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Comparison of three methods for susceptibility testing of *Mycobacterium avium* subsp. *paratuberculosis* to 11 antimicrobial drugs

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***Objectives*: To evaluate the BACTEC™ MGIT™ 960/MGIT Para TB (MGIT) system for drug susceptibility testing of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), a pathogen implicated in some forms of Crohn's disease.**

***Methods*: MICs of 11 drugs for 10 MAP strains were determined using the MGIT system, the BACTEC™460TB system (BACTEC) and conventional agar dilution methods.**

***Results*: MICs determined by MGIT methods showed 80%–100% agreement (± 1 $\log_2$ dilution) with those determined by the BACTEC and agar dilution methods for ciprofloxacin, levofloxacin, azithromycin and clofazimine. The MGIT and BACTEC methods showed 70%, 80% and 90% agreement (± 1 $\log_2$ dilution) for MICs of ethambutol, rifabutin and rifampicin; agreement for all drugs increased to 100% at 2 $\log_2$ dilution differences. For clarithromycin, the MGIT method had greater agreement with the agar dilution method (70% at the same dilution) than the BACTEC method (60% at ± 1 $\log_2$ dilution); agreement increased to 100% at ± 2 $\log_2$ dilutions in both cases. The MGIT and agar dilution methods agreed 60% and 100% for amikacin MICs at ± 1 $\log_2$ dilution and ± 2 $\log_2$ dilutions, respectively. By all methods MICs were higher than achievable serum concentrations for isoniazid and dapsone. There was 100% agreement between all three methods for azithromycin, clarithromycin and ciprofloxacin, and 80% agreement for rifampicin using published MIC thresholds available for *M. avium* complex strains.**

***Conclusions*: This study shows that the MGIT system can be used for rapid and reliable drug susceptibility testing of MAP.**

Keywords: agar dilution, BACTEC, MGIT, MIC

Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease (paratuberculosis), a chronic inflammatory bowel disease in ruminants. In recent years there have been several reports suggesting an association between MAP and Crohn's disease (CD), a chronic granulomatous inflammatory bowel disease in humans.[1–5]

The idea that MAP may contribute to some cases of CD is not new. Previous attempts (1982–94) to test this theory involved the treatment of CD patients with anti-tubercular drugs assumed to be efficacious against MAP.[6] Failure to observe significant clinical improvement dampened enthusiasm for the mycobacterial aetiology theory. Only later was it recognized that MAP is resistant to most first-line tuberculosis (TB) drugs.[7]

More recent clinical trials have treated CD patients with combinations of macrolides, rifabutin and clofazimine based on limited MAP antibiotic susceptibility data and on the assumption that MAP should be similar in susceptibility to other members of the *M. avium* complex (MAC).[8–14] Clinical outcomes of these studies were generally encouraging, but far from conclusive.[15,16]

In vitro drug susceptibility data specific for human clinical isolates of MAP are needed to better guide clinical trials targeting MAP in CD patients. However, at present there are no standard methods available for drug susceptibility testing of MAP. The few such studies available used a radiometric [BACTEC™ 460 TB (BACTEC); Becton Dickinson, Sparks, MD, USA] broth dilution method,[17–20] which is an accurate and reliable macrodilution method for susceptibility testing of MAC.[21]

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In clinical laboratories the radiometric system has largely been replaced with the new fully automated BACTEC™ MGIT (mycobacterial growth indicator tube) 960 system (MGIT) (Becton Dickinson) for the isolation[22,23] and susceptibility testing of pathogenic mycobacteria.[24,25] The MGIT system was optimized for detection of MAP with a MAP-specific culture medium, MGIT™ ParaTB medium, and has been successfully used for faster and sensitive isolation of MAP from clinical samples and also for quantification of MAP.[26] We previously described drug stability in the MGIT™ ParaTB medium[27] and *in vitro* susceptibility of MAP to thiopurine drugs.[28] In the present study, the MGIT 960 system–MGIT™ ParaTB medium was evaluated for susceptibility testing of MAP strains to different classes of drugs and the results were compared with those of radiometric (BACTEC) and agar dilution methods.

Materials and methods

MAP isolates

Ten clinical isolates of MAP (nine of human origin and one of bovine origin) were used (Table 1). The strains were verified as MAP by detecting IS*900* by PCR. All strains were grown in Middlebrook 7H9 broth containing 10% oleic acid–albumin–dextrose–catalase (OADC) supplement (Becton Dickinson) and 2 μg/mL mycobactin J (Allied Monitor, Fayette, MO, USA). Cultures (6–8 weeks old) were harvested, resuspended in fresh Middlebrook 7H9 medium–glycerol (20% final concentration) and transferred to screw-capped test tubes containing 8–10 glass beads (3 mm). Tubes were vigorously vortexed, and allowed to stand for 30 min. The supernatant was stored as aliquots at −80°C until used.

M. avium controls

Strains 101 and 104 were gifts from Dr Venkata Reddy (Sequella Inc., Rockville, MD, USA). Colonies from Middlebrook 7H10 agar plates were inoculated into Middlebrook 7H9 broth containing 10% OADC.

Preparation of inocula

Prior to susceptibility testing, the frozen MAP were inoculated into 4 mL of fresh Middlebrook 7H9 medium and incubated at 37°C for 2–3 days. The turbidity of the culture was adjusted with Middlebrook 7H9 broth to be equivalent to that of a No. 1 McFarland standard (for agar dilution method) or a 0.5 McFarland standard (for MGIT and radiometric methods) using a spectrophotometer (Biomate 3; Thermo Fisher Scientific, Waltham, MA, USA). Cultures (8–10 days old) of *M. avium* 101 and 104 strains were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard.

Drugs

Ciprofloxacin, levofloxacin, azithromycin, clarithromycin, amikacin, ethambutol, clofazimine, isoniazid and dapsone were purchased from Sigma-Aldrich (St Louis, MO, USA). Rifampicin and rifabutin were purchased from USP (Rockville, MD, USA).

Stock solutions of drugs were prepared using the most appropriate solvent: water (amikacin, ethambutol and isoniazid), 0.1 N sodium hydroxide (ciprofloxacin and levofloxacin), methanol (rifampicin and rifabutin), ethanol (azithromycin and clarithromycin) or methanol acidified by trace amounts of glacial acetic acid (clofazimine). The stock solutions were filter-sterilized, if required, and stored at −80°C for up to 2 months.

Prior to testing, each drug was freshly diluted in sterile deionized water. Each drug was tested at a suitable concentration range based on reported C_{max} values. Broadly, ciprofloxacin, levofloxacin, azithromycin, clarithromycin, rifabutin and isoniazid were tested at doubling dilutions in the range 32–0.125 mg/L. Amikacin was tested in the range 25–0.78 mg/L. Rifampicin was tested in the range 12–0.094 mg/L. Ethambutol, clofazimine and dapsone were tested in the range 20–0.156 mg/L.

Susceptibility testing by the MGIT method

The method was adapted from those for *Mycobacterium tuberculosis* and *M. avium* and described earlier for MAP.[28] Briefly, the final MGIT™ ParaTB medium was prepared according to manufacturer's instructions by adding 0.8 mL MGIT™ ParaTB supplement and 0.5 mL 50% egg yolk (Becton Dickinson) to 7 mL of MGIT™ ParaTB medium. Clofazimine and amikacin were tested in the absence of egg yolk since the MICs were found to be affected by egg yolk. Each tube received 0.1 mL of inoculum (bacterial suspension with a turbidity equivalent to that of a 0.5 McFarland standard) and 0.1 mL of drug. This inoculum resulted in cfu between 5×10^4 and 5×10^5/mL, as shown by plate counts. Growth control tubes

Table 1. MAP strains used in the study

Strain ID	Isolation source
ATCC 43015 (Linda)	human, Crohn's disease patient—ileum
ATCC 43544 (Ben)	human, Crohn's disease patient—intestinal tissue
ATCC 43545 (Dominic)	human, Crohn's disease patient—intestinal tissue
ATCC 49164 (Holland)	human, Crohn's disease patient—intestinal tissue
UCF3	human, Crohn's disease patient—ileum, UCF[a]
UCF4	human, Crohn's disease patient—ileum, UCF[a]
UCF5	human, Crohn's disease patient—ileum, UCF[a]
UCF7	human, Crohn's disease patient—ileum, UCF[a]
UCF8	human, Crohn's disease patient—ileum, UCF[a]
JTC 303	bovine, clinical case of paratuberculosis, JTC[b]

[a]Gift from Saleh Naser, University of Central Florida, Orlando, FL, USA.
[b]Isolated at the Johne's Testing Centre, Madison, WI, USA.

received 0.1 mL of sterile water instead of drug. The 1:100 growth control tubes received 1/100 of the standard inoculum. Tubes were incubated in the MGIT™ 960 instrument (Becton Dickinson) and time to detect (TTD) data were recorded. An experiment was considered valid only when the undiluted growth control tube became signal-positive between 2.5 and 4.5 days of incubation and the 1:100 inoculum control tube signalled positive between 7 and 10 days. MIC was defined as the lowest concentration of drug resulting in a TTD value greater than that of the 1:100 growth controls.

Susceptibility testing by the BACTEC method

The BACTEC™ 12B medium (Becton Dickinson) was modified to permit MAP growth, by adding 1 mL of 50% egg yolk suspension (Becton Dickinson) and 0.1 mL of mycobactin J (80 μg/mL) to each vial. Egg yolk was omitted for testing clofazimine. The medium was alkalized while testing azithromycin by adding 0.3 mL of 3% tri-potassium phosphate.[29] Drug dilutions were added in volumes of 0.1 mL. Drug-containing and growth control vials received 0.1 mL of MAP suspension equivalent in turbidity to that of a 0.5 McFarland standard, as recommended by the manufacturer for susceptibility testing of *M. tuberculosis*. The 1:100 growth control vials received 0.1 mL of a 100-fold dilution of the same MAP suspension. All vials were incubated at 37°C. Growth index (GI) readings were obtained using a BACTEC™ 460 TB instrument (Becton Dickinson). The 1:100 control vials were read on alternate days until the GI was ≥30 (considered positive MAP growth above background[30]) at which time all drug-containing vials were read that same day and again the following day. Differences in GI between consecutive days (ΔGI) were calculated for each culture. The MIC was defined as the lowest concentration of drug showing both a ΔGI and absolute GI lower than those of the 1:100 growth control.[31]

Susceptibility testing by the agar dilution method

Methods recommended by the CLSI (formerly the NCCLS) for *M. tuberculosis* and slow-growing mycobacteria were used with minor modifications.[29] Drug dilutions were placed in volumes of 0.1 mL at the centre of quadrant Petri plates and overlaid by molten, cooled, Middlebrook 7H10 agar (supplemented with OADC and mycobactin J). Plates were swirled gently to allow mixing of drug with the medium before solidification of agar. An inoculum suspension equivalent in turbidity to that of a No. 1 McFarland standard was diluted to 10^{-2} and 10^{-4}. Drug-containing and control quadrants were inoculated with 0.1 mL of the 10^{-2} dilution while the 1:100 control (1% control) quadrants received 0.1 mL of the 10^{-4} dilution. Plates were sealed and incubated at 37°C for 4–5 weeks.

Data analysis

Graph Pad Prism version 5 (GraphPad software Inc, San Diego, CA, USA) was used for non-parametric correlation (Spearman) analysis. $P < 0.05$ was interpreted as indicating significant correlation.

Results

Table 2 summarizes the agreement between MGIT and the other two methods in determining MICs of each of the drugs, except isoniazid and dapsone, for the 10 MAP isolates. Isoniazid and dapsone MICs by all methods were greater than the highest concentration tested. MICs determined by MGIT and BACTEC methods showed 80–100% agreement at ±1 $\log_2$ dilution (i.e. one doubling dilution) for ciprofloxacin, levofloxacin, azithromycin, rifampicin, rifabutin and clofazimine. The MICs of azithromycin in un-alkalized (pH 6.7) BACTEC™ 12B radiometric medium were >2-fold higher than the MICs determined in MGIT™ ParaTB medium (data not shown). MICs of azithromycin in MGIT™ ParaTB medium (pH 6.8) correlated better with those in alkalized (pH 7.3) BACTEC™ 12B medium. Clarithromycin was tested at pH 6.8 in both broth media and showed 60% agreement at ±1 $\log_2$ dilution and 100% agreement at ±2 $\log_2$ dilutions. However, for this drug,

Table 2. Agreement in MIC determinations by MGIT in comparison with BACTEC and agar dilution methods for nine antimicrobials against 10 MAP strains

	Percentage agreement in MIC determination between methods					
	MGIT and BACTEC			MGIT and agar dilution		
Drug	same dilution	±1 $\log_2$ dilution	±2 $\log_2$ dilutions	same dilution	±1 $\log_2$ dilution	±2 $\log_2$ dilutions
CIP	70	100	—	70	90	100
LVX	60	100	—	40	90	100
AZM[a]	90	100	—	40	100	—
CLR[b]	20	60	100	70	90	100
AMK[c]	ND	ND	ND	10	60	100
RIF	60	90	100	10	60	80
RFB	20	80	100	30	70	100
EMB	40	70	100	0	50	90
CLF[c]	30	90	100	10	90	100

CIP, ciprofloxacin; LVX, levofloxacin; AZM, azithromycin; CLR, clarithromycin; AMK, amikacin; RIF, rifampicin; RFB, rifabutin; EMB, ethambutol; CLF, clofazimine; ND, not determined.
[a]MICs at pH 6.8 in MGIT™ ParaTB medium and pH 7.3 in BACTEC™ 12B medium.
[b]MICs at pH 6.8.
[c]MICs in the absence of egg yolk.

there was better agreement between MGIT and agar dilution (70%, 90% and 100% at the same, ± 1 and ± 2 $\log_2$ dilutions, respectively). MICs of ethambutol showed 70% and 100% agreement between MGIT and BACTEC methods at ± 1 and ± 2 $\log_2$ dilutions, respectively. The MICs of ethambutol were higher by the BACTEC method for most strains. Non-parametric correlation analysis of MICs obtained by MGIT and BACTEC methods showed significant correlation ($P < 0.05$) for all 10 drugs compared (between 80% and 98% for 9 drugs and 73% for ethambutol).

Amikacin MICs determined in MGIT medium without egg yolk in comparison with those found by the agar dilution method showed 60% agreement at ± 1 $\log_2$ dilution and 100% agreement at ± 2 $\log_2$ dilutions; generally, slightly higher MICs were obtained for most strains by the agar dilution method. MICs found by MGIT and agar dilution methods showed 90%–100% agreement at ± 1 $\log_2$ dilution for ciprofloxacin, levofloxacin, azithromycin, clarithromycin and clofazimine. MICs of rifampicin, ethambutol and rifabutin showed 80%, 90% and 100% agreement between the MGIT and agar dilution methods at ± 2 $\log_2$ dilutions. The agar dilution method yielded higher MICs of ethambutol, compared with both broth culture methods. The MGIT and agar dilution methods showed significant MIC correlation for all drugs (64%–93%), except ethambutol (52%).

MIC range, MIC_{50} and MIC_{90} values determined by the MGIT method were compared with those by the BACTEC and agar dilution methods (Table 3). MIC ranges of ciprofloxacin, levofloxacin, azithromycin, clarithromycin, rifabutin, clofazimine (by all three methods) and amikacin (tested by two methods only) were either the same or differed by only one doubling dilution. The presence of egg yolk in broth caused >4-fold higher MICs of clofazimine and amikacin in broth methods compared with the agar method (data not shown). All strains tested were resistant to isoniazid and dapsone at the highest concentrations tested (higher than the respective C_{max} values) by all three methods. The upper limits of the MIC range of ethambutol and rifampicin were >2 $\log_2$ dilutions higher by agar dilution. There was good agreement between methods with respect to MIC_{50} and MIC_{90} values (minimum concentration of a drug that inhibited at least 50% and 90%, respectively, of the strains tested) for all drugs except ethambutol. The MIC_{90} of ethambutol was four times higher by the agar dilution as compared with the MGIT method. MGIT-derived MICs of all the drugs for *M. avium* strains 101 and 104 are shown in Table 4.

Table 3. Summary of MICs of 11 drugs for 10 MAP strains determined by three methods

				MIC_{50} and MIC_{90} by three methods					
	MIC (mg/L) range			MGIT		BACTEC		agar dilution	
Drug	MGIT	BACTEC	agar dilution	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
CIP	0.25–8	0.25–4	0.25–8	0.5	8	0.5	4	0.5	8
LVX	0.25–16	0.5–16	0.25–32	1	16	1	8	1	32
AZM[a]	0.5–8	0.5–8	0.5–8	2	4	2	4	1	8
CLR[b]	0.13–1	0.25–4	0.13–1	0.13	1	0.5	1	0.13	1
AMK[c]	0.78–6.25	ND	1.56–12.5	1.56	3.13	ND	ND	3.13	6.25
RIF	0.09–3	0.19–3	0.19–12	0.37	3	0.37	3	0.75	6
RFB	0.13–2	0.25–2	0.13–4	0.25	1	0.5	2	0.13	1
EMB	1.25–5	1.25–20	5–20	5	5	5	10	10	20
CLF[c]	0.16–1.25	0.16–1.25	0.16–0.63	0.62	1.25	0.16	0.63	0.16	0.63
DPS	>5	>5	>5						
INH	>8	>8	>8						

CIP, ciprofloxacin; LVX, levofloxacin; AZM, azithromycin; CLR, clarithromycin; AMK, amikacin; RIF, rifampicin; RFB, rifabutin; EMB, ethambutol; CLF, clofazimine; DPS, dapsone; INH, isoniazid; ND, not determined.
[a]MICs at pH 6.8 in MGIT™ ParaTB medium and pH 7.3 in BACTEC™ 12B medium.
[b]MICs at pH 6.8.
[c]MICs in the absence of egg yolk.

Table 4. MICs of various drugs for *M. avium* strains 101 and 104, as determined by the MGIT method

	MIC (mg/L)										
Strain	CIP	LVX	AZM	CLR	AMK	RIF	RFB	EMB	CLF	DPS	INH
MAC101	≤1	1	2	≤0.5	1.56	≤0.375	≤0.125	5	0.625	>5	2
MAC104	≤1	1	8	≤0.5	6.25	≤0.375	≤0.125	5	1.25	>5	2

CIP, ciprofloxacin; LVX, levofloxacin; AZM, azithromycin; CLR, clarithromycin; AMK, amikacin; RIF, rifampicin; RFB, rifabutin; EMB, ethambutol; CLF, clofazimine; DPS, dapsone; INH, isoniazid.

Table 5. Tentative classification[a] of MAP strains by the MGIT drug susceptibility method and comparison with that of the BACTEC and agar dilution methods

	MGIT and BACTEC methods							MGIT and agar dilution methods						
Drug	both S	both I	both R	S and I	R and I	S and R	agreement (%)	both S	both I	both R	S and I	R and I	S and R	agreement (%)
AZM[b]	10						100	10						100
CLR[b]	10						100	10						100
CIP[c]	7		3				100	7		3				100
AMK[c]				not compared				5			4	1		50
RIF[c]	5	3		2			80	5	2		2	1		70
RFB[c]		5	1	2	2		60	2	2		4	2		40
EMB[c]	2	3		1	4		50				1	7	2	0
CLF[c]	1		3	3	2	1	40	1		3	3	2	1	40

S, susceptible; I, intermediate; R, resistant; AZM, azithromycin; CLR, clarithromycin; CIP, ciprofloxacin; AMK, amikacin; RIF, rifampicin; RFB, rifabutin; EMB, ethambutol; CLF, clofazimine.
[a]Based on interpretive criteria for MAC recommended by: [b]the CLSI;[29] and [c]Heifets.[32]

Currently there are no criteria for drug susceptibility interpretation specific for MAP. However, for MAC organisms, the CLSI defines clinically significant resistance to two macrolides, azithromycin and clarithromycin, as MICs >256 mg/L and >32 mg/L, respectively, using the BACTEC™ 12B medium at pH 6.8.[29] For other drugs that are active against MAC, there are thresholds for MIC interpretation determined in 7H12 broth.[32] To evaluate the ability of the MGIT method to designate MAP strain drug susceptibility, the 10 MAP strains tested were classified as susceptible, intermediate or resistant to eight drugs based on the two aforementioned criteria. Drug susceptibility classification by the MGIT method showed 70%–100% agreement with the BACTEC and agar dilution methods for ciprofloxacin, azithromycin, clarithromycin and rifampicin (Table 5). Except for clofazimine and ethambutol, disagreement between the MGIT and the other two methods was observed whenever the other methods classified a strain as susceptible versus intermediate or resistant versus intermediate. For ethambutol, two strains, UCF3 and UCF8, were classified as susceptible by the MGIT method but resistant by agar dilution. One strain (Linda) was classified as resistant to clofazimine by the MGIT method but susceptible by the other two methods. There are no cut-offs proposed for three drugs tested in this study against MAC: levofloxacin, isoniazid and dapsone. For three MAP strains, the MICs of levofloxacin were at or above its C_{max},[33] while for all 10 strains the MICs were higher than the respective C_{max} for isoniazid and dapsone[34,35] by all three methods. Based on the same criteria, the MGIT-derived MICs classified the two MAC strains 101 and 104 as susceptible to ciprofloxacin, azithromycin, clarithromycin, rifampicin and rifabutin. Strain 101 was found susceptible to amikacin, while strain 104 showed intermediate amikacin susceptibility. Both strains showed intermediate susceptibility to ethambutol, but resistance to clofazimine and dapsone.

For MAP, the time required to determine MICs by the MGIT method was 7–10 days, while the BACTEC method required 10–12 days, depending upon the MAP strain being tested. However, azithromycin testing in BACTEC™ 12B medium at pH 7.2 required slightly longer (12–14 days), due to a slower MAP growth rate at the more alkaline pH. MIC determinations for MAP by the agar dilution method required 4–5 weeks.

Discussion

The fully automated, non-radiometric MGIT 960 system in conjunction with the MGIT™ ParaTB medium allows rapid and sensitive recovery of MAP from clinical specimens. The present study shows that the system can also be reliably used for drug susceptibility testing of MAP strains. MICs of all drugs compared between MGIT and BACTEC methods showed 60%–100% agreement at single doubling dilution or 100% agreement at two doubling dilution differences. MICs by MGIT and agar dilution methods agreed 60%–100% at single doubling dilution or 100% at two doubling dilution differences for all drugs except rifampicin and ethambutol. The higher MICs of rifampicin determined by agar dilution could be due to decreased stability of the drug in agar and the extended incubation time required to obtain results, as reported in other studies.[36] The MICs of ethambutol in Middlebrook 7H12 broth are known to be lower than in 7H11 agar plates and the difference is more pronounced for *M. avium* than *M. tuberculosis*.[37] The present study shows that the MIC_{90} of ethambutol for MAP is 2-fold higher by the BACTEC and 4-fold higher by the agar dilution method. However, Piersimoni *et al.*[38] showed 2-fold lower MICs of ethambutol for MAC in BACTEC™ 12B medium compared with MGIT. This discrepancy could be due to differences in media composition as the MGIT™ ParaTB medium is specifically enriched for growth of MAP. Moreover, observations with clofazimine and amikacin indicated that egg yolk in broth media results in $\geq$4-fold higher MICs of these drugs for MAP (data not shown). Decreased activity of clofazimine in the presence of egg yolk in the medium was observed in a prior drug stability study as well.[27] However, a similar effect of egg yolk on amikacin was not observed in that study, probably due to the test strain (*Escherichia coli*) used for amikacin.

Since the few published studies on drug susceptibility of MAP report data on a limited number of strains, few drugs and a

variety of methods, comparison of studies is difficult. However, results for ciprofloxacin, ethambutol, rifampicin and dapsone in the present study are comparable to those of Zanetti *et al.*,[39] who studied 12 MAP strains, of both human and animal origin, using the MGIT system. The discrepancies for clarithromycin and rifabutin (lower MICs found in the present study) could be due to changes in the broth medium employed as well as differences in the strains used. MICs of clarithromycin obtained in the present study correlate well with those of a study by Rastogi *et al.*[20] using the BACTEC method and five MAP isolates; minimal correlation for fluoroquinolones and rifampicin MICs was seen between the two studies.

A provisional antibiotic response classification of the 10 MAP strains as susceptible, intermediate or resistant to some of the drugs based on published interpretative criteria available for MAC showed 100% (macrolides and ciprofloxacin) and 80% (rifampicin) agreement between MGIT and the other two methods. For other drugs, the lesser agreement (40%–70%) observed between MGIT and other methods results mostly from classification of strains in the 'intermediate' category. Major discordance was observed for clofazimine against one strain, which was classified resistant by MGIT but susceptible by the other two methods. This discrepancy was probably due to the closer (one doubling dilution difference) MIC cut-offs used for clofazimine. It should be noted that clofazimine MICs determined by MGIT showed 90% agreement with those found by both the BACTEC and agar dilution methods at $\pm 1 \log_2$ dilution and 100% agreement at $\pm 2 \log_2$ dilutions. In comparison with reported susceptibility/resistance profiles for *M. avium*,[32] MAP are more resistant than *M. avium* to isoniazid, but more susceptible to amikacin and ciprofloxacin. Similar to *M. avium*, all MAP isolates tested were found susceptible to the macrolides azithromycin and clarithromycin. In conclusion, the MGIT 960™ system with MGIT™ ParaTB medium can be used for rapid and reliable drug susceptibility testing of MAP.

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Transparency declarations

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