In Vitro Activity of Amikacin Against Isolates of
Mycobacterium avium complex with Proposed MIC Breakpoints

And Finding of a 16S rRNA Gene Mutation in Treated Isolates

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Amikacin is a major drug in the treatment of Mycobacterium avium complex (MAC) disease but laboratory guidelines for susceptibility testing are not available. This study presents in vitro amikacin MICs for 462 consecutive clinical isolates of MAC using broth microdilution. Approximately 50% of isolates had amikacin MICs of 8 µg/mL and 86% had MICs of ≤16 µg/mL. Of the eight isolates (1.7%) with MICs of 64 µg/mL, five had an MIC of 32 µg/mL on repeat testing. Ten (2.1%) of isolates had an initial amikacin MIC of >64 µg/mL, of which seven (1.5%) were >64 µg/mL on repeat testing. These seven isolates had a 16S rRNA gene position 1408 A→G mutation and included M. avium, Mycobacterium intracellulare, and Mycobacterium chimaera. Clinical data were available for five of these seven, all of whom had received prolonged (>6 months) prior therapy, four with known amikacin. The 16S mutation was not detected in isolates with MICs of 64 µg/mL or lower. We recommend primary testing of amikacin against isolates of MAC and propose MIC guidelines for breakpoints identical to the CLSI guidelines for M. abscessus: ≤16 µg/mL, susceptible; 32 µg/mL, intermediate; and ≥64 µg/mL, resistant. If considered and approved by CLSI, this will be only the second drug recommended for primary susceptibility testing against MAC, and should facilitate its use for both IV and inhaled therapy.
Amikacin is considered a major drug in the treatment of *Mycobacterium avium* complex (MAC) lung disease, especially for patients who have cavitary disease or whose isolate is macrolide resistant (1,2,3). Its use is difficult in part because laboratory guidelines for susceptibility testing have not been established. The American Thoracic Society (ATS) and 2011 Clinical and Laboratory Standards Institute (CLSI) guidelines currently recommend reporting primary susceptibilities only for clarithromycin and secondarily for linezolid and moxifloxacin against isolates of MAC (1,4).

Although *in vitro* and in a murine model bactericidal and chemotherapeutic efficacy of amikacin and other aminoglycosides against MAC have been known for more than 20 years, drug toxicities and adverse events, including hearing loss, have limited the chemotherapeutic use of IV or IM amikacin, especially in elderly patients (1,5,6). The introduction of aerosolized amikacin has offered a potentially effective, practical, and less toxic alternative to parenteral administration of the antibiotic (7). The aerosolized form of amikacin has been touted as safer, and easier to tolerate, than the parenteral administration. Additionally, it may be possible to use a higher and thus presumably more effective dose than is usually tolerable with the IV or IM modality (7). This formulation is being increasingly used in patients with MAC and patients with *M. abscessus*.

We initiated a retrospective study of the *in vitro* MICs of amikacin in 462 consecutive isolates of MAC submitted for susceptibilities to the Mycobacteria/Nocardia Laboratory at UT Health Northeast (formerly The University of Texas Health Science Center at Houston).
The aim of our study was two-fold. We retrospectively examined the \textit{in vitro} MICs of amikacin in 462 consecutive isolates of MAC submitted to our laboratory for susceptibility testing to determine a resistance breakpoint. Additionally, prior amikacin use was examined in patients with initial and repeat amikacin MICs of >64 µg/mL and those with an \textit{rrn} gene mutation to help establish guidelines for an additional primary drug to treat MAC disease.
MATERIALS AND METHODS

Organisms. We studied 462 consecutive clinical isolates of MAC which had been submitted for susceptibility testing to the Mycobacteria/Nocardia Research Laboratory at UT Health Northeast between 2011 and 2012. This laboratory receives isolates for susceptibility testing from all over the U.S. Isolates were submitted as MAC, most of which had been identified as MAC by commercial probe, which according to the package insert has a sensitivity of 99.9% and specificity of 100%, although the manufacturer states that rare isolates of MAC may not be probe-positive. Isolates with initial and repeat amikacin MICs >64 µg/mL were identified as species of MAC by molecular identification using multiplex PCR and ITS sequencing as previously described (8,9).

For isolates with MICs of 64 µg/mL or greater, records were screened for additional isolates before or after the 64 µg/mL isolate.

Susceptibility testing. Antimicrobial susceptibility testing was performed using the CLSI recommended method of broth microdilution (CLSI) in cation adjusted Mueller-Hinton broth plus oleic acid-albumin-dextrose-catalase (OADC) using commercially available MIC panels (Thermofisher, formerly Trek Diagnostics, Cleveland, OH). Transparent colonies of MAC (if present) were selected from Middlebrook 7H10 agar plates. The colonies were inoculated into cation-adjusted Mueller-Hinton broth plus 5% OADC. Inoculum was standardized to match a 0.5 McFarland standard by using a nephelometer. Serial dilutions were performed to deliver a final concentration of approximately 1x10^5 to 5x10^5 CFU/mL in 0.1 mL volume. Susceptibilities were read
using a mirrored light box (Trek Diagnostics, Cleveland, OH) after incubation at 35°C in room air for 7 days. The end point (MIC) was complete inhibition of growth. Concentrations of amikacin tested ranged from ≤1 to 64 µg/mL. Isolates with amikacin MICs of 64 µg/mL or >64 µg/mL underwent repeat testing. For isolates with MICs of 64 or >64 µg/mL, the growth in the next lowest MIC well was reviewed and compared to the control growth.  

**Patients.** Clinical information about amikacin use and the testing of any additional isolates was sought in patients with MAC isolates with amikacin MICs of 64 µg/mL or >64 µg/mL. This study was approved by the UT Health Northeast and Duke University institutional review boards.  

**Variable Number Tandem Repeat (VNTR) Typing.** VNTR typing of the MAC isolates with MICs >64 µg/mL and prior isolates with MICs of ≤32 µg/mL from the same patient was performed using previously described methods (8,10,11). Isolates of *M. chimaera* were tested using *M. intracellulare* primers (8).  

**Quality Control.** Quality control was performed using *Mycobacterium avium* ATCC 700898 and incubation for 7 days as for clinical isolates. The acceptable range of MICs was derived by using values ± one dilution from the modal value in a series of 34 test values. Additional quality control was provided using *Pseudomonas aeruginosa* ATCC 27853. After the established 18-24 hour incubation for bacteria, the acceptable MIC range for *P. aeruginosa* was 1-4 µg/mL as previously defined by the CLSI (12).  

**PCR Restriction Endonuclease Analysis (PRA) of the 16S rRNA gene.** An approximately 520-bp sequence of the 16S rRNA gene that included bp position 1408 was selected for study and was amplified by PCR. Briefly, 25 microliters of reaction...
mix, which consists of 12.5 µl of Fail Safe Pre-Mix I, 2 µmol of each primer, 0.625 U of Fail Safe PCR enzyme (Epicentre, Madison, WI) and 1 µl of template DNA was placed in a thermocycler for 35 cycles. Primers 261 corresponding to 16S rRNA *Escherichia coli* position 1539-1520 (5'-AAGGAGGTGTCCAGCCGCA-3') and 297 corresponding to 16S rRNA *E. coli* position 1056-1075 (5'-TCCCTTGTGGCCTGTGTGCA-3' were used (13). The amplicon size was approximately 520 and the readable sequence was 480-bp.

Restriction endonuclease *Tsp*45I (New England Bio Labs, Ipswich, MA) failed to cut the 520-bp amplicon in the presence of the A1408→G mutation but cut the wild type sequence into two fragments of approximately 130 and 390-bp. PRA was performed on isolates with amikacin MICs >64 µg/mL. The use of this technique allows for detection of a mixed population of isolates with and without the A1408→G mutation (see Figure 2).

16S rRNA Gene Sequencing. Isolates were subjected to sequencing of the 16S rRNA gene region previously shown to confer resistance to amikacin in other mycobacteria (positions 1408, 1409, 1411) (*Escherichia coli* numbering system) (13).
RESULTS

Organisms. A total of 462 consecutive isolates from multiple states within the U.S. were tested. Isolates with initial and repeat amikacin MICs >64 were re-confirmed as MAC using a 16S multiplex PCR or commercially available DNA probes (AccuProbe, Hologic Gen Probe, San Diego, CA). All seven of the repeat isolates were identified to species of the *M. avium* complex by ITS sequencing (8). These results demonstrated the presence of one isolate of *M. avium*, six isolates of *M. intracellulare*, and one isolate of *M. chimaera*.

An additional 16 isolates from five of the seven patients with MAC isolates with initial and repeat MICs >64 µg/mL from different time periods before and after the MIC >64 µg/mL were identified and available for amikacin MIC and 16S rRNA gene analysis comparisons (see Table 1).

Susceptibility Testing. Almost one half (48.9%) of the 462 consecutive clinical isolates had amikacin MICs <8 µg/mL and almost 90% (85.7%) had MICs of <16 µg/mL. The MIC$_{50}$ was 16 µg/mL and MIC$_{90}$ at 32 µg/mL. Only 1.7% of the isolates had amikacin MICs of 64 µg/mL and only 2.1% of isolates had MICs >64 µg/mL (see Table 2). All eight isolates with amikacin MICs of 64 µg/mL had minimal (+/-) growth in the 32 µg/mL well. On repeat testing, five of eight isolates had MICs of 32 µg/mL. Of the eight patients with MAC MICs of 64 µg/mL, clinical histories were inadequate to determine if there was history of a prior amikacin treatment.

Of the 10 isolates with initial MICs >64 µg/mL, seven were reproducible on repeat testing (see Table 2). Growth in the 32 µg/mL and 64 µg/mL wells were
equivalent to the control growth. The remaining three isolates had repeat MICs of 64 µg/mL (± growth at 32 µg/mL).

**Patients.** Of the seven patients with MAC isolates with MICs >64 µg/mL, on both initial and repeat testing clinical histories were available on five. All five patients [Table 1, cases #1-5] had histories of prolonged (>6 months) prior drug therapy. Four patients were known to have received amikacin (three inhaled and IV, one inhaled only), and all were considered treatment failures. Not surprisingly, 5/7 isolates were also macrolide resistant with clarithromycin MICs of >64 µg/mL. The case summary of one patient is listed below.

**Case Summary.** Patient one was a 59 year old female with nodular MAC lung disease. She was treated for 5 years with clarithromycin, ethambutol, and rifabutin with subsequent cessation of treatment for approximately 5 years when the patient’s isolate became macrolide resistant. Subsequently, the patient clinically worsened and showed marked worsening of her nodular disease by pulmonary computed tomography (CT). Multiple sputa (6) over the past 3 years had 3+ to 4+ colony counts on 7H10 agar for MAC. At this time (see Figure 1), the patient was started on three times weekly 300 mg rifabutin and 35 mg/kg ethambutol. (The patient had never been on amikacin, but had been on the latter two drugs at the same doses for the first 5 years.) A regimen of 500 mg daily aerosolized amikacin was then added. The amikacin MIC was 8 µg/mL and had a wild type 16S rRNA gene at position 1408. At 6 months following initiation of aerosolized amikacin the patient’s sputum MAC colony counts on 7H10 agar decreased to <50 colonies and subsequently the patient had her first negative AFB culture in many years. However, at approximately 7 months after initiation of amikacin, the patient’s
MAC colony counts on sputum cultures rose to 3+ to 4+ on 7H10 Middlebrook agar and repeat amikacin MICs were >64 µg/mL. Follow-up amikacin MICs on additional isolates were also >64 µg/mL on both initial and repeat testing. Sequencing of the 16S rRNA gene showed the presence of an A→G mutation at position 1408 in the two relapse isolates. Clinically, however, the patient remained much improved and her chest CT showed dramatic clearing of her nodular lung disease. The patient remains stable as of this report. A graphic representation of the clinical treatment with amikacin related to the amikacin MICs and \textit{rrn} mutations is shown in Figure 1.

**Quality Control.** The amikacin MICs for the \textit{M. avium} control strain ranged from 4-16 µg/mL. The strain of \textit{M. avium} ATCC 700898 was tested 34 times. The modal MIC was 8 µg/mL (28 values) with five values at 4 µg/mL and 1 value at 16 µg/mL. The MICs for \textit{P. aeruginosa} ATCC 27853 were within the CLSI established acceptable range of 1-4 µg/mL (12).

**VNTR Typing**

Pre-treatment isolates with amikacin MICs of 8 µg/mL and post-therapy isolates of >64 µg/mL on initial and repeat testing in four patients (Table 1, cases 1, 2, 4, and 5), and one isolate obtained 4 months after an MIC of >64 µg/mL which was 32 µg/mL, underwent VNTR typing. All paired isolates belonged to the same species and all had the same VNTR type excluding the possibility of infection with a new recent isolate with an MIC >64 µg/mL or specimen contamination. Case 4 had two VNTR genotypes on initial testing (Table 1).

**PRA of the 16S rRNA Gene**

A total of 40 patient isolates with amikacin MICs of ≤32 µg/mL underwent PRA.
All exhibited a wild type bp position 1408. Of the eight isolates with initial amikacin MICs of 64 µg/mL (all with minimal + growth at 32 µg/mL), all exhibited a wild type bp at position 1408. Of the 10 isolates with initial amikacin MICs of >64 µg/mL, seven isolates from six patients had the same MIC on repeat testing. All seven of these isolates exhibited a mutation at 16S rRNA position 1408. The three isolates with a repeat MIC of <64 µg/mL were also wild type.

16S rRNA Gene Sequencing

A 16S rRNA gene mutation of bp 1408 A→G was observed in all seven of the isolates with a mutation pattern on PRA testing (see Figure 2). Upon sequencing of the 530-bp region, no other mutations were observed. Sequencing of seven isolates with MICs of 32 µg/mL and 64 µg/mL, and >64 µg/mL on only one of two MIC determinations also showed a wild type 530-bp region.
DISCUSSION

This study demonstrates that 96.2% of clinical MAC isolates submitted for susceptibility testing have MICs of \(< 32 \mu g/mL\) using the CLSI susceptibility method (4).

The amikacin MIC values are consistent within achievable serum and inhaled levels and with the clinical response to MAC seen when amikacin is used, especially in patients with cavitary disease or macrolide resistance (2). Prolonged exposure to amikacin was present in isolates with initial and repeat MICs of \(> 64 \mu g/mL\), which correlated with the development of a 16S rRNA gene A\(\rightarrow\)G mutation at position 1408. This was clearly demonstrated in case #1 (Figure 1).

The amikacin MIC results reported in each well for each isolate with MICs \(\geq 64 \mu g/mL\) were compared to the control growth in each panel. There are several possible explanations for the minimal amounts of growth seen at 32 \(\mu g/mL\) with isolates with amikacin MICs of 64 \(\mu g/mL\) (interpreted as + growth). These include the presence of an aminoglycoside modifying enzyme which is weakly inducible such as the \(erm\) gene seen in rapidly growing mycobacteria (14). Other possibilities are related to the presence of inoculum effects in drugs such as ethambutol or sulfonamides which need carefully controlled inocula to prevent trailing endpoints (3), or that the prior concentration with growth (i.e., 32 \(\mu g/mL\)) is very close to the real MIC, which still could be within achievable drug levels (especially for inhaled amikacin) (13,15,16).

The + growth interpretation at 32 \(\mu g/mL\) could also be attributed to reader interpretation. Multiple individuals read the amikacin MICs over the course of the study. A comparison of readings performed by readers with less experience in reading MAC
susceptibilities (<2 years) compared to readers with considerable experience (>5 years) showed that the wells recorded as “+” by the less experienced reader would have been recorded as “negative” by the more experienced reader (BBE, unpublished observations). This was supported by the finding that most of the isolates with initial amikacin MICs of 64 µg/mL had MICs of 32 µg/mL on repeat testing (5/8). Thus, just as CLSI guidelines currently recommend repeating amikacin MICs for isolates of *M. abscessus* with MICs >64 µg/mL, it is important to confirm amikacin MICs >64 µg/mL in isolates of MAC either by repeating the MIC or by sending to a qualified reference laboratory with specific experience in MAC susceptibility testing (4).

The assertion that an elevated amikacin MIC should be deemed amikacin-resistant (with treatment implications) may seem to contradict earlier work that found no association between aminoglycoside (streptomycin in this case) MICs and clinical outcomes in MAC pulmonary disease (17,18,19). However, the differences between the present findings and the earlier work are readily explicable. The patients in the prior studies had not been previously treated for MAC, and a relatively small number of isolates had streptomycin MICs of >64 µg/mL. If the resistance cutoff for streptomycin is similar to amikacin (which might be expected given similar pharmacokinetics and mechanism of action), a very small number of patients in these studies would be in the "resistant" range, providing limited power to discern differences. Variation of MICs within a “susceptible” range would not necessarily be expected to be associated with differences in clinical outcomes. Furthermore, all patients in these studies were treated with multiple drugs, so susceptibility or resistance to a single drug might have had limited impact on outcomes. Furthermore, heterogeneity in severity and extent of
Clinical disease reduces the power to detect significant associations between antimicrobial susceptibility and treatment outcomes. Larger, prospective studies will be needed to address these questions.

Additional mutations related to amikacin resistance other than \( rm \) 1408 A\( \rightarrow \)G have been demonstrated in other mycobacterial species (16). It is possible that MAC isolates with amikacin MICs \( \geq 64 \ \mu g/mL \) may harbor a mutation other than the 1408 mutation and this question remains under investigation.

We recommend that isolates of MAC be routinely tested against amikacin by broth microdilution. We further propose that the amikacin breakpoints for \( M. \) abscessus susceptibility be adopted for isolates of MAC: MICs \( \leq 16 \ \mu g/mL \) be considered as susceptible; 32 \( \mu g/mL \), intermediate; and \( \geq 64 \ \mu g/mL \), resistant. These values may allow for better usage of amikacin with either IV or inhaled drug, and recognition that MICs of \( >64 \ \mu g/mL \) on initial and repeat testing may develop with prolonged drug therapy as a consequence of a ribosomal mutation not seen among isolates with lower amikacin MICs. There are no data at present to suggest that breakpoints for inhaled amikacin for both \( M. \) abscessus and MAC may be different than those approved/proposed for intravenous administration. [There currently are no CLSI breakpoints for two other inhaled antibiotics (tobramycin and aztreonam) which are FDA approved for treatment of \( Pseudomonas \) aeruginosa in patients with cystic fibrosis.] The single case report (case 1) and other isolates from patients with available treatment histories suggest that higher inhaled concentrations of amikacin are not effective in the presence of the ribosomal mutation. The efficacy of inhaled therapy for isolates with MICs of 64 \( \mu g/mL \) will need evaluation, as their response for therapy is not known.
ACKNOWLEDGMENTS

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Figure 1. Graphic representation of Patient 1 (case 1) with *Mycobacterium intracellulare* lung disease and the microbiological response to addition of inhaled amikacin. The semi-quantitation of MAC sputum growth on 7H10 agar has previously been described (1). The cultures were in the 3+ to 4+ range in the 3 years from 2009 to 2011. Note the drop in colony counts of MAC over a 5 month period, then a return to 3+ positive culture associated with a rise in amikacin MIC to >64 µg/mL and the development of the ribosomal mutation. W.T. = wild type 16S rRNA gene at bp position 1408. Mut. = mutation at position 1408.
Table 1. Correlation of amikacin MICs and prior amikacin exposure available on five of the seven patients with a "Mycobacterium avium" complex isolate with an initial and repeat amikacin MIC of >64 µg/mL, and a 16S rRNA gene A→G mutation at bp-1408.

<table>
<thead>
<tr>
<th>Case</th>
<th>Culture ID</th>
<th>Type</th>
<th>Amikacin MIC (µg/mL) (Date)</th>
<th>Exposure to Amikacin bp-1408</th>
<th>Clinical History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MA-2778#4</td>
<td>-</td>
<td>32 (12/30/05)</td>
<td>Inhaled x 9 months (2012)</td>
<td>MAC disease x 11 years; Improved AFB culture</td>
</tr>
<tr>
<td></td>
<td>MA-5225</td>
<td>M. intra #16</td>
<td>8 (5/20/11)</td>
<td>W.T.</td>
<td>positivity, chest CT on inhaled amikacin, then relapsed microbiologically (see Figure 1)</td>
</tr>
<tr>
<td>2</td>
<td>MA-5812</td>
<td>-</td>
<td>16 (2/08/12)</td>
<td>W.T.</td>
<td>11/2008 – 1/20/10 on IV amikacin with other drugs;</td>
</tr>
<tr>
<td></td>
<td>MA-5826</td>
<td>&gt;64 (7/2012)</td>
<td>W.T.</td>
<td>Mut.</td>
<td>On 2/20/10 – 5/11/11 on inhaled amikacin;</td>
</tr>
<tr>
<td></td>
<td>MA-5278</td>
<td>M. intra #16</td>
<td>&gt;64 (10/20/12)</td>
<td>Mut.</td>
<td>drugs/doses at another institution;</td>
</tr>
<tr>
<td></td>
<td>MA-4437</td>
<td>-</td>
<td>8 (1/11/10)</td>
<td>W.T.</td>
<td>5/11/11 returned and cultures, CT improved</td>
</tr>
<tr>
<td></td>
<td>MA-5263</td>
<td>M. intra #40</td>
<td>8 (12/20/10)</td>
<td>W.T.</td>
<td>had worsened clinically;</td>
</tr>
<tr>
<td></td>
<td>MA-5263</td>
<td>-</td>
<td>8 (12/20/10)</td>
<td>W.T.</td>
<td>with switch to streptomycin</td>
</tr>
<tr>
<td></td>
<td>MA-5846</td>
<td>&gt;64 (5/11/11)</td>
<td>Mut.</td>
<td>5/11/11 returned and cultures, CT improved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MA-5222</td>
<td>M. intra #40</td>
<td>&gt;64 (12/05/11)</td>
<td>Mut.</td>
<td>had worsened clinically;</td>
</tr>
</tbody>
</table>


Table 1. Correlation of amikacin MICs and prior amikacin exposure available on five of the seven patients with a *Mycobacterium avium* complex isolate with an initial and repeat amikacin MIC of >64 µg/mL, and a 16S rRNA gene A→G mutation at bp-1408 (cont’d).

<table>
<thead>
<tr>
<th>Case</th>
<th>Culture ID</th>
<th>Species Type</th>
<th>Amikacin MIC (µg/mL) (Date)</th>
<th>Exposure to Amikacin</th>
<th>Exposure to rrn bp-1408</th>
<th>Clinical History</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Pre-Rx</td>
<td>Not available</td>
<td>On/off over 5 years (2005-2009)</td>
<td>CF; severe cavitary disease; also frequent use of inhaled tobramycin; Worsening, on lung transplant list</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MA-3675</td>
<td><em>M. intra</em></td>
<td>&gt;64 (7/25/07)</td>
<td>Mut.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pre-Rx</td>
<td>-</td>
<td>2 months IV, W.T.</td>
<td>Improved, then relapsed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MA-2799</td>
<td>#15, new</td>
<td>16 (4/07/03)</td>
<td>13 months inhaled over 4 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MA-5319</td>
<td>#15</td>
<td>&gt;64 (01/23/12)</td>
<td>Mut.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MA-5319</td>
<td>#15</td>
<td>&gt;64 (01/23/12)</td>
<td>Mut.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Correlation of amikacin MICs and prior amikacin exposure available on five of the seven patients with a *Mycobacterium avium* complex isolate with an initial and repeat amikacin MIC of >64 µg/mL, and a 16S rRNA gene A→G mutation at bp-1408 (cont'd).

<table>
<thead>
<tr>
<th>Case</th>
<th>Culture ID</th>
<th>Species Type</th>
<th>VNTR</th>
<th>Amikacin MIC (µg/mL)</th>
<th>Exposure to Amikacin</th>
<th>Amikacin bp-1408</th>
<th>Clinical History</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>MA-5215</td>
<td><em>M. chim</em></td>
<td>#41</td>
<td>8 (11/2011)</td>
<td>Unknown</td>
<td>W.T.</td>
<td>History of treated MAI “Treatment failure”</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>401</td>
<td>MA-5582</td>
<td><em>M. chim</em></td>
<td>#41</td>
<td>&gt;64 (6/2012)</td>
<td>Mut.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

W.T. = wild type *rrn* bp 1408

Mut. = A→G mutation at *rrn* bp 1408

Pre-Rx = Pre-treatment

Post-Rx = Post-treatment

M. intra = *M. intracellulare*

M. chim = *M. chimaera*
Table 2. Amikacin broth microdilution initial MICs (µg/mL) of 462 consecutive clinical isolates of *Mycobacterium avium* complex using the CLSI approved broth microdilution method (4).

<table>
<thead>
<tr>
<th>Amikacin Concentration</th>
<th>MICs (µg/mL)</th>
<th>Mode MIC (µg/mL)</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>No. of Isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with initial MICs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative % for each initial MIC value</td>
<td>(1.5)</td>
<td>(5.4)</td>
<td>(17.7)</td>
<td>(48.9)</td>
</tr>
</tbody>
</table>
REFERENCES


ERRATUM

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