Constructing a Cumulative Antibiogram
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November 2006

02R762A
Program Description

This program focuses on the development and use of cumulative antibiograms to guide empiric therapy of initial infections. This program will also detail the processes used to generate the data in the cumulative antibiogram. Participants will review the current guidelines for preparing and interpreting antibiograms including the test methods commonly used to perform antimicrobial susceptibility tests on individual patients. Additionally, this program will demonstrate how susceptible, intermediate, and resistant breakpoints are derived. The current Clinical and Laboratory Standards Institute (CLSI) guidelines for preparing cumulative antibiograms will be described and a discussion of potential problems that might be encountered during preparation of the report will be analyzed. Finally, suggestions for using cumulative antibiogram data will be discussed and the participant will be able to identify problems with antibiogram data.

Target Audience

This program has been designed to meet the educational needs of pharmacists and medical technologists interested in the development and interpretation of antibiograms.

Learning Objectives

Based on current CLSI standards, upon completion of this program the participants will be able to:

- Describe the methods commonly used for antimicrobial susceptibility testing and reporting in clinical laboratories and potential pitfalls of these methods
- Discuss the preparation of cumulative antibiograms and problems that might be encountered during this process
- Outline clinical applications of cumulative antibiogram data that might be used by various healthcare professionals
- Summarize factors contributing to increasing antimicrobial resistance
- Construct an antibiogram for your patient population

Faculty

Rebecca T. Horvat, PhD, D (ABMM)

Dr. Horvat is an Associate Professor of Pathology and Laboratory Medicine at the University of Kansas School of Medicine. For the past 15 years she has been director of the Clinical Microbiology, Virology, and Immunology sections at the University of Kansas Hospital. Dr. Horvat has a PhD in Medical Microbiology and is a diplomat of the American Board of Medical Microbiology. In addition to her clinical laboratory and teaching responsibilities, Dr. Horvat is an active researcher in the area of bacterial antibiotic resistance. Her scope of investigation includes assessment of new technology for detection of resistance, interaction of bacteria with host cells, investigation of molecular resistance mechanisms, and correlation of antimicrobial resistance with clinical outcomes. She is the recipient of numerous research grants and has authored many articles, book chapters, and abstracts.

Janet F. Hindler, MCLS, MT(ASCP)

Ms. Hindler has worked as a clinical microbiologist for over 30 years, the past 28 at UCLA Medical Center, Los Angeles, CA. Ms. Hindler has written and taught extensively in the area of antimicrobial susceptibility testing and has recently completed a four year contract (Interagency Personnel Agreement) with the Centers for Disease Control and Prevention’s (CDC’s) Division of Laboratory Systems where her focus was to develop and conduct training programs in antimicrobial susceptibility testing. She is now a consultant with the Association of Public Health Laboratories with support from the CDC to continue in this role. She is a member of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Subcommittee on Antimicrobial Susceptibility, fellow in the American Academy of Microbiology, Chair of ASM’s Waksman Foundation for Microbiology Lecturer Committee, Past Chair of ASM Division C, and Past President of the Southern California Branch of the American Society for Microbiology. She has served as a consultant to the World Health Organization and assisted in teaching individuals in developing countries about antimicrobial susceptibility testing and antimicrobial resistance.
Melinda L. Lacy, PharmD

Dr. Lacy is an Associate Professor of Pharmacy Practice at the University of Kansas School of Pharmacy. She has been on faculty at K.U. for the past 8 years and maintains a clinical practice at K.U. Medical Center in Kansas City with the Adult Infectious Disease Service. Dr. Lacy earned both a bachelor’s degree in Pharmacy and her Doctor of Pharmacy degree from the University of Kansas School of Pharmacy in Lawrence. She also completed a postdoctoral fellowship in Infectious Diseases Pharmacotherapy at Hartford Hospital in Hartford, Connecticut under the direction of Charles Nightingale, PhD, David Nicolau, PharmD, and Richard Quintiliani, MD. Her research interests are optimizing institutional antibiotic use, anti-infective pharmacokinetics and pharmacodynamics, clinical utilization of antibiogram data, and the influence of Gram-positive bacterial resistance on clinical outcomes. Dr. Lacy serves as a reviewer for many infectious disease and pharmacy journals and she has authored numerous journal publications, book chapters, and abstracts.

Disclosures:

Rebecca T. Horvat, PhD, D (ABMM) has received investigator initiated grants from Roche Pharmaceuticals, Inc., Pfizer, Abbott Laboratories, Ortho-McNeil, Inc., and Aventis Pharmaceuticals, Inc. She is currently on the speaker’s bureaus for Ortho-McNeil, Inc. and Roche Pharmaceuticals, Inc.

Janet F. Hindler, MCLS, MT (ASCP) is currently on the speaker’s bureaus for Ortho-McNeil, Inc. and Dade Behring, Inc.

Melinda L. Lacy, PharmD is currently on the speaker’s bureaus for Ortho-McNeil, Inc., Abbott Laboratories, and sanofi-aventis.

Unlabeled Use Disclosure: This activity will not include discussions of products or devices that are not currently approved for use by the Food and Drug Administration (FDA) or are currently investigational.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Susceptibility Testing Methods</td>
<td>2</td>
</tr>
<tr>
<td>Clinical Laboratory Standards Institute (CLSI)</td>
<td>5</td>
</tr>
<tr>
<td>Antibiogram Production</td>
<td>7</td>
</tr>
<tr>
<td>Clinical Utilization of Antibiogram Data</td>
<td>10</td>
</tr>
<tr>
<td>The Future of Antibiograms</td>
<td>12</td>
</tr>
<tr>
<td>Summary</td>
<td>13</td>
</tr>
<tr>
<td>References</td>
<td>14</td>
</tr>
</tbody>
</table>
Introduction

In an era of increasing antimicrobial resistance, the testing and dissemination of cumulative antimicrobial susceptibility or resistance data has become paramount. An antibiogram is a cumulative summary of antimicrobial susceptibility results over a prescribed time period. This summary is generated from the individual data of clinical isolates that are tested against a battery of antimicrobial agents for clinical purposes. Antibiograms provide important information to healthcare providers when they are properly prepared and interpreted. The development and presentation of antibiograms should be a collaborative effort between the clinical microbiology laboratory, pharmacy, physicians, and hospital policy committees such as infection control and/or anti-infective subcommittees. Antibiograms should be useful for initiating empiric therapy and may be able to track antimicrobial resistance trends over time within a healthcare system. Prior to 2000, there were no published instructions to guide preparation of cumulative antibiograms, and this resulted in a lack of standardization among reports prepared at various facilities. This lack of standardization likely had a negative impact on some empiric antimicrobial therapy decisions.

Then in 2000, the Clinical and Laboratory Standards Institute (CLSI, formerly known as NCCLS or the National Committee for Clinical Laboratory Standards) published a document entitled “Analysis and Presentation of Cumulative Antimicrobial Susceptibility Proposed Guideline (M39-P)” (1). This document was updated and published as an approved guideline (M39-A) in 2002. M39 defines and makes recommendations regarding analysis and presentation of cumulative antibiogram data.

An update of the M39 was released in January 2006 as M39-A2 (2). This new document defines a cumulative antibiogram as “the report generated by analysis of isolates from a particular institution(s) in a defined period of time that reflects the percentage of first isolates (per patient) of a given species that is susceptible to each of the antimicrobial agents routinely tested.” Thus the report is a summary of antimicrobial susceptibility results over a prescribed time period from clinical isolates that are likely to be contributing to an infectious process. M39-A2 also emphasizes that the report generated following recommendations in the guideline is to be used to guide empiric therapy of initial infections. Alternative ways of analysis and presentation of data may be required if the report is to be used for other purposes (e.g., infection control).

The current program will review common antimicrobial susceptibility testing methods, highlight important recommendations by CLSI, discuss the antibiogram preparation process along with challenges in data interpretation, and provide an overview of how antibiogram data may be applied to clinical practice.

Susceptibility Testing Methods

Clinical laboratories use a variety of antimicrobial susceptibility testing methods to determine the effectiveness of antimicrobial agents against clinical bacteria isolates. These testing methods have been standardized to provide accurate reproducible qualitative and/or quantitative results. Qualitative results are expressed as susceptible (S), intermediate (I), or resistant (R). Quantitative results expressed as minimal inhibitory concentration (MIC, µg/ml) values, are usually accompanied by an S, I, or R on the laboratory report.

In addition to procedures for preparing cumulative antibiograms, the CLSI publishes standards for performance of disk diffusion and MIC tests (3,4). Clinical laboratories can use either one of these CLSI reference methods or a commercial test system that has been Food and Drug Administration (FDA) cleared for testing clinical isolates. FDA clearance indicates that results obtained from the commercial test system have been shown to be comparable to those generated by a CLSI reference method. Most testing methods require a manual set up or inoculation step with either a manual or an automated reading and interpretation.

Automated antimicrobial susceptibility systems simplify and eliminate the subjectivity associated with reading, interpretation, and reporting of results. With semi-automated systems, the initial set up and incubation is done outside of the instrument, and the panel is placed in the instrument for the reading step. However, with fully automated systems, panels are inoculated, then placed in an incubator/reader, and then the instrument performs all additional tasks. When testing is complete, a report is automatically generated from the system’s computer. Currently there are four fully automated, antimicrobial susceptibility instruments (e.g. VITEK Legacy®, VITEK 2®, MicroScan® Walkaway, and BD Phoenix®) on the U.S. market. The methods most commonly used in clinical laboratories are listed on the next page with a brief description and a website that gives further information.
## Constructing a Cumulative Antibiogram

### Table 1. Methods used by clinical laboratories to measure antimicrobial susceptibility in clinical bacterial isolates

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLSI Reference Methods</strong></td>
<td></td>
</tr>
<tr>
<td>Disk diffusion</td>
<td>Filter paper disks impregnated with individual antimicrobial agents are placed on the surface of an agar plate that has been inoculated with a standard number of test bacteria. After overnight incubation, the zone of inhibition is measured and results are compared to a chart containing standard values for interpreting the zone reading as either S, I, or R.</td>
</tr>
<tr>
<td>MIC broth microdilution</td>
<td>Generally 12 antimicrobial agents are tested against a single isolate using the broth microdilution method. A series of two-fold dilutions of each antimicrobial agent in µg/ml concentrations are added to a liquid culture media in a microdilution tray. Each well is then inoculated with a standard number of test bacteria and the panel is incubated overnight. The minimum inhibitory concentration (MIC) of each antimicrobial agent is defined as the lowest concentration of drug that inhibits the visible growth of the bacteria. Any evidence of turbidity in the well indicates growth and a clear well indicates inhibition of growth. A well with broth inoculated with bacteria and no antimicrobial agent is included as a positive growth control.</td>
</tr>
<tr>
<td><strong>Commercial Manual Read MIC Methods</strong></td>
<td></td>
</tr>
<tr>
<td>Etest® (AB Biodisk)</td>
<td>The Etest® utilizes a plastic strip impregnated with a gradient concentration of antimicrobial agent on the bottom of the strip. The strip is placed on an agar surface that has been inoculated with the test bacteria as is done for the disk diffusion test. After overnight incubation, the MIC is read at the point where the ellipse of inhibition intersects the scale on the top part of the plastic strip. <a href="http://home.btclick.com/cosltd/etesthp.htm">http://home.btclick.com/cosltd/etesthp.htm</a></td>
</tr>
<tr>
<td>MicroScan (Dade Behring, Inc.)</td>
<td>MicroScan markets dried broth micro-dilution MIC panels (full range MIC or breakpoint panels) that are manually inoculated and incubated in a standard incubator and read manually. Alternatively and more commonly, the inoculated panels are placed in the MicroScan Walkaway. Combination panels containing identification and susceptibility tests are available as are panels with susceptibility tests alone. For the former, “breakpoint” MICs are determined because fewer dilutions of antimicrobial agents are available for the individual drugs. <a href="http://www.dadebehring.com/edbna2/ebusiness/home.jsp?lang=E">http://www.dadebehring.com/edbna2/ebusiness/home.jsp?lang=E</a></td>
</tr>
<tr>
<td>Sensititre® (Trek Diagnostics)</td>
<td>Trek Diagnostics offers broth microdilution panels that contain various dilutions of antimicrobial agents in a dried form. The dried antimicrobial agent does not contain any broth component, consequently the broth is added with the inoculum. This allows the use of a variety of broths as recommended for each type or organism tested (e.g., Mueller-Hinton for <em>E. coli</em>; Mueller-Hinton with lysed horse blood for <em>S. pneumoniae</em>). As with MicroScan, panels are incubated and read manually, however a reader/incubator instrument is also available. <a href="http://www.trekds.com/default.asp">http://www.trekds.com/default.asp</a></td>
</tr>
<tr>
<td><strong>Commercial Automated Read MIC methods</strong></td>
<td></td>
</tr>
<tr>
<td>Becton Dickinson (BD) Phoenix™</td>
<td>The BD Phoenix™ system uses a broth microdilution technology in a novel “cassette” format and growth of bacteria is detected with the aid of redox indicator in the liquid growth medium. Following inoculation, the cassette is placed in a reader/incubator instrument. The wells are examined automatically every 20 minutes by the instrument and when testing is complete, MIC results together with an S, I, or R interpretation are generated. A single cassette can be used for both identification and antimicrobial susceptibility testing. <a href="http://www.bd.com/ds/productCenter/PhoenixAutomatedMicrobiologySystem.asp">http://www.bd.com/ds/productCenter/PhoenixAutomatedMicrobiologySystem.asp</a></td>
</tr>
<tr>
<td>MicroScan Walkaway (Dade Behring, Inc.)</td>
<td>The inoculated MicroScan panels can be placed in the Walkaway reader/incubator. When the instrument determines the test is complete, results expressed as MIC with an S, I, or R interpretation are generated as a hardcopy printout or sent electronically to the laboratory information system. <a href="http://www.dadebehring.com/edbna2/ebusiness/home.jsp?lang=E">http://www.dadebehring.com/edbna2/ebusiness/home.jsp?lang=E</a></td>
</tr>
</tbody>
</table>

*continued*
The newer Vitek® 2 system uses test cards with 64 wells that can test up to 20 different antimicrobial agents. The optical system of the Vitek® 2 monitors each well of the card every 15 minutes and develops a kinetic profile of bacterial growth within each well. The interpretation of this data is computed and an MIC and S, I, or R, interpretation is generated for each drug. Identification results are available at 2-3 hours and susceptibility results between 5 and 14 hours.

Table 1. Methods used by clinical laboratories to measure antimicrobial susceptibility in clinical bacterial isolates, continued

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commercial Automated Read MIC methods</strong></td>
<td></td>
</tr>
<tr>
<td>Vitek® (bioMerieux)</td>
<td>For the Vitek® systems the wells in which the bacteria are tested with antimicrobial agents are miniaturized in cards that are slightly larger than a human palm. The card is inoculated with a suspension of the test bacterium by connecting the suspension and the card with a plastic straw. Using vacuum, the suspension is drawn up into the card to simultaneously inoculate and reconstitute the wells. The inoculated cards are placed in a reader/incubator where growth (indicated by turbidity) of bacteria is automatically monitored by the instrument and recorded in a computer. The older Vitek Legacy utilizes test cards with 45 wells which enable testing of up to 16 different antimicrobial agents.</td>
</tr>
<tr>
<td>Vitek® 2 (bioMerieux)</td>
<td>The newer Vitek® 2 system uses test cards with 64 wells that can test up to 20 different antimicrobial agents. The optical system of the Vitek® 2 monitors each well of the card every 15 minutes and develops a kinetic profile of bacterial growth within each well. The interpretation of this data is computed and an MIC and S, I, or R, interpretation is generated for each drug. Identification results are available at 2-3 hours and susceptibility results between 5 and 14 hours.</td>
</tr>
<tr>
<td>Sensititre® (Trek Diagnostics)</td>
<td>Sensititre® provides an automated reader/incubator where the inoculated broth microdilution panels can be placed. Growth is automatically monitored and when testing is complete, an MIC and S, I, or R interpretation is generated. <a href="http://www.trekds.com/default.asp">http://www.trekds.com/default.asp</a></td>
</tr>
</tbody>
</table>

**Disk diffusion testing, additional comments**

The zone diameter of growth inhibition is related to the ability of the drug to diffuse through the agar medium, the concentration of the drug in the disk, the number of bacteria applied to the agar plate, and the susceptibility of the bacterial isolate to the antimicrobial agent. One advantage of the disk diffusion method is flexibility in choosing the antimicrobial agents that are tested against each isolate. Generally, up to 12 disks are tested on a 150 mm diameter agar plate. Additionally, newly released antimicrobial agents will typically be available in the disk form before they are available in commercial MIC test systems. Camera box-type devices are available from commercial sources to enable electronic capture of images of zones of inhibition produced following disk diffusion testing. Zone measurements are transmitted to a computer containing software that interprets and stores data and facilitates reporting or results.

**Manual MIC testing by broth or agar dilution, additional comments**

Generally, a 6- to 8-well series of two-fold dilutions of each antimicrobial agent is prepared in liquid media in a 96-well broth microdilution panel (see Figure 2). Up to 12 antimicrobial agents can be tested per panel along with a positive broth control well that contains the bacteria but no antimicrobial agent and a negative control well that contains broth alone. The minimum inhibitory concentration (MIC) of an antimicrobial agent is defined as the lowest concentration of drug that inhibits the visible growth of the bacteria. Broth microdilution panels can be made in house but are generally purchased from a commercial source. Manual MIC testing can be performed in test tubes where 1 - 2 ml volumes of bacteria/antimicrobial solution are tested. This broth microdilution method is rarely used in clinical laboratories as the testing process is very labor intensive and there are no commercial suppliers of broth macrodilution tests.

**Manual MIC testing by Etest®, additional comments**

This method is used to determine the MIC of isolates that require different media such as chocolate agar (Haemophilus species) or blood containing agar (Streptococcus species). In the case that the bacterial strain lyses the blood in the media, care should be taken to interpret the MIC at the antimicrobial agent dose that inhibits bacterial growth and not the antimicrobial agent dose associated with the line of hemolysis.

**Automated MIC testing, additional comments**

Automated antimicrobial susceptibility systems simplify the interpretation of testing methods such as broth microdilution. These systems can be semi-automated in that the assay is set up manually and then read and interpreted by an instrument. Other systems are fully automated, and the testing is fully contained in the instrument.

The automated susceptibility systems generally use the same principle as the broth dilution system discussed above. The systems use various measures of automation such as optical measurement of test panels for detecting bacterial growth, computerization of result, interpretation, and reporting. All of the computers with these instruments can be interfaced with the laboratory or hospital information systems. However, this interface should be verified periodically to detect potential problems.
Selection of a Susceptibility Test Method

The susceptibility testing method used in microbiology laboratories will vary depending upon the organism tested. For example, the Etest® method is frequently used (40.3%) when determining the susceptibility of Streptococcus pneumoniae to penicillin, followed by disk diffusion (27.6%) and MicroScan® (23.2%). However, for Escherichia coli and Pseudomonas aeruginosa, automated commercial systems are used over 80% of the time, with the disk diffusion method accounting for the remainder (College of American Pathology Proficiency Testing Program).

Some bacterial strains such as Haemophilus influenzae are tested only for the production of beta-lactamase using a rapid method such as Cefinase®. Results are reported as positive or negative for beta-lactamase and the type of beta-lactamase, if present, is not specified. The percentage of strains that are beta-lactamase positive may then be included as a part of the cumulative antibiogram report. It is not uncommon for a clinical laboratory to use more than one method to accommodate the various types of bacterial strains requiring susceptibility testing. For example, a fully automated instrument may be used for routine testing of commonly encountered non-fastidious pathogens, a manual method for the isolates requiring specific growth conditions, and a rapid method for the detection of beta-lactamase. A cumulative antibiogram may contain data from results generated from several methods, and this may be referenced on the disseminated report.

Clinical Laboratory Standards Institute (CLSI)

The CLSI is a global, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. CLSI was originally known as the National Committee for Clinical Laboratory Standards and more recently was recognized by the letters NCCLS only. The new name, Clinical and Laboratory Standards Institute was adopted in 2005. Representatives from academia, government, healthcare industry, and practicing healthcare professionals participate in development of voluntary consensus standards and guidelines. In addition to documents for antimicrobial susceptibility testing, CLSI publishes documents that address many other processes that occur within the clinical laboratory and healthcare industry.

CLSI Standards for Antimicrobial Susceptibility Testing

The primary CLSI standards for routine antimicrobial susceptibility testing in clinical laboratories and a brief description of each are listed below. Included is the current edition (e.g., current edition of M100-S is “16” 2006) and schedule for updating.

M2-A9 - Performance Standards for Antimicrobial Disk Susceptibility Tests
This document contains detailed instructions for testing, reporting results, and performance of quality control using the disk diffusion test method. This standard is updated every 3 years.

M7-A7 - Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically
This document contains detailed instructions for testing, reporting results, and performance of quality control using the reference broth macrodilution, broth microdilution, and agar dilution MIC test methods. This standard is also updated every 3 years.

M100-S16 - Performance Standards for Antimicrobial Susceptibility Testing
This document provides tables containing: 1) recommendations for drugs to test and report; 2) interpretive criteria or breakpoints; 3) quality control ranges; 4) additional quality assurance aids; and, 5) a glossary of antimicrobial agents. M100 is used in conjunction with M2 and M7 and is updated annually, in January.

M23-A - Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters
This document addresses the required and recommended data needed for the selection of interpretive standards and quality control guidelines for antimicrobial agents. This standard is updated as needed with the most recent version published in 2001.
**Breakpoints**

A breakpoint can be defined as the minimum inhibitory concentration (MIC, µg/ml) or zone diameter (mm) value used to indicate a susceptible, intermediate, or resistant result. An alternate designation used throughout the CLSI documents to describe values that define susceptible, intermediate, or resistant is “interpretive criteria”. Breakpoints are specifically derived for each antimicrobial agent and organism group. An example of breakpoints for ampicillin and Enterobacteriaceae are listed below:

The interpretive criteria of susceptible, intermediate, or resistant are considered qualitative results while the actual MIC or zone diameter is considered quantitative. Sometimes the term “non-susceptible” is used to indicate that a particular bacterium is either “intermediate” or “resistant” to a specific antimicrobial agent. In the example above, an MIC of > 8 µg/ml is considered “non-susceptible” for ampicillin and Enterobacteriaceae. Breakpoints or interpretive criteria are listed in the pharmaceutical manufacturer’s package inserts for an individual antimicrobial agent in the “Susceptibility Tests” section and are also found in the CLSI M100 document. Generally, the FDA and CLSI breakpoints agree.

**Table 2. Breakpoints for Ampicillin with Enterobacteriaceae**

<table>
<thead>
<tr>
<th>Disk diffusion (mm)</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 17</td>
<td>14-16</td>
<td>≤ 13</td>
<td></td>
</tr>
<tr>
<td>MIC (µg/ml)</td>
<td>≤ 8</td>
<td>16</td>
<td>≥ 32</td>
</tr>
</tbody>
</table>

**CLSI Criteria for Establishing Breakpoints**

CLSI uses the following parameters to establish breakpoints: 1) graphs showing the distribution of MICs and zone diameter results for a large number of bacteria with varying susceptibility to the antimicrobial agent; 2) pharmacokinetic (PK) and pharmacodynamic (PD) data; and, 3) clinical outcome data. Pharmaceutical manufacturers petition CLSI for specific breakpoints by submitting data that complies with requirements listed in CLSI M23. The relationship between actual PK/PD parameter values (e.g. peak serum or plasma level to MIC ratio, drug AUC to MIC ratio, time above the MIC) and clinical efficacy data are reviewed. Correlating antimicrobial agent concentration and microbiologic susceptibility data with specific therapeutic outcomes (bacterial eradication, clinical cure, and clinical failure) is the cornerstone of the PK/PD evaluation process. All of these criteria are collectively evaluated in establishing breakpoints.

**Example of Breakpoint Changes:** Sometimes it is necessary to re-evaluate breakpoints when new resistance data, PK/PD data, or clinical outcome data become available. This occurred for several β-lactam agents and *Streptococcus pneumoniae* in January 2000. Examples of initial and modified MIC breakpoints for *S. pneumoniae* with two agents are shown below:

**Table 3. MIC Breakpoint changes for *S. pneumoniae***

<table>
<thead>
<tr>
<th></th>
<th>Initial Breakpoints(µg/ml)</th>
<th>Modified Breakpoints (µg/ml) (January 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>amoxicillin</td>
<td>≤ 0.5</td>
<td>1</td>
</tr>
<tr>
<td>amoxicillin/</td>
<td>≤ 0.5/0.25</td>
<td>1/0.5</td>
</tr>
<tr>
<td>clavulanic acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The initial pneumococcal breakpoints for cefotaxime and ceftriaxone related to concentrations of these agents attainable in CSF. Then in January 2002, they were modified to reflect MIC interpretations that would better guide the use of these drugs for treating meningitis versus non-meningitis infections. The breakpoints for non-meningitis reflect the higher concentrations attainable in blood and lung as compared to CSF. The initial and modified MIC breakpoints are shown on the next page:
Constructing a Cumulative Antibiogram

Table 4. Meningitis and non-meningitis MIC breakpoints for *S. pneumoniae*

<table>
<thead>
<tr>
<th></th>
<th>Initial Breakpoints (µg/ml)</th>
<th>Modified Breakpoints (µg/ml) (January 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>cefotaxime</td>
<td>≤ 0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ceftriaxone</td>
<td>≤ 0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CLSI lists similar breakpoints for meningitis and non-meningitis for cefepime, however, this agent is not approved for treating meningitis in the United States.

Although penicillin is sometimes used for treating pneumococcal meningitis or pneumonia, there are no separate meningitis and non-meningitis breakpoints for this agent. However, it is suggested that pneumonia due to isolates that have an “intermediate” interpretation for penicillin can be treated with high doses of penicillin or ampicillin. The rationale behind staying with a single set of breakpoints for penicillin is based on the variety of doses and forms of penicillin available and difficulties in fine tuning multiple sets of breakpoints to best represent these.

Antibiogram Production

Components of the Antibiogram

The CLSI M39-A2 document should be used to guide the preparation of cumulative antimicrobial susceptibility test data or cumulative antibiogram reports in healthcare facilities. Using this standardized approach for antibiogram development will improve the quality of antibiogram data and facilitate a more meaningful comparison of susceptibility data between institutions and across geographic areas.

The challenge for the microbiology laboratory is to regularly produce a concise antibiogram, which displays current, accurate, and clinically useful data. The development of sophisticated computer programs and improvements in laboratory information systems will aid this process. Interestingly, the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) recognizes the antibiogram document as a quality assurance measure for clinical laboratories fulfilling JCAHO Standard IM.8. The JCAHO IM.8 standard falls under the Management Information Section concerning Aggregate Data and Information and is defined as follows, “The hospital collects and aggregates data and information to support care and service delivery and operations.” Despite this, antibiograms are not produced by all hospitals, especially in those with fewer than 50 beds, and distribution policies vary widely among institutions where cumulative antibiograms are available.

The cumulative antibiogram should be prepared cooperatively between microbiology, pharmacy, and other medical clinicians. CLSI recommends that each laboratory have an internal mechanism to ensure that susceptibility results on individual bacterial isolates are accurate before they are reported to the patient’s chart or sent to the database for subsequent analysis for the cumulative antibiogram report. Before finalizing the cumulative antibiogram, the percent susceptible statistics should be carefully reviewed for errors or unusual results. Once completed, the cumulative antibiogram should be distributed to the Infection Control and Pharmacy and Therapeutics Committees, the microbiology laboratory, and all pharmacy and medical clinicians. Distribution of antibiogram data to clinicians is a critical and sometimes difficult step in the process since the reports can be easily misplaced. Displaying the antibiogram data on an institution’s private intranet can facilitate dissemination of information and assure that outdated antibiograms cannot be viewed.

Preparation of a cumulative antibiogram annually is recommended, however, some institutions elect to evaluate susceptibility data more frequently. Although most facilities compile all data into a single report, some may also present unit- or area-specific (i.e. MICU, SICU) data. In addition to the annual cumulative antibiogram that
Antibiogram Production, continued

is distributed for the purpose of guiding empiric therapy of initial infections, sometimes specific antibiograms are prepared for the Infection Control committee to help identify nosocomial outbreaks within certain areas of the hospital or in outpatient areas (i.e., dialysis centers)\(^8\). In order for these to be useful, they would have to be generated at the time the outbreak is suspected.

At a minimum, information required on an antibiogram includes: facility, time frame, specific organisms, antimicrobial agents tested, and percent susceptibility. Some medical centers may create an elaborate, pocket-sized document with various additional types of antibiotic information such as: formulary policies, criteria for using certain agents, dosing conversion charts, dosing nomograms, dosing alterations for organ impairment, antibiotic pricing, etc.

Specimen Site: Some institutions may include reports from subsets of data for isolates from select body sites in their antibiogram. For example, there may be a separate cumulative antibiogram report for isolates from urine or for “systemic” or blood culture isolates\(^9\). Antimicrobial agents that are only tested on urine isolates should be noted on the antibiogram. Isolates from surveillance cultures should be excluded from the cumulative antibiogram.

Antimicrobial Agents and Organisms: According to the M39-A2 document, antibiogram data should be organized into separate tables for Gram-positive, Gram-negative, and anaerobic organisms. The total number of isolates for each bacterial strain should be clearly listed, and all susceptibility data should reflect the percent of strains that were fully susceptible to each antibiotic. Thus, isolates with an “intermediate” classification should not be included as susceptible. Although not specifically recommended in the guidelines, antimicrobial agents can be organized in a variety of ways including alphabetically by generic name, grouped by classification, or even according to cost (e.g. least expensive to most expensive).

Potential Problems with Cumulative Anti-\(b\)ibiogram Reports: \(\text{Possible pitfalls in analysis and presentation of cumulative antibiogram data originate from several areas which include: analysis of multiple isolates from the same patient; reporting data for small numbers of isolates; reporting results from drugs that are selectively tested against subsets of isolates (e.g., more resistant isolates); and presenting data for shortened time period (monthly antibiograms).}

Multiple or Duplicate Isolates: \(\text{The inclusion of multiple isolates from the same patient during a given analysis period can significantly skew susceptibility data reported on the cumulative antibiogram. Generally, failure to eliminate duplicates will lead to falsely low percent susceptible values. “Duplicate isolates” is defined by CLSI as “two isolates that are the same based on defined phenotypic or genotypic characteristics.” Various methods can be used to remove duplicates from individual patients (e.g., include only the first isolate per hospitalization vs. first isolate per year vs. first isolate every 30 days, etc.), and these methods can result in considerable differences in the percent susceptible statistics generated\(^11\). CLSI M39-A2 guidelines recommend that only the “first isolate (per patient) of a given species per analysis period” be included “irrespective of body site, antimicrobial susceptibility profile, or other phenotypic characteristics”\(^11\).}

The mechanism for eliminating duplicates varies considerably among commercial software commonly employed to generate cumulative antibiogram data in clinical laboratories. This software might be provided with the laboratory information system (LIS) or a commercial susceptibility test system. Most commercial software will eliminate duplicates, but there are few systems that will eliminate duplicates following the current CLSI M39-A2 recommendations. It is important to know if and how duplicates are eliminated when critically reviewing cumulative antibiogram data. It would be impractical for clinical laboratories to manually compile data in order to comply with CLSI M39-A2, however, some laboratories with reasonable information technology resources might download data into a program (e.g., Access, Excel) that would allow them to follow CLSI M39-A2 recommendations. Improved LIS and other software analysis programs are needed to assist institutions in analysis and presentation of cumulative antibiogram data.

Number of Isolates: \(\text{In general, antibiograms that include larger numbers of isolates for a particular bacterial species provide a more accurate assessment of antibiotic susceptibility since the impact of unusual isolates is minimized.}

CLSI M39-A2 recommends reporting cumulative percent susceptible data for a single species only when there are 30 or more isolates analyzed within the reporting period.\(^2\) This differs from the original M39 document which suggested cumulative reporting of bacterial species with more than 10 isolates.\(^1\) If the number of isolates tested is clearly listed on the antibiogram for each species, then it is easier for the clinician to make a judgment as to the precision of the percent susceptible rates. The importance in strength of numbers becomes obvious when considering that a single fluoroquinolone-resistant isolate of *Streptococcus pneumoniae* at a small hospital would result in a 90% susceptible rate if only 10 isolates were tested. However, at a larger institution this would be 99% susceptible if 100 isolates were tested, a value consistent with current published surveillance studies for antipneumococcal fluoroquinolones\(^14\).\(^15\).
Antibiogram Production, continued

CLSI M39-A2 presents a statistical table to help determine 95% confidence limits for various sample sizes. If 10 isolates are tested against a drug and 90% are susceptible, the 95% confidence interval is 55-100% susceptible. This tightens up considerably to 82-95% susceptible if 100 isolates are tested (Table 5).

These formulae are then applied to situations where the number of bacterial isolates varies, but the confidence intervals and P value threshold are kept constant. The M39-A2 document includes a more comprehensive table that can be consulted when assessing the significance of a change in susceptibility rates.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>50% susceptible</th>
<th>90% susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>19 - 81</td>
<td>55 - 100</td>
</tr>
<tr>
<td>100</td>
<td>40 - 60</td>
<td>82 - 95</td>
</tr>
<tr>
<td>1000</td>
<td>47 - 53</td>
<td>88 - 92</td>
</tr>
</tbody>
</table>

Table 5. 95% confidence intervals for % susceptible with selected sample sizes

Combining Data from Different Patient Cohorts

Intuitively, the single value on a cumulative antibiogram represents the average susceptibility for a specific pathogen collected from several unique patient groups with pre-determined demographics. An example below illustrates the calculation of the antibiogram’s final percent susceptibility for *Escherichia coli* tested against antibiotic X.

Figure 1. Example of cumulative antibiogram data for multiple healthcare facilities

The cumulative antibiogram suggests that a percent susceptibility to drug X of 79% may indicate that the drug is suboptimal as an empiric therapy choice. However, if the clinician is a pediatrician using drug X in a child with a UTI, drug X performs very well (100% of isolates are susceptible). However, if the patient came into Hospital A from a long-term care facility such as LTCF B, selection of empiric therapy should be based upon data from LTCF B, which shows 25% of isolates are susceptible to drug X. Essentially, antibiograms are weighted average susceptibilities based upon the frequency of data inputs from multiple sources. Because an antibiogram is such a compilation, then antibiotic selection should be based upon individual antibiograms collected from well-defined patient groups. CLSI M39-A2 suggests separating percent susceptibilities for major pathogens into distinct patient cohorts, such as pediatrics, adult ICU, and non-ICU. For purposes of practicality, 2 to 3 separate individual antibiograms would be reasonable for major pathogens.
Constructing a Cumulative Antibiogram

Comparing Antibiogram Data

When comparing local antibiogram data to that from other institutions or to national surveillance data, the factors discussed above should be carefully considered. Both the number of isolates analyzed and the mechanism for elimination of duplicate isolates can significantly impact the percent susceptible statistics. Other factors that can impact percent susceptible data include: test method used in determining susceptibility and special patient populations at a given institution that may influence susceptibility results. For example, cystic fibrosis patients and Pseudomonas aeruginosa; long term care patients and Staphylococcus aureus and Enterococcus species; generation of data using isolates from all culture sites or from selected sites (e.g. urine, blood, respiratory specimens); generation of data from drugs selectively tested only when isolates are multi-drug resistant; and exclusion of isolates from surveillance cultures.

Combining Cumulative Antibiogram Data

While it is important for clinicians to become familiar with local susceptibility patterns from different institutions in a geographic area, not all investigators are in agreement that combining antibiogram data for institutions in a specific geographic area, especially concerning Streptococcus pneumoniae is useful[8,9,16-18]. White and colleagues reported that citywide antibiograms are not useful[9]. This study looked at Streptococcus pneumoniae isolates from 47 institutions in the U.S. using Etest® MIC values for penicillin, levofloxacin, and gatifloxacin. They concluded that citywide antibiograms concealed differences in susceptibility patterns that should be considered when making empiric antibiotic therapy decisions. Alternatively, Chin and colleagues compared penicillin, cefotaxime, trimethoprim-sulfamethoxazole, and erythromycin susceptibility for Streptococcus pneumoniae isolates using data obtained from two methods: active surveillance data from the community vs. aggregate antibiogram data from hospitals in Portland, Oregon[16]. They indicate that the percent susceptible results from two methods were comparable and that combining hospital antibiogram data does provide accurate, community-specific data for pneumococcus. Stein and colleagues supported these findings in a report that compared aggregate hospital antibiogram data with national surveillance data for Streptococcus pneumoniae[18]. They found that combining hospital antibiogram data taken from across the state was a “feasible, practical method for monitoring trends in pneumococcal susceptibility.” However, Lubowski and colleagues reported that the practice of combining antibiotic susceptibility data into integrated system-wide antibiograms should be discouraged[17]. The CLSI M39-A2 guidelines recommend the preparation of separate antibiograms for each hospital in a network, even when a single laboratory serves more than one healthcare facility.

Clinical Utilization of Antibiogram Data

The aim of any healthcare institution should be to develop a useful and accurate antibiogram based on the CLSI M39-A2 recommendations. This will ensure that physicians, microbiologists, pharmacists, infection control committees, and other healthcare workers can optimally utilize the information. It is important for clinicians to gain a greater understanding as to how to use their own antibiogram data when making empiric decisions regarding anti-infective therapy.

CDC 12-Step Program

Of interest, in March 2002 the CDC launched a campaign to prevent antimicrobial resistance in healthcare settings (http://www.cdc.gov/drugresistance/healthcare/ha/12steps_HA.htm). To accomplish this mission they have introduced a 12-step program centered on the following four major strategic areas: 1) prevent infection; 2) diagnose and treat infection; 3) use antimicrobials wisely, and, 4) prevent transmission. Interestingly, six of the 12 steps fall into the “use antimicrobials wisely” category. Step 6 in this category is “use local data” by knowing your local antibiogram, formulary, and patient population.

Thus, it is important for healthcare practitioners to be familiar with antibiogram issues and know how to apply the data in clinical practice. Lack of education on antibiogram function represents the current state of affairs in understanding resistance. The CDC and CLSI, through their respective 12-Step Program and M39-A2 document, along with government agencies, accreditation organizations, and regulatory agencies, attempt to focus on antibiogram standardization and resistance prevention. In addition, the CDC website has current information on problem pathogens and emerging resistance.
Constructing a Cumulative Antibiogram

Measuring and Expressing Susceptibility and Resistance Rates

The CLSI M39-A2 guidelines recommend that cumulative antibiogram data be reported as “percent susceptible” for each antimicrobial agent-organism combination. It is a simple mathematical task to determine the non-susceptible rates over the time period the data was collected. Resistance may also be examined from a longitudinal perspective. Monitoring for changes in percent susceptible over time can easily be accomplished by comparing data from year-to-year. However, as discussed above, if duplicate isolates from the same patient are included in antibiogram data, then calculated resistance susceptibility rates can be significantly understated. A year to year comparison is only valid if similar numbers of isolates are tested each year.

Empiric Therapy Decision-Making

Although clinicians may frequently rely on favorite antimicrobials for empirical therapy, antibiograms should be designed to suggest empiric regimens based on current, local surveillance data. Antibiograms provide a rational basis for establishing empirical therapy. If the CLSI M39-A2 recommendations are followed, antibiograms will become more useful in the selection of empiric antibiotics.

One example of using antibiogram data is for determining appropriate prophylactic use of vancomycin for surgical procedures involving prosthetic materials or devices. The Hospital Infection Control Practices Advisory Committee (HICPAC) have recommendations for the use of vancomycin in an attempt to prevent the spread of vancomycin resistance among Gram-positive bacteria. Included in the HICPAC guidelines is a list of five situations where the use of vancomycin in a hospital would be considered appropriate. One situation specifically deals with institutions that have high inherent rates of drug-resistant, Gram-positive cocci. The recent increased incidence of community-acquired MRSA (CA-MRSA) rates has complicated this situation. These types of epidemiological changes need to be addressed annually.

Formulary Decisions

In addition to data concerning efficacy, safety, and cost, it is important for local and regional bacterial resistance trends to be considered when making anti-infective formulary decisions. To accurately put local resistance patterns into perspective, it is optimal if the local data is interpreted within the context of regional trends along with published national susceptibility data. This is especially important when small numbers of certain pathogens have been observed over a given time period since the percent resistant can appear artificially inflated due to the small denominator as discussed above. In these cases it may be difficult to ascertain whether the resistance data is the result of outliers or if it is actually an emerging problem.

In response to increasing bacterial resistance trends, some hospitals have made extensive anti-infective formulary changes. A recent report describes significant decreases in the rates of ceftazidime-resistant Pseudomonas aeruginosa and Klebsiella pneumoniae, and piperacillin-resistant Pseudomonas aeruginosa infections in hospitalized patients after changing the formulary from ceftazidime and cefotaxime to cefepime.

To limit an outbreak of VRE and Clostridium difficile colitis, Quale et al. describes how they restricted the use of cefotaxime, clindamycin, and vancomycin and added beta-lactamase inhibitors to replace third generation cephalosporins. Six months after the change, fecal colonization of VRE was significantly reduced along with a decrease in the number of cultures that were VRE positive. In a follow-up report some of these same investigators reported a reduction in MRSA and ceftazidime-resistant Klebsiella pneumoniae after decreasing the usage of cephalosporins, imipenem, clindamycin, and vancomycin and increasing the use of beta-lactamase inhibitor antibiotic combinations. However, a significant increase in patients that were culture positive for cefotaxime-resistant Acinetobacter species was subsequently observed.

The true impact and consequences of antibiotic formulary changes
Constructing a Cumulative Antibiogram

Formulary Decisions, continued

on rates of resistance may not be observed within six months after a formulary change. Furthermore, one should bear in mind that antibiotic formulary changes are merely one of numerous factors that can ultimately impact rates of susceptibility reported on an antibiogram. Resistance is multifactorial, and limiting focus to antibiotic changes may result in “squeezing the resistance balloon”[24]. In a closed system, clinicians rapidly fill the “void” left by restricting one antimicrobial agent or class with another and may then lead to over-use of the new agent or class resulting in negative outcomes. For example, one institution banned use of extended-spectrum cephalosporins by 80% during an 18-month period due to a significant increase in ESBL-producing enterics[25]. These agents were replaced with imipenem-cilastatin and piperacillin-tazobactam. During the study period, a 44% decrease in ESBL-producing Klebsiella pneumoniae was observed, with an 87% reduction in the surgical ICU. Concurrently, imipenem use increased 140%, but imipenem-resistant Pseudomonas aeruginosa increased by 69%, although 85% of these isolates retained susceptibility to ceftazidime.

The resulting lesson is an important one. Inherently, antibiotic use leads to resistance, but the converse is not necessarily true – that decreasing antibiotic use leads to a return in susceptibilities. Other factors, as outlined in a recent publication from the Society for Healthcare Epidemiology of America (SHEA), are essential for preventing transmission of nosocomial pathogens, specifically multi-drug resistant strains of Staphylococcus aureus and Enterococcus spp.[26]. This report recommends active surveillance cultures for identifying reservoirs for spread of MRSA and VRE infections. Although this report is lengthy, it contains important information on understanding and controlling resistance. Thus, formulary decision-making based on surveillance should be tempered with a concurrent re-examination of infection control practices.

Publication and Dissemination of Antibiogram Data and Trending Results

A final aspect of antibiogram development is the decision of what to do with the antibiogram data and analyses. For instance, how are recommendations to be conveyed to prescribers, how is education to be conducted, and how will the antibiogram be distributed? Not infrequently, the development and publication of the antibiogram marks the endpoint of the process. Ernst et al. surveyed laboratory directors throughout the U.S. and found that although 95% of the hospitals compile an antibiogram, only 60% publish, update annually, and distribute the antibiogram to infection control staff and all physicians[27]. Antibiogram publication should represent the initial step in educating prescribers on the use of antibiotics. Hunter and Reeves pointed out that “the use of surveillance data to influence prescribing can be a two-edged sword, that making data available to clinicians without interpretive support may lead to inappropriate changes in prescribing behavior.”[28]. The ability to use a published antibiogram is based on 1) data presentation (i.e., data is easily read and interpreted); 2) the medical staff is receptive to education on antibiotic use based on data; and, 3) adequate venues for education and reinforcement.

The Future of Antibiograms

Antibiograms will continue to be an important tool for the detection and evaluation of resistance. Resistance will continue to increase because of poor antimicrobial selection, inappropriate use, inadequate dosing, and the rise of problem pathogens. Producing accurate antibiograms will remain a challenge with resistant pathogens such as S.aureus and P.aeruginosa and other multi-drug resistant bacteria.

Another deficit in the analysis of susceptibility data lies in the distribution of this data only to local prescribing healthcare providers in highly complex healthcare-delivery organizations. The potential audience and the mechanisms for interpretation and dissemination of the data, however, have never been greater. The challenge is creatively incorporating stratified antibiotic surveillance data into routine clinical practice at the local, state, and national level. Evidence-based medicine will evolve into guideline recommendations, and disclosure of hospital performance measures will increase. Consequently, government and accreditation agencies will intervene to improve outcomes. As physician order-entry and computer algorithms improve, a properly recorded and evaluated antibiogram needs to become incorporated into the empiric prescribing process. This type of practice is ever more important due to the increasing multi-disciplinary nature of hospital and community practice.

Antibiograms need to evolve from a paper surveillance study to an intervention. Relevant indicators using accurate susceptibility data need to be applicable to both unique physician-patient decision-making events and the public health aspects of detecting the emergence and spread of new resistance phenotypes. Critical resistance percentages for specific drug-pathogen combinations can
The Future of Antibiograms, continued

be combined with severity of infection and availability of therapies. The need to both micro-manage and macro-manage this data will become easier with bioinformatics platforms and portability tools.

The main goal of surveillance in the annual tracking of resistance is to detect problems and stimulate intervention. Surveillance “antibiogram hyper-networks” can evolve within a hospital setting to suggest or automatically generate an intervention specific for an ICU, a specific type of patient, and for treatment of a specific infection scenario. Clinicians at the bedside using antibiotic surveillance data will direct therapy based on these evaluations to improve clinical outcomes. Prevalence of resistance, antibiotic consumption, and risk factors for emergence of resistance, super-infections, and adverse events can be linked to suggested therapies. However, the lack of standardization limits the versatility of the antibiogram. Adoption of the M39-A2 guidelines will create antibiograms with applications to antibiotic selection, surveillance trending, and hospital and public policy-making. The goal of generating accurate antibiogram data is clear – it will assist in the application of antibiotic therapy which will promote prudent antibiotic usage.

Summary

Monitoring of resistance will always be required as long as humans and microbes coexist. Antimicrobial pharmacotherapy and susceptibility testing has evolved over the last 50 years and will continue to evolve as new antibiotics and procedures are used in medicine. Continued evolution of the CLSI evaluation process has improved standardization of testing and reporting of susceptibility data. The CLSI M39-A2 document contains recommendations that improve the quality of antibiograms as well as reporting susceptibility results on clinically important bacterial isolates. The use of these guidelines will result in greater utility of antibiograms when used to track resistance. It also will assist when making empiric antibiotic selection as well as formulary decisions.
References


(3) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; tenth informational supplement. National Committee for Clinical Laboratory Standards 20[M100-S10]. 2000.


