

# Frequency and Patterns of Multidrug-Resistant Organisms among Ventilated Patients During the Coronavirus Disease-19 Pandemic

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## ABSTRACT

**Objective:** To determine the frequency of multidrug-resistant (MDR) bacterial isolates in respiratory specimens obtained from ventilated patients admitted to critical care units at the National Institute of Cardiovascular Diseases (NICVD), along with COVID-19-positive cases.

**Study Design:** An observational study.

**Place and Duration of the Study:** National Institute of Cardiovascular Diseases, between November 2021 and March 2022.

**Methodology:** Tracheal aspirate and bronchoalveolar lavage (BAL) samples received for culture and sensitivity were obtained from the critical and surgical intensive care units during the specified period. The prevalence of MDR organisms was compared between gram-negative and gram-positive bacteria using the chi-square test to determine any statistical significant differences in their occurrence.

**Results:** Throughout the study duration, 357 patients were admitted to critical care units, yielding 662 samples of tracheal aspirate and BAL. Among these samples, 229 bacterial isolates were identified, comprising 171 gram-negative rods (GNR) and 58 gram-positive cocci (GPC). The overall prevalence of MDR was determined to be 22% in GNR and 38% in GPC. Additionally, the prevalence of MDR among COVID-19 patients was observed to be approximately 11%.

**Conclusion:** There was a notable frequency of MDR bacterial isolates in ventilated patients, particularly among GPC strains. Furthermore, the identified prevalence of MDR bacterial isolates in COVID-19 patients underscores the imperative for vigilant monitoring and intervention to address antibiotic resistance in this susceptible cohort.

**Key Words:** Ventilator-associated pneumonia, Bronchoalveolar lavage, Multidrug-resistant, Gram-negative rods, Gram-positive cocci, Coronavirus disease, Tracheal aspirate.

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## INTRODUCTION

Ventilator-associated pneumonia (VAP) represents a significant clinical challenge in the context of intensive care units (ICUs) and patients who require mechanical ventilation for a duration of at least 48 hours.<sup>1</sup> VAP, as a nosocomial infection, holds the distinction of being the most prevalent among hospital-acquired infections.<sup>2</sup> However, its incidence varies worldwide, contingent upon factors such as clinical diagnostic criteria, diagnostic methodologies employed, and the specific characteristics of the (ICU) environment.<sup>3</sup>

It is crucial to note that VAP is associated with a substantial burden of morbidity and mortality, with reported rates ranging from 10 to 30%.<sup>4</sup> Recent epidemiological studies have yielded divergent findings regarding VAP incidence. For instance, the International Nosocomial Infection Control Consortium surveillance study spanning from 2012 to 2017, encompassing 523 ICUs across 45 countries, reported an average VAP incidence of 14.1 cases per 1,000 ventilator days.<sup>5</sup>

The diagnosis of VAP poses challenges for healthcare providers, as it necessitates a comprehensive evaluation encompassing clinical, radiographic, and microbiological cultures before definitive confirmation.<sup>6</sup> It is worth emphasising that microbiological culture confirmation holds paramount importance in establishing the diagnosis of VAP.<sup>7</sup> To administer appropriate empirical therapy, it is imperative for healthcare providers to have a thorough understanding of the prevailing pathogen resistance trends within hospital settings.<sup>8</sup> While considerable research has been dedicated to elucidating the causative pathogens and their susceptibility patterns in VAP, a notable gap exists in the literature concerning the prevalence of colonisation by multi-

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drug-resistant (MDR) pathogens and their clinical significance in respiratory specimens.

The colonisation of patients with MDR gram-negative bacteria (MDR-GNB) has been associated with several risk factors, including prior use of broad-spectrum antibiotics, previous MDR-GNB infections, advanced age, mechanical ventilation, a high incidence of local antibiotic resistance, ICU admission, and states of immunosuppression.<sup>9</sup> The presence of these risk factors in ventilated patients can serve as valuable indicators for predicting the likely pathogens involved and their sensitivity patterns, aiding clinicians in making informed decisions regarding treatment strategies and antibiotic selection.

When clinical suspicion and radiological results support the bacterial infection, the coronavirus disease-positive ventilated patients also require empirical treatment.<sup>10</sup> Hence, the prevalence rate of MDR pathogens in hospital and ICU settings would help initiate appropriate antibiotics in these patients. Literature shows that there is an upsurge in MDR pathogen isolation in pneumonia cases worldwide during the COVID-19 pandemic.<sup>11</sup> Thus, this study provided important insight into MDR prevalence in COVID-positive patients and an opportunity to increase the understanding of the significance of positive bacterial culture findings and patient management. Because of practical constraints, this study cannot provide a comprehensive review of the patients' clinical parameters and other associated surgical risk factors.

The objective of this study was to determine the overall prevalence of MDR bacterial isolates in respiratory specimens collected from ventilated patients admitted to critical care units of National Institute of Cardiovascular Diseases Hospital. The second aim was to investigate the burden of MDR pathogens in respiratory specimens of COVID-positive patients admitted to NICVD.

## METHODOLOGY

This retrospective descriptive study was undertaken at the National Institute of Cardiovascular Diseases (NICVD), from November 2021 to March 2022. The investigation focused exclusively on tracheal aspirate and bronchoalveolar lavage (BAL) samples obtained from ventilated patients admitted to critical and surgical intensive care units within the hospital, with the exclusion of respiratory specimens from non-ventilated patients. All the samples were included from ventilated patients irrespective of the status of their infection.

Clinical data pertaining to the study cohort were meticulously retrieved from the data registry of critical care units within the NICVD hospital, while microbiological details were sourced from the institution's laboratory software. Tracheal aspirates and BAL specimens underwent quantitative processing in strict adherence to the guidelines outlined by the American Society for Microbiology (ASM).

Identification of bacterial pathogens was meticulously conducted using conventional biochemical tests and standardised techniques.<sup>12</sup> Antimicrobial susceptibility testing, particularly focusing on Colistin, was rigorously performed utilising both the disk diffusion method and broth microdilution technique, in accordance with recommendations provided by the Clinical and Laboratory Standards Institute (CLSI).<sup>13</sup> Confirmation of Methicillin-resistant *Staphylococcus aureus* (MRSA) was achieved through the cefoxitin disk sensitivity method, while penicillin sensitivity reporting was executed via oxacillin screening, aligning precisely with recommendations set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Ethical approval was acquired from the Ethics Review Committee at the aforementioned tertiary care hospital. Statistical analyses were performed using SPSS version 28, with a significance level set at  $p < 0.01$ . The Chi-square test was employed to assess the significance of MDR prevalence among gram-negative and gram-positive organisms. Categorical variables were presented as counts and percentages, while continuous variables were expressed as mean and standard deviation (SD).

## RESULTS

A total of 357 patients were admitted to critical care units from November 2021 to March 2022. A total of 662 samples of tracheal aspirate and BAL were collected during the study period. Out of 357 patients, 229 were males and 128 were females. Table I lists the baseline characteristics with diagnoses and comorbidities for the studied population.

During the study period spanning from November 2021 to March 2022, a variety of antibiotics were administered within the critical care unit for patient management. Notably, piperacillin-tazobactam was prescribed to 62 (17.3%) patients, ceftriaxone to 47 (13.16%) patients and meropenem to 19 (5.32%) patients. For the treatment of MDR gram-positive organisms, vancomycin was administered to 24 (6.72%) patients. It is important to highlight that patients who did not meet infection criteria were not subjected to antibiotic treatment, indicating discernment between colonisation and active infection.

During the specified timeframe, a total of 229 bacterial isolates were identified and reported, comprising 171 (74.7%) gram-negative rods (GNR) and 58 (25.32%) gram-positive cocci (GPC).

Among the GNR isolates, *Acinetobacter* species and *Pseudomonas aeruginosa* were the most frequently isolated, accounting for 19% each, with a diverse array of other GNRs also identified, as delineated in Table II.

The overall prevalence of multidrug-resistant organisms (MDRO) among GNRs was determined to be 22%, while among GPC isolates it was 38%, as illustrated in Table II. Multidrug resistance was defined as resistance to more than one drug within at least three classes of antibiotics. Notably, *Acinetobacter* species emerged as the most commonly reported MDRO representing 88% of cases.

**Table I: Baseline characteristics of the study population (n = 357).**

Parameters		N (%)
The age group (years)	<50 years	81 (23)
	>50 years	276 (77)
Gender	Male	229 (64)
	Female	128 (36)
Comorbidities	Type 2 diabetes	114 (32)
	Hypertension	111 (31)
	Cardiovascular diseases	44 (12.32)
	COPD	10 (3)
	TB	2 (0.5)
	Tumour (Myxoma)	1 (0.5)
	Other	4 (1)
	None	72 (20)
Diseases/Primary diagnosis	Myocardial infarction	104 (29)
	NSTEMI	58 (16.2)
	Heart failure	22 (6.1)
	Heart block	14 (4)
	Cardiogenic shock	6 (1.68)
	Pulmonary oedema	50 (14)
	Exacerbation of COPD	10 (2.8)
	COVID	9 (2.5)
	Kidney injury	6 (1.68)
	ARDS	5 (1.4)
	Atrial fibrillation	3 (0.84)
	Cardiomyopathy	3 (0.84)
	Mitral/aortic valve stenosis	9 (2.5)
	Pulmonary embolism/thrombosis	14 (3.9)
	Pericardial tamponade	3 (0.84)
	Pneumonia	5 (1.4)
	Sepsis	8 (2.24)
	Ventricular tachycardia	4 (1.1)

**Table II: The prevalence of MDR in gram-negative organisms isolated in respiratory samples from ventilated patients.**

Organisms	A total number (%)	MDR	Percent MDR
<i>Acinetobacter species</i>	33 (19.3)	29	88%
<i>Pseudomonas aeruginosa</i>	33 (19.3)	2	6%
<i>Enterobacter species</i>	23 (13.5)	2	9%
<i>E. coli</i>	23 (13.5)	1	4.3%
<i>Klebsiella species</i>	21 (12.3)	4	19%
<i>Hemophilus species</i>	20 (11.7)	0	-
<i>Moraxella species</i>	7 (4.09)	0	-
<i>Pseudomonas species</i>	5 (3)	0	-
Others	6 (3.5)	0	-
Total	171	38	22%

**Table III: Statistical association among gram-positive and gram-negative MDR isolates.**

Organisms	Total (N)	Prevalence of MDR		p-value*
		Negative	Positive	
Total (N)	229	169 (73.8%)	60 (26.2%)	-
Bacterial isolates				
Gram-negative isolates	171	133 (77.8%)	38 (22.2%)	0.019
Gram-positive isolates	58	36 (62.1%)	22 (37.9%)	
Gram-positive isolates				
<i>Staphylococcus aureus</i>	34	16 (47.1%)	18 (52.9%)	0.005
<i>Streptococcus pneumoniae</i>	24	20 (83.3%)	4 (16.7%)	
Gram-negative isolates				
<i>Acinetobacter species</i>	33	4 (12.1%)	29 (87.9%)	<0.001
Other species	144	135 (93.8%)	9 (6.3%)	

\*p-values are computed using chi-square test.

A total of 58 gram-positive bacterial isolates were reported from November 2021 to March 2022. *Staphylococcus aureus* was the most commonly reported organism (58%).

A total of 52% of isolates were Methicillin-resistant *Staphylococcus aureus* (MRSA). Comparison of MDR isolates in gram-negative organisms and gram-positive organisms was statistically significant ( $p$ -value  $<0.01$ ) as shown in Table III. The prevalence of MDROs was found to be significantly higher among gram-positive patients with a rate of 37.9% (22/58) vs. 22.2% (38/177,  $p = 0.019$ ) among patients with gram-positive and gram-negative, respectively. Likewise, the percentage isolation of MDRO in *Acinetobacter species* is significantly higher as compared to other species of gram-negative organisms as shown in Table III.

A total of nine bacterial isolates have been detected in COVID cases, and the isolation of MDR pathogens was low ( $n = 1$ ). Out of 357 patients, 69 died due to cardiovascular diseases, leading to 19.3% mortality.

## DISCUSSION

Amidst the backdrop of the COVID-19 outbreak, a comprehensive study was undertaken to evaluate the prevalence of bacterial isolation in ventilated patients at the esteemed Cardiovascular Disease Institute. The study cohort was characterised by a noteworthy demographic composition, with a substantial proportion of patients aged over 50 years, constituting 77% of the study population. Moreover, a gender disparity was evident, with 64% of the admissions representing males, while females accounted for 36% of admissions to critical care units. Within this patient population, prevalent comorbidities included diabetes and hypertension, collectively affecting approximately 32% of patients, a finding that aligns with previous research documented in various studies.<sup>14</sup> These epidemiological insights offer a foundational understanding of the patient demographics and comorbidity profiles, which are invaluable for contextualising the subsequent analyses and conclusions drawn from this study.

The analysis's most evident result is that the prevalence of gram-negative organisms is higher than that of gram-positive isolates, while the percentage of MDR organisms among gram-positive isolates is higher compared to MDRO in gram-negative isolates. The most effective management and appropriate treatment of these patients on ventilation depend on the pathogen confirmation and quantitative count through repeated endotracheal aspiration (ETA) cultures, along with their resistance pattern.<sup>15</sup>

*Acinetobacter species*, *K. pneumoniae*, *P. aeruginosa*, and *E. cloacae* have been detected as major GNRs in the current

study. The most frequently isolated MDROs ( $n = 29$ ) 76% are *Acinetobacter species*. Only Gentamicin ( $n = 4$ ) and Cotrimoxazole ( $n = 5$ ) were effective against these MDR *Acinetobacter species*. Ren *et al.* in their study also reported high prevalence of MDR *Acinetobacter* in ventilated patients.<sup>16</sup> A total of seven CREs (18%) were reported, and Fosfomycin was tested for four of these CRE isolates, three of which were Fosfomycin sensitive. Kassem *et al.* reported mechanical ventilation and in-hospital stay as major risk factors for CRE colonisation and subsequently these patients have high mortality as well.<sup>17</sup>

Providentially *Pseudomonas aeruginosa* was found to be highly susceptible to routinely used anti-pseudomonal medications, including Meropenem ( $n = 30$ ), Ciprofloxacin ( $n = 29$ ), and Amikacin ( $n = 28$ ). Furthermore, there was no evidence of colistin resistance in *Pseudomonas aeruginosa*, *Acinetobacter species*, and *Enterobacteriaceae*. In addition, multidrug resistance has also not been detected in community-acquired bacteria such as *Hemophilus* and *Moraxella species*.

Even though only 25% of the isolates were gram-positive cocci, a considerable number of *Staphylococcus aureus* ( $n = 34$ ) were identified, with 52% of the isolates being MRSA. These findings support the conclusions of much earlier research in ventilated patients.<sup>18,19</sup> Besides, 41% of the gram-positive cocci isolates were *Streptococcus pneumoniae*. The most important clinically relevant finding in *Streptococcus pneumoniae* is a low prevalence of penicillin resistance, which was found in only 4 isolates, and there is no evidence of ceftriaxone resistance.

Among the 357 patients admitted to the critical care unit during this period, 152 had received antibiotics. Piperacillin tazobactam was the most commonly used antimicrobial, which was given to 62 patients, followed by ceftriaxone given to 47 patients, and meropenem given to 19 patients. Among the 70 *Enterobacteriaceae* isolates, 49 (70%) of the isolates were sensitive to piperacillin-tazobactam compared to ceftriaxone, where only 23 (33%) of the isolates were sensitive. These results explain the more frequent use of piperacillin-tazobactam as an empirical and targeted choice of antibiotic. Although higher sensitivity for meropenem was seen in *Enterobacteriaceae* ( $n = 63$ , 90%) and *Pseudomonas aeruginosa* ( $n = 30$ , 90%) compared to *Acinetobacter species* ( $n = 3$ , 9%), infrequent and controlled usage of meropenem was observed. This reflects practices and policies in compliance with antimicrobial stewardship programmes and approaches to use antimicrobials carefully and preserve broad-spectrum antibiotics for highly resistant bacteria to counter increasing antimicrobial resistance.<sup>19</sup>

Positive respiratory cultures were obtained in a very limited percentage of patients with COVID-19 ( $n = 9$ ), and the prevalence of MDRO was very low ( $n = 1$ ). Despite being



stated in the literature review, the COVID-19 pandemic has driven up the frequency of healthcare-associated infections and subsequent antibiotic resistance, however, in this study, the authors found low rates of MDR isolates in respiratory specimens from COVID-positive patients.<sup>20,21</sup> A possible explanation for these results may be the lack of adequate infection-control practices among healthcare workers during the pandemic. Although there is adequate awareness among the HCWs, due to the limitation in the availability of hand-hygiene facilities and other necessary items for disinfection, infection-control practices have been compromised.

## CONCLUSION

The study revealed a higher frequency of gram-negative organisms compared to gram-positive counterparts. A notably low prevalence MDR organisms was seen among COVID-positive patients. This observation holds important implications for the optimisation of empirical antibiotic selection in clinical practice.

## ETHICAL APPROVAL:

This study was approved prior to the initiation of the research from the Institutional Review Board (IRB) of the National Institute of Cardiovascular Diseases, NICVD.

## PATIENTS' CONSENT:

Since it is a laboratory-based clinical study, patient consent statement is not applicable.

## COMPETING INTEREST:

The authors declared no conflict of interest.

## AUTHORS' CONTRIBUTION:

FR: Idealised and conceptualised the study.

TF, FR: Collected and managed the data, and drafted the manuscript.

FR: Performed statistical analysis and amended the results and tables.

FA, MN: Edited the manuscript.

NS: Critically reviewed the manuscript.

MN, NS: Revised the manuscript.

All authors approved the final version of the manuscript to be published.

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