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Comprehensive Analysis of Etiological Agents and Drug Resistance Patterns in Ventilator-Associated Pneumonia

Harendra K. Thakur [1,2], Bansidhar Tarai [2,*], Aradhana Bhargava [3], Pankaj Soni [4], Anup Kumar Ojha [5], Sudhakar Kancharla [6], Prachetha Kolli [7], Gowtham Mandadapu [6] and Manoj Kumar Jena [1,*]

1 Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144411, India; harendra.41900685@lpu.in
2 Department of Microbiology & Molecular Biology, Max Super Speciality Hospital, Saket, New Delhi 110017, India
3 Establishment & Strengthening NCDC Branches, New Admn Building, National Centre for Disease Control Dte.GHS, Ministry of Health & Family Welfare, Government of India, Delhi 110054, India; bhargavaaradhana@yahoo.com
4 Department of Internal Medicine, Max Super Speciality Hospital, New Delhi 110017, India; pankaj.soni@maxhealthcare.com
5 Indian Council of Medical Research Headquarters, New Delhi 110029, India; ojha.anup2011@gmail.com
6 Devansh Lab Werks, Homewood, AL 35209, USA; sudhakar@devlabwerks.com (S.K.); gowtham@devlabwerks.com (G.M.)
7 Microgen Health Inc., Chantilly, VA 20151, USA; prachetha@microgenhealth.com
* Correspondence: bansidhar.tarai@maxhealthcare.com (B.T.); manoj.20283@lpu.co.in (M.K.J.)

Abstract

Ventilator-associated pneumonia (VAP) develops in patients who stay on mechanical ventilation for more than 48 h. In the presence of causative pathogens, the patient develops clinical signs such as purulent tracheal discharge, fever, and respiratory distress. A prospective observational study was carried out in the Intensive Care Unit (ICU) of Max Healthcare Centre, New Delhi, from 2020 to 2023. The study comprised 70 samples from patients diagnosed with VAP. This study thoroughly examined VAP-associated microorganisms and resistance in the hospital ICU. *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were the most commonly reported pathogens. Significant drug resistance was seen in *P. aeruginosa*, *K. pneumoniae*, *A. baumannii* and *Staphylococcus aureus*. The heatmap also supported the antibiotic resistance data patterns obtained from conventional and automated systems of determination. Notably, *Serratia marcescens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Ralstonia insidiosa*, and *Ralstonia mannitolilytica*, showed 60 to 100% of resistance to a number of antibiotics. Among all VAP patients, 31.42% early-onset and 68.57% late-onset VAP cases were detected. Out of 70 patients, 43 patients died (mortality rate 61.4%); majority of them suffered from late-onset VAP. The study goal was to describe the antibiotic resistance patterns and microbial ecology of the pathogens that were isolated from VAP patients. According to the heatmap analysis, a varied VAP microbiome with high prevalences of MDR in *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* was identified. To address the increasing prevalence of MDR VAP, the study highlights the critical need for improved VAP monitoring, strong infection control, and appropriate antibiotic usage.

Keywords: ventilator-associated pneumonia; antibiotic sensitivity; bronchoalveolar lavage; VITEK-2 system; drug resistance

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1. Introduction

Ventilator-associated pneumonia (VAP) is defined as a disease where the patient is on mechanical ventilation for more than 48 h on the date of event, with day of ventilator placement being day 1, and the ventilator was in place on the date of event or the day before. If the ventilator was in place prior to inpatient admission, the ventilator day count begins with the admission date to the first inpatient location [1]. VAP usually occurs when the breathing machine, which uses a tube that is passed through the patient's mouth and windpipe for two or more consecutive days, carries harmful pathogens that infect the lungs [2]. VAP is a serious healthcare concern that affects ICUs all around the world. VAP is a significant challenge to healthcare professionals due to its elevated risk of mortality, morbidity, and extended hospital stays, along with hefty healthcare expenses [3]. Researchers have demonstrated that VAP is related with mortality rates of more than 24–50% and up to 76% in certain instances or when caused by high-risk pathogens, depending on the population studied and the underlying diseases [4,5].

Patients with VAP often have symptoms like fever, respiratory secretion, as well as radiological infiltrates. Despite of that VAP is difficult to diagnose due to the lack of distinct clinical signs and symptoms, and it frequently requires the thorough finding of clinical, radiological, and culture data [6]. Bacteria, viruses, and fungi are the etiological agents responsible for VAP. Usually, bacteria are the most common etiological agents, whereas viruses and fungi are implicated in a smaller number of cases [7,8]. *Staphylococcus aureus* (particularly methicillin-resistant *S. aureus*, or MRSA), *Streptococcus pneumoniae*, *Haemophilus influenzae*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., as well as *E. coli* are common bacterial infections that cause VAP [5,7]. However, the incidence of these etiological agents varies widely. This heterogeneity is determined by factors such as healthcare settings, patient demographics, and geographical regions. Understanding these local variances through surveillance data is crucial for guiding effective empirical treatment strategies, highlighting the dynamic nature of microbial epidemiology in VAP [5,7,9–11]. One of the most serious barriers to treating a VAP infection is the increasing rates of antibiotic resistance in the etiological agents. VAP primarily appears in the intensive care unit, which is supposed to be an ideal environment for the emergence of drug-resistant microorganisms. When clinicians encounter bacteria that are resistant to common antibiotics, it can be difficult to choose an empirical antimicrobial therapy because antimicrobial resistance (AMR) is a global health concern, whereas appropriate administration of empirical antimicrobial therapy is critically important for the treatment of VAP patients. The delayed administration of appropriate empirical antibiotics can lead to a high mortality rate of 75%, with the survival rate decreasing by 8% for each hour of delay [7,12]. So, the rapid diagnostic techniques are important to diagnose VAP infection as soon as possible.

The emergence of drug-resistant pathogens has recently made VAP management more difficult. The increasing prevalence of extensive-drug-resistant (XDR) and multidrug-resistant (MDR) bacteria has raised the therapeutic complexity of VAP [13]. Previous antibiotic treatments, poor infection control practices, and irrational antibiotic use may cause pathogens to become antibiotic-resistant [14,15].

Several variables increased the incidence of MDR VAP as compared to non-MDR VAP: recent intravenous antibiotic usage (within 90 days), a hospital stay of five or more days before VAP onset, septic shock at the time of VAP diagnosis, ARDS prior to VAP, and the necessity for acute renal replacement therapy before VAP [16]. Thus, determining the etiological agents causing VAP and related patterns of therapeutic resistance with underlying diseases has become essential for effective clinical therapy and infection management.

Due to the development of multidrug-resistant (MDR) and extensive-drug-resistant (XDR) strains of bacteria in patients, antibiotic treatment has become ineffective [2,17].

XDR bacteria are resistant to nearly all the major classes of antibiotic drugs available in the market, whereas MDR bacteria are resistant to more than three classes of drugs [17]. The prevalence of these resistant bacteria not only affects patient outcomes but also calls for the use of last-line antibiotics, which have been linked to higher toxicity and decreased efficacy.

There may be significant differences in the relative abundance of VAP pathogens between hospitals, as well as among different critical care units within the same hospital. Consequently, when choosing antibiotic therapies, the most recent European and American guidelines for addressing VAP recommend somewhat different strategies based on risk factors. While both guidelines employ strong methodology and are supported by current clinical studies, their practical implementation can be challenging.

The overuse of broad-spectrum antibiotics may occur in environments with a high frequency of risk factors and a lack of local epidemiological data, particularly in environments with a moderate or low prevalence of MDR pathogens [18]. Importantly, regardless of the time of intubation, the local ICU ecology is still a major risk factor for acquiring MDR infections and may contribute to the global increase in MDR pathogens [19].

The emergence of raising global antibiotic resistance in VAP pathogens poses a serious threat to the clinical practices used for the diagnosis and treatment. The current study focuses on the etiological agents responsible for VAP and their drug-resistance pattern evaluation by implying the robust combination of conventional and automated diagnostic systemic approach. The approach will significantly address the critical gaps in identification and drug resistance of the VAP pathogens. The study will also bring about the important insights to physicians, researchers, and policymakers to enhance the management and prevention of this lethal infection.

2. Materials and Methods

2.1. Study Design

A prospective observational study was conducted in the intensive care unit (ICU) of Max Healthcare Centre, New Delhi, from 2020 to 2023. This study comprised 70 samples from patients who had been diagnosed with VAP.

2.2. Inclusion and Exclusion Criteria

Inclusion criteria were as follows:

1. Participant older than 18 yrs
2. VAP-diagnosed participants, as per CDC guidelines [8].

Exclusion criteria were as follows:

1. Patients diagnosed with pneumonia before initiation of mechanical ventilation;
2. Patients without sufficient data/without a complete medical record;
3. Patients without informed consent (or whose guardians did not provide consent if the patient was unable to do so).

2.3. Patient Selection and Sample Collection

The inclusion and exclusion criteria listed above were used to choose the patients from three centres (a unit of Devki Devi Foundation, Max Healthcare Institute Limited, and Gujarmal Modi Hospital and Research Centre for Medical Sciences) of Max Healthcare Institutes, Saket, Delhi, India. The current study assessed hospital infections and quality control at an academic hospital that provides tertiary care with ethics committee approval. Patients over the age of 18 years who were placed on a mechanical ventilator for more than 48 h and had a Clinical Pulmonary Infection Score of more than 6 were included in the research. Also, the patients having symptoms (fever, tracheal secretions, leukocytosis, and fresh or progressive infiltrate on chest radiograph) and microbiological evidence from

respiratory samples were investigated to confirm the diagnosis of VAP according to CDC guidelines [8]. Trained medical personnel aseptically collected respiratory samples, bronchoalveolar lavage (BAL) fluid, and endotracheal aspirates (ET). Samples were processed immediately after being transferred to the microbiological lab.

2.4. Laboratory Methods

2.4.1. Identification of Bacterial and Fungal Pathogens

Bacterial Culture Identification

The semi-quantitative culture methods were performed by culturing the suspected bacterial pathogenic samples of Bronchoalveolar lavage (BAL) and endotracheal aspirate (ETA) in blood agar, MacConkey agar, and chocolate agar plates at 35 °C for a maximum period of 5 days. The organisms were identified using Vitek 2 automated system (Vitek 2 automated system, bioMérieux, Marcy-l' Etoile, France) and MALDI-TOF (Bruker Daltonics, Bremen, Germany) [20,21]. The bacteria were identified using colony morphology and Gram staining [20].

Fungal Identification

For the identification of filamentous fungal species, suspected samples were examined microscopically using wet mount and KOH (10–20%, *w*/*v*). Decontaminated samples were then inoculated to 1 Sabouraud Dextrose Agar (SDA) media plate and 4 SDA (supplemented with chloramphenicol/cycloheximide) slant tube. Inoculated 1 SDA media plate and one set of 2 SDA (supplemented with chloramphenicol/cycloheximide) slant tubes were placed into a 25 °C incubator and another set of 2 SDA (supplemented with chloramphenicol/cycloheximide) slants tube were placed into a 35 °C incubator for 21 days. Species of yeasts were identified using an automated system of VITEK 2 and MALDI-TOF MS. However, filamentous fungal species were also identified using the MALDI-TOF MS identification system.

Antibiotic Sensitivity

Gram-negative bacteria were examined with imipenem, ceftazidime, netilmicin, ticarcillin/clavulanic acid, piperacillin–tazobactam, amikacin, gentamicin, ciprofloxacin and levofloxacin. Furthermore, vancomycin, erythromycin, ciprofloxacin, clindamycin, amoxiclav, tetracycline, doxycycline, amoxicillin, and gentamicin were used to test for Gram's positive bacteria. Vancomycin, linezolid, teicoplanin, and mupirocin were used to screen for Gram's positive bacteria that were resistant to oxacillin. Gram-positive control organisms included *Enterococcus faecalis* ATCC29212 and *Staphylococcus aureus* ATCCBAA1026. For Gram-negative *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used [22].

Antifungal E-strips were used to determine the minimum inhibitory concentration (MIC) of several antifungal drugs against filamentous fungi on agar media. For yeast, the VITEK-2 identification system was used for antifungal susceptibility [23].

VITEK-2 System: Pure cultures were used to perform antibiotic susceptibility testing using the VITEK-2 system as per the instructions/guidelines given by manufacturer (bioMérieux, Marcy-l' Etoile, France). Briefly, pure culture colonies were suspended in sterile saline to achieve the appropriate McFarland turbidity according to the ID card (yeast: 1.80–2.20, bacteria: 0.50–0.63). The suspension was loaded on to specific reagent cards (ASTN405, ASTN406, ASTN235, ASTN628, ASTST03, and ASTYS08 panels). Panels were inserted into the VITEK-2 cassette (up to ten tests) followed by manual placement in the incubator (34.5–36.5 °C) after being inoculated with microbial suspensions. Up to 60 cards were incubated on a carousel, while optical measurements were taken every 15 min by the system, which also automatically sealed the cards and took out the transfer tubes. Different ID (identification) cards were used for various organism group detection, and AST was per-

formed using specific AST panels. Gram-positive cocci and non-spore-forming bacilli were tested using the ID-GP card; Gram-negative fermenting and nonfermenting bacilli were tested using the ID-GN card; yeasts and yeast-like organisms were tested using the YST card; and *Neisseria* and *Haemophilus* species were tested using the NH card. Anaerobic and corynebacterium species were represented by an ANC card, whereas endospore-forming organisms from the *Bacillaceae* family were represented by BCL. The VITEK-2 system analyzed the data to identify the organism and determine its antibiotic sensitivity, automatically recording and printing the results via the VITEK-2 Compact Software version 9.02.

The antibiotics (aztreonam—30 mcg, doripenem—10 mcg, levofloxacin—5 mcg, levonadifloxacin—10 mcg, nitrofurantoin—300 mcg, piperacillin/tazobactam—100/10 mcg cefepime tazobactam—30/10 mcg, ticarcillin/clavulanic acid—75/10 mcg, ampicillin/sulbactam—10/10 mcg, chloramphenicol—30 mcg, ceftazidime/avibactam—50 mcg, nitrofurantoin—300 mcg, netilmicin—30 mcg, minocycline—30 mcg and doxycycline—30 mcg) that were not associated with the panel of antibiotics in the VITEK 2 system were used to determine the sensitivity/resistance of the pathogens using the Kirby–Bauer disc-diffusion method [21,24]. Briefly, a standardized bacterial saline solution (0.85%, *w*/*v*) was prepared within an adjusted turbidity of 0.5 MacFarland. The suspension was spread uniformly over the Mueller–Hinton agar plate. The inoculated plates were then loaded with different antibiotics disc with specific concentrations. The antibiotic-loaded culture plates were incubated at 37 °C for 24–48 h. After the incubation, plates were observed for the zone of inhibition against bacterial growth. The sensitivity/resistance of the pathogens was evaluated by measuring the diameter of the inhibition zone [21,24].

2.4.2. Polymerase Chain Reaction

The BIOFIRE® FILMARRAY® Pneumonia plus Panel was used for quick and accurate automated testing of bacteria and viruses that cause pneumonia and lower respiratory tract infections (LRTI), as well as the detection of seven genetic markers for drug resistance. The hands-on time was only 2 min, with no pipetting necessary and a 1 h turnaround time for detecting 34 targets at once. A multiplex PCR technique was used by the PNplus Panel (BioFire Diagnostics, Salt Lake City, UT, USA) to identify a variety of respiratory pathogens and resistance genes. It was able to identify seven antibiotic resistance genes (CTX-M, KPC, OXA-48-like, NDM, VIM, IMP, and mecA/C/MREJ), eight viruses, three atypical bacteria, and fifteen typical bacteria (including species such as *Acinetobacter calcoaceticus-baumannii* complex, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). This approach showed a sensitivity of 96.3% and a specificity of 97.2% when tested with sputum samples [25].

Statistical Analysis

Origin 2024b and GraphPad Prism 8.0.1 were used for data analysis and graphical visualization, while a Microsoft Office 365 excel document was used for data input. The significance of qualitative data was assessed through the Chi-square test.

3. Result

The prospective observational study conducted in the intensive care unit (ICU) of three branches (a unit of Devki Devi Foundation, Max Healthcare Institute Limited, and Gujarmal Modi Hospital and Research Centre for Medical Sciences) of Max Healthcare Institutes, Saket, Delhi, India. A total of 70 patients were diagnosed with VAP, and their clinical and demographic data are shown in Table 1. Patients were categorized based on age, sex, residence, co-morbidities, and treatment received. The majority (n = 45) of patients were older than 60 yrs. In this study, the testing showed VAP among 28 diabetic patients. Patients with other co-morbidities, which are listed in the table, include renal

failure, heart disease, sepsis, hypertension, cancer, thyroid disorders, and liver disease. A significant number of patients (n = 34) received steroids as part of their treatment regimen. A total of 66 bacterial, 3 fungal and 1 viral infections of VAP were identified. The study highlighted most bacterial isolates detected in VAP patients from individuals with diabetes, hypertension, thyroid, COPD (conic obstructive pulmonary disease), kidney failure, heart disease, and sepsis (Table 2). The most common isolates found in patient groups with diabetes, hypertension, thyroid disorders, COPD, kidney failure, cardiac disease, and sepsis were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. Average hospital stay of VAP patients was 32.7 days.

Table 1. Demographic profile of patients and underlying diseases associated with the pathogens detected.

Demographic Profile of Patients with VAP				
Demography	**Bacterial**	**Fungal**	**Viral**	**Total**
Age				
<60 yrs	22	3	0	25
>60 yrs	44	0	1	45
Total	66	3	1	70
p value	0.0247	0.0865	0.319	0.0499
Sex				
Male	43	1	1	45
Female	23	2	0	25
Total	66	3	1	70
p value	0.0414	0.5678	0.319	0.0499
Residential				
Urban	60	2	1	63
Rural	6	1	0	7
Total	66	3	1	70
p value	<0.0001	0.5678	0.319	<0.0001
Co-morbidity condition				
Patients with single co-morbidities	12	0	0	12
Patients with ≥2 co-morbidities *	44	3	0	47
Patients with missing data/no co-morbidities	10	0	1	11
Total	66	3	1	70
p value	<0.0001	0.0541	0.371	<0.0001
Treatment given				
Intake of steroids	34	3	0	37
Intake of antibiotics	67	3	0	70
p value	0.0115	>0.9999		0.0154

* Co-morbid condition: immunocompromised, post-operative, lung disease, DM, COPD, kidney failure, accidental, heart disease, sepsis, hypertension, cancer, thyroid, liver, missing/no underlying disease.

Table 2. Number of patients infected with VAP causing pathogens with underlying disease; ND, not detected; HTN, hypertension; DM, diabetic mellitus; COPD, conic obstructive pulmonary disease. Values in the table signify the number of patients infected with pathogens with underlying co-morbid conditions.

Pathogens	DM	Kidney Failure	Accidental	Heart Disease	Sepsis	Respiratory Failure	HTN	Immuno-Compromised	Cancer	Thyroid	Motor Neuron Disease	COPD	Surgery	Liver
Acinetobacter baumannii	11	7	1	8	4	8	14	ND	3	5	1	2	1	ND
Pseudomonas aeruginosa	10	5	3	4	2	4	10	ND	2	4	1	3	ND	1
Klebsiella pneumoniae group	12	8	1	6	7	10	17	1	ND	3	ND	3	2	1
Escherichia coli	6	1	ND	2	1	1	4	ND	ND	1	ND	ND	ND	ND
Burkholderia cepacia	1	ND	ND	1	ND	ND	1	ND	ND	1	ND	ND	ND	ND
Staphylococcus aureus	5	2	1	ND	1	2	5	ND	1	1	ND	ND	ND	ND
Serratia marcescens	2	ND	ND	ND	ND	1	1	ND	1	2	ND	ND	ND	ND
Elizabethkingia meningoseptica	ND	ND	ND	ND	1	ND	ND	ND	ND	ND	1	ND	ND	ND
Candida tropicalis	3	3	1	2	2	1	3	ND	ND	ND	ND	ND	ND	ND
Candida albicans	ND	ND	ND	ND	ND	1	1	ND	1	ND	ND	ND	ND	ND
Ralstonia insidiosa	1	ND	ND	ND	ND	ND	ND	ND	1	ND	ND	ND	ND	ND
Stenotrophomonas maltophilia	ND	ND	ND	1	ND	1	1	ND	ND	ND	ND	ND	ND	ND
Ralstonia mannitolilytica	ND	ND	ND	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aspergillus	1	1	ND	1	1	1	1	ND	ND	ND	ND	ND	ND	ND
Streptococcus pneumoniae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

VAP associated pathogens (bacterial and fungal) detected with MALDI-TOF and VITEK *were Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* group, *Escherichia coli*, *Burkholderia cepacia*, *Staphylococcus aureus*, *Serratia marcescens*, *Elizabethkingia meningoseptica*, *Candida tropicalis*, *Candida albicans*, *Ralstonia insidiosa*, *Ralstonia mannitolilytica*, *Stenotrophomonas maltophilia*, *Aspergillus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Mycoplasma pneumoniae*.

The pathogens from bacterial and viral groups, detected with BioFire® FilmArray® multiplex PCR panel, were *Escherichia coli*, *Klebsiella pneumoniae* group, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus-baumannii* complex, *Staphylococcus aureus*, *Serratia marcescens*, and *Haemophilus influenzae*. Furthermore, viral pathogen, human rhinovirus/enterovirus, was also identified.

Based on the type of infection (bacterial, fungal, or viral) and the total infections, Table 1 showed the clinical and demographic characteristics of VAP patients. Both total VAP (p = 0.0499) and bacterial VAP (p = 0.0247) were significantly correlated with age. Similarly, certain VAP categories were significantly correlated with both sex and residential status. Steroid usage was significant for both bacterial and total cases, while co-morbidity conditions were highly significant for both bacterial and total VAP (p-value < 0.0001) indicating that their distributions were not random.

3.1. Pathogens Implicated to Both Late-Onset and Early-Onset VAP

Early-onset VAP was caused by many strains of the Enterobacteriaceae, *Candida albicans*, *Acinetobacter baumannii*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Serratia marcescens*, *Candida tropicalis*, *Ralstonia mannitolilytica*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Mycoplasma pneumoniae*. Late-onset VAP was mainly linked with *Acinetobac-*

ter baumannii, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and polymicrobial infections (Table 3).

Table 3. The pathogens causing both early- and late-onset VAP.

Pathogens	Early-Onset-VAP, *n* (%)	Late-Onset-VAP, *n* (%)	Chi sq.	*p* Value
Acinetobacter baumannii	4 (5.71)	5 (7.14)	0.1044	0.7466
Pseudomonas aeruginosa	4 (5.71)	7 (10)	0.7589	0.3837
Klebsiella pneumoniae group	1 (1.42)	9 (12.85)	5.99	0.0144
Escherichia coli	2 (2.85)	0	1.972	0.1602
Burkholderia cepacia	0	1 (1.42)	0.993	0.319
Staphylococcus aureus	0	1 (1.42)	0.993	0.319
Serratia marcescens	1 (1.42)	0	0.993	0.319
Elizabethkingia meningoseptica	0	1 (1.42)	0.993	0.319
Candida tropicalis	2 (2.85)	0	1.972	0.1602
Candida albicans	0	1 (1.42)	0.993	0.319
Ralstonia insidiosa	0	1 (1.42)	0.993	0.319
Stenotrophomonas maltophilia	0	1 (1.42)	0.993	0.319
Ralstonia mannitolilytica	1 (1.42)	0	0.993	0.319
Aspergillus	0	1 (1.42)	0.993	0.319
Streptococcus pneumoniae	1 (1.42)	0	0.993	0.319
Polymicrobial infection	3 (4.28)	19 (27.14)	10.16	0.0014
Haemophilus influenzae	1 (1.42)	1 (1.42)	0	>0.9999
Human Rhino/Enterovirus	1 (1.42)	0	0.993	0.319
Mycoplasma pneumoniae	1 (1.42)	0	0.993	0.319
Total	22 (31.42)	48 (68.57)	6.538	0.0106

Most pathogens responsible for VAP (both early- and late-onset) were *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* group. According to Table 3, late-onset VAP showed more frequently isolated pathogens than early-onset VAP, and the polymicrobial infection caused a higher number of late-onset VAP cases than monomicrobial infection. Overall number of pathogens responsible for early- and late-onset VAP showed significant *p*-values and chi sq. tests, however the *Klebsiella pneumoniae* group and polymicrobial infection showed significant *p*-values compared to other pathogens individually. Early-onset VAP affected 31.42% of the individuals, whereas late-onset VAP affected 68.57%. Early-onset VAP presented 19 cases of monomicrobial infection and 3 cases of polymicrobial infection whereas late-onset VAP showed 29 cases of monomi-

crobial infection and 19 cases of polymicrobial infection. Seventy clinical samples were identified as VAP, of which monomicrobial infections accounted for 68.58%, while polymicrobial infections contributed 31.42%. Out of 70 patients, 43 patients died; the majority of them suffered from late-onset VAP. This indicates that the mortality rate within the group was quite high—61.4%. In this study 31 patients died out of 48 cases of late-onset VAP, so the mortality rate of late-onset VAP was 64.5%.

The chi-square test revealed a statistically significant association between early- and late-onset VAP because of the *Klebsiella pneumoniae* group (Chi sq.—5.99, p-value < 0.0144) and polymicrobial infection (Chi sq.—10.16, p-value < 0.0014), indicating that their distributions were not random.

3.2. Pattern of Antibiotic Resistance of Isolated Pathogens Against Different Drugs

The commonly identified pathogens like *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* showed resistance to multiple antibiotics such as ceftazidime, piperacillin/tazobactam, meropenem, ticarcillin/clavulanic acid, levofloxacin, aztreonam, imipenem, ceftriaxone, ciprofloxacin, trimethoprim/sulfamethoxazole, cefotaxime, doxycycline. Resistance was observed in case of *Staphylococcus aureus* to ampicillin, benzylpenicillin, ciprofloxacin, clindamycin, erythromycin, and levofloxacin.

In this study the heat map analysis was carried out to check the resistance patterns of the pathogens detected in VAP patients (Figure 1). Gram-negative pathogens showed 100% resistance against some antibiotics, including polymyxin, ampicillin, third-generation cephalosporins, beta-lactamase, aminoglycoside, beta-lactam and carbapenem. *Serratia marcescens* and *Klebsiella pneumoniae* group showed 100% resistance against AMC- amoxicillin/clavulanic acid. *Serratia marcescens*, *Escherichia. coli*, *Klebsiella pneumoniae* group, *Ralstonia insidiosa*, and *Ralstonia mannitolilytica* were arising in a single cluster with a moderate to high resistance against colistion, polymixine B, ampicillin/sulbactam, amikacine, gentamicine, netilmicin, aztreonam, ceftazidime, meropenem, piperacillin/tazobactam and ticarcillin/clavulanic acid. However, a very low resistance percentage was observed in *Serratia marcescens* against tobramycin (TOB) and in *E. coli* against colistin, polymyxin B, amikacin, gentamicin and netilmicin, whereas in a single cluster of a *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus-baumanni* complex a high resistance was discerned in the heatmap.

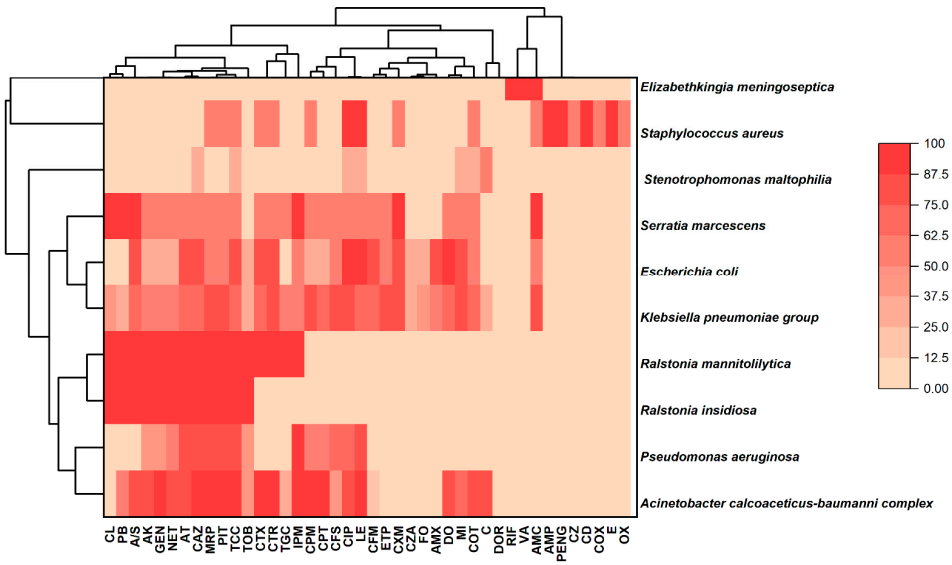


Figure 1. Cluster heatmap of VAP pathogens with antibiotic resistance pattern. Rows and columns of cluster heatmap plot represent pathogens and antibiotics, respectively. *CL* colistin, *PB* polymyxin B,

A/S ampicillin/sulbactam, *AK* amikacin, *GEN* gentamicin, *NET* netilmicin, *AT* aztreonam, *CAZ* ceftazidime, *MRP* meropenem, *PIT* piperacillin/tazobactam, *TCC* ticarcillin/clavulanic acid, *TOB tobramycin*, *CTX* cefotaxime, *CTR* ceftriaxone, *TGC* tigecycline, *IMP* imipenem, *CPM* cefepime, *CPT* cefepime-tazobactam, *CFS* cefoperazone/sulbactam, *CIP* ciprofloxacin, *LE* levofloxacin, *CFM* cefixime, *ETP* ertapenem, *CXM* cefuroxime, *CZA* ceftazidime/avibactam, *FO* fosfomycin, *AMX* amoxicillin, *DO* doxycycline, *MI* minocycline, *COT* trimethoprim/sulfamethoxazole, *C* chloramphenicol, *DOR* doripenem, *RIF* rifampicin, *VA* vancomycin, *AMC* amoxicillin/clavulanic acid, *AMP* ampicillin, *PENG* benzylpenicillin, *CZ* cefazolin, *CD* clindamycin, *COX* cloxacillin, *E* erythromycin, *OX* oxacillin.

A separate cluster was observed for *Elizabethkingia meningoseptica* (resistance against rifampicin, vancomycin, amoxicillin/clavulanic acid) and *Staphylococcus aureus* (resistance against amoxicillin/clavulanic acid, ampicillin, benzylpenicillin, cefazolin, clindamycin, cloxacillin, erythromycin and oxacillin). There was an absence of resistance reported in *Stenotrophomonas maltophilia* against the majority of antibiotics used in the current research investigation.

4. Discussion

This study included 70 samples from individuals who were diagnosed with VAP. The selected sample size was designed to create a robust and accurate dataset for precisely identifying prevalent diseases and monitoring resistance variations. The study evaluated resistance patterns in a wide variety of bacterial and fungal species according to the VITEK-2 system's extensive database, which covers a broad range of antibiotics.

The distribution of patients by age group revealed that the majority (n = 45) were beyond the age of 60, indicating a greater risk of VAP in the senior population. Another study also revealed that patients between the ages of 51 to 75 had a high incidence of VAP [26].

Forty-five patients were males and twenty-five were women, indicating a slight male dominance. In another study with a higher prevalence of men the researchers reported that gender seems to influence the risk of VAP [26], while other studies showed a higher prevalence in women [27]. These discrepancies might have occurred due to variations among study populations and methodologies.

The study investigated the etiological agents and resistance patterns of VAP in ICU patients. Suspected patient's samples were transferred to the lab and processed using the semiquantitative culture method for the identification of pathogens. Through these techniques positive growth was defined as reaching a threshold value of 10^4 colony-forming-unit (CFU)/mL when a BAL sample taken and $>10^5$ to 10^6 colony forming units for an ET sample. Another study also supported these threshold values of 10^4 colony-forming-unit (CFU)/mL for BAL and $>10^5$ to 10^6 CFU/mL for ET [28]. In this study the identification of pathogens was validated by the MALDI-TOF MS (Bruker Daltonics, Germany) and VITEK-2 systems (bioMérieux, France) for identification and susceptibility testing (ID/ST) [29,30]. Fungal isolation involved direct smears (KOH/lactophenol cotton blue) and cultures on Sabouraud dextrose agar. Candida was identified via Gram-positive pseudohyphae and growth on specific media, while filamentous fungi like *Aspergillus* were recognized by macroscopic and microscopic characteristics, with *Aspergillus niger* forming brown colonies with radiating conidial heads. Positive cultures at least 48 h after ventilation were used to diagnose bacterial/fungal superinfection in clinically suspected patients with previous negative findings [31].

Seventy clinical samples were identified as VAP, of which monomicrobial infections accounted for 68.58% of VAP cases, while polymicrobial infections contributed 31.42%. These results are comparable to another study which reported 72.2% and 27.8%, respectively [32].

The frequently isolated pathogens in late-onset VAP comprised *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. These findings were consistent with a previous study, apart from *Staphylococcus aureus* [33]. Development of late-onset VAP was mostly associated with drug resistant pathogens. In this study the mortality rate of late-onset VAP was high (64.5%) due to *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and polymicrobial infections. Previous studies also revealed that the mortality rate (11.11%) of VAP was greater in hospitals with previous *K. pneumoniae* infections [34]. Other retrospective observational study conducted during January 2010 to Jun 2015 also reported a high mortality rate as 39.8% [35]. Apart from *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa, Candida* species and *Aspergillus* were also detected in VAP patients. The study is also consistent with the study by Meawed et al. (2021), which reported that the most frequent bacterial and fungal pathogens isolated were pan-drug-resistant (PDR) *Klebsiella pneumoniae* and multidrug-resistant (MDR) *Acinetobacter baumannii*, *Candida* species, and *Aspergillus* [31]. These findings of VAP-associated pathogens were also consistent with another previous study, which identified *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *S. aureus*, *Candida albicans*, and *Candida tropicalis* as common isolates, confirmed the consistent epidemiological trends of these key VAP pathogens across different settings using MALDI-TOF and VITEK systems [36].

Furthermore, the identification of human rhinovirus/enterovirus in our cohort emphasized the rising role of viral pathogens in VAP, which is supported by studies showing rhinovirus's frequent occurrence in respiratory infections among critically ill patients. Other studies had implicated a broader range of viruses in severe pneumonia and VAP, including respiratory syncytial virus, influenza, adenoviruses, human metapneumovirus, herpes simplex virus (HSV), and cytomegalovirus (CMV) [37–42]. The findings of this study highlighted the importance of rapid multiplex PCR in revealing the polymicrobial nature of VAP and guiding comprehensive patient management.

The comprehensive identification of common Gram-negative and Gram-positive bacteria through Biofire FilmArray panel was consistent with the findings of various studies that evaluated this platform [43–47].

This study revealed that most prevalent underlying diseases to those with VAP were high blood pressure, diabetes, thyroid disorders, COPD, kidney failure, cardiac disease, and sepsis. These co-morbidities might have increased the chance of developing VAP. These findings are consistent with a previous study which identified COPD, high blood pressure and diabetes as the most common comorbidities [7]. In this study many patients ($n = 28$) had diabetes, emphasizing the link between diabetes and VAP risk. Meawed et al. (2021) also justified that the risk of fungal VAP was diabetes mellitus, chest disease, hypothyroidism and longer duration of mechanical ventilation [31]. Findings showed that polymicrobial infection in early-onset and late-onset VAP was 4.28% and 27.14%, respectively, whereas two grouped studies conducted in 2013 revealed that 50% of polymicrobial infections were identified in both late-onset and early-onset VAP [33].

More than 70% of the Gram-negative pathogen isolates in the current study were resistant to ampicillin and 3rd-generation cephalosporins. The combination of gentamicin with β lactam-β lactamase inhibitors (e.g., piperacillin–tazobactam) demonstrated about 50–75% resistance, while amikacin, imipenem, and meropenem showed 50–90% resistance. Other study of AMR among Gram-negative bacteria found that approximately 50–75% of isolates were resistant to gentamicin and combination of β-lactam–β-lactamase inhibitors, while over 75% became resistant to ampicillin and 3rd-generation cephalosporins. In decreasing order, levofloxacin, amikacin, imipenem, and meropenem showed the least resistance [7].

Individual Gram-negative bacteria had comparable levels of resistance, with the exception of *Acinetobacter baumannii*, which showed increased resistance to the highest-level drugs such as amikacin and carbapenems (imipenem and meropenem). *Staphylococcus aureus* was the only Gram-positive pathogen that showed 50% of methicillin resistant isolates. Also, higher levels of resistance to fluoroquinolones and aminoglycosides were observed. Another research found that resistance to anti-MRSA drugs such vancomycin, teicoplanin, and linezolid did not rise further in VAP patients, which is consistent with these investigations [7].

In this study, early-onset VAP accounted for 31.42% of all VAP cases (22/70), whereas late-onset VAP accounted for 68.57% (48/70). The majority of the pathogens that were isolated were *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*. On the other hand, 91.7% of patients in another study experienced early-onset VAP, and 8.3% experienced late-onset VAP, where *S. aureus*, *Enterococcus* species, *Acinetobacter* species, *K. pneumoniae*, and *P. aeruginosa* were among the organisms that were isolated [48].

A heat map analysis depicted a clear resistance pattern of the pathogens detected in VAP patients. In a study, a hierarchical cluster heat map analysis was carried out to analyze the differences in clustering patterns of common AMR profiles among pathogens [49]. Yudhanto et al. (2022) used hierarchical clustering to identify common AMR pattern and assist specific actions. This approach illustrates linkages by identifying groups of isolates with shared AMR pattern using tree-like arrangements. These clusters improve antimicrobial stewardship, enabling targeted surveillance and treatment methods [50].

5. Limitations of the Study

In the current study, we could not include the multiple underlying risk factors involved in the VAP patients due to a lack of patient metadata and ethical consent.

6. Conclusions

This study examines the pathogens and resistant patterns of antimicrobial agents linked with ventilator-associated pneumonia in the critical care unit. Findings from the study revealed a statistically significant association between early- and late-onset VAP, based on a chi-squared test, caused by the *Klebsiella pneumoniae* group (Chi sq.—5.99, p-value < 0.0144) and Polymicrobial infection (Chi sq.—10.16, p-value < 0.0014), indicating that their distributions were not random. Most of the underlying diseases associated with VAP pathogens were diabetes, kidney failure, heart disease, respiratory failure, and hypertension. The study also indicates the emergence of multidrug-resistant (MDR) bacteria, specifically *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. These pathogens showed high resistance to aminoglycosides, carbapenems, 2nd, 3rd, and 4th generations of cephalosporins, macrolides, penicillin, penicillin combinations with beta-lactamase inhibitors, sulfonamide/folate synthesis inhibitor combinations, and tetracyclines. The heatmap analysis revealed a complex microbial ecology of VAP, with different groups of pathogens exhibiting varying levels of antibiotic resistance. These findings emphasize the crucial need for vigilant VAP surveillance, the development of effective infection control measures, and careful administration of antibiotics to address the increasing risk of MDR organisms. Using local resistance patterns to guide targeted antimicrobial therapy is critical for improving outcomes of therapy and reducing the emergence of new resistance.

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Data Availability Statement: The datasets generated and/or analyzed during the current study are available in the manuscript itself.

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