Abstract:
Introduction Pneumonia is the most common nosocomial infection among ICU patients. Risk of developing pneumonia is 3-10 fold higher in mechanically ventilated patients. Objectives To assess the role of quantitative culture of Endotracheal aspirates in correlating the diagnosis of ventilator-associated pneumonia in ICU patients.

Materials and Methods About 542 ICU patients who were on mechanical ventilation were screened for clinical signs of VAP. VAP rate was 7.1, with 22 cases identified with ventilator associated pneumonia. Endotracheal aspirate culture sensitivity of those 22 cases of clinically diagnosed VAP were analyzed. Results Quantitative culture of endotracheal aspirates in all 22 patients diagnosed with VAP were 105 CFU ml. Conclusions This study shows that threshold values of quantitative endotracheal aspirate culture of 105 CFU ml correlates with the clinical diagnosis of VAP.

Keyword: VAP - Endotracheal Aspirate - 105 CFU ml

TITLE OF THE STUDY:
“CLINICAL AND MICROBIOLOGICAL CORROBORATION TO ASSESS THE ROLE OF QUANTITATIVE CULTURE OF ENDOTRACHEAL ASPIRATES IN THE DIAGNOSIS OF VENTILATOR-ASSOCIATED PNEUMONIA (VAP) IN ICU PATIENTS”

INTRODUCTION:
Patients in the intensive care unit (ICU) are not only at risk due to their critical illness but also from secondary processes such as nosocomial infections. Pneumonia is the single most common nosocomial infection among patients in ICUs. Rates of pneumonia are considerably higher among hospitalized patients, and the risk of developing pneumonia is 3-10 folds higher in ventilated patients. In critical care settings, within hours of intubation, the upper respiratory tract is colonized by potential pulmonary pathogens even when pneumonia is not present. Thus, if an organism is cultured or noted on Gram’s stain, one does not know if it
is the cause of the pneumonia or simply colonization. Ventilator-associated pneumonia (VAP) increases the crude mortality rate by 2-10 times, and the hospital costs by increasing the length of stay and the need for more expensive antibiotics.

**NEED FOR THE STUDY:**

Pneumonia is the most common nosocomial infection among ICU patients. Rates of pneumonia are considerably higher among hospitalized patients, and the risk of developing pneumonia is 3 to 10 times higher among mechanically ventilated patients. Ventilator-associated pneumonia (VAP) increases the crude mortality rate by 2-10 times, and the hospital costs by increasing the length of stay and the need for more expensive antibiotics. This study is designed to assess the role of quantitative endotracheal aspirate in the diagnosis of ventilator-associated pneumonia in our hospital.

**REVIEW OF LITERATURE:**

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after patients have been intubated and received mechanical ventilation. Diagnosing VAP requires a high clinical suspicion combined with bedside examination, radiographic examination, and microbiological analysis of respiratory secretions. VAP is usually suspected when the patient develops a new or progressive infiltrate on chest X-ray, leucocytosis & purulent tracheobronchial secretions. Aggressive surveillance is vital in understanding local factors leading to VAP and the microbiologic milieu of a given unit. Judicious antibiotic usage is essential, as resistant organisms continue to plague intensive care units and critically ill patients. After diagnosing VAP, the clinician should promptly institute therapy. Choosing an appropriate antibiotic regimen, defined by sensitivities of the organism cultured and by dosing regimen ordered, is paramount, as the first 48 hours is critical to patient survival. Starting ventilated patients on high end antibiotics especially if it is not a true pathogen, leads to development of MDR pathogens. The organisms recovered have an impact on outcome, with higher mortality rates seen in VAP caused by Pseudomonas aeruginosa, Acinetobacter spp., and Stenotrophomonas maltophilia. Numerous studies have showed that prior & concurrent antibiotic therapy decrease the accuracy, sensitivity & negative predictive value of Gram staining, including the % of cells containing intracellular organisms, as well as quantitative, semi-quantitative and non-quantitative cultures. To potentially improve the specificity of the diagnosis of VAP and the consequent unnecessary antibiotic use and its associated problems, numerous studies have investigated the role of quantitative cultures of respiratory secretions. These have included nonbronchoscopic methods such as quantitative cultures of ETAs (QEAs) and sampling of secretions from distal airways “blindly” via an endobronchial catheter. Blind bronchial sampling (BBS), PSB, protected telescoping catheter (PTC), BAL, and protected BAL (mini-BAL) samples can be obtained via the latter method. Bronchoscopic sampling methods permit quantitative cultures of PSB, PTC, and protected and nonprotected BAL specimens. For each of the quantitative culturing methods, threshold values have been derived and are expressed in CFU per milliliter. If the number of CFU/ml is equal to or exceeds the threshold values for the particular technique, a diagnosis of pneumonia is made. Threshold values often employed for diagnosing pneumonia by quantitative cultures are $10^5$ to $10^6$, $10^4$, and $10^3$ CFU/ml for QEA,
bronchoscopic BAL, and PSB, respectively, with $10^5$ CFU/ml being the most widely accepted value for QEA. For “blind” distal sampling, the thresholds are $10^3$ CFU/ml for PSB and mini-BAL and $10^4$ CFU/ml for cultures obtained with BBS and unprotected BAL. These “cutoff” values for diagnosing VAP are based in part on the findings of quantitative cultures obtained from infected lung tissue and the volume and dilution of the respiratory secretions retrieved by the technique. For instance, BAL collects approximately 1 ml of secretions in 10 to 100 ml of effluent. This corresponds to a dilution factor of 1/10 to 1/100. Several investigators have confirmed that with pneumonia, pathogens are present in lower respiratory tract inflammatory secretions at concentrations of at least $10^5$ to $10^6$ CFU/ml; contaminants are generally present at less than $10^4$ CFU/ml.

**OBJECTIVES:**
To assess the role of quantitative culture of Endotracheal aspirates in correlating with the diagnosis of ventilator-associated pneumonia in ICU patients.

**INCLUSION CRITERIA:**
All patients admitted in ICU who were on mechanical ventilation for 48 hrs

**EXCLUSION CRITERIA:**
Patients admitted in ICU who were not on mechanical ventilation

**METHODOLOGY:**
1. Identification of patients with a clinical diagnosis of VAP(ventilator-associated pneumonia)

2 Culture & sensitivity of organisms obtained from endotracheal aspirate.

3 Quantitation of culture aspirates

4 Corroboration of clinical diagnosis of VAP with quantitative culture of endotracheal aspirate $10^5$ CFU (colony forming units) / ml.

**STUDY DESIGN:** Prospective, observational study

**MATERIALS AND METHODS:**
All endotracheal aspirates were processed according to the Standard Operating Procedure mentioned in our laboratory manual. The steps were: 1. Smear was made on a clean glass slide and was allowed to air dry. 2. Gram stain was done and observed for the presence of pus cells and bacteria, and the findings were noted down. 3. 10µl of the uncentrifuged sample was drawn with a sterile pipette and was inoculated on sheep blood agar, 10µl on MacConkey agar and 10µl on chocolate agar.

4. In case of turbid samples, dilution of sample was done as follows: 100-fold dilution obtained by resuspending 10 µl of sample in 1 ml of sterile normal saline, and 1000-fold dilution was obtained by resuspending 100 µl of 100-fold diluted sample in 0.9 ml of sterile normal saline. 5. The mixture was vortexed well, and 10 µl each of corresponding dilutions (100-fold or 1000-fold) was inoculated consecutively in sheep blood agar, MacConkey agar and chocolate agar. 6. All the plates were kept in candle jar (5% CO$_2$) and incubated at 37 degC. 7. After 18-24 hours of incubation, the plates were examined for the growth of bacterial colonies. 8. Identification of bacteria was done by colony gram’s stain and routine biochemical reactions.

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9. Colony count (CFU/ML) of the pathogen was calculated as follows: For undiluted sample = number of colonies x 100 For 100-fold diluted sample = number of colonies x 10,000 For 1000-fold diluted sample = number of colonies x 1,00,000

10. Antibiotic susceptibility testing for all the isolates was done by Kirby–Bauer disc diffusion method on Mueller Hinton agar, and the sensitivity pattern was noted down.

RESULTS:
About 542 patients admitted in ICU who were on mechanical ventilation were included in the study. Those patients who were on ventilator support for more than 48 hours were screened for signs of ventilator associated pneumonia, using CPIS (clinical pulmonary infection score) - Rise in temperature, White blood cell count > 10,000/cu.mm, total number of ventilator days, development of new infiltrate shadows on chest X-ray, details of empiric antibiotics started, and presence of purulent tracheo-bronchial secretions. 22 patients were identified with ventilator-associated pneumonia based on the above mentioned criteria for diagnosing VAP. Quantitative culture of endotracheal aspirates in all 22 mechanically ventilated patients diagnosed with ventilator-associated pneumonia (VAP) were 10^5 CFU/ml. Total number of ventilator days: 3056 Number of patients with VAP: 22. VAP rate: 7.1 per 1000 vent-

<table>
<thead>
<tr>
<th>Pathogens isolated from endotracheal aspirates</th>
<th>No:of patients</th>
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<tr>
<td><strong>PATHOGEN ISOLATED</strong></td>
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<tr>
<td>Pseudomonas aeruginosa</td>
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<td>Klebsiella pneumoniae +Pseudomonas aeruginosa</td>
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<tr>
<td>Escherichia coli (ESBL)</td>
<td>2</td>
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<tr>
<td>Acinetobacter baumanii+ Klebsiella pneumoniae</td>
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<tr>
<td>Klebsiella pneumoniae –ESBL</td>
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<tr>
<td>Acinetobacter baumanii</td>
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<tr>
<td>Acinetobacter baumanii(carbapenemase)</td>
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DISCUSSION:
Diagnosing VAP requires a high clinical suspicion combined with bedside examination, radiographic examination, and microbiological analysis of respiratory secretions. On the first day, CPIS (clinical pulmonary infection score) can be useful, especially when combined with quantitative cultures. Antibiotic administration should be promptly initiated when VAP is suspected and quantitative cultures obtained and should be broad in coverage. Knowledge of local antibiograms should guide the choice of antibiotics, in addition to likelihood of organisms (early- or late-onset VAP). For patients already on antibiotics at the time of suspected VAP, the clinician should choose antibiotics from different classes, as it is likely that resistance to “in-use” antibiotics has developed. On the third day, assessment of quantitative culture results and sensitivities at this juncture is prudent, as it may permit early antibiotic de-escalation. To potentially improve the specificity of the diagnosis of VAP, and to avoid consequent unnecessary use of antibiotics & its associated problems like drug-resistance, various studies have investigated the role of quantitative cultures of respiratory secretions. These have included non-bronchoscopic methods such as quantitative cultures of endotracheal aspirates (QEA) and sampling of secretions from distal airways “blindly” via an endobronchial catheter. Threshold values often employed for diagnosing pneumonia by quantitative cultures are $10^5$ to $10^6$ CFU (colony forming units)/ml of pulmonary pathogens. Most studies have concluded that the sensitivities of nonbronchoscopic and bronchoscopic quantitative techniques are comparable. However, the overall concordance in some studies has been only approximately 80%. Superior specificity of quantitative compared to nonquantitative and semiquantitative culture techniques permits us to more confidently de-escalate antibiotics, including the potential for increased bacterial resistance.
In addition, a negative quantitative culture compels us to more aggressively search for other non-infectious and non-pulmonary infectious causes of the patient's presentation. Numerous factors can influence the results of quantitative cultures, including the timing of the pneumonia, the skill and experience of the operator, the adequacy of the specimen, technical aspects such as appropriate processing and delays in transport to the laboratory, special microbial populations in patients with chronic obstructive pulmonary disease (who may have relatively high bacterial counts without pneumonia), and prior or concurrent antibiotic therapy. Because of these potential limitations, it is important to bear in mind that a quantitative culture that exceeds a threshold value is not diagnostic of VAP by itself. False-positive quantitative cultures could be secondary to bronchiolitis, colonization, or oropharyngeal contamination. Likewise, a result below these threshold values does not rule out the presence of pneumonia, particularly in the setting of prior antibiotic therapy. While higher bacterial counts correlate with a higher likelihood of VAP, lower counts are associated with a lower probability. Consequently, rather than interpreting a quantitative culture as either "positive" or "negative," it is clinically more useful to utilize the exact number of CFU/ml.

In a study done by Chien Liang Wu et al., data regarding quantitative culture of endotracheal aspirates correlating with protected specimen brushings and broncho-alveolar lavage in patients with treatment failure diagnosed with ventilator-associated pneumonia have been analysed. QEA is non-invasive, and early investigation by QEA would be helpful to the clinician in decision making with regard to antibiotic use.

CONCLUSION:
This study does confirm the microbiological corroboration in predicting the diagnosis of ventilator-associated pneumonia, which is reinforced by the role of quantitative culture of endotracheal aspirates in ICU patients on mechanical ventilation for more than 48 hours. Aggressive surveillance is vital in understanding local factors leading to VAP and the microbiologic milieu of a given unit. Judicious antibiotic usage is essential, as resistant organisms continue to plague intensive care units and critically ill patients.

REFERENCES:
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4. Chen Liang Wu et al. CHEST 2002; 122: 662-668