ORIGINAL CONTRIBUTIONS

ROLE OF QUANTITATIVE ENDOTRACHEAL ASPIRATE AND CULTURES AS A SURVEILLANCE AND DIAGNOSTIC TOOL FOR VENTILATOR ASSOCIATED PNEUMONIA: A PILOT STUDY

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ABSTRACT

BACKGROUND: *Accurate diagnosis and appropriate treatment of ventilator associated pneumonia (VAP) is crucial for good outcomes. Endotracheal suctioning is performed in ventilated patients as part of routine care and for tracheal toileting.* ***AIM:*** *We evaluated if quantitative endotracheal aspirate (ETA) was a suitable alternative to bronchoalveolar lavage (BAL) for suspected VAP. In addition we assessed if surveillance ETA guided antibiotic selection for subsequent VAP.* ***SETTING AND DESIGN:*** *Prospective study in the surgical intensive care unit (ICU) of a tertiary hospital in India.* ***MATERIALS AND METHODS:*** *Two hundred consecutive patients with mean (standard deviation) APACHE II score of 12.3±5 and requiring mechanical ventilation beyond 48 hours underwent surveillance ETA cultures. A second ETA and BAL were performed if the patient developed features of VAP. The threshold for microbiological diagnosis of VAP was taken as 10^5 colony forming units/ml (cfu/ml) for ETA and 10^4cfu/ml for BAL.* ***STATISTICAL ANALYSIS:*** *The sensitivity and specificity of surveillance and concurrent ETA aspirate cultures were compared with BAL cultures.* ***RESULTS:*** *VAP was suspected clinically and corroborated radiologically in 27/177 patients (15.3%). Although microbiological support for VAP was obtained by ETA in 19 patients, bronchoscopy was possible only in 13 patients, 8 of whom had isolates at significant threshold. Of the 16 organisms isolated from BAL, 11 were of significant threshold with 9/11 (82%) BAL isolates having a similar antibiogram to a concurrent ETA. Only one BAL isolate (9%), at significant threshold, was not isolated on a concurrent ETA. On the other hand just 6/11 BAL isolates (55%) had an identical antibiogram to surveillance ETA. BAL had 3 additional isolates (27%) at significant threshold not isolated on surveillance ETA.* ***CONCLUSIONS:*** *Concurrent quantitative ETA could substitute BAL cultures for VAP. Surveillance ETA at 48 hours of ventilation does not appear to assist with antibiotic selection for a subsequent VAP.*

Key words: *Antibiogram, bronchoalveolar lavage, quantitative analysis, surveillance endotracheal aspirate, ventilator associated pneumonia*

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INTRODUCTION

Ventilator associated pneumonia (VAP) is the commonest intensive care unit (ICU) infection with an incidence ranging from 9 to 27% in intubated mechanically ventilated patients.[1] In the absence of a gold standard, VAP is assumed to be diagnosed more accurately by bronchoscopic sampling and microbiological cultures of the lower respiratory tract.[2] Bronchoscopy, being invasive, is not uncommonly associated with complications, especially in patients on high respiratory supports. This has paved the way for less invasive tests such as endotracheal aspirates (ETA) and quantitative ETA cultures with a threshold of 10^5 to 10^6 bacteria per milliliter of exudates that is considered as optimal for the microbiological confirmation of VAP.[3] More importantly, 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,[4-6] further strengthening the case for quantitative ETA as a diagnostic tool.

Once VAP is suspected, early, aggressive, empiric therapy with broad-spectrum agents targeted at likely pathogens has been shown to be associated with lower mortality rates.[7-9] Mortality is high even if an inappropriate empiric antibiotic is changed to a more appropriate one later in the course of therapy.[10] Therefore the role of initial or subsequent surveillance cultures in assisting antibiotic selection in patients developing VAP assumes importance. This study was thus undertaken to assess: (a) if quantitative ETA cultures at clinical suspicion of VAP could substitute for the more invasive bronchoscopic cultures in the microbiological isolation of the organism and (b) if surveillance ETA at 48 hours of ventilation enabled appropriate choice of antibiotics for subsequent VAP.

MATERIALS AND METHODS

Patient selection: This study was conducted in the surgical intensive care unit (SICU) of a tertiary care teaching hospital in a developing country. All adults aged 18 years or more and mechanically ventilated for at least 48 hours and willing to participate in the study were considered for inclusion. Immunocompromised patients and those considered at high risk for subsequent bronchoscopy were excluded from the study. These high-risk patients included patients with bleeding diathesis, recent acute myocardial infarction, seizures, raised intra-cranial pressure and those on very high respiratory supports. It was felt that immunocompromised patients were a unique cohort in whom the differential diagnoses of alveolar densities were protean, and hence they were excluded from the study. The institutional review board approved the protocol, and written consent was obtained from the patients or their next of kin prior to inclusion in the study.

All patients staying beyond 48 hours were recruited to the trial and the demographic data was abstracted to specific data abstraction forms. Surveillance ETA was performed in all patients at the time of recruitment, (i.e.) after 48 hours of mechanical ventilation. These patients were carefully followed up for signs of VAP. This included, apart from clinical examination, regular recording of body temperature, observance of tracheal aspirate appearance, PaO_2/FiO_2 ratio, leukocyte count and chest

radiographs. The diagnosis of VAP was based on the American College of Chest Physicians criteria[11] and was defined as the occurrence of new and persistent radiographic infiltrates following intubation along with the presence of at least two of the following criteria: (a) temperature > 38.3°C, (b) leukocytosis > 10,000 cells/mm^3 and/or (c) purulent tracheo-bronchial secretions. Patients with clinical suspicion of VAP (based on the above criteria) underwent concurrent ETA and BAL with former sample collected first, lest it be diluted during BAL.

Procedure of ETA: It was performed under aseptic precautions using sterile suction catheters and traps. 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. With quantitative analysis of ETA, the threshold for diagnosing VAP in this study was taken as 10^5 colony forming units/ml (cfu/ml).

Procedure of BAL: The procedure was carried out under aseptic precautions with adequate sedation and FiO_2 of 100% through the endotracheal tube via a specific adaptor. 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 for the diagnosis of VAP was taken as 10^4 cfu/ml.

Method of quantitative analysis: 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 $>10^4$ cfu/ml for BAL and $>10^5$ cfu/ml for ETA being suggestive of infection rather than colonization.[12,13]

Statistics: Sample size was calculated based on the assumption that 85% of patients with VAP would be accurately detected by quantitative analysis of the ETA (with assumed rate of VAP being 14%). This worked out as 200 patients assuming a difference of 5%. Mann-Whitney U test was performed to compare the ventilatory days between patients with or without VAP. The statistical analysis was carried out using SPSS 11.5.

RESULTS

The study was performed in the SICU over a

1-year period. Although 200 patients underwent surveillance ETA at 48 hours of mechanical ventilation, only 177 patients could be followed up for the development of VAP [Figure 1]. Six patients were excluded because of an underlying immunocompromised state, 12 patients were discharged from the ICU against medical advice and 5 patients succumbed due to other causes. VAP was suspected clinically and corroborated radiologically in 27 of 177 patients (15.3%) ventilated beyond 48 hours. Although 27 patients were initially suspected to have VAP based on the American College of Physicians criteria,[11] bronchoscopy was performed in only 13 patients. In 8 patients, bronchoscopy had to be abandoned due to the development of significant hypoxemia or hypotension. Bronchoscopy was not performed in 5 patients as they were on high respiratory supports and one patient was uncooperative during the procedure despite the use of sedative agents [Figure 1]. Of the 13 patients who underwent bronchoscopy, 8 patients had isolates at significant threshold on BAL and fulfilled the microbiologic criteria for VAP [Table 2]. Microbiologic criteria for the diagnosis of VAP were also fulfilled on ETA cultures in 11 of the 20 patients in whom bronchoscopy could not be performed [Figure 1].

Age and severity of illness (APACHE II score) did not predict the development of VAP in our cohort [Table 1]. However, female patients appeared to be at risk for the development of VAP ($P = 0.02$). VAP occurred after a mean of 6.0 ± 1.0 days of ventilation. All but one patient

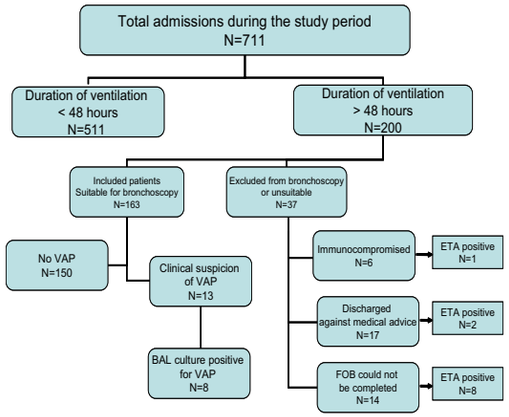

Figure 1: Flow chart of patient recruitment, inclusion and exclusion. Number of patients evaluated at each stage of the recruitment process and number of patients excluded from the trial with reasons for exclusion.
Among the patients who could not undergo bronchoscopy 14 had clinical suspicion of VAP. In this cohort, eight patients in whom FOB was abandoned due to development of complications had ETA culture suggestive of VAP. Two patients who were discharged against medical advice had positive ETA culture and one of the immunocompromised patients fulfilled VAP criteria by ETA culture.

***Causes for exclusion from bronchoscopy:** immunocompromised (6), uncooperative for FOB (1), high risk for FOB (5), procedure abandoned due to complications (8), discharged against medical advice (12), and died due to other reasons (5).

Table 1: Demographic data

Variables	*Patients with ventilator associated pneumonia N = 13**	*Patients without ventilator associated pneumonia N = 163*	*P value*
Sex (M/F)	6/7	123/40	0.02
Age ± SD (years)	46.1±19.76	42.84 ± 14.99	0.75
APACHE II (mean ± SD)†	13.6 ± 6	11.0 ± 5	0.83
Ventilation duration (mean ± SD) days	10.0 ± 5.35	6.71 ± 3.84	0.03

*Data provided only for the 13 patients in whom a bronchoscopy could be performed, †APACHE II scores were at the time of admission to ICU and not at the time of diagnosis of ventilator associated pneumonia

Table 2: Microbiological data of patients with ventilator associated pneumonia

Organism in ETA at 48 h	*Organism in ETA at n^{th} day†*	*Organism in BAL at n^{th} day†*	*n^{th} day†*	*Final outcome*
E. coli (S)	Klebsiella	*E. coli* (S)	7	Discharge
Klebsiella (S)	*E. coli* (S)			
Klebsiella (S)	Klebsiella (S)*	Klebsiella (S)*	5	Discharge
Pseudomonas*	Diphtheroids		7	Discharge
	Pseudomonas (S)*	Pseudomonas (S)*		
Pseudomonas (S)	Pseudomonas	No growth	6	Death
Enterobacter	Enterobacter	No growth	2	Death
Pseudomonas (S)*	Pseudomonas (S)*	Pseudomonas (S)*	5	Discharge
	Yeast	Yeast		
Pseudomonas (S)*	Pseudomonas (S)*	Pseudomonas (S)*	7	Death
Morganella (S)*	Morganella (S)*	Staph aureus (S)*		
Staph aureus (S)*	Staph aureus*	Proteus (S)		
No growth	Klebsiella (S)*	Klebsiella (S)*	10	Death
	E. coli (S)*	*E. coli* (S)*		
No growth	No growth	Pseudomonas	6	Death
Pseudomonas (S)*	Pseudomonas (S)*	Pseudomonas (S)*	5	Discharge
No growth	Klebsiella	Acinetobacter	6	Discharge
Acinetobacter (S)	Acinetobacter	Acinetobacter	5	Death
Alpha hem strep				
Pseudomonas (S)*	Pseudomonas (S)*	Pseudomonas (S)*	23	Death
	Acinetobacter	Acinetobacter		

S = Growth at significant thresholds, †n^{th} day = Day when VAP diagnosed, *= identical antibiogram

developed late (> 5 days) onset VAP [Table 2]; in the patient who developed early VAP, nosocomial pneumonia was diagnosed 48 hours after initiation of mechanical ventilation. The duration of ventilation, as expected, was longer in those developing VAP (10.0 ± 5.35 versus 6.71 ± 3.84, p = 0.03).

Microbiology: Gram negative organisms were the commonest causative agents identified by both ETA and BAL with a multi drug resistant pattern consistent with late onset VAP. Multi drug resistance was defined as a microorganism resistant to antibiotics considered "gold standard" for treatment of infection caused by that microorganism, or one that is only susceptible to antibiotics with more serious side effects than the standard ones.[14] Isolates from a concurrent ETA, performed at the time of diagnosis of VAP, were compared with isolates obtained from a BAL. A total of 16 organisms were isolated from the BAL and 19 organisms from the concurrent ETA [Table 2]. In 2 patients no organisms were isolated from the BAL whilst no organism was isolated from a concurrent ETA in one patient. Of the 11 organisms identified in significant threshold in the BAL, 9 organisms were also identified in the significant threshold in the concurrent ETA and one at a lower threshold in the concurrent ETA. Nine of the 11 isolates on the concurrent ETA (82%) had a similar antibiogram to the BAL isolates [Table 3]. BAL identified only one isolate at the significant threshold that was not isolated on a concurrent ETA (9%). Three other isolates with a similar antibiogram were identified at non-significant thresholds in both the concurrent ETA and BAL. Although a concurrent ETA identified 6 additional organisms that were not isolated on a BAL, only one was in the significant threshold. Thus if only isolates at a significant threshold were considered, then concurrent ETA compared with BAL has a sensitivity of 82%, specificity of 91% and positive predictive value

Table 3: Comparison of isolates obtained from a concurrent endotracheal aspirate and bronchoalveolar lavage performed at the time of diagnosis of ventilator associated pneumonia

	Significant growth† in BAL	*Non-significant growth or no growth in BAL*
Significant growth† in endotracheal aspirate	9	1
Non-significant or no growth in endotracheal aspirate	2	10

Overall sensitivity = 82%, Specificity = 91%, Positive predictive value = 90%

Concurrent endotracheal aspirate Vs BAL (expanded)

	Significant growth† in BAL	*Non-significant growth in BAL*	*No growth in BAL*
Significant growth† in ETA	9*	0	1
Non-significant growth in ETA	1†	3	5
No growth in ETA	1	2	0

*Of these, 8 isolates had the same antibiogram, †One organism in non-significant threshold on the ETA grew in significant threshold in the BAL and had the same antibiogram, † - indicates growth at significant thresholds of 10^5 and 10^4 colony forming units/ml for ETA and BAL respectively

of 90% in our cohort of patients.

On the other hand, of the 11 isolates grown in significant threshold in the BAL, only 6 organisms with an identical antibiogram (55%) were isolated in the surveillance ETA performed at 48 hours of mechanical ventilation [Table 4]. Four isolates grown in significant thresholds in the surveillance ETA did not grow in the subsequent BAL. BAL had 3 additional isolates (27%) at significant threshold not isolated on surveillance ETA [Table 2]. Thus the sensitivity of a surveillance ETA compared with BAL was 64% and specificity was 60% with a positive predictive value of 64%.

When assessing ETA cultures in patients in whom bronchoscopy could not be performed at the time of diagnosis of VAP, 17 isolates were identified in 11 patients at significant threshold. Initial surveillance ETA at 48 hours of mechanical ventilation in these patients identified 9 isolates in 5 patients. Only two isolates had an identical antibiogram compared with the ETA at the time of diagnosis of VAP. Antibiotics were initiated based on ETAs taken at the time of diagnosis of VAP in these 11 patients. One patient did not complete treatment and left against medical advice. Three patients including an immunocompromised patient died whilst seven patients were discharged to the ward.

Table 4: Comparison of isolates obtained from surveillance endotracheal aspirate (ETA) and bronchoalveolar lavage (BAL)

	Significant growth† in BAL	*Non-significant growth or no growth in BAL*
Significant growth† in ETA	7	4
Non-significant or no growth in ETA	4	6

Overall sensitivity = 64%, Specificity = 60%, Positive predictive value = 64%

Surveillance ETA Vs BAL (expanded)

	Significant growth† in BAL	*Non-significant growth in BAL*	*No growth in BAL*
Significant growth† in ETA	7*	1	3
Non-significant growth in ETA	1	0	2
No growth in ETA	3	4	0

*Of these isolates only 6 isolates had the same antibiogram, † - indicates growth at significant thresholds of 10^5 and 10^4 colony forming units/ml for ETA and BAL respectively

DISCUSSION

This study suggests that surveillance ETA was unable to assist in the appropriate identification of organism or choice of antibiotics in the majority of patients developing subsequent VAP. Only 6/11 BAL (55%) isolates obtained at the time of diagnosis of VAP had a similar antibiogram to surveillance ETA at 48 hours of ventilation, limiting the value of routine surveillance ETA. These results are consistent with published literature elsewhere[15] and CDC guidelines that restrict the role of routine surveillance of patients at high risk for health care associated pneumonia for specific clinical or epidemiologic or infection control objectives.[16]

The appropriate timing of surveillance ETA is unclear.[17-20] Although the most proximate ETA prior to VAP may be best suited, overall it appears that surveillance ETA cultures have a limited role given the need for repetitive sampling of the respiratory tract as well as changes in the antibiogram that may occur with time.

The results comparing concurrent ETA and BAL performed at clinical suspicion of VAP appear promising with a sensitivity of 82% (for identical antibiogram) and specificity of 91%. The Canadian Critical Care Trial group in a larger cohort reported similar results, but more importantly demonstrated that there were no significant differences in mortality, other clinical outcomes or the use of antibiotics between the two groups undergoing either an ETA or BAL as a diagnostic test.[21] An earlier meta-analysis by Shorr *et al*,[22] of four randomized trials of 628 patients, again suggested that invasive strategies do not alter mortality but affect antibiotic use and prescribing. Several other studies have again reported the utility and reliability of non-bronchoscopic methods in the diagnosis of VAP.[3,8,23] This study, which has local relevance, had a design that incorporated the relevance of quantitative analysis of ETA (compared to BAL) as well as evaluating the role of surveillance ETA in subsequent VAP.

Given the current body of evidence reiterated by this study, routine use of BAL for the diagnosis and management of VAP cannot be justified, particularly in the context of a developing country such as ours where added cost of a BAL over quantitative ETA (Rs. 2442/ USD 57 for BAL culture and sensitivity *vs* Rs. 519/ USD 12 for ETA culture and sensitivity based on current costs) is passed on to the patient. Lack of expertise in performing bronchoscopy in several centers also limits the use of BAL as a routine diagnostic tool for VAP.

Several other aspects of our study are worthy of mention. The incidence of VAP in our cohort was low (15.3%) compared with results published elsewhere. This may be a reflection of a predominantly surgical cohort of patients as the medical, neurology, pediatric and cardio-thoracic patients are managed elsewhere in separate dedicated ICUs within the hospital. The low incidence may have also been helped by a very strict hand hygiene protocol that was rigidly implemented during the period of the study as part of an international study on nosocomial infections. That the incidence of pneumonia was higher amongst females was surprising and could not be explained, as other studies have not reported a sex bias for VAP,[24] although female

sex has been shown to be a mortality predictor in VAP.[24]

This study however has several important limitations. This was a single center study confined to one of the five ICUs within the institution. Further, with a much lower incidence of VAP than predicted, the effects of ETA were probably under demonstrated. Although the plan was to recruit 200 patients, the final numbers were only 163 due to the exclusion of several patients and the study had to be terminated due to limitation of resources and funding. That most of our patients received antibiotics (third generation cephalosporin) prior to ICU admission may also have affected the sensitivity pattern. Finally, though the admission APACHE II score was not high, 13 patients could not undergo fiberoptic bronchoscopy due to anticipated risk (5/13) or development of procedural risk (8/13) i.e. hypotension and hypoxemia, further limiting the number of patients who ultimately had bronchoscopic evaluation.

Notwithstanding these limitations, we submit that this pilot study from a tertiary centre in our country has added to our current knowledge and understanding of the role of ETA in the diagnosis of VAP as well as in the choice of antibiotic selection. This probably is the first study in our country that has evaluated both ETA at clinical suspicion of VAP and surveillance ETA at 48 hours of ventilation. There are several implications that arise from the study that are relevant to India. Foremost are the financial implications. Bronchoscopy is expensive, particularly in terms of initial investment costs. The cost of a bronchoscopic evaluation was Rs. 1900 (USD 45) in our study and this can add to the financial burden of a critically ill patient. Moreover, expertise is required for this procedure, which may not be readily available in many centers. The microbiological spectrum of isolates from this study is again more applicable to our country that sees a predominance of gram negative organisms causing VAP rather than methicillin-resistant *Staphylococcus aureus.* Larger trials from this part of the subcontinent would be invaluable in the design of protocols for diagnosis of VAP in cost and resource constrained developing nations.

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