Introduction:
Less invasive technique such as quantitative culture of Endotracheal Aspirate (ETA) with a threshold of \(10^5\) to \(10^6\) CFU/ml of exudates is considered as optimal for microbiological confirmation of Ventilator Associated Pneumonia (VAP). Therefore, the present study was conducted to compare quantitative culture of ETA aspirates with qualitative culture in clinically suspected cases of VAP.

Methods:
Endotracheal aspirate (ETA) was collected under aseptic precautions. Qualitative as well as quantitative cultures of the endotracheal aspirate were performed as per standard methods. Even a single colony in plate of \(10^{-5}\) dilution was considered as infection.

Results:
Out of 130 ETA, 40.77% were VAP and 59.23% were non-VAP cases, according to clinical criteria. Though sensitivity of qualitative culture was 100% but specificity was only 22%. Sensitivity of quantitative culture was 94.34% and specificity was 88.31%. All VAP cases showed growth in qualitative culture (100%). Qualitative culture in the Non-VAP group showed growth in 77.92% cases, which significantly reduced to 11.69% in quantitative culture. All VAP cases showed growth in qualitative culture (100%).

Conclusion:
To potentially improve the specificity of the diagnosis of VAP, quantitative cultures of respiratory secretions should be done. Of the quantitative techniques, quantitative endotracheal aspirate culture is least invasive, most readily available, least expensive, requires least experience and is easily repeatable.

Keywords: Ventilator-associated pneumonia, Endotracheal aspirate, Quantitative culture.
patient population, exposure to antibiotics and changes over the time, emphasizing the need for timely, local surveillance data. More resistant microorganisms appear in late onset form of VAP, which occurs 5-7 days after mechanical ventilation.[3]

Centers for Disease Control and Prevention (CDC) provide guidelines which help in diagnosis of VAP. Also, the Clinical Pulmonary Infection Score (CPIS) aids in diagnosis of the same. Score calculated by quantifying amount of tracheal secretions on a subjective 0-4 scale multiple times per day, then summing all of a patient’s scores for the day. Total score of > 6 points suggests Ventilator Associated Pneumonia.[4] VAP is diagnosed more accurately by bronchoscopic sampling and microbiologic cultures of lower respiratory tract samples. Bronchoscopy being invasive is commonly associated with complications, especially inpatients on high respiratory support. This has paved the way for less invasive technique such as quantitative culture of Endotracheal Aspirate (ETA) with a threshold of $10^5$ to $10^6$ CFU/ml of exudates that is considered as optimal for microbiological confirmation of VAP.[5]

Moreover recent small trials have repeatedly shown that there is no advantage of bronchoscopic cultures over quantitative ETA cultures when mortality was considered as the end point, thus further strengthening quantitative ETA culture as a diagnostic tool. Qualitative ETA culture cannot differentiate colonization from infection. Thus, reliability of this technique is less.[5-9] Although the use of tracheal aspirates in VAP management is increasing, a study from Brazil has emphasised the usefulness of quantitative as opposed to qualitative cultures.[10]

Therefore, the present study was undertaken to compare quantitative cultures of ET aspirates with qualitative cultures in clinically suspected cases of VAP.

**Material and Methods**

It was a prospective study conducted in a tertiary care hospital, for a period of one and half years (April 2013 to October 2014), after taking permission from the Institutional Ethics Committee. A total of 130 cases were included in this study. All were adults > 14 years admitted in Intensive Care Units and fulfilling the criteria of VAP. Patients with respiratory malignancies and HIV positive patients were excluded from the study. Written informed consent was taken for each patient along with detailed clinical history.

Endotracheal aspirate (ETA) was collected under aseptic precautions using a sterile 30 cm long, 12F suction catheter. The secretions obtained were collected in a sterile 40-ml sputum trap. If no respiratory secretions were obtained on the first pass, 5 ml of sterile saline was injected through the endotracheal tube and the aspirate repeated. If the yield was <1 ml, the procedure was repeated following chest physiotherapy. The presence of epithelial cells of >10% implied contamination of the specimen, whilst <10% neutrophils suggested that the diagnosis of pneumonia was less likely.[11]

The sample collected was transported to the laboratory within two hours, 30 minutes being ideal.[12] Qualitative as well as quantitative cultures of the endotracheal aspirate was performed. Primary smear from the sample was made and Gram stain was done.

Semi-quantitative scoring of Gram stain was done based on the number of bacteria per oil immersion field ($\times1,000$):

- 0 = no bacteria per field
- 1+ = less than one bacterium per field
- 2+ = 1–5 bacteria per field
- 3+ = 6–30 bacteria per field
- 4+ = more than 30 bacteria per field

A significant correlation exists between the semi-quantitative Gram stain and quantitative culture results. Moreover, the semi-quantitative Gram stain score of 0 indicates a low probability of VAP, and score of 3+ or above indicates a high probability of VAP, defined as quantitative cultures with $10^5$ CFU/ml.[13]

For qualitative culture, the ETA was directly inoculated on blood agar, chocolate agar and MacConkey agar and plates were incubated overnight at 37°C. Chocolate agar plate was put in
candle jar. Any growth observed was identified using standard biochemical tests. For quantitative culture, samples with large mucus plugs were liquefied and homogenized by vortexing for one minute with glass beads, followed by centrifuging at 3000 rotations per minute for 10 minutes. Five autoclaved test tubes were taken and in each of them 0.9 ml of sterile normal saline was added. 0.1 ml of this aspirate was transferred to the first test tube, mixed thoroughly and 0.1 ml of this mixture was then transferred to second test tube already containing 0.9 ml of saline. Similarly, 0.1 ml of the ET aspirate was serially transferred in the subsequent three test tubes. The final dilutions of the five test tubes were $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$. Subsequently, leaving $10^{-1}$ dilution, the remaining four dilutions of tracheal aspirates were inoculated with a calibrated loop (10 µl) on blood agar and MacConkey agar plates and incubated overnight at 37°C. Next day, the number of colonies were counted on each plate and multiplied by appropriate dilution factor to express the colony count as CFU/ml. The CFU/ml considered as significant helps to discriminate colonization from infection. Threshold of > $10^5$ CFU/ml is suggestive of infection rather than colonization. Even a single colony in plate of $10^{-5}$ dilution was considered as infection.

Results

Tables 1 and 2 show the distribution of qualitative and quantitative culture results in VAP and Non-VAP cases. Though sensitivity of qualitative culture was 100% but specificity was only 22%. Sensitivity of quantitative culture was 94.34% and specificity was 88.31%.

Table 3 & Figure 1 show the comparison between qualitative and quantitative culture in VAP and Non-VAP cases. Qualitative culture in the Non-VAP group showed growth in 77.92% cases, which significantly reduced to 11.69% in quantitative culture. All VAP cases showed growth in qualitative culture (100%). Table 4 shows the growth pattern seen in qualitative and quantitative cultures of endotracheal aspirates in 53 patients. Same organism grew in both qualitative and quantitative cultures in 71.7% (38/53) cases of VAP. Total organisms grown in qualitative culture in VAP were 69 ($34+4\times2+12\times2+3$), whereas in quantitative culture it was 54 ($34+4\times2+12$).

Table 1: Distribution of Qualitative culture results in VAP and Non-VAP cases

<table>
<thead>
<tr>
<th>Qualitative culture</th>
<th>VAP (n=53)</th>
<th>Non-VAP (n=77)</th>
<th>Total (n=130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>53 (100.00)</td>
<td>60 (77.92)</td>
<td>113</td>
</tr>
<tr>
<td>No growth</td>
<td>00 (00.00)</td>
<td>17 (22.08)</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>77</td>
<td>130</td>
</tr>
</tbody>
</table>

Sensitivity = 100%, Specificity = 22.08%, Positive predictive value (PPV) = 46.90%, Negative predictive value (NPV) = 100%

Table 2: Distribution of Quantitative culture results in VAP and Non-VAP cases

<table>
<thead>
<tr>
<th>Quantitative culture (&gt;10^5 CFU/ml)</th>
<th>VAP (n=53)</th>
<th>Non-VAP (n=77)</th>
<th>Total (n=130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>50 (94.34)</td>
<td>09 (11.69)</td>
<td>59</td>
</tr>
<tr>
<td>No growth</td>
<td>03 (05.66)</td>
<td>68 (88.31)</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>77</td>
<td>130</td>
</tr>
</tbody>
</table>

Sensitivity = 94.34%, Specificity = 88.31%, Positive predictive value (PPV) = 84.74%, Negative predictive value (NPV) = 95.77%.

Table 3: Comparison between Qualitative and Quantitative cultures in VAP and Non-VAP cases

<table>
<thead>
<tr>
<th>VAP (n=55)</th>
<th>NON-VAP (n=77)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative culture</td>
<td>Growth</td>
</tr>
<tr>
<td>53 (100.00)</td>
<td>00 (00.00)</td>
</tr>
<tr>
<td>Quantitative culture</td>
<td>50 (94.34)</td>
</tr>
</tbody>
</table>

*Chi-Square test, p < 0.05 = Significant
Table 4: Growth pattern seen in Qualitative and Quantitative cultures of Endotracheal Aspirates in VAP patients (n=53)

<table>
<thead>
<tr>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single growth in both Qualitative and Quantitative cultures</td>
</tr>
<tr>
<td>Double growth in both Qualitative and Quantitative cultures</td>
</tr>
<tr>
<td>Double growth in Qualitative culture and Single growth in Quantitative culture</td>
</tr>
<tr>
<td>Growth in Qualitative culture but no growth in Quantitative culture</td>
</tr>
<tr>
<td>No growth in both Qualitative and Quantitative culture</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Discussion

Use of bronchoscopic techniques permits the physician to devise a therapeutic strategy that is superior to the one based on clinical evaluation. When bronchoscopy is not available, non-bronchoscopic procedures or a clinical score, e.g. Clinical Pulmonary Infection Score (CPIS) should be used. In both bronchoscopic, as well as non-bronchoscopic techniques, quantitative culture is more specific over qualitative culture, as it reduces false positivity due to colonization.[17] The present study was conducted in 130 clinically suspected cases of VAP and efficacy of quantitative cultures of Endotracheal Aspirates (ETA) and qualitative cultures was compared.

In a post-mortem study conducted by Fabregas et al.,[18] when findings on histologic analysis and cultures of lung samples obtained immediately after death was used as reference, the clinical diagnostic criteria of VAP which includes, a new and persistent (>48 hours) infiltrate on chest radiograph plus two or more of the following three criteria (i) fever >38.3°C (ii) leucocytosis of >12×10⁹/ml, and/or (iii) purulent tracheobronchial secretions had a sensitivity of 69% and a specificity of 75% for establishing the diagnosis of VAP.

Because of the poor specificity of the clinical diagnosis of VAP, Pugin et al. developed a clinical score, called the Clinical Pulmonary Infection Score (CPIS), based on six variables. Thus, with a CPIS score of >6, the probability of patient having VAP is more. Out of the total 130 clinically suspected cases of VAP, 53 cases i.e. 40.77% had a score >6 and were considered as VAP patients and the remaining 59.23% (77 cases) were classified as Non-VAP patients (Table 1).

Qualitative and quantitative culture of ETA was performed in all 130 cases. All VAP cases i.e. 53 cases showed growth in qualitative culture, whereas from 77 Non-VAP cases, growth was seen in 60 patients. Thus, the sensitivity of qualitative cultures of tracheal aspirates was 100% and specificity was 22.08%. The positive predictive value was 46.9% and negative predictive value was 100% (Table 1). For quantitative cultures, a threshold of 10⁵ CFU/ml was taken as significant. In the VAP group, 50 of the 53 cases had >10⁵ CFU/ml, while in Non-VAP group, it was only 9 out of the total 77 cases. Thus, the sensitivity of quantitative cultures of tracheal aspirates was 94.34% and specificity was 88.31%. The positive predictive value was 84.74% and negative predictive value was 95.77% (Table 2).

From the above values, it is obvious that, the specificity and positive predictive value of quantitative ETA are more as compared to qualitative ETA cultures, while sensitivity and negative predictive value of qualitative cultures are high. Thus, if no growth is seen in qualitative cultures, there is very little chance of the patient actually suffering from VAP.

In this study, three cases in VAP group didn’t show growth upto 10⁵ CFU/ml. This might be due to the effect of prior antibiotics in severely ill patients. Similar findings have been reported by Fernando et al. and they had shown that, for VAP diagnosis, sensitivity of quantitative cultures of tracheal aspirates are lower than those for qualitative cultures in severely ill patients receiving prior antibiotics. Thus, quantitative as well as qualitative cultures of tracheal aspirates should be done for the purpose of confirming a clinical diagnosis of VAP in such cases.[10]

Nine cases from the Non-VAP group showed growth in quantitative cultures with threshold above 10⁵ CFU/ml (Table 3). Out of these 9 cases, 5 patients had ARDS. In this study, CPIS of >6 was taken as a case of VAP. But, the sensitivity of clinical criteria for VAP is lower in patients with...
ARDS, where it may be difficult to detect new radiographic infiltrates. In the setting of ARDS, Bell et al reported a false negative rate of 46% for the clinical diagnosis of VAP.\(^{[19]}\) Also, a study conducted by Jean et al. showed that, 55% of the patients with ARDS develop VAP, as compared to 28% of the patients without ARDS. Thus, microbiological evaluation in the form of quantitative, invasive or non-invasive techniques helps in early diagnosis of VAP in patients with ARDS.\(^{[20]}\) Suspicion of VAP in the setting of ARDS should be high. The presence of even one of the clinical criteria for VAP, unexplained hemodynamic instability or an unexplained deterioration in arterial blood gases, should prompt consideration of further diagnostic testing.\(^{[19]}\)

In this study, 77 cases belonged to Non-VAP group. Ideally, they should show no growth in both, qualitative and quantitative cultures. But, in qualitative cultures, around 77.92% cases showed growth and only 22.08% had no growth. And when quantitative cultures with threshold of \(10^5\) CFU/ml were done, growth significantly reduced to 11.69% from 77.92% of that in qualitative culture (Table 4). Thus, false positivity rate was high (around 78%) with qualitative cultures. A study conducted by Estella et al.\(^{[21]}\) states that, qualitative tracheal aspirates should be stopped as a routine diagnostic method for VAP and replaced by bronchoscopic techniques or quantitative tracheal aspirates. Since the airways of most patients on mechanical ventilation are colonized by potentially pathogenic microorganisms, there might be risk of over-diagnosis of the cases of pneumonia, which in fact corresponds to tracheal colonization or tracheobronchitis. Based on the maximum evidence (Grade I), where evidence comes from well conducted, randomized control trials,\(^{[22]}\) both the American Thoracic Society (ATS) and the Spanish Society of Intensive Care Medicine advise against the routine use of qualitative tracheal aspiration for the microbiological diagnosis of VAP, because such samples are unlikely to allow differentiation between colonization and infection.\(^{[21]}\)

A study conducted in Spain\(^{[23]}\) compared the sensitivity and specificity of quantitative ETA cultures at threshold of \(10^3\) and \(10^6\) CFU/ml and Plugged Telescopic Catheter (PTC) at \(10^3\) CFU/ml. The sensitivity of ETA with cut off of \(10^6\) CFU/ml and PTC was significantly higher (71% and 68% respectively) than that of ETA with cut off of \(10^6\) CFU/ml (54%). But, the specificity of ETA \(10^6\) CFU/ml and PTC (75% each) was significantly higher than that of ETA \(10^6\) CFU/ml (58%). It therefore emphasizes the fact that, quantitative ETA can be accepted for diagnosis of VAP, when bronchoscopic techniques are not available. Table 5 shows the sensitivity and specificity of quantitative cultures of endotracheal aspirates in different studies. In a study conducted by Shin et al,\(^{[9]}\) the specificity and negative predictive value of quantitative ETA cultures at threshold of \(10^5\) CFU/ml was 89.5% and 97.1% respectively, which is almost similar to the present study. Sensitivity of quantitative culture in the present study is almost similar to the study by Wu et al.\(^{[11]}\)

Bronchoscopic techniques require skilled personnel to perform the procedure and the sensitivity of Protected Specimen Brush (PSB) decreases when sample collection is done even after 24 hours of starting or changing of antibiotic therapy.\(^{[24]}\) Moreover, the costs incurred are more and repeatability of sampling is difficult. On the other hand, endotracheal sample collection is cheap, easily performed and no qualified personnel are required for sample collection. Also, it has a good negative predictive value and is not associated with any complications during sampling like decline in PaO\(_2\), arrhythmia, bleeding, etc., which may be seen with bronchoscopic techniques.\(^{[11,19,24,25]}\)

Various studies have shown that, there is no advantage of using bronchoscopic methods over tracheal aspirate cultures, when mortality is an endpoint.\(^{[7,8,26]}\) When empirical antibiotic treatment is appropriately standardized, quantitative culture of samples obtained by invasive techniques does not improve the outcome of VAP in comparison with quantitative culture of samples obtained by non-
invasive techniques. Using invasive techniques for diagnosing VAP leads to more antibiotic changes but with no improvement in mortality or morbidity rate and with additional costs. Reduced costs and similar outcomes were reported using quantitative tracheal aspirates for guiding or deciding to de-escalate antibiotic treatment for VAP.

This may be due to the high correlation between tracheal aspirates and bronchoscopic cultures, when presence of VAP is highly suggestive.

Table 5: Sensitivity and specificity of quantitative cultures of endotracheal aspirates in different studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Year</th>
<th>Quantitative culture of ETA</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabregas et al.</td>
<td>1999</td>
<td>69%</td>
<td>18</td>
</tr>
<tr>
<td>Wu et al.</td>
<td>2002</td>
<td>92.8%</td>
<td>11</td>
</tr>
<tr>
<td>Shin et al.</td>
<td>2003</td>
<td>85.7%</td>
<td>9</td>
</tr>
<tr>
<td>Arango et al.</td>
<td>2003</td>
<td>71%</td>
<td>23</td>
</tr>
<tr>
<td>Present study</td>
<td>2014</td>
<td>94.3%</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusion
Where comparisons have been made, authors of most of the studies have concluded that, the diagnostic accuracies of non-bronchoscopic and bronchoscopic techniques are similar. Therefore, to potentially improve the specificity of the diagnosis of VAP and the consequent unnecessary antibiotic use, quantitative cultures of respiratory secretions should be done. Of the quantitative techniques, quantitative endotracheal aspirate culture is least invasive, most readily available, least expensive, requires least experience and is easily repeatable.

References


