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Multicenter Laboratory Validation of the BACTEC MGIT 960 Technique for Testing Susceptibilities of *Mycobacterium tuberculosis* to Classical Second-Line Drugs and Newer Antimicrobials

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The BACTEC MGIT 960 system, a fully automated, nonradiometric, noninvasive system for detection and drug susceptibility testing of mycobacteria, was evaluated for the ability to test susceptibilities to second-line drugs. In this study, which was carried out in three phases (phase I, mostly susceptible strains; phase II, mostly resistant strains; phase III, final testing of the optimal drug concentrations found in phases I and II), we established the critical concentrations for seven drugs to be tested in the BACTEC MGIT 960 system compared to the BACTEC 460TB system. The critical concentrations for the seven drugs used in the MGIT 960 system are as follows: amikacin, 1.0 μg/ml; capreomycin, 2.5 μg/ml; ethionamide, 5.0 μg/ml; protionamide, 2.5 μg/ml; ofloxacin, 2.0 μg/ml; rifabutin, 0.5 μg/ml; linezolid, 1.0 μg/ml. Our results demonstrate that the BACTEC MGIT 960 system is an accurate method for rapid testing of the susceptibilities of *Mycobacterium tuberculosis* to second-line drugs.

Drug susceptibility testing (DST) for both primary and secondary antituberculosis drugs with the broth-based radiometric BACTEC 460 TB system (Becton Dickinson Diagnostic Systems, Sparks, MD) is well established and is considered the "gold standard" (15). However, due to increasing concern about the use and disposal of radioactive material, there is a rapid trend toward using commercially available nonradiometric broth-based culture and susceptibility testing methods. BACTEC MGIT 960 (Becton Dickinson Diagnostic Systems) is a new nonradiometric system which is considered equivalent to the BACTEC 460 in performance. Recovery of mycobacteria from clinical specimens as well as DST for first-line drugs has been thoroughly studied for the MGIT 960 system (3, 4, 5, 7, 8, 10, 11, 12). However, no thorough multicenter study has been carried out establishing DST for second-line and newer drugs currently being used in the treatment of tuberculosis. According to the WHO reports, global drug resistance is an increasing concern (18). Some countries are reporting high resistance even against second-line drugs (1, 17). Therefore, it is important that nonradiometric broth-based systems should also offer DST procedures for drugs other than those considered first-line.

The primary aim of this multicenter study was to develop a basic protocol, establish critical test concentrations for seven second-line and newer drugs, including a few that have been introduced recently, and then test a large number of clinical isolates. For comparison, BACTEC 460 was used as the gold standard, since critical test concentrations of most of the drugs have already been established for this system (9). It is anticipated that this study will provide a guideline for rapid broth-based susceptibility testing not only of the drugs that have been included here but also of other drugs that are used in the treatment of tuberculosis or will be introduced in the near future.

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MATERIALS AND METHODS

Study sites. This study was carried out at three sites: (i) the National Reference Center for Mycobacteria, Forschungszentrum Borstel, Borstel, Germany (which acted as the principal investigator [PI]), (ii) the Mycobacteria Reference Center, Faculty of Medicine, University of Cordoba, Cordoba, Spain, and (iii) the Department of Microbiology, Royal Brompton Hospital, London, United Kingdom. An additional site, the Department of Medical Microbiology, Luzern General Hospital, Lucerne, Switzerland, was the arbiter.

Antimicrobial agents. All test drugs—amikacin (AK), capreomycin (CM), ethionamide (ETH), protionamide (PTH), ofloxacin (OFX), rifabutin (RBT), and linezolid (LIN)—were obtained in a chemically pure form (all from Sigma, Taufkirchen, Germany, except for RBT [Grunenthal GmbH, Aachen, Germany] and LIN [Pharmacia Corporation, Kalamazoo, MI]) and were distributed to other sites by the principal investigator. LIN, CM, and AK were dissolved in deionized (DI) water, ETH and PTH in ethylene glycol with subsequent dilution in DI water, OFX in 0.1 N NaOH with subsequent dilutions in DI water, and RBT in methanol with subsequent dilutions in DI water. All stock solutions were made at least 40 times more highly concentrated than the highest test concentration used for DST. Except for ETH and RBT, all stock solutions were sterilized using a 0.22-μm polycarbonate filter membrane, and the first 20% of the initial filtrate was discarded. Stock solutions were stored at −70°C in small aliquots. Frozen drug solutions were thawed once and then discarded. Test concentrations used are listed in Table 1.

Test methods and media. The Mycobacteria Growth Indicator Tube (MGIT) with BACTEC MGIT 960 growth supplement for DST was used in the MGIT 960 instrument (Becton Dickinson Diagnostic Systems, Sparks, MD). The standard protocol for DST in MGIT 960 was strictly followed as recommended for primary drugs. Culture suspensions for inoculation had to be well dispersed with no large clumps to avoid false-resistant results. After thorough mixing and homogenization of the culture suspensions, the tubes were allowed to rest for at least 15 min, and the supernatant was used to inoculate the drug-containing media and the control by following the manufacturer's instructions for DST to first-line drugs (3). All inoculated drug-containing MGIT 960 tubes were placed

TABLE 1. Drug concentrations used in MGIT 960

Drug	Drug concns (μg/ml)		
	Phase I	Phase II	Phase III
Amikacin	0.5, 1.0, 2.0	0.5, 1.0, 2.0	1.0, 2.0
Capreomycin	0.625, 1.25, 2.5	0.625, 1.25, 2.5	1.25, 2.5
Ethionamide	0.625, 1.25, 2.5	1.25, 2.5, 5.0	2.5, 5.0, 7.5
Protionamide		0.625, 1.25, 2.5	1.25, 2.5, 5.0
Ofloxacin	1.0, 2.0, 4.0	0.5, 1.0, 2.0	1.0, 2.0
Rifabutin	0.25, 0.5, 1.0	0.25, 0.5, 1.0	0.5, 1.0
Linezolid	0.5, 1.0, 2.0	0.5, 1.0, 2.0	1.0, 2.0

in the DST set carrier and entered into the MGIT 960 instrument as "unknown drugs" using the DST entry feature. For the DST set containing "unknown drugs," the instrument flagged the DST set "complete" when the growth control reached a growth unit (GU) value of 400. At that point, the GU values of drug-containing tubes were retrieved from the instrument by printing out a DST set report, and results were interpreted manually. If the GU of the drug-containing tube was more than 100 when the GU of the growth control was 400, the results were defined as resistant. If the GU values of the drug-containing tubes were equal to or less than 100, the results were considered susceptible. For comparison, 12B medium of BACTEC 460 was used according to the procedures specified in the BACTEC 460 procedure manual (13, 14). All media and reagents were supplied by the manufacturer.

Study design. The study was carried out in three phases. Phase I was designed to establish a basic test procedure and to determine the range of antimicrobial test concentrations. At least three concentrations of each drug were tested. The interlaboratory reproducibility of the test procedures by both methods was also determined. A total of 10 fully susceptible strains of *Mycobacterium tuberculosis* selected by the PI were tested by all three sites. Nine strains were clinical isolates, and the 10th was strain H37Rv (ATCC 27294; quality control [QC] strain). PTH, which is an analogue of ETH, was not tested in phase I.

In phase II, test concentrations were adjusted for some drugs based on the results of phase I. A set of 21 strains, with different degrees of resistance to the test drugs, were shared by all three sites. At least three concentrations of each drug were tested. A subset of these strains was sent to the arbiter site for proficiency testing.

Phase III consisted of the final testing of the optimal drug concentrations established in phase II. Clinical isolates collected at the individual sites were tested in this phase. These strains were not shared by the sites. Test concentrations were kept the same as in phase II, except that the lowest concentration was dropped and one higher concentration was added for ETH and PTH.

Selection and distribution of strains. In phases I and II, the PI site selected strains which originated from clinical specimens. In phase I, susceptible strains, and in phase II, as many resistant strains as possible, were selected and tested by two methods (BACTEC 460 and Löwenstein-Jensen [LJ] [9, 14]) to ensure the resistance profile. Only those strains yielding consistent results by the two methods were included in the study. The selected strains were subcultured on LJ medium. Once growth was obtained, these subcultures were shipped to the other sites. At all test sites, efforts were made to use the original LJ cultures provided by the PI. Subculturing was kept to a minimum. In phase III every site selected its own clinical isolates. Efforts were made by every site to select those clinical isolates that were multidrug resistant and were expected to have high drug resistance.

Quality control and resolution of discrepancy. *M. tuberculosis* H37Rv (ATCC 27294) was used in all three phases. If the QC strain showed a susceptibility result that differed from the expected result, all tests of that batch had to be repeated. In case of discrepant results between the two methods, retesting was done by both methods. Results of the repeat tests were used for the data analysis. If, upon repeat testing, discrepancies remained unresolved, these strains were sent to the arbiter for retesting (twice checked with BACTEC 460 and MGIT 960).

RESULTS

Phase I. The QC strain yielded consistently susceptible results throughout the testing. As shown in Table 2, the 10 strains tested for susceptibility to AK, OFX, and RBT showed high concordance among the three laboratories. All test strains were fully susceptible to AK, with a MIC of 0.5 μg/ml or lower. The MIC of CM was 1.25 μg/ml, while for ETH 2 out of 30 tests indicated resistance in the BACTEC 460 and 4 tests indicated resistance in the MGIT 960, even at 2.5 μg/ml. For LIN, only 1 out of 30 tests showed resistance at 2.0 μg/ml in the MGIT 960, while 77% and 67% of tests showed susceptibility at 0.5 μg/ml of the drug or a lower concentration in the BACTEC 460 and MGIT 960 systems, respectively.

TABLE 2. Drug susceptibility test results of phase I[a]

Drug and concn (μg/ml)	No. of strains for which the indicated concn is the MIC						Total no. (%) of tests	
	Site 1		Site 2		Site 3			
	MGIT	460	MGIT	460	MGIT	460	MGIT	460
Amikacin								
≤0.5	10	10	10	10	10	10	30 (100)	30 (100)
1.0	0	0	0	0	0	0	0	0
2.0	0	0	0	0	0	0	0	0
Capreomycin								
0.625	1	3	9	10	0	10	10 (33.3)	23 (76.7)
1.25	8	7	1	0	10	0	19 (63.3)	7 (23.3)
2.5	1	0	0	0	0	0	1 (3.3)	0
Ethionamide								
≤0.625	3	5	7	8	6	8	16 (53.3)	21 (70.0)
1.25	4	2	0	1	2	1	6 (20.0)	4 (13.3)
2.5	2	3	1	0	1	0	4 (13.3)	3 (10.0)
>2.5	1	0	2	1	1	1	4 (13.3)	2 (6.7)
Ofloxacin								
≤1.0	10	10	10	10	10	10	30 (100)	30 (100)
2.0	0	0	0	0	0	0	0	0
4.0	0	0	0	0	0	0	0	0
Rifabutin								
≤0.25	10	10	10	10	10	10	30 (100)	30 (100)
0.5	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0
Linezolid								
≤0.5	5	5	5	10	10	8[b]	20 (66.7)[b]	23 (76.7)[b]
1.0	5	5	4	0	0	1	9 (30.0)	6 (20.0)
2.0	0	0	1	0	0	0	1 (3.3)	0

[a] Ten strains were tested. MGIT, MGIT 960; 460, BACTEC 460.
[b] Results for one test missing.

Phase II. Phase II results are summarized in Table 3. Test strains were resistant to one or more second-line drugs, and thus results were very much indicative of different degrees of resistance. BACTEC 460 concentrations were kept the same as those established in a previous study except for ETH (9). According to the results achieved for BACTEC 460 in this study, the critical concentration for ETH is 2.5 μg/ml. Overall, the MICs of CM, ETH, and PTH were higher in the MGIT 960 than in the BACTEC 460. Therefore, for these three drugs a cutoff point for the critical concentration was established, which was one concentration higher than that defined for the BACTEC 460. The total tests carried out at the three sites (63 for all sites combined) were analyzed for each drug using the tentatively established breakpoints for critical concentrations. As shown in Table 4, the established critical concentrations yielded the most concordant results between the BACTEC 460 and the MGIT 960. For AK there were 16 resistant results by BACTEC 460 and 18 by MGIT 960, 2 being false resistant by MGIT 960. For CM there were 20 resistant results by BACTEC 460 and 21 by MGIT 960, 1 being false resistant. For ETH 45 strains were resistant by BACTEC 460 and 47 by MGIT 960, 3 being false resistant and 1 being false susceptible by MGIT 960. For PTH 32 strains were resistant by BACTEC

TABLE 3. Drug susceptibility test results of phase II[a]

Drug and concn (μg/ml)	No. of strains for which the indicated concn is the MIC						Total no. (%) of tests	
	Site 1		Site 2		Site 3			
	MGIT	460	MGIT	460	MGIT	460	MGIT	460
Amikacin								
≤0.5	11	12	14	15	11	10	36 (57.1)	37 (58.7)
1.0	4	3	1	1	4	6	9 (14.3)	10 (15.9)
2.0	1	1	1	0	1	0	3 (4.8)	1 (1.6)
>2.0	5	5	5	5	5	5	15 (23.8)	15 (23.8)
Capreomycin								
≤0.625	0	10	10	13	1	7	11 (17.5)	30 (47.6)
1.25	14	4	4	2	13	7	31 (49.2)	13 (20.6)
2.5	0	2	0	2	0	2	0 (0)	6 (9.5)
>2.5	7	5	7	4	7	5	21 (33.3)	14 (22.2)
Ethionamide								
≤1.25	3	5	3	5	3	6	9 (14.3)	16 (25.4)
2.5	1	0	0	2	1	0	2 (3.2)	2 (3.2)
5.0	1	6	1	9	3	6	5 (7.9)	21 (33.3)
>5.0	16	10	17	5	14	9	47 (74.6)	24 (38.1)
Protionamide								
≤0.625	4	6	2	7	3	7	9 (14.3)	20 (31.8)
1.25	0	4	2	4	4	3	6 (9.5)	11 (17.5)
2.5	4	2	4	5	5	5	13 (20.6)	12 (19.1)
>2.5	13	9	13	5	9	6	35 (55.6)	20 (31.8)
Ofloxacin								
≤0.5	8	9	11	15	8	6	27 (42.9)	30 (47.6)
1.0	9	8	6	2	9	11	24 (38.1)	21 (33.3)
2.0	0	1	0	1	0	0	0 (0)	2 (3.2)
>2.0	4	3	4	3	4	4	12 (19.1)	10 (15.9)
Rifabutin								
≤0.25	4	4	2	4	2	4	8 (12.7)	12 (19.1)
0.5	0	0	2	0	2	0	4 (6.3)	0 (0)
1.0	0	0	0	4	0	0	0 (0)	4 (6.3)
>1.0	17	17	17	13	17	17	51 (81.0)	47 (74.6)
Linezolid								
≤0.5	19	19	15	21	21	21	55 (87.3)	61 (96.8)
1.0	2	2	4	0	0	0	6 (9.5)	2 (3.2)
2.0	0	0	2	0	0	0	2 (3.2)	0 (0)
>2.0	0	0	0	0	0	0	0 (0)	0 (0)

[a] A total of 21 strains were tested. MGIT, BACTEC MGIT 960. 460, BACTEC 460.

460 and 35 by MGIT 960, 5 being false resistant and 2 being false susceptible by MGIT 960. For OFX 10 strains were resistant by BACTEC 460 and 12 by MGIT 960, 2 being false resistant by MGIT 960. For RBT both methods yielded 51 resistant results, while for LIN there was no resistance by BACTEC 460 but 2 resistant results by MGIT 960, both at site 2.

TABLE 4. Phase II discordant results between BACTEC 460 and BACTEC MGIT 960 using the best cutoff point for the critical concentrations[a]

Drug (concn [μg/ml])[b]	No. of strains with discrepant results[c]							
	Site 1		Site 2		Site 3		Total	
	S/R	R/S	S/R	R/S	S/R	R/S	S/R	R/S
Amikacin (1.0/1.0)	0	0	0	1	0	1	0	2
Capreomycin (1.25/2.5)	0	0	0	1	0	0	0	1
Ethionamide (2.5/5.0)	0	0	0	3	1	0	1	3
Protionamide (1.25/2.5)	0	2	0	3	2	0	2	5
Ofloxacin (2.0/2.0)	0	1	0	1	0	0	0	2
Rifabutin (0.5/0.5)	0	0	0	0	0	0	0	0
Linezolid (1.0/1.0)	0	0	0	2	0	0	0	2

[a] A total of 21 strains were tested.
[b] BACTEC MGIT concentration/BACTEC 460 concentration.
[c] BACTEC MGIT 960 result/BACTEC 460 result. S, susceptible; R, resistant.

TABLE 5. Drug susceptibility test results of phase III based on the established breakpoints

Drug (concn [μg/ml])[a]	Site	No. of strains with:				Total no. of strains
		S/S[a]	R/R[a]	R/S[a]	S/R[a]	
Amikacin (1.0/1.0)	Site 1	14	26	0	1	41
	Site 2	32	0	0	0	32
	Site 3	18	1	0	0	19
	All sites	64	27	0	1	92
Capreomycin (1.25/2.5)	Site 1	15	25	0	1	41
	Site 2	31	0	1	0	32
	Site 3	17	2	0	0	19
	All sites	63	27	1	1	92
Ethionamide (2.5/5.0)	Site 1	14	26	0	1	41
	Site 2	30	2	0	0	32
	Site 3	11	8	0	0	19
	All sites	55	36	0	1	92
Protionamide (1.25/2.5)	Site 1	20	17	2	2	41
	Site 2	30	2	0	0	32
	Site 3	12	7	0	0	19
	All sites	62	26	2	2	92
Ofloxacin (2.0/2.0)	Site 1	33	8	0	0	41
	Site 2	32	0	0	0	32
	Site 3	18	1	0	0	19
	All sites	83	9	0	0	92
Rifabutin (0.5/0.5)	Site 1	4	37	0	0	41
	Site 2	16	15	1	0	32
	Site 3	9	10	0	0	19
	All sites	29	62	1	0	92
Linezolid (1.0/1.0)	Site 1	38	3	0	0	41
	Site 2	32	0	0	0	32
	Site 3	21	0	0	0	19
	All sites	89	3	0	0	92

[a] BACTEC 460/BACTEC MGIT 960. S, susceptible; R, resistant.

Phase III. Since each site tested its own clinical isolates of *M. tuberculosis*, the number of cultures tested and number of resistant cultures differed from site to site (Table 5). For comparison, the critical concentration for BACTEC 460 was kept the same as in phase II.

As observed in phase II, breakpoint concentrations for AK, OFX, RBT, and LIN were the same by the two methods, while for CM, ETH, and PTH, higher concentrations in MGIT 960 yielded the most concordant results (Table 6). Results from all sites are listed in Table 5. Site 1 had the highest number of resistant strains (26 resistant to AK, 25 to CM, 26 to ETH, 19 to PTH, 8 to OFX, 37 to RBT, and 3 to LIN). By keeping the same breakpoints as those used in phase II, there were only two false-susceptible results by MGIT, both with PTH, while there were five false-resistant results, one each with AK, CM,

TABLE 6. Final critical concentrations (breakpoints) established for the BACTEC MGIT 960 system compared with the BACTEC 460

Drug	Critical concn (μg/ml)	
	BACTEC MGIT 960	BACTEC 460
Amikacin	1.0	1.0
Capreomycin	2.5	1.25
Ethionamide	5.0	2.5
Protionamide	2.5	1.25
Ofloxacin	2.0	2.0
Rifabutin	0.5	0.5
Linezolid	1.0	1.0

and ETH and two with PTH. Site 2 tested 32 strains, with few found resistant (0 to AK, 1 to CM, 2 to ETH, 2 to PTH, 0 to OFX, 16 to RBT, and 0 to LIN). Only two false-susceptible results (one each with CM and RBT) and no false-resistant results were found by MGIT. Site 3 tested 21 cultures. Results for two strains had to be eliminated, because these strains were found to be contaminated upon arrival at the arbiter's site. There were very few resistant strains tested at this site (1 resistant to AK, 2 to CM, 8 to ETH, 7 to PTH, 1 to OFX, 10 to RBT, and 0 to LIN) and no discrepant results between BACTEC 460 and MGIT 960.

Overall, 92 strains were tested in this phase, resulting in a total of 644 tests (Table 5). A large number of resistant strains were included in this testing (27 strains resistant to AK, 28 to CM, 36 to ETH, 28 to PTH, 9 to OFX, 63 to RBT, and 3 to LIN). Only four false-susceptible and five false-resistant results were observed by MGIT 960.

Strains with discrepant results in phase III were referred to the arbiter, which found four cultures, one each for AK and ETH and two for PTH, resistant by both methods (false susceptible by BACTEC 460 in the original testing) and one susceptible by both methods (false resistant by MGIT 960 in the original testing). The other four discrepancies remained unchanged. Only one culture with CM consistently gave false-resistant results with MGIT 960.

DISCUSSION

Only a few publications reported in the literature deal with MICs of second-line drugs in the MGIT medium. These, however, are not multicenter studies, and in addition, they focus on one or two drugs only (2, 6). Our multicenter study was designed to establish the critical test concentrations of second-line and newer antituberculosis drugs. Previously, a similar kind of study was carried out for the radiometric BACTEC 460 TB system (9). Since the MGIT 960 uses a richer medium, it was anticipated that different critical concentrations might be required for the new system, as it is well known that the MIC of a drug may vary due to many factors such as medium components, pH, and inoculum size. Several concentrations for each drug were tested to establish MICs and breakpoints for the critical concentrations. The critical concentration was considered the concentration that results in the least number of discrepant results upon testing of a large number of susceptible and resistant cultures.

For comparison, BACTEC 460 test results were taken as the gold standard, since this system is also broth based, and critical test concentrations for most of the drugs have already been established, with the exception of PTH and LIN. In the study of Pfyffer et al. (9), the range of ETH concentration for BACTEC 460 was 1.25 to 2.5 μg/ml, with 1.25 μg/ml recommended as the final test concentration. However, our experience and current data suggest that 2.5 μg/ml is more appropriate than 1.25 μg/ml in the BACTEC 460 DST.

This study was conducted in three phases, adjusting the concentrations according to the information gathered in the earlier phases. This enabled us to achieve a breakpoint which would yield the most concordant results. Since results for ETH and PTH were not very clear in phases I and II, it was decided to test with three concentrations in phase III.

In phase I, unambiguous MICs could be achieved for AK, OFX, and RBT, with the same values for both systems, while for some other drugs, such as ETH, a few strains yielded MICs, which were distributed more widely. Data of phases I and II clearly indicated that the critical test concentrations in the MGIT 960 should be higher than those in the BACTEC 460 for CM, ETH, and PTH.

Phase II data clearly indicated the working critical concentrations of the test drugs in MGIT 960, which would yield results equivalent to those in BACTEC 460 (Table 6). AK at 1.0 μg/ml yielded satisfactory results for both methods. CM at 1.25 μg/ml in BACTEC 460 compared best with 2.5 μg/ml in MGIT 960, and ETH at 2.5 μg/ml in BACTEC 460 compared best with 5.0 μg/ml in MGIT 960. With PTH, the inhibitory concentration was lower than that of ETH, but MGIT 960 required a higher concentration (2.5 μg/ml) than BACTEC 460 (1.25 μg/ml). Critical concentrations were found to be the same for OFX (2.0 μg/ml), RIF (0.5 μg/ml), and LIN (1.0 μg/ml). It was found that the majority of the differences in the DST results among the test sites were related to the presence of a low level of resistance for a particular drug, as the results indicated when the drug was tested at a level lower than the critical concentrations. Applying the tentative critical concentrations, among 441 tests and three sites in phase II, there were only 3 (0.7%) false-susceptible and 15 (3.4%) false-resistant results with MGIT 960. The highest discrepancies were seen with ETH and PTH. The data also indicate high interlaboratory reproducibility of MGIT 960 DST for these drugs.

Phase III represented the "work in the field," because each site had tested its own clinical isolates. Some of the sites were not able to select very many resistant strains. Overall, an impressive number of resistant strains was tested. The most concordant results were obtained when the critical concentrations established in phase II were applied. With a total of 644 data points, there were four false-susceptible (0.6%; very major errors) and five false-resistant (0.8%; major errors) results. Moreover, the arbiter's testing resolved five of the nine discrepancies. Thus, the concordance of results is remarkable.

Among the drugs, RFB and PTH have cross-resistance with rifampin and ETH, respectively. Eight cultures were found resistant to ETH but susceptible to PTH. However, in such situations there was probably an underlying low level of resistance to PTH, which needs further investigation.

For the quinolones we selected OFX as a representative. The MIC may be lower for newer quinolones, such as moxifloxacin, which is considered to be a drug of choice for the treatment of resistant tuberculosis (16).

One of the limitations of such studies is the small number of resistant cultures. In particular, we were able to find only three strains resistant to LIN, since it belongs to a novel drug class, which has been introduced very recently. With the established guidelines and defined critical concentrations, it is anticipated that more resistant strains will be tested in the future to validate our recommendations.

In our hands, susceptibility testing of second-line antituberculosis drugs with the MGIT 960 yielded reliable and reproducible results. On the other hand, there are some inconveniences, such as the following: (i) working solutions of the drugs are to be made by the user; (ii) susceptibility or resistance is not automatically defined by the instrument, as is the

case with primary drugs; and (iii) results have to be analyzed manually based on the GU values retrieved from the instrument. Compared with first-line DST with the MGIT 960, strict quality control measures (using susceptible and resistant strains) are even more necessary for second-line DST.

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