







REVIEW ARTICLE

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Pathogenesis, Diagnosis and Therapeutic Strategies for Ventilator-associated Pneumonia

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Abstract

Ventilator-associated pneumonia (VAP) is a major health care associated infection which usually emanates from aspiration, immigration of pathogens from aerodigestive tract, adulterated appliance uses or medications. The mortality rate due to VAP is approximately 13% and the causative organisms are bacteria, viruses, and fungi. Many studies have investigated the causative organisms as *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* with varying prevalence. Intensive Care Unit (ICU) admitted patients who are ventilated, are more prone to the infections where the pathogens adhere to the mucosa of lower respiratory tract of mechanically ventilated patients and start infections. Clinical diagnosis based on Clinical Pulmonary Infection Score (CPIS) has poor specificity and microbiological findings takes 48-72 hrs, that can delay the treatment of patients. Lymphopenia on complete blood count is a predictor of mortality in VAP patients, but decreased lymphocyte count occurs in various other infections too. Multiplex PCR is a better diagnostic technique for VAP which can even diagnose atypical bacteria along with other etiological agents. Effectively employing sampling techniques is a vital step in the diagnosis of VAP, enabling the identification of pathogens responsible for lung infections. Furthermore, the emergence of novel therapeutic options approved by regulatory bodies, adds significant advancements in VAP treatment. In this review article, we have performed an in-depth study on the pathogenesis, diagnosis and therapeutic strategies involved in VAP. This study will help the researchers working in this area to design their work appropriately with the updated knowledge on VAP.

Keywords: Ventilator Associated Pneumonia, Bacteria, Multiplex PCR, Pathogenesis, Therapy

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is a significant infection related to healthcare that occurs when a patient is on mechanical ventilation for two or more consecutive days, where the ventilator tube passes through the patient's mouth and windpipe. The machine that breathes for patients sometimes carries harmful pathogens which infect the lungs, causing pneumonia.¹ VAP is defined as a pneumonia where the patient is on mechanical ventilation for more than 2 days on the date of event, with the day of ventilator placement being day 1, and the ventilator was in place on the date of event or the day before.² VAP typically arises from various sources, including aspiration, the movement of pathogens from the aerodigestive tract, the use of contaminated equipment or medications.³ The mortality rate associated with VAP is around 13%, and diagnosing it early remains a challenge because there is no standard diagnostic method available.^{4,5} The clinical findings and the Clinical Pulmonary Infection Score (CPIS) have limited diagnostic accuracy.⁶ A recent study revealed that lung ultrasonography is a useful tool for assessing lung conditions in the ICU, and the Lung Ultrasound Score (LUS) can be used with confidence to diagnose patients with VAP.⁷

If VAP is not treated promptly and appropriately, it leads to a high mortality rate.⁸⁻¹² Microbial culture technology may take around 48-72 hr to diagnose the VAP, which causes delay in starting the antibiotic therapy, and can increase mortality rate.¹³⁻¹⁶ Besides, many other diagnostic techniques are not sensitive or specific enough to detect the causative agent at an early stage.¹⁷ In the present context, early diagnosis of VAP with suitable technique is very important to initiate the antibiotic therapy targeting the particular microorganisms and saving the life along with the avoidance of antibiotic resistance development.

In this review article, we have discussed thoroughly about the pathogenesis, diagnosis and therapeutic strategies involved in VAP which will help the researchers working in this area to design their future research work effectively with updated informations.

Different microorganisms associated with VAP

Different microorganisms associated with VAP are bacteria (typical and atypical bacteria), viruses and fungi (Table 1, 2, and 3). Many studies have investigated the causative organisms as *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* with varying prevalence.¹⁸⁻²⁰ One study showed that *Pseudomonas aeruginosa* was an etiological agent in 33.3% cases and *Klebsiella pneumoniae* in 20.8% cases of VAP infection, followed by *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus* each causing 8.3% of VAP infections.²¹

VAP usually develops by micro-aspiration of the microorganisms which colonize in the upper respiratory tract in ICU patients. The colonization by pathogens results in the replacement of normal mixed microbial flora by the virulent microorganisms in the upper respiratory tract, which may be monomicrobial or polymicrobial in nature. Studies have revealed that approximately 58% of the total infections are caused by Gram negative bacilli and 20% due to *Staphylococcus aureus* infection.²¹⁻²³ The most common multi-drug resistant (MDR) pathogens included in the VAP infection are *Pseudomonas* spp, *Acinetobacter* spp. and some *Enterobacteriaceae* strains which are extended spectrum β -lactamase (ESBL), AmpC β -lactamase or metallo- β -lactamase (MBL) producers. The aetiological agents vary according to the patient population and the hospital settings. 24 Most of the MDR pathogens have been isolated from late onset VAP compared to early onset VAP and the reason could be a prolonged stay in the hospital and associated prior antibiotic therapy.²¹

Pathogenesis

The ICU admitted patients who are ventilated, have several reasons like critical illness, comorbidities, and malnutrition that affect their immune system.^{46,47} Endotracheal intubations suppress the cough impulse, hamper muco-ciliary clearance, damage the tracheal epithelial surface and make a path for pathogenic bacteria which migrate from upper respiratory tract to lower respiratory tract.⁴⁸⁻⁵⁰

These pathogens then adhere to the mucosa of lower respiratory tract of mechanically

Table 1. List of different microorganisms (typical bacteria) causing ventilator associated pneumonia (VAP)

Pathogens	Specimens collected	Identification techniques	References
<i>E. coli</i>	NB-BAL (Nonbronchoscopic bronchoalveolar lavage), Tracheal aspirates, Blood and Sputum, sinus lavage	Using Microbiological techniques like NB-BAL Culture, Tracheal aspirates culture, Blood Culture, Sputum culture, Gram staining, standard biochemical test, and pleural fluid culture, Kirby-Bauer disc diffusion technique, VITEK® 2 automated system, MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry, and PCR.	(21, 24-40)
<i>Klebsiella</i> spp.	NB-BAL, Tracheal aspirates, Blood, Sputum, and Pleural Fluid.	Using Microbiological techniques like NB-BAL Culture, Tracheal aspirates culture, Blood Culture, pleural fluid culture, Sputum culture, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK® 2 automated system, MALDI-TOF mass spectrometry, and Molecular typing by ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus- Polymerase chain reaction) amplification.	(21, 25-34, 38, 39, 41, 42)
<i>Pseudomonas aeruginosa</i>	NB-BAL, Tracheal aspirates, Blood, pleural fluid, and sputum, sinus lavage	Using Microbiological techniques like NB-BAL Culture, Tracheal aspirates culture, Blood Culture, Sputum culture, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK® 2 automated system, and pleural fluid cultures, and MALDI-TOF mass spectrometry	(19, 21, 24-32, 34, 35, 37-44)
<i>Acinetobacter</i> spp.	NB-BAL, Tracheal aspirates, Blood, pleural fluid, Sputum	Using Microbiological techniques like NB-BAL Culture, Tracheal aspirates culture, Blood Culture by the Bactec method, Sputum culture, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK® 2 automated system, and pleural fluid cultures,	(21, 24-29, 32-35, 37-40, 42)
<i>Citrobacter</i> spp.	NB-BAL, Tracheal aspirates, Blood	Using Microbiological techniques like NB-BAL Culture, Tracheal aspirates culture, Blood Culture by the Bactec method, abdominal radiography,	(21, 25, 32,) 34, 37-40
<i>Staphylococcus</i> spp.	NB-BAL, Tracheal aspirates, Blood, Sputum, sinus lavage	Using Microbiological techniques like NB-BAL Culture, Tracheal aspirates culture, Blood Culture by the Bactec method, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK 2 system, MALDI-TOF mass spectrometry	(19, 21, 25-40, 42)
<i>Enterococci</i> spp.	Sputum, ETA (Endotracheal aspirates), Blood	Sputum culture, endotracheal aspirates culture, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK® 2 automated system, Blood culture	(26-28, 32, 33, 35-38)
<i>Enterobacter</i> spp.	Sputum, ETA, Blood, BAL, sinus lavage	Sputum culture, endotracheal aspirates culture, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK 2 system, MALDI-TOF mass spectrometry, Blood culture	(26, 27, 30, 31, 34, 35, 37, 38-40, 42, 43)
<i>Streptococcus</i> spp.	Sputum, ETA, BAL, sinus Lavage	Sputum culture, endotracheal aspirates culture, Gram staining, standard biochemical test, Kirby-Bauer disc diffusion technique, VITEK 2 system	(19, 24, 26, 27, 31, 37-40)
MRSA (Methicillin-resistant <i>S. aureus</i>)	Sputum, ETA, BAL	Sputum culture, endotracheal aspirates culture, Gram staining, standard biochemical test	(24, 26, 32, 40, 43)

Table 1. Cont...

Pathogens	Specimens collected	Identification techniques	References
MSSA (Methicillin-susceptible <i>S. aureus</i>)	Sputum, ETA, BAL biochemical test	Sputum culture, endotracheal aspirates culture, Gram staining, standard	(24, 26, 32, 40, 43)
<i>Morganella morganii</i>	Respiratory samples	Bacteria were cultured using standard microbiological methods, VITEK 2 system	(27)
<i>Proteus</i> spp.	ETA, BAL	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system, Endotracheal aspirates culture	(21, 24, 27, 32, 36-40)
<i>Pseudomonas fluorescens</i>	Respiratory samples	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system, Direct fluorescent antibody, Soluble antigen testing, PCR,	(27, 44, 40)
<i>Serratia marcescens</i>	Respiratory samples, Blood sample	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system, Blood culture	(27, 33, 35-40, 43)
<i>Listeria monocytogenes</i>	Respiratory samples	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system	(27)
<i>S. maltophilia</i>	Respiratory samples, sinuslavage	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system	(21, 27, 28, 31, 36-38, 40)
<i>Burkholderia pseudomallei</i>	Respiratory samples	VAP was diagnosed based on both the clinical and microbiological criteria	(21)
<i>Providencia</i> spp.	ETA	Endotracheal aspirates culture	(24)
Miscellaneous pathogens	Respiratory samples, sinuslavage	Microbiological culture, Kirby-Bauer disc diffusion technique, VITEK 2 system, sinus lavage culture	(21, 27, 28, 31-34, 36, 39, 40, 43,45)

Table 2. List of different microorganisms (atypical bacteria) causing ventilator associated pneumonia (VAP)

Pathogens	Specimens collected	Identification techniques	References
<i>Haemophilus</i> sp.	ETA, BAL, Blood, Pleural Fluid	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system, Tracheal secretion culture, Bronchoscopy, Blood culture, and pleural fluid cultures	(19, 24, 27, 36-41)
<i>Mycoplasma pneumoniae</i>	ETA, BAL, Blood, Pleural Fluid	Tracheal secretion culture, Bronchoscopy, Blood culture, and pleural fluid cultures	(36)
<i>Moraxella catarrhalis</i>	ETA, BAL, Blood, Pleural Fluid	Bacteria were cultured using standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system, Tracheal secretion culture, Bronchoscopy, Blood culture, and pleural fluid cultures	(27, 41)
<i>Burkholderia cepacia</i>	Respiratory samples, ETA	The microorganisms were identified by standard microbiological methods using MALDI-TOF mass spectrometry, Endotracheal aspirates culture, standard biochemical test, VITEK® 2 automated system	(24, 28, 30)
<i>Aeromonas salmonicida</i>	Respiratory samples	Microbiological culture techniques, VITEK® 2 automated system, standard biochemical test	(28)
<i>Cupriavidus pauculus</i>	Respiratory samples	Microbiological culture techniques, VITEK® 2 automated system, standard biochemical test	(28)
<i>Enterococcus faecalis</i>	Respiratory samples	Microbiological culture techniques, VITEK® 2 automated system, standard biochemical test	(28)
<i>Nocardia</i> spp.	BAL, Sputum, blood, serum,	Gram stain and culture, Mycological culture, Soluble antigen testing, PCR, Ziehl Neelsen stain, Modified Ziehl Neelsen or Kinyoun acid fast stain	(44)
<i>Mycobacterium tuberculosis</i>	BAL, Sputum, blood, serum,	Gram stain and culture, Mycological culture, Soluble antigen testing, PCR, Ziehl Neelsen stain	(44)
Miliary tuberculosis	BAL, Sputum, blood, serum,	Gram stain and culture, Mycological culture, Soluble antigen testing, PCR, Ziehl Neelsen stain, Modified Ziehl Neelsen or Kinyoun acid fast stain	(44)
<i>Legionella</i> spp.	BAL, Sputum, blood, serum,	PCR, culture method, Gram stain, Mycological culture, soluble antigen testing	(44, 45)
non-tuberculous mycobacteria	BAL, Sputum, blood, serum,	Gram stain and culture, Mycological culture, soluble antigen testing, PCR, Ziehl Neelsen stain, Modified Ziehl Neelsen or Kinyoun acid fast stain	(44)
<i>Bosea massiliensis</i>	Respiratory samples	PCR, Culture method.	(45)
<i>Mycoplasma hominis</i>	Respiratory samples	PCR	(36)
<i>Neisseria meningitidis</i>	BAL	Quantitative cultures performed	[40]
<i>Haemophilus parahaemolyticus</i>	BAL	Quantitative cultures performed	(40)

Table 3. List of different microorganisms (fungi) causing ventilator associated pneumonia (VAP)

Pathogens	Specimens collected	Identification techniques	References
<i>Pneumocystis jirovecii</i>	BAL, Sputum, blood, serum,	Gram stain and culture, Mycological culture, soluble antigen testing, PCR,	(44)
<i>Aspergillus</i> spp.	BAL, Sputum, blood, serum,	Gram stain and culture, Mycological culture, soluble antigen testing, PCR,	(44)
<i>Cryptococcus</i> spp.	BAL, Sputum, blood, serum,	India Ink Preparation, Gram stain and culture, Mycological culture, soluble antigen testing, PCR, Cryptococcal antigen.	(44)
<i>Candida</i> spp.	ETA, Blood sample, sinus lavage	Endotracheal aspirates culture, Blood culture, standard microbiological methods, Kirby-Bauer disc diffusion technique, VITEK 2 system, sinus lavage culture on Sabouraud's dextrose agar	(21,24,27, 31-33, 36, 42)

ventilated patients and start infections. Generally, four routes are responsible for providing access to microorganisms in order to produce VAP infections a) Aspiration from oropharynx or from stomach into oropharynx then into the lower respiratory tract,⁵¹⁻⁵³ b) Extension of contiguous infection,⁵⁴ c) Contaminated air or ICU aerosol inhalation,⁵⁵ and d) Vascular or urinary catheter-related blood-stream infection seeding the lungs.⁵⁶⁻⁵⁸

Few studies have revealed that use of contaminated respiratory therapy equipment in bronchoscopy and endoscopy can also be the reason for VAP infection.^{55,59} Critically ill patients in the ICU get exposed with these contaminated equipments and the ICU aerosol inhalation makes changes in their oral flora dramatically to aerobic gram-negative bacilli and *Staphylococcus aureus* microorganisms.⁶⁰ These pathogenic bacteria adhere to the orotracheal mucosa of ventilated patient, which is eased by reduced IgA and increased protease production, exposed and denuded mucous membranes, elevated airway pH, and increased number of airway receptors for bacteria.⁶¹

Some studies showed that gastric pathogens can also be the source for VAP infections.⁵¹ The number of pathogens enhances when the pH level increases inside the gastric tract.⁶²⁻⁶⁵ It is revealed that the gastric pathogens or gastric contents can be aspirated to the lower respiratory tract and cause direct or indirect VAP infections.⁶⁶⁻⁷⁰

Pathogens can also make biofilms near the endotracheal tube and become highly resistant to antibiotics and host defense mechanisms. Many antibiotic-resistant nosocomial pathogens

colonize in the biofilms.^{71,72} The presence of an endotracheal tube is one of the major culprits for VAP development: air flow moves pathogens toward the distal airways, while clearance of the trachea is blunted due to reduced tracheal ciliary movement and impaired cough.⁷³

It is found that unsterilized contaminated hospital water and air can also infect critically ill patients.⁶¹ Nosocomial infections can spread through water which is contaminated with bacteria, mycobacteria, fungi, and parasites.⁷⁴

Sampling methods for the diagnosis of VAP

Several sampling methods are available for the diagnosis of VAP, but the choice of sampling method may affect the accuracy and reliability of the results. In this context, we have discussed here the various sampling methods available for the diagnosis of VAP, their advantages and disadvantages, and the evidences supporting their use.

Endotracheal aspirate (ETA)

ETA is the most commonly used sampling method for the diagnosis of VAP. This method involves suctioning secretions from the endotracheal tube and is relatively easy to perform. ETA has high sensitivity and specificity, and it is less invasive than other sampling methods. However, ETA may not accurately reflect the lower respiratory tract microbiome, and contamination with oropharyngeal flora may lead to false-positive results.⁷⁵ The use of quantitative cultures can improve the accuracy of ETA-based diagnosis of VAP. A threshold of 10⁶ CFU/mL is often used to differentiate between contamination and

true infection.⁷⁶ A meta-analysis of 25 studies comparing the accuracy of ETA and BAL for the diagnosis of VAP showed that ETA had a sensitivity of 75.7% and a specificity of 67.9%, while BAL had a sensitivity of 71.1% and a specificity of 79.6%.⁷⁷ ETA remains a useful and practical method for the diagnosis of VAP in resource-limited settings.

Bronchoalveolar Lavage (BAL)

BAL involves the instillation and aspiration of sterile saline into the lower respiratory tract. BAL provides a more accurate representation of the lower respiratory tract microbiome and is more specific for the diagnosis of VAP than ETA.⁷⁸ However, BAL is more invasive and requires more expertise and resources to perform. A threshold of

10^4 CFU/mL is often used to diagnose VAP based on BAL samples.⁷⁶ A meta-analysis of 25 studies comparing the accuracy of BAL and PSB for the diagnosis of VAP showed that BAL had a sensitivity of 71.1% and a specificity of 79.6%, while PSB had a sensitivity of 61.4% and a specificity of 76.5%.⁷⁷ BAL is considered the gold standard for the diagnosis of VAP, but its use is limited by its invasiveness and the need for specialized equipment and expertise.

Protected Specimen Brush (PSB)

PSB involves the insertion of a sterile brush through the endotracheal tube into the lower respiratory tract. The brush is then rotated to collect a sample from the bronchial mucosa.

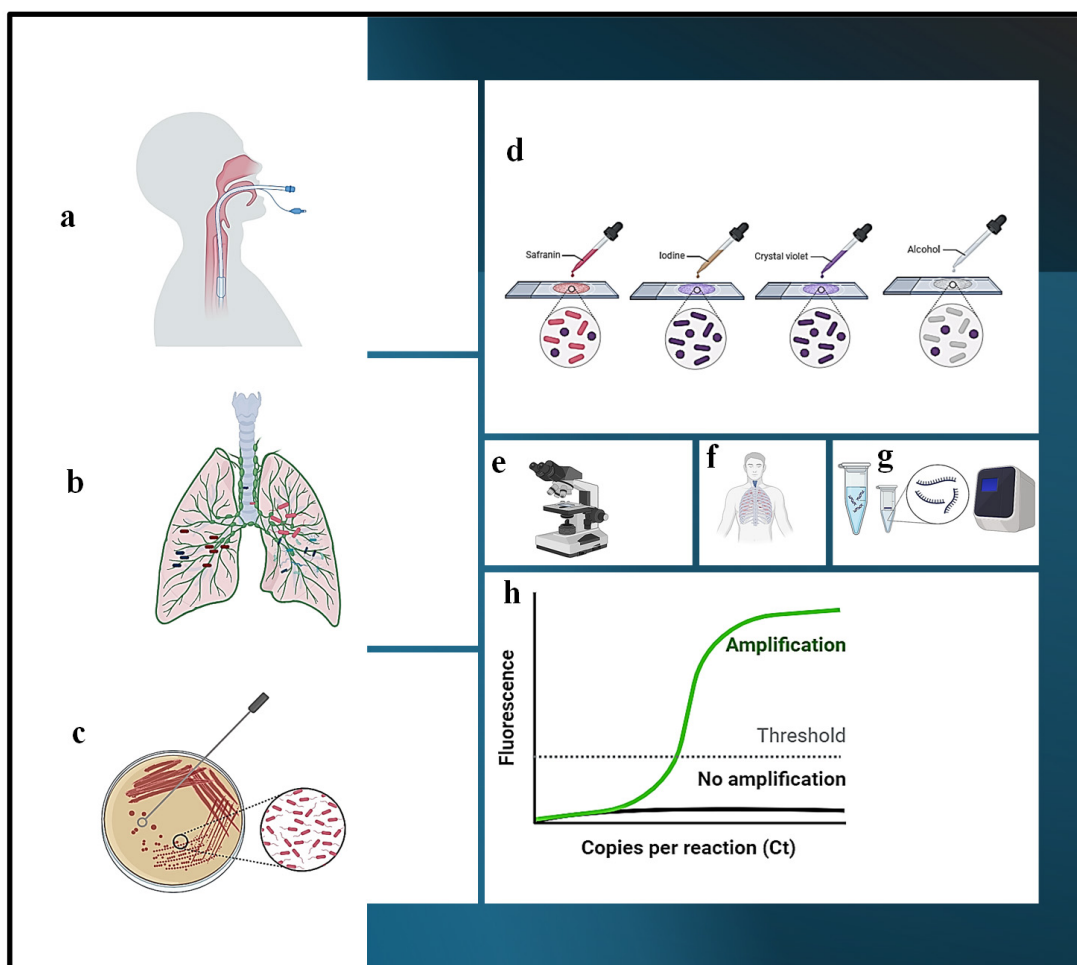


Figure 1. Diagrammatic representation of various diagnostic techniques for VAP infection: a) Intubated patient, b) Infected lung, c) Microbiological culture, d) Gram's staining, e) Microscopic examination, f) X-ray diagnosis, g) Polymerase Chain Reaction, h) Real-Time PCR

PSB has similar sensitivity and specificity to BAL, and it is less invasive than BAL. However, PSB may cause bleeding or trauma to the respiratory tract. A threshold of 10^4 CFU/mL is often used to diagnose VAP based on PSB samples. A meta-analysis of 25 studies comparing the accuracy of BAL and PSB for the diagnosis of VAP showed that BAL had a higher sensitivity than PSB but nearly similar specificity.⁷⁷

Mini-BAL

Mini-BAL is a modification of the BAL technique that uses a smaller volume of sterile saline to lavage the lower respiratory tract. Mini-BAL has similar sensitivity and specificity to BAL and is less invasive than BAL. However, it may be

less accurate than BAL in patients with high lung compliance. A threshold of 10^4 CFU/mL is often used to diagnose VAP based on mini-BAL samples. A randomized controlled trial comparing the accuracy of mini-BAL and BAL for the diagnosis of VAP showed that mini-BAL had a lower sensitivity and similar specificity than BAL.⁷⁹

Diagnostic methods for VAP

The diagnosis of VAP is typically made based on a combination of clinical and radiographic findings, as well as laboratory testing (Figure 1). It is crucial to diagnose the VAP as early as possible and adopt effective strategies to curb the infections and manage the condition (Figure

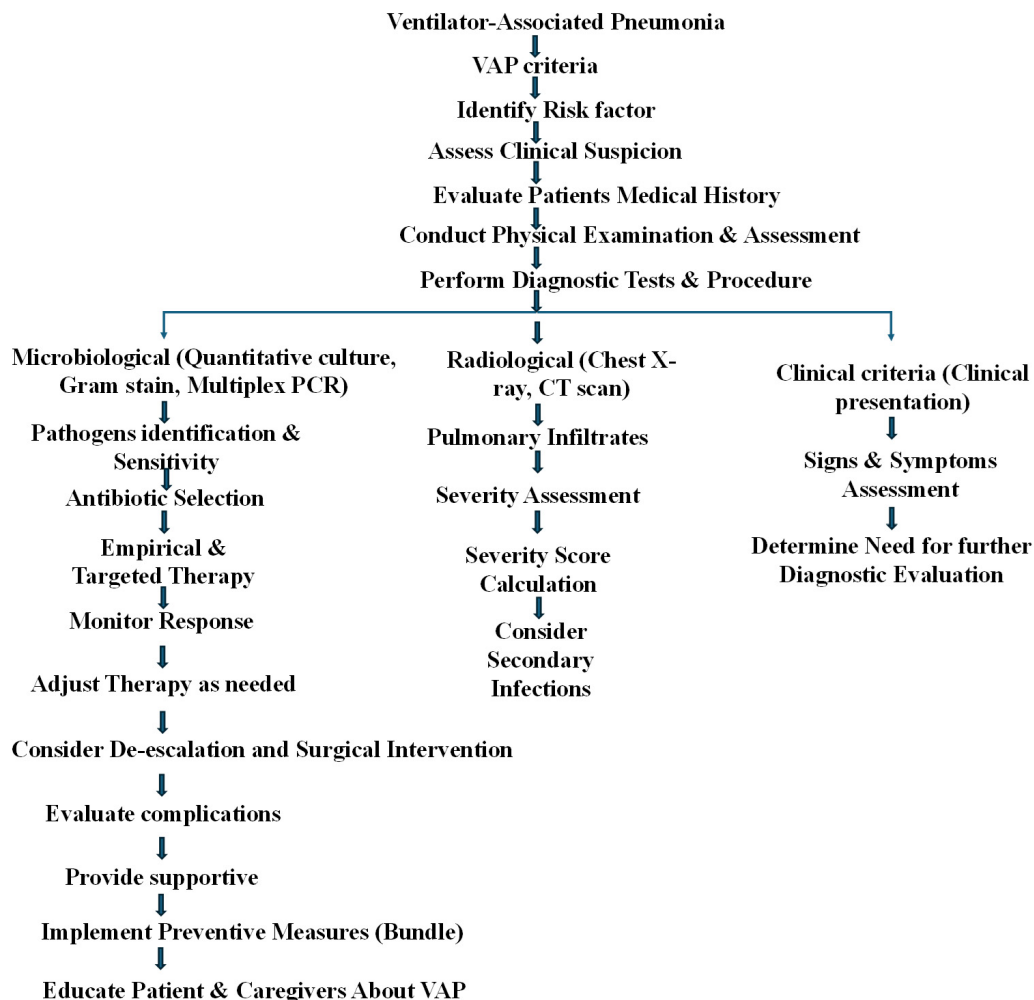


Figure 2. Illustrates VAP criteria, various diagnostic techniques, their associated risk factors, and patient management strategies

2). Some of the common diagnostic criteria used to diagnose VAP are discussed here.

Clinical, radiological, and microbiological criteria

Clinical criteria: The patient should have a new or progressive infiltrate on chest X-ray, along with two or more of the following: fever ($>38^{\circ}\text{C}$), leukocytosis ($>10,000$ cells/ mm^3), purulent sputum, or a decrease in oxygen saturation. **Radiological criteria:** The patient should have a new or progressive infiltrate on chest X-ray, with consolidation or cavitation, and/or a new or persistent infiltrate on computed tomography (CT) scan of the chest. **Microbiological criteria:** The patient should have a positive culture from a lower respiratory tract sample, such as bronchoalveolar lavage (BAL) or endotracheal aspirate (ETA), with a significant growth of pathogenic bacteria ($>10^4$ CFU/mL).⁷⁶

It is important to note that the diagnosis of VAP can be challenging, as many of the clinical and radiographic findings can be non-specific and may be present in other respiratory conditions. Therefore, it is essential to rule out other potential causes of respiratory symptoms, such as acute lung injury, acute respiratory distress syndrome, and pulmonary embolism. In addition to the above criteria, physicians should consider the patient's clinical history, risk factors, and response to treatment when making a diagnosis. Early diagnosis and prompt treatment with appropriate antibiotics are critical for improving outcomes in patients with VAP. Etiological diagnosis of VAP is very challenging and limited diagnostic tests are available so far (Figure 1). Broad differential diagnosis is performed for the confirmation of VAP infections in patients suffering from increasing oxygen requirements, leukocytosis, fever, and productive cough. Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) suggests that clinical pulmonary infection score (CPIS) can help determine the presence or absence of VAP and its management to some extent.^{80,81} The CPIS score of >6 (between 0-12) shows good amount of VAP infection.^{17,82}

VAP diagnosis through the clinical indicators like fever, purulent secretions, leukocytosis, chest radiography, routine aerobic cultures from ETA, PSB (protected specimen brush) and BAL samples, and CPIS had poor

diagnostic specificity. Presence of any of these indicators might misguide the antibiotic therapy to the patients. Clinicians need to have better tools along with these clinical findings which will help in proper antibiotic therapy.⁷⁸ Limited studies explain the usefulness of CPIS score for VAP detection. Based on CPIS score irrational antibiotic prescription may occur.⁸³⁻⁸⁶

Microbial culture

The microbiological diagnosis of VAP is typically made through the collection and analysis of respiratory secretions from the lower respiratory tract. The goal of microbiological diagnosis is to identify the causative organism(s) responsible for the infection, so that appropriate antibiotic therapy can be initiated. There are several methods used for collecting respiratory secretions in VAP, including bronchoalveolar lavage (BAL), protected specimen brush (PSB), and endotracheal aspirate (ETA).⁷⁶ Each method has its own advantages and disadvantages, and the choice of method may depend on factors such as the severity of illness, the patient's clinical status, and the availability of resources. Once respiratory secretions have been collected, Gram staining is performed and further the samples are cultured for antibiotic sensitivity testing. Gram staining involves staining a sample of respiratory secretions with crystal violet and iodine, then decolorizing with alcohol and counterstaining with safranin. The resulting staining pattern can help identify the type of bacteria present in the sample. Gram staining is quick and easy to perform, but it has a low sensitivity and specificity for diagnosing VAP, and it cannot identify specific bacterial species. The results of microbiological testing can take several days to become available. In the meantime, broad-spectrum antibiotic therapy is usually initiated based on the patient's clinical presentation and risk factors for infection. Once the results of microbiological testing are available, antibiotic therapy may be adjusted based on the specific pathogens identified and their susceptibility to antibiotics.^{87,88} Microbiological culture techniques are performed using different types of nutrient support such as nutrient agar, blood agar, chocolate agar, MacConkey agar, and sabouraud dextrose agar. Selective media is used for bacterial isolates and CHROM agar (Chromogenic culture

media) is used for fungi (*Candida* species is commonly isolated fungi). Microbial culture is the gold standard for diagnosis of VAP, but it can take several days to obtain results, and it may be subject to contamination with other microorganisms.⁸⁹

It is important to note that microbiological diagnosis of VAP can be challenging, as respiratory secretions may be contaminated with normal flora or other opportunistic pathogens. In addition, antibiotic therapy prior to sample collection may interfere with the ability to isolate and identify pathogens. Many organisms implicated in VAP are fastidious in nature and difficult to culture using routine culture methods. Viral agents require special cell lines, technical expertise and infrastructure for their detection. Hence, traditional culture methods are less sensitive and detect small range of etiological agents. Therefore, careful interpretation of microbiological results is important, and the diagnosis of VAP should be made based on a combination of clinical, radiographic, and microbiological findings.

Antigen testing

This technique involves detecting specific bacterial antigens in a sample of respiratory secretions using immunoassay methods. Antigen testing is quick and easy to perform, and it can provide results within hours. However, it has a lower sensitivity and specificity than culture or PCR, and it may be subject to cross-reactivity with non-pathogenic bacteria.

Multiplex PCR

Early Diagnosis of VAP with the help of multiplex PCR can resolve the time barrier for the treatment of VAP patients. Multiplex PCR can detect very low levels of pathogenic nucleic acid with high specificity and independent of viability of the target microbe. Moreover, supplementary information like presence of antibiotic resistance genes can also be gathered by this technique.⁹⁰ Previous antibiotic treatments, poor infection control practices and irrational antibiotic use may cause pathogens to become antibiotic resistant. Multidrug resistance due to production of ESBL, AmpC or MBL by these bacteria add to the morbidity, making the infection even more difficult to treat.^{28,91} About 50% of VAP is polymicrobial; making it difficult to define the bacteria having

particular role in VAP. Hence, appropriate and rapid diagnosis is important to adequately manage this condition.

Molecular techniques may overcome this problem. Over the past two decades PCR technology has become an important tool of molecular diagnosis of clinical pathogenic samples. Molecular methods are more sensitive and rapid so that the treatment can be initiated at an early. Apart from being expensive and highly sophisticated, one of the important limitations of molecular techniques is that they can detect both dead and as well as live organisms which demands clinical correlation of the test results. However, multiplex PCR technique has shown to be a valuable method for simultaneous identification of viruses, bacteria, fungi, and parasites and has the potential to save time and effort without compromising the accuracy.

According to Buchan *et al.*, standard culture methods are mostly unable to detect respiratory infectious pathogens because of the polymicrobial nature and simultaneous detection of viral agents is also necessary for urgent identification of hospital-acquired and ventilator-associated pneumonia.⁹² To get rid of all these problems scientists have implemented semiquantitative BioFire FilmArray Pneumonia panel (PN panel) test (multiplex PCR). All bacterial targets reported as $>10^5$ CFU/mL in culture were reported as $\geq 10^5$ genomic copies/ml by the PN panel.⁹² The BIOFIRE® FILMARRAY® Pneumonia plus Panel can be used for rapid and accurate automated testing of pneumonia by detection of LRTI (lower respiratory tract infections) causing bacteria, viruses along with seven genetic markers for antibiotic resistance. In this technique hands-on time is only 2 minutes and no pipetting is required. The turnaround time is 1 hr and it can detect 34 targets simultaneously. The sputum/BAL/ET samples could be analysed by PNplus Panel (BioFire Diagnostics, USA) system which is a multiplex PCR system used to determine 9 viruses (coronavirus, human metapneumovirus, enterovirus, influenza A and B virus, parainfluenza virus, respiratory syncytial virus, MERS virus, and adenovirus), 3 atypical pathogens (*C. pneumoniae*, *L. pneumophila*, and *M. pneumoniae*), 15 bacteria (*Acinetobacter calcoaceticus-baumannii* complex, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella*

oxytoca, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Moraxella catarrhalis*, *Proteus* spp., *Serratia marcescens*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*), and 7 resistance genes i.e., CTX-M, KPC (*Klebsiella pneumoniae* carbapenemase), OXA-48-like (oxacillinase), NDM (New Delhi metallo- β -lactamase), VIM (Verona Integron-encoded MBL), IMP (Imipenemase), *mecA/C/MREJ* (methicillin resistance geneA/methicillin resistance geneC/mec right-extremity junction). The sensitivity and specificity reported for this method by using sputum samples are 96.3% and 97.2%, respectively.⁹³

Study suggested that the Unyvero platform (Curetis AG, Holzgerlingen, Germany) is a multiplex PCR method used to diagnose severe infectious pathogens within 5 hrs. The Unyvero Hospitalized Pneumonia Multiplex PCR panel detects 21 bacteria (including Enterobacterales, *P. aeruginosa*, *S. maltophilia*, *S. aureus* etc.) with 21 antimicrobial resistant genes including ESBL (Extended-spectrum beta-lactamases), carbapenemases, *mecA* gene, *mecC*, blaOXA-23, blaOXA-24, blaOXA-48, blaOXA-58, blaVIM, blaIMP, blaKPC, blaNDM, and blaCTX-M. It is observed that multiplex PCR is suitable for the diagnosis of VAP/HAP (Hospital acquired pneumonia) by detecting the pathogens very fast and thus it can aid to early antimicrobial therapy.⁹⁴ The Unyvero pneumonia cartridges correctly detected the VAP causing pathogens for 73% of the episodes and identified the resistance mechanism in 67% of them with *P. aeruginosa* having the highest discordance rate.⁹⁵ Recently VAPs were diagnosed with various types of multiplex PCR platforms with different sensitivity and specificity. One of the studies performed to evaluate the performance of rapid screening test, GeneXpert MRSA/SA ETA (Methicillin-resistant *Staphylococcus aureus*/ *Staphylococcus aureus* Endotracheal aspirates) assay test (in patients assuming at risk of VAP) compared with 3 cultures based and nuc gene-based qRT-PCR test (thermostable nuclease gene based quantitative Realtime PCR) for the detection of *S. aureus* in ETA samples of mechanically ventilated patients. GeneXpert MRSA/SA ETA assay rapidly and accurately diagnosed *S. aureus* directly from ETA samples. *S. aureus* is a common

pathogen that causes VAP infections with increased mortality and morbidity rate. *S. aureus* (prior colonization with potential pathogens) plays a significant role for the development of nosocomial infections. The GeneXpert MRSA/SA ETA was a rapid and sensitive technology for the identification of *S. aureus* in ETA samples. GeneXpert assay machine (RMRS/SA-ETA-10, Cepheid, USA) detects sequences within the staphylococcal protein A (*spa*) gene, the methicillin resistance gene (*mecA*), and staphylococcal cassette chromosome (*SCCmec*) gene inserted in attB insertion site (*S. aureus* chromosome). Extraction of DNA, amplification and the detection of the targets (by GeneXpert machine) was completed within 66 min. The nuc gene-based qRT-PCR was performed to target nuc gene, with SANucF2 (TAAAGCGATTGATGGTGATACG), and SANucR2 (TTCTTTGACCTTTGTCAAACCTCG) primers (200 nM) and TaqMan probe (cy5-TGGTCCTGAAGCAAGTGCATTACg-BBQ) to identify the *S. aureus*.⁹⁶ The Xpert Carba-R detection is also performed with GeneXpert machine (Cepheid) to identify the resistance gene carbapenemase (from carbapenemase-producing organisms). The Xpert Carba-R (Carbapenem Resistance Molecular Test) detects 5 targets for carbapenemase-producing organisms (blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP-1).⁹⁷ One of the previous studies was aimed to determine the OXA resistance gene and ISAb-1 gene by molecular typing (the repetitive extragenic palindromic sequence-based PCR in short called 'rec-PCR'). In this study, the nosocomial MDR *A. baumannii* isolates were highly resistant to antibiotics and carry the blaOXA-23 resistance gene. The analysis of these isolates by rep-PCR revealed that they were very closely related strains of the same origin, recently separated from each other.⁹⁸ Study of Morris *et al.* has assessed the diagnostic accuracy of a novel application for 16S PCR. This study revealed that 16S PCR in BAL could be used as a rapid test in suspected VAP and may allow for judicious use of antibiotics.⁹⁹

Hematological assay

Complete blood count also aids to the diagnosis of VAP and many studies suggest that VAP patients have decreased lymphocyte count (lymphocytopenia) which predicts bad

outcome. When a patient acquires pneumonia from hospital, the marked changes observed in the patient are immunological changes, along with lymphocytopenia.¹⁰⁰⁻¹⁰³ Patients with sepsis also exhibit decreased lymphocyte count. This lymphopenia can be attributed to the recruitment of lymphocytes in the site of infection or their destruction at the site of inflammation or infection due to apoptosis.^{104,105} At the time of diagnosis, <595 cells/mm³ of lymphocyte count is considered as a risk factor for mortality (approx. 90 days) in VAP patients.¹⁰⁶

Flow cytometric analysis

Flow cytometric analysis in the pneumonia infected mouse revealed that CD4⁺ T-helper 17 (Th17) cells and interleukin (IL)-17A play an important role in clearing pathogens. Based on these observations, it has been hypothesized that number of Th17 cells and level of IL-17A implicate the risk of nosocomial pneumonia in humans. The Th17 cells could be a protective agent from development of nosocomial pneumonia in patients having mechanical ventilation.¹⁰⁷ Study by Ma *et al.* showed that hyper-IgE syndromic patients are deficient with healthy Th17 cells.¹⁰⁸ Similarly, Milner *et al.* observed depleted Th17 cell number in HIV patients.¹⁰⁹ A study by Paats *et al.* revealed that in community acquired pneumonia (CAP) patients, BAL and peripheral blood have significantly increased IL-17A⁺IL-22⁺ cells compared to healthy controls.¹¹⁰ In the early stage of sepsis, patients may suffer from systemic inflammatory response syndrome (SIRS) with a “cytokine storm” also called as CARS (Compensatory anti-inflammatory response syndrome), which may lead to immune paralysis. CARS may lead to increased susceptibility to secondary infections by immune cell apoptosis, impaired lymphocyte and phagocyte function, and the phenotypic shift from Th1 to Th2.^{107,111-113}

Mass spectrometry analysis

Mass spectrometry (MS) is an essential analytical technology for the modern health care laboratories because of its high sensitivity, selectivity and suitability for quantitative evaluations.¹¹⁴ Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

(MALDI-TOF MS) drastically shortens the time for identification of the pathogens, thereby initiating early antibiotic therapy. Management of VAP patients by early identification of causative pathogens is very crucial and mass spectrometry plays essential role in this aspect.¹¹⁵ MALDI-TOF MS identification can be failed if we proceed with complex, faintly multi-contaminated biological matrix. Moreover, this technique is not compatible with ETA samples for the identification of bacteria.¹¹⁵ ESI-MS (Electrospray Ionisation-Mass Spectrometry) has the potential to diagnose VAP samples within 6 hr and identify the species of bacteria that usually cause VAP, prior to clinical diagnosis. Periodical monitoring of pulmonary specimen through PCR/ESI-MS has clinical benefits for ventilated subjects by guiding appropriate and adequate initial level of antibiotic therapy to attain better outcomes and lower the use of broad-spectrum antibiotics.¹¹⁶

Gene and protein expression studies

Studies have shown that VAP is associated with up and down regulation of certain genes. It is found that overexpression of tumor necrosis factor- α (TNF- α) gene is associated with a 2.1 to 13-fold increase in the development of severe sepsis from various causes including pneumonia. PIK3R3 (Phosphoinositide-3-Kinase Regulatory Subunit 3) encodes for phosphoinositide 3-kinase regulatory subunit gamma (PI3Ky) and it is predominantly expressed in immune cells and plays a role in chemoattractant-induced cell migration. Furthermore, genome wide screening approach and microarray analysis would be useful for identification of new genetic factors that are associated with the development of infection in VAP. To identify differentially expressed genes in patients who develop VAP compared to similar patients who do not develop VAP, a logistic regression model was developed with five genes like PIK3R3, ATP2A1 (ATPase Sarcoplasmic/Endoplasmic Reticulum Ca²⁺ Transporting 1), PI3, ADAM8 (ADAM Metalloproteinase Domain 8) and HCN4 (hyperpolarization activated cyclic nucleotide-gated potassium channel 4) and was able to accurately categorize 95% of patients that developed VAP.¹¹⁷ ADAM8 gene encodes a disintegrin and metalloproteinase domain-

containing protein 8 which helps in cell adhesion. HCN4 encodes for the hyperpolarization activated cyclic nucleotide-gated potassium channel for the cardiac rhythm maintenance. The over-expression of HCN4 gene is significantly associated to the infectious complications in VAP patients. The gene ATP2A1 encodes for the sarcoplasmic reticulum calcium transporting ATPase (SERCA1) in the skeletal muscle. Lipopolysaccharides (LPS) can cause myocardial dysfunction in ICU patients and that could be the reason SERCA comes into the picture related to cardiac relaxation. In VAP positive patients ATP2A1 was found to be down regulated after LPS stimulation.^{117,118}

Therapeutic strategies

Antibiotic therapy

The use of antibiotics is the mainstay of treatment for VAP, with the aim of eradicating the causative organisms and reducing morbidity and mortality. However, the selection of appropriate antibiotics and the duration of treatment are critical to optimize outcomes while minimizing the emergence of antibiotic resistance. Appropriate antibiotic therapy in VAP patients depends on duration of mechanical ventilation, late onset VAP (> 4 days) or early onset VAP (< 4 days) and the type of pathogens detected in culture and molecular techniques. Selection of best possible antibiotics for the treatment of VAP patients is also assisted by clinical information, such as patient risk factors/comorbidities and previous antibiotic exposure.¹¹⁹

Proper antibiotic treatment is very challenging for VAP patients, because of the traditionally followed techniques for the diagnosis which takes 24-48 hrs for the confirmation of pathogenic agents. Treatment of patients empirically or without the knowledge of pathogens, can help pathogens to become multidrug resistant. Knowing all this, the American Thoracic Society (ATS) currently recommends antibiotic therapy based on the patients' risk of colonization by an organism with multidrug resistance. Inadequate initial antimicrobial treatment is directly associated with increased mortality and longer ICU stay. Study showed that the pathogens associated with initial inappropriate empiric antimicrobial therapy are *Pseudomonas aeruginosa*, *Acinetobacter* species, *Klebsiella pneumonia*, *Enterobacter* species, and MRSA. Delayed/inappropriate initial antibiotic

therapy can lead to fatality in critically ill patients with infections, including VAP.¹

Empirical treatment of VAP patients starts very soon, after the detection of pathogens through microscopic findings. However, the treatment should be readjusted according to culture findings as soon as the results are available. The criteria for the selection of antibiotic treatment to the patients is determined by the type of pathogens detected, patients' history of exposure to antibiotics and comorbidities, and compatibility with particular antibiotics type. The type of pathogens treated according to their role in different type of patients, like MSSA (Methicillin sensitive *Staphylococcus aureus*) is frequently seen in coma patients. The bacteria MRSA (Methicillin resistant *Staphylococcus aureus*) is not seen in patients who haven't exposed with antibiotics. Combination of antibiotic therapy should be prescribed in *Pseudomonas aeruginosa* infection. Antifungal therapy is given in fungal infections but not recommended in non-neutropenic patients, if intubated. Gram-positive bacteria should be treated with vancomycin in VAP infection.¹¹⁹ Combined treatment with meropenem and imipenem, ciprofloxacin and vancomycin seem to be appropriate and could cover all possible infective agents. To reduce mortality rate, reasonable prescription of antibiotics and corticosteroids could be effective.²⁶ Knowledge on local distribution of pathogens and their resistance pattern can also determine the empirical treatment of VAP patients. According to Grief and Loza, the antibiotics that can be prescribed with early onset VAP without associated MDR risk factors, are ceftriaxone, fluoroquinolone, ampicillin-sulbactam, and ertapenem.¹²⁰ They further advocated that for late onset/MDR patients, prescription should include individual or combination of antibiotics such as antipseudomonal cephalosporins (Cefepime, ceftazidime), antipseudomonal carbapenems (imipenem or meropenem), beta-lactam/beta-lactamase inhibitors (piperacillin-tazobactam) with an antipseudomonal fluoroquinolone (ciprofloxacin) or aminoglycoside plus linezolid or vancomycin (For MRSA), Telavancin is indicated for VAP for susceptible isolates of *S. aureus* (when other therapies are not suitable).¹²⁰

Antibiotics treatment duration

The optimal duration of antibiotic treatment for VAP remains a topic of debate among clinicians. Traditionally, a 10–14 day course of antibiotics has been recommended for VAP, but recent evidence suggests that shorter courses may be equally effective and associated with fewer adverse effects and less chance of emergence of antibiotic resistance. The Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) issued guidelines in 2016 that recommend a duration of 7 days for most patients with VAP, with the possibility of shorter courses (i.e., 5–7 days) in selected patients based on clinical and microbiological response.⁵ The guidelines suggest that the decision to shorten the duration of treatment should be based on the following factors:

1. The patient's clinical stability, as evidenced by resolution of fever, improvement in oxygenation, and stable vital signs.
2. The patient's microbiological response, as evidenced by clearance of the causative organism(s) from respiratory specimens.
3. The absence of comorbidities or immunosuppression that could increase the risk of treatment failure or relapse.
4. The presence of biomarkers (such as procalcitonin) that indicate a low risk of ongoing infection.

Several studies have investigated the efficacy and safety of shorter courses of antibiotics for VAP. A randomized controlled trial by Chastre *et al.* compared a 15-day course of antibiotics to a 8-day course in patients with VAP caused by Gram-negative bacilli. The study found no significant difference in clinical cure rates or mortality between the two groups, but the 8-day group had a lower incidence of superinfections and fewer days of antibiotic exposure.¹²¹

Another randomized controlled trial by Uranga *et al.* compared a 5-day course of antibiotics to a 10-day course in patients with VAP caused by Gram-negative bacilli. The study found no significant difference in clinical cure rates or mortality between the two groups, but the 5-day group had a shorter duration of mechanical ventilation and a lower incidence of antibiotic-related adverse effects.¹²²

However, it is important to note that shorter courses of antibiotics may not be appropriate for all patients with VAP, particularly those with comorbidities, immunosuppression, or polymicrobial infections. In addition, the decision to shorten the duration of treatment should be based on clinical judgment and individualized for each patient.

Antibiotics commonly used to treat VAP patients Cefoperazone–Sulbactam

Cefoperazone-sulbactam is a combination of a third generation cephamycin and a traditional β -lactamase inhibitor. It exhibits effectiveness against Enterobacterales and *Pseudomonas* spp. The inclusion of sulbactam in the combination extends its activity to include *Acinetobacter* and anaerobic bacteria. Additionally, sulbactam enhances the stability of cefoperazone against certain β -lactamases and reduces the impact of the high inoculum effect. The recommended dosage for treatment is 2g administered every 12 hours for a duration of 7 days.¹²³

Tedizolid

Tedizolid operates by attaching itself to a specific region called the 23S rRNA within the 50 S subunit of the bacterial ribosome. This binding action has the effect of preventing the formation of the 70 S initiation complex, which in turn disrupts the normal process of protein synthesis within the bacteria. As a result, the growth and proliferation of the bacteria are inhibited.¹²⁴

The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have both granted approval for the use of tedizolid in treating acute bacterial skin and skin structure infections (ABSSSIs). It can be administered either orally or intravenously, with a recommended daily dose of 200 mg, for a duration of six days.¹²⁵

Tedizolid demonstrates activity against a wide range of gram-positive pathogens, including vancomycin-resistant *Enterococcus* species, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *S. aureus*. When compared to another drug called linezolid, tedizolid has been found to be significantly more potent against various gram-positive pathogens, ranging from four to eight times more effective.¹²⁶⁻¹³⁰ It has

shown promising results as a therapeutic agent to treat infections caused by MRSA or methicillin-susceptible *S. aureus* in individuals with cystic fibrosis.¹³¹ At a minimum inhibitory concentration (MIC) of less than 0.5 mg/L, tedizolid has the ability to inhibit the growth of 99.7% of bacterial isolates tested.¹³² Furthermore, it is effective against *S. aureus* and *Streptococcus pneumoniae*, two commonly encountered bacteria in clinical infections.¹³³

Ceftaroline-Avibactam

Ceftaroline, a fifth-generation cephalosporin, exhibits strong efficacy against common respiratory pathogens such as MRSA, PRP, and non-ESBL producing Enterobacterial strains. However, it demonstrates limited effectiveness against anaerobes, ESBL and AmpC producing strains such as *A. baumannii*, and *P. aeruginosa*.¹³⁴ When ceftaroline is combined with avibactam, it effectively counteracts the breakdown caused by class A (including ESBLs and KPC), class C (AmpC), and certain class D beta-lactamases, while simultaneously preserving its activity against gram-positive bacteria. This medication is utilized for the treatment of HAP/VAP.¹²³

Plazomicin

This is a newly developed semisynthetic aminoglycoside that exhibits resistance to inactivation by aminoglycoside-modifying enzymes. It demonstrates effectiveness against a significant portion of carbapenem-resistant Enterobacterales (CRE),¹³⁵ specifically over 95% of Enterobacterales strains with a susceptibility breakpoint below 2 mg/L.¹³⁶ However, it is susceptible to the impact of 16S rRNA methyltransferase.¹³⁷ No efficacy has been observed against anaerobes, *Enterococcus*, *Streptococcus*, and *Stenotrophomonas*.¹³⁸ The drug has received FDA approval for intravenous administration at a dosage of 15mg/kg, for the treatment of complicated urinary tract infections (cUTI) caused by aerobic gram-negative bacteria, including AP (acute pyelonephritis).¹²³

Aztreonam-Avibactam

Aztreonam is the sole beta-lactam compound that exhibits activity against metallo-beta-lactamases (MBL). However, it is vulnerable to hydrolysis by the majority of ESBLs or AmpC

enzymes, which are frequently co-produced in carbapenem-resistant strains.¹³⁹ When combined with avibactam, aztreonam gains stability against most multidrug-resistant (MDR) pathogens, including those carrying class A, C, and D beta-lactamases.^{140,141} Aztreonam-avibactam effectively inhibits 99.9% of Enterobacterales, even in the presence of NDM, KPC, or OXA-48 producers.¹⁴²⁻¹⁴⁴ Over 75% of tested isolates of *P. aeruginosa* exhibited *in vitro* susceptibility to aztreonam-avibactam, with a MIC value below 8 mg/L.¹⁴² Although the drug has not yet received approval from the FDA or EMA, clinical trials are underway for the treatment of severe gram-negative infections. However, until more robust data is available, aztreonam has been utilized in combination with ceftazidime-avibactam to treat serious infections caused by MBL-producing strains.¹²³

Eravacycline

Eravacycline is a newly developed fluorocycline compound that shares a structural similarity with tigecycline. It is accessible in both oral and intravenous forms. Eravacycline demonstrates a broad spectrum of activity, encompassing both gram-positive and gram-negative bacteria, as well as anaerobic bacteria, except for *P. aeruginosa*. Notably, eravacycline exhibits effectiveness against *A. baumannii* isolates that are resistant to sulbactam. During treatment with eravacycline, there have been reports of a low incidence of *C. difficile* infection.¹²³

Some examples of newly developed drug combinations that are used for treatment of VAP with promising results, are ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, imipenem-relebactam cilastatin, and cefiderocol.¹²³

Phyto-therapeutic approach

Phyto-therapeutic treatment can be the best alternative treatment for antibiotics against VAP infection as they have no side effects, and no chance of development of antibiotic resistant microorganisms. Since the ancient times people are using plant products as medicines for eradication of lung infection, chronic bronchitis, sinusitis, and respiratory tract mycoses.¹⁴⁵⁻¹⁴⁷ The essential oil and phytochemicals derived from medicinal plants,

have found to play significant role against many diseases. Previous studies showed that essential oil has anti-bacterial, anti-viral and antifungal properties.^{148,168} Antibiotics target only one type of pathogen, but phytochemicals target many pathogens and are either water/buffer soluble, show lower leftover impact, and there is no molecular catalysis and cross reactivity. Essential oils easily bind to the lipids of bacterial cell membrane and mitochondrial cell membrane.¹⁴⁹ The stability of bacteria is affected when some important change occurs in its membrane structure through the interaction of essential oils.¹⁵⁰ Some acids present in medicinal plants like anacardic, gallic acid and flavonoids play very important role for the eradication of pathogenic microorganisms. Various studies are going on for the development of phyto-therapeutics that can reduce the use of antibiotics.¹⁵¹ Tea oil and thyme oil can stop spreading of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA).¹⁴⁸ Essential oils being volatile at room temperature used as aroma therapy that were effective against *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Staphylococcus aureus*.¹⁴⁷ It was seen that in liquid and solid media essential oil has antimicrobial activity and its diffusion capacity in agar shows inhibition of bacterial growth.¹⁵²⁻¹⁵⁵ Barley and wheat extracted thionin peptide contains 47 amino acid which is very toxic to yeast and Gram-positive bacteria.^{156,157} Febatin from faba beans, is effective against *E. coli*, *P. aeruginosa* and *Enterococcus hirae*. The ethanol soluble fractions of purple clove yield terpenoids, which show excellent activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*.^{158,159} Oil from the plant *Melaleuca alternifolia* showed activity against *E. coli*, *S. aureus* and skin pathogens.^{160,161} Few essential oils and their major constituents were found effective in gaseous state against *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*.¹⁶² The plant *Sida cordifolia* contains many volatile components which have antimicrobial effect against many bacteria like *S. aureus*, *Staphylococcus epidermidis*, *Candida guilliermondii* and *Trichosporon inkin*.¹⁶³ The phytotherapeutic agents derived from *Sida cordifolia*, are effective against many diseases

like rheumatism, inflammation, asthma and nasal congestion.¹⁶⁴ Broad spectrum pharmacological activities of Jupiner berry oil target against many bacteria and fungi.^{165,166} Currently antifungal medicines are also in use from some plants extracts like *Inula viscosa*, *Artemisia velotorum*, *Lavandula angustifolia*, and *Ocimum gratissimum*.¹⁶⁷ Some essential oils inhibit microbial DNA/RNA which ultimately affect the synthesis of proteins and stop their growth.¹⁶⁸ Essential oils extracted from various seed spices (*Anethum graveolens*, *Carum capticum*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Pimpinella anisum* and *Seseli indicum*) have shown antibacterial activity against many bacterial pathogens (*Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Streptococcus haemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* species and *Proteus vulgaris*). The oils extracted from *Carum capticum*, *Cuminum cyminum*, *Coriandrum sativum*, *Anethum graveolens*, and *Pimpinella anisum* can control hospital acquired infections/VAP/urogenital tract infections/diarrhoea (the associated pathogens being *P. aeruginosa*, *E. coli*, *Klebsella* species and *Proteus vulgaris*).¹⁵¹

Alternative therapeutic approach

Other than antibiotics therapy, some other approaches have been evaluated to control bacterial infections, like cytokines which can modulate phagocyte functions to control the infections. Cytokines, namely, interferon- γ , granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor and macrophage colony stimulating factor, have been used as adjunctive agents for treatment of infections. Archeocins (myxopyronin) are also an alternative antibiotic agent that inhibit bacterial enzyme like RNA polymerase, which lead to bacterial death. Preventive strategies can also be used to check the spread of infection through disinfectant.¹⁴⁸ Also, oral hygiene with Chlorhexidine (CHX), CHX body washing, selective oral decontamination (SOD) and/or digestive decontamination (SDD), multiple decontamination regimens, probiotics, subglottic secretions drainage (SSD), special cuff material and shape, silver-coated endotracheal tubes (ETTs), universal use of gloves and contact isolation, alcohol-based

hand gel, vaporized hydrogen peroxide, and bundles of care have been addressed as effective preventive approaches.¹⁶⁹

Bacteriophage therapy could be a good therapeutic strategy for the treatment of VAP patients because it was seen that bacteriophage treatment against *E. coli*, *Pseudomonas* spp., *Acinetobacter* spp., and *S. aureus* showed good efficacy.¹⁴⁸

BAL is considered a suitable sample for studying the cellular and biological changes induced by drugs. This means that it can be used in proof-of-concept studies during the clinical development of new drugs, allowing researchers to evaluate the effects of drugs on the respiratory system.¹⁷⁰

VAP associated with COVID-19

During the COVID-19 pandemic a lot of patients admitted in ICU, needed mechanical ventilation and nearly 1 out of 2 of these COVID-19 infected patients developed VAP with 42.7% mortality.¹⁷¹ ICU admitted COVID-positive patients were severely hypoxemic with microvascular and parenchymal lung damage.¹⁷² These patients were under high-risk zone to develop VAP, especially if compared with cohorts with ARDS (acute respiratory distress syndrome) patients and large mixed COVID⁺ ICU admitted patients.^{173,174} COVID Patients with severe lung damage had increased infection rate.¹⁷¹

Viruses have ability to damage immune cells such as lymphocytes, B cells, natural killer cells and T cells which lead to immune cell dysfunction, which is one of the reasons for bacterial and fungal coinfections. SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) damages the lung epithelial cells and also slows down ciliary clearance, thereby inviting bacteria as well as fungi infection (*Aspergillus* and *Mucor*).¹⁷⁵ This is the reason why, at the time of 2nd wave of COVID-19, there were multiple cases of mucormycosis reported in immunocompromised patients (uncontrolled diabetes mellitus and leukemia patients) or patients under treatment with corticosteroids and tocilizumab.^{176,177} COVID-19 infected patients showed cytokine storm due to increased level of IL-6. Tocilizumab (recombinant humanized monoclonal antibody) reduced the level of IL-6 in a patient's body to protect lung

damage and multiorgan failure.^{178,179} But at the same time blockage of IL-6 also had adverse consequences due to its central role in innate immunity and microbial clearance.¹⁸⁰ Tocilizumab had a higher risk of serious secondary bacterial and fungal infections with subsequent higher mortality.¹⁸¹

One of the studies reported that approximately 50% of COVID-19 positive patients were infected with both bacteria as well as fungal agents which were highly resistant to multiple antibiotics.¹⁸² This study was performed during the 2nd wave of COVID-19 when an unexpected high rate of VAP in mechanically ventilated COVID-19 cases were seen in the ICUs.¹⁷⁷ One of the studies conducted in Iran reported that every Covid 19 patient who were mechanically ventilated had also bacterial pneumonia infections, and all died in ICU except one.¹⁸³ China and UK also reported that 13.9% and 6.1% patients had COVID as well as secondary bacterial infections respectively.^{184,185}

Invasive and prolonged mechanical ventilation, use of immunomodulant therapies i.e., corticosteroids and anti-IL-6 drugs, overcrowded ICU during COVID-19 pandemic wave (inadequate staffing with more episode of cross-contamination) combinedly increased the risk for VAP development.¹⁸⁶⁻¹⁸⁷

CONCLUSION

Pathogens can adhere to the mucosa of the lower respiratory tract of VAP patients and start infections by aspiration from the oropharynx to the lower respiratory tract, contiguous infection extension, ICU aerosols inhalation and also by vascular or urinary catheter-related blood stream infection moving to the lung. Diagnosis of VAP is very challenging and there are limited diagnostic tests available. Clinical diagnosis has poor specificity and microbiological findings take 48-72 hrs which delays treatment of patients. Moreover, a lot of organisms implicated in VAP are fastidious in nature and are difficult to culture using routine aerobic culture methods. Molecular methods are more sensitive, rapid and specific in identification of the etiological agents, and help in initiating the early treatment. Multiplex PCR, flow cytometry, and mass spectrometry analysis are some of the sensitive techniques used,

although culture method is the gold standard for microbial identification. VAP treatment has witnessed notable advancements with the emergence of novel therapeutic options that have obtained approval from regulatory bodies like the Food and Drug Administration (FDA) or the European Medicines Agency (EMA). The availability of diverse treatment options presents new opportunities for improved patient outcomes in the management of VAP. Development of antibiotic resistance is the biggest challenge the world is facing now. Phyto-therapeutic approach, bacteriophage therapy, cytokine treatment could be some of the alternative therapeutic strategies to overcome this issue, although it needs in-depth research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BT, AB, and MKJ Conceptualized the study. HKT designed the figures and wrote the original draft. BT, AB, PS, PKR, BPM, and MKJ wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable

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