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

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ABSTRACT

Isolation of non-tuberculous mycobacteria (NTM) from the sputum of patients with cystic fibrosis (CF) is challenging due to overgrowth by rapidly-growing species that colonise the lungs of patients with CF. Extended incubation of *Burkholderia cepacia* selective agar (BCSA) has been recommended as an expedient culture method for isolation of rapidly-growing NTM in this setting. The aim of this study was to assess the efficacy of five selective media designed for the isolation of *Burkholderia cepacia* complex (BCC) along with two media designed for the isolation of mycobacteria (RGM medium and Middlebrook 7H11 agar) for their ability 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 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 seven samples using the most selective brand of BCSA (P = 0.023). RGM medium offers a superior option to other selective agars for 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 sputum samples submitted by patients with CF.

IINTRODUCTION

Burkholderia cepacia complex (BCC) and non-tuberculous 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 isolation of BCC (1) and several brands of such media are commercially available. Rapidly-growing mycobacteria represent a subset of NTM that generate colonies on solid culture media within seven 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 is increasing in the CF population (3, 5-7). This rapidly-growing species comprises three subspecies: *Mycobacterium abscessus* subsp. *abscessus*, *Mycobacterium abscessus* subsp. *bolletii* and *Mycobacterium abscessus* subsp. *massiliense*.

Culture of mycobacteria from sputum samples traditionally relies upon methods that were designed to accommodate slow growing mycobacteria – in particular *Mycobacterium tuberculosis*, which is comparatively rare in 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.* (2011) demonstrated that extended incubation of *Burkholderia cepacia* selective agar (BCSA) afforded an increased recovery rate of NTM from 0.7% to 2.8% using routine microbiological culture methods and 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

ability 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, focussing on the inclusion of species frequently recovered from the sputum of patients with CF. Two agar-based media designed for the isolation of 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 ability to isolate rapidly-growing NTM from 224 sputum samples from patients with CF.

MATERIALS AND METHODS

Materials. All five media for isolation of BCC were purchased as pre-poured plates from their respective manufacturers. *Burkholderia cepacia* selective agar (BCSA; Product Ref: 33631) and Cepacia selective agar (Product Ref: 44347) were purchased from bioMérieux, Basingstoke, UK or bioMérieux, Nürtingen, Germany. *Burkholderia cepacia* agar (Product Ref: PO0938) was purchased from Oxoid Ltd, Basingstoke, UK. BD Cepacia medium (Product Ref: 256180) and BD OFPBL (oxidation-fermentation polymyxin-bacitracin-lactose) medium (Product Ref: 254481) were purchased from BD Diagnostic Systems, Oxford, UK. Middlebrook 7H11 agar (Product Ref: PP4080) was obtained from E&O Laboratories, Bonnybridge, UK, as pre-poured plates. Blood agar was prepared from Columbia agar powder (Oxoid, Basingstoke, UK) and supplemented with 5% defibrinated horse blood (TSC Biosciences, Buckingham, UK). Sabouraud agar and bacteriological agar were purchased from Oxoid, Basingstoke, UK. A sample of 9-chloro-9-[4-(diethylamino)phenyl]-9,10-dihydro-10-phenylacridine hydrochloride (C-390) was kindly synthesized by Dr. Annette Johnston of Northumbria University, Newcastle upon Tyne, UK but is also available commercially from Biosynth, Staad, Switzerland. Colistin methanesulfonate, yeast extract and amphotericin B were kindly provided by bioMérieux,

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Craponne, France. All other chemicals and antibiotics were purchased from Sigma-Aldrich, Poole, UK.

Preparation of RGM medium. RGM (rapidly-growing mycobacteria) medium was designed and prepared by staff at the Freeman Hospital Microbiology Department (Newcastle upon Tyne, UK) and is an adaptation of Middlebrook agar described by Middlebrook and Cohn in 1958 (11). A tenfold-strength solution of Middlebrook 7H9 broth was prepared by dissolving the following ingredients in 960 ml of deionized water: ammonium sulphate (5 g), L-glutamic acid (5 g), di-sodium phosphate (25 g), mono-potassium 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), ferric ammonium citrate (0.4 g) and 40 ml of glycerol. The pH of the solution was adjusted to 6.6 and it was sterilized by autoclaving at 116°C for 20 minutes. One hundred milliliters of this solution was added to 750 ml of deionized water with 10 g of agar and 4 g of yeast extract and this was autoclaved as before and allowed to cool to 50°C in a waterbath. 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 of N-methyl-2-pyrrolidinone followed by 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 previously isolated by standard methods from sputum samples from patients with CF was used for evaluation of all media. These included *Mycobacterium abscessus* subsp. *abscessus*

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(*n* = 79), *Myobacterium 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 these isolates were obtained from the Microbiology Department, Freeman Hospital, Newcastle upon Tyne, UK and all were from distinct patients. Seventeen were kindly provided by St. Vincent's University Hospital, Dublin, Ireland and were also from distinct patients. The remaining 57 were consecutive clinical isolates kindly supplied by Public Health England, Newcastle upon Tyne, UK. The species and subspecies identity of all strains had been previously confirmed by sequencing of at least two of three housekeeping genes (*rpoB*, *hsp65* and *sodA*) as previously described (12).

The collection of non-mycobacteria was selected to represent a variety of species frequently recovered from the sputa of patients with CF. Non-mycobacterial strains (*n* = 185) were obtained from national culture collections (*n* = 23) or from the culture collection of the Microbiology Department, Freeman Hospital, Newcastle upon Tyne (*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),

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142 *Elizabethkingia miricola* (*n* = 1), *Geosmithia argillacea* (*n* = 1), *Haemophilus influenzae* (*n* =
143 1), *Moraxella catarrhalis* (*n* = 1), *Neisseria flavescens* (*n* = 1) and *Ochrobactrum* sp. (*n* = 1).
144
145 **Inoculation of isolates onto culture media.** Strains were previously stored at -20°C
146 in glycerol/skimmed milk and frozen isolates were subcultured onto Columbia agar with 5%
147 horse blood prior to testing. Each isolate was suspended in 1 ml of saline (0.85%) to a
148 turbidity equivalent to a McFarland 0.5 standard (approximately 1.5×10^8 CFU/ml) using a
149 densitometer. For NTM rough colony-types, where clumping occurred, vortexing with three
150 sterile 3 mm glass beads for 10 min effectively dispersed all clumps. A 1 µl aliquot of each
151 suspension of mycobacteria was inoculated onto each medium type and the inoculum was
152 spread using a loop. Filamentous fungi were inoculated in the same way. Suspensions of all
153 other isolates were inoculated onto media using a multipoint inoculator to deliver inocula of
154 approximately 1 µl per spot (i.e. approximately 1.5×10^5 CFU/spot). All plates were
155 incubated at 30°C and growth was recorded after four, seven days and ten days of incubation.
156 To demonstrate the viability of isolates, Columbia blood agar for bacterial isolates and
157 Sabouraud agar for fungal isolates were used as controls. All tests were performed in
158 duplicate on separate occasions.
159
160 **Comparison of RGM medium with BCSA (Ref: 33631) for isolation of**
161 **mycobacteria from sputum samples.** From the data generated in the experiments detailed
162 above, the most selective brand of BCSA (Ref: 33631) was evaluated against RGM medium
163 for isolation of mycobacteria from sputum samples. A total of 224 consecutive sputum
164 samples were prospectively collected from 133 adults and children with CF attending the
165 Christiane Herzog CF-Centre, University Hospital Frankfurt, Frankfurt am Main, Germany,
166 between July 2015 and January 2016. Samples were digested using Copan Sputum

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Liquefying solution in accordance with manufacturer's instructions. After vortexing for 30 s samples were left for 15 minutes. A 100 µL aliquot was then cultured onto RGM and BCSA (bioMérieux Ref: 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 11-12 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 VITEK-MS IVD system with knowledge database version 2.0 (bioMérieux, Nürtingen, Germany). Suspected isolates of mycobacteria were confirmed as acid-fast bacilli using a Ziehl-Neelsen stain and identified to species level by sequencing of the internal transcribed spacer (ITS) region. The ITS region was amplified with primers ITS1 5'-GATTGGGACGAAGTCGTAAC-3' and ITS2 5'-AGCCTGCCACGTCCTTCATC-3' (TIB MOLBIOL, Berlin, Germany) as previously described (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 of dATP, dGTP, dCTP, dTTP (Roche, Mannheim, Germany), 1.25 U of Taq polymerase (Invitrogen, Darmstadt, Germany) and an annealing temperature of 64°C for 40 cycles. The PCR product was sequenced in both directions using ITS1 and ITS2 and the resulting DNA sequence was aligned with NCBI sequence database.

Statistical analysis. Any difference in performance of the two media for 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 a probability (*P*) value of ≤ 0.05.

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RESULTS

Evaluation of seven selective agars for supporting the growth of mycobacteria. Clear differences were revealed between the five different brands of BCSA in terms of their ability to support the growth of mycobacteria. For example, on Cepacia selective agar (bioMérieux) 95.9% of mycobacteria generated growth within 4 days of incubation compared with only 40.1% of isolates on Oxoid *B. cepacia* agar (Table 1). After 10 days of incubation, ten isolates had still 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; Ref: 44347) whereas other selective agars for BCC failed to support the growth of between four and eight isolates. All isolates were recovered on Middlebrook 7H11 agar and RGM medium.

Evaluation of seven selective agars for inhibition of non-mycobacteria. Table 2 provides insights into the selectivity of the seven selective media with 185 non-mycobacteria. 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 three isolates were able to grow on Oxoid *B. cepacia* agar and bioMérieux BCSA (Ref: 33631). All brands of media for isolation of BCC showed a poor ability to inhibit the growth of fungi – particularly *Aspergillus* spp. and yeasts. Overall, bioMérieux BCSA showed the greatest selectivity and BD OFPBL showed the weakest selectivity against non-mycobacteria among the five brands tested.

Although Middlebrook selective medium is designed specifically for the isolation of mycobacteria from clinical samples, growth of non-mycobacterial species was common with

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75 out of 186 (40.3%) isolates able to grow. Overall, its selectivity was inferior to the two most selective media for BCC, although it 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 non-mycobacteria inhibited including all fungi and Gram-positive bacteria (Table 2).

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

Comparison of RGM medium with BCSA (Ref: 33631) for recovery of mycobacteria from sputum samples. A total of 17 isolates of mycobacteria were recovered from 224 sputum samples (Table 4). These 17 isolates were derived from a total of 12 patients (prevalence: 12/133; 9%). For four of these 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 seven (41%) recovered on BCSA (P = 0.023). For seven of 12 patients, mycobacteria were only detected 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 methods. Clearly this cannot be proven and moreover might be considered

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242 unlikely for slower-growing species such as *M. avium* complex. The average time-to-
243 detection for mycobacteria was 7.9 days using RGM medium (range 4-11 days) and 7 days
244 using BCSA (range 4-11 days).
245
246 Table 4 shows that RGM medium was much more selective than BCSA for the
247 inhibition of non-mycobacteria with only 17 isolates of non-mycobacteria recovered on RGM
248 medium from 224 sputum samples (compared with 59 on BCSA). This is highly likely to
249 have had an impact on the recovery of mycobacteria on BCSA. For example, for the ten
250 sputum samples shown to contain mycobacteria that were not recovered on BCSA, five of
251 these showed growth of other bacterial species ($n = 3$) or fungal species ($n = 2$) on BCSA. All
252 17 isolates of mycobacteria recovered on RGM medium were isolated in pure culture.
253
254 **DISCUSSION**
255
256 The accurate and prompt detection of rapidly-growing NTM for patients with CF is important
257 for treatment management and for infection control purposes. In the only previously reported
258 study with RGM medium, a comparison was performed with Cepacia selective agar
259 (bioMérieux; 44347) for the isolation of mycobacteria from 502 sputum samples.
260 Mycobacteria were detected in 54 samples using RGM medium and from only 17 samples
261 using Cepacia selective agar ($P \leq 0.0001$) (18). As media for isolation of *B. cepacia* have
262 been recommended for isolation of mycobacteria, this prompted us to examine different
263 commercial brands of such media to compare their ability to support the growth of rapidly-
264 growing mycobacteria and their selectivity against other flora associated with CF sputum
265 samples. Cepacia selective agar (bioMérieux; 44347) was at least as effective for the recovery
266 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 lack of inhibition of methicillin-resistant *S. aureus*. Cepacia selective agar was less selective than bioMérieux BSCA but more selective than BD OFPBL.

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 included 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 and OFPBL medium. In this medium, polymyxin B and crystal violet were retained as selective agents 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 and PC medium for supporting the growth of *B. cepacia* and suppressing the growth of other flora (22). In this study we re-affirm the high selectivity of BCSA, which was much more selective than OFPBL, however, six isolates of BCC were inhibited using BCSA. The selective agents exploited by various pre-poured media commercially available for isolation of BCC are detailed in Table 5.

Mycobacteria grow more slowly than most if not all of the other bacterial and fungal isolates commonly recovered from sputum samples from patients with CF; this means that high selectivity is extremely important to inhibit or restrict the growth of non-mycobacteria so that they 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

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than RGM medium. If BCC is excluded (as BCSA is designed to detect this species), 25 non-mycobacteria were able to grow on BCSA compared with only six on RGM medium (Table 1). A particular drawback of selective agars for BCC is their failure to inhibit fungi, and particularly *Aspergillus* species. On 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 where infection of mycobacteria has been associated with concomitant isolation of *Aspergillus* sp. (23, 24).

Middlebrook 7H11 agar, designed for isolation of mycobacteria, was better at inhibiting fungi, due to the inclusion of amphotericin (Table 5). However, other species, such as *Aspergillus terreus* and *Scedosporium apiospermum* remained uninhibited 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 (Ref: 33631) was found to the most selective of the five media designed for isolation of BCC (and more selective than Middlebrook 7H11 agar), it was compared with RGM 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 on RGM medium (P = 0.023). We believe that the use of RGM medium constitutes a simple, convenient method for 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 three times as many samples as BCC in

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this study. From our analysis we conclude that RGM medium offers a superior option compared with any of 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 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 slow-growing species of NTM (25).

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TABLE 1 Percentage of rapidly-growing mycobacteria recovered on various selective agars at 30°C.

	n	**BCSA**	**Cepacia selective agar**	***B. cepacia* agar**	**Cepacia medium**	**OFPBL**	**RGM medium**	**Middlebrook 7H11 agar**
		bioMérieux	bioMérieux	Oxoid	BD	BD	-	E&O Laboratories
		33631	**44347**	**PO0938**	**256180**	**254481**	**-**	**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 mycobacteria	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

TABLE 2 Number of non-mycobacteria isolates recovered on various selective agars after 10 days of incubation at 30°C.

	n	**BCSA**	**Cepacia selective agar**	***B. cepacia* agar**	**Cepacia medium**	**OFPBL**	**RGM medium**	**Middlebrook 7H11 agar**
		bioMérieux	bioMérieux	Oxoid	BD	BD	-	E&O Laboratories
		33631	44347	PO0938	256180	254481	-	PP4080
Gram Negatives	**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 sp.	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
Neisseria 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 Positives	**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
S. 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

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TABLE 3 No. of isolates of *B. cepacia* complex recovered on various selective agars after 5 days of incubation at 30°C.

	n	**BCSA**	**Cepacia selective agar**	***B. cepacia* agar**	**Cepacia medium**	**OFPBL**	**RGM medium**	**Middlebrook 7H11 agar**
		bioMérieux	bioMérieux	Oxoid	BD	BD	-	E&O Laboratories
		33631	44347	PO0938	256180	254481	-	PP4080
B. ambifaria	2	1	2	2	2	1	0	0
B. anthina	2	1	2	2	1	1	0	1
B. cenocepacia	11	11	11	10	9	10	3	11
B. cepacia	3	3	3	3	3	3	0	3
B. contaminans	1	1	1	1	1	1	0	1
B. dolosa	2	2	2	2	2	2	0	1
B. multivorans	12	10	10	7	10	11	2	10
B. pyrrocinia	2	2	2	2	2	2	1	1
B. stabilis	4	2	3	3	3	3	0	3
B. vietnamiensis	4	4	4	4	4	4	0	4
Total	43	37	40	36	37	38	6	35
% recovery		**86**	**93**	**84**	**86**	**88**	**14**	**81**

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

	RGM	**BCSA** (Ref: 33631)
Total mycobacteria	**17**	**7**
M. abscessus complex[a]	9	6
M. avium complex	1	0
M. chelonae	1	0
M. mucogenicum	2	0
M. simiae	3	1
Mycobacterium species	1	0
Sensitivity (%)	**100**	**41**
Total non-mycobacteria	**17**	**59**
Achromobacter sp.	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

458 [a]Sub-speciation of the *M. abscessus* complex was not possible using

459 the ITS sequencing method used for identification in Frankfurt.

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TABLE 5 Selective agents (per liter) included in various culture media as disclosed by manufacturers [a]

	BCSA	Cepacia selective agar	*B. cepacia* agar	Cepacia medium	OFPBL	RGM medium	Middlebrook 7H11 agar [b]
	bioMérieux	bioMérieux	Oxoid	BD	BD	-	E&O Laboratories
	33631	44347	PO0938	256180	254481	-	PP4080
Polmyxyn B	600000 U	300 000 U	150000 U	300000 U	300000 U	-	Included
Colistin	-	-	-	-	-	32 mg	-
Crystal violet	2 mg	1 mg	1 mg	1 mg	-	-	-
Bile salts	-	0.5 g	1.5 g	0.5 g	-	-	-
Ticarcillin	-	10 mg	100 mg	100 mg	-	-	Included
Gentamicin	10 mg	-	5 mg	-	-	-	-
Vancomycin	2.5 mg	-	-	-	-	-	-
Bacitracin	-	-	-	-	200 U	-	-
Trimethoprim	-	-	-	-	-	-	Included
Amphotericin B	-	-	-	-	-	5 mg	Included
Malachite Green	-	-	-	-	-	-	Included
Fosfomycin	-	-	-	-	-	0.4 g	-
C-390	-	-	-	-	-	32 mg	-

[a] The composition of these media may be adjusted by manufacturers to meet performance requirements.

[b] Concentrations are not published for selective agents in E&O Middlebrook 7H11 agar.