Molecular Diagnostics for Detection of Bacterial and Viral Pathogens in Community-Acquired Pneumonia

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Traditional microbiological methods for detection of respiratory tract pathogens can be slow, are often not sensitive, may not distinguish infection from colonization, and are influenced by previous antibiotic therapy. Molecular diagnostic tests for common and atypical causative pathogens of community-acquired pneumonia have the potential to dramatically increase the diagnostic yield and decrease the time required to render results. Unfortunately, these tests often lack standardization and are not widely available. Consideration should be given to the development and evaluation of companion molecular diagnostic tests for detection of respiratory pathogens in future clinical trials of antimicrobials intended to treat community-acquired pneumonia.

Despite advances in our knowledge of the etiology, treatment, and management of community-acquired pneumonia (CAP), it remains a major cause of morbidity and mortality worldwide. Although a wide variety of etiological agents can cause CAP, a limited number of agents are responsible for the vast majority of cases (table 1) [1]. The role of microbiological testing for patients with CAP is still a matter of debate. Unfortunately, a specific etiological agent is determined in only approximately half of CAP cases, even when the best available diagnostic methods are used. The conventional laboratory tests for CAP-causing pathogens are so poor that current clinical practice guidelines do not recommend testing for any but the most severely affected individuals [1]. Nucleic acid–amplification methods have the potential to improve the timeliness, sensitivity, and accuracy of the tests used to detect respiratory pathogens. Advances in real-time amplification systems, multiplex analysis, and liquid-bead arrays have been key to the development of individual-pathogen and multipathogen panel approaches to diagnosis of CAP.

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Streptococcus pneumoniae accounts for approximately two-thirds of cases of CAP for which an etiology is determined [2]. Early and accurate diagnosis of pneumococcal pneumonia is problematic because of the limitations of conventional methods. Sputum and blood cultures are slow and lack sensitivity, particularly for patients who have received previous antibiotic treatment. False-positive sputum culture results may occur as a result of oropharyngeal contamination, and Gram stains of sputum specimens are frequently unreliable. Pneumococcal urinary antigen tests offer the promise of increased sensitivity but are reported to have variable specificity depending on the comparators chosen, the way in which the test is performed and interpreted, and the population studied [3–7].

A number of investigators have developed PCR-based assays for detection of *S. pneumoniae* in respiratory tract samples, with varying degrees of success [8–10]. There are 2 major concerns with PCR-based assays for this application: the presence of target genes in closely related viridans group streptococci and asymptomatic colonization with *S. pneumoniae.* A variety of pneumococcal gene targets, including *lytA, ply, psaA,* and the Spn9802 fragment, have been used in PCR assays [10, 11]. It is now clear that all but *ply* are found exclusively in *S. pneumoniae.* The use of real-time PCR methods addresses the second concern. In real-time PCR, the amount of target nucleic acid in the sample is inversely related to the cycle threshold (C_T) value. This relationship can be used to establish a C_T

Table 1. The most common etiological agents of community-acquired pneumonia in different types of patients.

Patient type, disease etiology
Outpatient
Streptococcus pneumoniae
Mycoplasma pneumoniae
Haemophilus influenzae
Chlamydophila pneumoniae
Respiratory viruses[a]
Inpatient, non-ICU
S. pneumoniae
M. pneumoniae
C. pneumoniae
H. influenzae
Legionella species
Aspiration
Respiratory viruses[a]
Inpatient, ICU
S. pneumoniae
Staphylococcus aureus
Legionella species
Gram-negative bacilli
H. influenzae

NOTE. Adapted from [1], with permission from the University of Chicago Press. ICU, intensive care unit.

[a] Influenza viruses A and B; parainfluenza viruses 1, 2, and 3; adenovirus; respiratory syncytial virus; metapneumovirus; and coronaviruses.

value cutoff that provides optimal sensitivity and specificity by preventing false-positive results due to colonization with small numbers of pneumococci. Yang et al. [8] reported a sensitivity of 90% and a specificity of 80% for a quantitative real-time PCR assay for the *ply* gene—compared with a composite reference standard comprising Gram stains of sputum specimens and sputum and blood cultures—when a C_T value of 29.86 was used as the cutoff. The presence of the *ply* gene in closely related viridans group streptococci may explain the relatively poor specificity of their assay.

A panel of 6 real-time PCR assays targeting the *lytA* gene of *S. pneumoniae,* the *mip* gene of *Legionella pneumophila,* and the 16s rRNA genes of *Haemophilus influenzae, Streptococcus pyogenes, Mycoplasma pneumoniae,* and *Chlamydophilia pneumoniae* were used by Morozumi et al. [12] to analyze 429 clinical samples from children and adults with pneumonia. The analysis time was ~2 h, and analytical sensitivity ranged from 2 to 18 copies/reaction, depending on the pathogen. The sensitivity and specificity, relative to conventional cultures with clinical specimens, were as follows: 96.2% and 93.2% for *S. pneumoniae*; 95.8% and 95.4% for *H. influenzae*; 100% and 100% for *S. pyogenes*; and 100% and 95.4% for *M. pneumoniae*, respectively. Culture for *C. pneumoniae* was not performed, but positive PCR results were obtained for all 6 patients with serological evidence of infection. No patients had results positive for *Legionella* species by culture or PCR. In addition, they found an excellent correlation between semiquantitative culture results (1+ to 3+) and C_T values in the PCR assays for *S. pneumoniae* and *H. influenzae* in clinical specimens. All patients with positive PCR and negative culture results for these 2 pathogens had a history of previous antibiotic therapy.

There are commercial multiplex assays that use microwell hybridization for detection of *Bordetella pertussis, L. pneumophila, Legionella micdadei, M. pneumoniae,* and *C. pneumoniae* (Pneumoplex; Prodesse [13]) or *L. pneumophila, M. pneumoniae,* and *C. pneumoniae* (Chamylege; Argene [14]). The technology is more cumbersome than real-time methods are, because of separate amplification and detection steps and the longer time needed to complete the analysis. Neither assay has been validated with large numbers of clinical specimens, and they have not been cleared by the US Food and Drug Administration (FDA) for diagnostic use.

Molecular diagnostics hold much promise for detection of the common and atypical bacterial pathogens that cause CAP. Analysis can be completed in hours, rather than days, for detection of typical pathogens and weeks for detection of atypical pathogens. This approach eliminates concerns about decreased organism viability associated with transport of specimens and the effects of previous antibiotic therapy. Real-time PCR panels that include the common causative pathogens of CAP could substantially increase the diagnostic yield in clinical practice. Unfortunately, these assays are not standardized or widely available, and, except for *L. pneumophila* and *Mycobacterium tuberculosis* (table 2), no FDA-cleared nucleic acid–amplification assays for bacterial respiratory pathogens are available. One major limitation to the molecular diagnostic approach to detection of bacterial causes of pneumonia is that culture would still be required to obtain an isolate for antimicrobial-susceptibility testing. So, for the near future, these methods will supplement, rather than replace, culture-based methods for pathogens for which antibiotic resistance is a concern.

Currently, there are >200 known respiratory viruses, but accurate data on how many CAP cases are caused by viral pathogens are lacking. The severe acute respiratory syndrome coronavirus, the H5N1 strain of influenza virus, and, most recently, adenovirus serotype 14 [15] have focused attention on viruses as causes of severe lower respiratory tract infections. Historically, clinical virology laboratories have used cell culture and immunoassays to detect the 7 "usual suspects": influenza viruses A and B; parainfluenza viruses 1, 2, and 3; respiratory syncytial virus; and adenovirus. More recently, nucleic acid–amplification methods have been used to detect respiratory viruses, often with dramatic increases in sensitivity. However, the diversity and complexity of the viral flora present significant challenges for nucleic acid–based detection systems. The dis-

Table 2. US Food and Drug Administration–cleared diagnostic tests for pulmonary pathogens.

Pathogen, test method	Test name	Manufacturer
Mycobacterium tuberculosis		
Transcription-mediated amplification	AMPLIFIED *M. tuberculosis* direct test	Gen-Probe
PCR	AMPLICOR *M. tuberculosis* test	Roche Molecular Diagnostics
Legionella pneumophila		
Strand-displacement amplification	BD ProbeTec *L. pneumophila* amplified DNA test	Becton Dickinson
Respiratory viruses		
PCR, allele-specific primer extension, and tag sorting	xTAG respiratory virus panel[a]	Luminex Molecular Diagnostics
Multiplex real-time PCR	ProFlu+ assay[b]	Prodesse

[a] Viruses include influenza viruses A, A subtype H1, A subtype H3, and B; respiratory syncytial viruses A and B; metapneumovirus; parainfluenza viruses 1, 2, and 3; adenovirus; and rhinovirus.
[b] Viruses include influenza viruses A and B and respiratory syncytial virus.

covery of 6 new respiratory viruses since 2000—including metapneumovirus, the severe acute respiratory syndrome coronavirus, influenza virus strain H5N1, coronavirus strains NL63 and Hku1, and human bocavirus—has presented new challenges for comprehensive viral diagnostics. Multiplex PCR and microarray-based systems provide potential solutions to this complex diagnostic problem. However, the number of viruses detectable in a single multiplex PCR is relatively small, and, although microarrays can detect many more pathogens, this approach is impractical for routine diagnostic use.

Recently, 3 different, highly multiplexed PCR assays for respiratory viruses that use microsphere flow cytometry (Luminex Xmap technology) to decode the PCR products have been commercially developed (by EraGen Biosciences [16], Genaco/Qiagen [17], and Luminex/Tm Bioscience [18]), and one assay (Luminex/Tm Bioscience) has recently been cleared by the FDA for diagnostic use. These assays can detect up to 20 different respiratory viruses in a single PCR, with an analysis time of ~4 h. The Genaco/Qiagen product is unique, in that it provides separate RNA and DNA target panels. The RNA panel detects respiratory viruses, whereas the DNA panel detects bacterial pathogens, including *M. pneumoniae, L. pneumophila, C. pneumoniae, Neisseria meningitidis, S. pneumoniae, H. influenzae,* and *Acinteobacter baumannii,* and adenovirus serotypes 3, 7, and 21 [19].

All these assays provide high throughput and are much less labor intensive and more cost-effective than is performance of a similar number of single-target assays in parallel. These tests have sensitivities and specificities comparable to or better than those of culture-based methods, depending on the target, and similar to those of single-target PCRs for the individual panel targets. The real value in these systems is that they can expand the capabilities of diagnostic laboratories to detection of viruses not commonly sought, such as rhinoviruses, coronaviruses, and metapneumovirus, with little more effort than is currently expended to detect the "usual suspects." Expanded panels can also detect more mixed viral infections and more mixed infections with bacteria and viruses. Increased diagnostic yield for respiratory pathogens could translate into better management of lower respiratory tract infections.

However, few studies have addressed the impact of molecular diagnostics on management of pneumonia. Oosterheert et al. [20] conducted a randomized, controlled trial involving 107 adults with lower respiratory tract infection at 2 Dutch hospitals. All patients had respiratory tract samples tested by real-time PCR methods for common respiratory viruses and atypical pathogens, but only results for patients in the intervention group were reported to the attending physicians. Respiratory tract samples from the control group were also tested using standard microbiological methods. The authors found that real-time PCR methods significantly increased the diagnostic yield, compared with standard diagnostic tests alone, but did not reduce antibiotic use, duration of hospital stay, or treatment costs. More studies with larger numbers of patients in different settings need to be done to better understand the impact of these methods on patient care.

Molecular methods for detection of respiratory pathogens can dramatically increase diagnostic yield and, consequently, can better identify which patients would benefit most from antibiotic therapy in clinical trials. Unfortunately, these methods are not standardized, and there are few FDA-cleared diagnostic tests for respiratory pathogens (table 2). The debate about the role of microbiological testing in the diagnosis of CAP and the expense associated with clinical trials serve to discourage the diagnostic industry from developing new diagnostic tests for CAP. Partnerships between diagnostic and pharmaceutical companies to develop companion diagnostics along with new drugs for CAP could potentially benefit clinical trials and ultimately provide clinical laboratories with validated tests that could better assist clinicians in the care of individual patients with pneumonia.

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