Lower Respiratory Tract Infections: Precision Diagnostics

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KEYWORDS

• Critical care • Intensive care • Infection • Lower respiratory tract infection
• Pneumonia • Genomics in critical care • Molecular microbiology
• Polymerase chain reaction

KEY POINTS

- Lower respiratory tract infections are the most common infections in critically ill worldwide, and, when complicated by sepsis, carry the highest risk of mortality.
- Thoracic ultrasound has greater diagnostic accuracy for pneumonia than chest radiography; it can be used as a first-line tool by an experienced provider, and as a second-line option when computed tomography is not available or unsafe.
- Early identification of causative organisms through lower respiratory tract sampling is paramount, ideally prior to the start of antimicrobial therapy.
- Both invasive and noninvasive sampling can be used to establish a microbiological diagnosis, though culture methods must be carefully chosen to avoid false positives, particularly in chronically ventilated patients.
- Molecular tests are now available to supplement conventional microbiological tests, but careful attention to their limitations is essential when interpreting their results.

INTRODUCTION

Lower respiratory tract infections (LRTI) are among the leading causes of death worldwide and represent the most common cause of death from communicable disease, according to a World Health Organization (WHO) report.[1] Among critically ill intensive care unit (ICU) patients suspected of having an infection, 60% are believed to have the respiratory tract as the source.[2] In these patients, most had microorganisms isolated, with almost 40% diagnosed with a Gram-negative bacterium-induced LRTI. Rates of pathogen identification vary significantly across studies, ranging from as low as 36%

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Abbreviations	
ATS	American Thoracic Society
BAL	bronchoalveolar lavage
CMT	conventional microbiological test
CMV	Cytomegalovirus
COPD	chronic obstructive pulmonary disease
ELISA	enzyme-linked immunosorbent assay
HAP	hospital-acquired pneumonia
HIV	human immunodeficiency virus
HSV	herpes simplex virus type
ICU	intensive care unit
IPA	invasive pulmonary aspergillosis
LAM	lipoarabinomannan
LRTI	lower respiratory tract infection
MALDI-TOF MS	matrix-assisted laser desorption ionization TOF mass spectrometry
MMT	molecular microbiological test
mNGS	metagenomic NGS
MRSA	methicillin-resistant *S aureus*
NAAT	nucleic acid amplification test
NGS	next-generation sequencing
NTM	nontuberculous mycobacteria
OR	odds ratio
PCR	polymerase chain reaction
PJP	*Pneumocystis jirovecii* pneumonia
qPCR	quantitative PCR
RSV	respiratory syncytial virus
tNGS	targeted NGS
VAC	ventilator-associated complication
VAP	ventilator-associated pneumonia
WHO	World Health Organization

by culture when patients were receiving antibiotics at the time of sampling, to as high as 84% when supplemented with multiplex polymerase chain reaction (PCR) panels.[3]

LRTI in the ICU can be challenging to diagnose. By virtue of their communication with the external environment, the human respiratory tract can become colonized and infected relatively easily with a near limitless array of pathogens. As a result, predicting the infecting organisms and selecting the investigations that can accurately identify true pathogens while distinguishing them from colonizers is challenging and constitutes an inherent limitation for all diagnostic tests. Exposure to different ecosystems also affects the patients' microbiome, and some comorbidities can render patients vulnerable to otherwise harmless organisms. Each of these factors influences the presentation, microbiologic etiology, and, in many regards, the severity of the infection and its prognosis. Proper identification of the syndrome and the microbiology is critical to outcomes, ensuring appropriate antimicrobial therapy and effective treatment when indicated.

The development of molecular tests such as the PCR, and more recently, of next-generation sequencing (NGS) methods, represents a promising path to improving our ability to rapidly identify pathogens. The objective of this article is therefore to describe the diagnostic tools available to clinicians for LRTI. To that end, we will briefly highlight the definitions and classification of LRTI in the critically ill, before exploring the microbiological etiologies and investigation modalities available for their diagnosis. Throughout this article, the clinical data underpinning investigative measures in the ICU will be discussed.

This article is informed by a rapid literature review conducted on EBSCO (CINAHL Plus, ERIC, PubMed) and Ovid for publications on diagnostic modalities for LRTI in

the ICU. All studies published in English or French before August 2025 were screened. The search strategy is described in Supplementary Material 1. Of note, influenza, COVID-19, and respiratory syncytial virus (RSV) will not be discussed, as another article by Dr Andre C. Kalil and colleagues titled "*The Respiratory Triple Pandemic in the ICU: Epidemiology, Clinical Features and Management of COVID-19, Influenza and RSV,*" has been included in this issue.

DEFINITIONS AND CLASSIFICATION OF LOWER RESPIRATORY TRACT INFECTIONS

The lower respiratory tract includes all structures below the larynx, namely the trachea, bronchi, bronchioles, and alveoli. Thus, infection in at least one of these structures defines an LRTI (**Fig. 1**). The most frequent LRTI encountered in critically ill patients is pneumonia, an infection of the pulmonary parenchyma, which includes the bronchioles and alveoli. Pneumonia was defined in the 2005 guidelines by the American Thoracic Society (ATS) and the Infectious Diseases Society of America[4] as a new or progressive pulmonary infiltrate with clinical evidence of an infectious

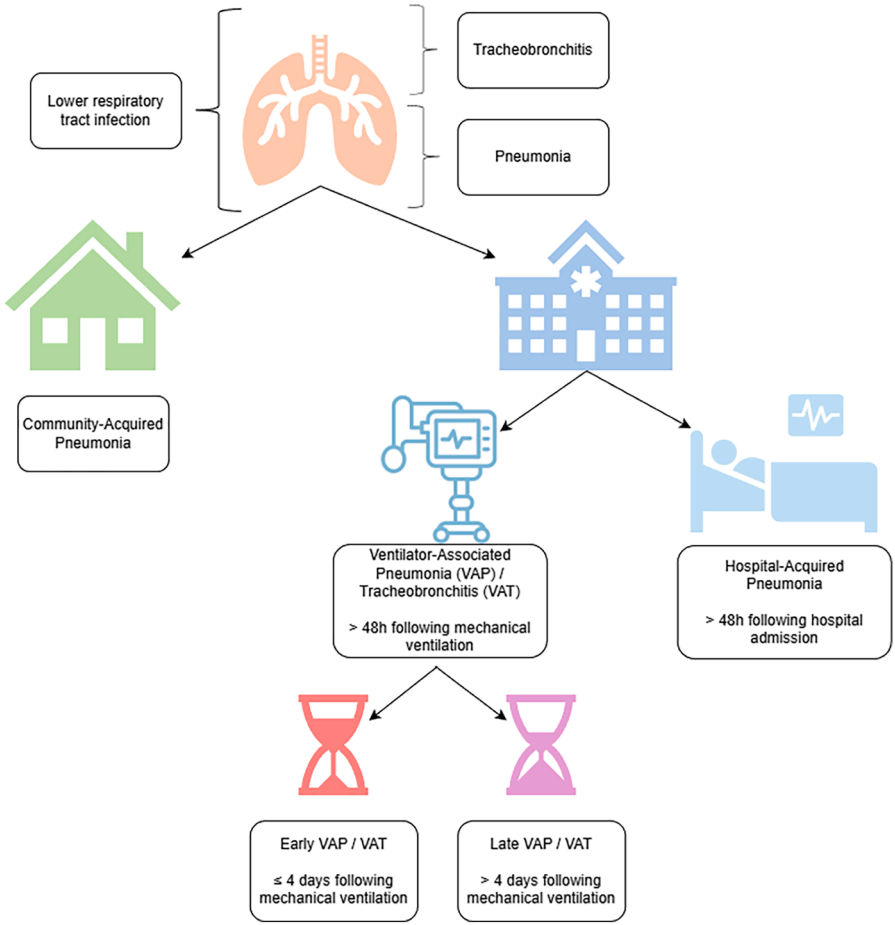

Fig. 1. Lower respiratory tract infections (LRTI) classification. VAP, ventilator-associated pneumonia; VAT, ventilator-associated tracheobronchitis.

origin (with at least one of the following: fever, leukocytosis, or purulent tracheal secretions) and decline in oxygenation.

Tracheobronchitis can also be encountered in critically ill patients and is defined as clinical evidence of a LRTI, such as fever, leukocytosis, purulent sputum, and a positive sputum culture, in the absence of a new lung infiltrate.[4] LRTI can further be classified according to the time of onset relative to hospitalization. Discrepancies between the European[5] and American[6] definitions can be found in the most recent guidelines. The European guidelines classify hospital-acquired pneumonia (HAP) as pneumonia caused by a pathogen present in the hospital setting, whereas the American guidelines define HAP as pneumonia that develops more than 48 h following hospital admission and was not thought to be incubating prior to admission.[6] This entity is referred to, in the European guidelines, as nosocomial pneumonia.[5] The lack of consensus also extends to the classification according to the presence of mechanical ventilation, as some define ventilator-associated pneumonia (VAP) as a type of HAP, with others, such as the Center for Disease Control and Prevention, complicate the definition by adding ventilator-associated complications (VACs).[7]

In this article, LRTI will be classified according to the American guidelines, with VAP and VAT treated as separate entities from HAP (see **Fig. 1**). Early VAP/VAT will be defined as appearing 4 days or less after initiation of mechanical ventilation, and late VAP/VAT will be defined as after 5 or more days.

THE "STANDARD" INTENSIVE CARE UNIT PATIENT

Once LRTI has been diagnosed, appropriate empirical antibiotics must be initiated based on epidemiologic data. In community-acquired pneumonia, the most frequent bacteria identified in high-income countries are *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*.[8–10] With increasing use of antibiotics and a higher prevalence of patients with multiple comorbidities, the microorganisms involved in LRTI in critically ill patients have shifted in recent years. A study published in 2025 from the MIMIC database now recognizes *Pseudomonas aeruginosa* as the second most common pathogen in CAP.[9] Furthermore, with the advent of molecular tools in clinical practice, viral LRTI has been increasingly identified in critically ill patients, with 10% to 49% of viral LRTI cases admitted to the ICU, depending on seasonality and the molecular modality used for diagnosis.[11–16] Bacterial coinfection complicates a viral LRTI in up to 39% of cases, leading to increased mortality and longer hospital length of stay.[11,12,14,17,18] As a result, the ATS now recommends continuing empirical antibiotics even when a viral pathogen is identified.[11,12,19] Low- and middle-income countries face a different challenge. A retrospective study from Africa on 931 patients admitted to the ICU identified tuberculosis as the most common organism involved in LRTI.[20] Given the heterogeneity of common pathogens across the world, empirical treatment must be adapted to the patient's environment and exposures.

This also holds true for hospital-acquired LRTI. A multicentric observational study conducted in 27 ICUs throughout Europe on 827 patients admitted to the ICU with either HAP or VAP revealed variable dominant isolates depending on the country.[21] *S. aureus* was most often identified in Spain, France, Belgium, and Ireland, whereas *P. aeruginosa* was most common in Italy and Portugal. *Acinetobacter* was responsible for most HAP and VAP in Greece and Turkey, and *Escherichia coli* was most frequently found in Germany. A more recent study conducted by the European Network for ICU-Related Respiratory Infections revealed that the microorganisms involved varied according to the presence or absence of invasive ventilation, with a higher prevalence

of *P. aeruginosa*, *Klebsiella* spp., and methicillin-resistant *S. aureus* (MRSA) in invasively ventilated patients.[22] Other frequently encountered organisms were *Acinetobacter baumannii*, methicillin-susceptible *S. aureus*, and *E. coli*. A study conducted in a Chinese ICU on patients undergoing noninvasive ventilation revealed similar pathogens to those in invasively ventilated patients.[23] Regarding VAP timing, most studies reported data from the time of intubation rather than from admission, which might explain why very similar organisms were identified in both groups, with the exception of Gram-negative bacteria such as *Stenotrophomonas maltophilia*, which were more frequent in the late-onset group.[24,25]

With increasing pathogen resistance, efforts have been made to predict MRSA pneumonia. Observational cohort studies have shown that nasal MRSA PCR screening has a high negative predictive value (89%–99%).[26–29] These findings may allow for rapid adjustment of antimicrobial therapy, although no randomized controlled trials on the subject were identified in this article, limiting specific recommendations. Similarly, early detection of *P. aeruginosa*, another high-risk resistant organism, has been described using liquid chromatography-mass spectrometry to detect specific urine metabolites.[30] Readily available and validated modalities for clinical use have yet to be released.

THE "ATYPICAL" INTENSIVE CARE UNIT PATIENT

Beyond the usual lower respiratory tract pathogens, critically ill patients may occasionally harbor atypical microorganisms that present unique diagnostic and therapeutic challenges. For example, viral HAP and VAP are uncommon but remain possible in the ICU setting. One study reported the incidence of viral VAP by prospectively assessing all intubated patients using PCR on tracheobronchial aspirates when VAP was suspected.[31] Of the 139 included patients, only 39 developed VAP, of which 13 cases were positive for a virus, with 11 solely due to herpes simplex virus type 1 (HSV1). The pathogenicity of HSV in nonlung transplant patients is yet to be established, as inconsistent effects on prognosis have been observed in patients with signs of pulmonary HSV reactivation.[32–36] The lack of changes in prognosis in treated patients, even after adjusting for severity of illness, challenges the existence of HSV pneumonia in non-immunocompromised patients.[34,35] Conversely, cytomegalovirus (CMV), although rare, remains a viral LRTI organism of concern in immunocompromised patients. A French prospective observational cohort study followed immunocompromised patients who underwent either open lung biopsies or autopsies and demonstrated an incidence of 29% of CMV pneumonia, with CMV being the sole pathogen responsible for HAP or VAP in 88% of these cases.[37]

While uncommon, pathogens such as *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella* spp. are increasingly identified as causes of severe pneumonia, with prevalence rates reaching 10%.[38] Although culture is often used as a gold standard, these pathogens are notoriously fastidious and require a considerable amount of incubation time.[39] Serology has been described as an alternative for *C. pneumoniae* and *M. pneumoniae*, but detection is highly dependent on the duration of the illness prior to testing. Molecular technology such as PCR has allowed rapid detection of these organisms.[40] However, as PCR platforms are specific to pathogens, not all are able to detect these organisms, and clinical suspicion must remain high in atypical presentations.[41] When comparing clinical signs of patients with one of the most frequent LRTI pathogens, *S. pneumoniae*, differences can be identified for *M. pneumoniae*, but *C. pneumoniae* has a similar clinical presentation.[42] *M. pneumoniae* is more frequently associated with hemolytic anemia, abdominal manifestations, and extensive pulmonary infiltrates.

Legionella spp. LRTI can also present with distinguishing features, and its presentation is often more severe than with typical organisms.[43] Clinically relevant distinctions are the association with diarrhea, hepatitis, hyponatremia, hypophosphatemia, and the presence of the Faget sign (pulse–temperature dissociation). Although distinct species of this Gram-negative bacilli exist, *Legionella pneumophilia* remains responsible for 90% of infections. *L. pneumophilia* is subdivided into different strains with the serogroups 1, 4, and 6 being the most virulent. As with other atypical organisms, the diagnostic sensitivity of culture is low, which is also the case for PCR. Additional diagnostic methods, such as serum antibody detection via enzyme-linked immunosorbent assays (ELISA), have been developed, but they typically require several weeks to yield results. Urine antigen testing, using an immunochromatographic assay, is not affected by the administration of antibiotics with a high sensitivity (70%–100%) and specificity (95%–100%) and can detect the serogroup 1 as early as 3 days from symptom onset.[42,44] Although its rate of positivity remains low, its use is recommended in severe CAP.[45] In cases where a high clinical suspicion remains and a negative urine antigen, clinicians should pursue culture and PCR while treating empirically for other serogroups and species.[42,44] Thriving in humid environments, *Legionella* has also been involved in HAP, namely associated with hospital water systems.[38,46] It should therefore remain in the differential diagnosis in patients with clinical features that may be compatible with this organism.

THE "GLOBAL" INTENSIVE CARE UNIT PATIENT

In contrast to global organisms, geographically influenced infections remain central considerations for critically ill patients. With the incidence of *Mycobacterium tuberculosis* (TB) surpassing the most frequent pathogens involved in LRTI in certain regions, careful travel history must be taken to ensure appropriate isolation and treatment, as imaging might not contribute to distinguishing pulmonary TB from other severe LRTI.[20,47] Although smear microscopy is frequently used as a first-line diagnostic test for pulmonary TB given its low cost and rapid turnaround, its sensitivity and specificity remain low, especially with poor quality specimens.[48] While a combination of solid and liquid cultures remains the gold standard for diagnosis of pulmonary TB, results typically require several weeks.[49]

Two molecular diagnostic tests, low-complexity automated nucleic acid amplification tests (NAAT), are now recommended by the WHO as first-line methods for the detection of pulmonary TB: Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, United States of America [USA]), and Truenat MTB Plus and Truenat MTB-RIF Dx (Molbio, Goa, India). Although they do not provide information on drug resistance beyond rifampin, their rapidity and low complexity allow for widespread implementation in low- and middle-income countries, with accessibility improving through WHO-supported interventions.[49] Another commercially available molecular diagnostic test is urine lipoarabinomannan (LAM) antigen testing, which detects a lipopolysaccharide from the cell wall of *M. tuberculosis*.[50] Sensitivity is limited overall, but performance improves substantially in patients living with human immunodeficiency virus (HIV), particularly when CD4 counts are low.[49,50] As HIV patients often struggle to produce adequate sputum, the WHO recommends urine LAM as an adjunct to low-complexity NAATs and culture in this population.[49]

A similar challenge arises in critically ill patients who frequently cannot expectorate sputum, making standard diagnostic sampling difficult. In this setting, invasive respiratory sampling such as tracheal aspirates, bronchoalveolar lavage (BAL), or blind bronchial sampling (mini-BAL) offers an alternative approach. Studies have shown

that these specimens can yield diagnostic performance comparable to sputum when subjected to NAATs.[51,52] While more invasive, they remain essential tools in the ICU to ensure timely diagnosis of pulmonary TB. Their use could be complemented by a urine LAM testing strategy, although this has not yet been validated in the ICU. Given the risk of false positives in patients with non-TB mycobacteria, urine antigen-based testing should be limited to patients with a high suspicion of pulmonary TB.

Beyond TB, other geographically restricted pathogens can cause LRTI. Endemic mycoses such as Blastomyces, Histoplasma, and Coccidioides demonstrate this pattern. Although their geographic distribution varies, the highest prevalence remains in the United States and Central America.[53,54] Clinically, these infections often mimic CAP and are frequently difficult to distinguish due to their prolonged incubation periods.[54,55] Diagnosis is challenging given the risk of cross-reactivity in antigen testing, which complicates interpretation. Culture and histopathology remain the only definitive diagnostic tests available for these endemic mycoses. This topic will be addressed in more detail in the section on Conventional Pathogen Identification.

THE "IMMUNOCOMPROMISED" INTENSIVE CARE UNIT PATIENT

Immunocompromised patients comprise approximately 30% ICU admissions with LRTI.[9] They often present with more severe disease and are at a higher risk of mortality during their hospital stay when compared with nonimmunocompromised patients.[9] Early etiologic pathogen identification can be lifesaving in this population, yet it is often more challenging as the organisms responsible for LRTI include not only the "Standard" and "Atypical" ICU pathogens. Central to identifying the potential pathogen is an understanding of the nature of the immunocompromise, as defects in different elements of the immune system will predispose patients to specific pathogens. For instance, patients with advanced HIV disease will be at elevated risk for *Pneumocystis jirovecii* and *Cryptococcus* but less so for invasive aspergillosis. In contrast, patients with hematological malignancies will be at higher risk for invasive mold infections. Patients with solid organ transplants are at elevated risks of a range of viral infections, including both typical respiratory viruses (eg, influenza) and atypical ones (eg, CMV, HSV). Similarly, patients with chronic steroid use may be at elevated risk for pulmonary nocardiosis, while patients with structural lung disease will have higher incidences of mycobacterial infection including the nontuberculous mycobacteria (NTM). As invasive fungal infections are the more common pathogens shared across immune-compromising conditions, as well as the more common opportunistic nonbacterial infection encountered in the critically ill, the following section focuses on LRTI caused by fungi, organized according to their microbiological classification. For viral pneumonias and mycobacterial diseases, several excellent review articles can be found.[56–58]

Candida and *Cryptococcus* spp. are 2 yeasts that have been described in LRTI.[59] Although Candida is often isolated from respiratory tract samples in ICU patients, it is almost always a contaminant and should not be treated unless other signs of invasive candidiasis are present.[60] Regarding *Cryptococcus* spp., LRTI is seldom an isolated clinical presentation, and additional infected sites, such as the central nervous system should be pursued for microbiological diagnosis.

Common opportunistic mycoses in LRTI, such as *Mucorales* spp., *Aspergillus* spp., and *Pneumocystis jirovecii* pneumonia (PJP), have variable presenting features depending on the host's type and degree of immunosuppression. Clinicians must remain vigilant when caring for patients with nonclassical host risk factors for mycoses including prolonged corticosteroids use, decompensated liver cirrhosis, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome, and

with viral infections from influenza and COVID-19.[61,62] The diagnostic steps for opportunistic pulmonary mycoses recommended by the European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC), the Invasive Fungal Diseases in Adult Patients in ICU (FUNDICU), and the German national guideline have been summarized in Fig. 2.[59,61,63] A proven mold infection requires either pathologic or conventional culture from a tissue sample obtained via biopsy or needle aspiration, provided it can be done without the possibility of contamination from a nonsterile site.[59] For PJP, the fungi must be detected through staining of the specimen or through molecular techniques as culture is technically very challenging and not performed in routine clinical settings.[59] In this regard, molecular detection is increasingly the routine for

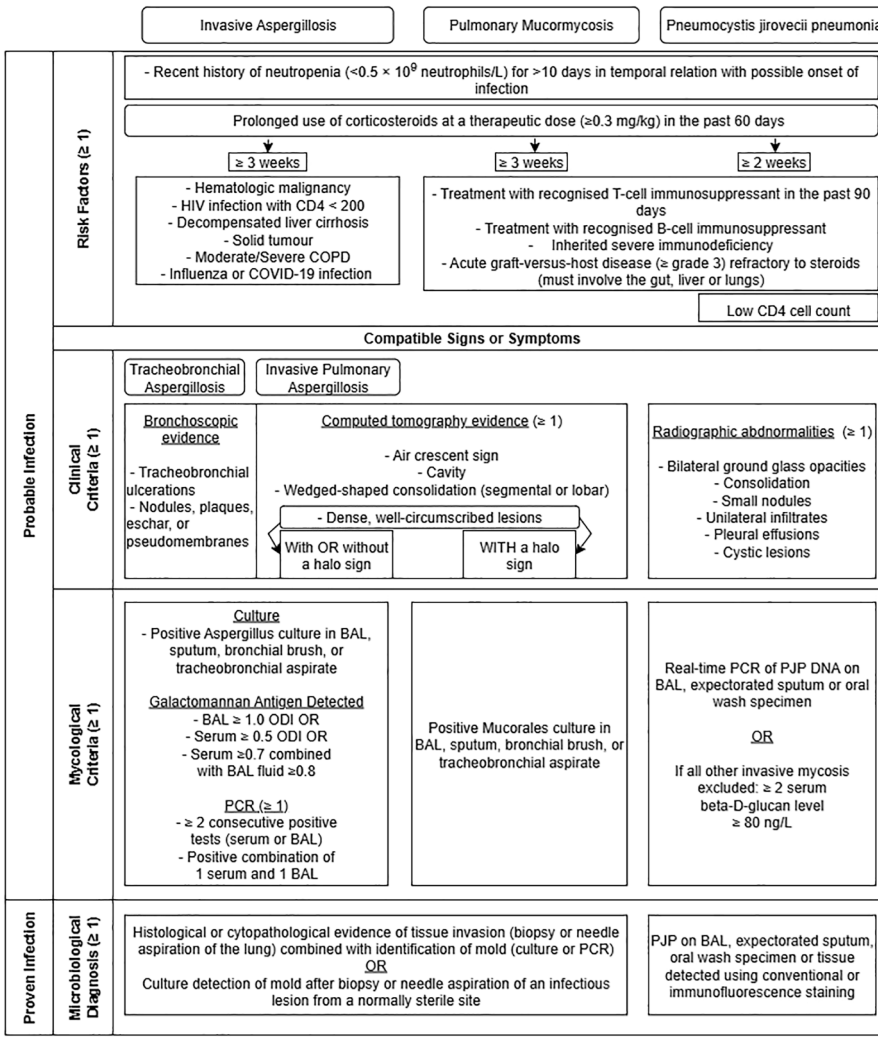

Fig. 2. Diagnostic steps for opportunistic pulmonary mycoses. BAL, bronchioalveolar lavage; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; ODI, optic density index; PCR, polymerase chain reaction; PJP, *Pneumocystis jirovecii* pneumonia.

diagnosis of PJP infection but poses the challenge of detection of airway colonization. PCR detection of *P. jirovecii* is highly sensitive but cannot differentiate colonization from disease. Colonization is common in patients with COPD, advanced liver disease, chronic steroid use, or in mechanically ventilated patients. Quantitative PCR (qPCR) cycle threshold (Ct) values or fungal load estimates help increase specificity: higher organism burdens (low Ct values) correlate with clinically significant PJP, whereas high-Ct/low-load detections often represent colonization. However, Ct thresholds vary by platform, and no universal cutoff is validated. Clinical interpretation therefore requires integrating the host's risk factors, imaging findings (classically bilateral ground-glass opacities), serum β-D-glucan (often markedly elevated in true PJP), and response to therapy.

A probable infection by one of these 3 opportunistic fungi requires at least one of each of the following diagnostic steps: (1) risk factor; (2) compatible sign or symptom (often nonspecific, particularly in ICU patients); (3) clinical criteria; and (4) mycological criteria. However, differences between pathogens must be highlighted.

Although the clinical criteria for mucormycosis and invasive pulmonary aspergillosis (IPA) are similar, the reverse halo sign is more specific to mucormycosis and is therefore sought when a circumscribed lesion is observed.[59] As for IPA, nonneutropenic critically ill patients present diagnostic challenges compared to classical hosts, as traditional radiological features are often lacking, and galactomannan and PCR demonstrate lower diagnostic accuracy. In this population, the radiological findings are often less specific such as lobar consolidation, bilateral ground glass opacities or "crazy paving."[64,65] Beta-D-glucan should not be used to aid IPA diagnosis due to its low specificity and frequent false positives.

Pathologic patterns also differ, with patients with severe bone marrow suppression expressing hyphae in a radial pattern with a circular band of hemorrhage surrounding coagulation necrosis areas, whereas other patients will express fungal growth in the alveoli with inflammatory exudates and liquefactive necrosis. Influenza- and COVID-19-associated IPA represent newly recognized nonclassical presentations linked to increased mortality.[66] These trends underscore the role of viral epidemics in contemporary opportunistic infection risks and highlight the importance of timely and accurate diagnostic approaches in critically ill patients.

SAMPLING TECHNIQUES

The diagnostic accuracy of a diagnostic test depends not only on its characteristics but also on the sample type and quality ("pre-analytical" considerations). Noninvasive sampling techniques (induced sputum and endotracheal aspiration) and invasive sampling techniques (BAL, protected specimen brush and mini-BAL) both allow potential identification of causative pathogens for LRTI. Notwithstanding, both approaches can lead to overestimation of pathogen involvement in LRTI, and are associated with specific disadvantages.

In nonintubated patients, noninvasive samples may be impossible, whereas invasive sampling may lead to significant adverse events in up to 35% of cases, although the associated risk of mortality remains similar.[21,67,68] In intubated patients, although access to invasive sampling is easier, risks of hypoxemia and clinical deterioration remain. Multiple observational and randomized-controlled studies have evaluated the accuracy of both techniques and have not demonstrated improvements in clinical outcomes such as mortality, length of stay, or duration of mechanical ventilation in VAP.[6,68–73] Nevertheless, the use and duration of antibiotics might be improved with invasive sampling.[70,72,73] According to these results, the American guideline on VAP

and HAP, and the French guideline on CAP, recommend using nonsampling, whereas the VAP and HAP European guidelines recommend using invasive sampling in intubated and stable patients and noninvasive sampling in others.[6,45,71]

Practice variability should be considered when selecting a diagnostic method. For endotracheal aspiration, semiquantitative measure can help differentiate pathogenic from nonpathogenic organisms, though optimal thresholds remain unclear, with negative predictive values ranging from 88% at 10^4 colony-forming units per milliliter (cfu/mL) to 97.3% at 10^6 cfu/mL.[74] In invasive techniques, quantitative thresholds of 10^3 cfu/mL for PBS, and 10^4 cfu/mL for BAL and mini-BAL have been suggested.[75,76] When comparing these diagnostic techniques, a meta-analysis found similar diagnostic accuracy for PBS and BAL in ventilated patients, although PBS can be affected by the administration of antibiotics.[77] To further improve diagnostic accuracy, bilateral BAL has been suggested.[78] As for variability in BAL dilution,[79] observational data indicate that this does not significantly affect diagnostic accuracy.[80] As for mini-BAL, it is an emerging technique involving blind advancement of a specialized catheter that allows the selection of the left or right lung, installation of normal saline, followed by aspiration, analogous to standard BAL.[18] Its advantage lies in feasibility outside regular hours by nonspecialized health care professionals, allowing for sampling prior to antibiotic use, while its main disadvantage is the inherent lack of directed sampling. Limited data indicate a good sensitivity but a lower specificity when compared to BAL.[76] Another new diagnostic technique is the electronic nose analysis of exhaled breath, which remains a research device, and thus far has demonstrated poor specificity of 56% and poor sensitivity of 76%.[81]

CULTURE-BASED PATHOGEN IDENTIFICATION

Diagnostic microbiology in the ICU spans culture-based assays, immunoassays, and molecular assays; in this section, we first review culture-based approaches historically referred to as "conventional' testing." Conventional microbiological tests (CMTs) typically include cultures and Gram staining of respiratory samples and blood, although the diagnostic yield of blood culture for LRTI is often poor.[45] Although sometimes informative, Gram stain should not be used to change antibiotic coverage given its low accuracy, particularly with Gram-positive cocci.[82,83] The current gold standard for pathogen identification remains quantitative culture of samples obtained directly from the lower respiratory tract. Bacterial identification of growing colonies used to rely on assessment of metabolic/biochemical reactions, but these are limited and time-consuming and can limit pathogen identification, namely nonfermenting Gram-negative bacilli.[84] More modern CMTs have been introduced to facilitate microbial identification such as matrix-assisted laser desorption ionization TOF mass spectrometry (MALDI-TOF MS), and ELISA. MALDI-TOF MS measures the molecular mass of bacterial cellular proteins obtained after exposure of previously isolated colonies through culture to a laser, causing desorption and ionization facilitated by a chemical matrix. The protein profile obtained through this method is then compared to a library of known pathogens to rapidly identify the organism. This technique remains reliant on the initial culture of the pathogen.

Immunoassays (including ELISA, immunochromatographic assays, and latex agglutination) rely on antibody–antigen interactions to detect either pathogen antigens (eg, pneumococcal urinary antigen, Histoplasma antigen) or host antibodies (eg, *Coccidioides* IgG/IgM). Lateral flow assays, such as the *S. pneumoniae* urine antigen test, and latex agglutination (eg, cryptococcal antigen) share similar biological principles but are operationally distinct from ELISA. Performance varies by antigen burden,

host immune status, and timing of infection. Cross-reactivity, particularly among endemic mycoses, remains an important limitation. Because they depend on the pathogen's antigen (most commonly, as opposed to host antibodies), it is usually more sensitive in immunocompromised patients with disseminated disease. However, cross-reactivity between pathogens with similar epitopes and the timing of testing relative to infection are critical points clinicians need to consider for all immunoassays. For instance, pneumococcal urinary antigen detects the C-polysaccharide antigen with variable sensitivity and Streptococcal vaccine within 5 days of the test might result in a false positive. With a specificity of more than 90%, its use is recommended in the French guidelines on CAP despite the low rate of positivity (4.2%).[45,85,86] The drawbacks of this technology explain the variable test properties (**Table 1**). Use of these diagnostic tests for LRTI in the ICU must be made with careful attention to their time limitations and risks of serologic interference.

MOLECULAR PATHOGEN IDENTIFICATION

In comparison to CMTs, molecular microbiological tests (MMTs) use DNA or RNA extracted from samples to identify potential pathogenic infectious organisms.[7,12] PCR is the most common type of MMT in clinical settings. It uses specific targets on nucleic acids (DNA or RNA) to identify specific organisms.[7] For organisms to be identified using PCR, a target-specific primer has to be added to the prepared sample to allow amplification of the desired nucleic acid sequence that is specific to these organisms, and the amplification occurs during thermal cycling (varying temperatures). The method is described in **Fig. 3** and compared to other MMTs such as loop-mediated isothermal amplification, NGS, and clustered regularly interspaced short palindromic repeats. PCRs can be single-plex (eg, the Cepheid Xpert SARS-CoV-2 assay) or multiplexed (eg, the BioFire respiratory panel), meaning they can be designed to amplify target sequences for one or multiple pathogens. Numerous commercial panels exist, and clinicians should be aware of what panels are used in their local institution. Given the increased use of NGS in clinical practice, only this MMT will be described in detail. Two major NGS approaches exist: metagenomic NGS (mNGS) and targeted NGS (tNGS). mNGS comprehensively sequences all genes in a given sample, whereas tNGS sequences only preselected organisms' genomic regions. While mNGS allows broad pathogen discovery, tNGS provides higher sensitivity for specific pathogens and can capture multiple overlapping reads, improving assembly and detection in defined contexts[12] to detect pathogens.

NGS sequences all nucleic acids present in a sample.[7] Unlike target-specific tests, mNGS is generally untargeted, whereas many clinically deployed NGS workflows—including tNGS and hybrid-capture methods—are targeted by design. Depending on the platform, host nucleic acids may be depleted, retained, or computationally accounted for. Although host-read depletion is common in bioinformatic pipelines, retaining host transcriptomic data can improve discrimination between infection, colonization, and noninfectious inflammation. Several studies demonstrate that combined microbial and host–response signatures outperform pathogen-only approaches in distinguishing true infection from colonization.[96,97] It may be preceded by an amplification step. During library preparation, adapters are ligated to nucleic acid fragments to enable amplification, sequencing, and sample multiplexing.

A final important form of PCR for clinicians to be aware of is the 16S and 18S PCRs, now being incorporated into clinical practice. These PCRs amplify conserved segments of ribosomal RNA within bacteria and eukaryotes, respectively, which allows for a restricted set of primers to amplify the nucleic targets from a wide array of

Table 1
Properties of Immunoassays for lower respiratory tract infection pathogens[50,85,87]

Diagnostic Test	Diagnostic Target	Sensitivity	Specificity	Time to Diagnosis	Sample	Additional Comments
Legionella pneumophilia serogroup 1-soluble antigen	Serogroup 1 antigen	70%–100%	95%–100%	30 min	Urine	Time to detection ≥3 d from symptom onset
Pneumococcal urinary antigen	C-polysaccharide antigen	50%–80%	>90%	15–30 min	Urine CSF	Can remain positive in up to 50% of patients for a month
Cryptococcal glucuronoxylomannan antigen (CRAG)	Capsular polysaccharide antigen	HIV patients • Serum: 70% • BAL 40%–80% Non-HIV with disseminated disease: • Serum: 85%–95% • BAL: 80%–98%	HIV patients • Serum: 99% • BAL 98% Non-HIV patients with disseminated disease: Serum and BA: >95%	4 h	Serum BAL	Detects all serotypes
Lipoarabinomannan antigen testing (Tuberculosis)	Glycolipid antigen	50%	84.2%	30 min	Urine	Recommended only in HIV (+) patients
Histoplasma serology	Antigen and IgM/IgG antibodies	Urine 83% Serum 82% BAL 94%	Urine 99% Serum 97% BAL 98%	Hours	Urine Serum BAL	Cross-reactivity with Blastomyces 93%–96%
(Pulmonary) coccidioidomycosis serology	IgM/IgG antibody detection	67%–87%, sequential testing increases to 84%–95%	70%–86%	Up to 1 d	Serum	Not used as poor diagnostic performance

Galactomannan[88–90]	Galactomannan polysaccharide component of cell wall	Neutropenic hosts BAL: 85%–93% Serum: 70%–90% Nonneutropenic hosts BAL: 50%–70% Serum: 22-<40%	BAL: 85%–95%	2 h	BAL Serum	Operating characteristics vary as a function of immunocompromise; numerous potential causes of false positive assays including contaminated dextrose solutions, recent IvIG administration, and some antibiotics including piperacillin-tazobactam (historic)
Pneumocystis jirovecii Direct fluorescence antibody	β-1,3-glucan-rich antigens on the outer cyst wall	BAL: 48%–100%	BAL: 82%–100%	1 h	BAL	Poorer performance in the HIV-negative population[91,92]

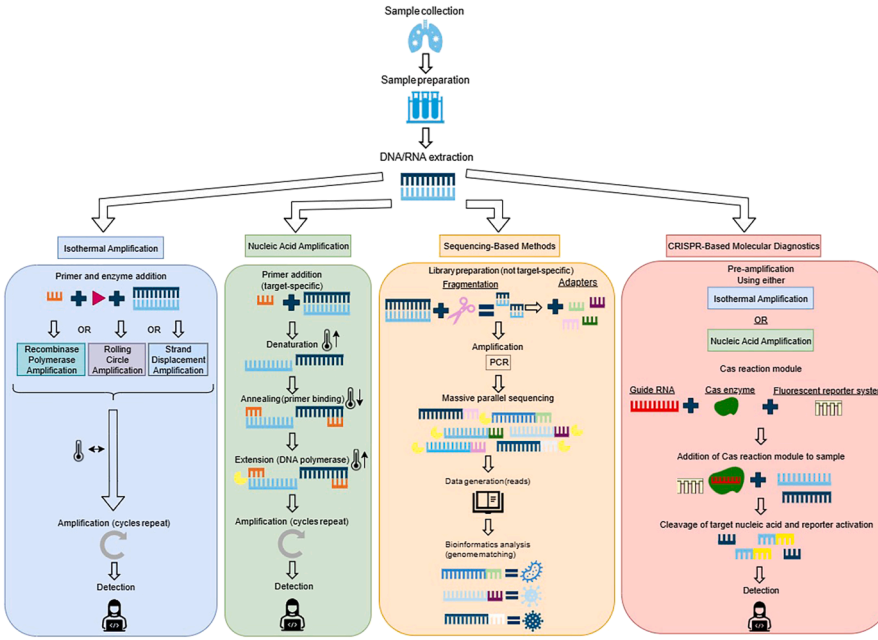

Fig. 3. Molecular diagnostic tests.[93–95] PCR, polymerase chain reaction.

species. However, unlike conventional PCRs, the amplified targets must undergo sequencing to identify the pathogens detected. The power of these techniques is in the breadth of potential pathogens that can be identified, while the main drawbacks are their complexity and increased chance of detecting environmental or contaminating microbes, which can particularly limit their use in respiratory samples.

Understanding of their mechanisms allows for comprehension of the limitations and disadvantages of these advanced modular diagnostic tests. As any modality, their diagnostic yield can be diminished when multiple targets are pursued and is vulnerable to the sample provided, mostly for the quality of the DNA and/or RNA. Accordingly, these novel strategies come at a higher cost than most CMT and are unable to distinguish live from dead organisms, which is sometimes an advantage for highly sensitive pathogens or when samples can only be obtained post-antibiotic initiation. Furthermore, as molecular tests rely on genomics, they are able to identify antibiotic-resistant genes, an asset given the increasing incidence of multidrug-resistant pathogens be encountered in critical care settings. **Table 2** summarizes the diagnostic properties of MMT.

Most of the MMT performed on BAL are limited to the research setting, but they yield promising results. A meta-analysis was conducted on mNGS and demonstrated an increased pathogen detection rate with an odds ratio (OR) of 6.81 when compared with conventional methods. This was also associated with a lower 30 day mortality (OR 0.35).[104] As all the articles identified were observational trials from China, additional data are required before the broad use of mNGS in the ICU. PCR technology is the only readily available MMT that is frequently used in the ICU, and multiple different panels are available from a single pathogen detection to the syndromic panels. Two systematic review articles on the use of a syndromic panel that detects 15 common bacteria, 3 atypical bacteria, 8 viruses, and 7 markers for resistance in

Table 2
Molecular microbiologic diagnostic test properties

Method	Pathogen Coverage	Sensitivity	Specificity	Validated Sample	Key Information
PCR	Single to multiple pathogens (bacteria, viruses, resistance markers)	Bacteria: 50%–100% Resistance genes: 91%	Bacteria: 93%–100% Resistance genes: 99%	Invasive and noninvasive sampling	Dependent on the pathogens in the panel
LAMP[98]	Multiple pathogens	73.7–94%	59%–100%	BAL	Only 1 study found on the detection of *S. aureus, E. coli, Pseudomonas aeruginosa, K.pneumoniae, S. maltophilia,* and *A. baumannii*
CRISPR[99]	Multiple pathogens	100%	87%	BAL	Only 1 study found on the detection of *A. baumannii, K. pneumoniae, P. aeruginosa, S. aureus,* and *S. maltophilia*
Non-commercial mNGS[100,101]	Unlimited (mNGS)	Nanopore: 93.5% Illumina 80.6%	Nanopore: 28.6% Illumina 42.9%	Plasma	Decreased specificity for mycobacteria and mycoses
Plasma cell-free DNA mNGS (eg, Karius Test)[102]	Broad, especially invasive/disseminated infections	~50%–70% for LRTI (higher when infection is angio-invasive or disseminated)	~85%–95%	Plasma only (validated sample)	Strongest validated mNGS platform for respiratory samples; excellent for viruses and atypical pathogens; lower specificity for *Aspergillus* and NTM due to colonization

(*continued on next page*)

Table 2
(*continued*)

Method	Pathogen Coverage	Sensitivity	Specificity	Validated Sample	Key Information
tNGS[103]	Specific to certain pathogens (bacteria, viruses, fungi, resistance markers)	63%–69%	98%	BAL	Very high depth of coverage for preselected targets; excellent for resistance gene detection; not broad—requires prior clinical suspicion

ICU patients have demonstrated a high sensitivity and specificity.[105] The clinical benefit of this type of MMT has not been demonstrated, but they remain recommended in the ATS guidelines on severe CAP, as it might reduce antibiotic consumption in the ICU setting.[106] Given its cost, the most recent French guidelines suggest a stepwise approach in CAP, starting with a quadruplex (influenza A/B, COVID-19, RSV), followed by a syndromic PCR in case of negative results.[45]

In contrast, most commercially available NGS-based infectious disease diagnostics (eg, Karius cell-free DNA test, University of Washington and UCSF mNGS services) use plasma as their standard sample type and not BAL, though the latter does have extensive validation. Plasma cfDNA testing offers advantages, including noninvasiveness, feasibility in hypoxemic patients, and detection of deep-seated or disseminated infections. However, plasma assays may underperform for pathogens confined to the airway lumen (eg, *Pseudomonas aeruginosa* VAP, *Aspergillus* bronchial disease). BAL-based mNGS has higher sensitivity for localized pulmonary infections but is more invasive and susceptible to upper-airway contamination and is only offered at select institutions.[107,108]

SUMMARY

The diagnosis of LRTIs in the ICU is a major clinical challenge due to the heterogeneity of pathogens, overlapping clinical features, and limitations of diagnostic methods. Advances in precision diagnostics are progressively changing the care of critically ill patients by enabling faster, more accurate pathogen identification. These tools not only enhance diagnostic accuracy but also hold the potential to refine antimicrobial exposure. Nevertheless, their optimal integration into clinical practice must be made in light of cost-effectiveness and real-world impact on patient outcomes. Ongoing trials on the integration of comprehensive molecular testing in the ICU setting are necessary to bridge the gap between technological innovation and bedside application.[109]

CLINICS CARE POINTS

- In high-income countries, viral lower respiratory tract infections (LRTIs) are increasingly recognized in critically ill patients, including in nosocomial settings.
- In low- and middle-income countries, tuberculosis remains the most frequently identified cause of LRTIs in critically ill patients.
- When pulmonary tuberculosis is suspected, molecular diagnostic tests are recommended as first-line investigations.
- Critically ill patients represent non-classical hosts and remain at risk for opportunistic mycoses.
- In patients with suspected LRTIs caused by fastidious organisms, molecular diagnostic techniques such as polymerase chain reaction (PCR) enable early and sensitive pathogen detection.
- PCR platforms are pathogen-specific; therefore, careful selection is required to ensure inclusion of the suspected organisms.
- Broad-range 16S and 18S PCR permit detection of a wide spectrum of organisms but are associated with an increased risk of identifying environmental contaminants.
- Immunoassays rely on antigen–antibody interactions and may therefore be subject to cross-reactivity.

DISCLOSURE

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SUPPLEMENTARY DATA

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