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Evaluation of various culture media for detection of rapidly-growing mycobacteria from patients with cystic fibrosis

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Abstract

Background: Isolation of non-tuberculous mycobacteria (NTM) from the sputum of patients with cystic fibrosis (CF) is challenging due to the overgrowth of 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.

Methods: Five commercially available pre-poured media for the isolation of *Burkholderia cepacia* complex (BCC) were assessed and compared with two media designed specifically for the isolation of mycobacteria (RGM medium and Middlebrook 7H11 selective agar). All seven media were challenged with 147 isolates of rapidly-growing mycobacteria, 43 isolates of BCC and 142 isolates belonging to other species. RGM medium was then compared with the most selective brand of BCSA for the isolation of NTM from 216 sputum samples from patients with CF.

Results: RGM medium supported the growth of all isolates of mycobacteria and was more selective against other bacteria and fungi than any other culture medium. NTM were recovered from 17 sputum samples using RGM medium compared with seven samples using the most selective brand of BCSA ($P = 0.023$).

Conclusions: RGM medium offers a superior option than any of the other selective agars for isolation of rapidly-growing mycobacteria from the sputum of patients with CF. Furthermore, the convenience of using RGM medium enables screening for rapidly-growing NTM in all sputum samples submitted by patients with CF.

Introduction

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 specifically designed for the isolation of BCC from the sputum of cystic fibrosis patients is recommended [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 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 relies upon traditional 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 BCSA afforded an increased recovery rate of NTM from 0.7% to 2.8% using routine culture methods and recommended this as an expedient method for culture of rapidly-growing species from patients with CF [10]. The aim of this study was to assess five commercially-available media 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 with a bias towards 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 selective agar) were included for comparison. The two media with the most potential to recover mycobacteria were compared for their ability to isolate NTM from 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 and RGM medium was prepared in house as previously described [11]. Blood agar was prepared from Columbia agar powder (Oxoid, Basingstoke, UK) and supplemented with 5% defibrinated horse blood (TSC Biosciences, Buckingham, UK). Sabouraud agar was prepared from Sabouraud dextrose agar powder (Product Ref: CM0041), provided by Oxoid, Basingstoke, UK.

Bacterial 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* ($n = 79$), *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* ($n = 28$), *Enterobacteriaceae* ($n = 11$), *Achromobacter xylosoxidans* ($n = 8$), *Ralstonia mannitolilytica* ($n = 7$), *Stenotrophomonas maltophilia* ($n = 4$), *Streptococcus* spp. ($n = 4$),

Aspergillus spp. ($n = 3$), *Bacillus subtilis* ($n = 1$), *Candida* spp. ($n = 3$), *Pandoraea* spp. ($n = 3$), *Acinetobacter* spp. ($n = 2$), *Enterococcus* spp. ($n = 2$), *Inquilinus limosus* ($n = 2$), *Scedosporium* spp. ($n = 2$), *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 media

Strains were previously stored at -20ºC in glycerol/skimmed milk and frozen isolates were subcultured onto 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 McFarland 0.5 standard (approximately 1.5×10^8 CFU/ml) using a densitometer. For NTM rough colony-types where clumping occurred, vortexing 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 four, seven days and ten 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 with BCSA for isolation of mycobacteria from sputum samples.

A total of 216 sputum samples were collected from 130 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 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 using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (VITEK-MS, bioMérieux, Nürtingen, Germany). Suspected isolates of mycobacteria were further confirmed as acid-fast bacilli using a Ziehl-Neelsen stain and identified to species level by ITS sequencing………further details (and / or reference) required.

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.

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 has 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 brands[S1] of BCSA failed to support the growth of between four and eight isolates. All isolates were recovered on Middlebrook 7H11 selective agar and RGM medium.

	n	BCSA	Cepacia selective agar	B. cepacia agar	Cepacia medium	OFPBL	RGM medium	Middlebrook 7H11 agar
Table 1: Percentage of mycobacteria recovered on various selective agars at 30°C.								
		bioMérieux	bioMérieux	Oxoid	BD	BD	N/A	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	N/A	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

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 of the five brands of BCSA 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. 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 among the five brands tested.

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

Performance of selective agars for isolation 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.

	n	BCSA	Cepacia selective agar	B. cepacia agar	Cepacia medium	OFPBL	RGM medium	Middlebrook 7H11 agar
		bioMérieux	bioMérieux	Oxoid	BD	BD	N/A	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 3: No. of isolates of *B. cepacia* complex recovered on various selective agars after 5 days of incubation at 30°C.

Comparison of RGM medium with BCSA for recovery of mycobacteria from sputum samples.

A total of 17 isolates of mycobacteria were recovered from 216 sputum samples (Table 4). These 17 isolates were derived from a total of 12 patients (prevalence: 12/130; 9.2%). 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 [s2]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 unlikely for slower-growing species such as *M. avium* complex. The average time-to-

detection for mycobacteria was 7.9 days using RGM medium (range 4-11 days) and 7 days using BCSA (range 4-11 days).

Table 4: Numbers of isolates of mycobacteria and other species recovered on BSCA and RGM medium from culture of 216 sputum samples.		
	RGM	BCSA
Total mycobacteria	**17**	**7**
M. abscessus complex	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	**16**	**56**
Achromobacter sp.	6	12
Burkholderia multivorans	5	6
Cryseobacterium sp.	0	1
Cupriavidus sp.	1	1
Proteus mirabilis	0	4
Pseudomonas aeruginosa	0	7
Serratia marcescens	0	2
Shingobacterium spiritivorum	0	1
Stenotrophomonas maltophilia	0	2
Aspergillus fumigatus	2	9
Aspergillus terreus	0	1
Candida sp.	1	7
Exophiala dermatitidis	0	1
Geotrichum sp.	1	1
Unidentified fungus	0	1
No growth	183	155

Table 4 shows that RGM medium was much more selective than BCSA for the inhibition of non-mycobacteria with only 16 isolates of non-mycobacteria recovered on RGM medium from 216 sputum samples (compared with 56 on BCSA). This is highly likely to have had an impact on the recovery of mycobacteria on BCSA. For example, for the ten sputum samples

shown to contain mycobacteria that were not recovered on BCSA, five of these 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 culture.

Table 4: Selective agents included in various culture media as disclosed by manufacturers.*							
	BCSA	**Cepacia selective agar**	**B. cepacia agar**	**Cepacia medium**	**OFPBL**	**RGM medium**	**Middlebrook 7H11 agar**
	bioMérieux	bioMérieux	Oxoid	BD	BD	N/A	E&O Laboratories
	33631	44347	PO0938	256180	254481	-	PP4080
Polmyxin B	600 000 U	300 000 U	150 000 U	300 000 U	300 000 U	ND	Included
Crystal violet	2 mg	1 mg	1 mg	1 mg	-	ND	-
Bile salts	-	0.5 g	1.5 g	0.5 g	-	ND	-
Ticarcillin	-	10 mg	100 mg	100 mg	-	ND	Included
Gentamicin	10 mg	-	5 mg	-	-	ND	-
Vancomycin	2.5 mg	-	-	-	-	ND	-
Bacitracin	-	-	-	-	200 U	ND	-
Trimethoprim	-	-	-	-	-	ND	Included
Amphotericin B	-	-	-	-	-	ND	Included
Malachite Green	-	-	-	-	-	ND	Included
*The composition of these media may be adjusted by manufacturers to meet performance requirements. ND: Not disclosed. Concentrations are not published for selective agents in E&O Middlebrook agar.							

[S3]

Discussion

The accurate and prompt detection of rapidly growing NTM for patients with CF is important for treatment management and for infection control purposes. In the only previously reported study, RGM medium was compared with Cepacia selective agar (bioMérieux; 44347) for the isolation of mycobacteria from 502 sputum samples. Mycobacteria were detected in 54 samples using RGM medium and from only 17 samples using Cepacia selective agar (P ≤ 0.0001) [12]. As media for isolation of *B. cepacia* have been recommended for isolation of mycobacteria, this prompted us to examine different commercial brands of such media to

compare their ability to support the growth of mycobacteria and their selectivity against other flora associated with CF sputum samples. Cepacia selective agar (bioMérieux; 44347) was at least as effective for culture 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 [17]. 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 [18]. 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 [19]. 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 [20]. 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 than RGM medium. If BCC is excluded (as BCSA is designed to grow this), 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. [21, 22].

Middlebrook 7H11 selective 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 selective 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 a result of these findings, RGM was compared with BCSA for the isolation of mycobacteria from 216 sputum samples. The data confirm the superior selectivity of RGM medium 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 this study. From our analysis we conclude that RGM

medium offers a superior option compared with any of the other selective agars for isolation of rapidly-growing mycobacteria from the sputum of patients with CF. It is anticipated that RGM medium will be made commercially available in due course but until then, the authors are committed to making the culture medium freely available to clinical laboratories who wish to independently verify the findings of this study. Further studies are required to compare the sensitivity of RGM medium with formal culture methods for acid-fast bacilli (i.e. 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.

References:

[1] Cystic Fibrosis Trust. Laboratory standards for processing microbiological samples from people with cystic fibrosis. Report of the UK Cystic Fibrosis Trust Microbiology Laboratory Standards Working Group. ISBN 0-9548511-4-5.

[2] Brown-Elliot BA, Wallace RJ. *Mycobacterium*: clinical and laboratory characteristics of rapidly growing mycobacteria. In: Versalovic J, Carroll KC, Jorgensen JH, Funke G, Landry ML, Warnock DW, eds. Manual of Clinical Microbiology. 10th ed. Washington, DC: ASM Press; 2011:525–538.

[3] Qvist T, Gilljam M, Jönsson B, *et al.* Epidemiology of nontuberculous mycobacteria among patients with cystic fibrosis in Scandinavia. J. Cyst. Fibros. 2015;**14**:46-52.

[4] Roux AL, Catherinot E, Ripoll F, *et al.* Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. J. Clin. Microbiol. 2009;**47**:4124-8.

[5] Seddon P, Fidler K, Raman S, *et al.* Prevalence of nontuberculous mycobacteria in cystic fibrosis clinics, United Kingdom, 2009. Emerg. Infect. Dis. 2013;**19**:1128-30.

[6] Bar-On O, Mussaffi H, Mei-Zahav M, *et al.* Increasing nontuberculous mycobacteria infection in cystic fibrosis. J. Cyst. Fibros. 2015;**14**:53-62.

[7] Foundation CF. Cystic Fibrosis Foundation Patient Registry Annual Data Report; 2010.

[8] Burns JL, Rolain JM. Culture-based diagnostic microbiology in cystic fibrosis: Can we simplify the complexity? J. Cyst. Fibros. 2013;**13**:1-9.

[9] Whittier S, Hopfer RL, Knowles MR, *et al.* Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol. 1993;**31**:861–4.

[10] Esther CR, Jr., Hoberman S, Fine J, *et al.* Detection of rapidly growing mycobacteria in routine cultures of samples from patients with cystic fibrosis. J. Clin. Microbiol. 2011;**49**:1421-5.

[11] Preece CL, Perry A, Gray B, *et al.* A novel culture medium for isolation of rapidly-growing mycobacteria from the sputum of patients with cystic fibrosis. J Cyst Fibros 2015 May 20. pii: S1569-1993(15)00117-4. doi: 10.1016/j.jcf.2015.05.002.

[12] Blauwendraat C, Dixon GL, Hartley JC, Foweraker J, Harris KA. The use of a two-gene sequencing approach to accurately distinguish between the species within the *Mycobacterium abscessus* complex and *Mycobacterium chelonae*. Eur. J. Clin. Microbiol. Infect. Dis. 2012;**31**:1847-53.

[13] De Soyza A, Hall AJ, Mahenthiralingam E, Drevinek P, Kaca W, Drulis-Kawa Z, Stoitsova SR, Toth V, Coenye T, Zlosnik JE, Burns JL, Sa-Correia I, De Vos D, Pirnay JP, T JK, Reid D, Manos J, Klockgether J, Wiehlmann L, Tummler B, McClean S, Winstanley C. 2013. Developing an international *Pseudomonas aeruginosa* reference panel. Microbiologyopen. **2**:1010-1023.

[14] Mahenthiralingam E, Coenye T, Chung JW, Speert DP, Govan JR, Taylor P, Vandamme P. 2000. Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* complex. J. Clin. Microbiol. **38**:910-913.

[15] Coenye T, Vandamme P, LiPuma JJ, Govan JR, Mahenthiralingam E. 2003. Updated version of the *Burkholderia cepacia* complex experimental strain panel. J. Clin. Microbiol. **41**:2797-2798.

[16] Vermis K, Coenye T, LiPuma JJ, Mahenthiralingam E, Nelis HJ, Vandamme P. 2004. Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. Int. J. Syst. Evol. Microbiol. **54**:689-691.

[17] Gilligan PH, Gage PA, Bradshaw LM, Schidlow DV, DeCicco BT. Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol. 1985 **22**:5-8.

[18] Welch DF, Muszynski MJ, Pai CH, Marcon MJ, Hribar MM, Gilligan PH, Matsen JM, Ahlin PA, Hilman BC, Chartrand SA. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. J. Clin. Microbiol. 1987 25:1730-4.

[19] Henry DA, Campbell ME, LiPuma JJ, Speert DP. Identification of *Burkholderia cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. J. Clin. Microbiol. 1997 35:614-9.

[20] Henry D, Campbell M, McGimpsey C, Clarke A, Louden L, Burns JL, Roe MH, Vandamme P, Speert D. Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol. 1999 **37**:1004-7.

[21] Verregghen M, Heijerman HG, Reijers M, van Ingen J, van der Ent CK. Risk factors for *Mycobacterium abscessus* infection in cystic fibrosis patients; a case-control study. J. Cyst. Fibros. 2012 **11**:340-3.

[22] Esther CR Jr1, Esserman DA, Gilligan P, Kerr A, Noone PG. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. J. Cyst. Fibros. 2010 **9**:117-23.