Rapid Molecular Detection of Macrolide Resistance in the Mycobacterium avium Complex: Are We There Yet?

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Macrolides are an important tool in the treatment of Mycobacterium avium complex infections. Here, we evaluate the use of 23S rRNA gene sequencing for the rapid detection of macrolide resistance. Routine sequencing of the 23S rRNA gene is highly specific for macrolide resistance but lacks in sensitivity.

The Mycobacterium avium complex (MAC) is comprised of Mycobacterium avium and Mycobacterium intracellulare and is implicated in various infections in the immunocompromised. Traditional antituberculosis antibiotics have not been very successful in the treatment of MAC infections, with in vivo activities as low as 1/100 the level of activity against tuberculosis. The introduction of the macrolides azithromycin and clarithromycin to the treatment regimen for MAC infections represented a major advance as these drugs showed good in vivo activity against the organisms (1).

Currently accepted methods for antibiotic sensitivity testing for MAC isolates are limited primarily to testing of the macrolides and are accomplished using broth macrodilution and microdilution (2). Broth methods for testing macrolide sensitivity of MAC strains are the only tests that have been correlated with clinical responses. Clarithromycin is the recommended class antibiotic for in vitro testing due to technical issues with azithromycin. With the elimination of the radiometric Bactec 460 system (Becton, Dickinson, and Co., Franklin Lakes, NJ), a significant method for macrodilution testing has been eliminated. Broth microdilution methods require a positive solid medium culture and a 7- to 14-day incubation period before the results can be successfully interpreted. Further, the Clinical and Laboratory Standards Institute (CLSI) recommends confirmation of resistant results from untreated patients by repeat testing prior to reporting (2). These factors can lead to a significant delay in the identification and reporting of macrolide resistance.

There have been many published reports correlating macrolide resistance with mutations at nucleotides 2058 and 2059 (Escherichia coli numbering) in the peptidyl transferase loop of the 23S rRNA gene (V domain). Previous studies have found these mutations in 80 to 100% of instances of high-level clarithromycin resistance although detection of lower-level resistance is much rarer, at between 10 and 20% (3–8). Using DNA sequencing for the detection of macrolide resistance could reduce turnaround times from 7 to 14 days from the availability of culture to 1 to 2 days. Liquid culture can also be used, which decreases the time to culture positivity. Based on the high level of correlation between 2058/2059 mutations, sequencing of the V domain of the 23S rRNA gene appears to be a viable option for the rapid detection of macrolide resistance in MAC organisms.

In an attempt to validate detection of 2058/2059 mutations for rapid detection of macrolide resistance in our laboratory, we sequenced the V domain of the 23S rRNA gene in 451 isolates previously tested for clarithromycin resistance by broth microdilution using the Sensititre SLOMYCO (slow-growing mycobacteria plate format; formerly, MAISLO) panels (Trek Diagnostic Systems Inc., Cleveland, OH) (8). Amplification and Sanger-based sequencing were performed as previously described (4). Isolates were determined to be resistant if they had an MIC value of ≥32 μg/ml, as recommended by the CLSI (2). All of the 394 sensitive isolates had wild-type sequences at the 2058/2059 position. Of the remaining 57 resistant isolates, only 28 (49%) showed a mutation at the 2058/2059 position of the 23S rRNA gene upon initial screening. Compared to findings reported in the literature, this value was very low, and further investigation was warranted. The 29 resistant isolates showing no detectable 23S rRNA mutations were resurrected from stock and subcultured to Bactec MGIT (Becton Dickenson) medium. These isolates were then subcultured to Middlebrook 7H10 agar containing 16 μg/ml clarithromycin and to nonselective Middlebrook 7H10 agar simultaneously. Eight isolates that did not grow in the presence of 16 μg/ml of clarithromycin were subsequently subcultured to agar containing 8 μg/ml clarithromycin, which resulted in growth of four of these isolates. The resulting cultures were sequenced for mutations in the V domain of the 23S rRNA gene. Six of the resistant isolates had a broth microdilution MIC value at the resistance breakpoint of 32 μg/ml; only two of these showed growth on Middlebrook 7H10 agar containing 16 μg/ml clarithromycin, and the remaining four isolates did not grow on 7H10 agar containing 8 μg/ml clarithromycin. None of these six isolates showed mutations at the 2058/2059 position whether they were grown on selective or nonselective medium.

Twenty-three isolates had MIC values for clarithromycin of ≥64 μg/ml but showed no 2058/2059 mutations. All of these strains could be grown on 7H10 agar containing clarithromycin. When grown on selective medium, 18/23 isolates had mutations at the 2058/2059 position. Six of these sequences indicated mixed populations of wild-type and nonwild-type strains, but this was...
not uncommon among the 28 strains with mutations on the initial screen. For the simultaneous cultures grown on nonselective 7H10 agar, the gene sequences for all 23 isolates were wild type. In one case, very small A2059C peaks were seen, but these were so small that they would not be distinguished from normal background during routine analysis. Two strains contained A2057T mutations. The clarithromycin MIC of one of these strains was 4 μg/ml (sensitive) and that of the other was ≥64 μg/ml (resistant). Further analysis of this mutation is required to determine its significance.

Our evaluation of 23 rRNA gene sequencing for the rapid detection of macrolide resistance in MAC organisms determined that this test does not identify resistance at the lower MIC limit for resistance determined by the CLSI. The significance of this finding and the investigation into an alternate mechanism of resistance in these strains showing low-level resistance require further exploration. After these six strains were eliminated from our analysis, 54.9% of clarithromycin-resistant MAC strains tested directly from nonselective culture were identified by 2058/2059 mutations. Mutations at positions 2058/2059 were ultimately found in 92.2% of resistant strains. While the latter value is in better agreement with available literature, growth on selective medium was required to isolate the resistant subpopulations so they could be detected by traditional PCR and Sanger sequencing. Detection of 2058/2059 mutations does provide 100% specificity, and therefore 2058/2059 mutations can be used to accurately identify resistant isolates. Due to the less optimal specificity of the test (49% when low-level resistance is included and 54.9% when low-level resistance is excluded) and its inability to detect lower level resistance, it can be used only to screen for resistance, but it cannot be used to the exclusion of phenotypic testing. Sequencing for 2058/2059 mutations could help reduce turnaround times in cases where repeat testing is required, especially if the resistant culture is available for testing. Currently available technology such as real-time PCR, next-generation sequencing platforms, or digital PCR may provide better sensitivity in the detection of these mixed populations. Further investigation into these methodologies that can detect low copy numbers of mutated sequences among much larger wild-type populations may lead to a more rapid assay for the detection of these mutations. The sensitivity of these technologies for the detection of the mixed populations in question is yet to be established. Implementation of these techniques will also be affected by the availability of technology in the clinical setting and cost effectiveness based on potentially low throughput for immediate screening of isolates.

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REFERENCES