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EVALUATION OF MYCOBACTERIA GROWTH INDICATOR TUBE FOR DIRECT AND INDIRECT DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS* FROM RESPIRATORY SPECIMENS

Pathology

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ABSTRACT

The manual Mycobacteria Growth Indicator Tube (MGIT) method was evaluated for performing direct and indirect drug susceptibility testing (DST) of Mycobacterium tuberculosis for isoniazid and rifampin on 101 strongly smear-positive sputum specimens in RIMS RAIPUR CG. Using the indirect method of proportion (MOP) as the "gold standard," the accuracies of isoniazid and rifampin susceptibility testing by the direct MGIT system were 97.0 and 94.1%, respectively. The accuracy of the indirect MGIT system was 98.0% for both drugs. The turnaround times from specimen processing to reporting of the DST results ranged between 4 and 23 (mean, 9.2) days by the direct MGIT method, 9 and 30 (mean, 15.3) days by the indirect MGIT method, and 26 and 101 (mean, 59.6) days by the indirect MOP. MGIT appears to be a reliable, rapid, and convenient method for performing direct and indirect DSTs in low-resource settings.

KEYWORDS

Liquid culture, Mycobacterium growth indicator tube, Tuberculosis

INTRODUCTION

Effective treatment and prevention of MDRTB rely upon the prompt availability of drug susceptibility testing (DST) results. Conventional mycobacteriological methods using solid media require more than 6 weeks on average to report identification and susceptibility results. Various commercial broth-based methods with sensitive growth-detection systems have been developed to improve this turnaround time (TAT), and multiple evaluations have demonstrated the performance of these methods to be essentially equivalent. Unfortunately, cost and the requirement for sophisticated equipment have prevented the use of these systems in the resource-poor settings where MDRTB is endemic and where these methods are most needed. Unlike many of these new technologies, the manual Mycobacteria Growth Indicator Tube (MGIT) system does not require additional instrumentation. The MGIT method uses a fluorescence quenching-based oxygen sensor embedded in the base of a tube containing a modified Middlebrook 7H9 broth. The fluorescence that indicates the presence of mycobacterial growth can be detected by transillumination with a 365-nm UV light (e.g., a simple Wood's lamp). In the present study we therefore evaluated the performance and practicability of MGIT for performing direct and indirect susceptibility tests for isoniazid and rifampin on strongly smear-positive sputum specimens.

MATERIAL AND METHOD

Smear-positive sputum specimens that had been collected for routine diagnosis or follow-up and that contained more than 10 acid-fast bacilli (AFB) in at least 20 high-power fields (i.e., grade 31 by the WHO scale were selected for inclusion in the study, which was conducted between AUGUST 2017 and AUGUST 2018.The final cohort of 101 specimens were processed and studied(who had failed the WHO-recommended category II treatment regimen). Sputum specimens were decontaminated and digested by using the standard N-acetyl-L-cysteine (NALC)–NaOH method , which provided exposure to 2% NaOH for 15 min. After centrifugation, the pellets were resuspended in 4 ml of sterile phosphate-buffered saline. DST. (i) Direct MGIT test. Three MGIT tubes were supplemented with 0.5 ml of OADC (oleic acid, bovine albumin, dextrose, and catalase), 0.1 ml of PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin), and 0.1 ml of test antibiotic; the third tube, being the growth control (GC), received no test antibiotic. The antibiotics were provided by and prepared as recommended by the manufacturer. The final concentrations in the test tubes were isoniazid at 0.1 mg/ml and rifampin at 1.0 mg/ml. Equal volumes (0.5 ml) of the Setting and specimens. Smear-positive sputum specimens that had been collected for routine diagnosis or follow-up and that contained more than 10 acid-fast bacilli (AFB) in at least 20 high-power fields (i.e., grade 31 by the WHO scale were selected for inclusion in the study, which was conducted between AUGUST 2017 and AUGUST 2018. The antibiotics were provided by and prepared as recommended by the manufacturer. The final concentrations in the test tubes were isoniazid at 0.1 mg/ml and rifampin at 1.0 mg/ml. Equal volumes (0.5 ml) of the Statistical analysis. The sensitivity (ability to detect true resistance), specificity (ability to detect true susceptibility), predictive value for resistance (PVR), predictive value for susceptibility (PVS), and accuracy (the rate of correct results) were calculated.

RESULTS

All 101 sputum specimens entered in the study grew M. tuberculosis isolates both in MGIT and on solid media. No specimens had to be excluded from the study because of bacterial contamination. Susceptibility testing by the indirect MOP found that 25 were susceptible to isoniazid and rifampin, 21 were isoniazid resistant rifampin susceptible, one was isoniazid susceptible rifampin resistant, and 54 were multidrug resistant . When compared with the above "gold standard" test, the direct MGIT system produced three false-resistant isoniazid results and six false-susceptible rifampin results, while the indirect MGIT system gave two false-resistant isoniazid and two false-susceptible rifampin results.When compared with each other, the direct and indirect MGIT DSTs showed only one discrepant isoniazid result and four discordant rifampin results (99.0 and 96.0% accuracies, respectively); the indirect test agreed with the MOP on all five occasions.The direct MGIT system provided DST results 2 to 13 (mean, 6.1) days sooner than the indirect MGIT method, which in turn produced results 9 to 91 (mean, 44.3) days earlier than the indirect MOP. The manufacturer instructs that indirect MGIT DSTs are invalid and should be repeated if the GC tube does not fluoresce by day 12. No such invalid indirect MGIT tests occurred in this study. A review of the data found that the GC tube became positive more than 12 days after inoculation for two (22.2%) of the nine specimens producing discordant isoniazid or rifampin results by the direct MGIT method compared with only 7 (7.6%) of 92 concordant specimens .

DISCUSSION

Both systems share the advantages of being rapid and of testing the actual mycobacterial population causing the patient's disease instead of a selected subset that is (preferentially) cultivated in vitro during primary isolation. Fortunately, the direct MGIT DST system does not appear to have some of the disadvantages that have limited the widespread use of direct radiometric BACTEC DST. For example, unlike the direct MGIT DST system which used a different "critical proportion" to define resistance than the indirect radiometric BACTEC DST, the criteria for defining resistance in the indirect MGIT DST also appears appropriate for the direct MGIT DST. The manufacturer stipulates that indirect MGIT DST results are only valid if the GC tube becomes positive within 12 days of inoculation. The present study found that discordant results tended to occur more frequently with the direct MGIT method among specimens incubated beyond this time. However, this association did not reach statistical significance, with only nine discordant results. Further experience with the direct MGIT DST method is required to define an upper limit for the incubation time that optimizes test performance. Contamination did not prove to be a problem in the direct MGIT DST despite the enriched Middlebrook medium that is used in the tubes. As in the previous direct radiometric BACTEC DST evaluations, PANTA antibiotic solution was added to limit contamination. The high concordance (i.e., 96 to 99.0%) between the direct and indirect MGIT methods suggests that the addition of PANTA has had little effect on the direct DST results. Finally, this evaluation found that the direct MGIT system produced DST results for both isoniazid and rifampin 2 to 13 (mean, 6.1) days earlier than when using MGIT for primary isolation and then an indirect DST. Though statistically significant, the actual clinical benefit of this 6-day time-saving remains to be defined. The present study does have some limitations. First, this initial evaluation of direct MGIT DST used only strongly smear-positive specimens to ensure that a significant quantity of acid-fast bacilli was present in each DST. Second, we only evaluated the direct MGIT system for obtaining isoniazid and rifampin susceptibility results. This approach was adopted because these two drugs are the key elements in short-course chemotherapy and provide the most robust DST results. Third, the study cohort contained only 26 isoniazid-susceptible specimens, so the estimated performance of the direct MGIT system for isoniazid susceptibility testing is inexact, with wide 95% confidence intervals (e.g., specificities of 69.8 to 97.6%). Direct agar dilution susceptibility testing is a recognized inexpensive alternative that can provide DST results within 3 to 4 weeks . However, direct agar DST can be confounded by bacterial contamination, under- or overgrowth in controls that invalidate about 15% of tests, and potential inactivation of the test drug during prolonged incubation. This evaluation has also confirmed that the excellent performance and rapid TAT reported for indirect MGIT DST in other studies can be reproduced in a low-resource setting. Cost is the only prohibitive factor

CONCLUSION

Our study has demonstrated that the nonautomated MGIT system is a dependable, rapid method for performing direct DST. This evaluation has also confirmed that the excellent performance and rapid TAT reported for indirect MGIT DST in other studies can be reproduced in a low-resource setting. Cost is the only prohibitive factor.

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