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Evaluation of Various Culture Media for Detection of Rapidly Growing Mycobacteria from Patients with Cystic Fibrosis

Clair L. Preece,[a,b] Thomas A. Wichelhaus,[c] Audrey Perry,[a] Amanda L. Jones,[b] Stephen P. Cummings,[b] John D. Perry,[a,b] Michael Hogardt[c]

Microbiology Department, Freeman Hospital, Newcastle upon Tyne, United Kingdom[a]; Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom[b]; Institute of Medical Microbiology and Infection Control, University Hospital Frankfurt, Frankfurt am Main, Germany[c]

Isolation of nontuberculous mycobacteria (NTM) from the sputum of patients with cystic fibrosis (CF) is challenging due to overgrowth by rapidly growing species that colonize the lungs of patients with CF. Extended incubation on *Burkholderia cepacia* selective agar (BCSA) has been recommended as an expedient culture method for the isolation of rapidly growing NTM in this setting. The aim of this study was to assess five selective media designed for the isolation of *Burkholderia cepacia* complex, along with two media designed for the isolation of mycobacteria (rapidly growing mycobacteria [RGM] medium and Middlebrook 7H11 agar), for their abilities to isolate NTM. All seven media were challenged with 147 isolates of rapidly growing mycobacteria and 185 isolates belonging to other species. RGM medium was then compared with the most selective brand of BCSA for the isolation of NTM from 224 sputum samples from patients with CF. Different agars designed for the isolation of *B. cepacia* complex varied considerably in their inhibition of other bacteria and fungi. RGM medium supported the growth of all isolates of mycobacteria and was more selective than any other medium. NTM were recovered from 17 of 224 sputum samples using RGM medium, compared with only 7 samples using the most selective brand of BCSA ($P = 0.023$). RGM medium offers a superior option, compared to other selective agars, for the isolation of rapidly growing mycobacteria from the sputum of patients with CF. Furthermore, the convenience of using RGM medium enables routine screening for rapidly growing NTM in all submitted sputum samples from patients with CF.

Burkholderia cepacia complex (BCC) and nontuberculous mycobacteria (NTM) are both recognized as potentially important pathogens when isolated from the lungs of patients with cystic fibrosis (CF). The use of a selective culture medium is recommended for the isolation of BCC (1), and several brands of such media are commercially available. Rapidly growing mycobacteria (RGM) represent a subset of NTM that generate colonies on solid culture media within 7 days of incubation (2). The predominant species of NTM within the CF population in Europe is *Mycobacterium abscessus* complex (MABSC) (3–5), and there is convincing evidence that the prevalence of infection by MABSC in the CF population is increasing (3, 5–7). This rapidly growing species comprises three subspecies, i.e., *Mycobacterium abscessus* subsp. *abscessus*, *Mycobacterium abscessus* subsp. *bolletii*, and *Mycobacterium abscessus* subsp. *massiliense*.

Culture of mycobacteria from sputum samples has traditionally relied on methods that were designed to accommodate slowly growing mycobacteria, particularly *Mycobacterium tuberculosis*, which is comparatively rare among cystic fibrosis patients. Such methods are laborious and expensive, as they involve chemical decontamination of samples and subsequent culture on both liquid and solid media (8, 9). Furthermore, contamination of cultures by faster growing microorganisms may mean that cultures have to be abandoned (9), and decontamination protocols may reduce the yield of mycobacteria (8).

Esther et al. demonstrated that extended incubation on *Burkholderia cepacia* selective agar (BCSA) afforded an increase in the recovery rate of NTM from 0.7% to 2.8%, using routine microbiological culture methods, and they recommended this as an expedient method for culture of rapidly growing mycobacteria from patients with CF (10). The aim of this study was to assess five commercially available media designed for the isolation of BCC for their abilities to support the growth of BCC ($n = 43$) and rapidly growing mycobacteria ($n = 147$). We also assessed the selectivity of these culture media against 142 other bacteria and fungi, focusing on the inclusion of species frequently recovered from the sputum of patients with CF. Two agar-based media designed for the isolation of mycobacteria (rapidly growing mycobacteria [RGM] medium and Middlebrook 7H11 agar) were included for comparison. The two media with the most potential to recover mycobacteria were compared for their abilities to isolate rapidly growing NTM from 224 sputum samples from patients with CF.

(Some of the data presented here were previously reported at the 29th Annual North American Cystic Fibrosis Conference, Phoenix, AZ, 2015.)

MATERIALS AND METHODS

Materials. All five media for isolation of BCC were purchased as prepoured plates from their respective manufacturers. *Burkholderia cepacia* selective agar (BCSA) (product no. 33631) and Cepacia selective agar (product no. 44347) were purchased from bioMérieux (Basingstoke,

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Address correspondence to John D. Perry, john.perry@nuth.nhs.uk.

United Kingdom, or Nürtingen, Germany). *Burkholderia cepacia* agar (product no. PO0938) was purchased from Oxoid Ltd. (Basingstoke, United Kingdom). BD Cepacia medium (product no. 256180) and BD oxidation-fermentation polymyxin-bacitracin-lactose (OFPBL) medium (product no. 254481) were purchased from BD Diagnostic Systems (Oxford, United Kingdom). Middlebrook 7H11 agar (product no. PP4080) was obtained as prepoured plates from E&O Laboratories (Bonnybridge, United Kingdom). Blood agar was prepared from Columbia agar powder (Oxoid) and supplemented with 5% defibrinated horse blood (TSC Biosciences, Buckingham, United Kingdom). Sabouraud agar and bacteriological agar were purchased from Oxoid. A sample of 9-chloro-9-[4-(diethylamino)phenyl]-9,10-dihydro-10-phenylacridine hydrochloride (C-390) was kindly synthesized by Annette Johnston (Northumbria University, Newcastle upon Tyne, United Kingdom), but the compound is also available commercially from Biosynth (Staad, Switzerland). Colistin methanesulfonate, yeast extract, and amphotericin B were kindly provided by bioMérieux (Craponne, France). All other chemicals and antibiotics were purchased from Sigma-Aldrich (Poole, United Kingdom).

Preparation of RGM medium. RGM medium was designed and prepared by the staff in the microbiology department of Freeman Hospital (Newcastle upon Tyne, United Kingdom) and is an adaptation of Middlebrook agar, as described by Middlebrook and Cohn in 1958 (11). A 10× solution of Middlebrook 7H9 broth was prepared by dissolving the following ingredients in 960 ml of deionized water: ammonium sulfate (5 g), L-glutamic acid (5 g), disodium phosphate (25 g), monopotassium phosphate (10 g), sodium citrate (1 g), magnesium sulfate (0.5 g), calcium chloride (0.005 g), biotin (0.005 g), copper sulfate (0.01 g), zinc sulfate (0.01 g), pyridoxine (0.01 g), and ferric ammonium citrate (0.4 g), with 40 ml of glycerol. The pH of the solution was adjusted to 6.6, and the solution was sterilized by autoclaving at 116°C for 20 min. One hundred milliliters of this solution was added to 750 ml of deionized water with 10 g agar and 4 g yeast extract, and this mixture was autoclaved as before and allowed to cool to 50°C in a water bath. Oleic acid-albumin-dextrose-catalase (OADC) supplement was prepared by dissolving 5 g bovine serum albumin, 2 g glucose, 0.004 g catalase, and 63 μl oleic acid in 100 ml deionized water. The OADC supplement was filter sterilized and added to the molten agar. Finally, the following additives were each dissolved in 10 ml sterile deionized water and added to make 1 liter of medium: 32 mg colistin (as colistin methanesulfonate), 400 mg fosfomycin, 25 mg glucose-6-phosphate, 5 mg amphotericin, and 32 mg C-390. Amphotericin required initial dissolution in 200 μl *N*-methyl-2-pyrrolidinone, followed by the addition of 9.8 ml sterile deionized water. The agar was immediately poured into 90-mm petri dishes.

Microbial strains. A collection of 147 isolates of rapidly growing mycobacteria that had been isolated previously, by standard methods, from sputum samples from patients with CF was used for evaluation of all media. The isolates included *Mycobacterium abscessus* subsp. *abscessus* ($n = 79$), *Mycobacterium chelonae* ($n = 43$), *Mycobacterium abscessus* subsp. *massiliense* ($n = 12$), *Mycobacterium abscessus* subsp. *bolletii* ($n = 3$), *Mycobacterium fortuitum* ($n = 3$), *Mycobacterium salmoniphilum* ($n = 3$), *Mycobacterium llatzerense* ($n = 2$), *Mycobacterium immunogenum* ($n = 1$), and *Mycobacterium mucogenicum* ($n = 1$). Seventy-three of the isolates were obtained from the microbiology department of Freeman Hospital (Newcastle upon Tyne, United Kingdom), and all were from distinct patients. Seventeen isolates were kindly provided by St. Vincent's University Hospital (Dublin, Republic of Ireland) and were also from distinct patients. The remaining 57 isolates were consecutive clinical isolates kindly supplied by Public Health England (Newcastle upon Tyne, United Kingdom). The species and subspecies identities of all strains had been previously confirmed by sequencing of at least two of three housekeeping genes (*rpoB*, *hsp65*, and *sodA*), as described previously (12).

The collection of nonmycobacteria was selected to represent a variety of species frequently recovered from the sputum of patients with CF. Nonmycobacterial strains ($n = 185$) were obtained from national culture collections ($n = 23$) or from the culture collection of the microbiology department of Freeman Hospital ($n = 162$) and included an international *Pseudomonas aeruginosa* reference panel ($n = 43$) (13) and a BCC experimental strain panel ($n = 26$) (14–16), as well as clinical isolates of both species. In total, the collection comprised *Pseudomonas aeruginosa* ($n = 55$), BCC ($n = 43$), *Staphylococcus aureus* ($n = 28$), various species of *Enterobacteriaceae* ($n = 11$), *Achromobacter xylosoxidans* ($n = 8$), *Ralstonia mannitolilytica* ($n = 7$), *Stenotrophomonas maltophilia* ($n = 4$), *Streptococcus* spp. ($n = 4$), *Aspergillus* spp. ($n = 3$), *Candida* spp. ($n = 3$), *Pandoraea* spp. ($n = 3$), *Acinetobacter* spp. ($n = 2$), *Enterococcus* spp. ($n = 2$), *Inquilinus limosus* ($n = 2$), *Scedosporium* spp. ($n = 2$), *Bacillus subtilis* ($n = 1$), *Delftia acidovorans* ($n = 1$), *Elizabethkingia miricola* ($n = 1$), *Geosmithia argillacea* ($n = 1$), *Haemophilus influenzae* ($n = 1$), *Moraxella catarrhalis* ($n = 1$), *Neisseria flavescens* ($n = 1$), and *Ochrobactrum* sp. ($n = 1$).

Inoculation of isolates onto culture media. Strains were previously stored at −20°C in glycerol-skim milk, and frozen isolates were subcultured on Columbia agar with 5% horse blood prior to testing. Each isolate was suspended in 1 ml of saline (0.85%) to a turbidity equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml), using a densitometer. When clumping occurred for NTM rough colony types, vortex mixing with three sterile 3-mm glass beads for 10 min effectively dispersed all clumps. A 1-μl aliquot of each suspension of mycobacteria was inoculated onto each medium type, and the inoculum was spread using a loop. Filamentous fungi were inoculated in the same way. Suspensions of all other isolates were inoculated onto media using a multipoint inoculator to deliver inocula of approximately 1 μl per spot (i.e., approximately 1.5×10^5 CFU/spot). All plates were incubated at 30°C, and growth was recorded after 4, 7, and 10 days of incubation. To demonstrate the viability of isolates, Columbia blood agar for bacterial isolates and Sabouraud agar for fungal isolates were used as controls. All tests were performed in duplicate on separate occasions.

Comparison of RGM medium and BCSA for isolation of mycobacteria from sputum samples. From the data generated in the experiments detailed above, the most selective brand of BCSA (product no. 33631) was evaluated against RGM medium for the isolation of mycobacteria from sputum samples. A total of 224 consecutive sputum samples were prospectively collected from 133 adults and children with CF attending the Christiane Herzog CF Centre, University Hospital Frankfurt (Frankfurt am Main, Germany), between July 2015 and January 2016. Samples were digested using Copan sputum-liquefying solution, in accordance with the manufacturer's instructions. After vortex mixing for 30 s, samples were left for 15 min. A 100-μl aliquot was then cultured on RGM medium and BCSA (bioMérieux product no. 33631), and the inoculum was spread to obtain isolated colonies. Both media were incubated for 10 days at 30°C and examined for growth after 4, 7, and 10 days of incubation. A minority of samples were read after 11 or 12 days of incubation, if the day of the final reading fell on a weekend.

Colonies were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry using a Vitek MS IVD system with knowledge database version 2.0 (bioMérieux, Nürtingen, Germany). Suspected isolates of mycobacteria were confirmed as acid-fast bacilli (AFB) using Ziehl-Neelsen staining and were identified to the species level by sequencing of the internal transcribed spacer (ITS) region. The ITS region was amplified with the primers ITS1 (5′-GATTGGGACGAAGTCGTAAC-3′) and ITS2 (5′-AGCCTGCCACGTCCTTCATC-3′) (TIB Molbiol, Berlin, Germany), as described previously (17). PCR was performed in a 50-μl reaction mixture with 0.4 pmol/μl of each primer, 1.5 mM $MgCl_2$, 0.2 mM (each) dATP, dGTP, dCTP, and dTTP (Roche, Mannheim, Germany), and 1.25 U of *Taq* polymerase (Invitrogen, Darmstadt, Germany). Samples were denatured at 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 20 s, annealing at 64°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR product was sequenced in both directions using ITS1 and ITS2, and the resulting DNA sequence was aligned with the NCBI sequence database.

TABLE 1 Percentages of rapidly growing mycobacteria recovered on various selective agars at 30°C

Type of mycobacteria and incubation time	Total no. of isolates	Recovery (%)						
		BCSA (bioMérieux 33631)	Cepacia selective agar (bioMérieux 44347)	*B. cepacia* agar (Oxoid PO0938)	Cepacia medium (BD 256180)	OFPBL medium (BD 254481)	RGM medium	Middlebrook 7H11 agar (E&O Laboratories PP4080)
MABSC	94							
Day 4		92.6	96.8	57.4	96.8	93.6	98.9	98.9
Day 7		98.9	98.9	91.5	98.9	98.9	98.9	98.9
Day 10		98.9	100	95.7	100	98.9	100	100
M. chelonae	43							
Day 4		97.7	100	9.3	95.3	100	100	100
Day 7		100	100	69.8	95.3	100	100	100
Day 10		100	100	93	97.7	100	100	100
Other species	10							
Day 4		10	70	10	70	40	90	70
Day 7		30	80	60	70	40	90	70
Day 10		30	100	70	70	70	100	100
Total	147							
Day 4		88.4	95.9	40.1	94.6	91.8	98.6	97.3
Day 7		94.6	98	83	95.9	95.2	98.6	97.3
Day 10		94.6	100	93.2	97.3	97.3	100	100

Statistical analysis. Any difference in the performance of the two media for the isolation of NTM from sputum samples was investigated for statistical significance using McNemar's test, with the continuity correction applied. Statistical significance was assigned to P values of ≤ 0.05.

RESULTS

Evaluation of seven selective agars for support of the growth of mycobacteria. Clear differences were revealed between the five different brands of BCSA, in terms of their abilities to support the growth of mycobacteria. For example, 95.9% of mycobacteria generated growth within 4 days of incubation on Cepacia selective agar (bioMérieux), compared with only 40.1% of isolates on Oxoid *B. cepacia* agar (Table 1). After 10 days of incubation, 10 isolates still had not grown on Oxoid *B. cepacia* agar, including MABSC ($n = 4$), *M. chelonae* ($n = 3$), *M. llatzerense* ($n = 2$), and *M. mucogenicum* ($n = 1$). All isolates were recovered on Cepacia selective agar (bioMérieux product no. 44347), whereas other selective agars for BCC failed to support the growth of 4 to 8 isolates. All isolates were recovered on Middlebrook 7H11 agar and RGM medium.

Evaluation of seven selective agars for inhibition of nonmycobacteria. Table 2 provides insights into the selectivity of the seven selective media with 185 nonmycobacterial isolates. All five media for BCC showed effective inhibition of *P. aeruginosa*, which is an essential attribute of such media. Inhibition of other species was more variable, however. For example, of 28 isolates of *S. aureus* (mainly methicillin-resistant strains), 21 (75%) were able to grow on BD OFPBL medium, whereas only 3 isolates were able to grow on Oxoid *B. cepacia* agar and bioMérieux BCSA (product no. 33631). All brands of media for isolation of BCC showed poor abilities to inhibit the growth of fungi, particularly *Aspergillus* spp. and yeasts. Overall, bioMérieux BCSA showed the greatest selectivity and BD OFPBL medium showed the weakest selectivity against nonmycobacteria among the five brands tested.

Although Middlebrook selective medium is designed specifically for the isolation of mycobacteria from clinical samples, growth of nonmycobacterial species was common, with 75 (40.3%) of 186 isolates being able to grow. Overall, its selectivity was inferior to that of the two most selective media for BCC, although the medium was able to inhibit the growth of *Aspergillus fumigatus*. RGM medium was by far the most selective of all of the agars tested, with 90% of nonmycobacteria, including all fungi and Gram-positive bacteria, being inhibited (Table 2).

Performance of selective agars for support of the growth of BCC. Of the five brands of media for BCC, none was able to support the growth of every BCC isolate within the standard incubation period of 5 days (Table 3). Cepacia selective agar (bioMérieux product no. 44347) showed the highest sensitivity (93%), with only 3 isolates being inhibited (1 *Burkholderia stabilis* isolate and 2 *Burkholderia multivorans* isolates), whereas the growth of 7 isolates was inhibited on Oxoid *B. cepacia* agar (1 *Burkholderia cenocepacia* isolate, 5 *B. multivorans* isolates, and 1 *B. stabilis* isolate). Extended incubation up to 10 days resulted in the recovery of 3 additional isolates on BD OFPBL medium but had no impact on other brands of BCSA.

Comparison of RGM medium and BCSA for recovery of mycobacteria from sputum samples. A total of 17 isolates of mycobacteria were recovered from 224 sputum samples (Table 4). The 17 isolates were derived from a total of 12 patients (12/133 patients [9%]). For 4 of those 12 patients, the same species was recovered from more than one sample.

All 17 isolates of mycobacteria were recovered on RGM medium, compared with only 7 (41%) being recovered on BCSA ($P = 0.023$). For 7 of the 12 patients, mycobacteria were detected only using RGM medium ($P = 0.023$). The calculation of sensitivity in Table 4 is for comparative purposes only and assumes that all mycobacteria were recovered by at least one of the two meth-

TABLE 2 Numbers of nonmycobacterial isolates recovered on various selective agars after 10 days of incubation at 30°C

Type of isolate	Total no. of isolates	No. of isolates recovered						
		BCSA (bioMérieux 33631)	Cepacia selective agar (bioMérieux 44347)	*B. cepacia* agar (Oxoid PO0938)	Cepacia medium (BD 256180)	OFPBL medium (BD 254481)	RGM medium	Middlebrook 7H11 agar (E&O Laboratories PP4080)
Gram-negative	141	54	60	55	59	72	18	63
Enterobacteriaceae	11	2	0	2	2	6	0	1
A. xylosoxidans	8	3	3	3	5	8	2	3
Acinetobacter spp.	2	0	0	0	0	0	0	0
B. cepacia complex	43	37	40	36	37	41	12	39
D. acidovorans	1	1	0	0	0	1	0	0
E. miricola	1	1	1	1	0	1	0	1
H. influenzae	1	0	0	0	0	0	0	0
I. limosus	2	0	2	0	2	0	1	2
M. catarrhalis	1	0	0	0	0	0	0	0
N. flavescens	1	1	1	1	1	1	1	1
Ochrobactrum sp.	1	0	1	1	1	1	0	1
P. aeruginosa	55	0	2	1	2	2	0	2
Pandoraea spp.	3	3	3	3	3	3	2	3
R. mannitolilytica	7	6	6	6	6	5	0	7
S. maltophilia	4	0	1	1	0	3	0	3
Gram-positive	35	3	11	3	14	21	0	7
B. subtilis	1	0	0	0	0	0	0	0
Enterococcus spp.	2	0	0	0	0	0	0	0
S. aureus	28	3	11	3	14	21	0	7
Streptococcus spp.	4	0	0	0	0	0	0	0
Yeast and fungi	9	5	8	9	8	8	0	3
A. fumigatus	2	2	2	2	2	2	0	0
A. terreus	1	1	1	1	1	1	0	1
Candida spp.	3	2	3	3	3	3	0	1
G. argillacea	1	0	0	1	0	0	0	0
S. apiospermum	1	0	1	1	1	1	0	1
Scedosporium prolificans	1	0	1	1	1	1	0	0
Total	185	62	79	67	81	101	18	73
Total excluding *B. cepacia* complex	142	25	39	31	44	60	6	34

ods. Clearly this cannot be proven and might be considered unlikely for slower growing species such as the *Mycobacterium avium* complex. The average times to detection for mycobacteria were 7.9 days using RGM medium (range, 4 to 11 days) and 7 days using BCSA (range, 4 to 11 days).

Table 4 shows that RGM medium was much more selective than BCSA for the inhibition of nonmycobacteria, with only 17 isolates of nonmycobacteria being recovered from 224 sputum samples on RGM medium (compared with 59 on BCSA). This is highly likely to have had an impact on the recovery of mycobacteria on BCSA. For example, of the 10 sputum samples shown to contain mycobacteria that were not recovered on BCSA, 5 showed growth of other bacterial species ($n = 3$) or fungal species ($n = 2$) on BCSA. All 17 isolates of mycobacteria recovered on RGM medium were isolated in pure cultures.

DISCUSSION

The accurate prompt detection of rapidly growing NTM for patients with CF is important for treatment management and infection control purposes. In the only previously reported study with RGM medium, a comparison was performed with Cepacia selective agar (bioMérieux product no. 44347) for the isolation of mycobacteria from 502 sputum samples. Mycobacteria were detected in 54 samples using RGM medium and in only 17 samples using Cepacia selective agar ($P \leq 0.0001$) (18). As media for the isolation of *B. cepacia* have been recommended for the isolation of mycobacteria, this prompted us to examine different commercial brands of such media, to compare their abilities to support the growth of rapidly growing mycobacteria and their selectivity against other flora associated with CF sputum samples. Cepacia selective agar (bioMérieux product no. 44347) was at least as effective for the recovery of pure strains of mycobacteria as any other selective agar for *B. cepacia*. It was less selective than some other agars, and much of this could be attributed to a lack of inhibition of methicillin-resistant *S. aureus*. Cepacia selective agar was less selective than bioMérieux BSCA but more selective than BD OFPBL medium.

In 1985, Gilligan et al. were the first to report the design of a selective culture medium for *B. cepacia* (PC medium) for use with sputum samples from patients with CF (19). Their medium in-

TABLE 3 Numbers of isolates of *B. cepacia* complex recovered on various selective agars after 5 days of incubation at 30°C

Species	Total no. of isolates	No. (%) of isolates recovered						
		BCSA (bioMérieux 33631)	Cepacia selective agar (bioMérieux 44347)	*B. cepacia* agar (Oxoid PO0938)	Cepacia medium (BD 256180)	OFPBL medium (BD 254481)	RGM medium	Middlebrook 7H11 agar (E&O Laboratories PP4080)
Burkholderia ambifaria	2	1	2	2	2	1	0	0
Burkholderia anthina	2	1	2	2	1	1	0	1
Burkholderia cenocepacia	11	11	11	10	9	10	3	11
Burkholderia cepacia	3	3	3	3	3	3	0	3
Burkholderia contaminans	1	1	1	1	1	1	0	1
Burkholderia dolosa	2	2	2	2	2	2	0	1
Burkholderia multivorans	12	10	10	7	10	11	2	10
Burkholderia pyrrocinia	2	2	2	2	2	2	1	1
Burkholderia stabilis	4	2	3	3	3	3	0	3
Burkholderia vietnamiensis	4	4	4	4	4	4	0	4
Total	43	37 (86)	40 (93)	36 (84)	37 (86)	38 (88)	6 (14)	35 (81)

cluded polymyxin B, ticarcillin, crystal violet, and bile salts as selective agents, and such agents are commonly exploited in commercial brands. At around the same time, Welch et al. evaluated the use of OFPBL medium, exploiting the use of polymyxin B and bacitracin as selective agents (20). Finally, in 1997, Henry et al. described *B. cepacia* selective agar (BCSA) and showed it to have greater selectivity than PC agar or OFPBL medium. Polymyxin B and crystal violet were retained as selective agents in this medium, with the addition of gentamicin and vancomycin (21). In a large trial with 656 clinical samples, Henry et al. concluded that BCSA was superior to OFPBL medium and PC medium in supporting the growth of *B. cepacia* and suppressing the growth of other flora (22). In this study, we reaffirm the high selectivity of BCSA, which was much more selective than OFPBL medium; however, 6 isolates of BCC were inhibited with BCSA. The selective agents included in various prepoured media that are commercially available for the isolation of BCC are detailed in Table 5.

TABLE 4 Numbers of isolates of mycobacteria and other species recovered from cultures of 224 sputum samples on BSCA and RGM medium

Species	No. of isolates recovered	
	RGM medium	BCSA (bioMérieux 33631)
Total mycobacteria[a]	17	7
M. abscessus complex[b]	9	6
Mycobacterium avium complex	1	0
M. chelonae	1	0
M. mucogenicum	2	0
Mycobacterium simiae	3	1
Mycobacterium sp.	1	0
Total nonmycobacteria	17	59
Achromobacter spp.	6	13
Burkholderia multivorans	5	7
Chryseobacterium sp.	0	1
Cupriavidus sp.	1	1
Proteus mirabilis	0	4
Pseudomonas aeruginosa	0	7
Serratia marcescens	0	2
Sphingobacterium spiritivorum	0	1
Stenotrophomonas maltophilia	0	2
Aspergillus fumigatus	2	9
Aspergillus terreus	0	1
Candida spp.	1	7
Exophiala dermatitidis	0	1
Geotrichum sp.	1	1
Trichosporon mycotoxinivorans	1	1
Unidentified fungus	0	1
No growth	190	160

[a] The sensitivities for mycobacteria were as follows: RGM medium, 100%; BCSA, 41%.
[b] Subspecies identification of the *M. abscessus* complex was not possible using the ITS sequencing method used for identification in Frankfurt.

Mycobacteria grow more slowly than most, if not all, of the other bacterial and fungal species commonly recovered from sputum samples from patients with CF; this means that high selectivity is extremely important, to inhibit or to restrict the growth of nonmycobacteria, so that mycobacteria do not remain undetected due to overgrowth by other species. Although BCSA was the most selective of the agars designed for recovery of BCC, it was much less selective than RGM medium. If BCC is excluded (as BCSA is designed to detect this species), then 25 nonmycobacteria were able to grow on BCSA, compared with only 6 on RGM medium (Table 1). A particular drawback of selective agars for BCC is their failure to inhibit fungi, particularly *Aspergillus* spp. With extended incubation of these media, the growth of *Aspergillus* can overwhelm the entire culture plate, severely compromising the isolation of mycobacteria. This is particularly problematic with sputum samples from CF patients, for whom infection with mycobacteria has been associated with concomitant isolation of *Aspergillus* spp. (23, 24).

Middlebrook 7H11 agar, designed for the isolation of mycobacteria, was better at inhibiting fungi, due to the inclusion of amphotericin (Table 5). Other species, such as *Aspergillus terreus* and *Scedosporium apiospermum*, remained uninhibited, however, and overall the selectivity of Middlebrook 7H11 agar was inferior to that of bioMérieux BCSA and Oxoid *B. cepacia* agar (Table 1). In contrast, no yeasts or fungi were able to grow on RGM medium.

As BCSA (product no. 33631) was found to be the most selective of the five media designed for the isolation of BCC (and more

TABLE 5 Selective agents included in various culture media, as disclosed by the manufacturers[a]

Component	BCSA (bioMérieux 33631)	Cepacia selective agar (bioMérieux 44347)	*B. cepacia* agar (Oxoid PO0938)	Cepacia medium (BD 256180)	OFPBL medium (BD 254481)	RGM medium	Middlebrook 7H11 agar[b] (E&O Laboratories PP4080)
Polymyxin B (U/liter)	600,000	300,000	150,000	300,000	300,000		Included
Colistin (mg/liter)						32	
Crystal violet (mg/liter)	2	1	1	1			
Bile salts (g/liter)		0.5	1.5	0.5			
Ticarcillin (mg/liter)		10	100	100			Included
Gentamicin (mg/liter)	10		5				
Vancomycin (mg/liter)	2.5						
Bacitracin (U/liter)					200		
Trimethoprim							Included
Amphotericin B (mg/liter)						5	Included
Malachite Green							Included
Fosfomycin (g/liter)						0.4	
C-390 (mg/liter)						32	

[a] The compositions of these media may be adjusted by the manufacturers to meet performance requirements.
[b] The concentrations of selective agents in Middlebrook 7H11 agar (E&O Laboratories) are not published.

selective than Middlebrook 7H11 agar), it was compared with RGM medium for further evaluation with 224 sputum samples. The study with sputum samples confirmed the superior selectivity of RGM medium (Table 4), and it is likely that this facilitated the significantly greater yield of mycobacteria recovered with RGM medium ($P = 0.023$). We think that the use of RGM medium constitutes a simple convenient method for the culture of mycobacteria that can be embedded within routine diagnostic methods, allowing the culture of all submitted sputum samples from patients with CF. A dedicated culture method for detection of BCC is accepted practice for sputum samples from patients with CF (1), and it is noteworthy that NTM were recovered in almost 3 times as many samples as BCC in this study. From our analysis, we conclude that RGM medium offers a superior option, compared with the other selective agars, for screening and monitoring of rapidly growing mycobacteria from the sputum of patients with CF. Further studies are required to compare the sensitivity of RGM medium with that of formal culture methods for acid-fast bacilli (AFB) (e.g., automated liquid culture). It would also be of interest to examine the utility of RGM medium in locations where slower growing species of mycobacteria, such as *M. avium* complex, may predominate. Until such data are available, formal AFB culture methods remain essential in order to detect slowly growing species of NTM (25).

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