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


Swedish Institute of Disease Control (SMI), Stockholm, Sweden; Clinical Microbiology, MTC—Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; Medical Microbiology, Department of Laboratory Medicine, Lund University, Malmö, Sweden; Department of Clinical and Experimental Medicine, Clinical Microbiology, Linköping University Hospital, Linköping, Sweden; Department of Clinical Microbiology, Växjö Hospital, Växjö, Sweden; Department of Infectious Diseases, Uppsala University, Uppsala, Sweden; and Department of Clinical Microbiology, Kalmar County Hospital, Kalmar, Sweden

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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 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. 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 mechanism of action for aminoglycosides and cyclic polypeptides is mainly the inhibition of protein synthesis (1). As a class member of the aminoglycosides, streptomycin was the first drug used for the treatment of tuberculosis in the 1940s (24). Until the recommendations were revised by the WHO, streptomycin was interchangeable with ethambutol as a first-line drug but was abandoned due to increasing resistance and the risks involved in administering injections in areas where HIV is endemic (7). Where afforded, amikacin is commonly the injectable drug of choice in the treatment of MDR TB. According to WHO recommendations (25), amikacin lacks a critical concentration for Middlebrook 7H10 medium, and no breakpoint in any DST method is available for viomycin (20, 25). Resistance to the aminoglycosides and cyclic polypeptides is associated with mutations in the 16S rRNA gene (rrs), and, in addition, mutations in the thrA gene also confer resistance to the cyclic polypeptides (8, 15). It has been suggested that isolates with low-level and intermediate-level kanamycin resistance can be susceptible to amikacin, whereas strains with high-level kanamycin resistance are resistant to both drugs (13, 15). Cross-resistance among the cyclic polypeptides has been reported between capreomycin and viomycin, but limited data are available (16).

DST against Mycobacterium tuberculosis is currently based on testing the susceptibility to single so-called critical concentrations of antibiotics (17). However, the scientific basis for defining these critical concentrations is weak, particularly for second-line drugs (25). Using inappropriate susceptibility breakpoints might lead to poor reproducibility and the incorrect reporting of susceptibility results to clinicians (25). In a first attempt to address this issue, we recently reported the problematic close
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 Antimicro-
bial Susceptibility Testing (EUCAST) when setting clinical
breakpoints (9). Surprisingly, published wild-type MIC distri-
butions for \textit{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 rea-
sible strains, were tested twice. Three consecutive isolates with
exceed one MIC dilution step (Table 1). H37Rv was tested
due to the conflicting reports of cross-resis-
tance within the aminoglycoside group, for which the lack of
background data in establishing the current critical concen-
trations 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 \textit{M. tuberculosis}
by comparing them to wild-type MIC distributions and avail-
able PK/PD data.

**TABLE 1. Reproducibility of MIC determinations between and within rounds\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Strain\textsuperscript{b}</th>
<th>Amikacin</th>
<th>Kanamycin</th>
<th>Streptomycin</th>
<th>Capreomycin</th>
<th>Viomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>H37Rv</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>08-622</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>SM1-15</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SM1-17</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>SM1-7</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>SM1-18</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SM1-14</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>07-10680</td>
<td>0.5</td>
<td>1</td>
<td>0.125</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>07-9206*</td>
<td>&gt;64</td>
<td></td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>08-2095*</td>
<td>&gt;64</td>
<td></td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>08-8079*</td>
<td>&gt;64</td>
<td></td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values shown are expressed as milligrams per liter.
\textsuperscript{b} All values shown are expressed as milligrams per liter. One patient.

**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
twice, and seven isolates, including resistant and suscept-
able 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.

Wild-type distributions in relation to current critical concen-
trations 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

**MATERIALS AND METHODS**

Strains of \textit{Mycobacterium tuberculosis} and quality control. To establish the
wild-type distribution, 95 consecutive clinical isolates from the clinical mycobac-
teriology laboratory at Karolinska University Hospital were included. Five iso-
lates 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 \textit{M. tuberculosis}
H37Rv strain (ATCC 27290) 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 per-
formed previously using the Bactec 460 method (B460) (Becton Dickinson). The
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.

\textbf{MIC determinations in Middlebrook 7H10 medium and Bactec 960 MGIT.}
Stock solutions (30.7 g/liter) in distilled water were prepared for amikacin,
kanamycin, capreomycin, streptomycin (all from Sigma, St. Louis, MO), and
viomycin (VWR International, Stockholm, Sweden). The methodology for MIC
determination is described in detail elsewhere (23). Briefly, bacterial suspensions of
all strains were transferred by using a 96-stick replicator to Middlebrook 7H10
agar plates with 1:2 serial dilutions from 0.002 to 512 mg/liter for all drugs.
Drug-free control plates with undiluted and 1:100 diluted bacterial suspensions
were replicated in the same way. Inoculated agar plates were incubated at 37°C
for 3 weeks. The MIC was defined as the first antibiotic concentration which
showed less growth compared to the 1:100 diluted controls of the corresponding
strain, i.e., the lowest concentration of drug that inhibited more than 99% of
the bacterial population. MIC testing against amikacin as a class representative
was performed on a subset of 10 isolates, including H37Rv in Bactec MGIT 960
(MGIT). Serial dilutions of amikacin were prepared as outlined above, and
MGIT tubes were inoculated on the day of the experiment in final concentrations
covering two MIC steps above and below the MIC obtained in the Middlebrook
7H10. The control strain \textit{M. tuberculosis} H37Rv was included in all
runs as a quality control.

Calculation of PD indices. The peak serum concentration (\(C_{\text{max}}\)) was chosen
as the important PK parameter based on data availability and the fact that it is
commonly used to define PD indices for other bacteria (10, 18). The \(C_{\text{max}}\),
divided by the MIC was calculated for amikacin, kanamycin, streptomycin, cap-
reomycin, and viomycin according to standard doses and previously published
pharmacokinetic data (3, 18, 19). The free fraction was calculated by multiplying
the percentage of protein-free drug fraction with the \(C_{\text{max}}\) and the free fraction
of this variable (\(fC_{\text{max}}\)) was divided by the corresponding MIC value (see Table
4 for the pharmacokinetic data used for calculations). All parameters were used
in the lower range of published data (3, 6, 18, 19).
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 cut off (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).
TABLE 2. Cross-resistance in isolates exhibiting resistance against any aminoglycoside or cyclic polypeptidea

<table>
<thead>
<tr>
<th>Cross-resistance pattern</th>
<th>Streptomycin (ECOFF: S ≤ 2)</th>
<th>Amikacin (ECOFF: S ≤ 1)</th>
<th>Kanamycin (ECOFF: S ≤ 4)</th>
<th>Capreomycin (ECOFF: S ≤ 4)</th>
<th>Viomycin (ECOFF: S ≤ 2)</th>
<th>No. of isolatesb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>4*</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>1*</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>1</td>
</tr>
</tbody>
</table>

a The isolates were classified as susceptible (S) or resistant (R) according to tentative ECOFFs (mg/liter).

b *, 5 isolates with an MIC for capreomycin (8 mg/liter) higher than the ECOFF (4 mg/liter) would have been interpreted as susceptible according to the critical concentration for capreomycin (10 mg/liter).

glycoside 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-
considering 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 \textit{M. tuberculosis} isolate combined with \textit{C}_{\text{max}}/\text{MIC} 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 \textit{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 \textit{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 otoxicity (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 \textit{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 \textit{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.

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REFERENCES


14. Reference deleted.


22. Reference deleted.

