Disk Diffusion Breakpoint Determination for Antimicrobial Susceptibility Testing

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Misuse of antibiotics is a major contributor to the growth of drug-resistant bacteria.

Two clinical tests assess susceptibility of bacterial pathogen to drug

- Drug Dilution (MIC) $\rightarrow$ concentration ($\mu$g/ml)
- Disk Diffusion (DIA) $\rightarrow$ zone diameter (mm)

Drug/Test breakpoints classify pathogen to be:

- Susceptible - very high likelihood of successful treatment
- Intermediate - marginal likelihood of successful treatment
- Resistant - low likelihood using FDA approved dosage
### Breakpoint Determination for Susceptibility Testing

Photograph of MIC Assay, After 18 Hour Incubation

<table>
<thead>
<tr>
<th>More Concentrated Antimicrobial</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
<th>1:256</th>
<th>1:512</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less Concentrated Antimicrobial</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- **E. coli** Tested In Rows A-D
- **S. aureus** Tested In Rows E-H
- The last dilution inhibitory to the microorganism is called the “MIC”
- For the assay pictured here, the MIC for the green antimicrobial is 1:8 for *E. coli*; 1:256 for *S. aureus*.
- These columns are negative and positive growth controls.
- Clear = No Growth
- Cloudy = Growth

www.AntimicrobialTestLabs.com
Properties of the Two Tests

- **Drug Dilution (MIC) test**
  - Considers set of two-fold dilutions
  - Will use $\log_2(\text{MIC}) = \ldots , -4, -3, -2, -1, 0, 1, 2, 3, 4, \ldots$
  - Observed MIC: lowest dilution with no visible growth
  - Test rounds up to nearest dilution (integer)

- **Disk Diffusion (DIA) test**
  - Observed DIA: diameter of clear zone surrounding disk
  - Rounded to the nearest mm

- Inverse relationship between true MIC and true DIA
Both MIC and DIA breakpoints are set by agencies such as the Clinical and Laboratory Standards Institute (CLSI) and the Food and Drug Administration (FDA).

- MIC breakpoints \((\log_2 \text{concentrations})\) based primarily on the pharmacokinetics and pharmacodynamics of the drug.

- Given the MIC breakpoints, the DIA breakpoints must be estimated.

- Relationship between DIA and MIC is drug/pathogen specific with unknown functional form.

- DIA is more often used in hospitals because it is faster and requires less lab space.
Problem

How to best determine appropriate DIA breakpoints?
The error rate bounded (ERB) method is currently used by agencies to determine DIA breakpoints.

Perform MIC & DIA tests on hundreds of strains of a particular pathogen and then find the DIA breakpoints that minimize an observed classification discrepancy index.

Discrepancies based on Very Major (VM), Major (M), and minor (m) errors

- Very Major: MIC test = R and DIA test = S
- Major: MIC test = S and DIA test = R
- Minor: One test = I
Example MIC DIA Test Data
Partition of MIC/DIA observations

- **Susceptible**
  - I, MIC
  - S, DIA (Minor error)

- **Intermediate**
  - R, MIC
  - I, DIA (Minor error)

- **Resistant**
  - S, MIC
  - R, DIA (Major error)

- **R, MIC**
  - S, DIA (Very major error)
Error Rate Bounded Method

![Graph showing MIC vs. DI/A with different categories represented by different markers.]

- Correct
- Minor
- Very Major
Concerns with ERB Method

- Simulation studies (Craig, 2000) show the ERB method:
  - Is biased
  - Lacks precision
- Focuses on observed test results
- Does not take into account MIC test characteristics:
  - Rounding Up
  - 3-fold variability
3-fold Variability

Table 1. Quality Control Study - MIC test: 10 Labs, 50 reps/lab

E. coli ATCC 25922

<table>
<thead>
<tr>
<th>MIC</th>
<th>LAB</th>
<th>All Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>-8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>-7</td>
<td>36</td>
<td>41</td>
</tr>
<tr>
<td>-6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>StDev</td>
<td>0.46</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Example of the rounding up effect on MIC data
MIC Model

- Distinguish **observed** test result, $x_i$, from **true** result, $m_i$

- To generate observed pair:

  $$x_i = \lceil m_i + \epsilon_i \rceil \quad \epsilon \sim \mathcal{N}(0, \sigma^2_m)$$

- Given MIC breakpoints $M_L$ and $M_U$ one can calculate the probability of correct classification:

  $$p_{MIC}(m) = \begin{cases} 
  \Pr(x \leq M_L) = \Phi \left( \frac{M_L - m}{\sigma_m} \right) & m \leq M_L \\
  \Pr(M_L < x < M_U) = \Phi \left( \frac{M_U - 1 - m}{\sigma_m} \right) - \Phi \left( \frac{M_L - m}{\sigma_m} \right) & M_L < m < M_U \\
  \Pr(x \geq M_U) = 1 - \Phi \left( \frac{M_U - 1 - m}{\sigma_m} \right) & m \geq M_U 
  \end{cases}$$
Probability of Correct MIC Classification
We propose an underlying model of the process that focuses on the probability of correct classification.

We develop a hierarchical model that uses latent MIC test means to link the two observed results as well as describe the probability of correct classification for each test.

Our model accounts for the inherent test variabilities and differing testing properties by linking the observed test pairs to a latent 1-1 function of 'true' test results.

This should result in better precision and increased accuracy.
The observed scatterplot of test results are used to estimate the model parameters using Bayesian inference.

Use model parameters to determine DIA breakpoints with best 'performance'.

More intuitive in the fact that the performance curves do not directly depend on the observed results and also provides a visual description of each test rather than a joint numerical summary.
Model Based Approach

Summarized in four steps:

1. Observed Data Given True Test Value
2. True Pathogen MIC Distribution
3. Relationship between True Test Values
4. Calibration based on Probability of Correct Identification
Distinguish observed test pair from true pair

Observed pairs: $x_i$ and $y_i$

True pairs: $m_i$ and $d_i$

Observed data are integers due to rounding

To generate observed pair:

$$x_i = \lceil m_i + \epsilon_i \rceil \quad y_i = \lfloor d_i + \delta_i \rfloor$$

$$\epsilon \sim \mathcal{N}(0, \sigma_m^2) \quad \delta \sim \mathcal{N}(0, \sigma_d^2)$$
True Pathogen MIC Distribution

- Describe underlying pathogen MIC distribution as a mixture of Normals:

\[ \pi(m_i|k, \mu, \sigma, p) = \sum_{j=1}^{k} p_j \mathcal{N}(m_i; \mu_j, \sigma_j) \]
Relationship, \( d_i = f(m_i) \), has been assumed to be linear (Rudrik et al., 1985) or logistic (Craig, 2000).

Here we propose a more flexible nonparametric approach using I-splines (Ramsey, 1988) which restricts the relationship to be monotonic.

Assume \( a = t_0 < ... < t_q = b \) is a partition of \([a, b]\) and \( y_1, ..., y_{2k+q-1} \) is the knot sequence of the M-spline of order \( k \). Then the \( i^{th} \) I-spline

\[
I_i(x; y_i, y_{i+1}, ..., y_{i+k}) = \int_a^x B_i(t)dt
\]

Where \( B_i(t) \) is the \( i^{th} \) M-spline basis function.
Example Splines

M-splines - knot locations: (0, .2, .4, .6, .8, 1)

Resulting I-splines
Example of Hierarchical Model
Probability of Correct Identification

- Probability model links observed MIC results to true MIC
- Can determine probability of correct identification
- Assume true MIC breakpoints same as test breakpoints
- Given breakpoints $M_L$, $M_U$, $D_L$, and $D_U$

\[
\begin{align*}
    P_{\text{DIA}}(m) = \begin{cases} 
    Pr(y \geq D_U) = 1 - \Phi \left( \frac{D_U - 0.5 - d}{\sigma_d} \right) & m \leq M_L \\
    Pr(D_L < y < D_U) = \Phi \left( \frac{D_U - 0.5 - d}{\sigma_d} \right) - \Phi \left( \frac{D_L + 0.5 - d}{\sigma_d} \right) & M_L < m < M_U \\
    Pr(y \leq D_U) = \Phi \left( \frac{D_L + 0.5 - d}{\sigma_d} \right) & m \geq M_U
    \end{cases}
\end{align*}
\]
'Calibrate' the two probability of correct identification curves

Use loss function:

\[ L = \int_{-\infty}^{\infty} \min(0, p_{\text{DIA}}(u) - p_{\text{MIC}}(u))^2 w(u) \, du \]
Given the MIC probability of correct identification curve (fixed), presented is the DIA probability of correct identification curve for a particular $D_U$ and $D_L$. 
Bayesian Inference

- Interested in the joint posterior of model parameters:
  \[ \theta = \{ I_{1...q}, k, \mu, \sigma, p \} \]
  \[ \pi(\theta|\{x, y\}) \propto \pi(\{x, y\}|\{m, d\}, \theta)\pi(\{m, d\}|\theta)\pi(\theta) \]

- Given fixed knot sequence, use Markov Chain Monte Carlo (MCMC) to estimate parameters:
  1. True test values:
     - \( m, d \)
  2. Underlying mixture of Normal parameters:
     - \( \mu, \sigma, p \)
  3. Spline Coefficients:
     - \( I_{1...q} \)
MCMC

- Use when posterior is analytically intractable
- Construct a Markov chain whose stationary distribution is the target distribution
- Metropolis et al (1953) showed how this can be done
- Method generalized by Hastings (1970)
Suppose the specified distribution has unnormalized density $h$. The Metropolis-Hastings update does the following:

1. When the current state is $x$, propose a move to $y$, having conditional probability density given $x$ denoted $q(x,.)$

2. Calculate the ratio:

$$r(x,y) = \frac{h(y)q(y|x)}{h(x)q(x|y)}$$

3. Accept the proposed move $y$ with probability:

$$a(x,y) = \min(1, r(x,y))$$

So the state after the update is $y$ with probability $a(x,y)$ and $x$ with probability $1 - a(x,y)$
- Use MH proposals to update each of the parameters during each iteration

- $\pi(\{x, y\}|\{m, d\}, \theta)$:

\[
\prod_{i=1}^{n} \frac{1}{\sqrt{2\pi \sigma_m^2}} \exp\left(\frac{-(x_i - m_i)^2}{2\sigma_m^2}\right) - \frac{1}{\sqrt{2\pi \sigma_m^2}} \exp\left(\frac{-(x_i - 1 - m_i)^2}{2\sigma_m^2}\right) +
\]

\[
\frac{1}{\sqrt{2\pi \sigma_d^2}} \exp\left(\frac{-(y_i + .5 - d_i)^2}{2\sigma_d^2}\right) - \frac{1}{\sqrt{2\pi \sigma_d^2}} \exp\left(\frac{-(y_i - .5 - d_i)^2}{2\sigma_d^2}\right)
\]

- $\pi(\{m, d\}|\theta)$:

\[
\prod_{i=1}^{n} \sum_{j=1}^{k} p_j \frac{1}{\sqrt{2\pi \sigma_j^2}} \exp\left(\frac{-(m_i - \mu_j)^2}{2\sigma_j^2}\right)
\]
Priors $\pi(\theta)$

- $\mu, \sigma, p$
  - $\mu$ - ordered: $k! \left( \frac{1}{\mu_u - \mu_l} \right)^k$
  - $\frac{1}{\sigma^2} \sim \text{Gamma}(3, 1)$
  - $p \sim \text{Dirichlet}(1, 1, ..., 1)$
- $\log(l_1...q)$
  - $\mathcal{N}(0, \sigma = 10)$
MCMC Process for Our Model

1. Update $m$
   - Proposal: $\mathcal{N}(m, .5)$

2. Update $\mu, \sigma, p$ based on new $m$
   - Propose new neighboring Normal components for $m$
   - Update $p$
   - Propose new $\mu'_i$s (proposal uniform between $\mu_{i-1}$ and $\mu_{i+1}$)
   - Update $\sigma$

3. Propose new or collapse two Normal components (RJMCMC)

4. Update Spline Coefficients ($I$)
   - Proposal: Adaptive multivariate normal: $I_{n+1} \sim \exp\{\mathcal{N}(\log(I_n), \Sigma \ast s)\}$

5. Update $d$
   - Deterministic update given $m$ and $I_{n+1}$

6. Update number and location of knots - work in progress
Can use RJMCMC to dynamically change number of normal components

Provides framework for MCMC in which the dimension of the parameter space can vary between iterations

Applications: change-point models, finite mixture models, variable selection, ...

Change from model \( (k, \theta_k) \) to \( (k', \theta_{k'}) \) with probability:

\[
\min \left( 1, \frac{\pi(k', \theta_{k'})|x)q(k' \rightarrow k)q_{d_{k' \rightarrow k}}(u') \delta_{g_{k \rightarrow k'}}(\theta_k, u)}{\pi(k, \theta_k)|x)q(k \rightarrow k')q_{d_{k \rightarrow k'}}(u) \delta(\theta_k, u)} \right)
\]
Dimensions and first and second moments of each model must match

Often must introduce random variables

Consider mixture of normals. Combine two mixtures:

\[ p_{j^*} = p_{j1} + p_{j2} \]
\[ p_{j^*}\mu_{j^*} = p_{j1}\mu_{j1} + p_{j2}\mu_{j2} \]
\[ p_{j^*}(\mu_{j^*}^2 + \sigma_{j^*}^2) = p_{j1}(\mu_{j1}^2 + \sigma_{j1}^2) + p_{j2}(\mu_{j2}^2 + \sigma_{j2}^2) \]

Similar idea for splitting one component into two but must introduce three random variables for dimension matching.
Simulation

- Used fixed set of model parameters
- Generated 300 scatterplots with 500 pathogens
- Computed DIA breakpoints for each scatterplot
- Summarize distribution of 'best' DIA breakpoints
Simulation 1 - Linear Relationship Results

Our Approach

ERB
Simulation 2 - Logistic Relationship

Example Data

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Breakpoint Determination for Susceptibility Testing
Simulation 2 - Logistic Relationship Results

Our Approach

ERB
Simulation 3 - Convex Relationship

Example Data

DIA vs. MIC Graph

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Breakpoint Determination for Susceptibility Testing
Simulation 3 - Convex Relationship Results

Our Approach

ERB
Conclusions

- Because of the increasing number of moderately susceptible and resistant isolates, choosing appropriate breakpoints has become more of a statistical problem.

- The ERB method, while simple to implement, is too sensitive to the variability in the observed frequencies caused both by the inherent experimental error in each test and the choice/location of the isolates relative to the intermediate zone.

- Our model approach has shown increased accuracy and better precision.

- Working with subgroup of CLSI for making method available to clinicians.
Additional Ongoing Work

- Real data sets often have censored values:
  - Ex. MIC $\geq 5$ instead of MIC = 5
  - Difficult to deal with in a nonparametric setting
Additional Ongoing Work

- Assumed the measurement errors for MIC and DIA are known
  - Reasonable given the abundance of past data
  - However would like to estimate these parameters as well

- We have presented this approach to CLSI (Nov 2011)
  - Interest shown in new approach
  - Actively developing software
Acknowledgments

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References: