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Can Thin Layer Agar Test Play a Key Role in the Diagnosis of Tuberculosis in Low-Resource Settings?

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Abstract

Background: Fast, reliable, and cost-effective tests are recommended for tuberculosis diagnosis and drug susceptibility testing, especially in resource-limited settings.
Objectives: This study aimed to evaluate the performance of thin-layer agar for tuberculosis diagnosis and drug susceptibility testing.
Methods: Samples were collected from patients with presumptive tuberculosis and tested using thin-layer agar for tuberculosis and drug susceptibility testing in parallel with Lowenstein Jensen culture method for tuberculosis diagnosis and proportion method for drug susceptibility testing as the gold standard. Receiver operating characteristic curve analysis was performed to calculate the performance parameters.
Results: Thin-layer agar method showed sensitivity and specificity values of 96.63% and 62.50%, respectively, for the isolation of *Mycobacterium tuberculosis* directly from specimens. Drug susceptibility results using thin-layer agar showed sensitivity values for isoniazid, rifampicin), ethambutol and streptomycin were 94.74%, 86.84%, 94.74% and 81.58%, respectively, while the specificity values were 100%, 100%, 86.27% and 100% for isoniazid, rifampicin, ethambutol and streptomycin, respectively. Results were available in a median time of 16 days for thin-layer agar and 25 days for the conventional method.
Conclusions: The thin-layer agar method is a relatively rapid, simple, and cost-effective method for the diagnosis and drug susceptibility testing of *M. tuberculosis*. It may be a useful tool for establishing tuberculosis laboratories in resource-limited settings because it does not require expensive equipment and a high level of training. Our study may help in choosing the appropriate treatment and control of tuberculosis.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, drug Susceptibility, Thin-Layer Agar

1. Background

Tuberculosis is an infectious disease and is a leading cause of death. According to the latest WHO report, 10 million new tuberculosis cases in 2019, including 1.2 million deaths, were attributed to tuberculosis (1). This increased incidence of tuberculosis cases is due to the multidrug-resistant, or at least rifampicin (RIF)- or isoniazid (INH)-resistant, multidrug resistant *Mycobacterium tuberculosis* (MDR- tuberculosis) strains, becoming a threat to tuberculosis control programs (2, 3). Early detection of resistance against first-line drugs, namely INH, RIF, ethambutol (EMB), streptomycin (STR) and pyrazinamide (PZA), is essential to control the spread of MDR- tuberculosis strains (4, 5). Moreover, the increased emergence of MDR- tuberculosis is a healthcare threat that has been reported (6, 7). Thus, there is an urgent need for rapid, cost-effective, and reliable methods for diagnosis and drug susceptibility testing in tuberculosis, especially in low-resource settings (8, 9).

The conventional methods for drug susceptibility testing of *M. tuberculosis* include time-consuming methods, such as the proportion method using Löwenstein Jensen medium or Middlebrook 7H10 agar and those involving heavily-mechanised equipment and radioactive materials such as the BACTEC TB-460 system (Becton Dickinson) (10). Recently, several methods have been proposed for the rapid diagnosis and drug susceptibility testing of tuberculosis (10, 11). Among these methods, the Mycobacterial Growth Indicator Tube (MGIT, Becton Dickinson) and molecular tools, such as the INNO-LiPA Rif. Tuberculosis (Line probe assay, Innogenetics, Ghent, Belgium) and Gene Xpert MTB/RIF (Cephid, USA), have been used extensively.

These methods are expensive and impractical for routine use (10).

Thin-layer agar is a fast test for the diagnosis and drug susceptibility testing of pulmonary and extrapulmonary tuberculosis (12, 13). This technique has been described previously as a cost-effective method, and it provides rapid results compared to conventional methods (14). Direct thin-layer agar can be used for simultaneous detection of *M. tuberculosis* and drug susceptibility testing (15), avoiding the intermediate step of *M. tuberculosis* isolation that reduces the time and need for a high level of biohazard containment (16). These features make thin-layer agar a convenient method for low-resource settings (17). A limited number of studies have evaluated the performance and applicability of thin-layer agar in routine practice (14).

2. Objectives

The primary aim of this study was to evaluate the performance of thin-layer agar in the fast detection of *M. tuberculosis* and drug susceptibility testing as first-line antituberculosis agents, applied to different sample types. The results were evaluated and compared with the reference standard.

3. Methods

3.1. Study Setting

This cross-sectional prospective study was conducted between May 2018 and October 2020 at Abbassaia Chest Hospital and Ain Shams University Hospital, Cairo, Egypt. Inclusion criteria were patients with clinical signs of tuberculosis. Exclusion criteria patients who started tuberculosis treatment for more than one week.

3.2. Specimen Collection and Processing

Sputum, stool, and body fluid samples were collected from patients with suspected tuberculosis. Samples were contained in new plastic containers prior to treatment. All collected samples were stored at 4°C and analysed within 24 h. Sputum samples were decontaminated with N-acetyl-L-cysteine sodium hydroxide and concentrated via centrifugation (3,000× g) for 30 min, followed by acid fast bacilli (AFB) microscopy and cultivation. Body fluid and abscess aspirates were collected under sterile conditions and used directly for AFB microscopy and cultivation.

3.3. Routine Laboratory Testing

The presence of AFB was noted and quantified using AFB staining (18). Both smear-positive and smear-negative samples were cultured using Lowenstein Jensen culture medium (Oxoid, Hampshire, United Kingdom), and drug susceptibility testing of isolates was performed using proportion method on 7H10 Middlebrook agar (Difco Laboratories, Detroit, USA), which was used as medium for the first-line drug according to the Clinical and Laboratory Standards Institute (CLSI) procedures and recommended critical concentrations (19).

3.4. Thin-Layer Agar Test

A 5 mL of 7H11 Middlebrook agar (Difco Laboratories) were poured onto Petri plates that are divided into quadrants. Per quadrant contains the following: growth control with no additions, with a quadrant containing a specific inhibitor of *M. tuberculosis* complex (0.5 mg/mL of paranitrobenzoic acid), a quadrant with 0.2 μg/mL INH, and a quadrant with 1 μg/mL of RIF. Other plates were prepared with 2 μg/mL of STR and 75 μg/mL EMB (20). Samples were decontaminated and diluted according to the AFB load on the smear as follows: > 250 AFB/field, 10^{-3} dilution, 25 - 250 AFB/field, 10^{-1} dilution, < 25 AFB/field, no dilution, while smear-negative samples were not diluted. Each plate quadrant was inoculated with 0.1 mL of diluted sample, tape-sealed, and incubated at 37°C in a 5% CO_2 incubator. Plates were observed using a conventional microscope at 100× magnification every two days over one month. The specimen was considered positive for *M. tuberculosis* when the growth was recorded in the control quadrant and resistant if growth was observed in quadrants containing RIF, INH, STR, and EMB as compared to the growth control. All results were read blindly to the reference tests.

3.5. Data Analysis

Receiver operating characteristic curve analysis was performed using MedCalc version 11.61 (MedCalc Software, Mariakerke, Belgium), and sensitivity and specificity were calculated for detection of *M. tuberculosis* and drug susceptibility testing by thin-layer agar. Key proportions were reported with 95% confidence (95% Confidence Interval (CI)), and a P-value of < 0.05 was considered statistically significant. The time for *M. tuberculosis* detection was recorded, and the time for drug susceptibility testing was calculated from sample processing until the appearance of sufficient growth to read the samples from thin-layer agar, Lowenstein Jensen culture method, and culture proportion method. The time was compared using Microsoft Excel version 365 (Microsoft, USA) for both thin-layer agar and conventional methods, and the uninterpretable samples were excluded.

4. Results

During the study period, 241 samples were collected, in which 2 samples were excluded, and 239 samples were eligible. Of the 239 samples, 123 were smear positive (105 were sputum, 12 body fluid, and 6 pus) and 116 were smear negative (107 were sputum and 9 body fluid). Detection of *M. tuberculosis* using thin-layer agar showed a 93.7% agreement between thin-layer agar and Lowenstein Jensen culture method (Table 1). Detection of *M. tuberculosis* using thin-layer agar showed a sensitivity of 96.63% and specificity of 93.50%, and the CI values were at 0.910 - 0.966 (95% CI) when compared to Lowenstein Jensen culture (Table 2). Drug susceptibility testing using thin-layer agar showed that the sensitivity level of specimens against INH, RIF, EMB, and STR were 94.74%, 86.84%, 94.74%, and 81.58%, respectively. Specificity levels of drug susceptibility testing using thin-layer agar were 100%, 100%, 86.27%, and 100% for INH, RIF, EMB, and STR, respectively (Table 2). The area under the curve (AUC) for detection of *M. tuberculosis* was 0.916, and drug susceptibility testing were 0.974, 0.934, 0.905, and 0.908 for INH, RIF, EMB, and STR, respectively (Table 2).

The median time for drug susceptibility testing from sample processing to readable results was 16 days, which was significantly faster than Lowenstein Jensen culture method - proportion method that had a median of 29 days ($P < 0.01$) (Figure 1).

5. Discussion

This study investigated the performance of thin-layer agar to detect *M. tuberculosis* and drug susceptibility testing to the first-line anti- tuberculosis agents. Our study reported a high positivity rate of *M. tuberculosis* detection because of the fast processing of the samples directly after collection and decontamination. Moreover, we showed a high positivity frequency, which may be due to selection bias, and the inclusion criteria were highly suspected cases of tuberculosis. Delay in testing of the specimens affects the positive results and increases the chance of contamination; thus, thin-layer agar is a suitable method for peripheral laboratories (21). Studies conducted in Switzerland showed a high positivity rate in the detection of *M. tuberculosis* using thin-layer agar in low-resource settings (21, 22). More *M. tuberculosis* -positive cultures were detected using thin-layer agar and MGIT than with Lowenstein Jensen culture method. Thin-layer agar and MGIT had a shorter detection time for *M. tuberculosis* growth (median 10 and 7.1 days, respectively) than Lowenstein Jensen culture method (median 22 days) and specimen bacillary load (23).

Finding a rapid, reliable, and cost-effective diagnostic method will be a key role in tuberculosis control, especially in low-resource settings. In our study, the median time for *M. tuberculosis* diagnosis and drug susceptibility testing using thin-layer agar was 16 days as compared when using Lowenstein Jensen culture method and proportion method (29 days). In a multi-centre study, BACTEC took approximately 11 days for direct drug susceptibility testing (24, 25). Although BACTEC takes a shorter time, we must consider that it is not available in low-resource settings. Molecular techniques provide drug susceptibility testing on the same day (26). Although molecular techniques are superior, their high cost and required level of training make them impractical in peripheral laboratories and low resource settings. In our study, using microscopic observations in thin-layer agar reduced the time compared to macroscopic detection. Supporting findings were reported by Welch et al. median detection time using thin-layer agar. M7H11medium was reported as 10 days, and the time was shorter in the case of smear-positive samples (27, 28).

In the current study, tuberculosis diagnosis using thin-layer agar showed a sensitivity of 96.63% and specificity of 93.5%. In concordance with our results, a recent study in Estonia found that thin-layer agar showed a sensitivity and specificity of 93.0% 99.4%, respectively, versus 62.5% and 99.3% for Xpert®MTB/RIF (16). Our results on the sensitivity and specifity rates on drug susceptibility testing using thin-layer agar were similar with Robledo et al., (23) wherein thin-layer agar showed 100% sensitivity and specificity for the RIF and INH resistance (23). Several studies used redox along with thin-layer agar. This method reduced the time, and the performance results were consistent with our findings. The coloured thin-layer agar test showed a sensitivity of 94.7% for levofloxacin, 95.8% for INH, and 97.3% for RMP. All tested drug specificities were > 97% (29). In Ethiopia, the colour test reported a sensitivity of 59%, 96%, and 95% for INH, RMP, and MDR- tuberculosis, respectively. Furthermore, it detected a specificity of 96%, 94%, and 98%, for INH, RMP, and MDR- tuberculosis, respectively (30).

Study limitations. This study aimed to evaluate the performance of thin-layer agar for the detection of *M. tuberculosis* and drug susceptibility testing and its implementation in low-resource settings. The high positivity rate in our study was due to selection bias and did not reflect disease prevalence. In this study, sensitivity calculations considered only patients with confirmed tuberculosis. The results of thin-layer agar were close to those of AFB, implying that a high positive rate would enhance thin-layer agar sensitivity. Only four smear-negative samples were positive using thin-layer agar, and this was not sufficient to evalu-

Table 1. Specimen-Wide Distribution of *Mycobacterium tuberculosis* Isolates

Test	Sensitivity	Specificity	95% CI	AUC
***Mycobacterium tuberculosis* detection**	96.63	93.50	0.910 - 0.966	0.916
Susceptibility testing				
Isoniazid	94.74	100.00	0.915 - 0.996	0.974
Streptomycin	81.58	100.00	0.828 - 0.959	0.908
Ethambutol	94.74	86.27	0.824 - 0.957	0.905
Rifampicin	86.84	100.00	0.861 - 0.976	0.934

Table 2. *Mycobacterium tuberculosis* Susceptibility Testing Against First Line Anti-tuberculosis Drugs Using Lowenstein Jensen Culture and Thin-Layer Agar Methods.

Specimen	No. of Samples	Both +ve	Both -ve	LJ +ve, TLA -ve	LJ -ve, TLA +ve	Agreement (%)
Sputum	212	71	129	1	4	96.7
Body fluid	21	7	12	0	2	90.5
Pus	6	2	3	0	1	83.3
Total	239	80	144	1	7	93.7

Abbreviations: TLA, thin layer agar; LJ, Lowenstein Jensen culture method.

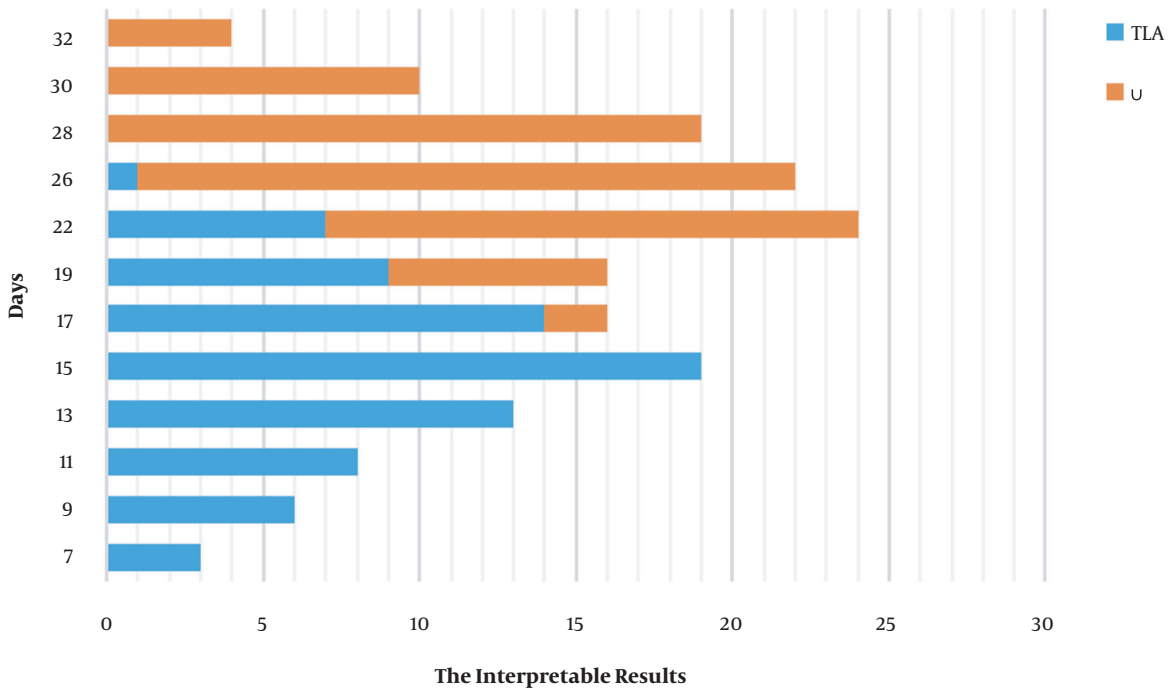

Figure 1. The turnaround time in days for drug susceptibility testing using Lowenstein Jensen culture method - proportion method and thin-layer agar methods.

ate its performance in drug susceptibility testing in smear-negative cases; thus, further studies are required. Furthermore, the study did not examine the performance of thin-layer agar for the phenotypic detection of new mutations that may result from inadequate treatment.

5.1. Conclusions

In conclusion, these results show that the thin-layer agar method is a rapid, simple, and cost-effective tool for the detection of *M. tuberculosis* and drug susceptibility testing. Our findings provide an approach to use thin-layer

agar in low-resource settings and peripheral labs. Further studies are required for the large-scale evaluation of thin-layer agar and its ability to detect new mutations in *M. tuberculosis* and extensive drug resistance *M. tuberculosis*.

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Footnotes

Authors' Contribution: All authors have shared in study design, data analysis, and writing of the manuscript.

Conflict of Interests: There was no conflict of interest.

Ethical Approval: Written approval was obtained from enrolled patients. The study was approved by the local ethics committee of the faculty of science, Sohage University according to principles of Helsinki declaration.

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References

1. WHO. *Health at a Glance: Asia/Pacific 2020 Measuring Progress Towards Universal Health Coverage: Measuring Progress Towards Universal Health Coverage*. OECD Publishing; 2020.
2. Lienhardt C, Glaziou P, Uplekar M, Lonnroth K, Getahun H, Raviglione M. Global tuberculosis control: lessons learnt and future prospects. *Nat Rev Microbiol*. 2012;**10**(6):407–16. doi: 10.1038/nrmicro2797. [PubMed: 22580364].
3. Pourostadi M, Rashedi J, Mahdavi Poor B, Samadi Kafil H, Kazemi A, Ahmadpour E, et al. Role of molecular epidemiology on Tuberculosis control in the middle east countries: A systematic review and meta-analysis. *Tanaffos*. 2018;**17**(4):223–32. [PubMed: 31143212]. [PubMed Central: PMC6534806].
4. Lemus D, Martin A, Montoro E, Portaels F, Palomino JC. Rapid alternative methods for detection of rifampicin resistance in Mycobacterium tuberculosis. *J Antimicrob Chemother*. 2004;**54**(1):130–3. doi: 10.1093/jac/dkh320. [PubMed: 15190018].
5. Pourostadi M, Rashedi J, Mahdavi Poor B, Samadi Kafil H, Kazemi A, Asgharzadeh M. Tuberculosis control and role of molecular epidemiology studies in Iran: A systematic review. *Tanaffos*. 2017;**16**(3):190–200. [PubMed: 29849672]. [PubMed Central: PMC5960223].
6. Sotgiu G, Sulis G, Matteelli A. Tuberculosis-A World Health Organization perspective. *Microbiol Spectr*. 2017;**5**(1). doi: 10.1128/microbiolspec.TNMI7-0036-2016. [PubMed: 28185618].
7. WHO. *Tuberculosis: Voices of the unheard*. World Health Organization,; 2008. Available from: https://apps.who.int/iris/handle/10665/119877?locale-attribute=ru&locale=es&null=.
8. Hurevich G. Belarus and drug-resistant tuberculosis. *Bull World Health Organ*. 2019;**97**(12):795–6. doi: 10.2471/BLT.19.021219. [PubMed: 31819286]. [PubMed Central: PMC6883274].
9. Fadaee M, Rashedi J, Arabi S, Poor BM, Kafil HS, Pourostadi M, et al. Stopping of the downtrend of Tuberculosis in Iran, a systematic review of associated risk factors. *Infect Disord Drug Targets*. 2020;**20**(3):367–73. doi: 10.2174/1871526519666181228162837. [PubMed: 30592256].
10. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, et al. Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. *Mol Biol Rep*. 2020;**47**(5):4065–75. doi: 10.1007/s11033-020-05413-7. [PubMed: 32248381].
11. Shi J, Tao B, Li Z, Song H, Wu J, Qiu B, et al. Diagnostic performance of GeneChip for the rapid detection of drug-resistant Tuberculosis in different subgroups of patients. *Infect Drug Resist*. 2021;**14**:597–608. doi: 10.2147/IDR.S297725. [PubMed: 33633456]. [PubMed Central: PMC7900445].
12. Schaberg T, Reichert B, Schulin T, Lode H, Mauch H. Rapid drug susceptibility testing of Mycobacterium tuberculosis using conventional solid media. *Eur Respir J*. 1995;**8**(10):1688–93. doi: 10.1183/09031936.95.08101688. [PubMed: 8586123].
13. Drobniewski F, Nikolayevskyy V, Balabanova Y, Bang D, Papaventsis D. Diagnosis of tuberculosis and drug resistance: What can new tools bring us? *Int J Tuberc Lung Dis*. 2012;**16**(7):860–70. doi: 10.5588/ijtld.12.0180. [PubMed: 22687497].
14. Ardizzoni E, Mulders W, Kotrikadze T, Aspindzelashvili R, Goginashvili L, Pangtey H, et al. The thin-layer agar method for direct phenotypic detection of multi- and extensively drug-resistant tuberculosis. *Int J Tuberc Lung Dis*. 2015;**19**(12):1547–52. doi: 10.5588/ijtld.15.0136. [PubMed: 26614200].
15. Wilson ML. Rapid diagnosis of Mycobacterium tuberculosis infection and drug susceptibility testing. *Arch Pathol Lab Med*. 2013;**137**(6):812–9. doi: 10.5858/arpa.2011-0578-RA. [PubMed: 23721277].
16. Bedi RS. *Manual on tuberculosis, HIV and lung diseases: A practical approach*. Jaypee Brothers Medical Publishers (P) Ltd; 2009.
17. Martin A, Munga Waweru P, Babu Okatch F, Amondi Ouma N, Bonte L, Varaine F, et al. Implementation of the thin layer agar method for diagnosis of smear-negative pulmonary tuberculosis in a setting with a high prevalence of human immunodeficiency virus infection in Homa Bay, Kenya. *J Clin Microbiol*. 2009;**47**(8):2632–4. doi: 10.1128/JCM.00264-09. [PubMed: 19494065]. [PubMed Central: PMC2725698].
18. Metchock BG, Nolte FS, Wallace RJ, Murray PR, Baron EJ, Pfaller MA. *"Mycobacterium," in manual of clinical microbiological laboratory*. 6th ed. Washington, DC: American Society for Microbiology; 1999.
19. Woods GL, Lin SG, Desmond EP. *Susceptibility Test Methods: Mycobacteria, Nocardia, and Other Actinomycetes*. Manual of clinical microbiology; 2015.
20. Weinstein MP, Lewis JS, Kraft CS. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: Background, organization, functions, and processes. *J Clin Microbiol*. 2020;**58**(3). doi: 10.1128/jcm.01864-19.
21. Ardizzoni E, Ariza E, Mulengwa D, Mpala Q, de La Tour R, Maphalala G, et al. Thin-layer-agar-based direct phenotypic drug susceptibility testing on sputum in eswatini rapidly detects Mycobacterium tuberculosis growth and rifampicin resistance otherwise missed by WHO-endorsed diagnostic tests. *Antimicrob Agents Chemother*. 2021;**65**(6). doi: 10.1128/AAC.02263-20. [PubMed: 33722892]. [PubMed Central: PMC8315964].
22. Mejia GI, Castrillon L, Trujillo H, Robledo JA. Microcolony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of Mycobacterium tuberculosis infection. *Int J Tuberc Lung Dis*. 1999;**3**(2):138–42. [PubMed: 10091879].
23. Robledo J, Mejia GI, Paniagua L, Martin A, Guzman A. Rapid detection of rifampicin and isoniazid resistance in Mycobacterium tuberculosis by the direct thin-layer agar method. *Int J Tuberc Lung Dis*. 2008;**12**(12):1482–4. [PubMed: 19017461].

24. Siddiqi S, Ahmed A, Asif S, Behera D, Javaid M, Jani J, et al. Direct drug susceptibility testing of Mycobacterium tuberculosis for rapid detection of multidrug resistance using the Bactec MGIT 960 system: A multicenter study. *J Clin Microbiol*. 2012;**50**(2):435–40. doi: 10.1128/JCM.05188-11. [PubMed: 22162558]. [PubMed Central: PMC3264138].
25. Ozma MA, Rashedi J, Poor BM, Vegari A, Asgharzadeh V, Kafil HS, et al. Tuberculosis and diabetes mellitus in northwest of Iran. *Infect Disord Drug Targets*. 2020;**20**(5):667–71. doi: 10.2174/1871526519666190715142100. [PubMed: 31322073].
26. Kimerling ME, Lambregts-Van Weezenbeek K, Jaramillo E. Programmatic control of multidrug-resistant tuberculosis. *Tuberculosis*. CRC Press; 2016. p. 265–94.
27. Irfan S, Hasan R, Kanji A, Hassan Q, Azam I. Evaluation of a microcolony detection method and phage assay for rapid detection of Mycobacterium tuberculosis in sputum samples. *Southeast Asian J Trop Med Public Health*. 2006;**37**(6):1187–95.
28. Welch DF, Guruswamy AP, Sides SJ, Shaw CH, Gilchrist MJ. Timely culture for mycobacteria which utilizes a microcolony method. *J Clin Microbiol*. 1993;**31**(8):2178–84. doi: 10.1128/jcm.31.8.2178-2184.1993. [PubMed: 8370748]. [PubMed Central: PMC265718].
29. Klaos K, Agejeva A, Kummik T, Laks S, Remets O, Sasi S, et al. A successful introduction to a non-expert setting of the thin-layer agar Colour Test as an indirect phenotypic drug susceptibility test for Mycobacterium tuberculosis. *Int J Infect Dis*. 2021;**104**:19–26. doi: 10.1016/j.ijid.2020.12.071. [PubMed: 33385582].
30. Shibabaw A, Gelaw B, Kelley HV, Tesfaye E, Balada-Llasat JM, Evans CA, et al. MDR/XDR-TB colour test for drug susceptibility testing of Mycobacterium tuberculosis, Northwest Ethiopia. *Int J Infect Dis*. 2020;**90**:213–8. doi: 10.1016/j.ijid.2019.10.041. [PubMed: 31689528].