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# The accuracy of gram stain of respiratory specimens in excluding *Staphylococcus aureus* in ventilator-associated pneumonia

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## ABSTRACT

*Objective:* To evaluate the Gram stain of deep tracheal aspirate as a tool to direct empiric antibiotic therapy, and more specifically as a tool to exclude the need for empiric antibiotic coverage against *Staphylococcus aureus* in ventilator-associated pneumonia

*Design:* A prospective, single-center, observational, cohort study

*Setting:* All wards at a community hospital.

*Patients:* Adult patients requiring mechanical ventilation, identified as having VAP in a 54-month prospective surveillance database.

*Interventions:* Sampling of lower airway secretions by deep endotracheal aspiration was taken from each patient who developed VAP**.** Samples were sent immediately for Gram stain and qualitative bacterial cultures. Demographic and relevant clinical data were collected, Gram stain, culture and antibiotic susceptibility results were documented, and outcome was followed prospectively.

*Measurements and Main Results:* The analysis included 114 consecutive patients with 115 episodes of VAP from June 2007 to January 2012. Sensitivity of Gram stain compared to culture was 90.47% for gram positive cocci, 69.6% for gram negative rods and 50% for sterile cultures. Specificity was 82.5%, 77.8%, and 79%, respectively. Negative predictive value was high for Gram positive cocci (97%) and sterile cultures (96%) but low for gram negative rods (20%)**.** *Acinetobacter baumanii* (45%), *Pseudomonas aeruginosa* (38 %) were the prevailing isolates. *Staphylococcus aureus* was found in 18.3% of the patients. Most isolates were multi-resistant.

*Conclusions:* Absence of Gram positive bacteria on Gram stain had a high negative predictive value**.** These data can be used to narrow the initial empiric antibiotic regimen, and avoid unnecessary exposure of patients to vancomycin and other antistaphyloccocal agents.

## INTRODUCTION

Ventilator-associated pneumonia (VAP) is a common and serious infection in mechanically ventilated patients.VAP is caused by a wide spectrum of bacterial pathogens and may be polymicrobial. Common pathogens include aerobic gram-negative bacilli, such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* species. Infections due to gram-positive cocci, such as *Staphylococcus aureus*, particularly methicillin resistant *S. aureus* (MRSA), have been rapidly emerging in the United States [1]. Pneumonia due to *S. aureus* is more common in patients with diabetes mellitus, head trauma, and those hospitalized in intensive care units (ICUs) [2].

While cultures of respiratory-tract specimens are useful to confirm the microbiologic diagnosis of VAP and to tailor antibiotic therapy, the results are not available until 48–72 hours after collection of specimens. Early effective therapy for VAP is associated with improved outcome whereas delays in the administration of appropriate antibiotic therapy for VAP have been associated with excess mortality [3,4]. Inadequate therapy during the initial 48 hours was associated with a mortality rate almost three-fold higher (91%) than with appropriate empiric therapy (38%) [3]. Hence, Administration of empiric antibiotics that provide adequate early coverage is critical. Consequently, once VAP is considered, samples for culture must be obtained quickly and broad spectrum empiric treatment initiated without delay.

Principles of choosing appropriate empiric therapy for VAP include onset of VAP (early or late), knowledge of organisms likely to be present and local resistance patterns within the ICU [5]. Any additional information that can assist to tailor the empiric regimen as early as possible in a way that assures optimal coverage on one hand and avoids unnecessary exposure to antibiotics on the other hand is of prime importance. Gram staining of respiratory specimens can provide rapid information, but the predictive value of this procedure for VAP and the concordance of Gram stain with subsequent culture results are unclear [6].

The recommended initial empiric therapy for VAP includes coverage of aerobic gram-negative bacilli and of *S. aureus* [1].Antibiotic selection for the individual patient is based on the risk factors for MDR pathogens. When MRSA is likely to be present the antibiotic coverage should include vancomycin or linezolid [1]. However, even in these circumstances, strategies to refine the choice of empiric antibiotics are warranted because vancomycin has limited efficacy against MRSA [7] and more so against MSSA [8], and because increased vancomycin exposure is strongly related to the emergence of resistant organisms, particularly vancomycin-resistant enterococci **[**9].

The aim of the present study was to examine whether we can use the results of bronchial secretions gram-stain in patients with VAP to refine the choice of initial empiric antibiotic therapy, and to avoid unnecessary exposure to vancomycin.

## PATIENTS and METHODS

### *Setting and design*

We conducted a prospective observational study of VAP patients in all wards of a community hospital in the center of Israel. Our aims were to characterize the patients, to depict the causative pathogens of VAP and their antibiotic susceptibility and to evaluate deep tracheal aspirate Gram stain as a tool to direct empiric antibiotic therapy. Consecutive adult patients with VAP (see definition below) were recruited prospectively during the daily Infectious diseases consultation rounds at the multidisciplinary ICU and other wards (Internal medicine, surgery). In Israel, mechanically ventilated patients are transferred to ICU according to bed availability. When there are no available beds in ICU, ventilated patients are treated in step-up units in non-ICU wards. We allowed for more than one episode of VAP per patient. Demographic, radiologic, clinical and microbiological data were recorded and patients were monitored throughout their entire hospitalization. The study was approved by the E. Wolfson Hospital Institutional Review Board. Informed consent was waved given the non-interventional design of the study.

***Variables and Definitions***

VAP was defined as a new/progressive infiltrate on chest X-ray in a patient who is intubated for at least 48 h, plus 2 or more of the following: temperature greater than 38°c; WBC ≥10,000/ml or ≤ 3,500/ml; increased purulence/frequency of tracheal secretions; reduced oxygen saturation [10]. Only patients whose antibiotic therapy was not started/changed in the 48 hours prior to VAP diagnosis were included. To better characterize the study population with VAP we also collected demographic, epidemiological and clinical data on the recruited patients; modified SOFA (Sequential Organ Failure Assessment) score from which we omitted the Glasgow Coma Scale due to the fact that many of our patients were sedated at the time of VAP diagnosis and could not be assessed neurologically; concurrent true bacteremia was defined as bacteremia during the 72 hours following VAP diagnosis excluding known contaminants such as CONS, *corynebacterium* spp. etc; outcome (7 and 30 days mortality) and date of death/hospitalization discharge were also recorded.

***Microbiology***

Lower airway secretions for qualitative bacterial cultures were obtained by deep endotracheal aspiration with a sterile suction catheter and suction trap. Specimens were transported to the laboratory immediately after collection. All samples were plated for culture within 1 hour. Gram stains were performed on all specimens using the usual technique. Cultures were performed using standard media. Organisms recovered were identified and their susceptibility was tested using standard techniques. Blood cultures were processed by the BACTEC 9240 blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). Commercial system (VITEK 2, bioMérieux, Marcy l'Etoile, France) was used for identification and susceptibility.

### *Statistical Methods*

The Statistical analysis was performed in R, version 2.15.2. (R Foundation for Statistical Computing, Vienna, Austria). We report two-sided p-values and 0.95 confidence intervals. Fisher's exact test for 2-by-2 contingency tables was used for computing p-values, estimating the Odds Ratio, and constructing confidence intervals for the Odds Ratio for association between dichotomous variables.

## RESULTS

### *Patients*

From July 1st 2007 to January 31st 2012, VAP was diagnosed in 114 patients at the E. Wolfson Hospital. One patient had 2 episodes of VAP (on the 13th and 55th days of his intubation) resulting in a total of 115 episodes of VAP. Most patients were males (58.7%), 40% in their 8th decade (mean age 72 years).The majority of them were admitted from the community (83.3%), and acquired VAP in the ICU (63.1%). Fifteen out of 114 VAP patients (13.1%) died within 7 days from the time VAP was first diagnosed. Another 32 patients died between day 8 and day 30 resulting in a total 30 days mortality rate of 41%.

The only significant difference between VAP cases that occurred in ICU (75 patients) and VAP cases on non-ICU wards (40 patients) was the younger age of ICU patients (mean age 68.2 years vs. 78.5 years, respectively; p-value = 0.0006; CI: 4.53 - 16). Gender, healthcare background, intubation to VAP period, modified SOFA score, 7 days mortality and 30 days mortality were not significantly different between ICU and Non-ICU patients. High modified SOFA score was the only factor associated with increased 7 days and 30 days mortality. True bacteremia was insignificantly associated with increased 7 days (OR = 2.2, P value = 0.23) and 30 days mortality (OR = 1.6, P value = 0.31).

***Pathogens and Antibiotic resistance***

Six deep tracheal aspirate cultures were sterile. Growth was poly-microbial in 50% of the remaining 109 positive deep tracheal aspirate cultures; in total 187 pathogens were isolated. *Acinetobacter baumanii* (45%) and *Pseudomonas aeruginosa* (38 %) were the prevailing isolates. *Staphylococcus aureus* was found in 18.3% of the patients. Thirty-five patients with VAP (31.3%) had concurrent bacteremia; nine patients had poly-microbial growth. In total, 45 pathogens were isolated from blood.

A high rate of antibiotic resistant pathogens was found in our cohort: 43 of 49 *Acinetobacter baumanii* isolates (87.75%) were MDR (defined as resistance to 3 or more antibiotics), as were 12 of 42 *Pseudomonas aeruginosa* isolates (28.5%). Forty of 64 enterobacteriacea (62.56%) were ESBL producers, and 13 of 20 *Staphylococcus aureus* (65%) were MRSA.

***Gram Stain***

101 out of 115 deep tracheal aspirates were Gram stained. Of the 101 tracheal samples that were cultured 6 yielded no growth. The comparison between Gram stain results and final culture results showed different performance of the Gram stain for different pathogens. Sensitivity of Gram stain (in descending order) was: 90.47% for Gram positive cocci (95% Confidence Interval (CI) 0.697-0.988), 69.6% for Gram negative rods (95% CI 0.591-0.787) and 50% for sterile cultures (95% CI 0.119-0.881). Specificity was 82.5% (95% CI 0.724-0.9), 77.8% (95% CI 0.4-0.971), and 79% (95% CI 0.694-0886), respectively. Negative predictive value was high for Gram positive cocci (97%, CI 0.898-0.996) and sterile cultures (96%, CI 0.892-0.991) but low for Gram negative rods (20%, CI 0.085-0.369). Positive predictive value was high for Gram negative rods (97%, CI 0.895-0.996), fairly low for Gram positive cocci (57.6%, CI 0.393-0.745) and extremely low for sterile cultures (13%, CI 0.028-0.335). See Tables 1-3.

In subset analysis of Gram stain in cultures that yielded *S. aureus* compared to cultures that yielded any gram positive cocci the Negative Predictive Value remained unchanged and high (97%).

## DISCUSSION

The main purpose of this study was to evaluate the accuracy of Gram stain in excluding *S. aureus* in VAP. We found a very high NPV (97%) of endotracheal aspirates Gram stains for Gram positive bacteria and *S. aureus*, meaning that in 97% of cases where no Gram positive bacteria were seen on Gram stain the final cultures did not grow Gram positive bacteria or *S. aureus*. Based on this high NPV, it is reasonable to withhold empiric coverage for Gram positive bacteria if the initial Gram stain from the endotracheal aspirates does not demonstrate any such bacteria. This was also the conclusion of Albert M. et al [11] who found a NPV of 93% in a retrospective analysis of data from a Canadian multicenter VAP study. These negative predictive values are relevant apparently for Gram positive Gram stains from endotracheal aspirates only; when Gram stains were performed on bronchoalveolar lavage specimens, the NPV for Gram positive organisms was substantially lower (86%, 83%) [11,12].The rate of *S. aureus* isolates was similar in the study by Albert M. et al [11] and in the present one: 18.6% and 18.5% respectively. In ICUs where the overall prevalence of Gram positive bacteria is higher, clinicians may still consider initial empiric Gram positive coverage.

Several methods are available for collection of respiratory-tract specimens for the microbiologic diagnosis of VAP [13]. Bronchoalveolar lavage with quantitative culture of the bronchoalveolar-lavage fluid and endotracheal aspiration with nonquantitative culture of the aspirate are associated with similar clinical outcomes and similar overall use of antibiotics [14]. On the basis of these data we have elected to use the nonquantitative endotracheal aspiration method in this study because it is less invasive, less cumbersome and less time consuming.

A recent meta-analysis [6] examined the role of respiratory specimen Gram stain in the diagnosis of VAP, and the correlation with final culture results. In 21 studies, pooled sensitivity of Gram stain for VAP was 0.79 (95% confidence interval [CI], .77–0.81; P < .0001) and specificity was 0.75 (95% CI, .73–.78; P < .0001). Negative predictive value of Gram stain for a VAP prevalence of 20%–30% was 91%, suggesting that VAP is unlikely with a negative Gram stain but the positive predictive

value of Gram stain was only 40%. Pooled kappa was 0.42 for gram-positive organisms and 0.34 for gram-negative organisms, suggesting fair concordance between organisms on Gram stain and recovery by culture. The authors concluded that positive Gram stain should not be used to narrow anti-infective therapy until culture results become available. We have adopted a different approach, asking a narrower and more specific question, namely the role of Gram stain in excluding *S. aureus* in VAP. As such our results are not in contradiction with those of the meta-analysis.

Our study has a few limitation:1) It is a single center series so unclear if it can be generalized to other settings, although similar results have been produced by a study from Canada [11]. 2) There were only 21 VAP cases with *Staph aureus* in this series hence the estimated sensitivity of Gram stain must lack some power, and 3) This is observational data alone. The next step would be to conduct an interventional trial demonstrating equivalent outcomes between empiric treatment with Gram positive cocci coverage versus empiric treatment without Gram positive cocci coverage for patients with negative Gram stains

## CONCLUSIONS

Gram stain of bronchial secretions is generally considered as a poor predictor of organisms recovered in culture [15], and a recent meta-analysis concluded that a positive Gram stain should not be used to narrow anti-infective therapy until culture results become available **[**6**].** Nevertheless, absence of Gram positive bacteria on Gram stain had a high negative predictive value as demonstrated by the present study and by others **[**11**].** These data can be used to narrow the initial empiric antibiotic regimen, and avoid unnecessary exposure of patients to vancomycin and other anti-staphylococcal agents.

Table 1: Gram stain results compared to Gram Positive Cocci (GPC) on culture

Table 2: Gram stain results compared to Gram Negative Rods (GNR) on culture

Table 3: Gram stain results compared to sterile cultures

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**Table 1: Gram stain results compared to Gram Positive Cocci (GPC) on culture***

| | **GPC seen on Gram stain** | | **Sum** |
|---|---|---|---|
| **GPC growth in culture** | Yes | No | |
| Yes | 19 | 2 | 21 |
| No | 14 | 66 | 80 |
| **Sum** | 33 | 68 | 101 |

*Sensitivity of Gram stain: 19/21= 90.47%

*Specificity of Gram stain: 66/80 = 82.5%

*Negative Predictive Value: 66/68 = 97%

*Positive Predictive Value: 19/33 = 57.6%

**Table 2: Gram stain results compared to Gram Negative Rods (GNR) on culture***

| | **GNR seen on Gram stain** | | **Sum** |
|---|---|---|---|
| **GNR growth in culture** | Yes | No | |
| Yes | 64 | 28 | 92 |
| No | 2 | 7 | 9 |
| **Sum** | 66 | 35 | 101 |

*Sensitivity of Gram stain: 64/92= 69.6%

*Specificity of Gram stain: 7/9 = 77.8%

*Negative Predictive Value: 7/35 = 20%

*Positive Predictive Value: 64/66 = 97%

**Table 3: Gram stain results compared to sterile cultures***

| | **No organisms seen on Gram stain** | | **Sum** |
|---|---|---|---|
| **sterile culture** | Yes | No | |
| Yes | 3 | 3 | 6 |
| No | 20 | 75 | 95 |
| **Sum** | 23 | 78 | 101 |

*Sensitivity of Gram stain: 3/6= 50%

*Specificity of Gram stain: 75/95 = 79%

*Negative Predictive Value: 75/78 = 96%

*Positive Predictive Value: 3/23 = 13%