Compliance of Clinical Microbiology Laboratories in the United States with Current Recommendations for Processing Respiratory Tract Specimens from Patients with Cystic Fibrosis

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Respiratory tract specimens from patients with cystic fibrosis (CF) require unique processing by clinical microbiology laboratories to ensure detection of all potential pathogens. The present study sought to determine the compliance of microbiology laboratories in the United States with recently published recommendations for CF respiratory specimens. Microbiology laboratory protocols from 150 of 190 (79%) CF care sites were reviewed. Most described the use of selective media for Burkholderia cepacia complex (99%), Staphylococcus aureus (82%), and Haemophilus influenzae (89%) and identified the species of all gram-negative bacilli (87%). Only 52% delineated the use of agar diffusion assays for susceptibility testing of Pseudomonas aeruginosa. Standardizing laboratory practices will improve treatment, infection control, and our understanding of the changing epidemiology of CF microbiology.

The most common cause of morbidity and mortality in patients with cystic fibrosis (CF) is chronic lung infections caused by Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae and, less commonly, by the Burkholderia cepacia complex. As the life expectancy for CF patients has increased, emerging pathogens such as Stenotrophomonas maltophilia, Achromobacter xylosoxidans, and nontuberculous mycobacteria are increasingly detected (21).

Accurate identification of potential pathogens from the respiratory tract of CF patients is critical to ensure appropriate treatment and infection control and to improve our understanding of the changing epidemiology of CF microbiology (3, 4, 14, 23). The present study assessed compliance of clinical microbiology laboratories associated with CF care sites in the United States with recent consensus guidelines for processing CF respiratory tract specimens (21).

In 2001, the Cystic Fibrosis Foundation (CFF) in the United States convened a multidisciplinary committee to update previous recommendations for clinical microbiology practices for CF respiratory tract specimens (20). Revised recommendations were categorized by the strength of published evidence (7); category IA recommendations were supported by well-designed experimental, clinical, or epidemiologic studies and category IB recommendations were supported by some experimental, clinical, or epidemiologic studies and a strong theoretical rationale. In May 2003, the new guidelines were published and disseminated to CF care site directors (21).

To assess compliance with category 1A and 1B recommendations, the study team sought to obtain a copy of the clinical microbiology laboratory protocols of 190 CF care sites by mailing a request to the clinical directors of 116 core CF care centers (those programs that met CFF criteria for clinical care, teaching, and research), 46 affiliate centers, 24 adult centers, and 4 outreach programs. As many as three requests were made from July 2003 to January 2004. If a site did not send its protocol, its laboratory was contacted. In a final attempt to secure protocols, the CFF sent as many as three e-mails from March to April 2005 to the CF care site directors.

The protocols were reviewed for laboratory methods that reflected specific category 1A and 1B recommendations, including the use of selective media for S. aureus, H. influenzae, and the B. cepacia complex (8, 9, 25, 26); prolonged incubation of specimens; agar-based diffusion assays for susceptibility testing of P. aeruginosa such as Kirby Bauer disks or Etests (1, 2, 16, 18); and the CFF Burkholderia cepacia Research Laboratory and Repository for molecular identification of the B. cepacia complex (11, 12).

Microbiology laboratory protocols were received from 79% (150/190) of CF care sites, including 85%, 63%, 88%, and 50% of core CF care centers, affiliate centers, adult centers, and outreach programs, respectively. Ten adult programs, one affiliate center, and one outreach program used the same laboratory as their affiliated CF care center. Thus, 138 unique protocols were reviewed.

Most of the laboratory protocols described practices consistent with current recommendations (Table 1). However, only 14% (19/138) of protocols had provisions to alert the CF care team if the B. cepacia complex was identified. The majority (86%) of protocols addressed susceptibility testing of P. aeruginosa, but fewer (52%) specified the methodology. Fifty percent of protocols indicated the use of the CFF Burkholderia cepacia Research Laboratory and Repository for molecular identification of the B. cepacia complex.

There has been an increasing appreciation of the importance of implementing appropriate laboratory practices for CF respiratory tract specimens. Past surveys have assessed the use of prolonged incubation for the B. cepacia complex (23) and the...
use of selective media for the *B. cepacia* complex and *S. aureus* (4, 23). As evidence, the use of selective media for the *B. cepacia* complex has significantly increased from 10% (11/113) of laboratories surveyed in 1988 by the Centers for Disease Control and Prevention, to 73% (104/142) of laboratories surveyed in 1995 by the Epidemiologic Study of Cystic Fibrosis project, to 99% (136/138) of laboratories today, as indicated in the present study. However, only a minority (14%) of protocols indicated that if the *B. cepacia* complex were detected, the CF care team would be alerted to facilitate the prompt implementation of appropriate infection control measures, i.e., a single patient room and contact precautions to prevent patient-to-patient transmission of these pathogens.

Currently, fewer (82%) laboratories are currently using selective media for *S. aureus*. The Epidemiologic Study of Cystic Fibrosis project demonstrated the importance of selective agar for this pathogen; only 65% of sputum cultures and 51% of throat cultures were plated on colistin-nalidixic acid and/or mannitol salt agar (23), but sites using complete protocols for *S. aureus* had a higher 2-year cumulative prevalence of *S. aureus* (54%) than sites not using complete protocols (48%; P < 0.001). At present, the national rate of methicillin-resistant *S. aureus* varies from 0 to 31.8% (mean, 11.8%) at individual CF centers (6), and these substantial differences in prevalence may be due to inconsistent use of selective media to detect *S. aureus*.

The majority of protocols (87%) addressed the challenges of identifying nonlactose fermenting gram-negative bacilli other than *P. aeruginosa* and the *B. cepacia* complex. Several studies have shown that conventional biochemical and/or automated, commercial microtiter assays can misidentify newly emerging pathogens such as *S. maltophilia* and *A. xylosoxidans* (10, 13, 17, 19, 22, 24). Thus, laboratories processing CF specimens need access to molecular identification strategies. It should be noted that the apparent decrease in the proportion of laboratories that identify all gram-negative bacilli and that report mucoidy than *Pseudomonas aeruginosa* and *B. cepacia* complex

Report mucoidy for *P. aeruginosa* NA NA 89

Agar-based *P. aeruginosa* susceptibility testing NA NA NA

Use of *B. cepacia* Research Laboratory and Repository NA NA NA

<table>
<thead>
<tr>
<th>Practice</th>
<th>1980s&lt;sup&gt;a&lt;/sup&gt; (n = 113)</th>
<th>1990s&lt;sup&gt;b&lt;/sup&gt; (n = 142)</th>
<th>2000s (n = 138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selective media; <em>Burkholderia cepacia</em> complex</td>
<td>10</td>
<td>73</td>
<td>99&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selective media; <em>Staphylococcus aureus</em></td>
<td>NA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65 sputum/51 throat</td>
<td>82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selective media; <em>Haemophilus influenzae</em></td>
<td>NA</td>
<td>90</td>
<td>89&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prolonged incubation; <em>B. cepacia</em> complex</td>
<td>NA</td>
<td>46</td>
<td>73</td>
</tr>
<tr>
<td>Identify non-lactose-fermenting gram-negative bacilli other than <em>Pseudomonas aeruginosa</em> and <em>B. cepacia</em> complex</td>
<td>NA</td>
<td>97</td>
<td>87</td>
</tr>
<tr>
<td>Report mucoidy for <em>P. aeruginosa</em></td>
<td>NA</td>
<td>89</td>
<td>73</td>
</tr>
<tr>
<td>Agar-based <em>P. aeruginosa</em> susceptibility testing</td>
<td>NA</td>
<td>NA</td>
<td>52</td>
</tr>
<tr>
<td>Use of <em>B. cepacia</em> Research Laboratory and Repository</td>
<td>NA</td>
<td>NA</td>
<td>50</td>
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</table>

<sup>a</sup> Data from reference 4.
<sup>b</sup> Data from reference 23.
<sup>c</sup> Some centers report using more than one selective agar: *Burkholderia cepacia*-selective agar (n = 35), oxidative-fermentative polymyxin B-bacitracin lactose agar (n = 40), or *Pseudomonas cepacia* agar (n = 70).
<sup>d</sup> NA, not available.
<sup>e</sup> Some centers report using more than one selective agar: Columbia colistin-nalidixic acid agar (n = 50) or mannitol salt agar (n = 82).
<sup>f</sup> Some centers report using more than one selective agar: chocolate agar with or without bacitracin (n = 114) or *Haemophilus* isolation medium (n = 18).

In conclusion, clinical microbiology laboratories are increasingly compliant with national recommendations for CF specimens. Future efforts should focus on further education and improving adherence. Such efforts could include a review of laboratory protocols during CFF site visits conducted every 3 years, monitoring the impact of appropriate laboratory processing on the prevalence of specific pathogens, and enhancing laboratory reimbursement in recognition of the high cost of processing CF specimens.

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REFERENCES


