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

Juyan Zhou,[1] Elizabeth Garber,[1] Manisha Desai,[2] and Lisa Saiman[1]*

Department of Pediatrics, Columbia University, New York, New York,[1] and Department of Biostatistics, Columbia University, New York, New York[2]

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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

* Corresponding author. Mailing address: Division of Infectious Diseases, Department of Pediatrics, Columbia University, College of Physicians & Surgeons, 650 West 168th St., PH 4 West Room 470, New York, NY 10032. Phone: (212) 305-9446. Fax: (212) 305-9491. E-mail: LS5@columbia.edu.

TABLE 1. Comparison of clinical microbiology laboratory practices utilized at CF care sites

Practice	% Of care sites reporting usage		
	1980s[a] (n = 113)	1990s[b] (n = 142)	2000s (n = 138)
Selective media; *Burkholderia cepacia* complex	10	73	99[c]
Selective media; *Staphylococcus aureus*	NA[d]	65 sputum/51 throat	82[e]
Selective media; *Haemophilus influenzae*	NA	90	89[f]
Prolonged incubation; *B. cepacia* complex	NA	46	73
Identify non-lactose-fermenting gram-negative bacilli other than *Pseudomonas aeruginosa* and *B. cepacia* complex	NA	97	87
Report mucoidy for *P. aeruginosa*	NA	89	73
Agar-based *P. aeruginosa* susceptibility testing	NA	NA	52
Use of *B. cepacia* Research Laboratory and Repository	NA	NA	50

[a] Data from reference 4.
[b] Data from reference 23.
[c] 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).
[d] NA, not available.
[e] Some centers report using more than one selective agar: Columbia colistin-nalidixic acid agar (n = 50) or mannitol salt agar (n = 82).
[f] Some centers report using more than one selective agar: chocolate agar with or without bacitracin (n = 114) or *Haemophilus* isolation medium (n = 18).

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 may be due to the different methodologies used in the different studies (Table 1). The previous study surveyed CF center directors who might overestimate the frequency of these desirable laboratory practices, while the present study reviewed written protocols, which might underestimate these practices if the written protocols do not accurately document actual practices.

Standardized methodologies for antimicrobial susceptibility testing are highly desirable to facilitate clinical management and to track trends in antimicrobial resistance in the CF population. *P. aeruginosa* strains from CF patients often have unique characteristics, including multidrug resistance, a mucoid phenotype which is associated with chronic infection and clinical deterioration, and slower growth rates (15); these bacterial characteristics can adversely impact the performance and interpretation of antimicrobial susceptibility testing with automated, commercial microtiter assays (1, 2, 18). While agar-based diffusion assays are recommended to minimize very major (false-susceptibility) and major (false-resistance) errors, only 52% of protocols specified their use (5, 16).

There were some limitations to the present study. Only 79% of protocols were reviewed; nonresponders may have lower rates of compliance with current recommendations. The actual use of the CFF *Burkholderia cepacia* Research Laboratory and Repository is most likely more widespread than indicated by the protocols (J. LiPuma, University of Michigan, personal communication). The methods used to process specimens for nontuberculous mycobacteria were not assessed. Only protocols from the laboratory that processed the majority of CF specimens from a center were analyzed, but some sites utilized more than one laboratory.

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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