INTRODUCTION

Mycobacterium avium Complex species, including strains of M. avium complex. They may be categorized by colony shape, elevation, margin, denticity, etc. Some of the most commonly reported morphotypes for clinical M. avium strains are: (1) a smooth, opaque, and domed type; (2) a smooth, transparent, and flat type; and (3) a rough type. Although morphotypes can be distinct, they can also overlap significantly between cultures tested. The recommended quality control (QC) organism for testing is M. avium ATCC 700898 (CLSI M-24A). In this study, M. avium ATCC 700898 stocks were tested for all four cultures. Up to eight different morphotypes were observed in three of the different antimicrobics. Four different stocks of M. avium ATCC 700898 were cultured between optimizing growth conditions to favor the most resistant morphotype for QC testing.

METHODS:

SLOMYCO Plate Inoculation cont.

- All inoculation of the plate, CFU counts were performed by selecting the positive growth control and using a sterile 1:10 loop, mixing with 37°C in a CO2 incubator and water.
- Plates were incubated up to 3-4 weeks at 34°C to 36°C. Colony counts and morphotype observations were recorded.
- Plates were covered with an adhesive seal and incubated at 34°C to 36°C in a CO2 incubator for four weeks.
- Results were read manually with a mirror box.

RESULTS

The four cultures contained the following morphotype ratios: approximately 90% smooth-dome, 7% flat- transparent, and 3% rough. M. avium ATCC 700898 stocks were tested for all four cultures. Data analysis was performed on cultures and two morphotypes, smooth-dome and flat-transparent, for a total of 136 data points.

RESULTS cont.

Figure 1. Smooth-Domed and Flat-Transparent with over light

Figure 3. Flat-Transparent with over light

DISCUSSION and CONCLUSIONS

Cultures of GC organisms M. avium ATCC 700898 show variable MIC susceptibility results in the Sensititre® SLOMYCO plate (TREK Diagnostic Systems) due to differences in clinical morphotype ratios. This finding corresponds to previously reported M. avium clinical strain colony variants and antimicrobial susceptibility trends of different morphotypes. Consequently, due to the variability of M. avium ATCC 700898, the parameters of MIC values for QC testing are wider for some isolates than for others such as was seen with the most resistant (flat-transparent) and susceptible (smooth-dome) morphotypes.

Since a wide range of MIC values can be obtained with M. avium ATCC 700898 for specific antimicrobics, it is not only important for QC testing to limit variability between cultures in order to maintain consistent results, but also to perform QC with a culture containing the most resistant morphotype to ensure the most significant difference in MIC values of end points of each antimicrobial tested. Until optimization of growth conditions can be determined, laboratories should use a flat-transparent variant to test for clinical isolates. There is published evidence that based on both macrophage and phagocytic cell experiments, the rate at which a smooth-dome to an opaque colony type transition in Mycobacterium avium. Antimicrobial Agents and Chemotherapy 12(3): 454-459. 3. It is common for smooth-dome to rough type transitions in M. avium to occur slowly, with a gradient of types present between the two end points. The rate of the transition has previously been reported to vary between 2-4 weeks. In this study, optimization of growth conditions was achieved when cultures began to change from flat- transparent to smooth-dome. The number of data points from cultures of flat- transparent and smooth-dome morphotypes are not reported here. Plates were read manually using a mirror box, and MICs were recorded and compared to previously observed in-house values.

Table 1 MIC Results for Antibiotics with Up to a 6 Fold Difference Between MICs.

Morphotype Smooth-Domed 6 3 12 17 34 3
Flatt-Transparent 0 0 0 0 0 0
Total Cultures 6 3 12 17 34 3
Culture A ~90% Smooth-Domed 37 1
Culture C ~equal % of each type 28 1
Culture D ~equal % of each type 4 28 4
Culture B ~70% Flat-Transparent 2 3
Total Cultures 4 22 66 18 1
Morphotype Smooth-Domed 11 8 1
Flatt-Transparent 21 0
Total Cultures 19 23 63 6
Morphotype Smooth-Domed 3 12 5
Flatt-Transparent 13 0
Total Cultures 16 14 30 6
Morphotype Smooth-Domed 3 8 4
Flatt-Transparent 16 0
Total Cultures 19 14 33 6
Morphotype Smooth-Domed 1 15 17
Flatt-Transparent 1 1 18
Total Cultures 2 16 19

Table 2 MIC Results for Antibiotics with Up to a 4 Fold Difference Between MICs.

Morphotype Smooth-Domed 4 2 11 17 34 3
Flatt-Transparent 0 0 0 0 0 0
Total Cultures 4 2 11 17 34 3
Culture A ~90% Smooth-Domed 28 10
Culture C ~equal % of each type 22 7
Culture D ~equal % of each type 3 1 19 15
Culture B ~70% Flat-Transparent 3 2 1
Total Cultures 97 12 2
Morphotype Smooth-Domed 8 1 17 17
Flatt-Transparent 0 0 0 0
Total Cultures 11 1 18 18
Morphotype Smooth-Domed 3 12 5
Flatt-Transparent 0 0 0 0
Total Cultures 15 15
Morphotype Smooth-Domed 1 15 17
Flatt-Transparent 0 0 0 0
Total Cultures 17 17
Morphotype Smooth-Domed 2 3 17 17
Flatt-Transparent 0 0 0 0
Total Cultures 22 22

REFERENCES


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Table 2. MIC Results for Antibiotics with Up to a 4 Fold Difference Between MICs.

Morphotype Smooth-Domed 4 2 11 17 34 3
Flatt-Transparent 0 0 0 0 0 0
Total Cultures 4 2 11 17 34 3
Culture A ~90% Smooth-Domed 28 10
Culture C ~equal % of each type 22 7
Culture D ~equal % of each type 3 1 19 15
Culture B ~70% Flat-Transparent 3 2 1
Total Cultures 97 12 2
Morphotype Smooth-Domed 8 1 17 17
Flatt-Transparent 0 0 0 0
Total Cultures 11 1 18 18
Morphotype Smooth-Domed 3 12 5
Flatt-Transparent 0 0 0 0
Total Cultures 15 15
Morphotype Smooth-Domed 1 15 17
Flatt-Transparent 0 0 0 0
Total Cultures 17 17
Morphotype Smooth-Domed 2 3 17 17
Flatt-Transparent 0 0 0 0
Total Cultures 22 22

RESULTS cont.