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## **Challenges and Progress Toward Determining Pneumonia Etiology**

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1 **Challenges and progress towards determining**  
2 **pneumonia etiology**

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21 **Keywords:** diagnosis; Gram stain; *Mycoplasma pneumoniae*; sputum; *Streptococcus*  
22 *pneumoniae*

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24 **Word count:** 1395

25 The World Health Organization estimates that lower respiratory tract (LRT) infection  
26 is the most common infectious cause of death worldwide and the fourth most  
27 common cause overall, with 3 million deaths attributed to LRT infection in 2016 [1].  
28 Timely and reliable identification of the underlying pathogen is critical for initiating  
29 effective and tailored antimicrobial treatment, but identifying the microbial etiology of  
30 pneumonia is challenging in many clinical settings. Community-acquired pneumonia  
31 (CAP) is an acute infection of the lung parenchyma acquired outside hospital or  
32 healthcare facilities. Microbiological testing to attempt an etiological diagnosis is  
33 generally recommended for CAP patients requiring hospitalization [2, 3].

34

35 The “gold standard” for determining pneumonia etiology is the detection of respiratory  
36 pathogens in specimens taken directly from the site of infection, the lungs, by  
37 bronchoalveolar lavage (BAL), pleural fluid sampling, or lung biopsy [4]. Sputum and  
38 tracheal aspirates are LRT specimens with a higher probability of upper respiratory  
39 tract (URT) contamination. Because BAL, pleural fluid sampling, and lung biopsy are  
40 invasive procedures, they are rarely performed in clinical practice. Therefore, the  
41 etiological diagnosis of CAP mostly depends on the detection of respiratory  
42 pathogens from specimens distant to the site of infection, such as URT samples,  
43 blood, and urine. Current recommendations for routine diagnostic testing in adult  
44 CAP patients include sputum Gram stain and culture, blood cultures, and urinary  
45 antigen tests for *Legionella pneumophila* and *Streptococcus pneumoniae*  
46 (pneumococcus) [2]. In children, sputum collection is hampered by difficulties with  
47 expectoration, and urinary pneumococcal antigen tests are not recommended.  
48 Testing for influenza virus, other respiratory viruses, and *Mycoplasma pneumoniae* in  
49 URT specimens by polymerase chain reaction (PCR) and/or rapid antigen tests may  
50 be done to evaluate children with CAP [3].

51

52 Recent large-scale studies performed extensive microbiological testing to determine  
53 the etiology of pneumonia in hospitalized adults (Musher et al. [5], U.S.,  $n=215$ ; CDC  
54 EPIC study [6], U.S.,  $n=2,259$ ; Gadsby et al. [7], U.K.,  $n=323$ ; CAPiTA study [8], the  
55 Netherlands,  $n=1,653$ ) and children (CDC EPIC study [9], U.S., <18 years old,  
56  $n=2,222$ ; Drakenstein Child Health study [10], South Africa, <2 years old,  $n=284$ ;  
57 PERCH study [11], Africa and Asia, <5 years old,  $n=1,769$ ) with a positive chest  
58 radiograph. A viral or bacterial pathogen was detected in 81–99% of pediatric and  
59 38–45% of adult CAP cases, except Gadsby et al. [7] reported a high detection rate  
60 in adults of 87%. Viruses accounted for the majority of detected pathogens [6, 7, 9-  
61 11], particularly in young children (>90%) [10]. Both viral and bacterial pathogens  
62 were detected in up to 90% of children [9-11], but <10% of adult patients [5, 6, 8].  
63 Conjugate vaccines have successfully reduced the burden of the former main causes  
64 of pneumonia, *S. pneumoniae* and *Haemophilus influenzae* type b, over the past  
65 three decades. *M. pneumoniae* is now the most commonly detected bacterial  
66 pathogen in children hospitalized with CAP in the U.S. [9].

67

68 Test results and epidemiological data must be carefully interpreted as no single  
69 diagnostic method applied to non-pulmonary specimens has both high sensitivity and  
70 high specificity for determining pneumonia etiology. Blood cultures are insensitive  
71 because they are positive in less than one-third of suspected bacterial pneumonia  
72 cases [5, 6, 8-11], but they are highly specific in determining pneumococcal  
73 pneumonia. In contrast, whole blood PCR and urinary antigen tests exhibit poor  
74 specificity, as they are also positive in patients who carry *S. pneumoniae* in the URT  
75 [11]. *S. pneumoniae* can be detected in the URT of up to 77% and 34% of healthy  
76 children and adults, respectively [11, 12]. In addition, *S. pneumoniae* carriage elicits

77 systemic antibody responses to pneumococcal antigens, limiting antibody detection  
78 as a diagnostic test to reliably determine pneumonia etiology [13]. This may also be  
79 true for *M. pneumoniae*, the detection of which by PCR in URT specimens and  
80 serology is not able to differentiate between infection and carriage [14]. Furthermore,  
81 the detection of many potential pathogens in the URT of a CAP patient represents  
82 carriage, URT infection, asymptomatic infection, or persistence of the pathogen after  
83 infection [11]. This complicates the assignment of causative pathogens in the URT  
84 for the pneumonia episode. In the PERCH study [11], more than half of childhood  
85 pneumonia cases (59%) and controls (54%) had  $\geq 4$  pathogens detected by multiplex  
86 PCR in URT specimens. Only RSV and *Bordetella pertussis* were rarely detected in  
87 URT specimens from healthy controls [10, 11].

88

89 Sputum has advantages in determining pneumonia etiology, including its origin from  
90 the LRT and non-invasive collection. Sputum induction, such as through hypertonic  
91 saline nebulization, may increase the likelihood of obtaining a valid sample and is  
92 especially useful in young children who are unable to expectorate spontaneously.  
93 Interestingly, testing of induced sputum is more sensitive than testing URT samples  
94 for the detection of several CAP pathogens in young children [10].

95

96 In this issue of *Clinical Infectious Diseases*, Ogawa et al. present a rigorous  
97 systematic review and meta-analysis of the literature on the diagnostic accuracy and  
98 yield of sputum Gram stain for diagnosing a bacterial pathogen in CAP [15]. Twenty-  
99 four studies were included ( $n=4,533$  adults), 22 on diagnostic accuracy and 4 on the  
100 diagnostic yield of this method. Consistent with previous studies, though on a larger  
101 scale, these new results suggest that Gram stain performed on good-quality sputum  
102 is highly specific for the diagnosis of *S. pneumoniae* and *H. influenzae* infections in

103 adult CAP patients (point estimates 0.91 and 0.97, respectively). Good-quality  
104 sputum was defined as the presence of <10 squamous epithelial cells and >25  
105 polymorphonuclear cells per low-power field [16, 17]. Data on other bacteria were  
106 limited. Sputum Gram stain diagnosed the bacterial pathogens in 36% of patients  
107 when sputum samples were collected successfully, increasing to 73% if only good-  
108 quality sputum samples were selected.

109

110 This study has three major strengths. First, the analyses were performed on studies  
111 with sputum Gram stain of good-quality samples. The applied sputum quality criteria  
112 may have helped detect URT contamination [16]. Second, the different (composite)  
113 reference standard tests of studies included in the work were extracted thoroughly  
114 and considered. This is essential, as BAL, pleural fluid sampling, and lung biopsy  
115 were rarely available, and the reference standard test (usually sputum culture) was  
116 imperfect in most cases. Third, the meta-analysis was performed using the Bayesian  
117 latent-class model, which accounts for the multiple imperfect reference standards.  
118 The study's major limitations are variations between included studies regarding pre-  
119 test symptoms and treatment, sample collection, transport, and processing methods,  
120 as well as the interpretation of results. Therefore, failure to detect pathogens on  
121 sputum Gram stain does not necessarily prove their absence. However, summary  
122 estimates across different subgroup analyses were consistent with those of the main  
123 analysis, and the major findings were confirmed by another recent review [18].

124

125 The results of this meta-analysis support current guidelines recommending prompt  
126 examination of pre-treatment sputum Gram stain and culture in hospitalized adults  
127 with CAP if good-quality specimens can be obtained and quality performance  
128 measures met [2]. Sputum Gram stain can be clinically useful in narrowing standard

129 (empirical) treatment decisions by providing immediate information about the  
130 potential causative pathogens, and sputum culture may enable pathogen isolation for  
131 sensitivity testing, which is the most important issue for many patients. Future studies  
132 on sputum Gram stain may focus on the possibility and consequences of URT  
133 contamination of even good-quality sputum and further elaboration of the diagnostic  
134 utility of induced sputum in children [10, 19-21].

135

136 Progress has been made towards determining the etiology and pathophysiology of  
137 pneumonia in recent years. Future challenges facing pneumonia etiology research  
138 include assigning a causative agent(s) from multiple potential pathogens detected in  
139 the URT during a pneumonia episode (even more so in children than adults),  
140 improving the pathogen-detection yield in adults, and tackling the pathophysiological  
141 significance of the lung microbiome, including putative pneumonia pathogens,  
142 moving away from a single-pathogen perspective [22, 23]. This can only be achieved  
143 with improved diagnostic methods. Thus, we need to advance current testing  
144 methods while exploring new and innovative approaches. Such an innovative and  
145 minimally invasive approach may be the detection of pathogen-specific antibody-  
146 secreting cells (**Figure 1**) [24], which allow differentiation between *M. pneumoniae*  
147 infection and carriage in children with CAP [14]. Other promising diagnostic  
148 approaches are exhaled breath analysis [25], novel biomarkers [26], new point-of-  
149 care and antigen detection assays [27], multidimensional (molecular) assessment of  
150 the host response [28], and new analytical approaches [11]. Efforts to determine the  
151 microbial etiology and understand the complex pathophysiology of pneumonia are  
152 key to reducing antibiotic overuse and resistance.

153

154

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157

158 **Potential conflict of interest:**

159 Nothing to disclose.



160 **References**

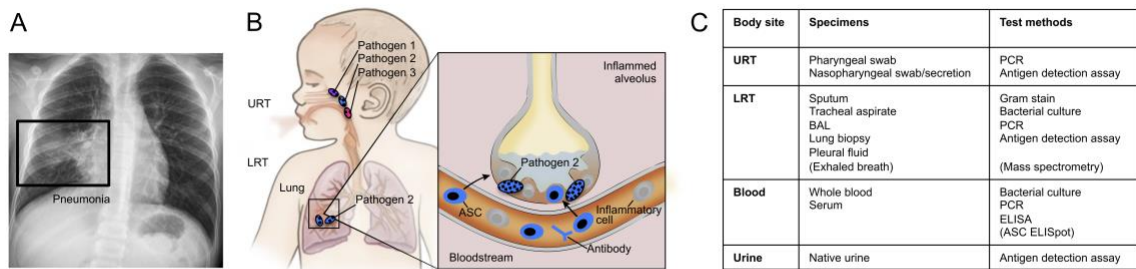
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251 **Figure 1. Pneumonia pathophysiology and testing methods.** *A:* Chest radiograph  
 252 of a child with CAP. The pulmonary infiltrate is indicated by the frame. *B:* Schematic  
 253 representation of the possible pathophysiology of childhood pneumonia. *C:* Overview  
 254 of different specimens and testing methods for pneumonia diagnostics.  
 255 Abbreviations: ASC, antibody-secreting cell; BAL, bronchoalveolar lavage; ELISA,  
 256 enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; LRT,  
 257 lower respiratory tract; PCR, polymerase chain reaction; URT, upper respiratory tract.  
 258



259