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Original Work

Study of Ventilator Associated Pneumonia in Neonatal Intensive Care Unit: characteristics, risk factors and outcome

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ABSTRACT: Ventilator Associated Pneumonia (VAP), the nosocomial pneumonia developing in mechanically ventilated patients after 48 hours of mechanical ventilation, is the second most common nosocomial infection. Therefore, there is a vital need to study the etiology and risk factors associated with VAP in neonates. Neonates admitted to neonatal intensive care unit (NICU), over a period of 1 year and who required mechanical ventilation for more than 48 hours were enrolled consecutively into the study. Diagnosis of VAP was made by the guidelines given by National Nosocomial infection Surveillance System (NNIS, 1996). Semi-quantitative assay of endotracheal aspirate was used for microbiological diagnoses of VAP. 10^5CFU/ml was taken as the cut off between evidence of pathological infection and colonization. The risk factors such as birth weight, prematurity (gestational age < 37 weeks), duration of mechanical ventilation, number of reintubations, length of hospital stay, primary diagnosis of neonate, postnatal age and small for gestational age (SGA) were studied for the development of VAP. Risk factors found significant on bivariate analysis were subjected to multiple regression analysis to determine the most important predictors of VAP. The study group comprised of 98 neonates out of which, 30 neonates developed VAP (30.6%). VAP rates were 37.2 per 1000 days of mechanical ventilation. Most common bacterial isolated from endotracheal aspirate of VAP patients was *Klebsiella spp* (32.8%), *E.coli* (23.2%) and *Acinetobacter* (17.8%) being the other two common organisms. Very low birth weight (<1500 grams), prematurity (gestational age < 37 week), duration of mechanical ventilation, number of reintubations and length of NICU stay were significantly associated with VAP in bivariate analysis. Multiple regression analysis revealed that duration of mechanical ventilation (OR 1.10, 95% CI 1.02, 1.21; P = 0.021) and very low birth weight (OR 3.88, 95% CI 1.05, 14.34; P = 0.042) were two single independent and statistically significant risk factors for predicting VAP. VAP developed in nearly one third of intubated neonates having gram negative organisms as predominant etiological agent.

KEY WORDS: Neonates; Nosocomial infection; Outcome; Risk factors; Ventilator associated pneumonia

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INTRODUCTION

Intensive care units have come to represent the most frequently identifiable source of nosocomial infections within the hospital, with the infection rates and rate of antimicrobial resistance several fold greater than the general hospital settings. Ventilator-associated pneumonia (VAP) is defined as nosocomial pneumonia in mechanically ventilated patients that develops more than 48 hours after initiation of mechanical ventilation (MV). VAP is the second most common nosocomial infection after urinary tract infection in pediatric intensive care unit patients accounting for 20% of nosocomial infection in this population[1]. Langer and co-workers divided VAP into early onset VAP which occurs within 5 days of mechanical ventilation and late onset VAP, which develops five or more days after initiation of mechanical ventilation.[2] The importance of segregating VAP into early and late is that, the pathogenesis, microorganisms responsible and outcome in these two groups are different and so the therapeutic implications also differ. Early onset VAP results from aspiration of endogenous community acquired organisms e.g. *S. pneumoniae*, *H. influenzae*, and other organisms (aerobic gram negative bacilli). Late onset VAP is more severe and results usually from aspiration of gastric/oropharyngeal secretions and caused by potentially drug resistant organisms like methicillin resistant staphylococcus aureus (MRSA) and Pseudomonas. The diagnosis of VAP is made by clinical criteria recently revised by National Nosocomial infection Surveillance System (NNIS) for pediatric patients[3]. It should be however noted that a "gold standard" for microbiological diagnosis of VAP does not exist. For microbiological diagnosis either bronchoscopic methods like bronchoalveolar lavage, protected specimen brushing (PSB) or non-bronchoscopic methods like quantitative endotracheal aspirate (ETA) is used in most intensive care units (ICUs).

In countries especially like India, clinical and radiological criteria in association with semi-quantitative ETA are accessible to most ICUs. VAP has been extensively studied in adults but there is great paucity of data of VAP in children especially in neonatal age, so this study was done.

METHODOLOGY

This is a prospective observational study conducted in a NICU of a tertiary care teaching hospital for a period of one year from September 2004 to August 2005 after seeking approval from the institute's Ethics committee. NICU of the hospital was 30 bedded with 11 ventilators at that time. All intubated patients admitted in the NICU during the study period were enrolled in the study consecutively. Neonates requiring MV for less than 48 hours and those who had pneumonia at the time of initiation of MV were excluded from study. The diagnosis of VAP was made on the basis of criteria given by National Nosocomial Infection Surveillance system (1996), the pediatric modification of the original guidelines given by Center for Disease Control and Prevention (CDC)[3]. Early onset VAP was defined as which develops within 5 days of MV and Late onset VAP, which develops five or more days after initiation of mechanical ventilation. The protocol that was followed in the NICU was as detailed below:

- All patients were ventilated by orotracheal tube, which was changed only if, blocked or displaced.
- Patients were ventilated in SIMV/CMV/ACV mode using Breathline digivent and Babylog 8000-plus Drager ventilator with heated humidification system.
- One set of disposable ventilation circuit was used for one patient. Open method of suction was used for suctioning of secretions.
- Patients were ventilated in supine position with frequent changing to right lateral and left lateral position and nasogastric tube was put in all patients.
- Patients were placed on Servo controlled warmer with skin temperature set on 37.0^0C with an audiovisual alarm on $\pm 1^0$C temperature.
- Any hyperthermia ($>38^0$C) or hypothermia ($<36^0$C) was rechecked by clinical thermometer by axillary method.
- There was no use of H_2 blocker among study cases.
- No prophylactic topical oropharyngeal antibiotics and selective gut decontamination was done in any of the patients.
- Baseline total leukocyte count, differential leukocyte count and chest X ray P-A view were done in all patients at the time of initiation of MV.
- Subsequent blood counts were done on day 3, day on which pneumonia was suspected by the onset of fever, new chest signs, increasing ventilator requirements.
- After Baseline chest X-ray, next chest X-ray was done after 48 hours of mechanical ventilation, on suspicion of pneumonia or

any other pulmonary complication, 48 hours after pneumonia was detected to check for persistence of infiltrates, otherwise routinely once weekly if no pneumonia was detected.

- Follow up included clinical examination for pneumonia and chest X-ray after one week of extubation.

Collection of endotracheal aspirate

After proper hand washing and wearing sterile gloves before suctioning, the endotracheal secretions were collected by instilling 1-2 ml of sterile normal saline into the endotracheal tube and then collecting it back with the help of sterile mucous trap. The specimen collected was immediately transported to the laboratory within one hour of collection. Sample collected at night was stored at 4 degree centigrade overnight and send to the laboratory by 10 am next day morning. Semiquantitative culture was done on endotracheal aspirate. Measured amount of aspirate was used for plating on Blood Agar Media and Bromthymol Blue agar media, colony characteristics were observed and identification was done in accordance with standard recommendations. Sensitivity of the organisms isolated was done by Disc diffusion method by Kurby Bauer (inhibition zone diameter-interpretive criteria adopted from laboratory procedures in clinical microbiology Washington, J.A. and manufactures). 10^5 CFU/ml of endotracheal aspirate was the cut off between organisms causing VAP and colonization. Blood cultures were collected whenever there was a suspicion of VAP. The site of sample was cleaned with povidone iodine and alcohol and allowed to dry for two minutes. Two ml of blood was added to bile broth and trypticase soya broth (1 ml in each bottle). After overnight incubation at 37^0 C it was inoculated on blood agar and McConkey's agar. The reporting of the X-ray was done by one consultant radiologist of our centre to minimize the interobserver bias.

Statistical Analysis

Data were analyzed using statistical software package, STATA 8.2. Proportions were compared using Chi squire statistics and were supported by 95% Confidence Limits wherever required. Fisher's exact p-value to see the difference between the mean of two different groups if data was normally distributed. If data was not found to be normally distributed, a non-parametric equivalent of two-sample t-test, the Mann Whitney test used to test the level of significance between two values. The difference between any two groups was considered to be significant if p value was < 0.05. The method for multiple regression analysis was 'Enter' method and this analysis was done using Stata 9.2 software, College Station, TX.

RESULTS

Of 248 mechanically ventilated patients, 98 fulfilled the inclusion criteria and formed the subjects. About 10% of these patients were given AMBU bag ventilation for variable periods before being provided with mechanical ventilator. **Table 1** shows the demographic profile of the study patients.

Incidence of VAP

VAP developed in 30 of 98 neonates (30.6% VAP rate). Incidence of VAP was 37.2 per thousand days of MV. The mean duration of ventilation before the onset of pneumonia was 5 days. 14 (46.6%) cases developed VAP within 5 days of mechanical ventilation and were classified as early onset VAP. Rest16 (53.3%) cases developed pneumonia $\geq$5 days of ventilation and cases categorized under late onset VAP.

Risk factors for VAP

Intubated neonates who developed VAP were compared with those who did not develop VAP with respect to post natal age, birth weight, prematurity (gestational ages < 37 weeks), duration of mechanical ventilation, number of reintubations, length of NICU stay, primary diagnosis of neonate and small for gestational age (SGA). Very low birth weight, prematurity, duration of MV, number of reintubations and length of NICU stay were significantly associated with VAP as observed by bivariate analysis. However, there were statistically no significant difference between the two groups with respect to post natal age (day on which pneumonia developed), sex and SGA. **(Table 2)**

Multiple logistic regression analysis revealed that duration of MV (OR 1.10; 95%CI 1.02, 1.21; P = 0.021) and very low birth weight (birth weight < 1500 gram) (OR 3.88; 95%CI 1.05, 14.34; P = 0.042) were two single independent and statistically significant risk factors for development of VAP. **(Table 3)**

Table 1: Demographic and baseline variables of neonates

	VAP(30)	Non VAP(68)	P value
Postnatal age on admission (days)	3.26±4	2.72±4.1	0.39
Birth weight (grams)	2249.3±797.1	2387.5±678.3	0.38
Male sex	22(73%)	39(57%)	0.09
SGA/AGA/LGA	2/28/0	4/62/2	0.63
Mean hemoglobin (gm%)	16.2	16.45	0.43
PROM	12(42%)	40(58%)	0.14
Resuscitation at birth:			0.95
Oxygen	10/30	25	
Tactile stimulation	1/30	1	
BMV	2/30	3	
IPPV	5/30	3	
Indication for MV			0.68
Pneumonia	9/30	24/68	
Apnoea	10/30	18/68	
Poor respiratory effort	5/30	8/68	
HMD	6/30	18/68	

(MV: Mechanical Ventilation; BMV: Bag and Mask ventilation; HMD: Hyaline Membrane Disease; IPPV: Intermittent Positive Pressure Ventilation; SGA: Small for Gestational Age; AGA: Appropriate for Gestational Age; LGA: Large for Gestational Age; PROM: Premature Rupture of Membrane - leaking per vagina > 24 hours)

Table 2: Risk factors for VAP (Bivariate Analysis)

Variables	Pneumonia (n=30)	No Pneumonia (n=68)	P value
Postnatal age (Early vs late neonatal period)	27/3	63/5	NS
Sex (M:F)	4:1	3:8	NS
Very low birth weight (birth weight < 1.5 kg)	10/20	6/92	0.002
Prematurity (EGA < 37weeks)	17/13	20/48	0.01
Small for gestational age (SGA)	2/28	4/64	NS
Length of NICU stay in days (mean ± SD)	32.7± 34.7	19.7 ± 23.9	0.0028
Number of reintubation (%)	18/30 (60%)	6/68 (8.8%)	<0.001
Duration of MV (hours) (mean ± SD)	300.2±174.6	138.04±93.1	<0.001

SGA: Birth weight less than 10th percentile for that gestational age; MV: Mechanical ventilation; EGA: estimated gestational age; NS: Not significant

Table 3: Multiple Logistic Regression Analysis for the risk factors of VAP

Factor	Odds Ratio (OR)	95% Confidence Interval (CI)	P value
Days on MV	1.10	1.02,1.21	0.021
Very low birth weight	3.88	1.05,14.34	0.042
Prematurity (gestational ages < 37 weeks)	1.16	0.74,6.21	0.157
Number of reintubations	0.75	0.45,1.23	0.249

Likelihood ratio χ^2 = 16.98; df = 4; p = 0.0019; Log likelihood = - 50.32; Pseudo r^2 = 14.44%

Etiological organisms

The organisms isolated by semiquantitative assay of endotracheal aspirate (ETA) of patients who developed VAP with their antibiotic sensitivity pattern are shown in **table 4** and **5**. Out of 232 ETA samples cultured during the study period, 69 samples yielded significant growth (>10^5 CFU/ml). Amongst these samples, 4 samples had polymicrobial growth of two organisms (one of *Klebsiella spp*. and *E. coli*; one of *E. coli* and *Pseudomonas*; 2 of *Coagulase-negative staphylococci* and *Acinetobacter*) thus yielding total of 73 isolates. Blood culture was sent in all cases. None of the VAP patient has recovered same organism from blood culture and ETA culture.

Most common bacterial isolate from ETA of VAP patient was *Klebsiella spp*. (32.87%). E. coli and *Acinetobacter* were the other two common organisms. *Klebsiella* was found to be most sensitive to Cefoperazone and Sulbactam combination (87.50%) and Amikacin (66.67%). Only 1 strain of 24 isolates was found to be sensitive for the antibiotics like Ampicillin, Amoxicillin, Cefotaxime and Gentamicin with resistance in 95.83% of the cases. Some of the strains were also sensitive to Netilmicin and Cefotaxime, Sulbactam combination.

On comparing the organism causing early VAP and late onset VAP, it was found that early onset VAP was most commonly caused by *Klebsiella* whereas equal number of *Klebsiella* and *Acinetobacter* (33.3% each) were the causative agent of late onset VAP. None of the early VAP was caused by *Enterobacter* which was a causative agent of late onset VAP only.

Outcome

The mean length of NICU stay was significantly longer in patients with VAP as compared to those with out VAP i.e. 232.7 days Vs 19.7 days (p = 0.028). Mortality rates were higher in patients with VAP (40%) and lower in non-VAP cases (22.06%) (p=0.058). Among the study population of 98 neonates, 27 expired (27.5%).

Table 4: Organisms grown from Endotracheal Aspirate

Organisms	No. of isolates grown	Percent
Klebsiella spp.	24	32.87
E. coli	17	23.28
Acinetobacter	13	17.80
Pseudomonas aeruginosa	8	10.95
Coagulase-negative staphylococci	05	6.84
Staphylococcus aureus	02	2.73
Candida	02	2.73
Enterobacter	02	2.73
	Total Isolates 73	100.00

DISCUSSION

The incidence of VAP in our study was 30.6 %. This incidence is comparable to the incidence of VAP in earlier neonatal studies such as Apisarnatharak et al[4] 28.3%, Petdachai[5] et al 50% and Yuan[6] et al 20.1%. This variation is due to difference in diagnostic criteria used, aseptic precautions in intensive care unit and variable sensitivity and specificity of diagnostic tests. Most studies on VAP have used objective diagnostic criteria based on the combination of quantitative culture samples obtained with fiberoptic bronchoscopy using the PSB or the bronchoalveolar lavage (BAL)[7-10]. Both these methods have been shown in literature to have a specificity and sensitivity of greater than 95% in the diagnosis of VAP[11,12]. The quantitative bacteriologic methods have increased the reliability of sputum specimens for the diagnosis of lower respiratory tract infections when compared with conventional qualitative cultures because of more careful collection of sputum and the method of dilution which eliminates contaminating oropharyngeal secretions.[13] However, Marquette[14] et al in their study comparing endotracheal aspirate (ETA) cultures with PSB found that the former technique could be used as a reliable alternative to PSB at the cut off value of 10^6 CFU/mL. These findings compare favorably with our study where we had used the semiquantitative cultures of the ETA aspirates at a cut off point of 10^5 CFU/mL. Since the technique of PSB and BAL are not readily available to us in our NICU, the ET aspirate semiquantitative technique can be used as an alternative diagnostic tool for VAP. It may be helpful in differentiating infection from colonization and can thus reduce the overuse of antibiotics in NICU. Moreover, it is a simple, inexpensive and non-invasive technique and suited to the set up where cost becomes a major limiting factor.

In our study the mean duration of ventilation before the onset of pneumonia was 5 days. Late onset VAP (> 5 days of MV) was more common than early onset VAP (< 5 days of MV) in the ratio of 53.3 % vs. 46.7 % which was statistically not significant. Very low birth weight (< 1500 grams), prematurity (gestational age < 37 week), duration of mechanical ventilation, number of reintubation and length of NICU stay were significantly associated with VAP in bivariate analysis. Multiple regression analysis revealed that duration of mechanical ventilation (OR 1.10; 95% CI 1.02, 1.21; P = 0.021) and very low birth weight i.e. birth weight < 1500 grams (OR 3.88; 95% CI 1.05, 14.34; P = 0.042) were two single independent and statistically significant risk factors for predicting ventilator associated pneumonia. The study conducted by Yuan[6] et al also showed the same results, according to which the risk factors of neonatal VAP were reintubation (OR 5.3; 95% CI 2.0, 14.0), duration of mechanical ventilation (OR 4.8; 95%CI 2.2, 10.4), treatment with opiates (OR 3.8; 95%CI 1.8, 8.5) and endotracheal suctioning (OR 3.5; 95% CI 1.6, 7.4). Apisarnthanarak et al[4] also found similar results showing high rates of VAP in extremely preterm neonate (EGA < 28 weeks). The incidence rate of VAP were 6.5 per 1000 ventilator days for patients with EGA < 28 weeks and 4 per 1000 ventilator days for EGA ≥ 28 weeks. In their study, they have included those neonates who were having birth weight ≤ 2000 gm and have been given MV for more than 48 hours. In a study conducted by Petdachai et al[5], stepwise logistic regression analysis identified 3 factors independently associated with VAP: umbilical catheterization (adjusted odds ratio (AOR) 2.5; 95% CI=1.3 to 4.7; P = 0.007), respiratory distress syndrome (AOR 2.0; 95% CI 1.0 to 3.9; P = 0.03) and insertion of orogastric tube (AOR 3.0; 95% CI 1.3 to 7.2; P = 0.01). Infants with VAP had longer duration on ventilator (14.2 days vs 5.9 days; p < 0.001) and longer hospital stay (28.2 days vs 13.8 days; P < 0.001) .

As far as etiological organisms are concerned, gram negative predominance was observed as a causative factor of VAP in our study. Earlier neonatal studies[4,5,6] reported similar finding. In the present study, most common causative organism was *Klebsiella spp.* (32.87%) followed by *E. coli* (23.28%) and *Acinetobacter* (17.80%. If we segregate organisms causing early and late onset VAP, we found that early onset VAP was caused by *Klebsiella spp.* (32.87%) followed by E. coli (23.28%) and *Acinetobacter* (17.8%) whereas late onset VAP was caused by equal number of *Klebsiella* and *Acinetobacter* (33.33%) each. Half of the patients (8) who developed VAP after 5 days of mechanical ventilation were caused by *Acinetobacter* species. Out of these 8 patients, 6 expired and 2 had very long NICU stay. This finding is comparable with the result observed by some authors[15] that community acquired organisms such as S*treptococcus pneumoniae, H. influenzae* and methicillin sensitive *staphylococcus aureus* (*MSSA)* were frequent causes of early onset VAP (developing within 5 days of hospitalization) and the resistant *Enterobacter, Pseudomonas* and *Acinetobacter* species

encountered in late onset VAP (developed after 5 days of hospitalization).

The isolated Klebsiella spp. was found to be most sensitive to cefoperazone and Sulbactam in combination (87.50%) and Amikacin (66.6%), and resistant to Ampicillin, Amoxycillin, Gentamicin, and Cefotaxime (95.83% each). This is because of the referred patients admitted to our NICU already received intravenous antibiotics for variable periods. In the present study only 5.4% of cultures were polymicrobial. The study of Petdachai et al[5] showed 12.9% cultures of polymicrobial in nature. In our study none of the VAP patients developed pneumonia caused by the same organism as their blood stream infection (BSI). The same findings were shown in the study conducted by Apisarnatharak et al[4]. Therefore, it is unlikely that VAP occurred as a direct consequence of BSI. A randomized controlled trial which was done to test the hypothesis that intubated infants positioned on their sides would be less likely to contract bacterial colonization in their tracheae, compared with those positioned supine, showed that compared with the lateral group, more infants in the supine group experienced increased colony counts or had new organisms in their tracheal aspirates over time (21 vs 8 infants). The most common organisms isolated from tracheal aspirates in both groups were Gram-negative rods[16]. A study was done to compare the airway pressure (NCPAP) treatment (1.8/1000 NCPAP days) rates of nosocomial pneumonia with nasal CPAP verses VAP among VLBW neonates and it showed that the ventilator associated pneumonia (VAP) rate (12.5/1000 ventilator days) seemed significantly higher than the pneumonia rate during NCPAP ($p = 0.04$)[17].

Limitation of study

There are several limitations in our study. The NNIS definition of VAP lacks specificity; also there is no current gold standard for defining or diagnosis of VAP. Cordero L et al[18,19] in their retrospective studies concluded that surveillance diagnosis of VAP in VLBW infants is difficult because current CDC definition are not specific for this population. To improve accuracy, surveillance diagnosis of VAP in special populations such as VLBW infants should be reformulated; meanwhile, infection control practitioners should seek consultation with experienced clinicians for interpretation of data[18]. Because tracheal aspirate was used as a means to identify organisms associated with VAP, we found some of our VAP patients (4 cases) had multiple organisms isolated and some were contaminated. Some of the VAP patients did not have purulent tracheal aspirate with no isolated organism, which suggests that some of these VAP episodes may represent colonization rather than true VAP. Nevertheless, this limitation was minimized because our NICU attending physician reviewed the radiographs of all the patients and radiological and clinical diagnosis was the primary diagnosis of VAP with microbiology as a secondary supportive part of it. Small sample size of this study limits the statistical power to detect other possible independent risk factors for VAP and for mortality of patients. Our data did not include the indication for reintubation which also may be a relevant risk factor for VAP. Because many of the NICU patients had concurrent nosocomial infections during their NICU stay, the attributable morbidity and mortality associated with VAP cannot be determined from our study.

CONCLUSION

The endotracheal aspirate of the patients on ventilator should be sent routinely for culture sensitivity and if the patient develops VAP, antibiotic should be changed as per report. By using aseptic precautions while handling ventilated patients and empirical antibiotics as per the endotracheal aspirate sensitivity pattern of our NICU in the patients who develop nosocomial pneumonia, we may be able to improve the outcome rate of the patients on mechanical ventilation. Additional studies are necessary to develop interventions to prevent neonatal VAP.

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