

Optimum Use of Selective Plated Media in Primary Processing of Respiratory Tract Specimens from Patients with Cystic Fibrosis

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A total of 258 respiratory tract specimens from patients with cystic fibrosis were inoculated onto nine different plated media, and the rates of recovery of potential pathogens were compared. Media included sheep blood agar, enriched chocolate agar, MacConkey agar for gram-negative bacilli, chocolate agar containing bacitracin for *Haemophilus* spp., bromcresol green agar for yeasts, cetrимide agar for *Pseudomonas* spp., sheep blood agar containing colistin and nalidixic acid for gram-positive cocci, mannitol salt agar for *Staphylococcus aureus*, and oxidation-fermentation agar containing 300 U of polymyxin B per ml and 2 U of bacitracin per ml (OF-PBL medium) for *Pseudomonas cepacia*. With two exceptions, all of these media proved useful in recovering potential pathogens from respiratory tract specimens from patients with cystic fibrosis. The two exceptions were cetrимide agar and colistin-nalidixic acid-supplemented sheep blood agar, which were found to be superfluous. In addition, the results of this study further delineated the prevalence of selected bacteria and fungi in respiratory tract secretions from patients with cystic fibrosis. In rank order of frequency of isolation, we recovered isolates of *Pseudomonas aeruginosa*, *Haemophilus parainfluenzae*, *Candida albicans*, *S. aureus*, *Haemophilus influenzae*, molds, members of the family *Enterobacteriaceae*, yeasts other than *Candida albicans*, miscellaneous gram-negative bacilli, beta-hemolytic streptococci, *P. cepacia*, and *Streptococcus pneumoniae*.

Several unanswered questions surround the laboratory evaluation of respiratory tract secretions from patients with cystic fibrosis (CF). These include defining the optimum specimen for establishing the etiology of lower respiratory tract infections (i.e., pharyngeal swab versus deep throat swab versus expectorated sputum), as well as determining the best way to process specimens upon receipt in the laboratory (i.e., liquefaction versus direct plating and quantitative versus qualitative cultures) (7-10, 12). However, one issue for which agreement appears to exist concerns the need to use selective media for the primary processing of specimens from CF patients, irrespective of the nature of the specimen (1-3, 5, 6, 9, 11, 13-15). Selective media have been advocated for the optimum recovery of isolates of *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, and other organisms. The intent of our investigation was to determine which of a variety of different media were of the greatest value in the routine processing of respiratory tract specimens from patients with CF.

A total of 258 consecutive specimens from patients known to have CF were included in this study. Among these, 157 specimens were throat swabs and the remaining 101 specimens were expectorated sputum specimens. The contents of throat swabs were eluted into 1.0-ml volumes of Trypticase soy broth, and then approximately 0.1-ml aliquots of these suspensions were transferred to the surfaces of primary plates with swabs and streaked for isolation. Expectorated sputum specimens were processed semiquantitatively after being liquefied. Briefly, equal aliquots of specimen and a solution containing *N*-acetyl-L-cystine and sodium citrate were mixed, vortexed for 60 s, allowed to stand for 15 min, and then mixed again. By

using a swab, the contents of this suspension were transferred to the surfaces of culture plates and streaked for isolation.

Primary plates included 5% sheep blood agar (BAP), enriched chocolate agar (CHOC), MacConkey agar (MAC), CHOC containing 300 µg of bacitracin per ml (CHOC-BAC), bromcresol green agar (BCG), cetrимide agar (CET), 5% BAP containing 10 µg of colistin per ml and 10 µg of nalidixic acid per ml (CNA), mannitol salt agar (MANN), and oxidation-fermentation agar containing 300 U of polymyxin B per ml and 2 U of bacitracin per ml (OF-PBL). All plates were purchased from Remel Laboratories, Lenexa, Kans. BAP and CHOC were included as general-purpose media. The following selective media were used to facilitate the recovery of the following organisms: MAC, gram-negative bacilli, in particular members of the family *Enterobacteriaceae*; CNA, gram-positive cocci; CHOC-BAC, *Haemophilus* spp.; BCG, yeasts; CET, *Pseudomonas* spp.; MANN, *S. aureus*; and OF-PBL, *P. cepacia*.

All primary plates were incubated at 35°C in 5 to 7% CO₂ for 3 days and examined daily for the presence of growth. Viridans group streptococci, aerobic non-spore-forming gram-positive bacilli, species of staphylococci other than *S. aureus*, and *Neisseria* species other than *N. meningitidis* were characterized when present but were not included in the data analysis unless they had been isolated in pure culture in quantities of at least 3+ (i.e., more than 30 colonies with growth into the third quadrant). All other organisms were identified to the species level by using conventional methods and criteria (1). When an individual species was recovered on more than one primary plate, a subjective assessment was made as to which one of the plates provided optimum growth (i.e., the largest number of well-isolated colonies) for the performance of identification procedures and susceptibility tests.

Among the 258 specimens included in this study, 2 yielded no growth and 8 yielded insignificant growth. The results of positive cultures with the remaining 248 specimens are sum-

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TABLE 1. Recovery of microorganisms from respiratory tract specimens from patients with CF by using different selective media

Organism(s)	No. of isolates	No. of isolates recovered on:										Medium used for characterization
		BAP	CHOC	MAC	BCG	CET	CNA	MANN	CHOC-BAC	OF-PBL		
<i>Pseudomonas aeruginosa</i>	137	135	136	137	15	113	2	0	86	19	MAC (92%), BAP (4%), CET (3%), and CHOC-BAC (1%)	
<i>Candida albicans</i>	115	87	108	16	113	0	58	32	82	72	CHOC (42%), BCG (51%), CHOC-BAC (5%), and BAP (2%)	
<i>Haemophilus influenzae</i> ^a	30	0	30	0	0	0	0	0	30	0	CHOC (37%) and CHOC-BAC (63%)	
<i>Haemophilus parainfluenzae</i>	119	0	119	0	0	0	0	0	119	0	CHOC (33%) and CHOC-BAC (67%)	
<i>Staphylococcus aureus</i>	64	62	62	0	0	0	60	64	49	0	BAP (55%) and MANN (45%)	
Members of the <i>Enterobacteriaceae</i> ^b	23	22	22	23	0	1	1	0	11	1	MAC (100%)	
Yeasts other than <i>C. albicans</i> ^c	20	14	19	5	20	0	6	12	15	18	CHOC (25%) and BCG (75%)	
Filamentous fungi ^d	24	7	14	1	6	1	4	2	10	6	Variable	
<i>Achromobacter anitratus</i>	6	6	6	6	0	0	0	0	2	0	MAC (100%)	
<i>Pseudomonas cepacia</i>	3	1	1	2	0	0	0	0	1	2	MAC (67%) and OF-PBL (33%)	
<i>Neisseria meningitidis</i>	1	1	1	0	0	0	0	0	1	0	CHOC (100%)	
<i>Moraxella catarrhalis</i>	1	1	1	0	0	0	0	0	0	0	CHOC (100%)	
Miscellaneous gram-negative bacilli ^e	7	4	5	7	0	1	0	0	4	2	MAC (100%)	
<i>Staphylococcus</i> spp. ^f	3	3	3	0	0	3	3	0	0	0	BAP (100%)	
<i>Streptococcus pneumoniae</i>	3	3	3	0	0	0	1	0	0	0	BAP (100%)	
Beta-hemolytic streptococci ^g	6	6	4	0	0	0	2	0	2	0	BAP (100%)	

^a Nine isolates were encapsulated type B strains (2 of which produced β-lactamase) and 21 were nonlysozyme (1 of which produced β-lactamase).

^b Isolates included *Escherichia coli* (n = 11), *Serratia liquefaciens* (n = 1), *Citrobacter freundii* (n = 1), *Citrobacter amalonaticus* (n = 1), *Klebsiella pneumoniae* (n = 3), *Enterobacter cloacae* (n = 5), and *Enterobacter agglomerans* (n = 1).

^c Isolates included *Candida tropicalis* (n = 8), *Torulopsis glabrata* (n = 7), and *Candida parapsilosis* (n = 5).

^d Isolates included *Aspergillus fumigatus* (n = 16), a *Trichosporon* sp. (n = 1), *Aspergillus flavus* (n = 5), *Aspergillus terreus* (n = 1), and a *Penicillium* sp. (n = 1).

^e Isolates included *Xanthomonas malophilia* (n = 5), *Pseudomonas fluorescens* (n = 1), and a *Flavobacterium* sp. (n = 1).

^f Other than *S. aureus*.

^g Two serogroup A, two serogroup B, and two serogroup G isolates.

marized in Table 1. *P. aeruginosa* was recovered most frequently (53.1% of specimens). *Haemophilus parainfluenzae* and *Candida albicans* were the next most common isolates (46.1 and 44.6% of specimens, respectively). *S. aureus* was recovered with intermediate frequency (24.8% of specimens). *H. influenzae* (11.6%), filamentous fungi (9.3%), members of the *Enterobacteriaceae* (8.9%), and yeasts other than *C. albicans* (7.7%) were recovered less often but with similar frequency. All other bacteria, including *Streptococcus pneumoniae*, beta-hemolytic streptococci, *P. cepacia*, *Acinetobacter calcoaceticus* subsp. *anitratus*, and other miscellaneous gram-negative bacilli, were isolated only infrequently.

In most instances, selective media proved to be of value in processing respiratory tract specimens from patients with CF (Table 1). BCG yielded 133 of 135 isolates of yeast. The next most productive medium for yeasts was CHOC, which yielded 127 of 135 isolates of yeast. In addition, BCG agar was judged to be the optimum medium for the performance of additional procedures, yielding 51% of the 115 isolates of *C. albicans* and all 20 of the isolates of yeast other than *C. albicans*. CHOC-BAC was helpful in isolating and characterizing *Haemophilus* spp. All 30 isolates of *H. influenzae* and all 119 isolates of *H. parainfluenzae* were recovered on CHOC-BAC. This was also true for CHOC lacking bacitracin; however, in two-thirds of the cases, supplemental tests were performed with growth from the CHOC-BAC plates. This reflects the selective nature of CHOC-BAC for *Haemophilus* spp., with resultant isolated colonies and little overgrowth (4). MAC agar plates were equally useful for recovery and characterization of members of the *Enterobacteriaceae*, *P. aeruginosa*, *A. calcoaceticus* subsp. *anitratus*, and other miscellaneous gram-negative bacilli (Table 1). MANN plates yielded all 64 isolates of *S. aureus*, and although 62 of these were also recovered on BAP and CHOC, growth from the MANN plate was used for isolate characterization in 45% of the cases.

In contrast, CET and CNA plates were of little value in processing respiratory tract specimens from patients with CF. CET plates yielded only 113 of 137 (82.5%) isolates of *P. aeruginosa* and were the source of growth for additional tests in only 3% of the cases. As noted above, MAC plates were the most useful for the isolation and characterization of *P. aeruginosa*. Similarly, the rate of recovery of gram-positive cocci other than *S. aureus* on CNA was only 50% (6 of 12), and in no case was growth from CNA plates used for additional studies. All 12 of these gram-positive cocci were isolated and then characterized from growth on BAP plates.

Unfortunately, the number of isolates of *P. cepacia* in the current study was too small to permit meaningful analyses of the utility of OF-PBL medium. It is clear from the literature, however, that optimum recovery of *P. cepacia* from CF patients requires the use of selective media (2, 3, 5, 11, 13, 15). Two different media, OF-PBL (13), as used in our investigation, and *P. cepacia* agar, have been most commonly employed (5). Two reports previously indicated that recovery rates with these two media are essentially comparable (2, 11).

The specimens included in the current study consisted of both throat swabs and expectorated sputa. There was no difference in the rates of recovery of individual organisms on the basis of specimen type, nor were there discernible differences in the relative yields of different media when analyzed according to specimen type.

Ascribing clinical significance to microorganisms recovered from respiratory tract secretions from CF patients is often difficult, if not impossible. This is due to the high frequency with which the lower airways of asymptomatic CF patients become colonized with potential pathogens. For these rea-

sons, it was not possible in the current study to analyze the results of cultures in light of their clinical significance.

In conclusion, the results of this investigation are consistent with the previous observations of others (7, 8, 12) regarding those microorganisms most commonly recovered from respiratory tract secretions from CF patients. Furthermore, in general, the previous recommendations of others (9, 14) concerning the need for the use of several selective media for the optimum processing of CF specimens were confirmed. We found little value, however, in the routine use of CET and CNA plates. A useful battery of primary plates might include BAP, CHOC, MAC, BCG, MANN, CHOC-BAC, and OF-PBL plates.

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