INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 18, Issue 1, 2026

Original Article

RISK FACTORS, MICROBIOLOGICAL SPECTRUM, ANTIBIOTIC SENSITIVITY AND OUTCOMES OF VENITILATOR ASSOCIATED PNEUMONIA IN NEONATES: A STUDY FROM A TERTIARY CARE HOSPITAL IN SOUTHERN ASSAM

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Received: 09 Oct 2025, Revised and Accepted: 28 Nov 2025

ABSTRACT

Objective: To determine the incidence, microbiological profile, antibiotic sensitivity, risk factors, and outcomes of VAP in neonates.

Methods: This prospective observational study, conducted for one year in a tertiary care NICU in Southern Assam, included 101 neonates ventilated for over 48 h. VAP was defined using CDC criteria, integrating clinical, Laboratory, radiological, and microbiological findings. Data on patient characteristics, VAP occurrence, microbiology, and antibiotic susceptibility were collected and analysed.

Results: Thirty-seven (36.7%) neonates developed VAP, resulting in an incidence of 41.11 per 1,000 ventilator days. Key risk factors significantly associated with VAP included prematurity, low birth weight, bronchopulmonary dysplasia, and asphyxia. Common clinical signs were new onset tachypnoea and increased respiratory secretions. VAP led to significantly longer hospital stays (38.1±17.82 vs. 21.4±14.3 d, p=0.0001) and mechanical ventilation durations (13.42±8.74 vs. 5.89±3.56 d, p=0.0001), alongside increased antibiotic use. Gram-negative organisms predominated (68.9% of 45 isolates), notably multi-drug resistant (MDR) Acinetobacter spp. and ESBL-producing *Klebsiella pneumoniae*/*oxytoca*. *Pseudomonas* isolates showed high resistance to Amikacin, Meropenem, and Piperacillin+Tazobactam. MRSA exhibited good Linezolid susceptibility. VAP is associated with increased morbidity and mortality.

Conclusion: Neonatal VAP imposes a substantial burden, driven by prematurity, severe underlying conditions, and a high prevalence of MDR Gram-negative pathogens. Our findings highlight the urgent need for robust infection control, vigilant surveillance, and culture-guided antibiotic therapy to optimize outcomes and combat antimicrobial resistance. Larger, multi-center studies are essential.

Keywords: Neonatal VAP, Antimicrobial resistance, Multi-drug resistant (MDR), NICU, Gram-negative bacteria

DOI: https://dx.doi.org/10.22159/ijcpr.2026v18i1.8008 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Ventilator-associated pneumonia (VAP) is a serious, frequent hospital-acquired infection in Neonatal Intensive Care Units (NICUs), notably impacting neonates on prolonged mechanical ventilation. Defined as pneumonia developing after 48 h of intubation, VAP increases morbidity, mortality, hospital stays, and healthcare costs [1, 2]. Neonates, especially preterm or low birth weight infants, are highly susceptible due to immature immune systems, fragile lungs, and invasive procedures [3, 4].

Despite VAP research in pediatrics, specific studies on neonates, particularly low birth weight infants, are limited [5]. Key risk factors include prolonged ventilation, frequent suctioning, reintubation, prematurity, low birth weight, and prior broad-spectrum antibiotic use [5, 6]. The microbiological landscape often features Gram-negative bacilli (e. g., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*) and Gram-positive cocci (e. g., *Staphylococcus aureus*) [7, 8]. Multidrug-resistant (MDR) organisms are increasingly problematic, emphasizing the need for antibiotic stewardship and targeted therapy [9].

Diagnosing neonatal VAP is complex due to non-specific clinical signs and lack of a gold standard. Criteria typically include new respiratory symptoms, radiological infiltrates, and microbiological confirmation from tracheal aspirates [10, 11]. Guidelines like the CDC's aim to standardize definitions for better accuracy and surveillance [12].

Outcomes of neonatal VAP vary from prolonged respiratory support to increased mortality, especially in extremely low birth weight and critically ill infants [13, 14]. Given its substantial clinical and economic burden, understanding risk factors, accurate diagnosis, and local microbial resistance patterns is vital for optimizing NICU protocols.

MATERIALS AND METHODS

Study design

This was a prospective, observational study aimed at determining the incidence, microbiological profile, antibiotic sensitivity, risk factors, and outcomes of VAP in neonates admitted to the NICU. The prospective design enabled real-time data collection and close monitoring of clinical, microbiological, and treatment variables. Standardized diagnostic criteria for VAP were applied.

Study setting

The study was conducted in the Neonatal Intensive Care Unit (NICU) of Silchar Medical College and Hospital, Assam, a tertiary care referral centre serving the southern Assam and Neighbouring Northeastern States. The hospital's high patient volume, well-equipped NICU, and comprehensive laboratory facilities provided a representative neonatal population and reliable microbiological diagnostics essential for this research.

Study duration

Study was conducted for 1 y (May 2024 to June 2025).

Study population

We included all neonates in the NICU admitted in our unit and who needed over 48 h of mechanical ventilation via an endotracheal tube. However, we excluded those born with major congenital malformations, those with an APGAR score below 4 at five minutes of birth, and any whose guardians didn't provide informed written consent.

VAP definition and identification

Neonatal VAP is defined as pneumonia developing ≥48 h after starting endotracheal mechanical ventilation. Diagnosis, as per CDC guidelines [10], relies on a combination of criteria: clinical symptoms (e. g., worsening respiratory status, temperature instability, altered tracheal secretions, apnea/bradycardia), laboratory findings (e. g., elevated CRP, abnormal white blood cell count, thrombocytopenia), and radiological evidence (new/progressive chest X-ray infiltrates). Isolation of pathogens from respiratory samples (tracheal aspirates, BAL, PSB), especially with purulent secretions, provides supportive microbiological confirmation.

Sample size and collection

Neonates born on the unit's emergency service days at Silchar Medical College, who met the inclusion criteria, were enrolled in the study after obtaining parental consent, irrespective of their delivery method. Total of 101 babies were included in the study during the study period. Consecutive sampling technique was chosen to minimize selection bias and to ensure that the sample accurately represented the overall population of neonates in the institution.

Data collection and statistical analysis

Data were collected via a structured proforma detailing patient history (gender, gestational age, birth weight, delivery mode, APGAR scores), ventilation specifics (duration, intubation), and VAP occurrence. Microbiological analysis involved tracheal aspirates and blood cultures. SPSS software was used for analysis: continuous variables were presented as means±SD, categorical as frequencies/percentages. Groups were compared using chi-square/Fisher's exact tests for categorical data and t-tests/Mann-Whitney U tests for continuous data, with p<0.05 considered significant.

Ethical clearance

Upon receiving approval and clearance from the Institutional Ethics Committee (vide no. SMC/ETHICS/MI/2024/34), the research was started.

RESULTS

Out of 101 neonates requiring mechanical ventilation for over 48 h, 37 (36.7%) developed VAP, an incidence rate of 41.11 per 1,000 ventilator days. Although males outnumbered females (62 vs. 39), females had a slightly higher VAP incidence (41% vs. 33.8%). The 64 neonates who didn't develop VAP served as the control group (table 1).

Lower gestational age significantly correlated with higher VAP and mortality rates (p=0.028). Neonates born at ≤28 w gestational age had the highest VAP incidence (41.7%) and mortality (58.3%), contrasting with those>37 w (35% VAP, 25% mortality). Intermediate gestational age groups (34-37 w and 28-34 w) showed a progressive increase in VAP and mortality as gestational age decreased.

Lower birth weight was significantly associated with higher VAP and mortality (p=0.035). Neonates weighing less than 1000g showed the highest mortality rate (37.5%), despite varying VAP percentages across weight groups. The VAP group, overall, had a lower mean birth weight (1851grams).

Several clinical conditions and interventions were significantly more prevalent in the VAP group. Bronchopulmonary dysplasia (BPD) affected 37.83% of VAP patients versus 15.62% of non-VAP (p=0.012). Asphyxia was also notably higher in VAP neonates (62.16% vs. 28.12%, p=0.0008). Additionally, a higher percentage of VAP patients received surfactant therapy (45.94% vs. 26.56%, p=0.012). Mode of delivery, however, showed no significant difference (p=0.321).

Neonates with VAP experienced significantly longer hospital stays (38.1±17.82 d vs. 21.4±14.3 d; p=0.0001) and prolonged mechanical ventilation (13.42±8.74 d vs. 5.89±3.56 d; p=0.0001). The VAP group also had a higher reintubation rate (29.7% vs. 12.5%), though this difference was not statistically significant (p=0.33).

Antibiotic utilization was significantly higher in the VAP group. Neonates with VAP received antibiotics for a much longer duration (34.34±11.73 d vs. 18.26±11.22 d; p<0.000001) and were administered a significantly greater number of different antibiotics (5.43±1.34 vs. 3.13±1.22; p<0.000001). Despite this increased antibiotic exposure and overall morbidity in the VAP group, mortality rates did not differ significantly between the VAP (37.8%) and non-VAP (39.1%) cohorts (p=0.90).

Table 1: Baseline and clinical characteristics of VAP vs non-VAP neonates

Variables	VAP(n=37)	Non VAP(n=64)	p-Value
Gender			
Male	21 (56.75%)	41 (64.06%)	0.46
Female	16 (43.24%)	23 (35.93%)	
Gestational Age			
>37 w	14 (37.8%)	26 (40.6%)	0.028
37-34 w	12 (32.4%)	21 (32.8%)	
34-28 w	6 (16.4%)	10 (15.6%)	
<28 w	5 (13.6%)	7 (11%)	
Birth weight			
>2500g	10(27.1%)	21(32.8%)	0.035
1500-2500 g	11 (29.7%)	17 (26.5%)	
1000-1500g	9 (24.3%)	17 (26.5%)	
<1000 g	7(18.9%)	9 (14.1%)	
Mode of delivery			
Caesarean section	20 (54.05%)	28 (43.75%)	0.321
Normal Vaginal delivery	17 (45.95%)	36 (56.25%)	
APGAR score			
At 1 min	4.9±2.8	5.1±2.7	0.73
At 5 min	7.2±2.6	7.9±3.1	0.23
Other parameters			
BPD	14 (37.83%)	10 (15.62%)	0.012
Asphyxia	23 (62.16%)	18 (28.12%)	0.0008
Received Surfactant	19 (45.94%)	17 (26.56%)	0.012
Length of Hospital stay (days)	38.1±17.82	21.4±14.3	0.0001
Mechanical Ventilation (days)	13.42±8.74	5.89±3.56	0.0001
Antibiotics Treatment (days)	34.34±11.73	18.26±11.22	0.000001
No of Antibiotics used	5.43 ±1.34	3.13 ±1.22	0.000001
Reintubation (n)	11 (29.72%)	8 (12.5%)	0.33
Deaths	14 (37.8%)	25 (39.1%)	0.90

Among 37 VAP cases, new onset tachypnea (45.95%) and increased respiratory secretions (40.54%) were the most common clinical signs, followed by tachycardia (32.43%) and new onset apnea (24.32%), Both increased temperature and color changes in respiratory secretions (R. S) were observed in 16.22% of cases. Lab results frequently showed elevated C-reactive protein (70.27%), an I/T ratio>0.2 (62.16%), and thrombocytopenia (48.65%). Radiologically, new patches on X-rays (56.76%) were typical, with progressive patches in 29.73%. Microbiologically, tracheal aspirate cultures were positive in 75.68%, and blood cultures (CS) in 56.76% of VAP patients (fig. 1).

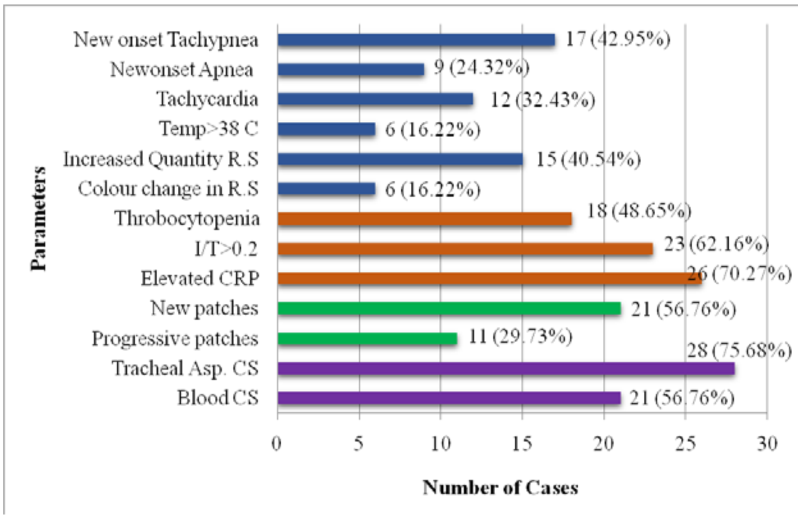

Fig. 1: Clinical, laboratory and radiological findings in VAP cases

From 37 VAP cases, 45 bacterial isolates were recovered, primarily from tracheal aspirates (24 isolates) and blood cultures (21 isolates). Gram-negative organisms predominated (68.9%), with *Acinetobacter spp.* (n=9), *Klebsiella pneumoniae* (n=9), and *Klebsiella oxytoca* (n=8) being the most common. Less frequent Gram-negative isolates included *E. coli* (n=2) and *Pseudomonas* (n=3). Gram-positive organisms made up 31.1% of isolates, mainly Staphylococcus species MRSA (Methicillin-Resistant *Staphylococcus aureus*) (n=6); and MSSA (Methicillin-Sensitive *Staphylococcus aureus*) (n=5) and *Enterococcus spp.* (n=3). This overall profile indicates a substantial burden of Gram-negative pathogens, particularly *Acinetobacter* and *Klebsiellasp.* in VAP among this neonatal cohort (fig. 2).

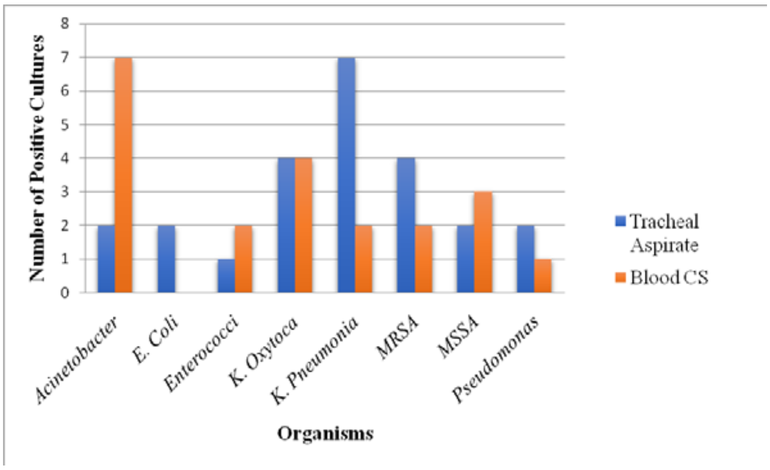

Fig. 2: Distribution of bacterial isolates from tracheal aspirate and blood cultures in neonates with VAP

Analysis of antibiotic susceptibility patterns for the 45 bacterial isolates revealed varied effectiveness. Among Gram-negative pathogens, *Acinetobacter spp.* (n=9) showed significant multi-drug resistance, with less than 50% susceptibility to Amikacin (44.4%), Amoxicillin+Clavulanic Acid (22.2%), Ceftriaxone (44.4%), Ciprofloxacin (33.3%), and Piperacillin+Tazobactam (44.4%). Resistance to Meropenem was lower, with 6 out of 9 isolates being susceptible.

K. pneumoniae (n=9) exhibited concerning resistance to third-generation cephalosporins, with 66.7% resistant to both Cefixime and Ceftriaxone, highly suggestive of ESBL production. It maintained better susceptibility to Meropenem (66.7% S) and Amikacin (66.7% S). *K. oxytoca* (n=8) displayed similar patterns to *K. pneumoniae*, with notable resistance to Cefixime (37.5% R) and Ceftriaxone (25% R, 12.5% I), but 62.5% susceptibility to Meropenem and 75% to Piperacillin+Tazobactam.

In contrast, the few *E. coli* (n=2) and *Pseudomonas* (n=3) isolates showed mixed susceptibility. For *E. coli*, 100% were susceptible to Meropenem, Piperacillin+Tazobactam, and Doxycycline, though one isolate was resistant to Ceftriaxone and Cotrimoxazole. For *Pseudomonas*, all isolates were resistant to Amikacin, and two out of three were resistant to Meropenem and Piperacillin+Tazobactam.

Regarding Gram-positive organisms, MRSA (n=6) showed good susceptibility to Linezolid (83.3%) but significant resistance to Amoxicillin+Clavulanic Acid (66.7% R), Azithromycin (66.7% R), Cefixime (50% R), and Ceftriaxone (83.3% R). MSSA (n=5) was highly susceptible to Linezolid, Ciprofloxacin, and Doxycycline (all 100% S), and showed good susceptibility to Amoxicillin+Clavulanic Acid, Cefixime, Ceftriaxone, and Levofloxacin (all 80% S). *Enterococcus spp*. (n=3) were 100% susceptible to Linezolid and Doxycycline. However, one out of three tested isolates was resistant to Amoxicillin+Clavulanic Acid and Ciprofloxacin, while no data was provided for Amikacin (table 2).

Table 2: Antibiotic susceptibility patterns of bacterial isolates from neonatal VAP cases

Antibiotics (µg)	**Organisms**							
	***Acinetobacter* (n = 9)**	***E. coli* (n = 2)**	***K. pneumoniae* (n =9)**	***K. oxytoca* (n = 8)**	**MRSA (n =6)**	**MSSA (n =5)**	***Pseudomonas* (n = 3)**	***Enterococcus* (n =3)**
	SIR	**SIR**	**SIR**	**SIR**	**SIR**	**SIR**	**SIR**	**SIR**
Amikacin (30)	4 2 2	2--	6-3	4 2 2	4-2	3-2	--3	-
Amoxicillin+Clavulanic Acid (20/10)	2-4	--1	2-2	2--	2-4	4-1	-	- 1
Azithromycin(15)	-	-	-	-	1-4	1-4	-	-
Cefixime(5)	-	2--	3-6	1-3	1-3	4--	2-1	-
Ceftriaxone(30)	4-3	1-1	3-6	1 1 2	1-5	4--	-	-
Ciprofloxacin(5)	3 2 2	2--	4 1 2	3-1	2 1 3	5--	2--	2-1
Cotrimoxazole (1.25/23.75)	5 1 1	1-1	5 3-	2-2	3 1 2	3-2	- 1	-
Doxycycline(30)	4-1	2--	6--	4-2	4-2	5--	1-2	3--
Linezolid(30)	-	-	1--	-	5--	5--	1-2	3--
Levofloxacin(5)	4-1	2--	4 3 1	4-2	4-2	4-1	1-2	-
Meropenem(10)	6-1	2--	6 1 1	5 2 1	3--	2--	1-2	-
Nitrofurantoin(300)	-	1--	3--	2--	1--	3--	-	-
Piperacillin+Tazobactam(100/10)	4--	2--	4-4	6--	1--	2--	1--	-

S-Susceptible; I-Intermediate; R – Resistant

DISCUSSION

The VAP incidence in this study was 41.11 per 1,000 ventilator days, it aligns with rates seen in developing countries (16.1 to 89 per 1,000 ventilator days), as reported by Mohamed *et al.* [2024] and Al-Kadri *et al.* [2024] [15]. Specifically, our findings are very similar to rates of 36.9 per 1,000 ventilator days in India (Mir *et al.,* 2017) [16] and 35.06 in Bulgaria (Georgieva *et al.*, 2022) [17], suggesting comparability with studies from similar resource settings.

This study highlights several critical risk factors for VAP in neonates, consistent with findings in other cohorts. Lower gestational age (p=0.028) and lower birth weight (p=0.035) were significantly linked to a higher incidence of VAP. This finding is consistent with a study by Singh *et al.* [2018] [20], which also observed that prematurity and low birth weight are significant risk factors for VAP due to immature immune systems and underdeveloped lungs in neonates. Similarly, Costa *et al.* [2020] [19] highlighted in their meta-analysis the strong correlation between lower gestational age and increased VAP risk.

Our study found bronchopulmonary dysplasia (BPD) (p=0.012) and a history of asphyxia (p=0.0008) were significantly more prevalent in neonates who developed VAP. These findings align with Singh *et al.* [2015] [21] and Van Der Pol *et al.* [2017] [22], who suggest that conditions prolonging ventilation compromise lung defenses, increasing VAP risk. Neonates with VAP also received surfactant therapy significantly more often (p=0.012), likely reflecting severe underlying respiratory distress, where invasive ventilation itself elevates VAP risk, as observed by Modi *et al.* [2017] [23].

Neonates who developed VAP had significantly longer hospital stays (averaging 38.1±17.82 d compared to 21.4±14.3 d for those without VAP; p=0.0001). They also required mechanical ventilation for a considerably longer duration (13.42±8.74 d versus 5.89±3.56 d; p=0001). These observations are consistent with research by El-Masri *et al.* [2020] [24], which similarly found that VAP leads to extended hospitalization and increased healthcare expenses.

Additionally, antibiotic use was much greater in the VAP group. These neonates received antibiotic therapy for significantly longer periods (34.34±11.73 d compared to 18.26±11.22 d in the non-VAP group; p=0.000001) and were given a higher average number of different antibiotics (5.43±1.34 versus 3.13±1.22; p=0.000001). While increased antibiotic use is expected for VAP, this trend, as noted by Kumar *et al.* [2016] [25], raises concerns about the potential for antibiotic resistance and adverse effects in this vulnerable patient population.

In this study, new onset tachypnea (45.95%) and increased respiratory secretions (40.54%) were the most common clinical VAP symptoms, consistent with findings from Georgieva *et al.* [2022] [17] and Al-Kadri *et al.* [2025] [15]. Laboratory tests frequently showed elevated C-reactive protein (70.27%) and an I/T ratio>0.2 (62.16%), indicating systemic inflammation, as supported by Al-Kadri *et al.* [2025] [15]. While challenging to interpret in neonates, chest X-ray changes like new infiltrates were crucial for diagnosis, a point highlighted by Georgieva *et al.* [2022] [17] and Li *et al.* [2022] [26].

Tracheal aspirate cultures had a high yield (75.68%), underscoring their importance for pathogen identification and targeted therapy (Tuteja *et al.*, 2022 [18]; Dey *et al.*, 2021) [27]. The significant rate of positive blood cultures (56.76%) suggests associated bacteremia, emphasizing the need for both local and systemic samples to guide antimicrobial management (Tuteja *et al.*, 2022) [18].

Analysis of antibiotic susceptibility revealed concerning Gram-negative resistance. *Acinetobacter spp.* showed broad multi-drug resistance to Amikacin, Amoxicillin+Clavulanic Acid, Ceftriaxone, Ciprofloxacin, and Piperacillin+Tazobactam, with Meropenem being relatively more effective. This high resistance in Acinetobacter is a recognized challenge in neonatal infections, limiting options and demanding potent empirical therapies, as Al-Kadri *et al.* [2025] [15] also reinforce. Similarly, *K. pneumoniae* and *K. oxytoca* exhibited substantial resistance to Cefixime and Ceftriaxone, strongly suggesting high ESBL production. Their good susceptibility to Meropenem highlights an increasing reliance on carbapenems for ESBL-producing strains, a trend noted by Mir *et al.* [2017] [16] in other NICU settings. Our *Pseudomonas* isolates showed alarming resistance, with 100% resistant to Amikacin and 66.7% resistant to Meropenem and Piperacillin+Tazobactam. This high-level resistance, common in *Pseudomonas aeruginosa*, complicates treatment and necessitates rapid susceptibility testing for effective neonatal VAP management [28, 29].

Among Gram-positive isolates, MRSA (n=6) exhibited good susceptibility to Linezolid, indicating its continued effectiveness against this resistant pathogen, consistent with general literature on Linezolid's efficacy. MSSA (n=5) showed broad susceptibility to most tested antibiotics, reflecting its generally less resistant nature. *Enterococcus spp.* (n=3) were entirely susceptible to Linezolid and Doxycycline but notably resistant to Amoxicillin+Clavulanic Acid, Ciprofloxacin, and Amikacin. The presence of MRSA, even in smaller numbers, underscores the ongoing need for vigilance and infection control, as discussed by Shah *et al.* [2023] [30]. The diverse susceptibility patterns observed across both Gram-negative and

Gram-positive pathogens, particularly the multidrug resistance in *Acinetobacter* and ESBL-producing *Klebsiella*, strongly emphasizes the critical importance of culture-guided antibiotic therapy in neonatal VAP management. This approach optimizes patient outcomes and mitigates further antimicrobial resistance development, a strategy supported by Smith *et al.* [2024] [29].

A recent study by Biswas N *et al.* [2025] [31] on pediatric UTIs demonstrated a similar pattern of MDR, with *Escherichia coli*, *Klebsiella spp.*, and MRSA emerging as predominant pathogens. The high resistance to Cephalosporins and Fluoroquinolones in both infections highlights the urgent need for continuous surveillance and antibiotic stewardship to guide effective empirical therapy.

LIMITATIONS

The relatively small sample size and its single-center origin mean the results may not be broadly applicable to all neonatal populations or different healthcare settings. Diagnosing VAP in neonates is inherently challenging due to the subjective nature of clinical, laboratory, and radiological criteria in this vulnerable group. Furthermore, our analysis of antibiotic susceptibility was confined to a specific set of agents, and we didn't explore broader resistance patterns or the underlying genetic mechanisms of resistance. Despite higher morbidity and antibiotic use in the VAP group, we found no significant difference in mortality rates compared to non-VAP neonates. However, without detailing the specific causes of death in either group, it's difficult to fully interpret this finding.

CONCLUSION

This study found neonatal VAP was linked to prematurity, low birth weight, bronchopulmonary dysplasia, and asphyxia. VAP led to longer hospital stays and ventilation durations, plus higher antibiotic use. We identified predominantly Gram-negative bacteria, like *Acinetobacter* and *Klebsiella*, with significant drug resistance. Despite the increased morbidity and mortality in this small, single-center study. Further research with larger cohorts is needed to confirm these findings and guide VAP management.

ACKNOWLEDGMENT

The authors expressed their appreciation to the Silchar Medical College and Hospital's Department of Microbiology for their significant contribution.

FUNDING

This study was entirely self-funded.

AUTHORS CONTRIBUTIONS

Dr. Veeranna Shivanand Chikkamath and Dr. M. Swathi Patil were involved in the conception and design of the study, patient recruitment, data collection, analysis, interpretation of results, and drafting of the manuscript. Dr. Nilanjan Biswas assisted with statistical support and contributed to the results section. Dr. Pranab Das performed literature review and provided inputs during revision of the discussion. Dr. Sujit Nath Choudhury offered overall supervision, critically reviewed the manuscript for important intellectual content. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

CONFLICT OF INTERESTS

There is no conflict of interests

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