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Original Research Article

Prevalence of multidrug-resistant gram-negative bacilli (MDR-GNB) in hospital-acquired and ventilator-associated pneumonia: A comparative study from a tertiary care center, Gujarat

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ABSTRACT

Background: Antibiotic resistance poses a formidable challenge to global healthcare, with Gram-negative bacteria emerging as a primary concern. Multidrug-resistant Gram-negative bacilli (MDR-GNB) have become a significant cause of nosocomial infections, particularly pneumonia, complicate therapy, and have a detrimental impact on patients' outcomes.

Aim and Objectives: This study aims to investigate the etiology, risk factors, and antibiotic resistance patterns associated with Gram-negative bacilli (GNB) isolated from nosocomial pneumonia cases.

Materials and Methods: This prospective cross-sectional study was conducted at the Microbiology laboratory of a tertiary care Hospital in Gujarat. Patients hospitalized for >48 hours with new lung infiltrates and at least two of the following clinical features: fever, leukocytosis/leukopenia, purulent secretions, or decreased oxygenation were included. The study was initiated after the ethical approval. Patient demographic and clinical details were noted in the preformed questionnaire. A total of 64 specimens [Sputum (n= 28) and Endotracheal aspirate (ET, n=36)] were cultured on MacConkey's agar and Blood agar and further species identification with Antimicrobial Susceptibility Pattern was done by automated Vitek-2 compact system.

Results: Ventilator-associated Pneumonia (VAP) was found in 14.6% of infected patients, with male predominance and common in the 30-50 years age group. Out of them, 72% were mainly associated with late-onset. Overall, the major isolates were *Pseudomonas aeruginosa* (20/64, 31%), followed by *Acinetobacter baumannii* (19/64, 29.6%) and *Klebsiella pneumoniae* (17/64, 26.5%) both as solitary and mixed infections.76% strains of *Klebsiella* and 85% of *E. coli* strains were resistant to carbapenems and 93.3% of *Acinetobacter baumannii* were resistant to cephalosporins and carbapenems. *Enterobacter cloaca* strains were 100% resistant to carbapenems.

Conclusion: The study recommends effective Infection control practices and strong antibiotic stewardship programs to reduce the morbidity and mortality of nosocomial pneumonia.

1. Introduction

Nosocomial pneumonia remains a significant healthcare challenge, with hospital-acquired pneumonia (HAP) and

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ventilator-associated pneumonia (VAP) representing major subsets.[1] Hospital-acquired pneumonia (HAP)[1,2] is defined as an infection of the pulmonary parenchyma in patients who develop the condition at least 48 hours after admission to the hospital, or within 14 days after discharge. HAP is mainly characterized by the presence of "new lung infiltrate on chest imaging plus clinical evidence that the infiltrate is of an infectious origin, and new-onset fever, purulent sputum, leukocytosis, also decline in oxygenation".[1,2] While Ventilator-associated pneumonia (VAP)[1,2] is defined as an infection of pulmonary parenchyma occurring at least 48 hours after endotracheal intubation, and includes the above clinical scenario of HAP. The pathogenesis, risk factors, diagnostic tools, and treatment options differ in both HAP and VAP. The incidence of ventilator-associated pneumonia is substantial, with rates falling between 13 and 51 cases per 1000 ventilator days.[3,4] Male gender, pre-existing medical conditions, and past injuries are known to contribute to the development of VAP.[3,5] Approximately 50% of antibiotics used in ICUs are prescribed to treat VAP.[3] VAP is further divided into Early onset if it occurs within four days of mechanical ventilation and late onset if it occurs after four days of mechanical ventilation.[3] Despite being generally less severe than VAP, HAP can still result in serious complications like empyema, septic shock, and multiorgan failure in about 50% of cases, especially among ICU patients. VAP was previously diagnosed solely based on clinical symptoms, often leading to inaccurate results. The Clinical Pulmonary Infection Score (CPIS) was developed to improve diagnosis.[6] Despite advancements in critical care, the emergence of multidrug-resistant Gram-negative bacteria (MDR-GNB) as causative pathogens has exacerbated the problem, leading to increased morbidity, mortality, and healthcare costs. Antibiotic resistance poses a formidable challenge to global healthcare, with Gram-negative bacteria emerging as a primary concern. Methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, along with extended-spectrum β-lactamase-producing *Enterobacteriaceae* spp., MDR or extensively drug-resistant (XDR)-*Acinetobacter baumannii* complex spp., and *Stenotrophomonas maltophilia*, are the predominant pathogens implicated in both HAP and VAP.[3,7] Along with them, Carbapenem-resistant *Enterobacteriaceae* remarkably emerged as an important concern.[3,7] Despite the substantial burden of both HAP and VAP, comparative studies exploring the differences in epidemiology, risk factors, and outcomes between these entities are limited. This study seeks to investigate the prevalence, causative pathogens, risk factors, and clinical outcomes of multidrug-resistant Gram-negative bacteria (MDR-GNB) in patients with hospital-acquired pneumonia (HAP) and Ventilator-associated Pneumonia (VAP) at a tertiary care center, Gujarat. Understanding these differences will inform targeted infection prevention and control strategies, ultimately improving patient outcomes.

2. Materials and Methods

2.1. Study design

A prospective cross-sectional study was conducted from January 2023 to June 2023 at the Microbiology laboratory of a tertiary care hospital in Gujarat.

2.2. Sample size

64 culture-positive endotracheal aspirate (n=36 and sputum (n=28 samples were used in the study.

2.3. Selection criteria

Patients of age group 10-80 years, with or without mechanical ventilation, hospitalized for >48 hours with new or progressive lung infiltrates and at least two of the following clinical features: fever, leukocytosis/leukopenia, purulent secretions, or decreased oxygenation were included (Based on IDSA guidelines).[8] Those patients with pre-existing pneumonia, radiological infiltrates due to other causes like pulmonary hemorrhage, edema, lung collapse, tumors, etc., or those who declined participation were excluded.

2.4. Data collection

After filling out the consent form, Demographic details (age/gender/BMI/smoking habit /alcohol consumption/reason for admission/ward/ICU, etc.) and comorbidities (Bronchial asthma/ Bronchiectasis / COPD/ Diabetes/ Hypertension/ Malignancy/ Chronic kidney disease/Autoimmune disease, etc.) were noted in a questionnaire. For the cases of VAP, additional information like the number of mechanical ventilation days, oropharyngeal care, peptic ulcer prophylaxis, use of antibiotics, the position of the patient, etc. was noted.

2.5. Microbiological analysis

1. Sample collection: First-morning sputum specimens were collected aseptically in a clean, sterile wide-mouth container for suspected cases of HAP, and endotracheal aspirate samples were aseptically collected by the trained nurse or physician from suspected VAP patients
2. Processing: Samples were immediately transported to the laboratory and were cultured on routine media like Blood agar, Nutrient agar, and MacConkey agar. Significant colony count was noted for ET aspirates ($\geq 10^5$ CFU/ml). Organisms were identified as Gram-negative bacilli by Gram staining and further identification of the isolate and antibiotic sensitivity testing was done by Automated Vitek-2 compact

system (BioMerieux India).

3. Data quality control: Quality check was done using the reference strains by American Type Culture Collection (ATCC) including *E. coli* (ATCC® 25922), *K. pneumoniae* (ATCC® 700603), *S. aureus* (ATCC® 25923) and *P. aeruginosa* (ATCC® 27853)..

2.6. Statistical methods used

Statistical analysis was completed by using Microsoft Excel 2019. The data was entered sequentially and the univariate analysis was presented as frequencies and percentages. VAP rates were calculated using the formula given in CDC-NHSN guidelines.[2]

1. VAP rates were calculated using the formula given in CDC-NHSN[2]

$$\frac{Number\ of\ VAP}{Number\ of\ ventilator\ days} \times 1000$$

3. Results

3.1. Demographic details of the patients enrolled in the study (Table 1)

Overall forty-six male (n=46,71.8%) and eighteen female (n=18, 28.2%) patients were included in the study. Most of the participants (34%) were over 60 years old, followed by 26.5% between 30 and 60 years old, and less than 12% under 20. Most patients (61%) were admitted to the Medical ICU, followed by the Surgical ICU (17.1%), Male Medicine Ward (12.5%), Paediatric ICU (4.7%), Female Medicine Ward (3%), and Respiratory Medicine (1.5%). Patients across wards and ICUs presented with diverse signs and symptoms. Among those on ventilators, 31% had lower respiratory tract infections with hypertension. Other common diagnoses included seizures with severe anaemia and diabetes (25%), diabetes with chronic kidney disease (11%), cerebrovascular accident (8%), alcoholic liver disease (5%), altered sensorium with glioblastoma (8%), COPD with hypertension (6%), and diffuse axonal injury (6%).

VAP rates were calculated using the prescribed formula (NHSN Guidelines).[2] The number of VAP cases was found in 36 patients with 2451 as the total number of mechanical ventilation days which; leads to the VAP rate of 14.6%. Out of that 72% of the VAPs were of late onset. VAP rates showed higher male predominance and were common in the 31-60 years age group (Table 1). Similarly, the incidence of Hospital-Acquired Pneumonia (HAP) was also higher in males. In patients with HAP, 70% had ICU stays exceeding seven days, while in VAP, 83% had stays longer than seven days before developing the symptoms. This suggests a direct correlation between length of stay and the risk of acquiring hospital-acquired infections. Among HAP patients, 39% had C-reactive protein (CRP) levels between 100 and 199 mg/L. and in VAP, 75% had (CRP) levels of more than 200 mg/L.

Table 2 summarizes the distribution of etiological agents in patients with hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). Overall, the Major isolates were *Pseudomonas aeruginosa* (20/64, 31%), followed by *Acinetobacter baumannii* (19/64, 29.6%) and *Klebsiella pneumoniae* (17/64, 26.5%) both as solitary and mixed infections.In HAP, *Pseudomonas aeruginosa* was the leading cause, followed by *Acinetobacter baumannii* and *Klebsiella pneumoniae*. In VAP, *Acinetobacter baumannii* was the predominant pathogen, with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* also frequently implicated. Notably, *Serratia marcescens* and *Stenotrophomonas maltophilia* were more prevalent in VAP, possibly reflecting the unique risk factors associated with mechanical ventilation. *Enterobacter cloacae* was the major isolate in the HAP cases in comparison with the VAP cases.The frequency of mixed infection was also higher in VAP cases (16.6%) as compared to HAP (10.7%), *Klebsiella pneumoniae* was the most common pathogen to cause mixed infections along with *Pseudomonas* species and *Acinetobacter baumannii*. In HAP, the most frequent amalgamations were *Pseudomonas aeruginosa and Klebsiella pneumoniae and Klebsiella pneumoniae with Acinetobacter baumannii.* While in VAP cases, the combination of *Klebsiella pneumoniae* was with *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa,* and *Pseudomonas fluorescence*. (Table 3).

3.2. Multidrug resistance (MDR) was prevalent among the fermenters and non-fermenters

1. The most effective antibiotics for *Klebsiella pneumoniae* were Ampicillin/Clavulanic acid and carbapenems like Ertapenem, Meropenem and Imipenem, Gentamicin, and Tigecycline. At the same time, 30% of *Escherichia coli* strains were sensitive to Cefoperazone/Sulbactam, Carbapenems, Minocycline, and Trimethoprim/Sulfamethoxazole and 58% were sensitive to Tigecycline. 60% of Enterobacter cloaca strains were sensitive to Ceftriaxone and Meropenems. (Table 4)
2. *Pseudomonas aeruginosa* demonstrated 90% resistance to Piperacillin-tazobactam, 80% resistance to Aztreonam, 70% resistance to Imipenem and Ciprofloxacin, 65% resistance to Ceftazidime, Cefoperazone/sulbactam, and Cefepime. Amikacin and Gentamycin were the effective drugs (45% sensitivity).
3. *Acinetobacter baumannii* exhibited 93.3% resistance to Ampicillin-clavulanic acid, Piperacillin-tazobactam, Cefuroxime, Ceftriaxone, Cefoperazone/sulbactam, Cefepime, and Ciprofloxacin, and 86.6% resistance to

Table 1: Comparison of Hospital-acquired Pneumonia (HAP) and Ventilator-associated Pneumonia (VAP) based on demographic features and hospital stay

Variables		Hospital-acquired Pneumonia n=28(%)	Ventilator-associated Pneumonia n=36(%)
Gender	Male(n=46)	18(64%)	28(78%)
	Female(n=18)	10(36%)	08(22%)
Age-Group	<30	5 (17.86%)	7 (19.44%)
	31-60	11 (39.29%)	13 (36.11%)
	>60	12 (42.86%)	6 (16.67%)
Socioeconomic status	Urban (n=20)	13(65%)	07(35%)
	Rural (n=44)	15(34%)	29 (66%)
Ward/ICU	MICU	17(60.7)	22(61.1)
	SICU	00	11(30.5)
	PICU	01(3.57)	02(5.5)
	MMW	07(25)	01(2.78)
	FMW	02(7.14)	-
	RM	01(3.57)	-
Length of hospital stay	<4 days	02(7.1)	01 (2.7)
	4 to 7 days	06(21.4)	05(13.8)
	> 7 days	20(71.4)	30(83.3)
C-Reactive Protein (CRP) level	<10 mg/L	03(10.7)	02(5.5)
	<50 mg/L	07(25)	04(11.1)
	<100 mg/L	05(17.86)	03(8.3)
	100-199 mg/L	11(39.29)	15(41.6)
	200-299 mg/L	02(7.14)	12(33.3)

Table 2: Evaluation of single etiological agents associated with HAP and VAP

Etiological agents	HAP (n=25/28, 89.2%)	VAP (n=30/36, 83.3%)	Total
Klebsiella pneumoniae	05(17.8%)	06(16.6%)	11+6 (mixed infections) =17
Escherichia coli	03(10.7%)	04(11.1%)	07
Enterobacter cloacae	04(14.2%)	01(2.7%)	05
Pseudomonas aeruginosa	07(25%)	08(22.2%)	15+5 (Mixed infections) =20
Acinetobacter baumannii	06(21.4%)	09(25%)	15+4 (mixed infections) = 19
Serratia marcescens	-	01(2.7%)	01
Stenotrophomonas maltophilia	-	01(2.7%)	02 (including mixed infections)

Table 3: Disparities of mixed infections in both HAP and VAP cases

Multiple etiological agents	HAP (3/28)-10.7%	VAP (6/36)- 16.6%
Pseudomonas aeruginosa + *Klebsiella pneumoniae*	01	-
Klebsiella pneumoniae + *Acinetobacter baumannii*	02	01
Klebsiella pneumoniae and *Stenotrophomonas maltophilia*	-	01
Pseudomonas aeruginosa and *Acinetobacter baumannii*	-	01
Pseudomonas florescence and *Klebsiella pneumoniae*	-	01
Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii	-	01
Pseudomonas aeruginosa+ *Klebsiella pneumoniae*+ *Acinetobacter baumannii*	-	01

Table 4: Antimicrobial resistance pattern of fermenter from VAP/HAP patients

Antimicrobial agents	*Klebsiella pneumoniae* n=17(%)	*Escherichia coli* n=7 (%)	*Enterobacter aerogenes* n=5 (%)
Ampicillin/Clavulanic Acid	13(76.4)	7(100)	4(80)
Piperacillin/Tazobactam	14(82.3)	6(85.7)	4(80)
Cefuroxime	17(100)	7(100)	4(80)
Cefuroxime Axetil	17(100)	7(100)	5(100)
Ceftriaxone	16(94.1)	7(100)	2(40)
Cefoperazone/Sulbactam	14(82.3)	5(71.4)	3(60)
Cefepime	15(88.2)	7(100)	4(80)
Ertapenem	13(76.4)	6(85.7)	5(100)
Imipenem	13(76.4)	5(71.4)	4(80)
Meropenem	13(76.4)	5(71.4)	2(40)
Amikacin	13(76.4)	5(71.4)	5(100)
Gentamicin	13(76.4)	6(85.7)	4(80)
Ciprofloxacin	15(88.2)	7(100)	5(100)
Tigecycline	12(70.5)	3(42.8)	4(80)
Trimethoprim/ Sulfamethoxazole	16(94.1)	5(71.4)	5(100)
Minocycline	16(94.1)	5(71.4)	5(100)
Cotrimoxazole	17(100)	-	4(80)

Imipenem and Meropenem.

4. Discussion

Device-associated healthcare-associated infections (DA-HAIs) in critical care units significantly increase patient morbidity and financial burden on healthcare facilities. Factors influencing DA-HAI incidence include ICU access, device usage frequency and duration, infection control measures, and patient immune status.[9] Laboratory surveillance, adhering to NHSN-CDC guidelines,[2] employed baseline and routine cultures to diagnose DA-HAIs, including ventilator-associated pneumonia (VAP), central line-associated bloodstream infections (CLABSI), and catheter-associated urinary tract infections (CAUTI).

Recent research has continued to delve into the prevention, diagnosis, and treatment of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). These conditions significantly contribute to inpatient morbidity and mortality, and their timely diagnosis in the intensive care unit is particularly challenging due to the myriad of other factors that can contribute to clinical deterioration in complex, critically ill patients. In this present study total of 64 clinically suspected patients were enrolled for the diagnosis of Nosocomial Pneumonia including both with and without ventilation, and were admitted for reasons other than Pneumonia. Overall, when we compare HAP and VAP, we found that in both cases we had male predominance. These findings align with the previous studies[3,10,11] that have identified a gender disparity in these infections (HAP-M: 64%, VAP-M: 72%). Age-group affected in HAP was mainly elderly, > 60 years (12/28, 42.8%), while in VAP the incidence was higher in the middle age group, 31-60 years (13/36, 36.1%). Similar reports have been shown by many studies[10,12] showing a higher incidence of HAP in the elderly group, possibly the reason could be the associated co-morbidities and declined immune status in the elderly. The VAP incidence in this study was approximately 14.6%, aligning with the 13.1 per 1000 mechanical ventilator (MV) days reported by the International Healthcare-associated Infection Control Consortium (INICC) for Southeast Asian countries during 2010-2015.[13] Variations in VAP incidence across ICUs may be attributed to differences in patient demographics, diagnostic methods, and standard management protocols.

The major causative agents in HAP were *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Klebsiella pneumoniae,* while in VAP cases the major organism was *Acinetobacter baumannii* followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E. coli* with additional *Serratia* and *Stenotrophomonas maltophilia.* The frequency of mixed infections was also higher in VAP cases. A study done by Mohd. Saif Khan et al.[14] 2015 in JIPMER, Puducherry, also shows *Pseudomonas aeruginosa* and Acinetobacter baumannii as the major pathogen in VAP. Duszynska et al.[15] 2020, from Poland, also found *Acinetobacter baumannii* and *Klebsiella pneumoniae* as a leading cause of VAP. Various factors attributed to *Acinetobacter baumannii* and *Pseudomonas aeruginosa* causing nosocomial infections include their biofilm-forming abilities, colonization in the healthcare environments, including sinks, drains, and medical equipment and their Multidrug resistance make them difficult to treat. The occurrence of specific pathogens causing VAP differs concerning several factors like hospitals, patient populations, geographic areas, duration of mechanical

ventilation, antibiotic dose, ventilator days, duration of ICU stay, and specific patient characteristics. The frequency of specific MDR pathogens causing VAP varies in hospitals, patient populations, prior use of antibiotics, and type of ICUs emphasizing the need for routine surveillance.

5. Conclusion

Our study emphasizes the continued importance of ventilator-associated pneumonia (VAP) as a healthcare-associated infection. Our findings highlight the increasing prevalence of non-fermenting Gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* as significant hospital-acquired pathogens. Accurate identification of these non-fermenters is essential, as their frequent antibiotic resistance can lead to unnecessary antibiotic use and infection control challenges. To address the morbidity and mortality associated with nosocomial pneumonia, we advocate for robust infection control practices and a comprehensive antibiotic stewardship program.

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