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Microbiological Diagnosis of Respiratory Illness: Recent Advances

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Infections of the respiratory tract are among the most common health problems in children worldwide, and are associated with substantial morbidity and mortality.[1] A wide variety of microorganisms are potential respiratory pathogens; knowledge about the likely etiologic agents of respiratory infections can help direct management and can also play an important role in disease surveillance. Beyond the identification of specific pathogens, the clinical microbiology laboratory can also provide valuable information on antimicrobial susceptibility and strain typing. Continued liaison between clinicians and laboratory staff is vital to facilitate the most cost-effective use of laboratory diagnostics.

Presently, we are still reliant on many traditional diagnostic tools that have been used for decades to determine the microbial etiology of respiratory infections.[2,3] However, these tools have been increasingly supplemented by newer methods, particular molecular diagnostic techniques, which have enabled the more rapid detection of many pathogens that were previously difficult to detect.[4] These advances have particularly led to improvements in the ability to detect respiratory viruses and other microorganisms that do not normally colonize the respiratory tract. Moreover, recent discussions about the existence of a lung microbiome have challenged traditional paradigms about the pathogenesis of respiratory infections.[5,6] The concept that the healthy lung may not be a sterile organ is reshaping our interpretation of laboratory diagnostics.

This chapter focuses on the use of the clinical microbiology laboratory to determine the microbial causes of respiratory infections in children. Diagnostic aspects of some specific respiratory infections, such as tuberculosis and pertussis, are also covered in other chapters.

Respiratory Pathogens and Syndromes

Tables 22.1–22.17 show the etiologic agents associated with respiratory infections broken down by respiratory syndrome. These lists represent our current understanding and have changed little over recent decades; there have been only a relatively small number of newly discovered pathogens. The latter include human bocavirus, human metapneumovirus, and a variety of coronaviruses (SARS-CoV, CoV-NL63, CoV-HKU1 and MERS-CoV).[7] Pathogen discovery efforts using unbiased next-generation sequencing methods have shown considerable promise but have not yet identified major new respiratory pathogens.[8,9]

In general, upper respiratory infections tend to be monomicrobial and are predominantly caused by viruses, with a few notable exceptions caused by specific bacteria (e.g., acute pharyngitis caused by *Streptococcus pyogenes*). Lower respiratory infections are caused by a wide variety of viral and bacterial pathogens. For pneumonia at least, sequential or concurrent polymicrobial infection may be relatively common, and the exact roles of individual microorganisms and how they interact in this context are still poorly understood.[10,11] The incidence of many respiratory infections follows a cyclical pattern aligned with the typical seasonal transmission of specific pathogens. Secular trends have also been noted for some vaccine-preventable infections, such as those caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, with decreasing burden following the successful implementation of vaccine programs.

Use of the Clinical Microbiology Laboratory

Before ordering a diagnostic test, it is important to be clear about the key clinical questions and expectations of diagnostic testing. Is knowledge about the cause of a particular respiratory infection important for patient treatment, outbreak management, epidemiological surveillance, or to reassure the clinician or caregiver of the child? It is also important to have an understanding about which specimens to collect, what tests are available, test limitations, and how to interpret results to appropriately integrate the findings into their clinical management.

The most useful specimens for diagnostic testing are those collected directly from the site of infection. Unfortunately, it is not always possible to collect these specimens, and this particularly applies to the lower respiratory tract, which is difficult to access safely in a manner that avoids contamination with colonizing organisms.

When bacteria are isolated from specific body sites, such as a throat swab, nasopharyngeal swab, or sputum, it is important to know which bacteria can be found as commensals or colonizers in the upper respiratory tract and which

ABSTRACT

A wide variety of microorganisms are potential respiratory pathogens, and the spectrum of known pathogens for each respiratory infection syndrome has not changed markers over recent years. Detection of likely etiologic agents of respiratory infections can help direct management and can also play an important role in disease surveillance. For this purpose, we are still reliant on many traditional diagnostic tools that have been used for decades in order to determine the microbial etiology of respiratory infections. However, these tools have been increasingly supplemented by newer methods, particular molecular diagnostic techniques, which have enabled the more rapid detection of many pathogens that were previously difficult to detect. These advances have particularly lead to improvements in the ability to detect respiratory viruses and also other microorganisms that do not normally colonize the respiratory tract. Recognition of the existence of the lung microbiome has challenged the traditional views of pneumonia pathogenesis and may provide the opportunity for new diagnostic tools that are focused on more than just detection of specific known pathogens. Continued liaison between clinicians and laboratory staff is vital in order to facilitate the most cost-effective use of laboratory diagnostics.

KEYWORDS

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Table 22.1 Etiologic Agents Associated With Pharyngitis

Viral	Bacterial	Fungal
Adenoviruses	*Streptococcus pyogenes*	*Candida* species
Coronaviruses	Other β-hemolytic streptococci	
Parainfluenza viruses	*Corynebacterium diphtheriae*	
Respiratory syncytial virus	*Corynebacterium ulcerans*	
Human metapneumovirus	*Arcanobacterium haemolyticum*	
Rhinoviruses	*Neisseria gonorrheae*	
Influenza viruses	Mixed anaerobes	
Epstein-Barr virus	*Treponema pallidum*	
Enteroviruses	*Chlamydophila pneumoniae*	
Herpes simplex viruses	*Mycoplasma pneumoniae*	
Measles	*Streptobacillus moniliformis*	
Rubella		
Cytomegalovirus		
HIV		

HIV, Human immunodeficiency virus.

Table 22.2 Etiologic Agents Associated With Croup

Viral	Bacterial
Parainfluenza viruses	*Mycoplasma pneumoniae*
Influenza viruses	
Respiratory syncytial virus	
Human metapneumovirus	
Coronaviruses	
Human bocavirus	
Adenoviruses	
Measles	
Rhinoviruses	
Enteroviruses	
Herpes simplex viruses	

Table 22.3 Etiologic Agents Associated With Sinusitis

Viral	Bacterial	Fungal
Rhinoviruses	*Haemophilus influenzae*	*Aspergillus* species
Influenza viruses	*Streptococcus pneumoniae*	*Alternaria* species
Parainfluenza viruses	Anaerobes	*Penicillium* species
Adenoviruses	*Moraxella catarrhalis*	Zygomycetes
	Staphylococcus aureus	
	Streptococcus pyogenes	
	Mycoplasma pneumoniae	

when found would indicate definitive infection. Table 22.15 outlines microorganisms that are regarded as part of the normal respiratory flora.[12–15] Importantly, given the right conditions, some bacteria that can harmlessly colonize the respiratory tract may also be respiratory pathogens. As will be discussed further in this chapter, several microbiological diagnostic tests employed in the diagnosis of childhood respiratory disease have limited ability to differentiate between colonization and disease and are therefore of limited value when considered in isolation.

Table 22.4 Etiologic Agents Associated With Acute Bronchitis

Viral	Bacterial
Adenoviruses	*Mycoplasma pneumoniae*
Influenza viruses	*Bordetella pertussis*
Parainfluenza viruses	*Bordetella parapertussis*
Respiratory syncytial virus	*Chlamydophila pneumoniae*
Rhinoviruses	*Haemophilus influenzae*
Coronaviruses	*Streptococcus pneumoniae*
Human metapneumovirus	*Moraxella catarrhalis*
Herpes simplex viruses	*Streptococcus pyogenes*
Enteroviruses	
Measles	
Mumps	
Human bocavirus	

Table 22.5 Etiologic Agents Associated With Bronchiolitis

Viral	Bacterial
Respiratory syncytial virus	*Mycoplasma pneumoniae*
Parainfluenza viruses	
Adenoviruses	
Influenza viruses	
Human metapneumovirus	
Rhinoviruses	
Enteroviruses	
Mumps	
Herpes simplex viruses	

Table 22.6 Etiologic Agents Associated With Pneumonia

Viral	Bacterial	Fungal
Respiratory syncytial virus	*Streptococcus pneumoniae*	*Pneumocystis jiroveci*
Parainfluenza viruses	*Haemophilus influenzae*	*Aspergillus* species
Influenza viruses	*Staphylococcus aureus*	Zygomycetes
Coronaviruses	*Mycoplasma pneumoniae*	*Coccidioides immitis*
Adenoviruses	*Bordetella pertussis*	*Cryptococcus neoformans*
Human metapneumovirus	*Legionella* species	*Histoplasma capsulatum*
Rhinoviruses	Enterobacteriaceae	
Epstein-Barr virus	*Pseudomonas aeruginosa*	
Enteroviruses	*Acinetobacter* species	
Human bocavirus	Mixed anaerobes	
Herpes simplex viruses	*Streptococcus agalactiae*	
Varicella zoster virus	*Chlamydophila pneumoniae*	
Measles	*Chlamydia psittaci*	
Rubella	*Chlamydia trachomatis*	
Cytomegalovirus	*Burkholderia pseudomallei*	
HIV	*Streptococcus pyogenes*	
	Neisseria meningitidis	
	Coxiella burnetii	
	Mycobacterium species	

HIV, Human immunodeficiency virus.

Table 22.7 Etiologic Agents Associated With the Common Cold

VIRAL

- Rhinoviruses
- Coronaviruses
- Parainfluenza viruses
- Respiratory syncytial virus
- Human metapneumovirus
- Adenoviruses
- Influenza viruses
- Enteroviruses
- Human bocavirus

Table 22.8 Etiologic Agents Associated With Epiglottitis

BACTERIAL

- *Haemophilus influenzae* type b
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Haemophilus parainfluenzae*
- Other streptococci

Table 22.9 Etiologic Agents Associated With Pleural Effusion and Empyema

BACTERIAL

- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Haemophilus influenzae*
- Gram-negative bacilli
- *Mycoplasma pneumoniae*
- *Mycobacterium* species

Table 22.10 Etiologic Agents Associated With Lung Abscess

Bacterial	Parasitic
Staphylococcus aureus	*Entamoeba histolytica*
Anaerobes	
Streptococcus pneumoniae	
Other gram-negative bacilli	
α-Hemolytic streptococci	

Table 22.11 Etiologic Agents Associated With Cystic Fibrosis

BACTERIAL

- *Staphylococcus aureus*
- *Haemophilus influenzae*
- *Pseudomonas aeruginosa*
- *Burkholderia cepacia*
- *Stenotrophomonas maltophilia*
- *Mycobacterium* species

Table 22.12 Respiratory Specimens and Diagnostic Testing

Specimen Type	Microbiological Investigations	Comment
Sputum/induced sputum	Microscopy; culture; susceptibilities; DFA; PCR	Provided it is a good-quality specimen, it can be a highly informative specimen; can be difficult to obtain in children
Nasopharyngeal aspirate/swab	Microscopy; culture; susceptibilities; DFA; PCR	Most useful in viral infections; requires a skilled operator to obtain specimen; in some ways, it is easier to obtain than a throat swab, because the nares are always accessible
Nasal swab	Microscopy; culture; susceptibilities; DFA; PCR	Limited usefulness as it only recovers organisms present in the nasal cavity and not beyond
Throat swab	Microscopy; culture; susceptibilities; DFA; PCR	Probably the most representative specimen for disease of the upper respiratory tract; many bacterial pathogens are also common colonizers at various stages of childhood; can be difficult to obtain without child and parent cooperation; may represent organisms present in the nose as well as the oropharynx
Endotracheal aspirate	Microscopy; culture; susceptibilities; DFA; PCR	Invasive specimen, but is likely to represent pathogens from the lower respiratory tract; can be contaminated by organisms present in the oropharynx that can make result interpretation difficult
Bronchoalveolar lavage fluid	Microscopy; culture; susceptibilities; DFA; PCR	Invasive specimen but is likely to represent pathogens from the lower respiratory tract; can be contaminated by organisms present in the oropharynx, which can make result interpretation difficult
Transthoracic needle aspiration	Microscopy; culture; susceptibilities; DFA; PCR	Highly invasive specimen; risk of complications; microbiologically of high value provided the correct area has been biopsied
Lung tissue	Microscopy; culture; susceptibilities; DFA; PCR	Highly invasive specimen; risk of complications; microbiologically of high value provided the correct area has been biopsied
Pleural fluid	Microscopy; culture; susceptibilities; DFA; PCR	Invasive specimen but is the specimen of choice in a child with empyema
Blood cultures	Microscopy; culture; susceptibilities;	Very helpful if positive, but the positivity rate in pneumonia is relatively low
Serum/whole blood	Immunoassays; DFA; PCR	Serology per se is of limited value, since a diagnosis is dependent on paired sera that then makes it a retrospective tool; a single high titer can occasionally be obtained in acute disease; PCR on whole blood may be helpful in severe disease to detect viremia, but viremia is generally short lived
Urine	Antigen detection tests; microscopy; culture	Antigen detection tests are of limited value in children; pathogen is rarely cultured from urine

DFA, Direct fluorescent antibody; *PCR,* polymerase chain reaction.

Table 22.13 Gram Stain Appearance of Bacterial Respiratory Pathogens

Pathogen	Typical Gram Stain Appearance	Likely to Be Significant
Streptococcus pneumoniae	Gram-positive lancet-shaped diplococci	Predominant pathogen in Gram stain with abundant neutrophils
Staphylococcus aureus	Gram-positive cocci in clumps	
Haemophilus influenzae	Small pleomorphic gram negative coccobacilli	
Streptococcus pyogenes	Gram-positive cocci in chains	
Arcanobacterium haemolyticum	Gram-positive diphtheroid-shaped bacilli	
Corynebacterium diphtheriae	Pleomorphic diphtheroid gram-positive bacilli; special stain (Loeffler's methylene blue stain) demonstrates typical club-shaped ends)	
Mycoplasma pneumoniae	Absence of organisms as they lack a cell wall and cannot be visualized on Gram stain	

Table 22.14 Screening of Respiratory Specimen Quality

Specimen	Acceptable for Culture
Sputum	<10 SEC/average 10× field
Endotracheal aspirate	<10 SEC/average 10× field and bacteria seen in at least 1 of 20 oil immersion fields
Bronchoalveolar lavage fluid	<1% of cells present are SEC

This table has been modified from Jorgensen JH, Pfaller MA, Carroll KC, et al. *Manual of Clinical Microbiology*, 11th ed. Washington, DC: American Society of Microbiology; 2015.

SEC, Squamous epithelial cells.

Table 22.15 Normal Respiratory Flora

Streptococcus species
- including *Streptococcus pneumoniae*

Staphylococcus species
- including *Staphylococcus aureus*

Corynebacterium species

Moraxella species
- including *Moraxella catarrhalis*

Neisseria species
- including *Neisseria meningitidis*

Haemophilus species
- including *Haemophilus influenzae*

Cardiobacterium species

Kingella species

Eikenella corrodens

Table 22.16 Molecular Assays Commonly in Use for the Diagnosis of Respiratory Diseases

Molecular Assay	Principle	Main Use	Comment
Singleplex PCR	Single DNA or RNA target that is amplified	Can be designed for the detection of any known DNA or RNA sequence	Generally higher sensitivity than multiplex PCR as the targets are not competing
Multiplex PCR	Simultaneous amplification of several DNA or RNA targets	Respiratory pathogens; immunocompromised protocols; detection of various pathogens in blood cultures	Wide coverage of pathogens in a single test informs clinical management in a timely manner
16S rRNA sequencing	Amplification of 16S ribosomal RNA followed by sequencing of the product	Used to detect bacterial species in a clinical specimen that has failed to detect pathogens in culture.	Covers a wide range of pathogens listed in accessible sequence databases
Next-generation sequencing	Sequencing of a whole bacterial or viral genome or simultaneous sequencing of multiple bacterial or viral genes	Resistance testing and outbreak investigations	Can offer multiple gene sequences simultaneously or whole genome sequencing as well as de novo sequencing; currently, high cost prohibits routine use

PCR, Polymerase chain reaction.

Table 22.17 Molecular Terms Commonly Used in Diagnostics

Molecular Term	Explanation
PCR	An in vitro chemical reaction that leads to the synthesis of large quantities of a target nucleic acid sequence.
Reverse transcriptase PCR	RNA targets are converted into cDNA that is then amplified. This is needed for the amplification of RNA viruses (most common respiratory viruses).
RT PCR	The target amplification and the detection step occur simultaneously in the same tube. These assays require special thermal cyclers.
SNPs	Useful markers of genetic differences between strains, e.g., in outbreak investigations.
Target amplification techniques	Copies of a specific target nucleic acid are synthesized, and the products of amplification are detected by specifically designed oligonucleotide primers that bind to the complementary sequence on opposite strands of the double-stranded targets.
Signal amplification techniques	The target itself is not amplified; instead, the concentration of labeled molecules attached to the target nucleic acid is increased and measured

PCR, Polymerase chain reaction; *RT,* real-time; *SNPs,* single-nucleotide polymorphisms.

CLINICAL SPECIMENS FOR RESPIRATORY PATHOGEN DIAGNOSIS

Detection of respiratory pathogens is dependent on the type and quality of specimen collected, the timing of collection after the onset of clinical symptoms, the age of the patient, and transportation and storage of the sample before being tested in the laboratory. Ensuring high-quality collection of the right specimens is essential for making an accurate and interpretable laboratory diagnosis.

A range of specimens can be used for identifying the microbial etiology of respiratory infections in children and are shown in Table 22.12. Not all specimens are easily obtainable, and the diagnostic utility varies with each specimen type. The inability to obtain good-quality specimens from the lower respiratory tract is a fundamental problem with pneumonia diagnostics, and obtaining representative and uncontaminated specimens from the lungs is a challenge. Specimens collected by sputum induction or bronchoscopy may be contaminated by normal respiratory flora. Transthoracic needle aspiration is the best technique to obtain specimens from the site of infection in pneumonia, but it is performed in few centers despite a good safety profile.[16]

Specimens should be collected as early as possible in the acute stage of an infection, preferably prior to administration of antimicrobial or antiviral drugs. During this period, higher pathogen concentrations are likely to be present; however, the duration of pathogen shedding depends on the microorganism involved and the severity of the infection and other factors. With uncomplicated influenza virus infections, virus shedding is usually 3 to 5 days following symptom onset; however, this may be extended in severe respiratory disease to 5 to 10 days.[17] Children may also shed for up to 10 days and many weeks in immunocompromised individuals.

Throat and Nasopharyngeal Specimens

The majority of respiratory tract specimens received in the diagnostic laboratory from children are aspirates or swabs obtained from the upper respiratory tract. Nasopharyngeal aspirates are generally superior to swabs for the detection of respiratory viruses, since large numbers of respiratory epithelial cells are aspirated during the collection process.[18] However, aspirates are more difficult to obtain, especially outside the hospital setting, as they require a specific suction device. A range of commercial swabs are available, which include rayon tipped swabs and polyurethane sponges with wooden, plastic, or wire shafts. The availability of flocked nylon swabs, designed for the collection of respiratory samples, allows for the improved collection and release of respiratory epithelial cells and secretions from both children and adults.[19] Their use for obtaining nasopharyngeal specimens has been shown to have a similar performance to nasopharyngeal aspirates for the detection of common respiratory viruses in children, and the technique is relatively noninvasive.[20]

Nasal or oropharyngeal samples are generally not recommended for routine diagnostic use. The combining of nasal and throat swabs has been trialed in children in hospital and community settings and shown to have a reduced sensitivity.[21] In general, viral loads are higher in the nasopharynx than in the oropharynx, but with some respiratory virus infections, avian influenza H5N1, for example, titers may be highest in the lower respiratory tract. There may also be a higher yield from throat swabs compared to other samples for the detection of *Mycoplasma pneumoniae.*[22]

Induced Sputum

Culture of sputum specimens is commonly used as part of the evaluation of pneumonia in adults. Despite difficulties with interpretation of results,[3] carefully collected and processed sputum specimens have been shown to be useful in some contexts.[23] Nonetheless, there is still ongoing controversy about the value of routinely examining sputum.[24–28] Furthermore, sputum microscopy and culture are not routinely performed in children due to difficulties in obtaining specimens in this age group who are typically unable to expectorate.[29]

To overcome specimen collection problems, methods such as hypertonic saline nebulization have been used to induce sputum production. Induced sputum is now widely used to investigate lower respiratory infections in immunocompromised adults, especially for diagnosing *Pneumocystis jirovecii* infection,[30] and has also been used to diagnose pneumonia in children from settings with a high prevalence of tuberculosis.[31] However, few studies have collected induced sputum routinely from children with pneumonia. Recent studies of children hospitalized with community-acquired pneumonia from Finland, Kenya, and New Caledonia showed that collection of induced sputum was well tolerated, with a diagnostic yield from culture ranging from 12% to 65% using different interpretative criteria.[32–34]

The most rigorous evaluation of induced sputum for the diagnosis of pneumonia in children was performed as part of the Pneumonia Etiology Research for Child Health (PERCH) study. In this large study, there was no clear evidence that isolation of specific potential pathogens by culture or detection by polymerase chain reaction (PCR) was associated with pneumonia case status.[35,36] In addition, for PCR, there was no evidence that induced sputum provided additional evidence over and above testing a nasopharyngeal specimen.[36] In contrast, a recent longitudinal study from South Africa found that testing of induced sputum in addition to nasopharyngeal swabs provided incremental yield for detection of *Bordetella pertussis* and several respiratory viruses.[37]

Bronchoscopy Specimens

Although obtaining a lower respiratory sample via bronchoscopy is more invasive than sputum collection and is only available in certain facilities, there are potential advantages in being confident that the sample actually comes from the lower respiratory tract and in the avoidance of upper airway contamination. However, despite best efforts to avoid contamination with normal upper airways flora (including with use of protected specimen brushes), this is often difficult to achieve and must be considered when interpreting routine bacterial culture findings.[38] In practice, the use of bronchoscopy to obtain specimens in the context of childhood respiratory infections is largely restricted to immunocompromised individuals and those with problematic cystic fibrosis or with persistent focally abnormal chest radiographic changes.[39] Bronchoscopy can also have an important role in the diagnosis and management of pediatric pulmonary tuberculosis.[40]

Endotracheal Aspirates

Despite widespread use, the value of endotracheal aspirates to diagnose the cause of ventilator-associated pneumonia is debatable. Even though quantitative culture methods have been recommended, tracheal aspirate microscopy and culture do not appear to distinguish between infection and colonization.[41] There is also evidence that specimens should be rejected from further processing if no organisms are seen on Gram stain.[42]

Transthoracic Lung Aspiration

Needle aspiration of an area of suspected pneumonia is theoretically most likely to obtain the ideal clinical specimen for determining microbial etiology of pneumonia. Experience with large numbers of procedures at some locations has demonstrated the good safety profile of this technique. In The Gambia, which has the greatest experience with transthoracic lung aspiration in children, a review of over 500 lung aspirates over 25 years reported complications in six patients (all transient) and no deaths from the procedure.[16] Diagnostic yield with both culture and nucleic acid detection methods is appreciable,[16,43] with about a two-fold increase in yield with nucleic acid detection over culture alone.[43] However, the interpretation of results from highly sensitive molecular diagnostic techniques needs to consider new concepts of the lung microbiome that question whether the lungs are normally sterile. Transthoracic needle aspiration is not indicated for all children with pneumonia and is only appropriate for peripheral lesions confirmed on chest radiography.

Lung Tissue

The use of lung tissue to determine the microbial etiology of pneumonia is largely restricted to postmortem studies.[44] These are rarely performed on children but may provide valuable information on the causes of fatal cases of pneumonia and can confirm antemortem microbiological diagnoses.

Blood Specimens

Blood can be collected for culture, serological testing and, occasionally, nucleic acid tests. The yield from blood cultures is enhanced by obtaining adequate specimen volume, collecting the specimen prior to antimicrobial therapy, and avoidance of skin contamination through good phlebotomy technique.[45,46] Although there is good evidence that yield increases with increasing blood volume, the optimal collection volume in children is unclear. One current guideline recommends the collection of 3% to 4% of total patient blood volume in patients weighing less than 12.7 kg and 1.8% to 2.7% in patients weighing greater than 12.8 kg.[47] Anaerobic blood culture is usually unnecessary in children.

Urine

The main reason to collect urine specimens as part of the workup of respiratory infections in children is to test for specific antigens. For this purpose, the timing of specimen collection in relation to antimicrobial therapy is less important than for urine culture for suspected urinary tract infection. Collection of acute phase urine specimens can be challenging in young children, and a variety of techniques have been deployed to enhance collection in a clinically relevant time frame.

Testing of urine for antimicrobial activity by simple bioassay methods has been a valuable tool for detecting prior antimicrobial administration in epidemiological studies, although the timely collection of urine samples in young children can be challenging.[48]

MICROBIOLOGICAL TOOLS

Microscopy

As part of the investigation of respiratory infections, specimens obtained from the lower respiratory tract, pleural space, or abscesses that are sent for bacterial culture are usually examined first by Gram stain microscopy. Microscopy provides information on specimen quality and can provide early clues about the cause of infection. For example, the presence of large numbers of polymorphonuclear leukocytes indicates an inflammatory response, while the presence of bacteria with characteristic morphology may provide an early indication of the culture result and give guidance about treatment. When performed by experienced microscopists, some findings can be very specific. For example, the detection of Gram-positive cocci in clusters in a pleural fluid sample is highly suggestive of *Staphylococcus aureus.* The presence of a predominance of small gram-negative pleomorphic coccobacilli in a good-quality sputum sample is suggestive of infection with *H. influenzae.* Table 22.13 lists the typical Gram stain picture of three commonly found pathogens.

Microscopy is also an important tool to assess the quality of lower respiratory samples, which itself has a large impact on the interpretation of culture results.[49] Specimens from the lower respiratory tract can be contaminated by upper respiratory secretions during collection. Also, a poorly collected "lower respiratory" specimen may be predominantly composed of upper respiratory secretions. Either situation can lead to incorrect interpretations of culture results. To overcome this issue, it is standard practice for diagnostic laboratories to assess the quality of lower respiratory samples before they are cultured. This typically involves assessing the number of squamous epithelial cells (SECs) and polymorphonuclear cells (PMNs) in a Gram-stained smear of the specimen.[50,51] The presence of low numbers of SECs and high numbers of PMNs per low-power field are regarded as being indicative of a high-quality specimen.[52] Conversely, specimens with relatively low numbers of PMNs and high numbers of SECs are likely to represent oropharyngeal contamination and are rejected for routine culture. Detection of a potential pathogen in such a specimen that is contaminated with oropharyngeal flora may represent nothing more than the patient's oropharyngeal microbiota.

Table 22.14 summarizes some commonly used criteria for assessment of lower respiratory specimens. Other rejection criteria have been described that also include the presence of PMNs,[51] and it is the responsibility of the laboratory to have a standard operating procedure that specifies rejection criteria. Although there is a paucity of data from children, quantity of SECs alone was demonstrated to be a useful quality measure for induced sputum from young children with pneumonia.[53] Notable exceptions to sputum rejection criteria are specimens for detection of *Legionella* spp.[54] and *Mycobacterium tuberculosis;* any specimen submitted for investigation of legionellosis or tuberculosis should be processed by the laboratory regardless of the specimen quality.

Culture

Traditional bacterial culture techniques continue to be a fundamental diagnostic tool in diagnostic laboratories. In contrast, viral culture is now infrequently performed as a routine test, as it is time-consuming, requires a specialist laboratory area and has been largely superseded by molecular diagnostic techniques.

Although most important bacterial pathogens grow on standard laboratory media, such as sheep blood agar, special media environmental conditions are required to optimize the growth of some bacteria. For example, chocolate agar is the usual medium used to isolate *H. influenzae,* an atmosphere of 5% CO_2 is required to isolate *S. pneumoniae,* and special media are required for culturing *Legionella* species and *B. pertussis.* As a rule of thumb, it takes most bacterial pathogens 24 to 48 hours to grow in culture, and a further 24 to 48 hours are required to perform antimicrobial susceptibility testing.

The recent availability of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized the workflow in diagnostic laboratories.[55–57] MALDI-TOF MS allows the rapid identification of cultured microorganisms at a relatively low cost. The identification is based on the generation of mass spectra from whole cell extracts that are then compared to a library of well-characterized protein profiles. Although this method still

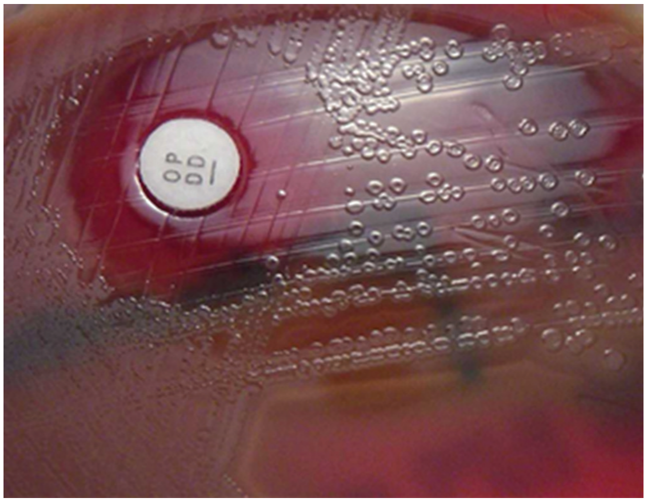

Fig. 22.1 Typical "draughtsman" phenomenon (ringed colonies with raised edges and depressed centers) and optochin-susceptibility of *Streptococcus pneumoniae*.

relies on traditional culture methods to obtain a pure isolate, MALDI-TOF MS provides full identification within minutes, including for all major bacterial respiratory pathogens.[58]

When reporting respiratory tract culture results, which are typically mixed cultures, laboratory scientists will focus on predominant organisms and those known to be important pathogens, such as *S. pyogenes* in throat swabs and *S. pneumoniae* in sputum samples (Fig. 22.1). Background oropharyngeal flora will normally not be worked up or reported in any detail, as this information will not contribute to patient care and, indeed, may give the false impression that they need to be treated.

The isolation of a bacterial organism from blood conclusively provides evidence of the cause of severe respiratory disease in children. The drawback is that recovery of bacterial pathogens from blood cultures in the context of respiratory infections is very low in children.[59,60] The typical blood culture yield in children admitted to hospital with community-acquired pneumonia is under 10%.[49,61] As discussed previously, the yield is greater when larger volumes of blood are collected, careful measures are taken to avoid skin contamination, and when samples are collected before antibiotics are commenced.[46]

Antigen Detection Assays

A variety of antigen detection assays for respiratory pathogens have been introduced into routine use by diagnostic laboratories. Direct fluorescent antibody (DFA) assays have been used for many years as rapid tests for respiratory viruses, although they have now been largely replaced by molecular methods or rapid antigen detection tests (RADTs). DFA looks for characteristic fluorescent staining patterns in cellular material from clinical specimens. Currently available assays detect respiratory syncytial virus (RSV), influenza A and B, parainfluenza viruses 1, 2, and 3, human metapneumovirus and adenovirus with sensitivities of about 80% to 90% and very high specificity.[62] DFA requires particular technical expertise but has the advantages that sample quality can be directly evaluated, and test results can be available within 60 minutes.

Among the most common antigen detection assays for respiratory infections are the RADTs for respiratory viruses, particularly influenza viruses and respiratory syncytial virus. The usefulness of RADTs is limited by variable and often suboptimal sensitivity, typically 50% to 98% for RSV and 10% to 85% for influenza viruses, although specificities are generally high (80% to 100%).[63,64] The clinical usefulness of these tests is affected by disease prevalence, being poor when there are few cases in the community (positive predictive value is low and false-positive cases are more likely). During peak virus circulation, although the positive predictive value approaches 100%, the negative predictive value is lower and false-negative results are more likely.[65,66] Due to concerns about poor sensitivity, most authorities recommend that RADTs for influenza are only used with caution outside the influenza season and only when a result will influence patient management; they emphasize that negative RADT results do not exclude influenza in patients with typical signs and symptoms.[67,68]

Other commonly used antigen detection assays for respiratory infections are those that detect *S. pneumoniae* and *Legionella pneumophila* serogroup 1 antigens in urine. These assays, typically in immunochromatographic test format, provide results within a short time frame, but are almost exclusively used on adults with suspected pneumonia. The specificity of currently used pneumococcal urinary antigen tests in children is poor, with frequent false positives due to nasopharyngeal carriage of *S. pneumoniae.*[69–71] This has limited the clinical utility of this test in children, but there may be some value as a diagnostic adjunct in cases with radiologically confirmed pneumonia. There is considerable interest in the development of serotype-specific pneumococcal urinary antigen tests.[72–74] Early assessments in children indicate that these next-generation assays may have some diagnostic value, at least in epidemiological studies, but assay cutoffs need to differ from adults to distinguish between carriage and disease.[75] Pneumococcal antigen detection assays have also been successfully applied to pleural fluid samples in children with pleural effusion or empyema.[76,77] A positive test result has high specificity in this context.

RADTs are also available for the diagnosis of *S. pyogenes* in throat swab specimens. These tests have high specificity for detection of *S. pyogenes,* but have relatively low sensitivity (70% to 90%), which is even lower in those with less severe disease.[78] As a consequence of suboptimal sensitivity, it is commonly recommended to perform bacterial culture on any samples that test negative by RADTs.[79,80]

Serology

Serological testing for respiratory pathogens was commonly performed in the past, relying either on the detection of immunoglobulin M (IgM) in the acute phase of the disease or the demonstration of seroconversion. More recently, the use of serological testing has largely been replaced by molecular-based assays that provide a rapid diagnosis with greater sensitivity and specificity.

Serological assays still have a limited place in the diagnosis of childhood respiratory disease. Detection of IgM antibodies is still a routine diagnostic tool for *M. pneumoniae* infection. However, older children may not mount an IgM response because of reinfection rather than primary infection, and IgM antibodies may persist for months after the acute infection.[81,82] For detection of *B. pertussis* infection, IgG antibody responses to pertussis toxin may be an indicator of infection, although these assays cannot differentiate between an immune response induced by infection and that due to vaccination. Serological diagnosis of pertussis has largely been replaced by molecular-based assays.[83,84] The serological diagnosis of *Chlamydophila pneumoniae* infection is complicated by the lack of species-specific tests and the resultant potential of cross-reactions in the assay. A single positive IgM response in any disease investigation may represent possible cross-reactivity or nonspecific interference in the assay and needs to be interpreted with caution and in the context of the clinical presentation.

Although detection of antistreptolysin O (ASO) and deoxyribonuclease (DNase) antibodies can be used when investigating the potential complications of *S. pyogenes* infections, such as glomerulonephritis and rheumatic fever, they are not useful for the diagnosis of acute *S. pyogenes* pharyngitis.[85]

Molecular Methods

The development and implementation of molecular methods is the single biggest recent advance in the diagnostics of respiratory infections.[4] While nucleic acid detection tests (NATs), such as PCR, have been used to detect respiratory pathogens for over two decades, the widespread adoption of these tests by diagnostic laboratories has occurred only recently, largely due to the increased availability of commercial assays. Table 22.16 discusses some of the more commonly used molecular assays, and Table 22.17 gives explanation of commonly used terms in molecular diagnostics.

NATs have several advantages over other diagnostic tools, including rapid turnaround time, the ability to detect low levels of all known pathogens, the lack of dependence on the viability of the target microorganism, little influence of antimicrobial therapy on diagnostic sensitivity, and the ability to be automated.[4] NATs may also provide additional information, such as antimicrobial susceptibility data and strain typing.

For the diagnosis of respiratory infections, the most widely used NATs are those that detect respiratory viruses and noncolonizing bacteria (e.g., *M. pneumoniae, Legionella* species, *B. pertussis*). For these microorganisms, detection in a respiratory sample from a child with a compatible clinical syndrome is regarded as sufficient evidence to assign causation. In contrast, NATs for other bacteria that may also be found in normal respiratory flora, including some of the most important pneumonia pathogens (e.g., *S. pneumoniae, H. influenzae,* and *S. aureus*), have struggled for a role outside research laboratories. As with culture-based methods, the problem with detection of these targets by NAT is the inability to distinguish colonization and carriage from disease.

NATs have particularly revolutionized the diagnosis of viral respiratory tract infections,[86,87] and are now the testing method of choice. Respiratory viruses are now commonly detected by large multiplex panels that typically include influenza A and B viruses, respiratory syncytial virus, parainfluenza viruses, human metapneumovirus, human rhinoviruses, enteroviruses, parechovirus, adenoviruses, human bocavirus, and several coronaviruses (OC43, 229E, NL63, HKU1). There are now many commercial multiplex assays available in a variety of formats, and the landscape is continually changing. In the right clinical context, the detection of a respiratory virus in a respiratory sample is generally regarded as being sufficient to assign causation.[88] However, this assumption is not always reliable as there is uncertainty about the pathogenic role of some viruses,[89] leading some to question the wisdom of using large multiplex NAT panels as first-line tests for respiratory pathogens, given potential problems with interpretation of positive results.[90] Furthermore, respiratory viruses are often detected in a similar proportion of both subjects with and without pneumonia in childhood pneumonia etiology studies,[32,91] although this observation typically does not apply to influenza A and B viruses, respiratory syncytial virus, and human metapneumovirus, which are disproportionately associated with case status.

NATs have also been used to detect *S. pyogenes* in throat swab samples, although these methods have not been used widely.[92–94] This situation is likely to change with the recent increased availability of commercial methods[92,94] and the motivation to improve turnaround times to better guide antimicrobial therapy.

Detection of microbial load by quantitative molecular methods has been explored in the effort to help distinguish infection from contamination or colonization. Microorganisms detected in greater quantities may be more likely to be clinically significant. Quantitative multiplex PCR has been used to determine the etiology of community-acquired pneumonia in adults using cutoffs developed for interpretation of culture results from lower respiratory tract specimens.[95,96] Greater confidence in the diagnostic cutoffs will be needed before this approach can be introduced into routine diagnostic use. Quantitative approaches using NATs have also been applied to nasopharyngeal specimens. Among human immunodeficiency virus (HIV)-infected adults in South Africa, quantitative PCR testing of nasopharyngeal samples distinguished between pneumococcal pneumonia and asymptomatic pneumococcal colonization with reasonable diagnostic accuracy.[97,98] Nasopharyngeal pneumococcal load also distinguished colonization from microbiologically confirmed pneumococcal pneumonia in a large pediatric study, although the diagnostic accuracy was inadequate for clinical use.[99]

NATs have also been applied, with limited success, to nonrespiratory specimens for determining the microbial etiology of respiratory infections in children. There has been particular interest in the testing of blood for *S. pneumoniae* by PCR. Among Italian children, blood PCR detected invasive pneumococcal disease with high specificity.[100–104] However, in other populations positive results have been reported in control participants who do not have suspected pneumococcal disease,[105] with false-positive results being relatively common in children from developing countries where there is a high prevalence of pneumococcal carriage.[106]

The potential application of whole genome sequencing in the diagnostic laboratory is still being realized. This method is already being increasingly used for strain characterization of bacterial isolates as part of epidemiological investigations.[107] However, its precise role in determining the etiology of respiratory infections is uncertain.

Antimicrobial Susceptibility Testing

Most antimicrobial susceptibility testing methods are performed on pure live bacterial cultures using a variety of standard methods.[108] Several guidelines have been established for interpretation of findings; the most commonly used guidelines are produced by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[109] and the Clinical and Laboratory Standards Institute (CLSI).[110] These guidelines are comparable, and it is essential that each diagnostic laboratory chooses an approved guideline for interpretation of their antimicrobial susceptibility test results. Increasingly, molecular methods with rapid turnaround times are being used to detect specific antimicrobial resistance mechanisms.[111] This trend is likely to continue given the constant demands for rapid identification of resistant pathogens.

Antiviral susceptibility testing against respiratory pathogens is rarely indicated and has mainly focused on influenza viruses.[112]

DIAGNOSTIC APPROACH BY SYNDROME

Common Cold

Manifestations of the common cold are so typical that diagnostic testing is usually unnecessary. If there is a reason to determine the specific virus involved, testing a nasopharyngeal specimen for respiratory viruses by NAT is the current test of choice.

Pharyngitis

The main reasons to diagnose the cause of acute pharyngitis are to detect cases caused by *S. pyogenes* and to identify the occasional case due to less common causes, such as *Arcanobacterium haemolyticum* and *Corynebacterium diphtheriae.* Throat swab culture is still the mainstay although antigen detection assays are available. In future, molecular point of care tests are likely to become available to clinicians in primary care.

Croup

The diagnosis of croup is usually based on the characteristic clinical picture (fever, hoarseness, barking cough, inspiratory stridor, and varying degrees of respiratory distress) and epidemiology. Identification of specific microbial causes can be accomplished by testing a nasopharyngeal specimen for respiratory viruses by NAT.

Sinusitis

Diagnostic testing is not usually performed on cases of acute sinusitis as the microbial etiology is well described. However, sinus puncture should be performed to obtain specimens for bacterial culture in patients with severe sinusitis, in those who have not responded to empiric antibiotics, and in patients with severe immunosuppression.

Epiglottitis

H. influenzae type b is isolated in cultures of blood and/or epiglottis in most children with epiglottitis. Direct visualization of the epiglottis should be performed in a setting where immediate securing of the airway is possible.

Bronchiolitis

A specific diagnosis of the causative agent of bronchiolitis can be made by testing a nasopharyngeal specimen for respiratory viruses by NAT.

Pneumonia

Determining the microbial etiology of pneumonia in children remains challenging, largely due to difficulties obtaining a sample from lungs.[2] Current guidelines for the management of community-acquired pneumonia in children generally recommend that diagnostic tests should mainly be used on patients with severe disease, with a focus on blood cultures and detection of respiratory viruses.[61,113] The development of improved urinary antigen tests and quantitative molecular assays holds hope for the future.

Pleural Effusion and Empyema

Gram stain and culture of fluid aspirated from the pleural cavity is indicated in patients for whom a diagnosis of infection is considered. The sample can also be tested for pneumococcal antigen by a RADT and nucleic acid detection methods. Testing of pleural fluid increased the yield of *S. pneumoniae* detection by 31% in South African children with empyema.[114]

Lung Abscess

Needle aspiration provides the best opportunity to identify the microbial cause of an abscess. Abscess fluid may also be recovered by bronchoscopy if it has ruptured. Blood cultures should also be performed in children with suspected lung abscess.

Infections Associated With Cystic Fibrosis

There is often a close working relationship between clinicians caring for patients with cystic fibrosis and laboratory scientists. Special attention is given by the laboratory to lower respiratory specimens from patients with cystic fibrosis with a particular focus on classic pathogens associated with this disease, such as *Pseudomonas aeruginosa, Burkholderia cepacia* complex, and *S. aureus.*[115] The use of synergy testing to assess antimicrobial combinations is often used in cystic fibrosis patients with multiresistant organisms, although the value of this practice has been questioned.[116]

Microbiome

Recognition of the possible existence of the lung microbiome has been a major recent revelation in respiratory medicine.[6] Until recently, the lungs in health were regarded as sterile, but the use of modern culture-independent techniques has consistently found evidence of bacteria in the lower airways.[6] Most of these studies have been performed on bronchoscopic specimens, which may be susceptible to contamination, but there is certainly mounting evidence supporting the non-sterility of the lung.

The existence of the lung microbiome has challenged our traditional paradigm of pneumonia pathogenesis, as the traditional view is that pneumonia is caused by a single invasive pathogen in a normally sterile site. There is increasing recognition that bacteria and viruses frequently interact in the causative pathway to pneumonia,[117,118] and the common finding of polymicrobial infection[10] adds further complexity to our understanding of how pneumonia develops. The traditional bacterial versus viral pneumonia concept may be too simplistic. Consequently, we are likely to need more sophisticated approaches to pneumonia diagnosis and interpretation of laboratory results than simply using assays that target single specific putative pathogens.

We have a lot to learn about the lung microbiome and are only just beginning to understand changes in the lung ecosystem during acute infections.[6,119–121] Analysis of the lung microbiome may provide insights into pneumonia etiology and reveal novel markers for pneumonia prognosis and treatment guidance.[122] The following are some examples of recent findings about the respiratory microbiome that may have clinical implications, and they give an indication of the applications that may be available in the future.

Using 16S ribosomal RNA sequencing, a recent study showed that certain taxa in the respiratory microbiota were associated with the clinical course of pediatric pneumonia.[123] In children aged 6 months to 5 years, high relative abundance in sputum of *Actinomyces*, *Veillonella*, *Rothia*, and Lactobacillales was associated with decreased odds of length of stay ≥4 days, and high relative abundance of *Haemophilus* and Pasteurellaceae was associated with increased odds of intensive care unit admission. In children aged 5 to 18 years, high relative abundance in sputum of Porphyromonadaceae, Bacteriodales, Lactobacillales, and *Prevotella* was associated with increased odds of length of stay ≥4 days.

In another recent study, the composition of the nasopharyngeal bacterial community of children was related to the prior history of acute sinusitis.[124] History of acute sinusitis was associated with significant depletion in relative abundance of taxa including *Faecalibacterium prausnitzii* and *Akkermansia* spp. and enrichment of *Moraxella nonliquefaciens.* Children who experienced more frequent upper respiratory infections had significantly diminished nasopharyngeal microbiota diversity.

Other recent data indicate that interactions between RSV and nasopharyngeal microbiota might modulate the host immune response, potentially affecting clinical disease severity,[125] that the nasopharyngeal microbiome at the time of upper respiratory viral infections during infancy may contribute to the ensuing risk for development of asthma,[126] and that the microbiome of children with cystic fibrosis is susceptible to environmental influences, suggesting that interventions to preserve the community structure found in young patients and slow disease progression might be possible.[127]

We can expect to see an exponential increase in publications on the role of the respiratory microbiome in health and disease over the next few years. The extent to which these findings can be readily translated into clinical applications is uncertain.

Future Prospects

The trend towards increased use of molecular diagnostic tools will probably continue with increased availability of point of care testing. It is also likely that measurement of bacterial and viral pathogen load will be part of those developments, both for distinguishing between colonization and disease and for monitoring response to treatment. Any future developments in diagnostics for respiratory infections must incorporate new knowledge about the lung microbiome. For lower respiratory infections, there is likely to be a move away from the detection of specific known pathogens to measurement of markers of change in the lung microbial ecology during disease. The development of new and better urinary antigen tests would be welcome, as these can be readily adapted to point of care testing.

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Access the reference list online at ExpertConsult.com.

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