

# Analysis of Minimum Inhibitory Concentrations

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In response to the threat presented by AMR, it is critically important to find methods for effectively interpreting minimum inhibitory concentration (MIC) tests. A wide array of techniques for analysis of MIC data exist, which require different ways of modifying the MIC data for use as the dependent variable in regression and analysis. For use as the outcome in logistic regression, MIC data is categorized using clinical breakpoints and epidemiological cutoff values (ECOFF). Clinical breakpoints classify isolates as susceptible (S), resistant (R), or an intermediate category based on expected clinical outcomes of treatment with a specific antimicrobial. The ECOFF classifies organisms as wild type (WT) or non-wild type (non-WT) based on the absence or presence of phenotypically-detectable acquired resistance mechanisms to the specific antimicrobial. Dichotomization of MIC data results in information loss, as only changes in the proportions of WT/non-WT or S/R are observed in dichotomized data. As a result, changes in MIC distributions that do not cross the threshold may be unobserved using approaches with dichotomized outcomes. Other modeling approaches for MIC data may attempt to avoid information loss by not dichotomizing the outcome when using regression to analyze MIC data.

Keywords: antimicrobial resistance ; minimum inhibitory concentration ; epidemiological cutoff ; clinical breakpoints ; mic creep

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## 1. Introduction

The World Health Organization has deemed antimicrobial resistance (AMR) one of the most urgent health threats of our time <sup>[1]</sup>. Antimicrobial-resistant bacteria are implicated in approximately 2.8 million cases of clinical infections and an additional 35,000 human deaths annually <sup>[2]</sup>. In addition to clinical burdens, the estimated annual economic cost of AMR is approximately \$3 trillion in GDP loss due to excess health care and loss of productivity <sup>[3]</sup>. The issue of AMR affects humans, animals, and the environment alike, prompting the World Health Organization to recommend the coordinated monitoring of resistance in bacteria from food, food animals, and clinically ill patients to understand the patterns of AMR and how it affects the entire food production chain <sup>[4]</sup>. As a part of this effort, the ability to monitor AMR phenotypically and genotypically with respect to resistance is an important methodological tool in public health and food production <sup>[5]</sup>.

To address the threat of AMR, national and international surveillance systems conduct widespread antimicrobial susceptibility testing to track resistance levels in microbial populations. The U.S. National Antimicrobial Resistance Monitoring System (NARMS) assesses resistance levels of enteric bacterial isolates from retail meats, livestock, and humans <sup>[6]</sup>. For this purpose, surveillance programs measure resistance levels of isolates to each antimicrobial using minimum inhibitory concentrations (MIC) of selected antibiotics. The MIC quantifies the lowest tested concentration at which an isolate's growth is inhibited by a specific antimicrobial <sup>[6]</sup>. The MIC is determined for a given isolate and antimicrobial using automated instrument systems or manual testing methods, including agar dilutions, antimicrobial gradient method (e.g., E-tests), and broth dilutions <sup>[7][8]</sup>. These tests expose the isolate to a series of concentrations of antimicrobials on a two-fold scale, and the lowest concentration where inhibition of bacterial growth is present is reported as the MIC for that isolate and the corresponding antimicrobial compound.

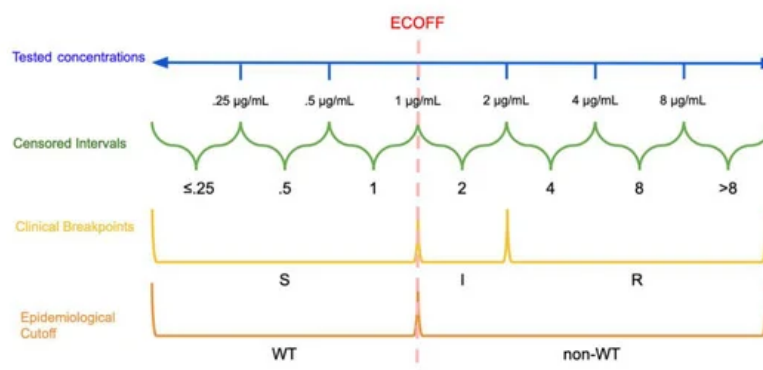
## 2. Epidemiological Cutoffs and Clinical Breakpoints

There are a number of different ways that MIC data is modified for use as the dependent variable in regression and analysis. In general, different modeling approaches utilize data handling methods that balance model simplicity with information loss. Logistic regression requires a dichotomous outcome, and the breakpoints are carefully selected to divide observations into separate meaningful outcome categories.

One method for selecting this dividing value on the MIC scale is to use the epidemiological cutoff value, which separates the distribution of MICs of wild type (WT) isolates, which lack phenotypically-detectable acquired resistance mechanisms, from the non-wild type (non-WT) organisms that possess phenotypically-detectable acquired resistance mechanisms. <sup>[9]</sup>  
<sup>[10]</sup>. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) sets epidemiological cutoff values

(ECOFF) based on the consensus of visual and mathematical analyses of observed MIC distributions performed by identifying WT and non-WT distributions in the bimodal distribution of MICs [11][12]. The mathematical determination of the ECOFF for each antimicrobial agent is done using a tool called the ECOFFinder, which fits a cumulative log normal curve to the WT MICs and identifies cutoffs that classify 97.5% and 99% of the WT isolates correctly [13]. A similar method is employed by the Clinical and Laboratory Standards Institute (CLSI) for its own epidemiological cutoff values (ECV) [11][14].

The other predominant categorization method involves the establishment of clinical breakpoints. This process partitions MIC values into distinct classes of bacterial susceptibility based on clinical outcomes. CSLI defines these classes as “susceptible” (S), “resistant” (R), and either “intermediate” (I) or “susceptible-dose dependent” (SDD) [15]. Note that EUCAST defines its intermediate breakpoint as “susceptible, increased exposure” (I) [16]. See Figure 1 for a visual comparison of MIC concentrations, censored MIC intervals, clinical breakpoints, and epidemiological cutoffs. [16][17]. These breakpoints are established by organizations such as the CLSI, EUCAST, and the U.S. Food and Drug Administration Center for Drug Evaluation and Research [17]. Different organizations use different names and definitions for intermediate classes that allow for some ambiguity in interpretation and specific information on these categories is typically available on each organization's website [16][17]. Classifications based on an ECOFF are not immediately related to classifications based on clinical breakpoints, an isolate identified as non-WT will not necessarily be clinically resistant, instead it may still be clinically susceptible [9].



**Figure 1.** Dichotomization and Categorization of Minimum Inhibitory Concentration (MIC) data: In this hypothetical example, the continuous scale of concentrations is divided into intervals based on the tested concentrations. The epidemiological cutoff (ECOFF) divides wild type (WT) and non-wild type isolates at 1 µg/mL while the clinical breakpoints divide the MICs into susceptible (S): ≤1 µg/mL, susceptible, increased exposure (I): >1 µg/mL and ≤2 µg/mL, and resistant (R): >2 µg/mL. Note that the ECOFF and S breakpoint need not be the same.

The clinical breakpoints and ECOFF seek to avoid splitting the WT isolates into multiple categories to avoid accidental classification of a WT isolate to a higher resistance category. This approach must account for about one dilution of variation in measurement introduced by testing procedures in breakpoint or cutoff determination, as well as inter-laboratory variance [8][11][18]. As a result, the ECOFF must account for this variation as it attempts to separate this distribution of isolates from the non-WT bacteria that are higher in the range of tested concentrations.

Though this approach simplifies the modeling approaches necessary to look for trends and increases the overall ease of interpretation, a portion of the information contained by the MIC value is lost when it is dichotomized or categorized, which will be further discussed later in the review [19][20][21]. Additionally, breakpoints may be subject to change over time in response to changes in protocols, resistance patterns, and guidelines [22].

### 3. Information Loss and Minimum Inhibitory Concentration (MIC) Creep

Information loss is a byproduct of the dichotomization of MIC data, as interval-censored, continuous data is simplified to being either above or below a threshold value (see ECOFF, ECV, and clinical breakpoints above), which results in data loss [19][20]. With dichotomized MIC data, only changes in the proportions of MIC values that fall on each side of the threshold are captured via logistic regression. In other words, only fluctuations in the proportions of WT and non-WT, or S and R, are captured by logistic regression, and all fluctuations of values within each category are lost. As a result, surveillance of dichotomized AMR data can result in the loss of critical information such as changes in trends in the mean MICs of the S and R, or WT and non-WT, groups if those changes do not cross threshold values. This phenomenon where changes in the mean MIC are not detected by dichotomized analysis of MIC data is referred to as “MIC creep” and “MIC decline.” In response to information loss through dichotomization, a wide array of methods of analysis incorporating additional information from MIC data have been developed. These methods examine the entire scale of MICs to gain

insight into the distributions of MICs above and below the cutoff values [23]. As a practical example, MIC creep has become a relevant concern for surveillance of increasing resistance to vancomycin among methicillin-resistant *Staphylococcus aureus*, though individual study results differ with regards to the nature of this trend [24][25][26].

In another study, two nonparametric approaches and logistic regression were used on subsets of NARMS retail and slaughter data to demonstrate significant changes in the distributions of MICs from consecutive years. MIC creep was identified via the nonparametric tests but not detected by logistic regression. In contrast to logistic regression, the nonparametric approaches treat each interval as a separate unordered category, then evaluate whether the distribution of MICs among these categories changes between two time points either a year apart or between slaughter and retail [19]. The results of the study suggest that the distributions of MIC data may vary without being detected by methods that dichotomize the data. Therefore, these data indicate that the entire scale of values needs to be incorporated in the analysis of MIC data [19].

In the interest of retaining more of the information lost through dichotomization, there are many modeling approaches for MIC data that do not dichotomize the data, several of which will be discussed later in this review. An alternative proposed approach allowing for mixed linear regression is to ignore censoring of the MIC data and instead treat MICs as observations on a continuous scale. However, this interpretation leads to an overestimation of the means and underestimation of the standard errors, increasing the likelihood of type I error [27][28].

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