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Recovery of Mycobacteria from Patients with Cystic Fibrosis

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Despite decontamination, overgrowth by pseudomonads renders cultural isolation of mycobacteria from respiratory specimens of patients with cystic fibrosis (CF) difficult or impossible. We performed a prospective study by comparing levels of reduction of overgrowth and recovery of mycobacteria using either pretreatment with *N*-acetyl-L-cysteine (NALC)–NaOH alone or pretreatment with NALC-NaOH and then with oxalic acid. From 406 specimens of 148 CF patients, 11 specimens were positive for mycobacteria, 5 of which grew mycobacteria after decontamination by either procedure. Three specimens grew mycobacteria only after decontamination with NALC-NaOH, whereas three specimens grew mycobacteria only after treatment with NALC-NaOH followed by oxalic acid but were overgrown after decontamination with NALC-NaOH. Thus, inactivation of mycobacteria by the more aggressive oxalic acid treatment offsets its beneficial effect of reducing the proportion of cultures overgrown with microorganisms other than mycobacteria.

Once considered quite rare, nontuberculous mycobacteria are being recovered with increasing frequency from respiratory specimens of adolescent and adult patients suffering from cystic fibrosis (CF) (1, 4). In particular, *Mycobacterium chelonae*, *Mycobacterium abscessus*, and *Mycobacterium fortuitum* appear to play an important role in this group of patients, although other nontuberculous mycobacteria such as *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium kansaii* have been isolated as well (2, 3, 5).

A variety of other microorganisms colonize or infect the respiratory tracts of CF patients and often hamper the recovery of mycobacteria, since they rapidly overgrow mycobacterial cultures. *Pseudomonas aeruginosa*, in particular, is present in the respiratory tracts of up to 80% of CF patients. Typically, it survives routine sputum decontamination with NALC-NaOH (*N*-acetyl-L-cysteine–2% sodium hydroxide). Thus, for eradication of overgrowing bacteria, the most commonly used laboratory manual recommends treatment with 5% oxalic acid for samples from the respiratory tracts of CF patients (7).

Whittier and colleagues demonstrated that NALC-NaOH treatment followed by oxalic acid for initial decontamination significantly reduced overgrowth by *P. aeruginosa* (8). In a subsequent report those authors evaluated their results in a multicenter study involving 20 participating laboratories by sending out for mycobacterial culture a panel of five specimens seeded with pseudomonads and with mycobacteria (9). From their results Whittier et al. concluded that decontamination of the seeded specimens with NALC-NaOH and oxalic acid yielded a significant reduction in bacterial overgrowth and improved recovery of mycobacteria. Thus, pretreatment with NALC-NaOH and then with oxalic acid prior to culturing for mycobacteria was recommended as a decontamination procedure for specimens from the respiratory tracts of CF patients.

In their original studies Whittier and colleagues pointed out that the combination of NALC-NaOH and oxalic acid, although very effective in eradicating overgrowing bacteria, might also reduce recovery of mycobacteria, especially from low-titer specimens. Thus, we designed a prospective study to examine the recovery rate of mycobacteria from respiratory specimens of CF patients following the use of either of two decontamination methods: treatment with NALC-NaOH or treatment with NALC-NaOH followed by oxalic acid.

Specimens were obtained from 148 patients attending the CF clinic of the Medical School Hannover between May and November 1998. Respiratory specimens, including sputa, tracheal aspirates, and specimens recovered by bronchoscopy, were processed in the Department of Medical Microbiology for acid-fast bacilli by smearing and culturing. All mycobacterial specimens were cultured in BACTEC MGIT 960 (Becton Dickinson, Sparks, Md.). Positive cultures were subjected to species identification by PCR-mediated amplification of the 16S rRNA gene and direct sequence determination as described previously (6). A total of 428 specimens were studied: 14 specimens in an initial pilot study and 414 specimens in a subsequent prospective study.

The recovery rate of *M. chelonae* that had been inoculated into clinical specimens contaminated with various microorganisms was assessed in the initial pilot study. Fourteen specimens from CF patients that grew either pseudomonads (11 specimens), proteins (1 specimen), molds (1 specimen), or both pseudomonads and molds (1 specimen) were seeded with *M. chelonae*. A cell suspension of *M. chelonae* was adjusted to match a 0.5 McFarland standard, and 0.05 ml of this volumen was added for every 1.0 ml of specimen volume. Each specimen was divided into two equal aliquots and decontaminated with NALC-NaOH or NALC-NaOH followed by oxalic acid. For the NALC-NaOH method, specimens were combined with an equal volume of 0.5% NALC–2% NaOH and vortexed for at least 15 min at room temperature. The specimen was washed with distilled water, and one half was used for inoculation of MGIT vials and staining for acid-fast bacilli, whereas the other half was subjected to further decontamination with oxalic acid. An equal volume of 5% oxalic acid was added to the second fraction. This mixture was vortexed and washed with phosphate-buffered saline. With pH paper, the specimen was neutralized by addition of 4% NaOH to a pH of about 7 prior to being stained and cultured.

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TABLE 1. Profiles of CF patients whose respiratory specimens (collected between May and November 1998) grew *Mycobacterium* species[a]

Patient	Gender	Age (yr)	Smear result	Result after decontamination with:		Species isolated
				NALC-NaOH	NALC-NaOH and oxalic acid	
204426	Female	18	Negative	Growth	No growth	*M. interjectum*
204486	Female	21	Positive (1+)	Growth	Growth	*M. chelonae*
204942	Female	36	Positive (2+)	Growth	Growth	*M. chelonae*
204652	Female	36	Positive (2+)	Overgrown by other bacteria	Growth	*M. chelonae*
204975	Male	40	Negative	Growth	No growth	*M. intracellulare*
205324	Female	36	Negative	Growth	Growth	*M. simiae*
205382	Male	22	Negative	Overgrown by other bacteria	Growth	*M. chelonae*
205608	Female	25	Positive (3+)	Growth	Growth	*M. simiae*
205768	Female	36	Positive (3+)	Growth	Growth	*M. chelonae*
206260	Female	19	Negative	Growth	No growth	*M. avium*
206160	Male	40	Negative	Overgrown by other bacteria	Growth	*M. intracellulare*

[a] Specimens were obtained from 148 CF patients over a period of 6 months (May to November 1998) and were decontaminated with either NALC-NaOH or NALC-NaOH followed by oxalic acid, before being stained for acid-fast bacilli and cultured at 37°C in the MGIT automated culture system. A total of 414 isolates were examined after both treatments. Each method revealed eight specimens that were culture positive for mycobacteria. The combined number of isolates positive by both procedures was 11, of which 5 were also smear positive. Of the remaining 406 specimens, 237 and 106 exhibited bacterial overgrowth after treatment with NALC-NaOH and NALC-NaOH plus oxalic acid, respectively, leaving 169 and 300, respectively, that showed no bacterial growth. The densities of acid-fast bacilli on the smear-positive specimens are indicated as 1+ to 3+, indicating 4 to 10, 11 to 100, or 100 to 500 acid-fast bacilli per smear, respectively. Species identification was achieved by PCR-mediated amplification of the 16S rRNA gene and direct sequence determination.

After decontamination with NALC-NaOH, 3 of 14 specimens (21%) grew exclusively *M. chelonae*. Almost 80% of the specimens remained contaminated. In contrast, after decontamination with NALC-NaOH followed by oxalic acid, none of the specimens was contaminated but all grew *M. chelonae*. Thus, the rate of overgrowing bacteria was dramatically reduced whereas the viability of *M. chelonae* appeared to be unchanged. This result prompted us to compare the yields of mycobacteria by both decontaminating procedures in a prospective study. For a period of 6 months all specimens from the respiratory tracts of CF patients sent to our department were divided and subjected either to standard decontamination with NALC-NaOH or to decontamination with NALC-NaOH followed by oxalic acid.

Of 406 specimens, 169 did not show any growth after decontamination with NALC-NaOH (42%) whereas 237 were contaminated (58%). In contrast, after treatment with NALC-NaOH followed by oxalic acid, no bacteria were recovered from 300 specimens (74%) whereas bacteria were recovered from 106 samples (26%). Overall, decontamination with NALC-NaOH followed by oxalic acid significantly reduced the proportion of contaminated specimens. We next wished to address the question of whether the reduced proportion of overgrown specimens after decontamination with NALC-NaOH followed by oxalic acid resulted in an increased recovery of mycobacteria. Of the 414 specimens investigated, 11 were positive for mycobacteria (Table 1). However, only five specimens were positive after either procedure. Three specimens showed growth of mycobacteria after treatment with NALC-NaOH but remained sterile after treatment with NALC-NaOH followed by oxalic acid. Three specimens became positive after treatment with NALC-NaOH followed by oxalic acid but were contaminated when NALC-NaOH alone was used.

This finding supports the assumption that oxalic acid may cause false-negative results, especially from low-titer specimens. Although the contamination rate was significantly reduced with NALC-NaOH and oxalic acid, the overall sensitivity of mycobacterial recovery remained the same: three specimens were positive only after treatment with NALC-NaOH followed by oxalic acid but were overgrown with other microorganisms when NALC-NaOH alone was used; however, three specimens grew mycobacteria only after treatment with NALC-NaOH and revealed no bacterial growth when both NALC-NaOH and oxalic acid were used. These three specimens were also smear negative, demonstrating that oxalic acid poses a particular problem for low-titer specimens. Thus, the negative effect on the recovery rate of mycobacteria by the more aggressive oxalic acid treatment offsets its beneficial effect in reducing the proportion of cultures overgrown with microorganisms other than mycobacteria.

In summary, we provide evidence that bacterial decontamination with NALC-NaOH followed by oxalic acid results in an impressive reduction of bacterial overgrowth. Overall recovery of mycobacteria, however, was not improved. Based on this experience, we are currently investigating a two-step decontamination procedure. In the first step, respiratory specimens from CF patients are decontaminated with NALC-NaOH and, without further decontamination, the specimens are inoculated for culture. In the second step, only those samples overgrown with microorganisms other than mycobacteria (about half of all specimens) are subjected to a second decontamination with oxalic acid.

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