Isolation of Mycobacteria from Healthy Persons' Stools

TO THE EDITOR:

In 1986, Collins published in this JOURNAL a paper "*Mycobacterium avium*-complex infections and development of the acquired immunodeficiency syndrome: casual opportunist or causal cofactor?" ([4]) One question brought up in his paper concerned the possible gastrointestinal origin of *Mycobacterium avium-intracellulare* (MAI) infections in some acquired immunodeficien-

cy syndrome (AIDS) patients. Indeed, the MAI complex is frequently a cause of disseminated infection in patients with AIDS ([19]), and the isolation of MAI from stools of AIDS patients has been reported ([1, 6, 8, 10, 14, 21–23, 28]). However, little is known about the presence of mycobacteria in the stools of healthy individuals. We therefore decided to study the mycobacterial flora of the stools of 50 healthy European volunteers and to identify the mycobacteria by both conventional tests and by lipid analysis. All specimens were examined by microscopy after Ziehl-Neelsen staining. The mycobacteria were isolated after decontaminating the specimens as described by Portaels, *et al.* ([20]), i.e., a modified procedure of Wolinsky and Rynearson ([27]). The decontaminated samples were inoculated onto four tubes of Ogawa egg yolk medium, incubated at 30°C for 6 months, and observed for growth every week. When a tube was suspected of being positive for mycobacteria, each colony was subcultured on Ogawa medium and checked for acid fastness by Ziehl-Neelsen staining. The mycobacteria were identified according to well-established methods ([12, 25]), and their fatty acid and mycolic acid compositions were analyzed by gas chromatography (GC) ([13, 15, 16]) and thin-layer chromatography (TLC) ([5, 7, 18]).

No acid-fast bacilli were observed in any of the 50 stool specimens after direct Ziehl-Neelsen staining. Twenty-six specimens (52%) were culture-positive for mycobacteria; the remaining 24 (48%) were negative. The number of colonies observed on the media varied between one and three. Six positive primary cultures failed to grow in subcultures, and from the 20 remaining positive specimens, 26 different mycobacterial strains were cultivated. From 14 specimens only one mycobacterial species was isolated, while from 6 specimens two different species were cultivated (The Table).

Fourteen of the 26 strains were identified as *M. simiae*. They produced α-, α′- and ketomycolates like *M. simiae* but were nonchromogenic. Six of the strains were also analyzed by GC; all contained tuberculostearic acid but were devoid of secondary alcohols (2-octadecanol and 2-eicosanol).

Five strains identified as *M. gordonae* contained α-, methoxy- and ketomycolates. The presence of a branched chain C14 and a hydroxylated C20 acid as well as the absence of tuberculostearic acid and secondary alcohols—all characteristic features of this species—were established by GC analysis.

THE TABLE. *Mycobacterial cultures from healthy persons' stools.*

Mycobacterial strains (26)	No. positive specimens (20)
M. simiae	8
M. gordonae	3
MAI[a]	2
M. malmoense	1
MAI[a] + *M. simiae*	3
M. gordonae + *M. simiae*	2
M. malmoense + *M. simiae*	

[a] MAI = *M. avium-intracellulare* complex.

Five nonchromogenic strains were classified as belonging to the MAI complex. The results of the TLC studies revealed the presence of α-, keto- and ω-carboxymycolates. Tuberculostearic acid and the secondary alcohols were identified by GC.

Two nonchromogenic strains which both grew poorly at 37°C were identified as *M. malmoense* according to the results of the TLC and GC studies. Their mycolate patterns were identical to those of *M. malmoense* and *M. simiae* (α-, α′- and ketomycolates), but, uniquely for *M. malmoense*, both strains contained appreciable amounts of 2-methyl eicosanoic and 2,4,6-trimethyl tetracosanoic acid ([24]).

The isolation of mycobacteria from human intestinal tissues ([2, 3, 9]) and from AIDS patients' stools ([1, 6, 8, 10, 14, 21–23, 28]) has been reported repeatedly. While a variety of different species (MAI, *M. fortuitum* complex, *M. chelonei*) have been found in intestinal specimens from patients with Crohn's disease, ulcerative colitis, and noninflammatory bowel diseases ([2, 3, 9]), the predominance of MAI strains from intestinal tissues and stools of AIDS patients is striking ([1, 6, 8, 10, 14, 21–23, 28]). The present study shows that mycobacteria can also be isolated from more than 50% of the stools from healthy individuals. We found a predominance of *M. simiae* followed by MAI strains, *M. gor-*

donae, and *M. malmoense.* Our findings of *M. simiae* and *M. malmoense,* both recognized as causes of human diseases ([11, 17, 26]), are particularly noteworthy. Environmental isolates of these species have not been reported although the epidemiology of the diseases for which they were responsible strongly suggests their presence in the environment. The mycobacteria might enter the host via contaminated food or water and then colonize the gastrointestinal tract. As suggested by Collins ([4]), it is possible that some of these mycobacteria (e.g., MAI) possess factors which enable them to attach to the intestinal mucosae, colonize the membranes, and invade them when the AIDS virus has depleted the T-cell defenses. To evaluate whether the mycobacteria isolated from stools originate from an occasional colonization of the intestinal tract or whether they form a part of the permanent flora of some individuals, samples should regularly be studied from the same subjects on a long-term basis. Additional studies on stools from healthy persons, from persons at high risk for developing AIDS, and from AIDS patients with and without disseminated MAI infections are also required for confirmation of the hypothesis that intestinal colonization precedes mycobacterial infection.

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