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Importance of Selective Media for Recovery of Yeasts from Clinical Specimens

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We compared the recovery of yeasts from clinical specimens cultured on routine bacteriological media to the recovery of yeast from specimens cultured on a selective fungal medium (Sabouraud agar). The use of Sabouraud agar was especially important in cases of mixed cultures, since in such cases yeast was recovered on bacteriological media from only 50% of 44 yeast-positive pus specimens and from 22.5% of 22 yeast-positive throat specimens. The use of a selective fungal medium is therefore necessary to ensure the detection of yeast in specimens containing a mixture of bacteria and yeasts. As a result, clinicians must request yeast isolation when clinically indicated, and the microbiological laboratory must add a selective fungal medium when clinically significant yeasts are likely to be encountered. It is also important that selective fungal media be used in clinical studies of yeast infections.

Yeasts are able to grow on routine bacteriological media, such as blood agar and chocolate agar plates. If yeasts are present together with a mixed bacterial population, it is, however, possible that bacteria will suppress yeast growth. It is therefore generally accepted that a selective medium such as Sabouraud agar should be used for the cultivation of yeasts from clinical specimens (6). Microbiological laboratories include such media if a fungal or yeast culture has been requested by the clinician, but if such a request has not been made, selective media are often omitted.

In order to assess the importance of including a selective fungal medium, we compared the efficiency of yeast recovery from routine bacteriological media and from Sabouraud agar. The design of the study was quite simple, as we only wanted to investigate the impact of selective fungal medium on the recovery of yeasts from clinical specimens. No attempt was made to study the clinical importance of the yeast isolates. In addition, we evaluated the methods used and the ability of Norwegian microbiological laboratories to detect yeast in mixed cultures by including specimens containing both bacteria and yeasts in two recent distributions in the Norwegian external quality assessment program for medical bacteriology, mycology, and parasitology (10).

Comparative studies. A total of 558 specimens, submitted as pus specimens to the microbiological laboratory at the Norwegian Radium Hospital, were included in the study. The origins of the specimens were quite heterogeneous, including specimens from abdomens, abscesses, wound and drain secretions, etc. The specimens were cultivated on blood, chocolate, and lactose agar plates and on Sabouraud agar (Acumedia Manufacturers, Inc., Baltimore, Md.). Streptomycin (40 μg/ml) and penicillin (13 μg/ml) were added to the Sabouraud agar to inhibit bacterial growth. Anaerobic media were included if appropriate. The bacteriological media were always inoculated before the Sabouraud agar plate. All media were incubated at 37°C for 48 h, and the growth of yeasts was reported semiquantitatively as light, moderate, or heavy.

Yeasts were detected in 50 of the 558 specimens (9%) (Table 1). Of these, six (12%) were pure cultures, and the yeasts from these cultures were recovered on all media. The remaining 44 yeast-positive specimens were mixed cultures, and for half of these specimens, yeasts were recovered on Sabouraud agar only. Sabouraud agar proved to be most useful when the specimens in addition to the yeasts consisted of aerobic, gram-negative bacteria, such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., or *Pseudomonas* spp. (Table 1), irrespective of the amount of yeast recovered. On rare occasions, gram-negative rods grew on the Sabouraud agar, and it is, of course, possible that yeasts were suppressed on some of these occasions. Antibiotics other than streptomycin and penicillin could be added to the Sabouraud agar if necessary.

A total of 41 throat samples were cultured on blood and chocolate agar plates and on Sabouraud agar. All samples proved to be mixed cultures, and yeasts were recovered from 22 (54%) of the specimens, of which 17 (77%) were detected on Sabouraud agar only (Table 2).

Of the 72 yeast strains recovered in this study, 42 strains were identified to species level. The species were as follows: *Candida albicans* (32 isolates), *Candida glabrata* (four isolates), *Candida parapsilosis* (four isolates), *Candida krusei* (one isolate), and *Saccharomyces cerevisiae* (one isolate).

External quality assessment specimens. Twenty-three laboratories participate in the Norwegian external quality assessment program. In 1998, a simulated abdominal pus specimen consisting of a mixture of *C. albicans*, *Proteus mirabilis*, and *Bacteroides fragilis* from a patient with peritonitis following an intra-abdominal perforation was included in the program. Fungal culture was not specifically requested, and in this case, only two laboratories included a selective fungal medium. These two laboratories detected *C. albicans*. Of the remaining 21 laboratories, which used no selective media, only eight (38%) detected the yeast. In 1999, a similar specimen (*C. albicans*, *E. coli*, and *Enterococcus faecalis*) was distributed. Twelve laboratories used a selective fungal medium to assess that sample. All of these, but only three (27%) of the 11 laboratories which did not use selective media, recovered *C. albicans*.

Discussion. Yeasts in pure cultures are consistently isolated on bacteriological media. However, yeasts are frequently present in mixed cultures with different bacteria, and on such occasions, it is possible that bacteria will suppress yeast growth. Yeasts might, on the other hand, suppress growth of slow-

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TABLE 1. Comparison of bacteriological media and Sabouraud agar for the recovery of yeast from 50 yeast-positive pus specimens

Medium	Type of bacteria recovered	No. of specimens from which yeast was recovered	No. of specimens yielding yeast growth rated:		
			Heavy	Moderate	Light
Bacteriological medium plus Sabouraud agar	No bacteria detected	6	1	3	2
	Aerobic gram-negative rods[a]	12	3	8	1
	Aerobic gram-positive cocci	10	1	2	7
Sabouraud agar only	No bacteria detected	0	0	0	0
	Aerobic gram-negative rods[a]	18	4	9	5
	Aerobic gram-positive cocci	4	0	1	3
Total		50	9	23	18

[a] In some cases, aerobic gram-positive cocci were also detected.

growing bacteria. In this study, yeast was recovered on bacteriological media from only 50% of 44 yeast-positive pus specimens and from 22.5% of 22 yeast-positive throat specimens consisting of mixed cultures. These results show that yeasts in specimens submitted to the microbiological laboratory often are suppressed by bacteria and are, therefore, not detected on the routine bacteriological media used. The reason for this might be that yeasts are overgrown by bacteria or are killed by bacterial toxins (4, 5). It is therefore important that clinicians specifically request yeast isolation when clinically indicated and that microbiological laboratories employ a selective fungal medium when clinically significant yeasts are likely to be encountered. In our opinion, a selective fungal medium should, for instance, always be included when processing abdominal pus specimens from patients with intra-abdominal perforations. The results of several studies indicate that yeasts are important pathogens in such situations (1, 7, 12).

Many studies on the significance of the recovery of yeasts from clinical specimens unfortunately lack information on the media used for the detection of yeasts (2, 3, 8, 9, 11). This omission of information concerning methods makes the interpretation of such studies difficult or impossible. The use of selective fungal media may have important implications on the results and conclusions of a study.

TABLE 2. Comparison of bacteriological media and Sabouraud agar for the recovery of yeast from 22 yeast-positive throat specimens

Medium	No. of specimens from which yeast was recovered	No. of specimens yielding yeast growth rated:		
		Heavy	Moderate	Light
Bacteriological medium plus Sabouraud agar	5	2	2	1
Sabouraud agar only	17	3	4	10
Total	22	5	6	11

The results of this study show the importance of using selective media for the recovery of yeasts and the importance of specifying the methods used for yeast recovery when reporting clinical studies of yeast infections.

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