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Journal of Microbiological Methods 62 (2005) 181–197
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Rapid mycobacteria drug susceptibility testing using Gel Microdrop (GMD) Growth Assay and flow cytometry

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Received 29 September 2004; received in revised form 11 February 2005; accepted 11 February 2005
Available online 29 March 2005

Abstract

The control of multi-drug-resistant tuberculosis has been hampered by the lack of simple, rapid and sensitive methods for assessing bacterial growth and antimicrobial susceptibility. Due to the increasing incidence and high frequency of mutations, it is unlikely that culture methods will disappear in the foreseeable future. Therefore, the need to modernize methods for rapid detection of viable clinical isolates, at a minimum as a gold standard, will persist. Previously, we confirmed the feasibility of using the Gel Microdrop (GMD) Growth Assay for identifying sub-populations of resistant Mycobacteria by testing different laboratory strains. Briefly, this assay format relies on encapsulating single bacterium in agarose microspheres and identifying clonogenic growth using flow cytometry and fluorescent staining. In this study, we modified the GMD Growth Assay to make it suitable for clinical applications. We demonstrated the effectiveness and safety of this novel approach for detecting drug susceptibility in clinically relevant laboratory strains as well as clinical isolates of *Mycobacterium tuberculosis*. Correlation between results using the GMD Growth Assay format and results using two well characterized methods (Broth Microdilution MIC and BACTEC 460TB) was 87.5% and 90%, respectively. However, due to the inherent sensitivity of flow cytometry and the ability to detect small (<1%) sub-populations of resistant mycobacteria, the GMD Growth Assay identified more cases of drug resistance. Using 4 clinically relevant mycobacterial strains, we assessed susceptibility to primary anti-tuberculosis drugs using both the Broth Microdilution MIC method and the GMD Growth Assay. We performed 24 tests on isoniazid-resistant BCG, *Mycobacterium tuberculosis* $H_{37}Ra$ and *Mycobacterium avium* strains. The Broth Microdilution MIC method identified 7 cases (29.1%) of resistance to INH and EMB compared to the GMD Growth Assay which identified resistance in 10 cases (41.6%); in 3 cases (12.5%), resistance to INH and EMB was detected only with the GMD Growth Assay. In addition, using 20 *Mycobacterium tuberculosis* clinical isolates, we compared results using BACTEC 460TB method performed by collaborators and the GMD Growth Assay. Eight of 20 (40%) clinical isolates, which were not identified as drug-resistant using the conventional BACTEC 460TB method, were resistant to 1, 2, or 3 different concentrations of drugs using the GMD Growth Assay (13 cases of 140 experiments). In one case (isolate 1879), resistance to 10.0 μg/ml of STR detected using BACTEC 460TB method was not confirmed by the GMD Growth Assay. Thus, the overall agreement between these methods was 90% (14 discrepant results of 140

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0167-7012/$ - see front matter
doi:10.1016/j.mimet.2005.02.012

experiments). These data demonstrate that the GMD Growth Assay is an accurate and sensitive method for rapid susceptibility testing of *Mycobacterium tuberculosis* for use in clinical reference laboratory settings.

Keywords: *Mycobacterium tuberculosis*; Gel Microdrop (GMD) Growth Assay; Drug susceptibility to isoniazid (INH); Streptomycin (STR); Ethambutol (EMB); Rifampin (RIF); Flow cytometry

1. Introduction

The emergence of multi-drug-resistant strains of *Mycobacterium tuberculosis,* which are essentially untreatable, is a serious public health problem. In the past decade, antimicrobial resistant infections have become rampant worldwide, increasing the morbidity, mortality, and cost associated with disease (Bloch et al., 1994; Cohn et al., 1997; McGray et al., 1997; Corbett et al., 2003; Iademarco and Castro, 2003; Raviglione, 2003). Nosocomial transmission of multi-drug-resistant tuberculosis (MDRTB) among HIV-infected individuals is dramatically changing the overall epidemiology of tuberculosis (Mugerwa, 1998; Aliyu and Salihu, 2003). Diagnosis of tuberculosis is usually confirmed by culturing mycobacteria from patient specimens (McGray et al., 1997; Moore et al., 1997; Lauzardo and Ashin, 2000; Loddenkemper et al., 2002; Seaworth, 2002). Efforts to control tuberculosis are severely hampered by the time required for growth, identification, and susceptibility testing (Tenover et al., 1993). The emergence of drug-resistant strains of *M. tuberculosis* is a particularly serious public health problem (Wallace et al., 1990; Moore et al., 1997; Loddenkemper et al., 2002; Seaworth, 2002; Espinal, 2003). Tuberculosis (TB) remains the leading cause of death in the world (Murray et al., 1992; Fatkenheuer et al., 1999). It is estimated that deaths from TB will reach 5 million by the year 2005 (Davis, 2000; Raviglione, 2003).

As recommended by the National MDR TB Task Force, in order to combat multi-drug-resistant tuberculosis, antimicrobial susceptibility testing must be performed on all initial and follow-up *M. tuberculosis* isolates from each patient (Centers for Disease Control and Prevention, 1992). Rapid detection of *M. tuberculosis* strains resistant to anti-tuberculous drugs is a key factor for minimizing the spread of this infection. Among currently available methods for drug susceptibility testing, the agar proportion method (MOP) is universally accepted as the "gold standard" (Woods, 2000). However, it generally takes at least 21 days for a result after assay set up. Automated systems including the BACTEC 460TB radiometric system and a Micobacteria Growth Indicator Tube 960 (MGIT 960, Becton Dickinson Microbiology Systems, Sparks, MD), a fully automated non-radiometric system, have reduced the time required for growth and antibiotic susceptibility testing from 21–35 days to 7–11 days. Drug susceptibility results obtained using both methods correlated with results using the MOP procedure (Siddigi et al., 1981; Ardito et al., 2001; Bemer et al., 2002; Tortoli et al., 2002). The most common reported problems associated with BACTEC 460TM include: the risk of needle puncture, the need to dispose radioactive waste, and potential contamination of test samples (Bemer et al., 2002; Tortoli et al., 2002). Other systems, such as the Septi-Chek AFB (Becton Dickinson), do not use radioisotopes, but require a longer detection time. ESP Culture System II and VersaTREK (TREK Diagnostics Systems, Inc., Cleveland, Ohio) are among the new liquid medium-based fully automated, aerosol-free systems that detect mycobacterial growth by automatically monitoring (every 24 min) the rate of oxygen consumption within the headspace of the culture bottle. These systems not only recover mycobacteria from clinical specimens, but also determine antimicrobial susceptibility to INH, RIF, and EMB more rapidly than the current methods. Definitive identification of the bacteria, however, may still require conventional culture and biochemical testing. Recent improvements have been directed toward integrating automated methods with probe technology, such as Amplified Mycobacterium Tuberculosis Direct Test developed by Gen-Probe (San Diego, CA), PCR-based MicroSeq 500 16S ribosomal DNA bacterial sequencing kit (Applied Biosystems, Foster City, CA), and high-performance liquid chromatography (HPLC) of mycolic acids to identify mycobacterial species or