

Impact of Microbiology Practice on Cumulative Prevalence of Respiratory Tract Bacteria in Patients with Cystic Fibrosis

MICHAEL R. SHREVE,^{1*} STEVEN BUTLER,² HALEY J. KAPLOWITZ,² HARVEY R. RABIN,³
DENNIS STOKES,⁴ MICHAEL LIGHT,⁵ AND WARREN E. REGELMANN¹ FOR NORTH
AMERICAN SCIENTIFIC ADVISORY GROUP AND INVESTIGATORS FOR
THE EPIDEMIOLOGIC STUDY OF CYSTIC FIBROSIS

University of Minnesota School of Medicine, Minneapolis, Minnesota¹; Genentech, Inc., South San Francisco,² and
University of California, San Diego, La Jolla,⁵ California; Health Sciences Center, University of Calgary,
Calgary, Alberta, Canada³; and Vanderbilt University Medical Center, Nashville, Tennessee⁴

Received 14 September 1998/Accepted 17 November 1998

Investigators participating in the Epidemiologic Study of Cystic Fibrosis project began to collect microbiological, pulmonary, and nutritional data on cystic fibrosis (CF) patients at 180 North American sites in 1994. Part of this study was a survey undertaken in August 1995 to determine microbiology laboratory practices with regard to pulmonary specimens from CF patients. The survey included a section on test ordering, completed by a site clinician, and a section on test performance and reporting, completed by each site's clinical microbiology laboratory staff. Seventy-nine percent of the surveys were returned. There was intersite consistency of microbiology laboratory practices in most cases. The majority of sites follow most of the CF Foundation consensus conference recommendations. There were differences in the frequency at which specimens for culture were obtained, in the use of selective media for *Staphylococcus aureus* and *Haemophilus influenzae*, and in the use of a prolonged incubation for *Burkholderia cepacia*. These variations in practice contribute to prevalence differences among sites and may result in differences in clinical care.

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane regulator gene resulting in abnormal chloride ion transport across epithelia. Affected individuals have defective cyclic AMP-regulated chloride channel activity involving several organs, including the pancreas, biliary tract, gastrointestinal tract, and airways. Pathological findings in the lung include retention of secretions, harboring a variety of microorganisms, in the distal airways. When cultured from the respiratory tracts of CF patients, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* are associated with increased morbidity and mortality (22). Marked inflammation occurs in these airways, causing progressive bronchiectasis until the loss of functioning airways results in respiratory insufficiency and, eventually, death.

Investigators participating in the Epidemiologic Study of Cystic Fibrosis (ESCF) project began to collect microbiological, pulmonary, and nutritional data on CF patients at 180 North American sites in 1994 in order to describe the disease burden and course, current practice patterns, and safety and effectiveness of dornase alfa (Pulmozyme) in the general CF patient population. All CF patients were invited to enroll regardless of whether they were receiving this medication. Enrollment at the time of the survey included more than 18,000 of the estimated 20,000 to 30,000 CF patients living in North America. The present survey of microbiology practice was undertaken in August 1995 to determine, for each site, the frequencies of performance of sputum and throat cultures, the culture techniques used, and the frequency with which certain organisms are tested for antibiotic susceptibility. These micro-

biological practice patterns were then correlated with the reported prevalences of *S. aureus*, *P. aeruginosa*, and *B. cepacia* at the participating sites.

Coincident with this survey, the recommendations of a CF Foundation consensus conference on microbiological procedures for evaluation of infectious diseases in CF patients were published (8). This permitted a comparison of the current practices in the participating CF patient care sites with these guidelines as well as those previously published for CF specimens (20).

MATERIALS AND METHODS

Data on the microbiology practices of the ESCF sites were obtained by survey. Surveys were mailed to the 180 enrolled ESCF sites in August 1995. Survey part A, which dealt with clinical data and indications for specific cultures and sensitivities, was completed by the principal investigator and the study coordinator. Part B, which consisted of questions regarding specific culture media, was completed by, or in conjunction with, the director of the site's clinical microbiology laboratory. Completed surveys were blinded as to site and then forwarded for data management, processing, and analysis. Site confidentiality was maintained throughout the survey.

Of the 180 surveys sent, 142 (79%) were returned within 6 months. These 142 sites represent 15,328 enrolled patients.

The 2-year cumulative prevalences of *S. aureus*, *P. aeruginosa*, *P. aeruginosa* of mucoid phenotype, and *B. cepacia* were calculated from data on the case report forms submitted to the ESCF by each participating site for the period from 12 months prior to enrollment to 1 year after enrollment in the ESCF. On the ESCF case report form completed at the time of enrollment were recorded the presence or absence of these bacteria for the 12 months prior to enrollment, the source of the cultured sample (throat or sputum), and other clinical information. On the case report form completed at each subsequent encounter were recorded the presence or absence of these bacteria, the source, and clinical information at the time the patient visited the site. A report of at least one culture in each of two successive years (12 months prior to and 1 year following enrollment) was needed for the patient to enter the prevalence analysis. This analysis included patients who enrolled before March 1996. The predicted forced expiratory volume in 1 s (FEV₁) percentage was calculated from the FEV₁, gender, and height for those 6 years or older at the time of enrollment, using Knudson standards (13). The highest value reported for the period from 6 months prior to enrollment to 1 year after enrollment was used. The age used was that at the midpoint of the period. These data were available for 14,577 patients.

To assess the association between clinical and laboratory practices and ob-

* Corresponding author. Mailing address: Department of Pediatrics, Division of Pulmonary and Critical Care Medicine, University of Minnesota School of Medicine, Box 742, 420 Delaware St. S.E., Minneapolis, MN 55044. Phone: (612) 626-4440. Fax: (612) 624-0696. E-mail: shrev002@gold.tc.umn.edu.

served bacterial prevalence rates, the sites were divided into two groups, those using a complete protocol and those using a partial protocol. To be categorized as using a complete protocol, a site had to report on its survey the use of the following elements: (i) the procurement and culture of throat swab specimens from patients who did not produce sputum; (ii) inoculation of specimens onto media selective for *S. aureus*, *P. aeruginosa*, and *B. cepacia*; (iii) performance of additional biochemical tests on, or referral to a reference laboratory for, organisms that the site's routine identification system failed to identify; and (iv) reporting of all isolates of *S. aureus*, *P. aeruginosa*, mucoid *P. aeruginosa*, and *B. cepacia* to the clinic. These sites were compared with those that did not employ one or more elements of this complete protocol. Because the selective media differed for each organism, a site might use a complete protocol for *S. aureus* but not *P. aeruginosa* or *B. cepacia*; hence the sites were categorized for each of these bacteria and the results were compared. To assess the association of culture frequency with observed prevalence, the culture data from only the year after enrollment were used. The chi-square test of independence was used to determine the significance of differences among the observed prevalences.

RESULTS

Clinical practices. Sixty-four percent of the sites sent 100% of their respiratory tract specimens from CF patients to an on-site laboratory for culture. Of the ESCF sites that sent out respiratory specimens for culture, 64% sent out 25% or less.

All sites requested culturing of CF patients' sputum specimens. In cases in which sputum could not be produced, 89% of sites requested a throat swab culture. Clinicians at 97% of the sites indicated on the laboratory request form that the specimen was from a CF patient.

Clinicians requested cultures specifically for yeast and fungi at 85% of the sites, for mycobacteria at 86% of the sites, and for *Aspergillus* species at 68% of the sites, primarily when clinically indicated for CF patients. Cultures for yeasts and fungi were requested routinely during all CF patient hospitalizations and clinic visits at 21% of the sites, while such specimens were routinely cultured for mycobacteria at 10% of the sites and for *Aspergillus* species at 4% of the sites.

Clinicians received antibiotic susceptibility reports for all respiratory tract bacteria other than normal flora without a specific request at 85% of the sites, at which 80% of the 15,328 patients were enrolled. At more than 93% of the sites, enrolling 91% of the patients, susceptibility results were received on all *S. aureus*, *P. aeruginosa*, and *B. cepacia* isolates without request. At 88% of the centers, enrolling 83% of the patients, susceptibility results on all other gram-negative organisms were received without request.

Microbiology laboratory practices. While clinicians at 96% of responding sites indicated on the laboratory request form that the respiratory cultures were from CF patients, only 65% of the laboratories, covering 72% of the patients, required a clinical diagnosis.

Media and culture conditions likely to support the growth of or select for common CF-associated pathogens (6, 9, 10) were used at most sites for all CF patient respiratory specimens (Table 1). More than 90% of the laboratories used sheep blood agar for all sputum and throat swab specimens from CF patients. More laboratories used selective media for sputum than for throat swab specimens: 65% (sputum) versus 51% (throat swab) for *S. aureus*-selective media and 95% (sputum) versus 80% (throat swab) for gram-negative-selective media. Importantly, only 17% of laboratories cultured sputum specimens on media or under conditions selective for *Haemophilus influenzae*. This was reduced to 13% for throat cultures. A much larger proportion of laboratories used chocolate agar under aerobic conditions, which will allow *H. influenzae* to grow but will also allow overgrowth by *P. aeruginosa* (5). Seventy-three percent of laboratories used *B. cepacia*-selective media for primary culture of sputum, but only 46% carried out longer-term incubation at a reduced temperature. All CF respiratory

TABLE 1. Percentage of laboratories using specified media on all cystic fibrosis respiratory specimens

Medium	% of laboratories using the specified medium for specimens with source of:	
	Sputum	Throat swab
Sheep blood agar	94	90
<i>S. aureus</i> selective ^a	65	51
Gram-negative selective ^b	95	80
<i>H. influenzae</i> selective ^c	17	13
<i>H. influenzae</i> supportive ^d	90	77
<i>B. cepacia</i> selective ^e		
Initial inoculation	73	65
Subsequent culture	46	41

^a Colistin-nalidixic acid agar and/or mannitol salt agar.

^b Eosin-methylene blue agar and/or MacConkey agar.

^c Either *Haemophilus* agar or chocolate agar, incubated under anaerobic conditions.

^d Includes *H. influenzae*-selective agars or chocolate agar, incubated under an atmosphere of ambient air or 5 to 10% CO₂.

^e *B. cepacia* agar and/or oxidative-fermentative polymyxin B-bacitracin-lactose agar, with an initial culture time of 24 to 96 h at 30 to 37°C and a subsequent culture time of 24 to 96 h at 30 to 37°C.

specimens were cultured on media selective for yeasts and fungi at 11% of sites, while at 87% of the sites, fungal media were used only if requested.

Responding laboratories routinely employed several systems to identify gram-negative organisms. Automated systems were used by 80% of the laboratories, biochemical strips were employed by 45%, and standard biochemical methods were used by 35% of the laboratories. Many of the sites used multiple systems routinely. When the routine systems failed to identify organisms, laboratories performed the following practices: 66% set up additional biochemical tests involving incubations of 48 h or more, 51% set up additional biochemical tests without extended incubation, 50% sent the organisms to a reference laboratory, 25% used information from prior isolates, and 4% conducted no further tests. Most sites (96%) used combinations of these practices.

Specimens for culture of mycobacteria, including those classified as atypical, were sent to a reference laboratory by 66% of the site laboratories. Fifty-eight percent of these sites indicated that the specimens were from CF patients and were likely to have high numbers of *P. aeruginosa*.

Several methods for bacterial quantification were reportedly used. A semiquantitative estimation for a swabbed primary plate was performed by 92% of the laboratories routinely and by 2% of the laboratories only upon request. Four percent of the laboratories performed serial dilutions of sputum routinely, while 8% used this method upon request only.

Most sites routinely reported the common pathogens associated with CF as being distinct from normal flora (Table 2). *S. aureus*, *P. aeruginosa*, *B. cepacia*, and *H. influenzae* isolates were reported routinely by at least 94% of the laboratories. Distinction between mucoid and nonmucoid *P. aeruginosa* isolates was reported routinely by 89% of the laboratories, only on request by 4%, and not at all by 4% of the laboratories. Mucoid *Pseudomonas* isolates that could not be speciated were reported routinely by 62% of the laboratories, only on request by 4%, and not at all by 18%. Fungi and yeasts were reported routinely by varying numbers of laboratories, with as many as 15% of sites reporting these organisms only upon request and up to 5% not reporting them at all.

All but two laboratories reported growth of other nonfer-

TABLE 2. Percentage of laboratories reporting specific organisms as distinct from normal flora in CF patient respiratory tract cultures

Organism	% of laboratories reporting specified organism			
	Routinely	Only upon request	Never	Frequency unknown
<i>S. aureus</i>	100	0	0	0
Methicillin-resistant <i>S. aureus</i>	98	2	0	0
<i>P. aeruginosa</i>	94	1	1	4
<i>P. aeruginosa</i> , mucoid strains	89	4	4	3
<i>Pseudomonas</i> species, mucoid strains	62	4	18	16
<i>Pseudomonas</i> species other than <i>P. aeruginosa</i>	88	2	4	6
<i>B. cepacia</i>	97	1	0	2
<i>H. influenzae</i>	96	4	0	0
<i>Stenotrophomonas maltophilia</i>	96	1	0	3
Group A beta hemolytic streptococci (<i>S. pyogenes</i>)	99	1	0	0
<i>Streptococcus pneumoniae</i>	92	6	0	2
<i>Candida albicans</i>	77	15	2	6
<i>Candida</i> species	72	15	5	8
Yeast	81	9	5	5
<i>Aspergillus</i> species	92	8	0	0

mentative gram-negative rods, such as *Stenotrophomonas (Xanthomonas) maltophilia* and *Alcaligenes* and *Acinetobacter* species, with 97% identifying them to the genus or species level. All but three sites reported growth of fermentative gram-negative rods (*Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Serratia* spp., etc.), with 96% identifying them to the genus or species level.

Association of clinical and laboratory practices with observed bacterial prevalences. The reported 2-year cumulative prevalences of several organisms of clinical interest in CF were significantly different for sites using complete protocols and those using partial protocols. For the purposes of this analysis, we focused on *S. aureus*, *P. aeruginosa*, and *B. cepacia* since patients whose airway secretions yielded these organisms upon culture have been documented to have increased airway obstruction compared with similar CF patients in whom these organisms have not been detected (1, 7, 12, 15, 16, 21, 23, 24).

For *S. aureus*, 35% of the sites used complete protocols and 65% did not. The 2-year cumulative prevalence was 54% at sites using complete protocols, versus 48% at sites using partial protocols ($P = 0.0001$). The largest difference in *S. aureus* cumulative prevalence occurred in patients aged 6 to 12 years with more-severe airway obstruction ($FEV_1 < 70\%$ [predicted]). The prevalence of *S. aureus* in these patients, at sites using complete protocols, was 58.2%, versus 49.7% at centers using partial protocols (Table 3).

For *P. aeruginosa*, 57% of the sites used complete protocols. The 2-year cumulative prevalence was 66% for sites using complete protocols, versus 63% for those using partial protocols ($P = 0.0001$). This difference in prevalence was most pronounced in those aged 6 to 12 years with FEV_1 of $\geq 70\%$ (predicted). Those sites using complete protocols detected *P. aeruginosa* in 60.3% of these patients, compared with a detection rate of 55.6% for these patients at sites using partial protocols (Table 3).

The 2-year cumulative prevalence rates for mucoid *P. aeruginosa* isolates were 44 and 36% at sites performing complete and partial protocols, respectively ($P = 0.0001$). Complete protocols were used at 55% of the sites. The differences in prevalence of mucoid *P. aeruginosa* isolates were greatest in those under 17 years of age with more-severe airway obstruction (Table 3).

Sites performing complete protocols for *B. cepacia* had a prevalence rate of 4.6%, versus 3.6% for sites using a partial protocol ($P = 0.005$), with the greatest differences being in the

6- to 12-year age group and those over 18 with less-severe airway obstruction (Table 3).

For *S. aureus* and mucoid *P. aeruginosa*, only part of these differences was accounted for by differences in culture frequency alone. After adjustment for the number of cultures performed, sites that used complete protocols still detected more patients with *S. aureus* or mucoid *P. aeruginosa* than those that did not. Twenty percent more *S. aureus* cultures per patient per year and 12% more *B. cepacia* cultures per patient per year were performed by sites using complete protocols than by sites using partial protocols. In the case of *P. aeruginosa*, essentially all of the difference in prevalence rates between sites performing complete protocols and those using partial protocols was explained by differences in culture frequency. Forty percent more cultures for *P. aeruginosa* (2.3 versus 1.6 cultures) were performed per patient per year at sites using complete protocols for that organism than at sites using partial protocols.

DISCUSSION

This survey describes the variation in the microbiology practices of 142 of 180 sites participating in a large multicenter study of CF. This variation in practice affects detection of these bacteria and, hence, the prevalence rates of microbes important in CF and must be taken into account when such rates are estimated and used to assess the impact of bacteria on disease status in CF patients. In particular, denominator data for epidemiological studies of the impact of *H. influenzae*, mycobacteria, yeasts, and fungi on disease progression in CF patients must be limited to sites performing routine culturing for the bacteria in question on selective media under optimal conditions.

All of the 142 sites request culturing of sputum produced by CF patients. However, if sputum cannot be produced, many sites do not request a throat swab culture. This is an important issue, since throat swab and sputum culture yields are similar for *S. aureus* and *P. aeruginosa* (9) and the frequency of detection of *S. aureus* and/or *P. aeruginosa* by throat swab culture in children under 2 years is associated with significantly increased morbidity and mortality in the following 10 years (12).

Ninety-six percent of the sites specify that the specimen is from a CF patient, although only 65% of the laboratories (representing 72% of the patients) require a clinical diagnosis. It is important that the diagnosis of CF be specified so that the

TABLE 3. Comparison of 2-year cumulative prevalences of sites using complete protocols and those using partial protocols to detect bacteria of interest

Bacterium	Patient age (yr)	Site protocol ^b	% of patients with FEV ₁ of $\geq 70^c$	P ^a	% of patients with FEV ₁ of $< 70^c$	P ^a
<i>S. aureus</i>	6–12	C	57.9 (858/1,481)	0.01	58.2 (153/263)	0.04
	6–12	P	53.6 (941/1,756)		49.7 (175/352)	
	13–17	C	60.6 (409/675)	NS	60.3 (190/315)	0.03
	13–17	P	58.5 (473/809)		51.9 (196/378)	
	>18	C	54.4 (289/531)	NS	45.9 (440/958)	0.001
	>18	P	51.6 (437/847)		38.7 (561/1,451)	
<i>P. aeruginosa</i>	6–12	C	60.3 (1,260/2,088)	0.009	84.2 (326/387)	NS
	6–12	P	55.6 (639/1,149)		82.0 (187/228)	
	13–17	C	71.5 (690/965)	NS	89.6 (388/433)	NS
	13–17	P	70.3 (365/519)		87.3 (227/260)	
	>18	C	76.2 (615/807)	NS	89.0 (861/968)	NS
	>18	P	73.0 (417/571)		89.0 (1,278/1,441)	
Mucoid <i>P. aeruginosa</i>	6–12	C	36.1 (770/2,135)	0.001	66.9 (273/408)	0.001
	6–12	P	28.8 (317/1,102)		47.3 (98/207)	
	13–17	C	49.3 (487/987)	0.003	73.9 (340/460)	0.001
	13–17	P	41.3 (205/497)		52.8 (123/233)	
	>18	C	56.8 (453/797)	0.01	73.1 (1,059/1,448)	0.001
	>18	P	50.1 (291/581)		61.2 (588/961)	
<i>B. cepacia</i>	6–12	C	2.4 (29/1,232)	0.03	5.4 (13/243)	NS
	6–12	P	1.4 (27/2,005)		4.0 (15/372)	
	13–17	C	3.8 (21/560)	NS	8.4 (20/237)	NS
	13–17	P	4.1 (38/924)		6.8 (31/456)	
	>18	C	8.1 (47/578)	0.01	9.3 (88/945)	NS
	>18	P	4.8 (38/800)		9.1 (133/1,464)	

^a P values are for comparisons between prevalences at sites using complete protocols and sites using partial protocols. NS, not significant.

^b C, complete protocol used; P, partial protocol employed.

^c Values in parentheses are number of patients with the bacterium/total number of cultures.

laboratories are alerted to use appropriate media to enhance the detection of clinically important organisms (25).

Tablan et al. (20) showed that *B. cepacia* was isolated from simulated CF sputum by 95% of the laboratories using *B. cepacia*-selective medium (*P. cepacia* agar or oxidative-fermentative polymyxin B-bacitracin-lactose agar) but was detected by only 22% of the laboratories not using a selective medium. Our data show that the use of *B. cepacia*-selective media has increased significantly in the past 9 years, from 12% in Tablan's study to 73% in our study. Unfortunately, over half of the laboratories still do not carry out long-term incubation at a reduced temperature, conditions which may increase the yield of *B. cepacia* by allowing slower-growing colonies to become apparent. Further complicating matters, other organisms, such as *Stenotrophomonas maltophilia*, can grow on *B. cepacia*-selective media and be difficult to distinguish from *B. cepacia*, even with further biochemical testing (2). Early detection of *B. cepacia* in CF patients is extremely important with respect to both the individual patient and the CF population, since this organism appears to be spread from person to person among CF patients. Non-culture-based methods of identifying *B. ce-*

pacia and *P. aeruginosa*, such as detection of species-specific DNA by PCR (4, 17), have been developed and may have more-widespread application in the future.

Routine culturing for organisms of clinical and prognostic importance in CF (*S. aureus*, *P. aeruginosa*, and *B. cepacia*) is carried out at most, but not all, sites. Most laboratories report these organisms routinely as being distinct from normal flora, and they almost all routinely report antimicrobial agent susceptibilities for these organisms. Mucoid *Pseudomonas* species are not reported as distinct from *Pseudomonas* species by over one-third of the laboratories. The presence of mucoid *Pseudomonas* species in respiratory secretions may have significant clinical implications (11, 14), making it potentially important to recognize these isolates as a distinct entity.

This survey indicates the degree to which practice currently follows the CF Foundation consensus conference recommendations. The sites follow these recommendations to various degrees. For example, 81% of the sites use protocols recommended for detection of *P. aeruginosa*, but only 65% follow the recommendation to use *S. aureus*-selective media for all CF patient sputum specimens. Less than half of the centers em-

ploy *B. cepacia*-selective culture media or incubate the cultures for extended periods, both of which improve the yield of this bacterium. These variations in laboratory practices contribute significantly to differences in the observed 2-year cumulative prevalences of several clinically important organisms in CF patients. These differences are most pronounced in the young. The contribution of each specific part of the complete protocol that accounted for the differences cannot be entirely determined because a site that completed one part of the protocol often completed the other parts as well and sample sizes allowing comparison of sites that used one part, with all other parts being equally employed or not employed, became too small. One specific part did, however, independently account for much of the variance: the more frequently cultures were obtained, the higher the observed prevalences for these bacteria. It appears likely that these differences in practice will affect estimates of the effect of these organisms on the clinical outcome.

This is the first large-scale survey of actual microbiological practices at a large number of CF patient care sites, representing over 14,000 CF patients. Several conclusions can be drawn from this survey. Substantial between-site differences exist in the protocols used for culturing the respiratory tracts of CF patients. This is particularly true for the use of throat swabs and thus affects the detection of *S. aureus*, *P. aeruginosa*, and *B. cepacia* most in the young CF patient. This results in significant differences in 2-year cumulative prevalences of bacteria between sites using complete protocols and those using partial protocols. Early detection of these organisms in this young CF patient population may significantly alter the therapy prescribed. In recent antibiotic trials, the degree of reduction of sputum bacterial colony counts correlated with the degree of reversal of airway obstruction in CF patients (18, 19). Hence, early detection of those microbes associated with greater airway obstruction at an earlier age in these patients (12) may motivate earlier appropriate antibiotic intervention and slow the development of airway obstruction in these patients. Finally, further communication among sites to routinely employ standard practices, as recommended by the CF Foundation and by a recent study by Burns et al. (3), will be valuable in improving the rates of detection of potentially pathogenic organisms associated with CF and may lead to improved infection control practices and allow for more-accurate measurement of the impact of new antimicrobial therapies in CF.

ACKNOWLEDGMENTS

This work was financially supported by Genentech, Inc. We thank Paul Quie and C. Carlyle Clawson for their assistance with this article.

REFERENCES

- Abman, S. H., J. W. Ogle, R. J. Harbeck, S. N. Butler, K. B. Hammond, and F. J. Accurso. 1991. Early bacteriologic, immunologic, and clinical courses of young infants with cystic fibrosis identified by neonatal screening. *J. Pediatr.* **119**:211-217.
- Burdge, D., M. Noble, M. Campbell, V. Krell, and D. Speert. 1995. *Xanthomonas maltophilia* misidentified as *Pseudomonas cepacia* in cultures of sputum from patients with cystic fibrosis: a diagnostic pitfall with major clinical implications. *Clin. Infect. Dis.* **20**:445-448.
- Burns, J., J. Emerson, J. Stapp, D. Yim, J. Krzewinski, L. Loudon, B. Ramsey, and C. Clausen. 1998. Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin. Infect. Dis.* **27**:158-163.
- Campbell, P., J. Phillips, G. Heidecker, M. Krishmanani, R. Zahorchak, and T. Stull. 1995. Detection of *Pseudomonas* (*Burkholderia*) *cepacia* using PCR. *Pediatr. Pulmonol.* **20**:44-49.
- Campos, J. M. 1995. *Haemophilus*, p. 556-565. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. ASM Press, Washington, D.C.
- Canadian Cystic Fibrosis Foundation. 1993. Microbiologic processing of respiratory specimens from patients with cystic fibrosis. Recommendations of the Clinical Subcommittee of the Medical/Scientific Advisory Committee of the Canadian CF Foundation. *Can. J. Infect. Dis.* **4**:166-169.
- Corey, M., and V. Farewell. 1996. Determinants of mortality from cystic fibrosis in Canada, 1970-1989. *Am. J. Epidemiol.* **143**:1007-1017.
- Cystic Fibrosis Foundation. 1994. Microbiology and infectious disease in cystic fibrosis, p. 1-26. In Cystic Fibrosis Foundation, concepts of care, vol. V, section 1. Cystic Fibrosis Foundation, Rockville, Md.
- Doern, G. V., and B. Brogden-Torres. 1992. Optimum use of selective plated media in primary processing of respiratory tract specimens from patients with cystic fibrosis. *J. Clin. Microbiol.* **30**:2740-2742.
- Gilligan, P. H. 1991. Microbiology of airway disease in patients with cystic fibrosis. *Clin. Microbiol. Rev.* **4**:35-51.
- Govan, J. R., and J. W. Nelson. 1992. Microbiology of lung infection in cystic fibrosis. *Br. Med. Bull.* **48**:912-930. (Review.)
- Hudson, V. L., C. L. Wielinski, and W. E. Regelman. 1993. Prognostic implications of initial oropharyngeal bacterial flora in patients with cystic fibrosis diagnosed before the age of two years. *J. Pediatr.* **122**:854-860.
- Knudson, R. J., M. D. Lebowitz, C. J. Holberg, and B. Burrows. 1983. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am. Rev. Respir. Dis.* **127**:725-734.
- Kulezcyki, L., T. Murphy, and J. Bellanti. 1978. *Pseudomonas* colonization in cystic fibrosis. *JAMA* **240**:30-34.
- Lewin, L. O., P. J. Byard, and P. B. Davis. 1990. Effect of *Pseudomonas cepacia* colonization on survival and pulmonary function of cystic fibrosis patients. *J. Clin. Epidemiol.* **43**:125-131.
- Muhdi, K., F. P. Edenborough, L. Gumery, S. O'Hickey, E. G. Smith, D. L. Smith, and D. E. Stableforth. 1996. Outcome for patients colonised with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. *Thorax* **51**:374-377.
- O'Callaghan, E., M. Tanner, and G. Boulnois. 1994. Development of a PCR probe test for identifying *Pseudomonas aeruginosa* and *Pseudomonas* (*Burkholderia*) *cepacia*. *J. Clin. Pathol.* **47**:222-226.
- Ramsey, B. W., H. L. Dorkin, J. D. Eisenberg, R. L. Gibson, I. R. Harwood, R. M. Kravitz, D. V. Schidlow, R. W. Wilcott, S. J. Astley, M. A. McBurnie, et al. 1993. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N. Engl. J. Med.* **328**:1740-1746.
- Regelman, W. E., G. R. Elliott, W. J. Warwick, and C. C. Clawson. 1990. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. *Am. Rev. Respir. Dis.* **141**:914-921.
- Tablan, O. C., L. A. Carson, L. B. Cusick, L. A. Bland, W. J. Martone, and W. R. Jarvis. 1987. Laboratory proficiency test results on use of selective media for isolating *Pseudomonas cepacia* from simulated sputum specimens of patients with cystic fibrosis. *J. Clin. Microbiol.* **25**:485-487.
- Tablan, O. C., W. J. Martone, C. F. Doershuk, R. C. Stern, M. J. Thomassen, J. D. Klinger, J. W. White, L. A. Carson, and W. R. Jarvis. 1987. Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis. Risk factors and outcomes. *Chest* **91**:527-532.
- Thomassen, M., C. Demko, and C. Doershuk. 1987. Cystic fibrosis: a review of pulmonary infections and interventions. *Pediatr. Pulmonol.* **3**:334-351.
- Webb, A. K. 1995. The treatment of pulmonary infection in cystic fibrosis. *Scand. J. Infect. Dis. (Suppl.)* **96**:24-27. (Review.)
- Whiteford, M. L., J. D. Wilkinson, J. H. McColl, F. M. Conlon, J. R. Michie, T. J. Evans, and J. Y. Paton. 1995. Outcome of *Burkholderia* (*Pseudomonas*) *cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. *Thorax* **50**:1194-1198.
- Whittier, S., R. L. Hopfer, M. R. Knowles, and P. H. Gilligan. 1993. Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. *J. Clin. Microbiol.* **31**:861-864.