Original Research Article

Study of quantitative bacterial cultures of non-bronchoscopic samples in ventilator associated pneumonia

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Received: 23 May 2018

Accepted: 26 June 2018

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ABSTRACT

Background: Diagnosis of VAP based on non bronchoscopic samples-ETA, NB-BAL culture. The aim is to study quantitative culture of the non-bronchoscopic sampling techniques such as Blind Broncho-alveolar lavage (NB-BAL) and endotracheal aspirates (ETA) in Ventilator Associated Pneumonia. It is a hospital based, observational study conducted in SPMCHI, Jaipur from September 2015 to September 2016.

Methods: Seventy patients who were under mechanical ventilation for more than 48 hours and clinically suspected for VAP were included in the study and divided into early and late onset VAP. The NB-BAL and ETA were obtained from these patients and quantitative cultures were performed.

Results: Out of the 70 samples analysed, 60 patients were found positive in BAL and 61 positive in ETA. The agreement between NB-BAL and ETA is 86.8%. GNBs remain the main burden of both early and late onset VAP. Most common organisms isolated were Enterobacter and Acinetobacter in early onset and Pseudomonas and Acinetobacter in late onset VAP. All the GNB isolates were sensitive to Polymyxin and Colistin and were resistant to majority of routinely used antibiotics.

Conclusions: The quantitative culture of non-bronchoscopic samples is a useful alternative to bronchoscopy, in the diagnosis of VAP in resource deprived centers. MDR gram negative bacilli are the main causative agents of VAP.

Keywords: ETA, NB-BAL, Non-bronchoscopic sample culture, VAP

INTRODUCTION

Ventilator associated pneumonia (VAP), an important form of hospital acquired pneumonia (HAP), refers to pneumonia that occurs 48 hours or thereafter following mechanical ventilation.¹ VAP continues to complicate the clinical course of patients receiving mechanical ventilation. VAP requires a rapid diagnosis and initiation of the appropriate antibiotics since many studies have shown adverse effects of inadequate antibiotic treatment on the patient's prognosis.² For many years, VAP was diagnosed by clinical criteria such as fever, leukocytosis and purulent tracheobronchial secretions supported by the

radiological evidence of new or persistent pulmonary infiltrates.³ These clinical criteria are non-specific and microbiological evaluation is necessary for confirmation of VAP.⁴ This is done by microbiological investigation wherein samples (bronchoscopic/ non-bronchoscopic) obtained from the lower respiratory tract are cultured quantitatively or semi quantitatively.

At the present time, bronchoscopic sampling of lower airways, using either a protected specimen brush (PSB) or bronchoalveolar lavage (BAL) is accepted as the most accurate method of diagnosing VAP.⁵ However, bronchoscopy is not readily available in many settings

and there is a growing need to evaluate other sampling techniques for collecting respiratory secretions. The collection of blind, non-bronchoscopic samples is an appealing alternative. They require less expertise and more readily available.

There are many studies, which compared the diagnostic values of quantitative cultures of non-bronchoscopic samples in VAP, whereas little data is available in Indian pediatric population.^{6,7}

Therefore, this study was aimed at evaluation of the usefulness of quantitative cultures of non-bronchoscopic samples in the management of VAP in the intensive care unit (ICU) of a tertiary care hospital.

The objectives are to identify and quantify bacterial growth and antibiotic susceptibility in VAP; to compare the efficacy of ET aspirate with NB-BAL sample in identification of the organisms causing VAP; to compare the spectrum of organisms in Early onset v/s Late onset VAP.

METHODS

It was a prospective, observational type of study conducted over a period of one year in PICU, SPMCHI, attached to SMS medical college, Jaipur. The PICU has 25 beds and staffed with experienced consultants, residents and nurse: patient ratio of 1:3 available around the clock. Permission from ethical committee was taken prior to the study. Sample size was calculated to be 37 subjects at 95% confidence level and relative allowable error 20% assuming sensitivity of the test to be 73% and authors included 70 patients in the study.

Inclusion criteria

Patients of age 1 month to 18 years, with primary non-respiratory disease and mechanically ventilated for more than 48 hours with:

- Clinical suspicion for VAP with modified CPIS8 (m-CPIS) score ≥ 5
- Informed consent.

Table 1: Modified CPIS score (m-CPIS).

Parameters	Score		
	0	1	2
Tracheal secretion	Rare	Abundant	Abundant or purulent
Chest X-ray	No infiltrate	Diffuse	Localized
Temperature	>36.5 and <38.4°C	>38.5 and <38.9°C	>39 or <36°C
TLC/mm3	>4000 and <11000	<4000 or >11000	<4000 or >11000 OR band forms >500
PaO ₂ / FiO ₂	>240 or ARDS		< 240 and no evidence of ARDS

CPIS: Clinical pulmonary infection score

The clinical suspicion of VAP is based on the following criteria:

New or progressive radiographic infiltrates any 2 of the following:

- Temperature >38°C or <35°C,
- Leucocytosis or Leucopenia,
- Purulent tracheal secretions.

Exclusion criteria

Patients with primary infective respiratory disease.

Data collection

Once the inclusion was made after getting informed consent from the parents, baseline data including age, gender, date of admission in ICU, underlying disease, date of intubation/ tracheostomy, duration of mechanical ventilation before inclusion in the study, date of inclusion in the study, m-CPIS score were recorded in the designed

proforma. Duration of prior antibiotic therapy and a short description of initial radiological findings were also recorded. For each patient, ETA samples and NB-BAL samples were collected and sent immediately for processing. The subjects were assigned in to 2 groups-early onset and late onset VAP based on the duration of mechanical ventilation at the time of inclusion in the study.

Sample collection

Endotracheal aspiration was done using sterile suction catheters of sizes appropriate for the different sized ETTs connected to the mucus trap unit. Suctioning was performed with wall mounted suction and the aspirate from the tip of the ETT was collected in a sterile mucus trap.

NB-BAL samples were collected by the technique based on the description by Morrow et al⁹. Patients were pre-oxygenated with 100% inspired oxygen for approximately 5 - 10 minutes prior to NB-BAL sample

collection. A manual resuscitation bag, with oxygen flow turned on and three 50 ml syringes pre-filled with aliquot volume of 0.9% saline were kept ready at the patient's side. A suction catheter of appropriate size (10 Fr / 8 Fr) was introduced in to the ET tube and advanced up to the tip of the ET tube. A NG tube of size 5 Fr or 6 Fr with its tip cut with a sterile surgical blade was telescoped through the suction catheter and advanced as far distal as possible. A lavage volume of 5ml for the patients of weight 5-15 kg and 10 ml for those weighing > 15 kg of 0.9 % normal saline was infused and the lavage was aspirated manually using 50 ml syringe. Two further lavages of similar volume were performed through the same NG tube in same position. The first aliquot was discarded, and the remains aliquot were collected in a single sterile container and sent for microbiological analysis.

If a patient's SpO₂ dropped to <90% at any stage during the procedure, the tubes were withdrawn immediately while attempting to suction as much fluid back as possible in the process. If immediate improvement did not occur, the patient was manually ventilated with 100% oxygen until SpO₂ returned to pre-lavage levels and then the procedure was continued.

All the samples collected were labeled and transported to the laboratory immediately and inoculated for culture within an hour of collection. Both Gram staining and quantitative cultures, using the calibrated loop method, were performed on common media, using standard techniques. Microbiological examination for unusual organisms, such as Mycoplasma, Chlamydia, Pneumocystis carinii, and viruses, did not form a part of this study. Also, sensitivity patterns were studied with disc diffusion, minimal inhibitory concentration methods and additionally with automatized systems.

Statistical analysis

The statistical software namely SPSS 16.0 was used for the analysis of the data and Microsoft word and excel have been used to generate graphs, tables.

RESULTS

During 1-year period of study, 70 patients were included in the study after applying inclusion and exclusion criteria. The mean age of the patient in the study group was 49.71±49.85 months and ranged from 1 to 168 months. Authors included patients who had predominantly non-pulmonary infectious diseases (78.1%) as the primary disease responsible for mechanical ventilation.

Other causes for mechanical ventilation included neuromuscular disorders (5.7%), ESRD (7.2%), hepatic encephalopathy (4.3%), bleeding disorders (2.8%) and DKA (1.9%). Out of 70 subjects, 47 had early onset VAP and 23 had late onset VAP.

The mean duration of mechanical ventilation before the clinical suspicion of VAP in early onset VAP was 3.55 days and in late onset VAP was 7.26 days (Table 2).

Table 2: Demographic and clinical characteristics of the study patients (n=70).

Parameter	Value
Mean age	49.71±49.85 months
<1 year	23 (33%)
1-5 year	26 (37%)
5-10 year	13 (18.5%)
> 10 year	8 (11.5%)
Male	50 (71.4%)
Female	20 (28.6%)
Cause of admission	
Infectious diseases	55
DKA	2
ESRD	5
Neuromuscular disease	4
Hepatic encephalopathy	3
ICH	1
EOV	47 (67%)
LOV	23 (33%)
Mean m-CPIS in culture negative cases	5.2±0.44
Mean m-CPIS score in culture positive cases	5.90±0.53
Mean CPIS score based on ETA microbiology report	7.90±0.54
Mean CPIS score based on NB-BAL microbiology report	7.86±0.64

Authors used modified CPIS scoring system (m-CPIS) in the initial evaluation of the patient and, complete CPIS scores were calculated for both ETA and NB-BAL samples based on the microbiology report.

Table 3: Pathogens isolated from endotracheal aspiration and non-bronchoscopic BAL specimen cultures.

Pathogen	ETA	NB-BAL
<i>Acinetobacter</i>	15	19
<i>Citrobacter</i>	1	1
<i>Cons</i>	3	2
<i>E. coli</i>	3	3
<i>Enterobacter</i>	18	20
<i>Klebsiella</i>	5	5
<i>Pseudomonas</i>	9	10
<i>Commensals</i>	3	0
<i>Polymicrobial</i>	4	0
Sterile	9	10

The mean m-CPIS score of the cases in present study was 5.81. CPIS based on ETA report was 7.52 and that of NB-BAL report was 7.47. Out of 70 patients, 52 patients had m-CPIS score ≥ 6 and 96% of them had positive culture and 18 patients had m-CPIS score < 6 of which 61% of them had positive culture. The difference in culture positivity of these 2 groups was statistically significant.

In present study, out of 70 patients, 61 samples were found to have positive reports in ET aspirate samples and 60 in NB-BAL samples.

Out of total positive reports, 53 had similar organisms isolated from both ETA and NB-BAL samples and the agreement between NB-BAL and ETA was 86.8%.

Among the remaining 8 samples, 3 ETA samples showed commensal growth and 4 ETA samples showed polymicrobial growth. The corresponding 7 NB-BAL samples had a single organism isolated in culture. Only one patient had pathogenic organism grown in the ETA and no growth in the NB-BAL sample (Table 3).

Out of 70 subjects, 61 samples of ETA showed positive growth and 9 samples were sterile. Among the 61 samples, single pathogenic organism was isolated in 54 samples.

Among the remaining 7 samples, 3 samples showed commensal growth and 4 samples showed polymicrobial growth. No commensal growth or polymicrobial growth was reported in the NB-BAL samples (Table 4).

Table 4: Comparison of ETA and non-bronchoscopic BAL culture results.

ETA +ve/ NB-BAL +ve (same organisms in both samples)	ETA +ve/ NB-BAL +ve (different growth in ETA and NB-BAL samples)	ETA +ve/ NB-BAL -ve	ETA -ve/ NB-BAL +ve	ETA -ve/ NB-BAL -ve
53	7	1	0	9

On comparing with NB-BAL technique, ETA technique had a sensitivity of 100%, specificity of 90%, PPV of

98.3%, NPV of 100%. The difference in outcome of the ETA and NB-BAL technique was statistically not significant.

Table 5: Organisms causing early onset VAP.

Type	<i>Acinetobacter</i>	<i>Citrobacter</i>	<i>Cons</i>	<i>E. coli</i>	<i>Enterobacter</i>	<i>Pseudomonas</i>	<i>Klebsiella</i>	<i>Sterile</i>	<i>Commensal</i>
ETA N=48	12 (25)	1 (2.1%)	2 (4.17)	2 (4.17)	16 (33.3)	4 (8.3)	3 (6.25)	6 (12.5)	2 (4.17)
NB-BAL N=47	12 (25.5)	1 (2.1)	1 (2.1)	2 (4.3)	16 (34.0)	5 (10.6)	3 (6.4)	7 (14.9)	0

Table 6: Organisms causing late onset VAP.

Type	<i>Acinetobacter</i>	<i>Citrobacter</i>	<i>Cons</i>	<i>E. coli</i>	<i>Enterobacter</i>	<i>Pseudomonas</i>	<i>Klebsiella</i>	<i>Sterile</i>	<i>Commensal</i>
ETA N = 26	7 (26.9)	0	1 (3.8)	1 (3.8)	3 (11.5)	6 (23)	3 (11.5)	3 (11.5)	1 (3.8)
NB-BAL N = 23	7 (30.4)	0	1 (4.3)	1 (4.3)	4 (17.4)	5 (21.7)	2 (8.7)	3 (13)	0

In present study, authors observed 7 different species isolated from the samples-with predominance of Gram Negative Bacilli (GNB)-91%. The organisms grown were *Acinetobacter* (29.2%), *Enterobacter* (30.7%), *CoNS* (4.6%), *E. coli* (5.1%), *Klebsiella* (9.2%), *Citrobacter* (1.5%), *Pseudomonas* (15.3%) and commensal growth (4.6%). The most common organisms that caused early onset VAP were *Enterobacter* and *Acinetobacter*

accounting for 33.3% and 25% in ETA samples respectively and 34% and 25.5% in NB-BAL samples respectively (Table 5). The most common organisms that caused late onset VAP in present study were *Acinetobacter* followed *Pseudomonas* accounting for 30.4% and 21.7% respectively in the NB-BAL samples and 26.9% and 23% in the ETA samples respectively (Table 6).

Table 7: Antibiotic susceptibility of the gram-negative organisms isolated in the study.

Antibiotics	<i>Enterobacter</i> n=20	<i>Acinetobacter</i> n=19	<i>Pseudomonas</i> n=10	<i>Klebsiella</i> n=5	<i>E. coli</i> n=3	<i>Citrobacter</i> n=1
Polymyxin and colistin	20/20	19/19	8/10	5/5	3/3	1/1
Levofloxacin	7/20	0/19	4/10	0/5	0/3	0/1
Imipenem	14/20	4/19	5/10	1/5	2/3	0/1
Piperacillin+tazobactam	4/20	1/19	5/10	0/5	0/3	0/1
Netilmycin	9/20	6/19	0/10	2/5	2/3	0/1
Aztreonam	0/20	0/19	5/10	0/5	0/3	0/1
Tigecycline	4/20	2/19	1/10	1/5	0/3	1/1
Ceftazidime	0/20	0/19	2/10	0/5	0/3	0/1
Cefaperazone+sulbactam	2/20	1/19	5/10	0/5	0/3	0/1
Doxycycline	3/20	4/19	0/10	0/5	0/3	0/1
Gentamycin	3/20	0/19	3/10	0/5	0/3	0/1
Cefepime	3/20	0/19	1/10	0/5	0/3	0/1
Tobramycin	0/20	2/19	7/10	0/5	0/3	0/1

In present study, all the Gram-negative isolates were sensitive to Polymyxin B. Majority of the isolates in present study were resistant to the routinely used antibiotics like aminoglycosides, cephalosporins and fluoroquinolones. Imipenem showed good activity against 70% of *Enterobacter*, 67% of *E. coli*, 50% of *Pseudomonas* isolates in present study. Netilmycin showed good sensitivity against 45% of *Enterobacter*, 66% of *E. coli*, 50% of *Pseudomonas* and 40% of *Klebsiella* isolates in present study. Levofloxacin also showed good activity against 35% of *Enterobacter* and 40% of *Pseudomonas* isolates in present study (Table 7). All the CoNS (in 3 patients) in present study were sensitive to Vancomycin and Linezolid.

DISCUSSION

Ventilator Associated Pneumonia (VAP) is one of the most common infection acquired in intensive care units which carries substantial morbidity and mortality. The identification of causative organisms of VAP is crucial for the selection of appropriate antibiotics, which in turn is an important determinant of survival of the patients and to curb the menace of antibiotic resistance. The quantitative culture of lower respiratory tract samples obtained by non-bronchoscopic techniques is highly useful in early diagnosis and appropriate management of VAP.¹⁰ Authors tested 2 types of non-bronchoscopic techniques- ETA and NB-BAL in 70 patients. The quantitative cultures were considered positive for a diagnostic threshold of 10^5 CFU/mL for ETA and 10^4 CFU/mL for NB-BAL samples.¹¹

Authors included patients with m-CPIS ≥ 5 in present study. In present study, 96% of the patients with m-CPIS score ≥ 6 had definite VAP. Multiple studies in the past also showed m-CPIS >6 is a reliable tool in the diagnosis of VAP.^{12,13} In present study, NB-BAL technique was done using 2 tubes (NG tube telescoped through a suction

catheter). For BAL, the lavage volume is not standardized for pediatric age group. Ratjen et al suggested the lavage volume of 1 ml/kg body weight of 0.9% saline at room temperature for better lavage results.¹⁴ Authors used a lavage volume of 5ml for the patients of weight 5-15 kg and 10 ml for those weighing >15 kg. Similar volume of lavage was used by Atikar Y et al.¹⁵

The important issue regarding the practical application of NB-BAL was the safety of the technique. In present study out of 70 patients, transient desaturation ($SpO_2 < 90$) was noted in 7 patients and blood-stained lavage was noted in 4 patients and they need no special intervention. No other significant side effects were noted in present study.

In present study, commensal growth was noted only in ETA samples (3 samples) but not in the NB-BAL samples. This shows the superiority of NB-BAL over the ETA technique. In present study, the concordance between ETA and NB-BAL was 86.8%. In a study by Artukz et al, 27% of ETA samples had commensal growth and agreement between ETA and NB-BAL was 50% only.¹⁶ The low percentage of commensals in present study on comparing with other studies may account for high agreement between ETA and NB-BAL in present study.

In present study, the most common organisms isolated in both early and late onset VAP were similar and it included predominantly GNBs. The most common organisms isolated were *Enterobacter*, *Acinetobacter* and *Pseudomonas*. Previously it was thought that early onset VAP was predominantly caused by community acquired organisms and late onset VAP by nosocomial agents.²⁰ But this difference was not found in present study due to changing epidemiological profile of organisms and antibiotic use policies in ICUs.¹⁷ The similar microbiological profile has been noted in a recent study by Golia S et al who documented that GNBs account for 85% of VAP and also in a study by Girish et al which

showed 84.5% of VAP episodes were caused by GNBs.^{18,19}

In present study, majority of the isolates were resistant to the routinely used antibiotics. All the organisms isolated in present study were sensitive to Polymyxin B and Colistin only. Most of the organisms isolated in present study showed moderate sensitivity to Imipenem. Fluoroquinolones showed moderate activity against *Pseudomonas* and *Enterobacter* in present study. Netilmycin showed moderate activity against *E. coli*, *Klebsiella* and *Pseudomonas* in present study. The only gram-positive organism isolated in present study was CoNS (in 3 patients) and all of them were sensitive to Vancomycin and Linezolid. Golia S et al also noted that 40% of the *Pseudomonas* and *Acinetobacter* isolates in their study were resistant to all the group of antibiotics including carbapenems.¹⁸ Mohanty D et al also noted that all the GNB isolates in their study were highly sensitive to Polymyxin B, Colistin and Imipenem.²⁰

The major limitation of this study is the non-inclusion of a gold standard method (bronchoscopic BAL) for diagnosis of VAP which prevents from reaching certain conclusion regarding the standard technique. Another limitation is the small sample size. This is a case-based study validating the diagnostic tests for VAP with no impact on outcome.

CONCLUSION

Present study concludes that the quantitative cultures of non-bronchoscopic samples (NB-BAL and ETA) is a simple, non-invasive and useful alternative for invasive bronchoscopic BAL in the diagnosis of VAP.

NB- BAL is a better sample than ETA as the chance of contamination with upper airway flora is less with the NB-BAL technique. m-CPIS score ≥ 6 is a reliable indicator of VAP. GNB remains the main burden of VAP which are resistant to most of the antibiotics. Polymyxin and Colistin were the antibiotics with good activity against these drug resistant VAP pathogens and seems promising. Other options include Imipenem, Fluoroquinolones and Netilmycin with moderate activity against most of the VAP organisms.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Azarudeen M, Sharma BS, Jain PK, Goyal AK, Malhotra B. Study of quantitative bacterial cultures of non-bronchoscopic samples in ventilator associated pneumonia. *Int J Contemp Pediatr* 2018;5:1837-43.