Nontuberculous Mycobacteria

I: Multicenter Prevalence Study in Cystic Fibrosis

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Nontuberculous mycobacteria (NTM) are potential respiratory pathogens in cystic fibrosis (CF). To assess the species-specific prevalence and risk factors for acquisition, we conducted a prospective, cross-sectional study of the prevalence of NTM and clinical features of patients at 21 U.S. centers. Almost 10% of patients with CF who were 10 years or older were included (n = 986). The overall prevalence of NTM in sputum was 13.0% (range by center, 7–24%). *Mycobacterium avium* complex (72%) and *Mycobacterium abscessus* (16%) were the most common species. When compared with patients with CF without NTM, culture-positive subjects were older (26 vs. 22 years, $p < 0.001$), had a higher FEV_1 (60 vs. 54%, $p < 0.01$), higher frequency of *Staphylococcus aureus* (43 vs. 31%, $p < 0.01$), and lower frequency of *Pseudomonas aeruginosa* (71 vs. 82%, $p < 0.01$). Molecular typing revealed that almost all patients within each center had unique NTM strains. In summary, NTM are common in patients with CF, but neither person-to-person nor nosocomial acquisition explained the high prevalence. Older age was the most significant predictor for isolation of NTM. The clinical significance of NTM in CF is incompletely defined, but patients with these organisms should be monitored with repeat cultures.

Keywords: cystic fibrosis; nontuberculous mycobacteria; mycobacterium infections; *Mycobacterium avium-intracellulare*; *Mycobacterium abscessus*

Cystic fibrosis (CF) is a genetic disorder affecting ∽ 30,000 people in the U.S. Defects in the Cystic Fibrosis Transmembrane Conductance Regulator gene product result in abnormally thickened airway secretions, chronic bacterial airways infection, bronchiectasis, and early death (1). The dramatic improvement in survival over the past 50 years, reflecting intensive therapy to clear airways secretions, improved nutrition, and more aggressive use of antibiotics (2, 3), has been accompanied by the emergence of new potential pathogens, such as *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans*, and nontuberculous mycobacteria (NTM) (4–6).

NTM have been increasingly reported in North America as pulmonary pathogens among nonimmunocompromised persons (7–20). In addition to previously described upper lobe cavitary disease in older, male smokers, recent reports have noted an association of NTM with nodular bronchiectatic disease in middle-aged, nonsmoking women without apparent predisposing conditions and patients with CF (6, 10, 21–27). Multiple nosocomial outbreaks of NTM have been described (28), but the mode of acquisition for persons with CF has not been defined.

This study, using standardized sampling, specimen processing, and organism identification techniques, provides new information regarding the overall and species-specific prevalence, associated clinical characteristics and/or acquisition risk factors, and mode of acquisition of NTM in CF.

METHODS

Subject Recruitment

Patients with CF who were 10 years or older were recruited at 21 centers (29). Subjects were not excluded because of prior recovery of NTM. We enrolled 1,186 subjects; 986 had complete data (38 died, 26 received transplants, 40 moved, and 96 withdrew). The study was approved by Institutional Review Boards at participating sites.

Period, Cross-sectional Study Design

At enrollment, clinical data were collected (*see* model), spirometry measured; sputum was collected for culture at two additional visits.

Identification and Molecular Typing

Specimens were processed for mycobacteria at 21 study sites, using validated techniques (30). Smears were performed (fluorochrome or Ziehl-Neelson); after decontamination, specimens were placed on Lowenstein-Jensen slants and in BacTec 7H12B vials with PANTA (31). The duration between Cultures 1 and 2 was 177 ± 99 days and 152 ± 77 days between Cultures 2 and 3. Of 2,958 specimens, 3% had insufficient volume and 4% had *Pseudomonas aeruginosa* overgrowth (32).

Mycobacteria were typed at study sites and sent to the reference laboratory (University of Texas Health Center, Tyler) for final identification. Slowly growing mycobacteria were speciated by polymerase chain reaction and restriction digest (33). Rapidly growing mycobacteria were identified by a modified technique (34). Isolates of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *avium*-complex X-strains were confirmed using commercial RNA/DNA probes (Accuprobe; Gen-Probe, Inc., San Diego, CA) (35, 36). Molecular typing of *M. avium* complex and *Mycobacterium abscessus* used published techniques (37–40).

(*Received in original form July 9, 2002; accepted in final form November 4, 2002*)

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Supported by a grant from the Cystic Fibrosis Foundation, CFF A914 and collaborative grants from participating General Clinical Research Centers, Center for Research Resources, National Institutes of Health (individual grant numbers cited in Appendix).

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Defense or other Departments of the U.S. Government.

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This article has an online supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

Am J Respir Crit Care Med Vol 167. pp 828–834, 2003
Originally Published in Press as DOI: 10.1164/rccm.200207-678OC on November 14, 2002
Internet address: www.atsjournals.org

TABLE 1. COMPARISON OF STUDY SUBJECTS AND REFERENCE POPULATIONS

Characteristic Study Subjects	Patients with CF with Three Cultures (*n = 986*)	Patients with CF at Study Sites† (*n = 2,970*)	All Patients with CF in CFF Registry‡ (*n = 11,356*)
Age, yr*	23 ± 9	21 ± 9	21 ± 9
Male, %	53	53	54
Race, %			
White	97	97	97
FEV_1, % predicted*	55 ± 22	68 ± 27	66 ± 27
BMI, kg/m^2*	19 ± 3	19 ± 5	19 ± 6
Use of pancreatic enzymes, %	94	91	92
Sweat chloride, mM/L*	107 ± 18	104 ± 19	104 ± 19
Pseudomonas aeruginosa, %	80	70	74
Staphylococcus aureus, %	33	36	35

Definition of abbreviations: BMI = body mass index; CF = cystic fibrosis; CFF = Cystic Fibrosis Foundation.
* Mean ± SD.
† Patients age ⩾ 10 years, seen at a CF center study site in 1994; not all variables available for all patients.
‡ Patients age ⩾ 10 years, seen at any CF center in the U.S. in 1994; not all variables available for all patients.

Data Analysis

Prevalence was defined as subjects having at least one positive culture divided by all subjects. To estimate validity, the study population was compared with subjects who were 10 years or older at study sites in 1994 and subjects who were 10 years or older in the National Registry in 1994.

The primary analysis used dichotomous outcome of NTM culture-positive versus culture-negative. Primary predictors were age and FEV_1. Other variables included (*1*) sweat chloride (41), (*2*) *P. aeruginosa*, *Staphylococcus aureus*, (*3*) CF genotype ("mild," pancreatic sufficient and "severe," pancreatic insufficient mutations) (29, 42), (*4*) nutritional status (body mass index (43), pancreatic enzyme use, and insulin use), and (*5*) nosocomial and antibiotic exposure (days hospitalized, outpatient visits, and intravenous antibiotics). The effect of "burden" of NTM was assessed by comparing culture-positive subjects who met microbiologic criteria for pulmonary disease (three positive cultures or two positive cultures with one positive smear) (44) with those having a lesser burden of organisms. The association of NTM with socioeconomic status (educational level; median family income) and population density was assessed by linking census-based characteristics to subjects using zip codes (45, 46). Data were analyzed using SAS Version 6.12 (SAS Institute, Cary, NC). Univariable comparisons used Pearson χ^2 for categoric variables, and Student's *t* test and multiple linear regression analyses or Kruskal-Wallis rank sum test for continuous variables.

A logistic regression model was developed that treated cross-sectional data as a case–control study, with culture-positive versus culture-negative as the dichotomous response variable and FEV_1% as the primary predictor variable. Model building included backward elimination and forward addition of variables and appropriate interaction terms. A 10% change in the main effects parameter estimates or a p value of less than 0.10 (using −2Log Likelihood Ratio test) were criteria for retaining variables or interaction terms in the model (47).

RESULTS

Demographic and Clinical Characteristics

Study subjects were of similar race, ethnicity, and nutritional status when compared to those of the populations of the study sites and all U.S. Cystic Fibrosis Centers. Study subjects were older (∽ 2 years), had a lower FEV_1 (∽ 11%), lower prevalence of *S. aureus* (∽ 2%), and a higher prevalence of *P. aeruginosa* (∽ 6%) (Table 1) than patients with CF at all centers in the U.S.

Prevalence and Geographic Distribution of NTM

The prevalence of NTM was 12.98% (95% confidence interval = 12.96, 13.00). Overall, 140 mycobacterial strains were isolated from 128 subjects; 116 subjects had a single species and 12 subjects had two species, including 4 subjects with both *M. avium* and *M. intracellulare* (Figure 1). The most common organisms were *M. avium* complex (72%) and *M. abscessus* (16%) (Figure 1). Five subjects grew only *Mycobacterium gordonae* on a single culture and three subjects with *M. avium* complex also grew *M. gordonae*.

Of the 128 culture-positive subjects, 90 (70%) had one of three, 21 (16%) had two of three, and 17 (13%) had all three cultures positive. Smears were positive in 33 (26%) culture-

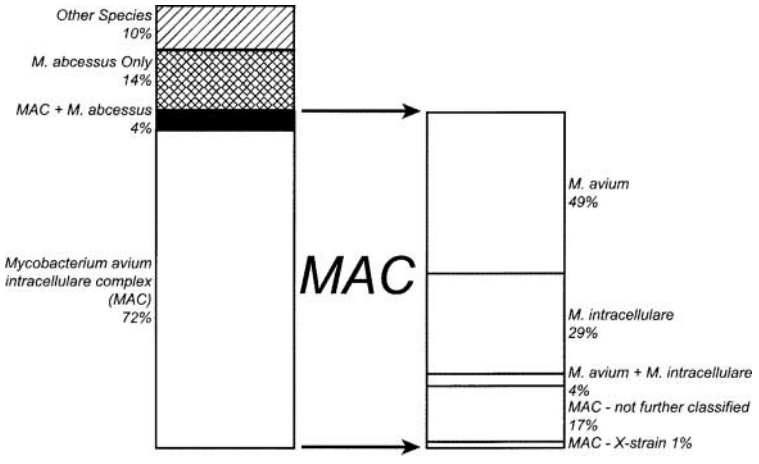

Figure 1. Frequency of NTM species by subject (n = 128) (*left bar*). Most subjects had only species of the *M. avium* complex (MAC) isolated (72%) (two of these also had *M. gordonae* isolated). The 13 subjects with "other species" included five with *M. gordonae*, two each with *M. kansasii*, *M. lentiflavum*, and *M. peregrinum* (formerly *M. fortuitum* biovar *peregrinum*), and one each with *M. malmoense* and *M. terrae*. Of the subjects with MAC, most had *M. avium* (49%) or *M. intracellulare* (29%) (*right bar*).

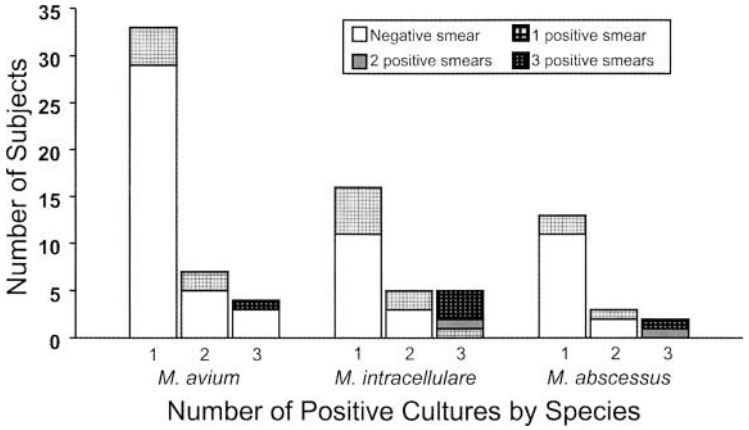

Figure 2. The number of subjects having positive mycobacterial smears and cultures. The number of positive cultures is listed on the abscissa grouped according to species recovered. The frequency of one, two, or three positive mycobacterial smears is shown as the *shaded partitions* within each *bar.*

positive subjects (Figure 2). Twenty-five subjects (20% of culture-positive subjects, 3% of all study subjects) met the American Thoracic Society microbiologic criteria for nontuberculous mycobacterial pulmonary disease (44). The cumulative frequency of recovering mycobacteria from three serial sputum specimens was 7, 10, and 13%.

Subjects with *M. intracellulare* were more likely to have positive mycobacterial smears than those with *M. avium* (46 vs. 18%; $p = 0.01$). *M. intracellulare* also tended to be isolated from multiple specimens more commonly than *M. avium* (39 vs. 23%; $p = 0.18$) (Figure 2).

Molecular DNA typing of isolates of *M. avium* complex (188 isolates from 118 subjects, average 5.9 subjects per center) and *M. abscessus* (47 isolates from 24 subjects, average 1.2 subjects per center) revealed that each subject within study sites had unique genotypes of organisms, except for two instances. One sib-pair was cultured on the same day, had the same genetic strain of *M. avium* complex; subsequently, each sib had two negative cultures for mycobacteria. In the other instance, two patients whose cultures were analyzed 3 days apart at the same site had the same molecular type of *M. avium* complex; each of these patients subsequently had at least six negative cultures for mycobacteria.

NTM were recovered from subjects at all participating sites, but the prevalence varied by geographic location, ranging from 7% in Boston to 24% in New Orleans. Despite the marked variation in prevalence, the distribution of species among sites was similar, with *M. avium* complex accounting for ∽ 75% of the isolates (Figure 3). With the exception of three sites (Madison, WI; Denver; and Salt Lake City), all centers with prevalences greater than 15% were in coastal states.

Associated Clinical Characteristics

When compared with culture-negative subjects, those with NTM were significantly older (4 years), had a higher FEV_1 (6%), a lower frequency of *P. aeruginosa* (10%), and a higher frequency of *S. aureus* (12%). Both groups were similar with respect to sex, sweat chloride, body mass index, use of pancreatic enzymes, and requirement for insulin. Culture-negative and culture-positive groups had a similar frequency of available genotypes (61 vs. 58%) and similar frequencies of mild and severe genotypes (Table 2). Subjects with NTM had no greater exposure to medical centers in the year before enrollment than culture-negative subjects, as assessed by frequency of hospitalizations and attendance at outpatient clinics (Figures 4A and 4B).

The prevalence of NTM increased in a nonlinear fashion with age, with a marked increase in persons 40 years or older (Figure 5A). Of course, the absolute number of study subjects diminishes as age increases. The prevalence also increased with FEV_1 (Figure 5B).

Culture-positive subjects were 1.7 times more likely (95% confidence interval 1.1, 2.6, $p = 0.01$) than culture-negative subjects to live in areas with a greater proportion of college graduates. There was also a trend toward culture-positive subjects

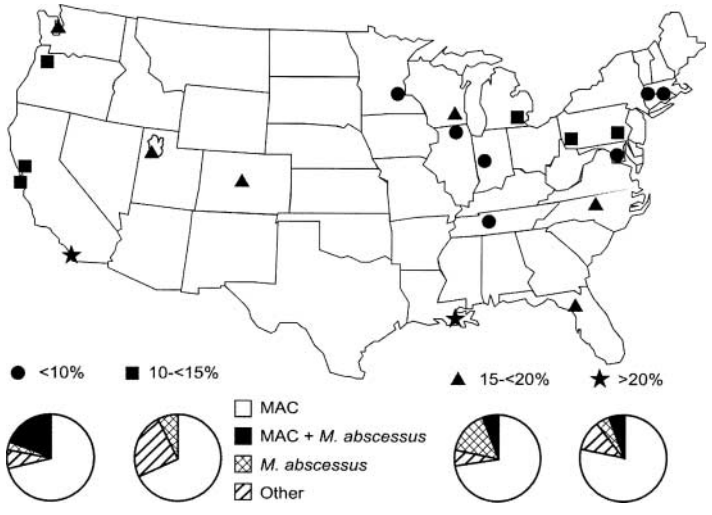

Figure 3. Prevalence of NTM by sites. The geographic location of participating sites is indicated with a symbol representing the prevalence of NTM (< 10% [*circle*], 10 to < 15% [*square*], 15 to < 20% [*triangle*], and > 20% [*star*]) at each site. The *pie charts* at the *bottom* show the relative distribution of *M. avium* complex (MAC), *M. abscessus*, and other species of NTM by the categorizations of prevalence.

TABLE 2. UNIVARIABLE ANALYSIS OF PREDICTORS BY NONTUBERCULOUS MYCOBACTERIAL CULTURE STATUS

	NTM Culture*		Microbiologic Criteria†		Organisms	
Predictor	Negative (*n = 858*)	Positive (*n = 128*)	Nondiagnostic (*n = 103*)	Diagnostic (*n = 25*)	MAC (*n = 92*)	*Mycobacterium abscessus* (*n = 18*)
Age, yr‡	22 ± 8	26 ± 11a	25 ± 11	29 ± 10e	26 ± 11	23 ± 10
Male, %	53	52	52	52	55	44
FEV_1, % predicted‡	54 ± 22	60 ± 23b	60 ± 24	58 ± 21	61 ± 24	61 ± 22
Sweat chloride§, mM/L‡	107 ± 18	106 ± 19	105 ± 20	109 ± 15	106 ± 19	106 ± 21
BMI, kg/m^2‡	19 ± 3	19 ± 3	19 ± 3	20 ± 3	20 ± 3	19 ± 3
Insulin requiring diabetes, %	8	6	7	0	4	6
Use pancreatic enzymes, %	94	92	93	88	92	94
Pseudomonas aeruginosa, %	82	71c	74	60	66	78
Staphylococcus aureus, %	31	43d	42	48	42	50
Severe genotypes§, %	95	93				

Definition of abbreviations: BMI = body mass index; MAC = *Mycobacterium avium* complex; NTM = nontuberculous mycobacteria.

* p Values for NTM (−) vs. NTM (+): a = 0.0002, b = 0.007, c = 0.006, d = 0.009.

† p Values for nondiagnostic vs. diagnostic (American Thoracic Society microbiologic criteria): e = 0.07.

‡ Continuous variables presented as mean ± SD.

§ For the following: sweat chloride n = 902; genetically tested for cystic fibrosis transmembrane conductance regulator n = 758; mutations identified on both alleles n = 598, of which 6% (of total) were mild and 94% severe (71% homozygous ΔF508) mutations.

living in areas with a median family income over $50,000 (odds ratio 1.4, 95% confidence interval 0.8, 2.6, p = 0.2) and in nonrural areas (odds ratio 1.5, 95% confidence interval 0.7, 2.9, p = 0.2).

Multivariable logistic regression demonstrated that subjects with NTM were significantly more likely to be older, have a higher FEV_1, a lower body mass index, and have *S. aureus* isolated; they were less likely to have *P. aeruginosa* (Table 3). A subject with multiple associated factors (e.g., age > 30 vs. < 18 years, FEV_1 > 90 vs. < 40%, *S. aureus*, no *P. aeruginosa*) would be over 50-fold more likely to have NTM.

Subjects meeting microbiologic criteria for disease (44) tended to be older (4 years, p = 0.07) but with roughly equivalent FEV_1% when compared with subjects with a lesser burden of

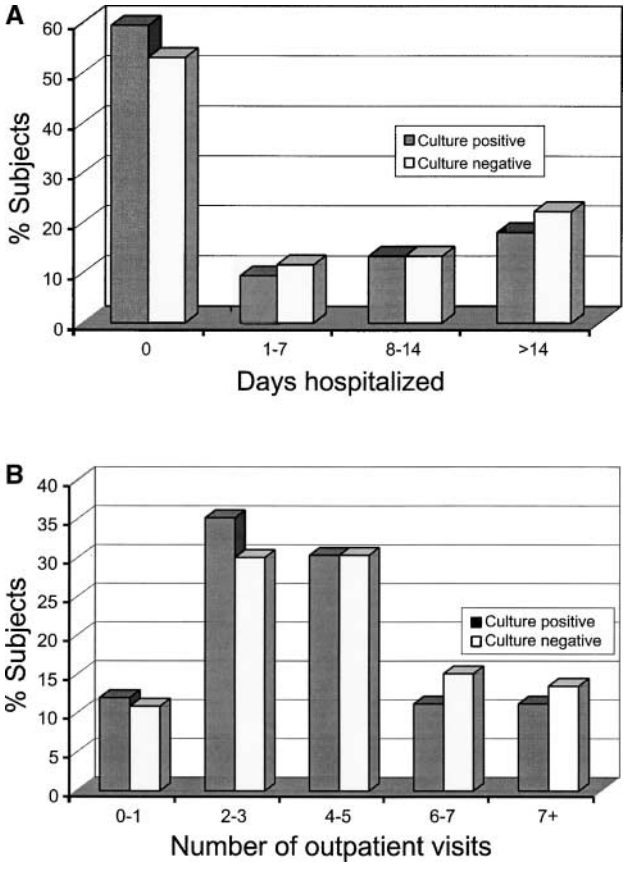

Figure 4. Percentage of subjects with positive or negative cultures for NTM grouped by the number of (*A*) days spent in hospital and (*B*) outpatient visits for each during the year before enrollment in the study.

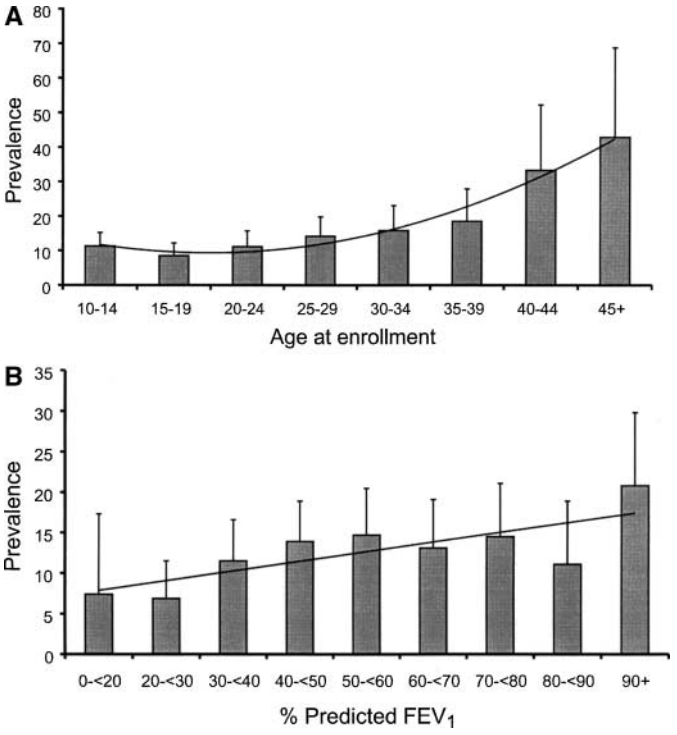

Figure 5. Prevalence of subjects with at least one positive culture for NTM from each (*A*) age category and (*B*) categorization of lung disease by FEV_1 (% predicted). The *error bars* represent the upper 95% confidence interval around the point prevalence in each category. The regression lines represent the (*A*) polynomial (r^2 = 0.97, p = 0.0002) and (*B*) linear (r^2 = 0.61, p = 0.01) predicted distributions.

TABLE 3. MULTIVARIABLE LOGISTIC MODEL OF CHARACTERISTICS ASSOCIATED WITH NONTUBERCULOUS MYCOBACTERIA

Characteristics	OR Association with Culture Positivity*	95% CI for Odds Ratio
FEV_1, % predicted		
Severe, < 40	1.0†	
Moderate, 40 to < 70	1.8	1.1, 3.0
Mild, 70 to < 90	2.0	1.1, 3.8
Normal, ⩾ 90	3.3	1.6, 7.0
Age at enrollment, yr		
Pediatric, 10 to < 18	1.0†	
Adult, 18 to < 30	1.8	1.1, 3.0
Adult, ⩾ 30	4.1	2.3, 7.5
Pseudomonas aeruginosa	0.7	0.4, 1.1
Staphylococcus aureus	1.6	1.1, 2.4
BMI, kg/m^2		
Normal–obese, ⩾ 18.5	1.0†	
Malnourished, < 18.5	1.9‡	1.2, 3.0

Definition of abbreviations: BMI = body mass index; CI = confidence interval; OR = odds ratio.

* OR of an association of a given characteristic with subject having positive cultures, relative to subject with negative cultures for nontuberculous mycobacteria, controlling for all other characteristics in model.

† Reference group for odds ratio comparisons.

‡ In univariable stratified analysis, this association was only seen in the pediatric population: OR (95% CI) for age 10 to < 18 years = 3.62 (1.31, 10.83); for age ⩾ 18 years = 0.87 (0.49, 1.53). Interaction term for age × BMI, however, was not significant in model building process.

organisms (Table 2). No significant differences were noted between *M. abscessus* and *M. avium* complex (or between species within the complex—data not shown) for any of the predictor variables.

DISCUSSION

NTM are emerging pathogens capable of causing disease not only in "high-risk" groups, such as persons infected with human immunodeficiency virus, but also in the general population. *M. avium* complex pulmonary infection was initially described as upper lobe, cavitary disease in older, male smokers who had disruption of local host defenses from underlying airways disease and/or impairment of systemic host defenses (8). More recently, up to 75% of all nontuberculous mycobacterial pulmonary cases have been reported in middle-aged to elderly nonsmoking females without obstructive lung disease or other recognized predisposing factors (10–13, 15, 17, 48, 49).

Before 1990, recovery of NTM from the lower airways of persons with CF was rarely reported (6), but over the last decade, multiple North American and European CF centers have reported case series with site prevalence of 2 to 28% (21–25, 27). Overall, NTM were isolated from 91 (12%) of 750 patients in those studies. The many limitations of these studies included the following: most were retrospective; prevalence was often based on only a single sputum specimen; culture techniques were inadequate (high rates of bacterial overgrowth) (27); methods for species identification were not standardized; patients were screened only when acutely ill; there was a focus on pediatric patients, who may have limited ability to expectorate sputum spontaneously, which excluded the older population with a more diverse lower airway flora; demographic and clinical information was incomplete; and there was no assessment of mode of acquisition.

This is the first comprehensive study of NTM among persons with CF. Almost 10% of all patients with CF of age 10 and more in the U.S. were sampled repeatedly, with standardized and validated techniques. Overall, the prevalence of mycobacteria was 13%, with more than 25% of the culture-positive subjects having positive smears and 13% having mycobacteria recovered on all three specimens.

The rare isolation of mycobacteria from patients with CF before 1990 may have been due to failure to obtain mycobacterial cultures, inadequate culture techniques, a younger CF population, or lack of person-to-person transmission or nosocomial acquisition. The use of newly developed and validated culture techniques that reduce *P. aeruginosa* contamination and improve mycobacterial recovery, together with sequential sampling, improved mycobacterial isolation. For example, by obtaining three specimens instead of one, the prevalence of detected mycobacteria almost doubled (7 to 13%). In addition, less than 3% of the almost 3,000 specimens processed had insufficient volume for optimal processing and less than 5% had overgrowth by *Pseudomonas*.

Person-to-person transmission of pathogens is well studied among patients with CF, including oxacillin-resistant *S. aureus*, *P. aeruginosa*, and *B. cepacia* (50), which raises concern about possible transmission of mycobacteria. Multiple nosocomial outbreaks of mycobacteria (28) also raise concern that patients with CF may acquire mycobacteria during hospital visits. Our molecular analyses revealed that almost all patients tested had unique mycobacterial strains, and cross-contamination in the laboratory could be implicated in the two instances in which the same molecular type of *M. avium* complex was seen in the same center (49). Thus, person-to-person transmission and nosocomial acquisition are very uncommon, or nonexistent. This finding is supported by the lack of correlation between nontuberculous mycobacterial culture status and the number of hospitalizations, days hospitalized, or outpatient visits.

The strong association between NTM and significantly older subjects suggests the high prevalence may be a phenomenon of increased life span in CF. The association of NTM with mild lung disease suggests either patients with more severe CF disease die before having enough exposure time to acquire and retain the organisms, or some factor associated with the ability to reach older age with relatively mild disease may predispose to the presence of NTM. No association was found between nontuberculous mycobacterial infection and genotype. Residence in nonrural areas, reported previously in association with NTM (9), and the presence of proxies of higher socioeconomic status may relate to better access to and adherence with care and therefore milder disease; the converse association of proxies of lower socioeconomic status and worsened clinical disease has also been reported (45). Other possible factors include the bacterial flora associated with milder disease. Although *P. aeruginosa*, inversely associated with NTM in this study, can interfere with recovery of mycobacteria from sputum, our low frequency of overgrowth suggests this was not a factor. It is possible the presence of *S. aureus*, positively associated with NTM in this study, may create conditions that either favor the presence of NTM in the airways or growth on culture in the laboratory, though more likely it is a marker of mild disease.

Our study is the first to examine rigorously the geographic distribution of NTM in a defined population. Prior epidemiologic data based on skin test reactivity (which lacks specificity), or surveillance data of cultures voluntarily submitted to state public health laboratories, have suggested a higher prevalence of *M. avium* complex in the Southeastern U.S. (9, 51, 52) Our data, coded using the classification for Centers for Disease Control groupings, (53) demonstrated a more widespread geographic distribution: Southcentral 22%, Mountain 18%, Southeastern 15%, Pacific 15%, Northcentral 10%, and Northeast 8%. Al-

though all participating sites are in the network of national Cystic Fibrosis Centers, we did not use standardized CF management protocols in this study; therefore, the effect of variations in the use of antibiotics, steroids, and other treatments on NTM prevalence is not known. Although clinical practice may vary among centers, we believe the geographic variation in patients with CF results from differences in exposure risk rather than host factors. Regardless of geographic variations in prevalence, the relative proportion of *M. avium* complex recovered at each site was similar (Figure 3).

M. intracellulare may be more significant than *M. avium* in this population. The finding that *M. intracellulare* was more often smear-positive than *M. avium* suggests a heavier burden of organisms. Other studies using comparable (DNA probe) methods of identification in cavitary (more severe) pulmonary disease have shown greater proportions of *M. intracellulare* (54, 55), which supports the possible greater pathogenicity of this organism. In contrast, studies in patients with nodular bronchiectasis (milder disease) have reported a higher relative prevalence of *M. avium* (55, 56).

This study was not designed to assess the clinical impact of nontuberculous mycobacterial infection. In patients infected with human immunodeficiency virus, NTM commonly cause clinically important morbidity. In nonimmunocompromised persons with *M. avium* complex and nodular bronchiectasis, serial computed axial tomography has demonstrated progression in the absence of drug therapy (54, 56). The clinical significance of this question will likely increase as the CF population continues to age. Indeed, the trend toward subjects with a heavy burden of organisms being older than subjects with a light burden of organisms may suggest that, over time, subjects with a single positive culture may progress to a more significant burden of organisms.

In summary, NTM are prevalent in the lower respiratory tract of adolescent and adult patients with CF. Although prevalence varied across sites, it was present throughout the U.S. Neither patient-to-patient nor nosocomial transmission account for acquisition of NTM. The greater burden of organisms recovered from patients with *M. intracellulare* suggests that this pathogen may be of more concern than *M. avium*. Patients who are culture-positive for NTM initially appear to have milder disease, though the burden of organisms appears to increase with age, and thus, perhaps, with time. The clinical impact of these organisms was assessed in a separate, short-term longitudinal study (57).

Acknowledgment: The authors thank Stephan Thiede, M.D., Bryan Jones, and Craig Swainey for assistance in study coordination and data management; Peter Gilligan, Ph.D., Stacey C. FitzSimmons, Ph.D., Steve Ostroff, M.D., Lisa Saiman, M.D., C. Fordham von Reyn, M.D., and James Yankaskas, M.D., for advice in the design and conduct of this investigation; Richard Sidley, M.S., Clarence Potter, and Eugene Orringer, M.D., for providing data network assistance, advice, and workspace; and the site investigators and study coordinators for their longstanding contributions. The authors thank James Yankaskas, M.D., Moira Aitken, M.D., and Lisa Saiman, M.D., for their thoughtful reviews of the manuscript. They also thank Lisa Brown for her graphic design and editorial assistance.

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APPENDIX

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