Ibrahim Card Med J 2014;4(2): 49-55

Efficacy of Blind Tracheal Aspirate in Comparison to Bronchoalveolar Lavage for Microbiological Diagnosis of Nosocomial Pneumonia in Patients on Ventilator

ASM Areef Ahsan[1], Mohammad Omar Faruq[2], Kaniz Fatema[3], Fatema Ahmed[4], Shakera Binte Hassan[5]

ABSTRACT

Background and Objectives: For diagnosis of nosocomial pneumonia in patients on ventilator, invasive procedure like bronchoscopy for microscopy and quantitative cultures of lower respiratory tract samples is useful but not always possible for potential risk of the procedure and the associated cost. The non-bronchoscopic sampling of the lower airways and quantitative cultures of tracheal aspirate may offer simple and readily available alternative to bronchoscopy with promising results. This study was done to evaluate the efficacy of blind tracheal aspirate in the microbiological diagnosis of nosocomial pneumonia occurring in intubated patients on mechanical ventilator.

Materials & Methods: This cross-sectional study was carried out in the Intensive Care Unit in the Department of Critical Care Medicine, BIRDEM Hospital, Dhaka over a period 16 months starting from January 2010 to April 2011. A total of 54 clinically diagnosed cases of nosocomial (hospital acquired) pneumonia who were on ventilator were consecutively included in the study based on predefined enrolment criteria. All the 54 cases were subjected to blind endotracheal aspirate (BTA) followed by bronchoalveolar lavage (BAL) for quantitative cultures of specimens and isolation of causative microorganisms from them.

Result: The present study showed that the mean age of the patients was 61 years (range: 24-86 years). Males were predominant in the series with male to female ratio being 7:3. Majority of the patients was haemodynamically stable as indicated by mean blood pressures, heart rate, temperature and respiratory rate. Most (83.3%) of the cases showed significant growth of microbes on culture of blind tracheal aspirates at cut-off value of $\geq 10^5$ colony forming unit/ml (cfu/ml), while 87% of the cases exhibited positive growth on culture of bronchoalveolar lavage at cut-off value of $\geq 10^4$ cfu/ml. *Acinetobacter baumannii* was the predominant organism isolated from BTA (73.3%) followed by *Pseudomonas aeruginosa* (33.3%). An almost similar pattern of growth was evident in BAL with more than 70% being *Acinetobacter baumannii* and about 30% *Pseudomonas aeruginosa. C. albicans. Klebsiella sp., E. coli,* and *Flavobacter* were less commonly observed in either group. The Kappa test revealed a good agreement (70.7%) between the two procedures suggesting that the two diagnostic modalities are almost comparable in diagnosing pneumonia in patients admitted in ICU ($p < 0.001$).

Conclusion: The study concluded that the accuracy of blind tracheal aspirate and bronchalveolar lavage in the diagnosis of nosocomial pneumonia was fairly comparable. The strength of agreement between the two diagnostic modalities is also good encouraging its use instead of more invasive procedures like BAL in the diagnosis of hospital-acquired pneumonia who are on mechanical ventilator.

Key words: Blind Tracheal Aspirate, Bronchoalveolar Lavage, Microbiological Diagnosis, Nosocomial Pneumonia, Ventilator.

ORIGINAL ARTICLE

INTRODUCTION

In hospitalized patients particularly who are mechanically ventilated, pneumonia is the leading cause of death from nosocomial infection.[1] Pneumonia that develops 48 hours after mechanical ventilation is called ventilator

Authors' Information:

[1] ***Dr. ASM Areef Ahsan,*** *FCPS (Medicine), MD (Chest), MD (CCM), Associate Professor and Head, Dept. of Critical Care Medicine, BIRDEM General Hospital, Dhaka*

[2] ***Prof. Dr. Mohammad Omar Faruq,*** *MD, FACP, FACEP, FCCM, FCPS, Prof. of Critical Care Medicine, BIRDEM General Hospital, Shahbag, Dhaka*

[3] ***Dr. Kaniz Fatema,*** *FCPS (Medicine), MD (CCM), Assistant Professor, Dept. of Critical Care Medicine, BIRDEM General Hospital, Dhaka*

[4] ***Dr. Fatema Ahmed,*** *FCPS (Medicine), MD (CCM), Assistant Professor, Dept. of Critical Care Medicine, BIRDEM General Hospital, Dhaka*

[5] ***Shakera Binte Hassan,*** *SMO, Green Life Hospital, Dhaka*

Address of Correspondance: ***Dr. ASM Areef Ahsan,*** *Associate Professor and Head, Dept. of Critical Care Medicine, BIRDEM General Hospital, Shahbag, Dhaka-1000, Bangladesh,* ***Cell:*** *01715765210,* ***E-mail:*** *dr_asmareef@yahoo.com*

associated pneumonia (VAP). Similarly, hospitalized patients who develop life threatening nosocomial pneumonia (NP) or hospital acquired pneumonia (HAP) often require mechanical ventilator support. A newly defined entity, health-care associated pneumonia (HCAP), is a form of NP that arises in patients who have been in contact with environments such as nursing homes and hemodialysis centers that expose them to the multidrug-resistant bacteria present in the hospital.[2,3] Antibiotic-resistant organisms may add to the mortality rate of VAP, not because of increased virulence but rather because these organisms are often not anticipated and, when present, are often initially treated with ineffective antibiotic regimens.[4] Accurate diagnosis of VAP, allowing for the timely administration of antibiotic therapy, may improve survival and reduce ventilator days in this group of patients.[5] Clinical diagnosis of pneumonia has been notoriously inaccurate, and the addition of quantitative cultures to the diagnostic work-up of pneumonia has added a significant degree of accuracy. The purpose of diagnostic testing is to confirm HAP and identify the likely pathogen. Diagnostic testing involves radiographic imaging and analysis of lower respiratory tract secretions, including Gram stain and culture. Lung biopsy can be diagnostic, but is seldom performed because of its invasiveness.

All patients suspected of having VAP should, therefore, undergo lower respiratory tract sampling, followed by microscopic analysis and culture of the specimen.[6] There are a variety of methods for sampling material from the airways and alveoli, including bronchoscopic and nonbronchoscopic (i.e. blind) techniques. Invasive bronchoscopic sampling techniques, using protected specimens processed by quantitative culture techniques, such as the protected specimen brush (PSB) and bronchoalveolar lavage (BAL) are currently considered as the most reliable sampling techniques to recover organisms infecting the lower respiratory tract.[7]

Bronchoscopic and nonbronchoscopic sampling for suspected HAP particularly VAP have been compared in several studies. At least one study has demonstrated that more invasive means of culturing the airways, such as bronchoalveolar lavage (BAL), may lead to improved outcome.[5] The quantitative deep endotracheal aspirate (QDEA) has been proposed as a less invasive and less expensive means of obtaining a specimen for culture in the diagnosis of pneumonia in the ventilated patients.[8,9] Endotracheal aspiration is the simplest method of obtaining secretions in patients receiving MV. Endotracheal aspirates are rarely negative in patients receiving MV who have fever though great number of false-positive results (mainly gram-negative bacilli) lead to over diagnosis of pneumonia and irrational use of antibiotics.[10,11] However, nonbronchoscopic sampling has its merit in that it does not require to be supervised by a clinician. This reduces cost, allows specimens to be obtained quickly, and facilitates serial sampling when necessary. Several studies comparing diagnostic value of bronchoscopic and nonbronchoscopic sampling for suspected VAP indicate that bronchoscopic sampling does not improve mortality, length of hospital stay, duration of mechanical ventilation, or length of intensive care unit stay.[12-14]

So early diagnosis is very important for reducing the morbidity, mortality, cost and duration of ICU stay. In a resource limited country like Bangladesh where cost and non availability of ICU bed is an issue, it is important to evaluate whether blind tracheal aspirate is as effective as bronchoalveolar lavage for early microbiological diagnosis of nosocomial pneumonia occurring in intubated patients who are on mechanical ventilator. If the efficacy of these two diagnostic modalities is same then blind tracheal aspirate can be a good alternative to BAL for accurate

diagnosis as it is less costly, non-invasive and expertise is not needed for sample collection.

MATERIALS AND METHODS

This cross-sectional study was carried out in the Intensive Care Unit in the Department of Critical Care Medicine, BIRDEM Hospital, Dhaka over a period of 16 months starting from January 2010 to April 2011. A total of 54 clinically diagnosed cases of nosocomial (hospital acquired) pneumonia who were on ventilator were consecutively included in the study based on predefined enrolment criteria. All the 54 cases were subjected to blind endotracheal aspirate (BTA) followed by bronchoalveolar lavage (BAL) for quantitative cultures of specimens and isolation of causative microorganisms from them. Instruments required to obtain the specimen were endotracheal tube, suction catheter, flexible bronchoscope (OLYMPUS CV-160) and Specimen Trap (busse brand, made in USA). The materials used in the study were normal saline, neuromascular blocking agent and sedatives. Endotracheal aspirate was collected under aseptic precautions using sterile suction catheters and traps. The presence of epithelial cells of >10% implied contamination of the specimen whilst <10% neutrophils suggested that the diagnosis of pneumonia was less likely. With quantitative analysis of endotracheal aspirate (ETA), the threshold for diagnosing NP in this study was taken as 10^5 colony forming units/ml (cfu/ml). The BAL procedure was carried out under aseptic precautions with adequate sedation and FiO_2 of 100% through the endotracheal tube. No topical anesthesia or endobronchial suctioning was used during the advance of the bronchoscope. The scope was wedged into the orifice of the bronchus draining the segment likely to be involved, as judged radiologically, and the sample was collected after instilling three aliquots of 50 mL sterile saline. The sample was sent immediately for culture. The presence of >1% squamous epithelial cells suggested a highly contaminated specimen. The microbiological threshold BAL fluid for the diagnosis of NP was taken as 10^4 cfu/ml.

Quantitative analysis of ETA was done according to gram stain smear interpretation. Depending on the number of organisms seen on direct smear, the clinical sample was diluted in 1 in 100 or 1 in 1000 and subsequently 10 µl of diluted sample was uniformly inoculated on to blood agar, chocolate agar and McConkey agar. If no organism was seen on direct smear, an undiluted sample was inoculated on the agar plates. After overnight incubation the number of colonies were counted on each plate and multiplied by the appropriate dilution factor to express the colony count as cfu/ml. 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. The cfu/ml considered as significant in this study helps discriminate colonization from infection, with thresholds of $\geq 10^4$ cfu/ml for BAL and $\geq 10^5$ cfu/ml for ETA being suggestive of infection rather than colonization.

Collected data were analysed using SPSS (Statistical Package for Social Sciences) for Windows, version 11.5 (SPSS, Inc., Chicago, IL). The test statistics used to analyse the data were descriptive and kappa statistics. Kappa analysis was used to assess the strength of agreement between the two diagnostic modalities used in this study to diagnose nosocomial pneumonia. While a kappa value of '1.0' indicates perfect agreement, a value of '0.0' indicate no agreement between the two diagnostic modalities. In between 0 – 1, the strength of agreement will be categorized as < 0.2 = poor, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = good and 0.81 – 1.0 = very good agreement.[17] Level of significance was set at 0.05 and 'p' value less than 0.05 was considered significant.

RESULTS

The patients were predominantly older (60 or > 60 years old) (61.1%) with mean age of the patients being 61.4 ± 15.6 years (range: 24-86 years). Over two-third (69%) of the patients were male giving a male to female ratio of roughly 7:3 (Table I). Over 40% of the cases had diabetes mellitus (DM), 31.5% had hypertension (HTN), 11.1% Chronic kidney disease (CKD), 9.3% ESRD and 7.4% COPD (Table II). Baseline haemodynamic state and total count of WBC and ESR are illustrated in Table III. The mean values of systolic and diastolic pressures, heart and respiratory rates and temperature indicate that most of the patients were haemodynamiclly stable. The mean total count of WBC was much higher than the upper limit of normal range. The lowest count of WBC was even higher than the upper limit of normal range. The mean ESR was 57 at the end of 1st hour.

TABLE I: Distribution of patients by their demographic characteristics

Age (years)	Frequency	Percentage
< 30	04	7.4
30 – 40	01	1.9
40 – 50	06	11.1
50 – 60	10	18.5
≥ 60	33	61.1
Sex		
Male	37	69.0
Female	17	31.0

***Mean age** = 61.4 ± 15.6; **range** = 24 - 86 years.*

TABLE II: Presence of associated comorbidities (n = 54)

Associated comorbidities	Frequency	Percentage
DM	22	40.7
HTN	17	31.5
COPD	04	7.4
CKD	06	11.1
ESRD	05	9.3

TABLE III: Baseline characteristics of the patients.

Baseline characteristics	Mean ± SD	Range
Systolic blood pressure (mmHg)	113.9 ± 18.7	90 - 160
Diastolic blood pressure (mmHg)	71.5 ± 10.1	60 - 95
Heart rate (beats/min)	112 ± 10	80 - 140
Respiratory rate (...../min)	26 ± 4	20 - 42
Temperature (^{0}C)	102.4 ± 0.8	101 - 106
WBC count (...../cu-mm of blood)	16750 ± 4280	11700-31000
ESR (mm at the 1st hr)	57 ± 27	10 - 130

Quantitative culture of blind tracheal aspirates showed a significant growth (≥ 10^5 cfu/ml) of microbes in 45(83.3%) out of 54 cases (Table IV). The predominant organism grown on cultures of blind TA was *Acinetobacter sp.* (73.3%) followed by *Pseudomonas sp.* (33.3%) and *Klebsialla sp.* (15.6%). *E. coli* and *Flavobacter* were each found in 9.3% cases. *C. albicans* was the least grown microorganism and *S. aureus* was rarely found.

TABLE IV: Growth of microbes on culture of blind tracheal aspirate (n = 54)

Quantitative culture of BTA	Frequency	Percentage
Growth of microorganisms (n = 54)		
Growth	45	83.3
No growth	9	16.7
Pattern of growth (n=45)		
S. aureus	01	2.2
Acinetobacter sp.	33	73.3
Pseudomonas sp.	15	33.3
Klebsiclla Sp.	07	15.6
C. albicans	04	7.4
E. coli	05	9.3
Flavobacter	05	9.3

** Total will not correspond to 100% for multiple response.*

Quantitative culture of bronchoalveolar lavage (BAL) demonstrated a significant growth (≥ 10^4 cfu/ml) of offending microorganisms in 47(87%) out of 54 cases (Table V). Over 70% of the cases exhibited growth of *Acinetobacter sp.* on quantitative culture of BAL. *Pseudomonas sp.* was

the second leading microorganism (29.8%) *C. albicans. Klebsialla sp., E. coli,* and Flavobacter each comprised 12.8% of the cases. *S. aureus* was the least grown microbes (4.2%) (Table V).

The strength of agreement between blind TA and BAL in diagnosing pneumonia was calculated using kappa statistics. The test revealed a good agreement (kappa-value = 0.707 or 70.7%) between the two procedures suggesting that the two diagnostic modalities were almost comparable in diagnosing nosocomial pneumonia in patients admitted in ICU ($p < 0.001$).

TABLE V: Growth of microbes on culture of bronchoalveolar lavage (n = 54)

Quantitative culture of BAL	Frequency	Percentage
Growth of microorganisms (n = 54)		
Growth	47	87.04
No growth	07	12.96
Pattern of growth (n = 47)		
S. aureus	02	4.2
Acinetobater sp.	33	70.2
Pseudomonas sp.	14	29.8
Klebsiclla Sp.	6	12.8
C. albicans	6	12.8
E. coli	6	12.8
Flavobacter	6	12.8

**Total will not correspond to 100% for multiple response.*

TABLE VI: Strength of agreement between BTA and BAL.

Disease studied	Diagnostic modalities		k-value	Strength of agreement
Nosocomial Pneumonia	BTA	BAL	0.707	Good

DISCUSSION

Despite the importance of prompt diagnosis of pneumonia in critically ill patients with ventilator support, several factors render this diagnosis difficult. Patients admitted in intensive care unit generally have abnormal chest roentgenograms, whether or not lung infection is present. Similarly, fever and leucocytosis are common in critically ill patients irrespective of pneumonia. Even purulent tracheobronchial secretions may not differentiate tracheobronchitis from pneumonia. So the clinical approach to nosocomial pneumonia treatment frequently leads to overuse of antibiotics. Accurate diagnosis is very important for early patient recovery and for rational use of antibiotics, avoiding over treatment and associated problems of drug resistance. Finally, accurate diagnosis allows for a detailed knowledge of the pattern of infection at specific institutions and allow more accurate empiric antibiotic selection.

The clinical diagnosis in this population is often inaccurate and many investigators have suggested that invasive methods of diagnosis are inappropriate. Recently quantitative cultures have added an impetus to the diagnostic work up of pneumonia with fair degree of accuracy. There are several methods of quantitative culture. Of them blind tracheal aspirate (BTA) and bronchoalveolar lavage (BAL) are more often used. But debate continues as which of them should be adopted, particularly in the context of our country keeping in mind the diagnostic accuracy and invasiveness of the procedures. There is approximately 1% incidence of lung parenchymal injury or pneumothorax as a result of BAL. If blind tracheal aspirate assures similar efficacy without inducing the above risks, it would be preferable.

In the present study, the mean age of the patients was 61 years and the youngest and the oldest patients were 24 and 86 years old respectively. A male preponderance was observed in the series. Majority of the patients were haemodynamically stable as indicated by mean blood pressures, heart rate, temperature and respiratory rate. Most (83.3%) of the cases showed significant growth of microbes on culture

of blind tracheal aspirates at cut-off value of $\geq 10^5$ cfu/ml, while 87% of the cases exhibited positive growth on culture of bronchoalveolar lavage at cut-off value of $\geq 10^4$ cfu/ml. *Acinetobacter baumannii* was the predominant organism isolated from blind TA (73.3%) followed by *Pseudomonas auriginosa* (33.3%). An almost similar pattern of growth was evident in BAL with more than 70% being *Acinetobacter baumannii* and about 30% *Pseudomonas aeruginosa. C. Albicans. Klebsialla sp., E. Coli,* and *Flavobacter* were less commonly observed in both groups.

In the diagnosis of nosocomial pneumonia, the "Gold Standard" is open lung biopsy. But it is not practical in large number of patients. Many investigators viewed BAL as "Gold Standard", for earlier studies had demonstrated a fewer false negative results (at a rate of 7 - 10%).[15,16] supporting its use as nearer to "Gold Standard".[17] Yet, in the present study we did not consider BAL as "Gold Standarad" and hence comparison between the two diagnostic modalities was made by Kappa statistics. The Kappa test revealed a good agreement (70.7%) between the two procedures suggesting that the two diagnostic modalities were almost comparable in diagnosing nosocomial pneumonia in patients admitted in ICU ($p < 0.001$). The strength of agreement in our study was observed to be good (61- 80%) but in Mondi's study[17] it was moderate (41 - 60%). Most workers found moderate agreement between the two procedures. Conversely, Jourdin et al.[18] in their evaluation of patients with VAP, found ETA to be of limited value, with only a 40% agreement when compared with findings of bronchoscope-directed protective brush (BDPB) samples thus discouraging its use in the diagnosis of VAP.

Many studies have so far been conducted using BAL as "Gold Standard." Using a cut-off value of $\geq 10^5$ cfu/ml, Mondi et al.[17] demonstrated 90% sensitivity and a 68% specificity of QDEA in diagnosing the causative microorganism of VAP. The sensitivity increased to 95% with a decrease in specificity by 10% as cut-off value is lowered to $\geq 10^4$ cfu/ml. Meanwhile the rate of false-positive increased from 31% to 42% when cut-off value of QDEA was decreased from $\geq 10^5$ cfu/ml to $\geq 10^4$ cfu/ml. French investigators found QDEA to have a sensitivity 89.5% and a specificity of 66.7% compared with protected specimen brush.[9] Velencia et al[8] found similar sensitivity and specificity for QDEA when compared against protected specimen brush in the diagnosis of VAP. Similar results were noted by Wu et al[19] in a population of medical patients who were already receiving antibiotics. El-Ebiary and associates,[20] however, found a relatively low sensitivity (70%) and low specificity (72%) of ETA using a cut-off value of ETA $\geq 10^5$ cfu/ml when compared with BAL.

To date the issues of diagnosis and therapy of nosocomial pneumonia particularly VAP remain complex and controversial. Most of the studies conducted to see the diagnostic accuracy of endotracheal aspirate claimed its high sensitivity and low specificity as compared against BAL with a higher yield of false positivity and over use of antibiotics. Empiric over-use of antibiotics has led to the emergence of resistant microbes and increased health-care costs. But in the present study, though sensitivity and specificity is not done as BAL is not 'Gold standard', Kappa analysis showed good agreement between BTA and BAL which encourages us to reconsider their use in the diagnosis of hospital-acquired pneumonia. However, the study has several limitations which deserve mention before drawing conclusion.

As the sample size was small, the findings derived from study cannot be generalized to reference population and the data should be interpreted with utmost caution. This study was carried out in an adult intensive Care Unit (ICU). So pediatric group of population was not included in the study.

CONCLUSION

Finally, based on the findings, the study concluded that the diagnostic efficacy of blind tracheal aspirate compared to bronchoalveolar lavage in the diagnosis of nosocomial pneumonia is appreciable. There is good agreement between the these two diagnostic modalities in diagnosing pneumonia in critical patients or ruling it out. So blind tracheal aspirate could be used instead of more invasive procedures like BAL in the diagnosis of nosocomial pneumonia patients on ventilator. This will reduce cost, allow specimens to be obtained quickly, and facilitate serial sampling when necessary.

REFERENCES

1. Campbell GD, Niederman MS, Broughton WA. Hospital-acquired pneumonia in adults: diagnosis, assessment of severity, initial antimicrobial therapy, and preventative strategies: a consensus statement. *Am J Respir Crit Care Med* 1996;153:1711-25.
2. Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD, Dean N, File T, Fine MJ, Gross PA. Guidelines for the management of adults with community-acquired pneumonia: diagnosis, assessment of severity, antimicrobial therapy, and prevention. *Am J Respir Crit Care Med* 2001;163:1730-54.
3. Niederman MS, Craven DE, Bonten MJ. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;171:388-416.
4. Kollef MH. Inadequate antimicrobial treatment: an important determinant of outcome for hospitalized patients. *Clin Infect Dis* 2000;31(Suppl 4):131-38.
5. Fagon JY, Chastre J, Wolff M. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia: A randomized trial. *Ann Intern Med* 2000;132:621-30.
6. Guidelines for the management of adults with hospital-acquired, ventilator associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005,171:388-416.
7. Pingleton SK, Fagon JY, Leeper KV. Patient selection for clinical investigation of ventilator-associated pneumonia Criteria for evaluating diagnostic techniques. *Chest.* 1992;102(Suppl 11):553-56.
8. Valencia AM, Torres MA, Insausti OJ. Diagnostic value of quantitative cultures of endotracheal aspirate in ventilator-associated pneumonia: a multicenter study. *Arch Bronconeumo* 2003;39:394-99.
9. Fangio P, Rouquette-Vincenti I, Rousseau JM. Diagnosis of ventilator associated pneumonia: a prospective comparison of telescoping plugged catheter with endotracheal aspirate. *Ann FrAnesth Re´anim* 2002;21:184-92.
10. Lambert RS, Vereen LE, George RB. Comparison of tracheal aspirates and protected brush catheter specimens for identifying pathogenic bacteria in mechanically ventilated patients. *Am J Med Sci* 1989; 297:377-82.
11. Salata RA, Lederman MM, Shlaes DM. Diagnosis of nosocomial pneumonia in intubated, intensive care unit patients. *Am Rev Respir Dis* 1987;135:426-32.
12. Sanchez-Nieto JM, Torres A, Garcia- Cordoba F, El-Ebiary M, Carrillo A, Ruiz J, Nuñez ML, Niederman M. Impact of invasive and noninvasive quantitative culture sampling on outcome of ventilator-associated pneumonia: a pilot study. *Am J Respir Crit Care Med* 1998;157:371-76.
13. Shorr AF, Sherner JH, Jackson WL, Kollef MH. Invasive approaches to the diagnosis of ventilator-associated pneumonia: a meta-analysis. *Crit Care Med* 2004;33:46.
14. Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. *N Engl J Med* 2006;355:2619.
15. Miller PR, Meredith JW, Chang MC. Optimal thresold for diagnosis of ventilator-associated pneumonia using bronchoalveolar lavage. *J Trauma* 2003;55:263-7.
16. Croce MA, Fabian TC, Waddle-Smith L. Utility of Gram's stain and efficacy of quantitative cultures for post-traumatic pneumonia: a prospective study. *Ann Surg* 1998;227:743-51.
17. Mondi MM, Chang MC, Bowton DL, Kilgo PD, Meredith JW, Miller PR,. Prospective comparison of bronchoalveolar lavage and quantitative deep tracheal aspirate in the diagnosis of ventilator-associated pneumonia. *J Trauma* 2005;59:891-6.
18. Jourdin B, Novara A, Joly-Guillou M. Role of quantitative cultures of endotracheal aspirates in the diagnosis of nosocomial pneumonia. *Am J Respir Crit Care Med* 1995;152:241-6.
19. Wu, C.L., Yang, D.I., Wang, N.Y., Kuo, H.T., Chen, P.Z., 2002. Quantitative culture of endotracheal aspirate in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. *Chest;* vol. 122, pp. 662-82.
20. El-Ebiary M, Torres A, Gonzalez J, de la Bellacasa JP, Garcia C, de Anta MTJ, Ferrer M, Rodriguez-Roisin R. Quantitative cultures of endotracheal aspirates for the diagnosis of ventilator-associated pneumonia. *Am Rev Respir Dis* 1993;148:1552-7.