Why Selecting the Appropriate Bacterial and Fungal Isolates in your Drug Development Program Matters

JMI LABS

Introduction

According to the US Centers for Disease Control and Prevention (CDC) report entitled *Antibiotic Resistance Threats in the US (2019):*

More than 2.8 million antibiotic-resistant infections occur in the US each year, and more than 35,000 people die as a result.

To highlight the seriousness of the antimicrobial-resistance threat, the CDC and the World Health Organization (WHO) annually list the biggest threats to effective prevention and treatment of bacterial and fungal infections, separating organisms into urgent, serious, and concerning categories. The CDC and WHO also provides a watch list for organisms that might develop clinically significant antimicrobial resistance (AMR). Currently, the following species and groups represent the most serious threats to public health in terms of AMR: carbapenem-resistant *Acinetobacter*, carbapenem-resistant *Enterobacterales, Candida auris, Clostridioides difficile,* and drug-resistant *Neisseria gonorrhoeae.*

Your work to counter the public health menace of AMR is commendable. However, as has been well publicized, it is a long and expensive journey from the beginnings of an antimicrobial drug-development project to the submission of US Food and Drug Administration (FDA) regulatory filing documents.

Various estimates suggest that 12 years and $1.5 billion dollars may be required to create a new antimicrobial drug.

As part of a New Drug Application (NDA), you will be required to submit data that demonstrate that your new antimicrobial is both safe and efficacious for its intended indication(s). In this paper, we are concerned with the generation of data for the *in vitro* microbiology section of your regulatory submission. We will discuss some of the FDA guidelines to industry that are relevant to the development of new systemic antimicrobials, with a particular focus on the selection of microbiological isolates and the requirements for *in vitro* tests beyond the minimal inhibitory concentration (MIC) assay.

Selecting Microbial Isolates

In 2018, the FDA published a document entitled Microbiology Data for Systemic Antibacterial Drugs — Development, Analysis, and Presentation Guidance for Industry that includes guidance on the generation of data intended to be submitted in the NDA, Investigational New Drug (IND) applications, or related filings.

Remember that this document constitutes recommendations rather than absolute requirements. It may be unnecessary or impossible for your drug development program to complete each study discussed in the document. Talk to the FDA as soon as possible to clarify regulatory expectations. Understand that the FDA has published additional guidance documents that may provide critical information to your drug development program that are not considered here. Finally, recall that the expectations of non-FDA regulatory agencies such as the European Medicines Agency may differ in detail to what is discussed here.

FDA Guidance on Isolate Selection Summary

To establish the *in vitro* antimicrobial activity of a potential new antimicrobial, the FDA requires:


Species intended to be included in the package insert


Clinical isolates collected within the last 3 years


At least 75% of the isolates from US hospitals


Isolates that reflect the phenotypic and genotypic profiles of target pathogens


Isolates that reflect the susceptibility profiles of standard-of-care antimicrobials that would be used to treat the intended indication(s)

What JMI Laboratories Can Do for You

JMI Laboratories has vast experience in antimicrobial testing assays that help define the *in vitro* activity of new compounds. Selecting clinical isolates for your experiments is a critical step of this process. You can be assured that the depository of clinical isolates available at JMI Laboratories from the SENTRY Program will satisfy your scientific needs and parameters.

SENTRY Antimicrobial Surveillance Program

Since 1997, JMI has hosted the SENTRY Antimicrobial Surveillance Program, a longitudinal surveillance collection and testing program. This pivotal antimicrobial resistance monitoring program has been a stalwart of drug development studies for decades by providing access to contemporary, relevant organisms from its isolate collection.

> ***JMI Laboratories collects between 40,000-50,000 clinical isolates each year, including over 2,000 fungal clinical isolates annually.***

SENTRY isolates are:

Prevalence-Based: Each clinical site submits a fixed number of isolates selected based each target infection type (e.g. bloodstream infection) rather than by species type.

Geographically Diverse: Isolates come from sites in all US census divisions, most countries in Europe, and select countries in Latin America and the Asia Pacific region.

From Multiple Sources: Sites provide isolates from a wide range of common infection types, including bloodstream, intraabdominal, community-acquired and nosocomial pneumonia, skin and skin structure, and the urinary tract.

Include Special Collections: When requested, JMI creates special collections like anaerobes, fungal isolates including yeasts and moulds, isolates from cystic fibrosis patients, patients with endocarditis, or the genital tract.

Antimicrobial Susceptibility Testing

SENTRY isolates are tested for MIC values against ~30 clinically relevant antimicrobials, including antimicrobial combination agents, using reference methods published by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Molecular Characterizations of Resistance Mechanisms

SENTRY isolates with unique phenotypic profiles are genetically characterized to provide information on potential resistance mechanisms and their effects on your new agent. JMI Laboratories incorporates next generation sequencing technologies into its molecular characterization assays, and our bioinformatics scientists have created a proprietary pipeline of software application tools to help us analyze each isolate sequence.

Including this information within your set of challenge organisms permits the evaluation of spectrum of activity, potential pre-existing resistance, and dosing requirements. Genetically characterizing isolates may also help identify specific resistance mechanisms, including altered permeability, bypass of metabolic pathways, compound inactivation, and target modification.

These phenotypic and genotypic characterizations to SENTRY isolates provide drug developers access to a relevant pool of wild-type and challenge organisms to be included in initial and subsequent drug development assays. Additionally, these data are stored in a searchable database that can be easily and rapidly accessed and selected for your program.

The Microbiology Visualization Platform Tool

JMI Laboratories created the Microbiology Visualization Platform (MVP) application to let drug developers, infectious disease experts, and scientists query and visualize surveillance data from the SENTRY Program platform. Data within MVP can be easily filtered to focus on subsets of data for specific antimicrobials, collection years, infection sources, or geographic regions.

> ***For example, an MVP query could be easily run to provide data on MRSA prevalence trends in each of the 9 US census divisions during the last 5 years.***

Users can view up-to-date information utilizing this free tool. Its intuitive interface allows you to better understand MIC and resistance profiles so that you can analyze spectrum of activity and identify ideal locations for clinical trials. Significantly, MIC data can be filtered on any of the parameters important to your project—including pathogen, collection year, geography, infection type, infection source, and age and sex of the patient—and then reviewed and exported in various formats such as antibiograms, charts, and cumulative percentage susceptibility tables.

What Others Have to Say

SENTRY data are integral to our global health work on estimating the burden of antimicrobial resistance. SENTRY data are particularly useful for estimating the distribution of pathogens responsible for the infectious syndromes with the greatest burden of disease. We are very pleased with the quality of data from SENTRY and the technical support provided by the team at JMI Laboratories.

Andy Stergachis, PhD
Professor of Pharmacy & Global Health
Adjunct Professor of Health Metrics Science
Co-Investigator, Global Research on Antimicrobial Resistance (GRAM) Project
University of Washington, Seattle, WA USA

In Vitro Antimicrobial Study Types Needed to Support Regulatory Filings

The FDA's Microbiology Data for Systemic Antibacterial Drugs — Development, Analysis, and Presentation Guidance for Industry describes a long list of potential *in vitro* antimicrobial assays that could be included in a prospective regulatory filing. We describe the most important of these assays below.

MIC Studies

MIC testing is the workhorse of antimicrobial drug development, typically carried out using broth microdilution or agar dilution using reference methods published by CLSI and EUCAST. MIC methods have two main steps: (1) exposure of a fixed number of bacterial cells to increasing concentrations of compound and (2) identification of the lowest concentration of compound that completely inhibited the growth of the inoculum. This compound concentration is the MIC. MIC values typically are read visually. Occasionally, these values can be difficult to interpret due to incomplete inhibition of growth. MIC studies usually are undertaken to understand a compound's spectrum of activity against contemporary collections of pathogen species relevant to the target indication. Often, the isolate sets chosen for initial MIC testing will include randomly collected isolates as well as specific subsets known to contain certain resistance mechanisms.

Compound-Binding Studies

The FDA recommends investigating the impact of bodily fluids and on MIC values exhibited by the compound. Typically, a special MIC study would be conducted that compared MIC values obtained under standard conditions with MIC values obtained in the presence of human serum, human urine, or pulmonary surfactant.

Bactericidal Studies

MIC data do not provide any information on whether a novel antimicrobial agent is bactericidal (actively kills cells) or bacteriostatic (prevents growth of cells without killing). The FDA recommends conducting specific *in vitro* studies to determine whether your antimicrobial exhibits bacteriostatic and bactericidal activity. In general, minimal bactericidal concentration (MBC) and time-kill assays would be conducted to generate data for regulatory filings.

Cross-Resistance Studies

As part of an overall assessment of the potential for target species to develop resistance to the compound, the antimicrobial activity of the compound should be tested against panels of strains with known resistance mechanisms; this step is particularly is important when the new agent is a member of an established antimicrobial class.

Mechanism of Action Studies

A detailed analysis of an agent's antimicrobial mechanism of action is ideal for new drug developers to provide a thorough explanation of how a new molecule kills or stops the growth of target bacterial cells. For new compounds of established antimicrobial classes, the target(s) will already be known. For novel compounds, mechanism of action studies will likely identify 1 or more specific target(s) to better understand the potential for resistance development to the antimicrobial. In general, resistance may develop more readily for compounds that have a single target as opposed to multiple targets. More studies are needed for novel antimicrobials than for new members of established antimicrobial classes.

Drug-Interaction Studies

The FDA also recommends conducting *in vitro* studies that investigate the potential for your new antimicrobial to exhibit synergy or antagonism when mixed in combination with currently marketed antimicrobial drugs that are used to treat potential target indications. Standard methodologies for studying the interactions of two antimicrobial compounds include time-kill kinetic assays and the so-called "checkerboard" titration with results evaluated by sum fractional inhibitory concentration analysis. The purpose of such studies is the early identification of drug-drug interaction signals that may impact human treatment.

Diagnostic Development Studies

Quality control testing parameters are necessary to ensure manufactured diagnostic reagents can be utilized clinically, including in clinical trials and validation studies. These assays are conducted by measuring testing results against established ranges that are developed utilizing CLSI guidelines. Other susceptibility testing device development studies, such as those for disk diffusion, also rely on selecting appropriate clinical isolates. These selection criteria especially are important to make sure that the susceptibility testing device can appropriately segregate the wild-type isolates from those isolates with resistant mechanisms near or above the projected breakpoint.

Methodology

Whenever possible, microbiological studies should be carried out using standard reference methods published by established organizations like CLSI and EUCAST. In general, such methods include extensive quality control processes and guidance that will help ensure the reproducibility of findings.

The use of non-standard MIC testing media and bacterial strains with unusual characteristics should be avoided if possible, because these non-standard methods may increase the time or cost of your developmental program. If bacterial strains with unusual characteristics are essential for MIC testing of your compound, you will need to make them available at a strain repository.

Conclusion

The development of a novel antimicrobial requires a significant risk and investment of time and resources. A critical aspect of the developmental process is the generation of high-quality *in vitro* data to support regulatory filings. The choice of a laboratory partner is vital in the drug development pathway. It is important to work with a team with vast experience in all aspects of assay development that has access to a relevant and diverse bank of clinical isolates collected through AMR surveillance networks. JMI Laboratories' curated data, catalogued isolates, and scientific expertise will help assure your antimicrobial drug development efforts are maximized.

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For further information, please contact:

info@jmilabs.com
www.jmilabs.com

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