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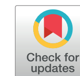
Research article

Evaluation of a novel rapidly-growing mycobacteria medium for isolation of *Mycobacterium abscessus* complex from respiratory specimens from patients with bronchiectasis



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A R T I C L E I N F O

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A B S T R A C T

This single center study assessed the performance of a novel solid rapidly-growing mycobacteria (RGM) medium for the recovery of nontuberculous mycobacteria (NTM), especially *Mycobacterium abscessus* complex, in patients with underlying bronchiectasis. A total of 297 mycobacterial sputa from 116 patients were plated directly on RGM medium and, following decontamination, onto an agar biplate [Middlebrook 7H11 and Mitchison (selective) agar] and into broth media (VersaTrek). The recovery of *M. abscessus* complex was increased by approximately 12% by implementation of the RGM medium. Contamination was reduced to 2% from 48% and 95% on routine solid media and broth cultures respectively. Our study corroborated previous studies in that recovery of *M. abscessus* complex was enhanced and contamination was virtually eliminated without the need for specimen decontamination when utilizing RGM medium.

1. Introduction

The number of patients with nontuberculous mycobacteria (NTM) infections is rapidly increasing in the United States and globally [1]. Traditional culture methods for isolation of NTM have involved the same methods with only slight modifications of decontamination of respiratory samples that are used for the isolation of *Mycobacterium tuberculosis*. However, often these methods used to eliminate contamination are too harsh, either decreasing or completely nullifying the yield of NTM [2, 3, 4]. Contamination of cultures with fungal or other bacterial species is also often problematic.

Recently an agar-based medium designed for the isolation of rapidly-growing mycobacteria (RGM), specifically the *Mycobacterium abscessus* complex, has been introduced [5]. Previous studies have shown that the medium, designated RGM medium, has improved the recovery of RGM and other NTM including *Mycobacterium avium* complex (MAC) although studies with large numbers of MAC and other NTM have not yet been reported [6, 7].

Our hospital and clinic are a major outpatient referral clinic for patients with bronchiectasis and NTM. We undertook an evaluation of the RGM medium and compared it to our routine detection method using a biplate containing Middlebrook 7H11 agar and Mitchison 7H11 agar (with antibiotics including carbenicillin, trimethoprim and amphotericin B) and the VersaTrek broth detection system in accordance with current Clinical and Laboratory Standards Institute (CLSI) guidelines [8].

2. Materials and methods

2.1. Materials

Middlebrook 7H11 agar and Mitchison selective (with carbenicillin, trimethoprim and amphotericin B) 7H11 agar biplates (Becton-Dickinson, Sparks MD) were used for routine acid-fast bacilli (AFB) culture along with the VersaTrek automated broth detection system (Thermofisher, Cleveland, OH). RGM medium was prepared at the Freeman Hospital, Newcastle upon Tyne, UK, as previously described [9] and shipped to the University of Texas Health Science Center at Tyler where specimen cultures were performed.

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2.2. Patient samples

A total of 297 respiratory samples from 116 patients with cystic fibrosis (CF) and/or non-CF bronchiectasis were cultured. A total of 173/297 specimens that required double processing for contamination were submitted for mycobacterial culture between October 2017 and August 2018, due to prior incidences of contamination during culture for AFB. During this time, 124 specimens that underwent single standard processing from 20 patients were also included. Fourteen of the 116 (12%) patients (with a total of 29 cultures) were known to have CF.

2.3. Sample processing

Each sample was divided into two parts. One aliquot was digested using the AlphaTec NAC-PAC Unitary system (Alpha-Tec Systems Inc., Vancouver, WA) with N-acetyl-L-cysteine (0.5%) and decontaminated with sodium hydroxide (2%). After centrifuging the samples at 3000 rpm for 15 min the supernatant was then decanted. For selected samples (see section 2.2), a second decontamination procedure using 5 ml of 5% oxalic acid was then added to each sample and incubated for 30 additional minutes at room temperature, vortexing the samples every 10 min. After neutralizing the samples with buffer, they were centrifuged at 3000 rpm for 15 min. The supernatant was decanted, and the pellets were resuspended using the pellet resuspension buffer. The samples were inoculated onto the biplate containing Middlebrook 7H11 and Mitchison selective agar (approximately 0.3 μl onto each agar) as directed for AFB processing [8]. Additionally, 0.5 ml of the concentrate was added to the VersaTrek bottle. A 0.5 mL aliquot of MycoPVNA (polymyxin B, vancomycin, nalidixic acid and amphotericin B) and 1 mL of growth supplement were added to the bottle as directed by the manufacturer. The broth and agar plates were incubated at 35 °C up to 6 weeks and 3 weeks respectively.

For the second aliquot of the untreated sample, a 100 μl aliquot of specimen was pipetted directly (without digestion or decontamination) onto the RGM medium and streaked for isolation. The plates were then incubated at 30 °C for 28 days.

2.4. Species identification

Isolates of RGM were identified to species level (and for *M. abscessus* complex to subspecies level) using sequencing of region 5 of the *rpoB* gene [10]. Isolates of MAC were identified using the AccuProbe system (Hologic Inc, San Diego, CA). Slowly growing mycobacteria which were not identified as MAC underwent partial *16S rRNA* gene sequencing [11]. Statistical analysis was performed using the chi square method in GraphPad. p values <0.05 were considered statistically significant.

This study was approved under an exempt protocol by the University of Texas Health Science Center at Tyler Institutional Review Board. Patient consent was not required since the testing performed was part of the standard of care for patients with suspected *M. abscessus* lung disease.

3. Results

A total of 297 AFB cultures were processed using standard methods with the addition of the RGM medium. Of the 297 cultures, 124 were from a subset of patients who were involved in a specific treatment protocol for treatment of *M. abscessus* complex refractory NTM lung disease. A total of 129/181 (71%) total cultures were positive for *M. abscessus* complex and 28/181 (15%) grew MAC (Table 1). The sensitivity of RGM medium was 94% for recovery of *M. abscessus* complex compared with 66% for Middlebrook 7H11/Mitchison agar and 61% for broth culture ($p = <0.05$).

A total of 32/181 (18%) isolates of slowly growing mycobacteria (SGM) including 17 isolates of MAC, one isolate of *M. paraffinicum*, and 7/181 (4%) other RGM (including those not identified to species levels) were recovered from routine solid agar media (Middlebrook and Mitchison agar) compared to 5/181 (3%) of SGM (MAC only) and 17/181 (9%) of RGM (not *M. abscessus* complex) on RGM medium alone (Table 1). Recovery of SGM (at 35 °C) was superior in broth media which recovered 19/181 (11%) MAC; 1/181 (0.6%) *M. paraffinicum,* and 3/181 (2%) *M. gordonae*. One isolate (0.6%) of RGM (not identified to species) and a single *M. mucogenicum* were also recovered in broth medium only. The recovery of MAC by RGM medium was surprisingly low in this study when compared with at least one large previous study [13] which emphasizes the importance of performing studies in different geographical regions using a range of comparator methods.

Overall, contamination (bacterial, fungal and/or yeasts) of all types of culture set ups was present in 116/297 (39%) of sputum cultures. Fifty-six of 297 (19%) specimens that were plated on Middlebrook 7H11 and Mitchison agar were contaminated compared to only 2/297 (0.6%) with direct plating of sputum on RGM medium. Thirty-seven percent (110/297) of the total cultures in broth medium were also contaminated. Of the 116 cultures with contamination, 48% and 95% on routine agar media and broth cultures respectively were contaminated compared to only 2% contamination from the unprocessed samples cultured on RGM medium ($p < 0.05$, Table 1).

4. Discussion

Traditional methods for *M. tuberculosis* from sputum culture have hampered the recovery of NTM species and made the clinical diagnosis of NTM disease more difficult. The RGM medium significantly enhances the recovery of *M. abscessus* complex while streamlining the culture process as there is no need for any kind of specimen processing, thus reducing

Table 1
Numbers of organisms recovered and rate of contamination from 297[a] respiratory samples cultured by different methods.

Species	Total isolates	7H11/Mitchison selective agar		RGM medium		Broth medium		p
		Isolates (n)	% isolated	Isolates (n)	% isolated	Isolates (n)	% isolated	
RGM ($n = 149$)								
M. abscessus complex	129	85	66	121	94	79	61	<0.05
M. chelonae	1	0	0	1	100	0	0	NA
M. mucogenicum	1	0	0	0	0	1	100	NA
RGM (NOS)[b]	18	7	39	17	94	1	6	NA
SGM ($n = 32$)								
M. avium complex	28	17	61	5	18	19	68	<0.05
M. gordonae	3	0	0	0	0	3	100	NA
M. paraffinicum	1	1	100	0	0	1	100	NA
Total NTM	**181**							
Contaminated samples	116	56	48	2	2	110	95	<0.05

p values calculated by Chi-Square using GraphPad. Values < 0.05 were statistically significant.
Abbreviations: NA = not applicable.
[a] There were 102 cultures with no growth.
[b] NOS = not speciated but excluded as *M. abscessus* complex. RGM: Rapidly-growing mycobacteria. SGM: Slowly-growing mycobacteria.

labor time and costs.

The currently recommended CLSI mycobacterial processing and culture method involves the use of N-acetyl-L-cysteine-NaOH decontamination and culture on both solid and liquid media for a minimum of 6 weeks [8]. As previously mentioned, the decontamination process often has substantial deleterious effects on the viability and recovery of NTM, especially for *M. abscessus* complex [8]. Not only can this prevent, or delay treatment strategies, but it may also under-estimate the prevalence and clinical significance of RGM in patients with chronic lung disease [5].

This relatively simple implementation of the RGM medium into the mycobacterial laboratory work flow may not only increase the yield of *M. abscessus* complex in cultures with previous low numbers of *M. abscessus* complex but may also identify *M. abscessus* complex in patients that are not identified by routine AFB culture alone. For all cultures, the overall dramatic reduction of contamination in this study (only 2% on the RGM medium) compared to approximately 48% contamination on routine agar media and 95% in broth culture greatly influenced the recovery of *M. abscessus* complex from otherwise uninterpretable cultures. The relatively high contamination rate observed in this study is partly attributable to the fact that 58% of samples (173/297) were from patients who had previously submitted samples that had led to contamination during routine culture for AFB. Although the number of patients and cultures in the current studies is less than some previous studies, our number of isolates of *M. abscessus* complex was higher than any study we are aware of to date [5, 6, 7, 12, 13].

There are major significant advantages to the use of RGM medium as noted above. However, our study is not without limitations. Our study population was small and some of the patients were well-known to our institution, thus there was a high level of suspicion of significant NTM, including *M. abscessus* complex and the *M. avium* complex in the majority of the AFB cultures.

Another limitation of our study was the lack of comparison of growth at 30 °C for the routine cultures as this is not the typical procedure for recovery of RGM from respiratory cultures in our laboratory, which cultures several thousand isolates of *M. abscessus* complex annually. However, incubation of routine cultures at 30 °C would be unlikely to reduce the contamination rate.

Additionally, we did not compare the growth of isolates on the RGM medium at 35 °C. The lack of incubation of the RGM medium at 35 °C may account for the recovery of reduced numbers of SGM (e.g., MAC). Although this may also be due to the fact that an un-concentrated sample was used to inoculate RGM medium.

Although we were not able to prove enhanced recovery of NTM other than the *M. abscessus* complex on RGM medium, the major aims of our study, including the increased recovery of the *M. abscessus* complex and the reduction of contamination of cultures, were achieved. When *M. absccesus* was recovered on both RGM and conventional agars, the time taken for colonies to develop was comparable (data not shown). The use of RGM medium does not obviate the necessity for conventional AFB cultures in order to detect other mycobacterial species including *M. avium* complex.

In conclusion, our study corroborates previous studies in patients with chronic pulmonary (bronchiectatic) disease where RGM medium shows potential for transitioning the current AFB sputum culture processing scheme into a less tedious, less time-consuming and more effective work flow with the expectation of enhanced recovery of NTM, especially the *M. abscessus* complex [4, 5, 6, 7, 11, 12].

Declarations

Author contribution statement

Barbara A. Brown-Elliott, Richard J. Wallace Jr.: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

John D. Perry: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Susan Molina, Travis Fly, Ousman Nije, Patricia Stribley: Performed the experiments.

Dominic Stephenson: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare the following conflict of interests: John Perry; the RGM media is a proprietary product of bioMérieux, France. It was kindly provided by John Perry at the Newcastle Upon Tyne Hospital Microbiology Department, Freeman Hospital, United Kingdom who receives funding from bioMérieux for the development and evaluation of diagnostic products including culture media.

Additional information

No additional information is available for this paper.

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References

[1] J.E. Stout, W.-J. Koh, W.W. Yew, Update on pulmonary disease due to non-tuberculous mycobacteria, Int. J. Infect. Dis. 45 (2016) 123–134.

[2] F.-C. Bange, E.C. Böttger, Improved decontamination method for recovering mycobacteria from patients with cystic fibrosis, Eur. J. Clin. Microbiol. Infect. Dis. 21 (2002) 546–548.

[3] P.C.A.M. Buijtels, P. Petit, Comparison of NaOH-N-acetyl cysteine and sulfuric acid decontamination methods for recovery of mycobacteria from clinical specimens, J. Microbiol. Methods 62 (2005) 83–88.

[4] C.R. Esther, S. Hoberman, J. Fine, S. Allen, K. Culbreath, K. Rodino, A. Kerr, P. Gilligan, Detection of rapidly growing mycobacteria in routine cultures of samples from patients with cystic fibrosis, J. Clin. Microbiol. 49 (2011) 1421–1425.

[5] C.L. Preece, A. Perry, B. Gray, D.T. Kenna, A.L. Jones, S.P. Cummings, A. Robb, M.F. Thomas, M. Brodlie, C.J. O'Brien, S.J. Bourke, J.D. Perry, A novel culture medium for isolation of rapidly-growing mycobacteria from the sputum of patients with cystic fibrosis, J. Cyst. Fibros. 15 (2016) 186–191.

[6] I. Eltringham, J. Pickering, H. Gough, C.L. Preece, J.D. Perry, Comparison of mycobacterial growth indicator tube with culture on RGM selective agar for detection of mycobacteria in sputum samples from patients with cystic fibrosis, J. Clin. Microbiol. 54 (2016) 2047–2050.

[7] R. Plongla, C.L. Preece, J.D. Perry, P.H. Gilligan, An evaluation of RGM medium for isolation of nontuberculous mycobacteria from respiratory samples from patients with cystic fibrosis in the United States, J. Clin. Microbiol. 55 (2017) 1469–1477.

[8] Clinical and Laboratory Standards Institute, Laboratory Detection and Identification of Mycobacteria. CLSI Guideline M48. CLSI Document M48, 2018.

[9] C.L. Preece, T.A. Wichelhaus, A. Perry, A.L. Jones, S.P. Cummings, J.D. Perry, M. Hogardt, Evaluation of various culture media for detection of rapidly growing mycobacteria from patients with cystic fibrosis, J. Clin. Microbiol. 54 (2016) 1797–1803.

[10] T. Adékambi, P. Colson, M. Drancourt, *rpo B*-based identification of nonpigmented and late pigmented rapidly growing mycobacteria, J. Clin. Microbiol. 41 (2003) 5699–5708.

[11] U. Edwards, T. Rogall, H. Blöcker, E. M, E.C. Böttger, Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA, Nucleic Acids Res. 17 (1989) 7843–7853.

[12] S. Rotcheewaphan, O.E. Odusanya, C.M. Henderson, D. Stephenson, K.N. O, J.D. Perry, A.M. Zelazny, Performance of RGM medium for the isolation of nontuberculous mycobacteria from respiratory specimens from non-cystic fibrosis patients, J. Clin. Microbiol. 57 (2019) e01519-18.

[13] D. Stephenson, A. Perry, M.R. Appleby, D. Lee, J. Davison, A. Johnston, A.L. Jones, A. Nelson, S.J. Bourke, M.F. Thomas, A. De Soyza, J.L. Lordan, J. Lumb, A.E. Robb, J.R. Samuel, K.E. Walton, J.D. Perry, An evaluation of methods for the isolation of nontuberculous mycobacteria from patients with cystic fibrosis, bronchiectasis and patients assessed for lung transplantation, BMC Pulm. Med. 19 (2019) 1–12.