, five of these isolated *Staphylococcus aureus* in culture. There was no statistical correlation between the presence of gram positive cocci in chains and growth of *Staphylococcus aureus* in culture.

From all *Staphylococcus aureus* identified, 71.1% were methicillin-resistant.

**Discussion**
In our study, we found a Positive Predictive Value of 82.1% and a Negative Predictive Value of 92.8% for Gram stain to identify *Staphylococcus aureus* in tracheal aspirate samples.

Our findings of high Negative Predictive Value are in accordance with Blot et al results. When considering microbiologically proven VAP, they found that the sensitivity and specificity of Gram stain examination were, respectively, 89% and 56% for tracheal aspirate. The negative and positive predictive value of Gram stain examination of tracheal aspirate were 90%, and 53% respectively. Their results strongly suggest that when the Gram stain examination of EA is negative, the diagnosis of VAP is very unlikely[16]. Similarly, Fagon et al demonstrated that the Gram stain examination of tracheal aspirate showed a good sensitivity (88.9%) but lacked specificity (59.6% false positives) for the diagnosis of VAP[10].

Still, our results differ from others. Namias et al. showed poor overall correlation of Gram positive cocci with the aspirate culture results in a study in which Gram stain was used to guide empiric antibiotic treatment of pneumonia in surgical ICU patients[17]. Tetenta and Metersky, found that the Gram stain had a sensitivity of 68%, a specificity of 72%, a negative predictive value of 80% and a positive predictive value of 59% for *Staphylococcus aureus*. However, they did not attempt to correlate the Gram stain findings with the presence of VAP. Therefore, some of the patients may have had low level colonization and not *Staphylococcus aureus* pneumonia[14].

With these findings, the discussion becomes how much risk the care team supports taking in a disease in which mortality can vary from 25 to 76%[5, 7]. It is well established that early and appropriate empirical treatment reduces mortality[18-21]. The results of culture tests usually take 72 hours to be available. So the question becomes, can we wait 72 hours to adjust treatment?

There is evidence that waiting for culture may have important consequences. Iregui et al demonstrated that patients with diagnostic criteria for VAP that waited for culture results had a significant delay to receive antibiotic treatment. The mean time interval until the administration
of antibiotic treatment was 28.6 h among patients that waited for culture results, compared to 12.5 h for all other patients ($p < 0.001$). Delayed administration of antibiotic treatment was an independent risk factor for mortality (odds ratio, 7.68; 95%CI, 4.50 -13.09; $p < 0.001$)[19]. Luna et al demonstrated that when adequate antibiotic therapy is initiated very early (before performing bronchoscopy), mortality rate is reduced (38%), when compared to inadequate or no therapy given (91%) ($p<0.001$). When patients were changed from inadequate antibiotic therapy to adequate therapy, based on the results of BAL, mortality was comparable to those who continued to receive inadequate therapy[21]. Kollef and Ward showed that changing or modifying initially inadequate antibiotic treatment did not improve outcome. The hospital mortality rate of patients who have their antibiotic therapy changed or whose therapy started following culture was 61%, while the mortality rate of patients with no change in their antibiotic management was 33%. Changes probably occurred too late in the course of the illness to have a beneficial effect[20]. Rello and coworkers found a significant increase in mortality as a result of inappropriate early antibiotic therapy (37 versus 15%) despite guided changes in therapy after the results of cultures[22].

Based on these reports, it is unquestionable that antibiotic treatment must be started early and there is no clear advantage of late escalation. At this early moment Gram stain could be an useful tool.

Our findings demonstrate that to discard the presence of Staphylococcus aureus, Gram stain offered a Negative Predictive Value of 92.8%. But microbial ecology of the unit, including the frequency of cases of S aureus should also be considered in the therapeutic decision.

Our study has some limitations. It is a single center study. We did not include patients suspected of VAP with negative cultures. Similar to other studies, many samples of the lower respiratory tract were obtained from patients who had received previous or current antibiotics,
potentially influencing the culture results. On the other hand, to establish Positive Predictive Value and Negative Predictive Value it is necessary to have the true prevalence, which means a population with microbiological confirmed VAP. However, our study has several valuable points, including its prospective nature, and the presence of a clinical diagnosis of VAP with CPIS ≥ 7 confirmed by positive culture in all patients.

Conclusions
Gram stain of tracheal aspirate could discard the presence of Staphylococcus aureus in patients with clinical diagnosis of VAP with a 92.8% Negative Predictive Value. Therefore, 7.2% of patients with S aureus would not be covered by an empiric treatment that precludes the use vancomycin when there is not identification of Gram-positive cocci in clusters in tracheal aspirate.

Key messages
- Absence of Gram positive cocci in clusters in tracheal aspirate could discard the presence of Staphylococcus aureus in patients with clinical diagnosis of VAP with a 92.8% Negative Predictive Value.
- 7.2% of patients with S aureus would not be covered by an empiric treatment that precludes vancomycin when Gram-positive cocci in clusters are absent.

List of abbreviations
CFU, colony-forming unit
CPIS, clinical pulmonary infection score
ICU, intensive care unit
MRSA, methicillin-resistant Staphylococcus aureus
*S. aureus, Staphylococcus aureus*

VAP, ventilator-associated pneumonia

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

RS contributed substantially towards the conception and design, drafting the manuscript, analysis and interpretation of data, drafting and critically revising it for the intellectual content and final approval of the version. BGSS involved in drafting and revising the work for important intellectual content and final approval of the version. LRK contributed substantially towards the conception and design of the work, acquisition, analysis and interpretation of data. RPS contributed substantially towards conception, design, acquisition of data, analysis, interpretation of data. All authors read and approved the final manuscript.

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**References**


Figure 1. The study design
Table 1. Crosstabulation of Gram stain and *Staphylococcus aureus* in tracheal aspirate

<table>
<thead>
<tr>
<th></th>
<th>Staphylococcus</th>
<th>Aureus</th>
<th>Total</th>
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<tbody>
<tr>
<td>Gram Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in clusters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>52</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>309</td>
<td>333</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>316</td>
<td>392</td>
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</tbody>
</table>
Table 2. Accuracy of Gram stain to predict *Staphylococcus aureus* in tracheal aspirate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>68.4</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.8</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>88.1</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>92.8</td>
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<tr>
<td>Positive Likelihood Ratio</td>
<td>31.1</td>
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<td>Negative Likelihood Ratio</td>
<td>0.3</td>
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<tr>
<td>Pre-Test Probability</td>
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<tr>
<td>Post-Test Probability – Positive</td>
<td>88.2</td>
</tr>
<tr>
<td>Post-Test Probability - Negative</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Pearson Chi Square $p = 0.0001$
399 patients suspected of VAP with CPIs ≥ 7 performed Tracheal Aspirate

7 patients did not have Gram

392 patients had Gram stain and culture

Presence of Gram positive cocci in clusters 59

Absence of Gram positive cocci in clusters 333

Presence of S. aureus 52

Absence of S. aureus 7

Presence of S. aureus 24

Absence of S. aureus 309

76 patients read S. aureus
54 were MRSA

316 patients did not read S. aureus
54 were MRSA

VAP, ventilator-associated pneumonia; CPIS, clinical pulmonary infection score; MRSA, methicillin-resistant *Staphylococcus aureus*; *S. aureus*, *Staphylococcus aureus*. 