Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:52:e20180267: 2019
doi: 10.1590/0037-8682-0267-2018

Short Communication

Increase in the diagnosis of mycobacteremia after the implementation of semi-automated blood culture at a Brazilian public health laboratory

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Abstract

Introduction: The prevalence of hematogenous dissemination of mycobacteria is high in immunosuppressed patients. The isolation of mycobacteria in culture remains the standard procedure. **Methods**: This is a cross-sectional study based on the results of solid (Löwenstein–Jensen medium) and semi-automated liquid (BACTEC 9240) blood cultures, obtained from the Lacen-GO database. **Results**: The implementation of a semi-automated procedure resulted in an increase of 61.5% and 350.0% in the positive results for *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria, respectively. This technique also accelerated the detection of positive results. **Conclusions**: Semi-automated liquid blood culture showed a better performance in the diagnosis of mycobacteremia.

Keywords: Blood culture. Immunosuppression. Mycobacteria.

The clinical-epidemiological importance of mycobacteria mainly lies in the pathogenicity of some species that cause disease, such as tuberculosis[1]. This infection is spread through aerosols that contain the *Mycobacterium tuberculosis* complex (MTC), which consists of the species *M. tuberculosis, M. africanum, M. bovis, M. microti,* and *M. canetti*[2]. In about 11% of cases, tuberculosis is related to coinfection with human immunodeficiency virus (HIV). Although tuberculosis predominantly affects the lungs, immunodeficiency substantially increases the risk of disease and favors disseminated and extrapulmonary tuberculosis[3].

The rapid and effective detection of MTC associated with lung infections helps prevent its dissemination. Early diagnosis is a crucial strategy for the tuberculosis control program worldwide, especially due to the emergence of drug-resistant mycobacteria strains and the consequences of such infections in patients infected with HIV[4]. Therefore, sensitive and specific methods that are simultaneously easy, fast, and cost-effective, are essential for the diagnosis of pulmonary tuberculosis and disseminated infections[5].

Nontuberculous mycobacteria (NTM) also cause serious infections that are difficult to treat. Their pathogenic potential was highlighted by the emergence of HIV and the development of more sensitive and specific detection techniques used in laboratories. NTM are widely distributed in nature and can be isolated from water and soil samples. The different species present distinct levels of virulence and patterns of drug susceptibility, being associated with different clinical aspects[6].

The *Mycobacterium avium* complex (MAC) is composed of slow-growing aerobic bacteria. It is estimated that more than 50% of patients with acquired immunodeficiency syndrome (AIDS) develop a MAC infection during their lifetime[1]. Therefore, the detection of mycobacteremia is a priority, especially in immunocompromised patients. Semi-automated blood culture in liquid media is recommended because it favors the growth of MTC and NTM. Despite the advances in mycobacteria diagnostic methodologies, their cost and accessibility remain limiting factors[5]. Use of liquid cultures

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Received 21 June 2018
Accepted 8 January 2019

should be carefully considered, because they are expensive and prone to contamination, despite being semi-automated and highly sensitive[7].

Solid culture in Löwenstein–Jensen (LJ) is a well-established and reliable methodology[4]; therefore, it is convenient to test the benefits of automation. Analyzing the results obtained in a public health laboratory could help to identify and define the methodologies with the best performance. The objective of this study was to evaluate the improvement in the diagnosis of mycobacteremia due to the implementation of semi-automated liquid blood culture.

This is a cross-sectional epidemiological study using results obtained from the isolation of mycobacteria in both solid (LJ medium, In-house produced) and semi-automated liquid (BACTEC 9240; Becton Dickinson Microbiology Systems, Sparks, MD, USA) blood cultures. The results were drawn from the Mycobacteria section of the database of the Public Health Laboratory Dr. Giovanni Cysneiros (Lacen-GO), a health-care facility that is a reference in diagnosing mycobacteria in the state of Goiás, Brazil.

Solid and semi-automated liquid cultures were analyzed from January to December 2014 and from June 2015 to June 2016, respectively. In both periods, no results were excluded from the analysis. The following variables were considered: positive and negative results for mycobacteria, contamination, time for growth detection, mycobacterial species, and HIV coinfection.

This study was approved by the Ethics and Research Committee of the Hospital of Tropical Diseases Dr. Anuar Auad (HDT/HAA), and the Brazilian Platform (Assessment No. 2100342).

Blood samples were collected from HDT/HAA patients after disinfection of the puncture site with 70% alcohol. For each sample, the blood was both seeded in LJ medium, in a laminar flow cabin, and immediately inoculated in a MYCO/F LYTIC culture flask from the BACTEC system. The samples were conditioned and sent to the reference laboratory Lacen-GO.

LJ media seeded with 1 to 2 mL of blood were incubated at 36 °C ± 1.0 in a bacteriological incubator for a maximum of 56 days. Growth was visually inspected after 48 h, and thereafter, once a week. MYCO/F LYTIC vials containing 3 to 5 mL of blood were incubated at 35 °C ± 1.5 °C on the BACTEC 9240 equipment with a non-invasive fluorescent detection system to carry out readings every 10 min. Samples were considered negative after 42 days of incubation without growth detection.

Positive results for mycobacteria were confirmed by the presence of acid-fast bacilli (BAAR) determined by Ziehl-Neelsen staining. MTC was identified by selective growth inhibition in LJ medium with p-nitrobenzene acid (PNB), and detection of the MPT64 antigen. NTM were identified from growth in LJ medium containing PNB, and the negative results were attested by MPT64 antigen testing. The NTM isolates were referred to Prof. Hélio Fraga Reference Center for typification.

Blood cultures that showed growth of microorganisms, but were negative by smear microscopy for mycobacteria, were considered contaminated. HIV coinfection was confirmed using the patient registry associated with each sample that was sent to Lacen-GO.

The data were tabulated and analyzed in Microsoft® Office Excel 2013. Chi-square test was used to analyze the association between the variables (such as, frequencies of positivity for MTC and NTM, frequencies of negativity, and contaminations) using different methodologies. A t test was used to analyze the differences in the average time for detection of positive results, using each methodology. All analyses were performed using Bioestat 5.3.

The results from the solid blood cultures in LJ medium (n = 1375) showed 13 (0.9%) positive samples for MTC, 1 sample identified as *M. bovis* BCG (Bacillus Calmette-Guérin), and 4 samples (0.3%) isolated as NTM and posteriorly identified as MAC. Additionally, 1210 (88.0%) samples were negative and 148 (10.8%) were contaminated.

The semi-automated liquid blood cultures performed on the BACTEC 9240 (n = 1000) equipment identified 21 (2.1%) positive samples for MTC and 18 (1.8%) for NTM. MAC was identified in 15 (83.3%) of the NTM samples. Other identified species were *M. colombiense, M. haemophilum,* and *M. intracellulare / M. chimaera*. Additionally, 844 (84.4%) samples were negative and 117 (11.7%) were contaminated.

There was an increase of 61.5% and 350.0% in MTC and NTM positive results, respectively, after the implementation of semi-automated liquid blood culture. The semi-automated liquid blood culture showed higher rate of positive results ($p<0.01$). The solid blood culture showed higher rates of negative results ($p=0.01$). There was no difference between the contamination rates of the two methodologies ($p=0.51$). It was observed that 96% of mycobacteria positive samples were from patients coinfected with HIV and diagnosed with AIDS.

The solid blood culture required 13 to 53 days (mean of 25 ± 10 days) and 22 to 49 days (mean of 36 ± 11 days) for the detection of MTC and NTM, respectively. The semi-automated liquid blood culture required 10 to 50 days (mean of 18 ± 6 days) and 9 to 38 days (mean of 21 ± 9 days) for the detection of MTC and NTM, respectively. The difference between the time required for growth of MTC and NTM in solid and liquid blood cultures was statistically significant ($p<0.01$). Therefore, semi-automated culture for different mycobacteria species was more efficient in detecting positive results, especially for NTM. **Figures 1 and 2** represent the percentage of positive results for MTC and NTM regarding time in days, respectively.

The culture of mycobacteria is considered a standard procedure for the confirmation of MTC/NTM infection and is required for the susceptibility test. Several culture methodologies are available using solid and liquid media[4]. Results obtained from the blood cultures analyzed by Lacen-GO corroborate with the literature, showing that the rates of positive results for MTC and NTM increased significantly with the use of semi-automated liquid blood culture due to its greater sensitivity.

Studies have evaluated the performance of different cultures, but the majority did not include blood samples or included a

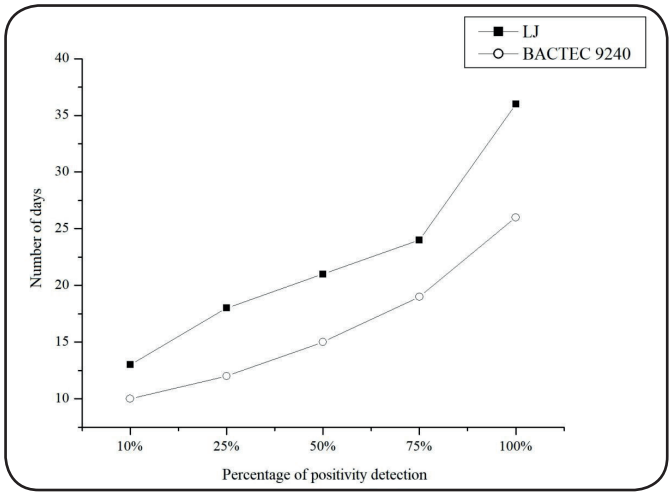

FIGURE 1: Percentage of *Mycobacterium tuberculosis* complex positivity detection in relation to time. **LJ:** Löwenstein–Jensen.

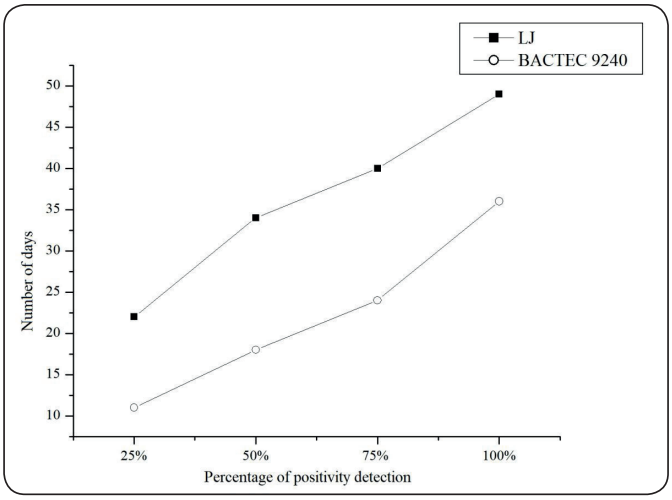

FIGURE 2: Percentage of Nontuberculous mycobacteria positivity detection in relation to time. **LJ:** Löwenstein–Jensen.

low sample number[7,8]. In this study, we included a large number of blood samples, therefore, our results can be considered trustworthy.

The detection of mycobacteremia is a priority, especially in AIDS patients. Blood culture in these patients has become a routinely tool for diagnosis. Additionally, the use of semi-automated methodologies is considered more sensitive as blood is considered a paucibacillary sample[9]. A study comparing the positivity of different clinical blood specimens using different methodologies found 2.1% of samples positive for mycobacteria, and showed that the semi-automated methodologies were more sensitive[10].

The semi-automated liquid blood culture for mycobacteria with the MYCO/F LYTIC medium in the BACTEC 9240 equipment is fast, simple, safe, and has high efficiency[8]. Despite the benefits, the use of this culture medium may result in higher contamination rates. However, we did not find a significant difference in contamination rates between the two methodologies.

Considering MTC species, we identified *M. bovis* BCG from vaccine reaction. Disseminated infection is a rare complication that can result in death, and its occurrence rate varies from 0.19 to 1.56 per million people vaccinated. Disseminated infection usually occurs in children with congenital or acquired immunodeficiency who are inadvertently vaccinated[11].

The detection of NTM increased significantly with the use of semi-automated liquid blood culture. Different species were isolated, demonstrating that the semi-automated method facilitates the growth of NTM. The rate of positive results was higher for MAC, corroborating another study conducted by a public health laboratory, in which 28 MAC isolates were obtained from blood cultures performed between 1996 and 2005[12]. The frequency of MAC dissemination was also high (358 isolates) in HIV-positive patients of a study carried out between 1991 and 1997 in the State of São Paulo[13].

The MTC and NTM detection was significantly faster in the liquid blood culture. This result provides evidence for the better performance of the liquid blood culture described in the literature[10,14]. It also corroborates a study that detected mycobacteria in the blood of AIDS patients using automated blood culture, in which the average time for detection was 24 and 15 days for MTC and NTM, respectively[15].

A high rate of HIV co-detection occurred in the samples received by Lacen-GO, as most of them came from AIDS patients hospitalized in HDT/HAA. The public health laboratory of Goiás frequently carries out this examination due to the prevalence of mycobacteria disseminated infections, justifying the use of this high sensitive semi-automated methodology despite its high cost. The use of this equipment in laboratories that do not handle similar profiles of patients might not be financially justified.

The major limitation of this study was the analysis of the two blood culture methodologies at different periods, as according to the laboratory´s standard procedure, they are not performed simultaneously. Each type of blood culture was analyzed during a one-year period; the months were chosen to assure the continuity of the methodology during that year. However, the semi-automated liquid blood culture was suspended for some periods due to lack of inputs. The difference in the absolute number of samples processed by each methodology was considered using appropriate statistical analyses to deal with the unbalanced sampling.

These data show the importance of using a methodology with a greater sensitivity for mycobacteria detection in blood. This methodology could also be applied reliably in studies on the prevalence of mycobacteria dissemination in AIDS patients.

The implantation of the semi-automated liquid blood culture, in reference laboratories, for HIV/AIDS patients allows a significant increase in the number of diagnosed cases of mycobacteremia and a reduction in the detection time. This methodology allowed the Lacen-GO to improve patient care by providing an early and accurate diagnosis.

Acknowledgements: Thanks to the Secretary of Health of the state of Goiás and Lacen-GO, mainly the department of Mycobacteria for assisting with the study achievement.

Conflict of interest: The authors declare no conflict of interest.

Financial Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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