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■ BRIEF COMMUNICATION ■

Clarithromycin Susceptibility Testing of *Mycobacterium avium* Complex Using 2,3-Diphenyl-5-thienyl-(2)-tetrazolium Chloride Microplate Assay with Middlebrook 7H9 Broth

A series of 119 *Mycobacterium avium* complex isolates were subjected to clarithromycin susceptibility testing using microplates containing 2,3-diphenyl-5-thienyl-(2)-tetrazolium chloride (STC). Among 119 isolates, 114 (95.8%) were susceptible to clarithromycin and 5 were resistant according to the new and the standard method. STC counts the low cost and reduces the number of procedures needed for susceptibility testing.

Key Words : *Tetrazolium; Microtiter Method; Mycobacterium Avium Complex; Clarithromycin; Susceptibility Testing*

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Non-tuberculous mycobacteria (NTM) account for almost a tenth of specimens smear positive for acid-fast bacilli in Korea (1). Of these, half are *Mycobacterium avium* complex (MAC) (2). Clarithromycin, a macrolide drug, forms the cornerstone of MAC treatment (3) and is the only drug recommended by the Clinical and Laboratory Standards Institute (CLSI) for drug susceptibility testing (DST) of MAC (4).

The oxidation-reduction dye, 2,3-diphenyl-5-thienyl-(2)-tetrazolium chloride (STC) has advantages for rapid and convenient DST. The compound allows easy identification of positive cultures by producing dark precipitates that change the solution from colorless to pink after solubilization; further, even when added to media before inoculation, STC has no inhibitory effect on microorganisms (5). Some studies present the advantages of STC-containing media to identify the growth of microorganisms in 96-well microplates (5, 6). Therefore, we applied a modification of standard methods to test the drug susceptibility of slowly growing mycobacteria (4) by adding STC.

A total of 119 MAC strains (56 *M. avium* and 63 *M. intracellulare*) isolated from clinical specimens, which had been stored at -70°C at the Korean Institute of Tuberculosis (KIT), were used. All isolates were subcultured initially on a slant of Löwenstein-Jensen medium and thereafter in Middlebrook 7H9 broth (DIFCO Laboratory, Detroit, MI, U.S.A.) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC; BBL, Becton Dickinson, Sparks, MD, U.S.A.) at 37°C for 5 days before DST. The bacterial suspension was adjusted to McFarland No. 0.5 by adding sterile medium according to CLSI guidelines (4). For susceptibility testing, STC-containing and STC-free 7H9 broth was dispensed into microplate wells. The STC was purchased from Tokyo Kansei Kogyo (Tokyo, Japan). Then, clarithromycin (Hanmi Pharm. Co., Ltd, Seoul, Korea) stock solution was added to the wells followed by serial dilutions to create the final concentrations of 64 to 1 μg/mL. The last three wells of each lane were filled with 7H9 broth alone without clarithromycin for growth control. Later, 100 μL of bacterial suspension was added to the

Table 1. MICs of clarithromycin for 119 *M. avium* complex isolates determined by STC-containing and STC-free 7H9 microplate methods

MIC (μg/mL) / Species	No. of isolates	≤1	2	4	8	16	32	≥64
M. avium	56	49	4					3
M. intracellulare	63	60				1		2
Total	119	109	4			1		5

MIC, minimal inhibitory concentration.

wells. The final volume of each well was 200 μL, and the final concentration of bacterial inoculum was approximately 1.5 $\times 10^5$ CFU/mL. In STC-containing medium, the final STC concentration was 50 μg/mL. Before incubation, a suspension of one antibiotic-free well was plated onto Middlebrook 7H10 agar (DIFCO) supplemented with 10% OADC (BBL) to determine the inoculum size. Then, the microplates were incubated at 37°C until the control media wells (6-9 days) developed dark precipitates secondary to STC reduction. At that point, the bacterial growth in the antibiotic-containing wells was determined by adding 50 μL of solubilizing agent. Susceptibility was defined by minimal inhibitory concentration (MIC): susceptible at ≤16 μg/mL, intermediate at 32 μg/mL, and resistant at ≥64 μg/mL at a broth pH of 6.8, as recommended by CLSI (4). The MIC was defined as the lowest drug concentration that prevented a change in the medium from colorless to pink.

The inoculation quantities of all 119 MAC isolates were optimal according to confirmation on 7H10 agar media (data not shown). The STC-containing and STC-free media resulted in completely identical MICs in all tested strains. Among the 119 MAC isolates, 114 (95.8%) were inhibited at concentrations ≤16 μg/mL and therefore were classified as clarithromycin-susceptible with no significant difference between *M. avium* and *M. intracellulare*. Among these susceptible isolates, 109 were inhibited at 1 μg/mL, 4 at 2 μg/mL, and only 1 at 16 μg/mL. Only 5 MAC isolates were resistant (Table 1). The decision for microbial growth in the microplate wells was easier in STC-containing medium than in STC-free medium because of the color indicator.

Initial isolates from patients with previously untreated MAC lung disease should be tested for clarithromycin susceptibility to establish baseline values, and many strains isolated from different situations are reserved for DST (7, 8). Although the MAC response to some antimicrobial agents may not be predicted reliably on the basis of current in vitro susceptibility test methods, clinical response correlates with DST for the macrolides (azithromycin and clarithromycin) (8). Therefore, CLSI recommends that susceptibility of MAC isolates should be evaluated for these drugs only (4). In the current study, among 114 clarithromycin-susceptible isolates, all except one were inhibited at ≤2 μg/mL of clarithromycin. This is consistent with the finding of Kawata et al. (9). STC, unlike other tetrazolium compounds, can be added to the medium before inoculation of bacteria (5) and avoids cumbersome processes such as putting tetrazolium into the well to view cultures after growth and adding extraction buffer to confirm color change (10).

In conclusion, we demonstrate that >95% of MAC isolates from Korea are susceptible to clarithromycin, and that modification of the CLSI-recommended microplate DST method using STC is useful.

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