Wild-Type MIC Distributions for Aminoglycoside and Cyclic Polypeptide Antibiotics Used for Treatment of Mycobacterium tuberculosis Infections

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The emergence of multidrug-resistant (MDR) tuberculosis (TB) and extensively drug-resistant (XDR) Mycobacterium tuberculosis creates higher demands for accurate and reproducible antimicrobial drug susceptibility testing (DST). The epidemiological cutoff value (ECOFF) separating wild-type susceptible strains from non-wild-type strains is an important but rarely used tool for indicating susceptibility breakpoints against Mycobacterium tuberculosis. In this study, we established wild-type MIC distributions on Middlebrook 7H10 medium for amikacin, kanamycin, streptomycin, capreomycin, and viomycin using 90 consecutive clinical isolates and 21 resistant strains. Overall, the MIC variation between and within runs did not exceed ±1 MIC dilution step, and validation of MIC values in Bactec 960 MGIT demonstrated good agreement. Tentative ECOFFs defining the wild type were established for all investigated drugs, including amikacin and viomycin, which currently lack susceptibility breakpoints for 7H10. Five out of seven amikacin- and kanamycin-resistant isolates were classified as susceptible to capreomycin according to the current critical concentration (10 mg/liter) but were non-wild type according to the ECOFF (4 mg/liter), suggesting that the critical concentration may be too high. All amikacin- and kanamycin-resistant isolates were clearly below the ECOFF for viomycin, and two of them were below the ECOFF for streptomycin, indicating that these two drugs may be considered for treatment of amikacin-resistant strains. Pharmacodynamic indices (peak serum concentration [Cmax]/MIC) were more favorable for amikacin and viomycin compared to kanamycin and capreomycin. In conclusion, our data emphasize the importance of establishing wild-type MIC distributions for improving the quality of drug susceptibility testing against Mycobacterium tuberculosis.

The aminoglycosides and cyclic polypeptides are essential drugs in the treatment of multidrug-resistant tuberculosis, underscoring the need for accurate and reproducible antimicrobial drug susceptibility testing (DST). The epidemiological cutoff value (ECOFF) separating wild-type susceptible strains from non-wild-type strains is an important but rarely used tool for indicating susceptibility breakpoints against Mycobacterium tuberculosis. In this study, we established wild-type MIC distributions on Middlebrook 7H10 medium for amikacin, kanamycin, streptomycin, capreomycin, and viomycin using 90 consecutive clinical isolates and 21 resistant strains. Overall, the MIC variation between and within runs did not exceed ±1 MIC dilution step, and validation of MIC values in Bactec 960 MGIT demonstrated good agreement. Tentative ECOFFs defining the wild type were established for all investigated drugs, including amikacin and viomycin, which currently lack susceptibility breakpoints for 7H10. Five out of seven amikacin- and kanamycin-resistant isolates were classified as susceptible to capreomycin according to the current critical concentration (10 mg/liter) but were non-wild type according to the ECOFF (4 mg/liter), suggesting that the critical concentration may be too high. All amikacin- and kanamycin-resistant isolates were clearly below the ECOFF for viomycin, and two of them were below the ECOFF for streptomycin, indicating that these two drugs may be considered for treatment of amikacin-resistant strains. Pharmacodynamic indices (peak serum concentration [Cmax]/MIC) were more favorable for amikacin and viomycin compared to kanamycin and capreomycin. In conclusion, our data emphasize the importance of establishing wild-type MIC distributions for improving the quality of drug susceptibility testing against Mycobacterium tuberculosis.

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relationship between the wild-type MIC distribution and the current critical concentration for ethambutol (23). In the determination of clinical breakpoints for DST with regard to most other bacterial pathogens, wild-type MIC distributions represent a significant and necessary tool (9). The definition of a wild-type strain is a microorganism without acquired and mutational resistance mechanisms to a certain drug. The wild-type cutoff, which is commonly labeled the epidemiological cutoff value (ECOFF), is one important tool in addition to pharmacokinetic and pharmacodynamic (PK/PD), as well as clinical, data used by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) when setting clinical breakpoints (9). Surprisingly, published wild-type MIC distributions for M. tuberculosis are limited, but the lack of such data is commonly discussed (4, 11, 21, 25). Clinical outcome data in relation to MIC determinations are very difficult to achieve for second-line drugs against MDR TB because several drugs are used in combination. As a result, wild-type MIC distributions and PK/PD data are essential in order both to establish reasonable breakpoints for M. tuberculosis and to be able to predict susceptibility patterns for novel anti-TB drugs. Such data could shed some light on the conflicting reports of cross-resistance within the aminoglycoside group, for which the lack of background data in establishing the current critical concentrations could be a significant cause of the variable results (8, 15, 16).

In the present study we aimed to evaluate the current critical concentrations for injectable aminoglycosides and cyclic polypeptides used in the treatment of M. tuberculosis by comparing them to wild-type MIC distributions and available PK/PD data.

### MATERIALS AND METHODS

**Strains of Mycobacterium tuberculosis and quality control.** To establish the wild-type distribution, 95 clinical isolates from the clinical mycobacteriology laboratory at Karolinska University Hospital were included. Five isolates were excluded due to clustering according to the restricted fragment length polymorphism (RFLP) database at the Swedish Institute for Disease Control as previously described (23). Additionally, 21 poly- and multidrug-resistant strains were included. The MIC determinations were performed in two separate runs after blinded renumbering. In each run, the pan-susceptible M. tuberculosis H37Rv strain (ATCC 27294) was tested in duplicate. In the second run, six strains were tested in duplicate along with three consecutive strains from the same patient.

**Drug susceptibility testing.** Susceptibility testing to first-line drugs was performed previously using the Bactec 460 method (B460) (Becton Dickinson). The results were replicated in the same way. Inoculated agar plates were incubated at 37°C for four times, and seven isolates, including resistant and susceptible strains, were tested twice. Three consecutive isolates with aminoglycoside resistance, representing separate cultures within a year, from an MDR TB patient, showed excellent reproducibility for viomycin and capreomycin, while the MIC for the other tested drugs were above 64 mg/liter at all occasions.

**Wild-type distributions in relation to current critical concentrations and cross-resistance.** For all injectable drugs investigated (streptomycin, viomycin, capreomycin, kanamycin, and amikacin), the MICs of consecutive susceptible clinical isolates formed a distinct normal distribution—the wild-type MIC distribution (Fig. 1a to e). Tentative ECOFFs separating susceptibility to amikacin (1.0 mg/liter) was tested on isolates that were resistant to any first-line drug, while the susceptibility to capreomycin (1.25 mg/liter) and streptomycin (2 mg/liter) was tested on MDR strains.

### RESULTS

**Quality control and reproducibility of MIC determinations.** Overall, the MIC variation between and within runs did not exceed one MIC dilution step (Table 1). H37Rv was tested four times, and seven isolates, including resistant and susceptible strains, were tested twice. Three consecutive isolates with aminoglycoside resistance, representing separate cultures within a year, from an MDR TB patient, showed excellent reproducibility for viomycin and capreomycin, while the MICs for the other tested drugs were above 64 mg/liter at all occasions.

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**TABLE 1. Reproducibility of MIC determinations between and within rounds**

<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Amikacin</th>
<th>Kanamycin</th>
<th>Streptomycin</th>
<th>Capreomycin</th>
<th>Viomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC 1</td>
<td>MIC 2</td>
<td>MIC 1</td>
<td>MIC 2</td>
<td>MIC 1</td>
</tr>
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<td>H37Rv</td>
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<td>0.5</td>
<td>0.5</td>
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<td>&gt;64</td>
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<td>&gt;64</td>
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<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>07-9206&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>&gt;64</td>
<td>0.5</td>
<td>0.5</td>
<td>&gt;64</td>
</tr>
<tr>
<td>08-2095&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;64</td>
<td>&gt;64</td>
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<td>&gt;64</td>
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<tr>
<td>08-8079&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values shown are expressed as milligrams per liter.

<sup>b</sup> *, consecutive isolates from the same patient.
FIG. 1. Panels a to d show MIC wild-type distributions for injectable drugs against *M. tuberculosis*. (a) Amikacin MIC wild-type distribution. Consecutive susceptible clinical isolates were used to define the wild-type MIC distribution (*n* = 88, indicated by bars filled with vertical lines). MIC levels for amikacin- and kanamycin-resistant strains (*n* = 7) and isolates resistant against any drug are shown. The epidemiological wild-type cutoff (ECOFF) is indicated by an arrow (1 mg/liter). (b) Kanamycin MIC wild-type distribution. Consecutive susceptible clinical isolates were used to define the wild-type MIC distribution (*n* = 88). MIC levels of kanamycin- and amikacin-resistant strains (*n* = 7) and isolates resistant against any drug are shown. The currently recommended critical concentration (5 mg/liter) and the epidemiological wild-type cutoff (ECOFF, 4 mg/liter) are indicated by arrows. (c) Streptomycin MIC wild-type distribution. Consecutive susceptible clinical isolates were used to define the wild-type MIC distribution (*n* = 86). MIC levels of streptomycin-resistant strains (*n* = 15) and isolates resistant against any drug are shown. The currently recommended critical concentration and the epidemiological wild-type cutoff (ECOFF) are indicated by arrows (both are 2 mg/liter). (d) Capreomycin MIC wild-type distribution. Consecutive susceptible clinical isolates were used to define the wild-type MIC distribution (*n* = 88, indicated by bars filled with vertical lines). MIC levels of capreomycin-resistant strains (*n* = 7) and isolates resistant against any drug are shown. The currently recommended critical concentration (10 mg/liter) and the epidemiological wild-type cutoff (ECOFF, 4 mg/liter) are indicated by arrows. Five isolates with an MIC of 8 mg/liter were susceptible to capreomycin according to the critical concentration (10 mg/liter) but had a higher MIC than ECOFF (4 mg/liter) and showed cross-resistance to kanamycin and amikacin. (e) Viomycin MIC wild-type distribution. Consecutive susceptible clinical isolates were used to define the wild-type MIC distribution (*n* = 88, indicated by bars filled with vertical lines). The MIC level of the viomycin-resistant strain (*n* = 1) is shown, and isolates resistant against any drug are shown. The epidemiological wild-type cutoff (ECOFF) is indicated by an arrow (2 mg/liter).
wild-type from non-wild-type strains were defined for all investigated drugs (Table 2), including viomycin and amikacin, for which no recommended critical concentration for solid medium is available. The ECOFFs (i.e., the highest MIC of the wild-type distribution) were defined as 4 mg/liter for kanamycin and capreomycin, 2 mg/liter for streptomycin and viomycin, and 1 mg/liter for amikacin. Five out of seven amikacin- and kanamycin-resistant isolates were classified as susceptible to capreomycin according to the current critical concentration for 7H10 (10 mg/liter) but had MIC levels at 8 mg/liter, which was non-wild type according to the ECOFF (4 mg/liter). All seven amikacin- and kanamycin-resistant isolates were clearly below the ECOFF (2 mg/liter) for viomycin (Table 2). Two isolates clearly below the ECOFF (2 mg/liter), as well as below the critical concentration, for streptomycin were resistant to capreomycin, kanamycin, and amikacin but below the ECOFF for viomycin (Table 2).

The MIC results were confirmed with a subset of strains using Bactec MGIT 960 and Bactec 460. A comparison of the results from MIC determination for amikacin in the Middlebrook 7H10 medium using the tentative ECOFF (1 mg/liter) with susceptibility results in B460 (1 mg/liter) showed full agreement (29/29, including 7 resistant isolates). This was also the case for streptomycin, for which a total agreement between Middlebrook 7H10 and B460 was observed (11/11, including 10 resistant isolates according to the ECOFF and the critical concentration). Regarding capreomycin, the agreement was 80% (8/10), for which the two discordant isolates resistant to capreomycin in B460 had MICs of 8 mg/liter in Middlebrook 7H10, which would be interpreted as susceptible according to the current critical concentration (10 mg/liter) but non-wild type according to the ECOFF for capreomycin (4 mg/liter). Furthermore, these two strains were highly resistant to kanamycin and amikacin but had MICs below ECOFF to viomycin (Table 2 and Fig. 1a to e). There was a good agreement of MIC determinations for amikacin between Bactec MGIT 960 and Middlebrook 7H10 for susceptible and resistant strains (n = 9; Table 3).

A pharmacodynamic target of an fCmax/MIC value of ≥10 was achieved in the majority of the wild-type strains and was more favorable for amikacin, streptomycin, and viomycin. The results from the PK/PD calculations are shown in Table 4. It should be noted that no pharmacodynamic targets have been determined for M. tuberculosis, although a Cmax/MIC at a minimum of 8 to 10 is a generally accepted target for the aminoglycoside group (10, 18). The Cmax/MIC target for cyclic polypeptides is unknown. In general, the selected PD index (fCmax/MIC) was most favorable for amikacin, followed by streptomycin and viomycin, since a higher fCmax/MIC ratio among wild-type isolates was achieved for these drugs than for kanamycin and capreomycin (Table 4). For both kanamycin and capreomycin, not all wild-type strains were covered by the target of an fCmax/MIC value of ≥10 (Table 4).

**DISCUSSION**

In an effort to reevaluate the current critical concentrations used in drug susceptibility testing for *Mycobacterium tuberculosis*, we established wild-type MIC distributions of the most commonly used aminoglycosides and cyclic polypeptides using a highly reproducible method.

As an example of the usefulness and importance of the MIC wild-type distributions, five isolates with an MIC of 8 mg/liter for capreomycin would be regarded as susceptible according to the present critical concentration (10 mg/liter) in Middlebrook 7H10 but were defined as non-wild type according to the ECOFF (4 mg/liter) (Fig. 1d). The fact that these strains were resistant to amikacin and kanamycin makes it questionable whether they are fully accessible for treatment with standard doses of capreomycin. This is also supported by a calculated fCmax/MIC value close to zero at a MIC of 8 mg/liter for capreomycin (Table 4). Although we used Cmax levels in the lower range and there are variations in pharmacokinetics among individuals, it seems that the current critical concentration in 7H10 for capreomycin is too high. Truly resistant isolates may thus be reported as susceptible, which subsequently can lead to a susceptibility pattern with an overestimation of therapeutic options and in the end to the development of resistance to other drugs. We suggest that the clinical breakpoint (critical concentration) for capreomycin in Middlebrook 7H10 should be revised to 4 mg/liter.

Both MIC data as such and PD indices were more favorable for amikacin and viomycin than kanamycin and capreomycin. Although the pharmacodynamic target for *M. tuberculosis* is unknown, using the commonly accepted target for Gram-positive and Gram-negative bacteria of an fCmax/MIC value of ≥8 to 10 (10, 18), we could show that wild-type isolates below the ECOFF for amikacin, streptomycin, and viomycin were readily covered (Table 4). This supports the validity of our data, con-
Sidering streptomycin is one of the few drugs for which clinical outcome data in relation to MIC levels and critical concentrations are available (7). Of particular importance for the aminoglycosides, therapeutic drug monitoring is possible by MIC determination of the M. tuberculosis isolate combined with C\text{max} determinations and is available in most reference hospitals.

Today, critical concentrations for 7H10 are available for kanamycin, streptomycin, and capreomycin, but no critical concentrations are defined for amikacin and viomycin (25). According to our data, we suggest clinical breakpoints (critical concentrations) for amikacin and viomycin at the tentative ECOFFs (1 mg/liter for amikacin and 2 mg/liter for viomycin). It is possible, however, that adding MIC data for more strains using data from several laboratories could shift the suggested ECOFFs one MIC dilution step upwards. When we compared the MIC levels for amikacin obtained by Middlebrook 7H10 and Bactec 960 MGIT of nine M. tuberculosis strains, we found good agreement, indicating that the ECOFFs for both methods are likely to be similar. From this perspective, it is interesting to note that there was a large span of critical concentrations used in a survey of supranational reference laboratories for capreomycin in B460 (from 1.25 to 10 mg/liter) and that there was an 8-fold difference in the critical concentrations between the 7H10 (10 mg/liter) and Bactec 460 (1.25 mg/liter) methods, whereas there are no or very small differences for streptomycin (2 mg/liter versus 2 mg/liter) or kanamycin (4 mg/liter versus 5 mg/liter) (12, 25). If wild-type MIC distributions had been used to define these breakpoints, such variations could have probably been avoided.

A novel genotyping test based on known resistance mutations is reported to have an 85% sensitivity to detect resistance to amikacin and capreomycin in the rrs gene (5). However, these tests are based on known mutations found on well-characterized resistant strains, and, considering the increasing resistance against the injectable drugs, it is of importance to have a drug susceptibility method which could screen for strains with MICs that are higher than the ECOFF in order to detect and characterize novel resistance mutations.

The number of aminoglycoside- and cyclic polypeptide-resistant strains in our study is limited, since the primary aim was to define the wild-type MIC distributions and the ECOFFs. Still, regarding cross-resistance, it is interesting to note that two isolates that were clearly below the ECOFF and the critical concentration for streptomycin were resistant to amikacin, kanamycin, and capreomycin. These data underline that streptomycin should be tested with and considered a treatment alternative for XDR TB, even if the isolate is classified as resistant to other aminoglycosides and cyclic polypeptides. The seven isolates that were resistant to capreomycin, kanamycin, and amikacin according to the ECOFFs were all susceptible to viomycin, implying that cross-resistance is not as common as previously reported (16). Five of these isolates had a MIC higher than the ECOFF (4 mg/liter) for capreomycin but would have been classified as susceptible using the recommended critical concentration in Middlebrook 7H10 medium (10 mg/liter).

Compared to capreomycin, viomycin is not as well described in the literature as a treatment alternative for TB (6, 16, 20). Previous publications report that viomycin shares the major side effects of other aminoglycosides, such as nephro- and ototoxicity (20). Some authors claim that the nephrotoxicity might limit its use and recommend intermittent regimens of 1 or 2 g three times a week (20). The side effects should be possible to avoid by careful drug monitoring of the patient, which could be important in the treatment of drug-resistant TB (19, 20). Our in vitro data indicate that viomycin could be a good choice for treatment of aminoglycoside drug-resistant TB, as overall resistance rates were low and cross-resistance to other injectable drugs among the M. tuberculosis strains tested was very limited.

In conclusion, wild-type MIC distributions of aminoglycosides and cyclic polypeptides were determined, including tentative epidemiological wild-type cutoffs. Our data clearly suggest that the critical concentration for capreomycin should be reconsidered and that viomycin and streptomycin should be considered treatment options for MDR and XDR TB, even in the case of resistance to other class representatives.

ACKNOWLEDGMENTS

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<table>
<thead>
<tr>
<th>Wild-type MIC (mg/liter)</th>
<th>Amikacin\textsuperscript{c}</th>
<th>Kanamycin\textsuperscript{d}</th>
<th>Capreomycin\textsuperscript{d}</th>
<th>Viomycin\textsuperscript{e}</th>
<th>Streptomycin\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\text{max}/MIC</td>
<td>n</td>
<td>C\text{max}/MIC</td>
<td>n</td>
<td>C\text{max}/MIC</td>
<td>n</td>
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<tr>
<td>0.125</td>
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<td>0</td>
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</tr>
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<td>0.250</td>
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<td>79</td>
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</tr>
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<td>2</td>
<td>3</td>
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</tbody>
</table>

\textsuperscript{a} Values in bold represent the C\text{max}/MIC level for the ECOFF. The asterisk represents the lowest MIC level with a PD target of at least 10 for the C\text{max}/MIC, which is generally accepted for aminoglycosides (3, 18, 19).
\textsuperscript{b} Normal dosage, 1 g/day; protein binding, 10%; C\text{max}, 21.6.
\textsuperscript{c} Normal dosage, 1 g/day; protein binding, 10%; C\text{max}, 19.8.
\textsuperscript{d} Normal dosage, 1 g/day; protein binding, 20%; C\text{max}, 20.0.
\textsuperscript{e} Normal dosage, 1 g/day; protein binding, 20%; C\text{max}, 20.0.
\textsuperscript{f} Normal dosage, 1 g/day; protein binding, 10%; C\text{max}, 22.5.
REFERENCES

14. Reference deleted.
22. Reference deleted.