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ORIGINAL ARTICLE

Clinical, radiological and microbiological corroboration to assess the role of endotracheal aspirate in diagnosing ventilator-associated pneumonia in an intensive care unit of a tertiary care hospital, India

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Abstract

Early and accurate diagnosis and follow up of Ventilator-associated pneumonia (VAP) varies considerably with the clinical, radiological and microbiological criteria employed. This study was aimed to correlate serial clinico-radiological findings with microscopy and quantitative culture of consecutive Endotracheal aspirate (ETA) from 50 mechanically ventilated patients along with the antibiogram and risk factor assessment. The results revealed the incidence of VAP to be 42% with a rate of 116/1000 ventilator days in Multidisciplinary Intensive Care Unit (MICU). Early onset VAP was seen in 8 patients and late onset VAP in 13, with no significant age or sex preponderance. The attributable mortality rate was 61.9% which rose with duration of stay. The independent risk factors were multi-organ failure, re-intubation and pleural effusion. The most commonly isolated organisms were Multidrug resistant (MDR) *Acinetobacter baumannii* (76%) and *Pseudomonas aeruginosa* (42%). All enterobacterial isolates were Extended spectrum beta lactamases (ESBL) producing organisms and all *Staphylococcus aureus* isolates were methicillin resistant. Colonization on day 1 resulted in development of VAP on day 4 in 66% of the population studied. Gram stain findings had a significant correlation with the quantitative culture of ETA. Quantitative culture by itself showed a significant progressive increase of specificity in diagnosing VAP on day 7. The strength of association between Clinical Pulmonary Infection Score (CPIS), the microbiological findings and the clinical diagnosis was found to be strong. This study concludes that Gram stain and quantitative culture of ETA can be considered useful for the diagnosis of VAP and a combined clinical, radiological and microbiological approach can be used successfully in the management and follow up of VAP with emphasis on stepping up the VAP prevention bundle protocols.

Key words

Endotracheal aspirate, India, Intensive Care Unit, Ventilator associated pneumonia

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Introduction

Pneumonia is the single most common nosocomial infection among patients in ICUs.[1,2] Rates of pneumonia are considerably higher among hospitalized patients, and the risk of developing pneumonia is 3-10 folds higher in ventilated patients. The prevalence of VAP varies from 6-52% depending on the population studied, the type of ICU and the diagnostic criteria used.[3-5] Moreover, 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.[6,7] The clinical prediction about the presence and absence of VAP is accurate only in 62% and 84% respectively.[6,8] However, subsequent corroboration of this diagnosis by serial clinical, microbiological and radiological examinations may not identify the patients truly having VAP. One popularly accepted criterion for diagnosing VAP is m-CPIS, which includes microbiological diagnosis also.[9-11] Since there is no accepted microbiological gold standard for diagnosis,[12-14] the simple, cheap and non-invasive quantitative culture of ETA may be considered as a better indicator of prognosis. This study was planned with the aim to assess ETA as a suitable microbiological sample for the diagnosis of VAP and to correlate it clinically and radiologically.

Materials and methods

Patient population

The prospective observational study was conducted at the MICU during the period of November 2007 to April 2008. A total of 137 ETAs from 50 intubated patients with informed consent (patient's or close relatives') were collected. First collection was on the day of ventilation in every patient and it continued with consecutive sampling on the 3rd, 5th, 7th and 10th day with the evaluation of CPIS scores. 35 males and 15 females were enrolled for the study. Patients of age >18 years (grouped as 18-30, 30-49 and >50 yrs) and those who have received mechanical ventilation for minimum of 48h were included for the study as suspects of VAP. Patients were grouped under medical and surgical causes. Patients diagnosed with any other definite source of infection were excluded from the study.

Definitions

A case of VAP was defined according to the ATS guidelines.[14] VAP occurring before and after 96h were considered as early and late onset VAP respectively. The clinical diagnostic criterion for VAP used was CPIS scoring.[15] Sequential Organ Failure Assessment (SOFA) score was calculated depending on the clinical status of the patient in the initial 24h of admission.[16] Chronic obstructive pulmonary disease (COPD), asthma, neurological diseases, leptospirosis etc. were classified as medical causes while malignancy, perforation of internal organs, pancreatitis etc. under surgical causes.

Microbiological processing

All the samples were collected aseptically with mucous extractor by gentle aspiration without instillation of saline. Smears prepared from samples were Gram stained and graded from 1+ to 5+ depending on the Polymorphonuclear cells (PMN). Samples were then mechanically liquefied, homogenized and serially diluted with 0.9% sterile saline solution with final dilutions of 10^{-2},10^{-3},10^{-4} and quantitatively cultured on agar plates, incubated overnight at 37°C aerobically.[17,18] Organisms were identified and antibiotic susceptibility including test for ESBL production and screening for MRSA were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[19,20] Growth >10^5 cfu/ml was considered as an infection and any growth <10^5 cfu/ml was assumed to be colonization. Individually, the clinical and radiological evaluation was done as mentioned by Niderman.[21] Patients were categorized as improved, expired and discharged against medical advice. The radiological improvement was assessed by the breakdown of pneumonic consolidation or resolution of infiltrates in the serial chest radiographs, clinical improvement with chest signs and microbiological improvement with reduction in the colony counts in subsequent ETA cultures.

Statistical analysis

SPSS version 12 was used to analyze the data. ANOVA, two-tailed Fisher's exact test, Mann Whitney U test, univariate analysis, logistic regression, were employed to analyze the data as applied.

Results

Patient characteristics analysis

Of the total number of 21 patients who developed VAP in MICU, 8 patients had early onset VAP and 13 had late onset VAP. Male to female ratio was 3.2:1. The incidence of VAP at our ICUs was 42% (21 out of 50), with a VAP rate of 116/1000 ventilator days during the study duration. The mean age for the development of VAP was 45 years. There was no significant difference among different age groups (table I).

Risk factors analysis

The median duration of ICU stay for patients with VAP was 12.5 days, where 42.5% patients were severely ill at the time of admission. Of the 9 patients who stayed in ICU for >20 days, 7 of them developed VAP (p=0.04). The attributable mortality rate was 61.9%, which rose with the longer duration of ventilation (p=0.001). The other significant risk factors of developing VAP were duration of ICU stay (p=0.04), presence of multi-organ failure by SOFA score (p=0.002), re-intubation (p=0.002) and pleural effusion (p=0.021). The other risk factors like IV sedatives, pre-existing lung diseases, trauma and major surgery, tracheostomy, supine body position, uremia had no significant effect (table I). Underlying medical illness was seen in 61.9% illness while the remaining 38.1% had surgical disease.

Microbiological results and its clinico-radiological corroboration

Gram stain finding of 3+ had a significant correlation for the microbiological diagnosis of VAP (p=0.013). The sensitivity and specificity of Gram stain alone were 67% and 77% respectively when compared with culture. There were 124 samples (90.5%) with monomicrobial growth and 13 samples (9.4%) with polymicrobial growth. The organisms isolated were *A. baumannii* (76%), *Ps. aeruginosa* (43%), *E. coli* (14%), *Klebsiella pneumoniae* (14%), Methicillin Resistant *Staphylococcus aureus* (MRSA) (9%) and *Candida albicans* (28%). *A. baumannii* was multi-drug resistant showing susceptibility only to netilmicin (89%) and cefoperazone-sulbactam (51%). Two isolates of *Ps. aeruginosa* were pan-drug resistant, i.e. resistant to all antipseudomonal antibiotics including colistin. All enterobacterial isolates were ESBL producing and all *S. aureus* isolates were methicillin resistant (tables II and III).

The sensitivity and specificity of CPIS scores and quantitative culture of ETA ranged from 50-88.2% over the 7-day period (table IV). However, 12 patients who had $>10^5$ cfu/ml but no clinical and radiological evidence of VAP, were not included for statistical analysis. The mean CPIS scores on day 1, day 4 and day 7 were 4.19, 6.0, and 5.3 (Mann Whitney U test) suggesting that day 4 scoring to be a close significant day of diagnosing VAP (p=0.001, z=4.06). The mean persistence of organisms in consecutive quantitative culture sample was 86% at the count of $>10^5$ cfu/ml. Almost all the organisms uniformly showed a high persistence at $>10^5$ threshold as logarithm of quantitative culture (table V). Significant correlation noted between the day 1 of colonization ($<10^3$) and day 4 of microbiological diagnosis of VAP ($>10^5$) (p=0.017). 66.7% colonized on day 1 developed VAP on day 4. Significant correlation was also noted between earlier to day 4 of microbiological and radiological diagnosis of VAP (p=0.013). However, as days progressed the significance dropped and correlation was lost. Day 4 of clinical diagnosis correlated strongly to day 7 of radiological diagnosis (p=0.01, r=0.507). The strength of association between microbiological diagnosis and CPIS diagnosis was found to be strong (gama = 0.847) and the association between clinical diagnosis and CPIS diagnosis was again found to be strong (gama = 0.595, data not shown)

Discussion

VAP is the most important hospital acquired infection in the ICUs. Incidence, despite slight decrease in last 3 years is still noted to be on the higher side as compared to western countries.[22,23] The important bacteria isolated were MDR *Acinetobacter spp*. and *Ps. aeruginosa*, which were suggestively hospital environmental strains as revealed by our routine microbiological survey. The other organisms in the decreasing order are ESBL producing *E. coli* and *K. pneumoniae*, and MRSA and *C. albicans*. The results agree with other studies in our country that MDR gram-negative bacilli as the major etiological agents causing VAP.[22, 24]

Role of risk factors

In our study, age, sex, medical and surgical cause had no preponderance of VAP as against other studies.[25, 26] All the patients had received stress ulcer prophylaxis and usage of Heat-Moisture (HME) filter, once a week

Table I: Patient characteristics and risk factors association with VAP

Patient characteristics and Risk factors	VAP (n=21)	No VAP (n=29)	*p value*
Mean Age (years)	45	52	
Age 18-30	5	5	
Age 31-40	8	8	0.491
Age >50	8	16	
Men (%)	16	19	0.416
Medical causes (%)	13(61)	14(48)	0.34
Surgical causes (%)	8(38)	15(51)	
Outcome (improved)	6	23	
Outcome (expired)	13	3	0.001*
Outcome (dama@)	2	3	
Duration of stay in ICU (<10 days)	12	20	
Duration of stay (10-20 days)	2	7	0.04*
Duration of stay (>20 days)	7	2	
Re-intubation	9	2	0.002*#
IV sedatives	18	20	0.17
Pre-existing lung diseases	4	4	0.61
Pleural effusion	10	5	0.021*
Stress ulcer prophylaxis	21	0	-
HME filters	21	0	-
Tracheostomy	11	11	0.31
Major surgery	7	14	0.56
Trauma	4	4	0.6
Uremia	8	8	0.43
Supine body position	16	26	0.2

* Significant variables from univariate analysis.

Significant p value by multivariate analysis as independent risk factors after adjusting odds ratio (data not shown)

@ Discharged against medical advice

Table II: Resistance pattern of Gram-negative bacilli

PATHOGENS	No.	AM	AMC	GEN	NET	AK	CAZ	SXT	CTX	CIP	CAZ	PIP	TBR
A. baumanni	47	100	100	100	11	100	100	100	100	86	-	-	-
Ps. aeruginosa	30	-	-	86	60	60	-	-	-	60	50	50	60
E. coli	12	100	100	100	75	50	100	50	100	75	-	-	-
K. pneumoniae	11	100	100	72	75	45	100	50	100	73	-	-	-

PATHOGENS	TZP	IMP	SCF	TCC	CFP	ATM
A. baumanni	100	74	49	100	100	100
Ps. aeruginosa	30	30	40	45	45	-
E. coli	25	0	0	25	100	100
K. pneumoniae	45	0	0	45	100	100

Table III: Resistance pattern of Gram-positive cocci (%)

PATHOGEN	No.	AM	AMC	SXT	CHL	GEN	ERY	OXA	CEF	CIP
S. aureus	6	100	100	33	33	67	33	100	100	67

AK-amikacin, AM-ampicillin, AMC-amoxi-clavulanic acid, ATR-aztreonam, CAZ-ceftazidime, CFP-cefpirome, CIP-ciprofloxacin, CTX-ceftriaxone, CZ-cefazolin, DOX-doxycycline, ERY-erythromycin, GEN-gentamicin, IMP-imipenem, NET-netilmicin, OXA-oxacillin, PIP-piperacillin, SCF-cefoperazone-sulbactam, SXT-Cotrimoxazole, TCC-Ticarcillin-clavulanic acid, TOB-tobramycin, TZP-piperacillin-tazobactam

Table IV: Sensitivity and specificity of CPIS scoring* and quantitative culture of ETA

Days	Sensitivity (%)	Specificity (%)
1	59	33.3
4	64.3	75
7	50	88.2

* taken as true positive

Table V: Quantitative culture results of bacterial isolates

Isolates	No. of patients	No. of positive samples	Median log cfu/ml	Range log cfu/ml
A. baumannii	16	47	6.9	4.8 - 9.0
Ps. aeruginosa	9	30	5.8	4.5 - 7.1
K. pneumoniae	3	11	4.6	3.0 - 6.0
E. coli	3	12	5.1	4.0 - 6.0
MRSA	2	6	4.9	3.9 - 5.9
C. albicans	6	18	5.5	4.5- 8.6

change of ventilator tubing.[8] The important risk factors are multi-organ failure, re-intubation, prolonged stay and pleural effusion (95% CI, OR 3.55). The crude mortality rate (61.9%) quoted however is noncommittal and remains inconclusive because of other existing premorbid illness.

Role of Quantitative cultures

Quantitative ETA achieves a better specificity (~70%) at the cost of reduced sensitivity (~70-80%) than quantitative culture of lower respiratory secretions.[27, 28] This approach is noninvasive, inexpensive and widely available. Even most post mortem studies have found ETA culture to be at least equally valid as bronchoscopic techniques.[29 - 31] In our study, quantitative culture of ETA turned out to be an acceptable sample for VAP as the specificity rose over a high of 88.2% on day 7. In developing countries, where bronchoscopic Brochoalveloar lavage (BAL), mini-BAL, Protected specimen brush (PSB) may cause a financial burden to the patient and need an expert to carry out the techniques ETA can be a suitable alternative to those techniques as shown in our study. Moreover, controversies surround the expensive invasive techniques like BAL, mini-BAL, PSB, Plugged telescopic catheter (PTC), which have different sensitivity and specificity.[32 - 34] ETA can be a suitable alternative to those techniques, as showed in our study. Our study substantiates colonization as an important indicator of impending infection since maximum percentage of day 1 colonization resulted in development of infection on day 4. This study conveys the importance of collection of sample at the earliest, which helps the attending team to institute adequate measures, in turn narrowing down the chances of impending infection. This study also proved that Gram stain could be a useful adjuvant for the quantitative culture, though the role of intracellular organisms could not be assessed. The sensitivity and specificity of Gram stain were 67% and 77%. This study agrees with the modified Singh's m-CPIS scoring criteria that bacteriological conformation of the quantitative culture of ETA with the Gram staining may need to be employed before confirming the diagnosis.[22] Quantitative cultures of ETA alone have demonstrated a high comparative result with the scoring. It suggests that m-CPIS in conjunction with quantitative cultures could be a better method of diagnosis (88.5% specificity on day 7).

Corroborative role

VAP is almost always an easily over-diagnosed disease clinically in the recent years since clinical criteria for diagnosing of VAP as judged by a new or progressive lung infiltrates and at least two of the following criteria: temperature more than 38°C, leucocyte count more than 10,000 cells / mm^3 and purulent respiratory secretion, have a limited diagnostic accuracy.[6,8] Whereas some authors have advocated an approach relying strictly on the results of invasive bronchoscopic diagnostic testing,[35] others have insisted on an approach that keeps clinical and microbiological criteria in balance, not withholding antimicrobial treatment in the presence of cultures below the thresholds, but clinically suspected VAP.[36] Radiographic signs of VAP also have limited sensitivity and specificity.[37, 38] The presence of air bronchograms was the only radiographic sign that correlated with VAP, correctly predicting 64% of pneumonias.[38] In our study, the strength of association between microbiological diagnosis and CPIS diagnosis was found to be significant and strong, implying that microbiological diagnosis could be a contributory addendum for definition ($p=0.021$, $r=0.259$) and the association between clinical diagnosis and CPIS diagnosis was again found to be significant which reassures the existing scoring ($p=0.01$, $r=0.507$). The study demonstrates that CPIS scoring has come out to be strongly associated, valid and reproducible clinically and radiologically (gama= 0.595, data not shown) and also there is an excellent association between the clinical and microbiological data existing in the patients on the same days (gama value=0.847, data not shown). The change and cycling of antibiotic considered after the culture reports shows that it has played a key role in the outcome of illness in terms of improvement. A significantly high number of VAP patients (74.1%, $p=0.035$) had a good outcome with recovery.

Prevention of VAP

With our staggering results, a lot needs to be done to step up the VAP prevention protocol bundle. VAP is a multifaceted diagnosis and many controversies continue regarding the epidemiology, diagnosis, prognosis and its management. However, the universally agreed fact is that, a significant proportion of VAP could be prevented by improving the quality of care of the patient. Hence, we recommend a combined clinical, microbiological and radiological approach,

which include accurate investigation, invaluable input from the microbiological laboratory, rational and early antibiotic therapy, timely surveillance, strict infection control measures, monitoring risk factors and finally the knowledge of the treating physicians about the local epidemiological data and susceptibility pattern of isolates. Measures to prevent VAP includes ensuring adequate pressure in the endotracheal cuff, early extubation, timely subglottic drainage, oral intubation, drainage of the condensate from the ventilator circuits, and humidification with HME. Health care providers should wear gowns and gloves, provide adequate nutritional support, and limit the magnitude of aspiration by placing patients in semi-upright position. Strict barrier precautions and contact isolation are emphasized in ICU set ups to lessen the burden of transmission of colonized MDR organisms, while waiting for the culture reports. More such local studies from the developing countries on the risk factors for VAP, combined with knowledge of causative pathogens and their antibiotic sensitivity pattern are potentially useful in formulating the multimodal preventive strategies.

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Ethical Clearance

The Ethical Committee of Manipal University has approved the study.

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