

Antibiotic-Resistant Microbe Panel for the Evaluation of New Antimicrobial Agents

Introduction

Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a direct result of these infections. The discovery and development of new antibiotics that will be effective against rapidly evolving strains of drug-resistant microorganisms is one of the greatest challenges in drug development today.

To facilitate new antibiotic development, ImQuest BioSciences offers MicroSENS, a full service antimicrobial development platform uniquely combining *in vitro*, *ex vivo* and *in vivo* efficacy and toxicity evaluations. The platform includes preclinical services necessary for the discovery and IND-directed development of antimicrobial agents. With MicroSENS, the sensitivity of test compounds can be rapidly assessed using a diverse panel of microorganisms and candidate compounds can be prioritized to expedite preclinical development.

An essential component of the MicroSens platform is a microbial library of clinically important and genetically defined microorganisms spanning a broad range of resistant gram-negative and gram-positive organisms as well as medically relevant fungal organisms. Custom panels of resistant organisms in the library can be assembled for *in vitro* screening, animal model validation, or *in vivo* testing. Panels can be supplemented with relevant microbes for the development of topical microbicides.

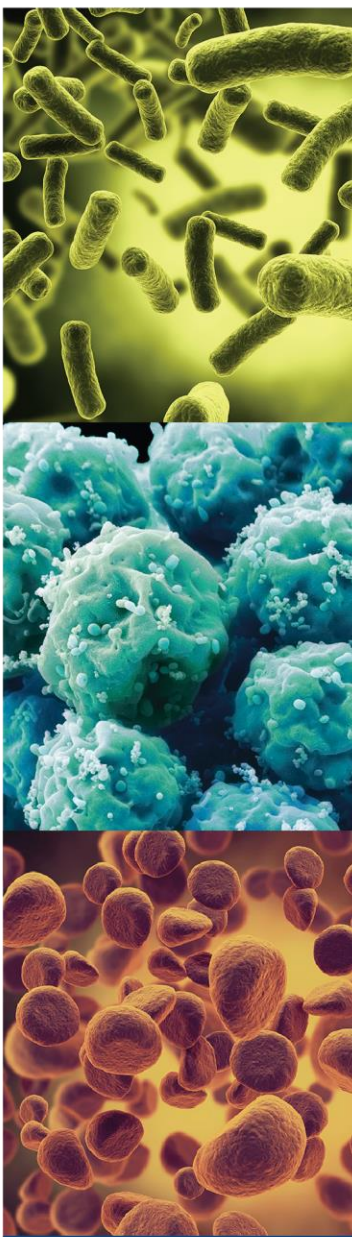
For each compound to be tested, these panels are used to evaluate the range and mechanism of antimicrobial activity, determine resistance potential and mechanisms, and examine the efficacy of combinations of antimicrobial agents.

A panel of antibiotic-resistant organisms was selected from the ImQuest microbial library and their sensitivity to control antibiotics was examined. The results are reported herein.

Method and Results

The sensitivity of a representative selection of organisms in our library of antibiotic-resistant organisms to control antibiotics was determined using the CLSI broth micro-dilution method. The minimal inhibitory concentration (MIC) of control antibiotics was then calculated.

The results demonstrated that the test organisms fell into distinct and expected categories of resistant phenotypes, with sensitivity to positive control compounds and resistance to the negative control compounds.



ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products. We specialize in the development of drugs, vaccines and biologic products for the treatment and prevention of infectious disease, cancer and inflammatory disease.

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MIC of Control Antibiotics against Representative Antibiotic-Resistant Microbes

Organism	Strain	Positive Control		Negative Control	
		Antibiotic	MIC (µg/mL)	Antibiotic	MIC (µg/mL)
Extended Spectrum β-Lactamase (ESBL) Producers					
Enteric Group 137	BAA-72	Meropenem	0.46	Penicillin	50
<i>Klebsiella pneumonia</i>	700603	Meropenem	15.00	Penicillin	50
<i>Escherichia coli</i>	BAA-196	Meropenem	3.75	Penicillin	50
New Delhi Metallo-β-Lactamase 1 (NDM-1) Positive Organisms					
<i>Enterobacter cloacae</i> (Carbapenam & MDR)	CDC 1000654	Tigecycline	8.00	Penicillin	50
		Colistin	4.00	Meropenem	60
<i>Escherichia coli</i> (Carbapenam & MDR)	CDC 1001728	Tigecycline	8.00	Penicillin	50
		Colistin	4.00	Meropenem	60
<i>Klebsiella pneumonia</i> (Carbapenam & MDR)	BAA-2147	Colistin	4.00	Penicillin	50
		Tigecycline	8.00	Meropenem	60
Penicillin Resistant					
<i>Streptococcus pneumonia</i>	700677	Ceftazidime-Clavulanic Acid	0.25	Penicillin	64
<i>Proteus mirabilis</i> (with ESBL)	BAA-856	Meropenem	0.50	Penicillin	64
Methicillin Resistant					
<i>Staphylococcus aureus</i> (CA, PVL +)	NRS 192	Clindamycin	0.0625	Penicillin	16
<i>Staphylococcus aureus</i> (CA)	NRS 384	Vancomycin	1.00	Penicillin	16
<i>Staphylococcus aureus</i> (CA)	NRS 123	Clindamycin	0.0625	Penicillin	16
<i>Staphylococcus aureus</i> (HA)	NRS 382	Gentamicin	1.00	Penicillin	32
<i>Staphylococcus aureus</i> (HA)	NRS 383	Vancomycin	4.00	Penicillin	32
		Vancomycin	1.56	Methicillin	100
<i>Staphylococcus aureus</i> (HA)	33591	Diocloxacillin	3.13	Methicillin	>100
		Vancomycin	0.78	Methicillin	>100
<i>Staphylococcus aureus</i> (HA)	33592	Diocloxacillin	3.13	Methicillin	>100
		Vancomycin	3.13	Methicillin	>100
<i>Staphylococcus aureus</i> (Mu50)	700699	Vancomycin	6.25	Oxacillin	>25
Vancomycin Resistant					
<i>Staphylococcus aureus</i> (VISA)	HIP 5836 (NARSA)	NRS4 Virginiamycin M1	2.00	Penicillin	32
	VRS1(NARSA)				
<i>Staphylococcus aureus</i> (VRSA)	HIP11714	Virginiamycin M1	1.00	Penicillin	32
Linezolid Resistant					
<i>Staphylococcus aureus</i>	NRS119	Vancomycin	4.00	Ciprofloxacin	32
Multi-Drug Resistant					
<i>Acinetobacter baumannii</i>	19606	Tigecycline	4.00	Penicillin	50
<i>Enterococcus faecium</i>	51559	Tigecycline	4.00	Penicillin	50
<i>Klebsiella pneumonia</i>	51503	Tigecycline	2.00	Penicillin	50
<i>Streptococcus pneumonia</i>	700677	Ceftazidime-Clavulanic Acid	0.25	Penicillin	64
<i>Staphylococcus aureus</i>	14154	Tigecycline	4.00	Penicillin	50

Conclusion

Custom panels of these representative strains and additional antibiotic resistant and quality control organisms can be assembled to define the effectiveness of new compounds for inhibition of resistant organisms.



MicroSENS for Rapid Antibiotic Discovery & Development

The MicroSENS platform includes preclinical services necessary for the discovery and IND-directed development of antimicrobial agents.

Essential elements of the platform include well-established validated assays and analytical tools for *in vitro* evaluations and an extensive microbial library of clinically important and genetically defined microorganisms spanning a broad range of resistant gram-negative and gram-positive organisms. Test agents can be evaluated for inhibition of pathogenic biofilms and the Minimal Biofilm Inhibitory Concentrations (MBIC) defined.

The MicroSENS platform also includes the evaluation of antimicrobial agents in well-defined animal models, including [peritonitis-sepsis](#) and [neutropenic thigh models](#), as well as other more unique and specific animal models.

ImQuest offers customized animal model evaluations depending on the nature of the product and indication to be treated. These models can be performed using antibiotic sensitive or resistant organisms.

ImQuestSUCCESS

Select drug candidates with the highest probability of clinical success

The ImQuestSUCCESS preclinical services platform is used to critically evaluate the potential of a test compound and to assure that its efficacy, toxicity, and pharmaceutical properties are evaluated in a comprehensive and interactive way. Successful completion of platform objectives provides significant confidence in the potential of a test compound to transition to human clinical trials, enhances the robustness of drug development efforts and reduces the risk of expensive clinical development failures by the exclusion of candidates which are likely to fail during advanced preclinical and clinical development at early (and less expensive) time points.

Microbe Libraries

- Indication-specific microbes
- Clinically-relevant Gram (-) & Gram (+) organisms
- Anaerobic organisms
- Sexually transmitted organisms

In Vitro Assays & Tools

- CLSI broth micro-dilution for MIC and MBC determination
- Kill-curve analysis
- Analysis of bactericidal versus bacteriostatic activity
- Post-antibiotic effect
- Inhibition of biofilm development
- Combination antimicrobial evaluations
- Resistance selection
- Mechanism of action studies:
 - *In vitro* generation & characterization of spontaneous resistant mutants
 - Radiolabeled precursor incorporation analysis
- Microbial quantification and identification

In Vivo Models

- Neutropenic thigh model
- Peritonitis-sepsis model
- Systemic sepsis model
- Custom models (on request)
- CLSI-based serum bactericidal effect (for some models)



PrevSENS for the Development of Microbicides to Prevent & Treat Infections

The PrevSENS platform includes preclinical services necessary for the discovery and IND-directed development of microbicides.

ImQuest BioSciences has the capability to expand the development of antimicrobial compounds to include evaluation of their use as topical agents to prevent or treat sexually transmitted infections (STI) and other skin and wound infections.

Evaluations of topical antimicrobial agents include efficacy and toxicity assays to define lead candidates, range and mechanism of action assays, combination microbicide product development strategies, resistance evaluation, formulation development, and the evaluation of safety and efficacy in *ex vivo* 3-dimensional tissue models. Inhibition of biofilm formation by test agents can be evaluated with organisms which form biofilms in the vagina or on damaged skin (wounds and burns).

All of our efficacy and toxicity defining assays are performed under conditions that closely mimic the environment in which they are designed to act, e.g., in the presence of vaginal and seminal fluids.

Additional range of action assays can be performed to assess the inhibitory potential of a compound against other viral and bacterial pathogens that might be present during the sexual transmission of HIV and HSV.

Toxic effects of a microbicide to the normal vaginal flora as well as to the cells that comprise the vagina or rectum may reduce the natural capacity of the tissue to prevent infection. For this reason, ImQuest BioSciences evaluates:

- The toxicity of microbicide products in the appropriate cells and tissues.
- The induction of pro-inflammatory cytokine expression in these cells and tissues.

STI panel for evaluation of activity against:

- *Chlamydia trachomatis*
- *Trichomonas vaginalis*
- *Neisseria gonorrhoeae*
- Vaginosis Causative Microbes:
Bacterioides fragilis
Gardnerella vaginalis
Mobiluncus curtisii
Candida albicans

Toxicity evaluations with:

- Cervical, vaginal, and rectal cell lines
- Epivaginal tissue
- Normal vaginal flora (including bacteriostatic and bactericidal activity):
Lactobacillus crispatus
Lactobacillus jensenii
Lactobacillus acidophilus

