

ORIGINAL ARTICLE

VENTILATOR ASSOCIATED PNEUMONIA: THE ROLE OF TYPICAL, ATYPICAL BACTERIA AND FUNGI; COMPARISON BETWEEN ENDOTRACHEAL ASPIRATE AND BRONCHOALVEOLAR LAVAGE

By

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Ventilator associated pneumonia (VAP) is defined as pneumonia occurring after the first 48 hours of starting mechanical ventilation. The incidence of VAP varied between 7-70% in different studies. The pathogenesis of VAP usually requires the occurrence of two important processes: bacterial colonization of the aerodigestive tract and the aspiration of contaminated secretion into the normally sterile lower respiratory tract. The principal promise of bronchoscopy for the diagnosis consists of the ability to retrieve uncontaminated lower respiratory secretions and consequently doing quantitative cultures, this should allow a valid differentiation of colonization from infection. The aim of this study was to determine the role of legionella species, mycoplasma pneumoniae, Chlamydia pneumoniae, mycobacterium tuberculosis, fungi, and anaerobes as etiologic agents of VAP and to compare between quantitative Endotracheal Aspirate (EA) and bronchoalveolar lavage(BAL) techniques for the diagnosis of VAP. The study was conducted on 30 patients admitted to the critical care medicine department of Alexandria main university hospital who required invasive mechanical ventilation for at least 48 hours and developed clinical manifestations of VAP.

Gram negative bacteria were the most common etiologic pathogens, followed by atypical bacteria. There was no statistically significant difference between both EA and BAL regarding the aetiologic pathogens.

INTRODUCTION

Ventilator associated pneumonia (VAP) is defined as pneumonia occurring after the first 48 hours of starting mechanical ventilation. The incidence of VAP varied between 7-70% in different studies.

VAP is associated with a prolongation of the duration of mechanical ventilation, ICU hospital stay, and increased treatment cost and mortality.^(1,2)

Dividing patients with VAP into group with early and late onset had been shown to be of paramount importance. Early-onset VAP is believed to be related to microaspiration of the habitual flora of the oropharynx or endogenous community acquired pathogens such as staphylococcus aureus, streptococcus pneumoniae and Haemophilus influenzae, with endotracheal intubation and impaired consciousness being the main risk factors. Conversely, late - onset pneumonia is most often caused by aspiration of microorganisms that pathologically colonize the pharynx or stomach.^(3,4)

The pathogenesis of VAP usually requires the occurrence of two important processes: bacterial colonization of the aerodigestive tract and the aspiration of contaminated secretions into the normally sterile lower respiratory tract.⁽⁵⁾

Clinical and bacteriological diagnosis of VAP in mechanically ventilated patients is still difficult. Up till now, no generally accepted optimal strategy or gold standard for the diagnosis of VAP has been recommended.⁽⁶⁾

The gold standard of the diagnosis is combined histopathological and microbiological examination of the lung tissue showing both an inflammatory response and microorganisms. Clearly this is rarely possible in critically ill patients when an antemortem diagnosis is required.⁽⁷⁾

A classic histopathologic definition includes abscess formation or area of accumulation of neutrophils, plus a positive quantitative culture of lung parenchyma (>10⁴ microorganisms per gram of lung tissue).⁽⁸⁾

VAP is a diffuse, polymicrobial and dynamic process, with heterogeneous distribution of lesions, showing different degrees of histological evolution predominating in the dependent lung zones, in which microbiology and histology can be dissociated.⁽⁹⁾

Lower respiratory tract secretion can be sampled

either bronchoscopically [protected specimen brush (PSB) and bronchoalveolar lavage (BAL)] or nonbronchoscopically [blind PSB, blind BAL, or Endotracheal Aspirate (EA)].⁽¹⁰⁾

The principal promise of bronchoscopy for the diagnosis consists of the ability to retrieve uncontaminated lower respiratory secretions and consequently doing quantitative cultures, this should allow a valid differentiation of colonization from infection. The PSB and BAL represent two principal bronchoscopic techniques for bacteriologic diagnosis of VAP. Quantitative culture of EA is a good diagnostic test when a non invasive test has been chosen for the diagnosis of VAP.⁽¹¹⁾

Compared to conventional PSB and /or BAL, nonbronchoscopic techniques are less invasive, can be performed by clinicians not qualified to perform bronchoscopy, are associated with less compromise of gas exchange during the procedure, and can be performed even in patients intubated with small endotracheal tubes. Disadvantages include the potential sampling errors inherent in a blind technique and the lack of airway visualization.⁽¹²⁾

Aim of the work:

The aim of this study was to determine:

1. The actual role of Legionella species, Mycoplasma pneumonia, Mycobacterium tuberculosis, fungi, and the subsequent influence on antimicrobial guidance.
2. The characteristics of endo- tracheal aspirate quantitative culture for the diagnosis of pneumonia in mechanically ventilated patients compared to bronchoalveolar lavage technique.

PATIENT AND METHODS

The study was conducted on 30 patients admitted to the critical care medicine department of Alexandria main university hospital who required

invasive mechanical ventilation for at least 48 hours and developed clinical manifestation of VAP (presence of a persistent new and / or progressive infiltrates with at least two of the following: fever or hypothermia, leukocytosis or leucopenia or purulent tracheal aspirates).

For every patient a simple blind EA was obtained followed by bronchoscopic BAL. EA and BAL were divided into two parts ; part 1 for quantitative conventional cultures for bacteria (aerobes and anaerobes) , fungi, and tuberculosis , and part 2 for polymerase chain reaction (PCR) studies for mycoplasma pneumoniae, Chlamydia pneumoniae , and legionella species DNA.

The growth of a potential pathogen in count $\geq 10^6$ CFU/ml and $\geq 10^4$ CFU/ml for EA and BAL fluid respectively was taken as the diagnostic threshold for VAP; quantitative cultures were done immediately, while part 2 was stored at -20 C for PCR study at one sum.

RESULTS

The mean age of patients was 42.07 ± 16.87 years. Males represented 56.7% of patients while females represented 43.3%. The APACHE II score of the entire study ranged between 8 and 42 with a mean of 19.93 ± 9.73 . Most of the patients received antibiotics before the diagnosis of pneumonia (83.3%).

Table 1. Values [mean \pm S.D or number & (%)] of demographic data, APACHE II score durations (days) of hospital stay, ICU stay, mechanical ventilation, and outcome of the studied patients.

Variable	Values
Age:	
Range	13 - 70
Mean \pm S.D	42.07 ± 16.87
Sex [number & (%)]:	
Male	(56.7 %)
Female	13 (43.3%)
Smoking [number & (%)]:	
Yes	15 (50.0%)
No	15 (50.0%)
APACHE II score:	
Range	8 - 42
Mean \pm S.D	19.93 ± 9.73
Hospital stay:	
Range	3 - 62
Mean \pm S.D	14.10 ± 13.80
ICU stay:	
Range	2 - 62
Mean \pm S.D	11.27 ± 12.52
Duration of MV:	
Range	2.0 - 45
Mean \pm S.D	9.83 ± 10.32
Outcome:	
Discharge	12 (40%)
Died	18 (60%)

Table 2. Distribution of primary diseases for admission, chronic and pre-existing lung diseases and associated outcome in the studied patients. Values are number & (%).

Variable	(%) N = 30	Survived	Died
Primary disease:			
Toxic	9 (30.0%)	4	5
Respiratory failure	6 (20.0%)	2	4
Cardiac	5 (16.7%)	1	4
Neurological	4 (13.3%)	3	1
Trauma	3 (10.0%)	1	2
Surgical	1 (3.3%)	1	-
Renal failure	1 (3.3%)	-	1
Post arrest	1 (3.3%)	-	1
Chronic diseases:			
DM	6 (20.0%)	1	5
Heart failure	3 (10.0%)	1	2
RHD	2 (6.7%)	1	1
Hypertension	2 (6.7%)	-	2
HCV	2 (6.7%)	-	2
SLE	1 (3.3%)	-	1
CRF	1 (3.3%)	-	1
None	17 (56.7%)	9	8
Pre-existing lung disease:			
COPD	4 (13.3%)	-	4
Bronchial asthma	1 (3.3%)	1	-
TB	1 (3.3%)	-	1
Pulmonary fibrosis	1 (3.3%)	-	1
None	23 (76.6%)	17	16

Table 3. Relative frequency of distribution of microorganisms isolated from conventional culture (below and above cutoff values) and positive PCR for atypical bacterial DNA of both EA and BAL.

Organisms	EA		BAL		P
	N	%	N	%	
Gram+ve bacteria					
Staphylococcus aureus	2	6.25	3	9.4	0.31
Gram-ve bacteria					
Klebsiella pneumoniae	12	37.5	9	28.1	0.41
Pseudomonas aeruginosa	12	37.5	12	37.5	
E. coli	3	9.4	0	0.0	
Proteus vulgaris	-	-	1	3.1	
Fungi					
Candida albicans	1	3.1	2	6.25	0.45
Atypical bacteria					
Mycoplasma pneumoniae	0	0.0	3	9.4	0.021*
Legionella spp	1	3.1	2	6.25	
Chlamydia pneumoniae	1	3.1	1	3.1	
Polymicrobial	5	16.7	5	16.7	0.55

Gram negative bacteria were the most common aetiologic pathogens, 50% and 70% of EA and BAL respectively, followed by atypical bacteria 6.6% and 20% respectively while, gram positive bacteria represented 6.7 % and 10% respectively. VAP was polymicrobial in 10% and 13.3% of patients diagnosed by EA& BAL respectively .There was no statistically significant difference between both EA and BAL regarding the aetiologic pathogens.

Pseudomonas aeruginosa had the highest

prevalence, (26.7% and 40% of EA and BAL respectively), followed by *Klebsiella pneumoniae* (20% and 26.7%), MSSA (6.7% and 10%), *Mycoplasma pneumoniae* (0.0% and 10%), *Legionella* species (3.3% and 6.7%), and *Candida albicans* (0.0% and 6.7%). Meanwhile, *Chlamydia pneumoniae*, *E.coli* and *Proteus vulgaris* represented only (3.3%, 3.3%), (3.3%, 0.0%), and (0.0%, 3.3%) respectively *Pseudomonas aeruginosa* was significantly recovered from BAL in comparison to EA.

Table 4. Aetiologic pathogens of pneumonia in order of frequency of occurrence as detected in EA & BAL in the studied group.

Organisms	EA		BAL		P
	N	%	N	%	
<i>Pseudomonas aeruginosa</i>	8	26.7	12	40.0	0.041*
<i>Klebsiella pneumoniae</i>	6	20.0	8	26.7	>0.05
<i>Staphylococcus pneumoniae</i>	2	6.7	3	10.0	>0.05
<i>Mycoplasma pneumoniae</i>	0	0.0	3	10.0	-
<i>Legionella</i> spp	1	3.3	2	6.7	>0.05
<i>Candida albicans</i>	0	0.0	2	6.7	-
<i>Chlamydia pneumoniae</i>	1	3.3	1	3.3	-
<i>E coli</i>	1	3.3	0	0.0	-
<i>Proteus vulgaris</i>	0	0.0	1	3.3	-

Table 5. Association of atypical bacteria detected by PCR with conventional bacteria detected by culture of EA and BAL, Values are number (%).

	EA		BAL	
	Significant Growth of Conventional Bacteria	Insignificant Growth of Conventional bacteria	Significant Growth of Conventional Bacteria	Insignificant Growth of Conventional bacteria
<i>Mycoplasma pneumoniae</i>	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)
<i>Legionella</i> species	1 (3.3%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
<i>Chlamydia pneumoniae</i>	0 (0.0%)	1 (3.3%)	1 (3.3%)	0 (0.0%)
Total	1	1	1	5

When BAL was considered the gold standard; VAP was diagnosed in 28 patients (93.3%) based on BAL conventional culture at $\geq 10^4$ cfu/mL and positive PCR results. Diagnostic efficiency of EA was then analyzed at the threshold of $\geq 10^6$ cfu/mL. EA coincided with BAL in 16 cases (15 positive and 1 negative culture findings).

Disagreement was seen in 14 cases (EA positive with negative BAL in one case only, and EA negative with positive BAL in 13 cases). The sensitivity and specificity of EA were 53.6 and 50.0%, respectively. Positive Predictive value was 93.8% and negative predictive value was 7.14% with an overall accuracy of 53.3%.

Table 6. Aetiologic pathogens of pneumonia as detected by PCR and significant bacterial culture count from EA and BAL.

Techniques	Organisms	EA ($\geq 10^6$ cfu/ml)		BAL ($\geq 10^4$ cfu/ml)		P
		N	%	N	%	
Culture:	Gram+ve bacteria					
	Staphylococcus aureus	2	6.7	3	10.0	0.65
	Gram-ve bacteria					
	Pseudomonas aeruginosa	8	26.7	12	40.0	
	Klebsiella pneumoniae	6	20.0	8	26.7	
	E.coli	1	3.3	0	0.0	0.36
	Proteus vulgaris	0	0.0	1	3.3	
	Fungi:					
	Candida albicans	0	0.0	2	6.7	
PCR	Atypical:					
	Mycoplasma pneumoniae	0	0.0	3	10.0	0.51
	Legionella species	1	3.3	2	6.7	0.41
	Chlamydia pneumoniae	1	3.3	1	3.3	0.68
Conventional Culture + PCR	Polymicrobial	3	10.0	4	13.3	0.12

The mortality rate was 60%; 18 patients died and 12 have been discharged outside the ICU unit. The risk factors significantly associated with mortality were: a high APACHE II score ($p=0.001$), older age ($p=0.001$) and multidrug resistant organisms.

($p=0.021$). Chronic health diseases, the aetiologic organisms of VAP, and inappropriate empirical antibiotics were not significantly associated with mortality from VAP.

DISCUSSION

In the present study, Gram negative bacteria were the most common aetiologic pathogens recovered from EA and BAL (50% and 70 %) respectively

, followed by atypical bacteria 6.6 % and 20 % ,while , Staphylococcus aureus represented 6.7 % and 10 % ,followed by Candida albicans (0.0 %,6.7 %).VAP was polymicrobial in 10 % and 13.3 % of EA and BAL samples respectively .Among gram negative bacteria ; Pseudomonas aeruginosa had the highest prevalence, (26.7 % and 40 %) of EA and BAL respectively , followed by Klebsiella pneumoniae (20 % and 26.7 %).

The result of the present study agree with the results reported by Singhal et al⁽¹³⁾ who retrospectively reviewed microbiological results of non bronchoscopic BAL samples of patients with clinical diagnosis of VAP over one year .The quantitative threshold was $> 10^4$ cfu/ml. Among

the recovered 192 isolated, 190 were gram negative bacilli (GNB) and 2 were *Staphylococcus aureus*. *Acinetobacter* species were commonest (44.8 %) followed by *Pseudomonas* species (40.1 %) *Klebsiella pneumoniae* (5.7 %), *E. coli* (4.2 %), *Citrobacter* species (2.1 %), *Enterobacter* species (1.6 %) and one isolate of *S. marcescens*. The authors attributed these results to the heavy administration of antibiotics in their ICU patients. Similarly, Fagon et al⁽¹⁴⁾ found that 67.6 % of VAP isolates based on PSB sampling were GNB.

Again, our results agree with the results of the study conducted by Trouillet et al⁽¹⁵⁾ in which the distribution of the causative bacteria isolated from the 135 episodes of VAP were analyzed according to four groups of patients defined by prior duration of MV (< 7 or ≥ 7 d) and presence or absence of antibiotics during the 15 days preceding the event. Early -onset pneumonia in patients who had not received prior antimicrobial treatment were mainly caused by sensitive *Enterobacteriaceae*, *Haemophilus influenzae*, *MSSA*, and *Streptococcus pneumoniae*, late -onset pneumonia in patients who had recently received antimicrobial treatment were mostly caused by potentially resistant pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *MRSA*.

In comparison, Woske et al⁽¹⁶⁾ identified the aetiologic agents of VAP by quantitative culture of three bronchoscopic techniques BAL, PSB and bronchoscopic tracheobronchial aspirate. *Staphylococcus aureus* was the commonest aetiologic agent identified (37.7 %) followed by *Haemophilus influenzae* and *Pseudomonas aeruginosa* (each 10.4 %), *Klebsiella* species (9.1 %) and *E. coli* (7.8 %), *Streptococcus pneumoniae* (5.2 %); totally Gram positive bacteria represented 49.4 % while gram negative bacteria represented 50.6 %. But the vast majority of patients with VAP were not on antibiotics at the time of bronchoscopy and the authors endorsed the reason of the high frequency of Gram positive microorganisms to the restrictive policy on the use

of antibiotics. A policy that was not in place during a previous study in the same ICU, when *Pseudomonas aeruginosa* was the predominant microorganism.

The high incidence of Gram negative bacteria in the present study can be explained by two factors. The first is the predominance of the late onset VAP (76.6 %) in the present study in comparison to early onset VAP (23.3%), this was proved by the significantly higher incidence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among patients with late onset VAP. The second is the high incidence of prior antibiotics administration among patients in the present study, as 83.3 % of patients received antibiotics prior to the diagnosis of VAP which has probably led to infection with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These bacteria were significantly higher among patients who received prior antibiotics.

In the present study, atypical bacteria were diagnosed by PCR technique to avoid the aforementioned difficulties associated with serology and culture and to improve sensitivity. Atypical bacteria DNA was found in 7 patients of the studied group (23.3%), EA - PCR was positive in 2 patients and BAL - PCR was positive in 6 patients. Both EA and BAL were positive in one patient.

In the present study VAP was polymicrobial in 10 % and 13.3% of EA and BAL samples respectively. Almost the same results were reported by Bedewy et al⁽¹⁷⁾ and Meduri et al⁽¹⁸⁾ as they reported 12% and 13% respectively.

The diagnostic threshold for diagnosing pneumonia using Quantitative Endotracheal Aspirate (QEA) varies among different studies, mostly ranging from ≥105 to ≥ 106 cfu /ml. In the present study QEA was considered positive at the threshold of ≥ 106. Jourdain et al⁽¹⁹⁾ assessed the reliability of quantitative cultures of EA to diagnose VAP. They studied 57 episodes of

suspected VAP with no recent changes in antimicrobial chemotherapy. VAP was diagnosed based on PBS sampling yielding ≥ 103 cfu/ml and/or $\geq 5\%$ of cells containing intracellular bacteria on direct examination of BAL. The operating characteristics of EA cultures were calculated over a range of cutoff values (from 103 to 107 cfu/ml) and the threshold of 106 cfu/ml appeared to be the most accurate.

In the present study there was a poor agreement between EA and BAL regarding the isolated aetiologic agents of VAP; only 38.5% of the aetiologic agents were concomitantly identified by positive PCR and growth from conventional cultures above the diagnostic thresholds of EA and BAL. Taking into consideration concordant negative results, the agreement value equals 40.0%. In the same way, Jourdain et al⁽¹⁹⁾ reported that only 40% of the microorganisms cultured from the EA were concomitantly isolated from PSB above the cutoff value.

CONCLUSIONS

From the present study we can conclude the following:

1. Gram negative bacteria are the most common aetiologic agents of VAP in our ICU.
2. Atypical bacteria are more common than thought, they can result in monomicrobial as well as polymicrobial VAP.
3. Anaerobes, Mycobacterium Tuberculosis, and Fungi (other than Candida) seem not to play a role as aetiologic agents for VAP in our ICU.
4. The previously assumed false positive results of the clinical approach for diagnosis of VAP are not always truly false as detected by PCR.
5. In case of unavailability of bronchoscope, quantitative endotracheal aspirate at cutoff value of ≥ 106 cfu/mL would achieve an acceptable sensitivity, specificity and could limit the unnecessary antibiotic prescription.

6. Inappropriate empirical antibiotic prescription for treatment of VAP is high among our patients.
7. Multidrug resistant Gram negative bacteria represent a major threat in our unit and it could be attributed to the overusage of prior antibiotics.

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