Recovery of Pathogenic Fungi from Clinical Specimens Submitted for Mycobacteriological Culture

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A total of 332 major pathogenic fungi were isolated from specimens cultured onto both fungal culture media and media used for culturing mycobacteria from January 1968 to June 1975. Only 72 (21.7%) fungi were recovered on media used for culturing mycobacteria. The effect of sodium hydroxide treatment was evaluated and shown to be detrimental to the recovery of fungi. It is recommended that clinical laboratories not rely on mycobacteriological cultures to recover fungal pathogens.

The question of whether pathogenic fungi can be recovered from clinical specimens that have been concentrated and cultured onto media used for culturing mycobacteria often arises. Studies by Ajello et al. (1), Neimeister et al. (3), Sanford et al. (6), and Reep and Kaplan (4, 5) have shown that fungi are generally intolerant to the action of chemical agents used to treat specimens prior to culturing, particularly when sodium hydroxide is one of the ingredients. However, these studies did not deal with the recovery of organisms from patients having mycotic infections but rather with the recovery of organisms from specimens inoculated with laboratory reference strains. The purpose of this paper is to present information concerning the recovery of pathogenic fungi from clinical specimens submitted for mycobacteriological culture. All results represent the recovery of organisms from patients having mycotic infections.

MATERIALS AND METHODS

In a retrospective fashion, culture data from the mycology and mycobacteriology laboratories (Mayo Clinic and Mayo Foundation, Rochester, Minn.) from January 1968 to June 1975 were reviewed for the recovery of pathogenic fungi. Only those specimens that were cultured simultaneously by both laboratories were compared.

Fungal cultures. From 1968 to 1971, Sabouraud dextrose agar (Emmons modification) and inhibitory mold agar (BBL, Cockeysville, Md.) were used as culture media. From 1971 to the present, the following media were used: (i) Sabouraud dextrose agar (Emmons modification); (ii) inhibitory mold agar; (iii) brain heart infusion agar containing 16 μ g of chloramphenicol and 5 μ g of gentamicin per ml and 10% sheep blood; and (iv) brain heart infusion agar containing 16 μ g of chloramphenicol, 5 μ g of gentamicin, and 0.5 mg of cycloheximide per ml and 10% sheep blood.

All specimens were inoculated into petri dishes containing the above media and incubated at 30 C for 30 days. Cultures were examined triweekly, and all pathogens appearing on any of the media were identified by their morphological or biochemical characteristics (2).

Mycobacteriological cultures. In 1968, Lowenstein-Jensen slants were used; however, from 1969 to the present, Lowenstein-Jensen and Middlebrook 7H10 slants were used for culturing all specimens.

All specimens except tissues, cerebrospinal fluid, bone marrow, and body fluids were digested and concentrated prior to being cultured. Equal volumes of 2% sodium hydroxide and specimen were mixed and allowed to stand for 15 to 20 min. This mixture was neutralized to pH 7.0 by the addition of 1.25 N hydrochloric acid and centrifuged, and the sediment was inoculated onto the surface of the above-mentioned media. Cultures were incubated for 8 weeks at 35 C in an environment containing 5% CO₂. Each was examined weekly, and all fungi were referred to the mycology laboratory for identification.

RESULTS

A review of cultures during the study period showed that 101,998 and 60,361 specimens were received by the mycology and mycobacteriology laboratories, respectively. Only those specimens cultured by both laboratories were compared for the recovery of pathogenic fungi, and during this time 332 major pathogens were isolated. The results are summarized in Table 1 and show that fungal culture media had a statistically significant higher recovery rate ($P < 0.001$) than media used for culturing mycobacteria. There were significant differences in isolation rates between the two media, except for *Sporothrix schenckii* and *Nocardia asteroides*. There were too few isolations of *S. schenckii* to make a valid comparison; however, more were recovered on fungal media than on

TABLE 1. Number of organisms recovered by medium

Organism	Positive recovery			P
	Both media	Fungal culture only	Mycobacteriological culture only	
<i>Cryptococcus neoformans</i>	2	113	0	<0.001
<i>Coccidioides immitis</i>	31	48	1	<0.001
<i>Histoplasma capsulatum</i>	6	53	0	<0.001
<i>Blastomyces dermatitidis</i>	4	23	0	<0.001
<i>Sporothrix schenckii</i>	2	9	0	<0.10
<i>Nocardia asteroides</i> ^a	17	14	9	NS ^b

^a Not a fungus; however, it is commonly isolated on fungal and mycobacteriological cultures.

^b NS, Nonsignificant.

media used for culturing mycobacteria. The number of isolations of *N. asteroides* was virtually the same for both media, and the difference was not significant.

There was no difference in the recovery rate of organisms on Lowenstein-Jensen and 7H10 media. There was only one instance where mycobacteria and fungi were recovered concomitantly from the same specimen; this occurred with *Cryptococcus neoformans* and *Mycobacterium tuberculosis*.

DISCUSSION

The results of this study show that fungi were isolated more frequently on fungal culture media than on media used for culturing mycobacteria. A total of 332 fungi were isolated, and only 72 (21.7%) were recovered on media used for culturing mycobacteria. Of those 72 recovered, 57 (79.2%) were not treated with sodium hydroxide since these were either cerebrospinal fluid or tissue specimens. *N. asteroides* accounted for 14 of 15 of the isolates in the treated group in addition to one isolate of *Histoplasma capsulatum*. Nine of the *N. asteroides* isolates were not recovered on fungal media because of overgrowth by other organisms or inhibition by antibiotics in the media.

When one considers the total number of specimens from which fungi were isolated (332), 91.8% of these isolates were not recovered in the treated group, and 61.5% were not recovered in the untreated group. The difference between the recovery of fungi in the treated and untreated groups is statistically significant ($P < 0.001$).

Ajello et al. (1) reported that *N. asteroides* is resistant to alkaline treatment, and this has been further verified by Uesaka (7). Our findings are in agreement with this, and it is depicted by the fact that the only organisms that were recovered after sodium hydroxide treatment were the 14 isolates of *N. asteroides* and one isolate of *H. capsulatum*. Our findings are not in agreement with Ajello et al. (1) in regard to the recovery of *C. neoformans*. None were recovered after sodium hydroxide treatment, although their study showed a 79.7% recovery rate after treatment with 4% sodium hydroxide.

It is clear that even mild treatment of organisms by sodium hydroxide (2%) is detrimental to their recovery. This is in agreement with the work by Neimeister et al. (3); however, none of the strict pathogenic fungi were isolated in their study. Ajello et al. (1), Sanford et al. (6), and Reep and Kaplan (4, 5) also agree, but their studies did not deal with the isolation of fungi directly from clinical specimens taken from patients having mycotic infections.

Currently, most digestion procedures have sodium hydroxide as one of the ingredients, and it is our feeling that clinical laboratories should not rely on mycobacteriological cultures to recover fungal pathogens.

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