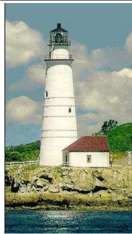

Northeast Branch Newsletter

Number 135 Winter 2011

46th Annual Region I Meeting

The Northeast Branch of the American Society for Microbiology was pleased to host the Region I Meeting this year, which was sponsored in conjunction with the Connecticut Valley, Eastern New York, and New York City Branches. The Region I Meeting was held at The Lantana conference center in Randolph, MA on October 26-27, 2011 and had over three hundred attendees. It attracted microbiologists and over sixty undergraduates, graduate students and postdoctoral fellows from surrounding states. We would like to thank our sponsors and exhibitors for their most generous support, and all the conveners and speakers for the exciting programs.

The title of the Meeting, *Microbiology at the Crossroads: Bad Bugs/Global Health and Ecology/New Technologies*, focused on emerging infectious diseases and evolving diagnostic technologies. Sessions were dedicated to hospital-associated infections caused by *Clostridium difficile* and gram negative rods, food microbiology, tuberculosis, marine microbiology, stimulating cognitive skills, global microbiology, molecular diagnostics and host-pathogen interactions.

Prof. Edward Carney (3rd from L) and Students from Norwich University, VT

David C. Hooper, MD, President, American Society for Microbiology, Presenting the Keynote Lecture

Exhibitors were invited to showcase new technologies in a session entitled “Innovative Diagnostics: An Industry Perspective”.

The keynote speaker was ASM President David C. Hooper, MD, Division of Infectious Diseases, Massachusetts General Hospital who spoke on “Microbial Ingenuity and the Challenge of Antimicrobial Resistance”.

(Continued on page 3)

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NEB Council Meetings

Council Meetings this year will continue to be held at the State Laboratory Institute in Jamaica Plain. Members and all interested microbiologists and scientists are welcome to attend. Please notify Irene George at (508) 785-0126 in advance.

Membership Notes

Dues reminders for 2012 are currently being sent to the membership e-mail. Members who did not provide an e-mail address will be contacted by postal service. Membership forms may be found on the NEB website or you may join the both the ASM and the Northeast Branch online through the ASM eStore. Please make the necessary corrections to your demographics and return dues to the Treasurer. Emeritus members need to reply if they wish to remain on the mailing list. Changes only may be e-mailed to: NEBranch-ASM@comcast.net. Please check mailing labels on postal correspondence as they reflect existing information. Although membership in the national branch automatically makes you a member of the local branch in some organizations, this is NOT the case in the ASM. *To be both a National Member and a NEB member, you have to join each individually.* The Northeast Branch currently has 271 members.

Council Election Results

Congratulations to the following NEB members whose terms as Branch Officers run from July 2011-June 2012: James E Kirby, President; Alfred DeMaria, Jr., President-Elect; Irene H. George, Secretary; National Councilor, Paulette M. Howarth; Alternate National Councilor, Frank J. Scarano, and our three Local Councilors are Gail S. Begley, Nancy S. Miller and Grigoriy Urman. We are looking forward to working with them!

Vote Regarding Emeritus Status

The membership voted this year on a motion made by the NEB Council to define emeritus membership in the NEB as a member in good standing for 20 consecutive years who is retired from their profession. The motion was passed in April 2011 and will be added to the NEB Constitution.

FUTURE PROGRAMS

Local Programs:

Announcements of Local Meetings and registration materials are posted on our website:
http:/www.asm.org/branch/brNoE/index.shtml

March 15, 2012
Identifying Human Chemical Exposures- The Role of Mass Spectrometry in the Public Health Laboratory
Speaker: Julianne Nassif, MS, Director, Division of Analytical Chemistry, Wm A. Hinton State Laboratory Institute, MA Dept. of Public Health, Boston, MA
Location: Hilton Garden Inn, 420 Totten Pond Road, Waltham, MA
This program is cosponsored with the Northeast Section of the American Association for Clinical Chemistry.

Contacts for Local Programs: Irene George at NEBranch-ASM@comcast.net

National Meetings:

June 16-19, 2012
112th ASM General Meeting,
San Francisco, CA.
Contact: ASM, Tel: (202) 737-3600
See: www.asm.org/asm2012

June 14-17, 2012
19th ASM Conference for Undergraduate Educators (ASMCUE), San Mateo, CA.
Contact: ASM, Tel: (202) 942-9317
See: www.asmcue.org

September 9-12, 2012
Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2012)
San Francisco, CA.
See: www.icaac.org

For additional information on ASM Meetings and Conferences please contact: (Tel) 202-942-9248, meetingsinfo@asmusa.org

Region I Meeting (continued)

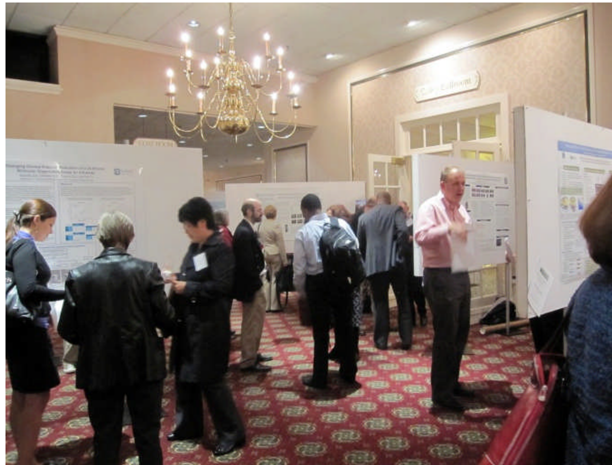
James E. Kirby, President, Northeast Branch, Susan M. Reverby, PhD, Wellesley College, and Alfred DeMaria Jr., MD, President-Elect, Northeast Branch

Late Wednesday afternoon included a wine and cheese reception with the exhibitors and poster presentations with authors in attendance. The evening dinner lecturer was Susan M. Reverby, PhD, Professor in the History of Ideas and Professor of Women's and Gender Studies at Wellesley College, Wellesley, Massachusetts who presented "Escaping Melodramas: Reflections on the U.S. Public Health Service Infamous Studies in Tuskegee and Guatemala". She spoke of what happened in these studies and provided reflections on the dangers and safeguards needed for human subjects.

Twenty poster presentations were accepted for presentation at the Meeting and three undergraduate and three graduate students received awards for their outstanding work (see article following).

We would like to thank many of the speakers who have allowed us to post their presentations on our website (in pdf format): http://www.asm.org/branch/brNoE/index.shtml

Region I Meeting
Student Poster Presentations



Awards were presented to the following students for their outstanding work and presentations:

Undergraduate Students:

Third prize undergraduate: **Tiffany Damiani**, Trinity College, Hartford, CT
"The Effects of Smoking on Bacterial Communities of the Upper Respiratory Tract".
T. Damiani and L. Foster

Second prize undergraduate: **Lindsay Musgrove**, Quinnipiac University, Hamden, CT
"Shopping Carts as a Fomite: What bacteria is on your Shopping Cart?"
Lindsay Musgrove, Yefrik Manni, Danielle Leahy, Natasha Dave, Dr. Lisa Cuchara

First prize undergraduate: **Danielle Davis**, University of Maine, Orono, ME
"Gold-nanoparticle-modified Carbon Electrode Biosensor for the Detection of *Listeria monocytogenes*".
D. Davis, X. Guo, and V.C.H. Wu

Graduate Students:

Third prize graduate: **Allison Lacombe**, University of Maine, Orono, ME
"The effect of blueberry-enriched diets on the microbial composition of the rat proximal colon detected by metagenomics."
Alison Lacombe, Robert W. Li, Dorothy Klimis-Zacas, Aleksandra S. Kristo, Shravani Tadepalli, Emily Krauss, Ryan Young, and Vivian C.H. Wu

Second prize graduate: **James Brooks**, University of Connecticut, Storrs, CT
"Cloning and Characterization of a Tryptophan Halogenase Involved in Antibiotic Biosythesis in *Frankia* sp. CcI3".
James M. Brooks, John M. Ngunjiri, Nicholas C. Butzin, and David R. Benson

First prize graduate: TIE
Steven Bryant, University of Massachusetts Boston, Boston, MA
"The Occurrence and Distribution of *Enterococcus* (ENT) species in an Urban Coastal Watershed".
Steven Bryant, Michie Yasuda and Michael P. Shiaris

and

Calvin Williams, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA
"Identification of Novel Antimicrobials that Target Host Pathogen Interaction using the Model Pathogen, *Legionella pneumophila*".
Calvin L. Williams, Lucius Chiaraviglio, Sylvine Raverdy and James E. Kirby

46th Annual Region I Meeting

Students from the Eastern New York Branch

Global Microbiology Speakers:
Frances Ingersoll and Leonard LaFazia

Hospital-Associated Infections – C. difficile
Speaker Stephen Brecher

Evolving Technologies Speakers: Sandra Smole,
Nancy Miller and Catherine Klapperich:

Global Microbiology Conveners:
Catherine Brown and Frank Scarano

Eastern New York Branch Speakers (L to R):
Terry Means, ENY Branch President Timothy Sellati,
Paul Wahome, Egil Lien, Samuel Behar, and
Kathleen McDonough

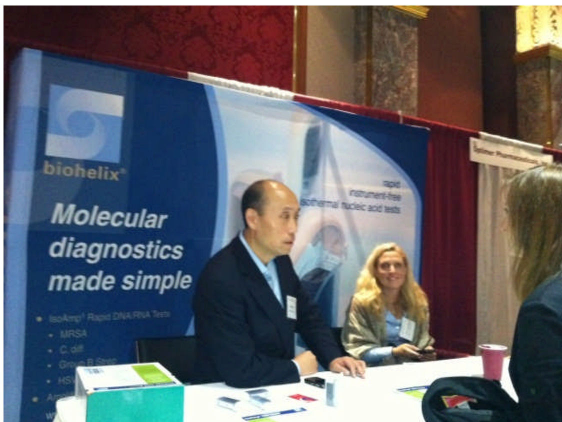
46th Annual Region I Meeting Exhibitors

Advanced Instruments, Inc.

Hardy Diagnostics

Catherine Papagno & Stacey Clapp both of BD Diagnostics; Edina Reiszner, Faulkner Hospital

Forest Pharmaceuticals, Roche Molecular Diagnostics and Cubist Pharmaceuticals



BioHelix Corporation

Integrated DNA Technologies

46th Annual Region I Meeting

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Programs in Review - 2011

Culture Shock! The Shift to New Technologies for In-Vitro Diagnostics in Clinical Microbiology

The third NEB dinner-meeting of 2011 was held on June 8, 2011. Nancy S. Miller, M.D., presented "*Culture Shock! The Shift to New Technologies for In-Vitro Diagnostics in Clinical Microbiology*". Dr. Miller is Medical Director of Clinical Microbiology & Molecular Diagnostics at the Department of Laboratory Medicine, Boston Medical Center (BMC), and Assistant Professor, Department of Pathology and Laboratory Medicine at the Boston University (BU) School of Medicine in Boston.

Dr. Miller is a clinical Principal Investigator for various translational research projects including collaborations with the BU School of Bioengineering. She is frequently invited to discuss diagnostic product development, including ad hoc advising for the BU Office of Technology Development. Under her direction the BMC Clinical Microbiology Laboratory also participates in preclinical and clinical testing of new diagnostics.

Conflict of interest disclosure for this presentation included that: 1) Dr. Miller has engaged in research collaborations with some companies mentioned in this talk; 2) The BMC Clinical laboratory uses some of the products mentioned; 3) Some technologies mentioned have not been FDA-cleared for diagnostic use in the United States.

Dr. Miller began by reviewing the advantages and disadvantages of familiar gold standard phenotypic methods. Then she discussed factors that are driving changes in diagnostics. This was complemented by a presentation of several notable or paradigm-changing technologies with a focus on platforms and assays that are currently available or within reach of many routine diagnostic laboratories. Challenges and considerations posed by these new methods were also discussed.

In brief, culture-based methods and bio-phenotypic inquiry have long been the "comfort zone" for microbiologists. These methods are often visual, inexpensive, and employ well-characterized assays. However, they can be labor intensive, time consuming, and subjective. Other limitations include reproducibility issues and a lack of utility for specimens with low organism burden or an inability to be cultured in vitro. The morphological expertise required for phenotypic assay is unlikely to be replaced by laboratories that are facing an aging retiring work force and fewer technologists.

Our new era of clinical microbiology is characterized by concerns for biosafety and infection control and the need for rapid detection of pathogens (many drug resistant) in order to direct optimal clinical outcome. In addition, new therapeutics (e.g., in virology) demand novel assays particularly for HIV, hepatitis and transplant medicine. All these factors are driving the need for new diagnostic methods. Research and industry have recognized these needs and in the past decade there has been an explosion of new diagnostic approaches that have finally come to the laboratory or are on their way.

To set the stage for newer technologies, Dr. Miller reviewed more traditional molecular methods such as first-generation hybridization probes and conventional polymerase chain reaction (PCR). Then she discussed optimized next-generation peptide nucleic acid (PNA) probes, transgenic cell lines, and clever improvements to PCR that have culminated in user-friendly, fully-integrated real-time PCR assays and platforms. These methods can help bring standardized patient care testing to all laboratory shifts regardless of molecular expertise. Dr. Miller also noted new

Culture Shock (continued)

technologies that feature expanded multiplex capability beyond that of real-time PCR – thus highlighting the need in microbiology to do more with less clinical sample in order to streamline workflow, decrease turnaround time and costs. While highly beneficial, Dr. Miller noted that the new technologies present challenges as well. For example, method validations often need to account for new technologies that are more sensitive than the predicate gold standard. Laboratory budgets may need to reconcile the costs of new technologies with their downstream clinical benefits. And choosing an assay or platform can challenge a laboratory's consideration of what is "best" versus a "best fit" for their specific needs.

Next, Dr. Miller turned to advances in database-dependent methods such as those technologies used for strain typing and microbial identification. Compared to the older labor-intensive and non-portable methods, the new methods offer speed, digital information portability, convenient workflow, and an expanding library of public and proprietary databases. Again, Dr. Miller pointed out important considerations for laboratories looking to embrace these new tools – including validating systems, understanding the nature of database-dependent results, and having resources maintain database quality.

From the pre-analytical perspective, Dr. Miller celebrated that we are now in the age of automated microbiology. There are now several available systems that optimize efficiency and workflow by using robotics to automate specimen processing. We can finally look forward to a virtual automated microbiology laboratory that uses various instruments that are docked or connected by interface to create open-ended efficiency. This is now becoming a reality. With increased frequency, vendors are offering advanced middleware to interface platforms, improve data management and enable remote monitoring.

In closing Dr. Miller presented some personal favorites among the recent novel, creative approaches to diagnostics. These included a phenotypic bacteriophage-based test for bacterial identification and susceptibility testing; an isothermal, non-instrumented helicase-dependent PCR test system; and a biosensor system that identifies microbes by electrochemical detection of organism-specific nucleic acid. Finally, Dr. Miller discussed emerging excitement about the validation of mass spectrometry biosensor platforms for microbial identification.

In summary, Dr. Miller noted that the adoption of new diagnostics presents us with a variety of challenges and considerations. Embracing these new tools requires a synthesis of details regarding assay design, scientific literature, technical options, workflow efficiencies, resources and regulatory requirements, just to name a few. We need to understand the advantages and disadvantages of each technology, how each can be used, and how results will be interpreted and applied to patient care. This is truly an exciting time for clinical microbiology and microbiologists.

Dormancy Mechanisms Shed Light on Old Microbiology Puzzles: Persisters and Uncultured Bacteria

(L to R) James Kirby, President, NEB and Speaker Kim Lewis, PhD)

The second dinner-meeting of 2011 was held on March 21, 2011, with speaker Kim Lewis, PhD, Professor of Biology and Director, Antimicrobial Discovery Center at Northeastern University in Boston, MA. Dr. Lewis is an author on over 100 papers and is an inventor of several patents related to the topics of discussion. These include a general method to

Persisters (continued)

grow previously unculturable bacteria that make up more than 99% of biodiversity on the planet and the discovery of the culprit of recalcitrant biofilm infections, drug-tolerant persister cells. Today Dr. Lewis spoke on *Dormancy Mechanisms Shed Light on Old Microbiology Puzzles: Persisters and Uncultured Bacteria.* In his introductory remarks he mentioned that when he became Director of the Antimicrobial Discovery Center it occurred to him that it meant that he had to discover at least one useful antibiotic before he retired. The problem with that lofty proposition was that the last time a useful antibiotic was discovered in academia was in1944, when Salman Waksman and his graduate student, Albert Schatz, discovered streptomycin. The difficulty in discovering and developing new antibiotics is one of the major challenges we are facing today, while the need in the context of emerging antimicrobial resistance is dire.

Dr. Lewis spoke of basic science involved with drug discovery and working on platforms for drug discovery. He was always been fascinated with puzzles in general and particularly the profound paradox of chronic infections. It fascinated him that pathogens that are susceptible to antibiotics often cannot be effectively treated if they are allowed to reach a stage of "chronic" infection. Most of these infections are associated with biofilms. However, there are other infections such as tuberculosis, where there is a drug susceptible pathogen, but the organism cannot be easily eradicated. For example, a fully susceptible strain of *E. coli* can settle on a urinary catheter and form a biofilm around it. Paradoxically, these infections are very difficult to eradicate with antibiotics even though the drugs penetrate well into the biofilm. There is a long list of infections associated with biofilms, perhaps accounting for half of the infections physicians see in clinical practice in the developing world. Indeed, antibiotics have a limited efficacy against susceptible cells in 60-65% of all infections: as with *H. influenzae* in middle ear infections in children; dental disease involving streptococci and Actinomyces; endocarditis; indwelling devices such as prostheses in which *S. aureus* and various other staphylococci are found; urinary catheters where various species of staphylococci are seen; Pseudomonas pulmonary infections in patients with cystic fibrosis; and tuberculosis.

One of the discoveries made about a decade ago was shown in a simple killing experiment using different pathogens from chronic infections related to biofilms. Dr. Lewis explained what happens in a relapsing biofilm infection. When a population is hit with an antibiotic it collapses, but a subpopulation of organisms (persisters) in a biofilm will survive and repopulate the biofilm; both viable and stationary phases produce up to 1% persisters. The paradox now becomes what it is that makes these cells essentially invincible to being killed by antibiotics. A simplified view of a chronic disease is that when a biofilm is formed, perhaps on a catheter, antibiotics will kill off the regular cells and the immune system will destroy any persisters in the bloodstream. Persisters in the biofilm will survive however, because the biofilm serves as a protective habitat and prevents the penetration of immune components into it; the patient feels well now and may discontinue the antibiotic. When the antibiotic concentration drops, the persisters repopulate the biofilm causing a relapsing infection.

Dr. Lewis explained one of the simplest experiments that can be done to search for genes underlying complex functions in bacteria, which is to screen a knockout library. A complete ordered library of all knockouts is available for *E. coli*. A high dose of antibiotic was added (ofloxacin) that killed all regular cells; only persisters survived. A total of 4000 clones were screened using this *E. coli* library. Surprisingly, they all showed the same persister phenotype: all strains showed only decreased levels of persisters in the stationary population. No strain was found that did not produce persisters. This was very disappointing as it did not point to a common control pathway for persister formation; the speculation was that perhaps many redundant or parallel pathways were involved, and left a scientific experimental conundrum, in that we would need to isolate something you knew nothing about.

That left a need to develop an educated guess or hypothesis, and test it. Dr. Lewis then proposed the theory that persisters are dormant because they are known to neither grow nor die in the presence of antibiotics. Dormancy was

proposed as a mechanism to avoid antibiotic killing effects, as the pathways that antibiotics attack general rely on metabolic activity for their effects to be manifest. If the "persisters" were in fact dormant cells, then we could take advantage of a strain of *E. coli* that has degradable GFP under a ribosomal promoter. In a population that is growing, regular cells express GFP and are a bright green fluorescent color; these are "quitters" and are should die when exposed to beta lactam antibiotics. However, if one of the cells goes dormant, protein synthesis should diminish and the cell should become dim due to GFP degradation; these cells should also survive antibiotic treatment because of a "tolerant" dormant state. Cells can thus be sorted into distinct populations: viable and metabolically active (GFP positive), dormant and metabolically active (GFP negative). The dim cells were also tolerant to ofloxacin, identifying them as persisters. Dr. Lewis emphasized that these phenotypically distinct populations are a clonal bacterial population, formed from a single cell, in which cells that are genetically identical split into two phenotypically different sub-populations. This is called bistability; i.e. when a population splits into two and goes down two different developmental pathways. Apparently there is some stochastic expression of persister genes that enables a small number of cells to go down a pathway to become specialized survivors or persisters; however they forfeit their ability to propagate, at least for the short term.

Dr. Lewis then explained why dormancy is an especially useful adaptive strategy for survival in high antibiotic concentrations. He proposes that there are two distinct mechanisms of bacterial survival in the presence of antibiotics. One is classic antimicrobial resistance. There are many such resistance mechanisms, such as efflux of antibiotics, destruction, mutation of target, etc. All essentially do the same thing and prevent the antibiotic from interacting with its native target. In the presence of elevated antibiotic, bacteria will grow, and we see a higher minimal inhibitory concentration (MIC). Bactericidal antibiotics kill by corrupting a critical cellular activity that is usually integral and active during a bacteria's normal state. This is true of fluoroquinolones that target DNA, beta-lactams which prevent cell wall resynthesis and cause self-destruction through the activity of native autolysins, aminoglycosides which cause toxic misfolded proteins etc. However, these same cellular activities are quiescent during the dormancy of persisters. Therefore antibiotics are ineffective and the bacteria cells cannot be killed by antibiotics until they awaken.

On the basis of this theoretical construct, his lab began analyzing the *E. coli* transcriptome for mechanisms that can stop essential functions in a cell. Advanced screens showed knockouts with a 10-fold decrease in persister formation. The majority of hits were in a number of global regulators which could affect expression of several potential persister genes at the same time. Persister transcriptome analysis showed that the overexpression of toxin/antitoxin (TA) module genes was indeed able to stop essential functions in a cell and perhaps cause dormancy; this could be reversed by expressing the antitoxins. These systems were of particular interest. Toxin/antitoxins were originally discovered as mechanisms for plasmid maintenance. The toxin is stable; while the antitoxin is unstable. If the bacterium loses the plasmid the toxin persists longer than the antitoxin and the bacterium dies. Therefore, the population with the plasmid has a select advantage and persists at the expense of the population that loses plasmids at some frequency. It was always a mystery as to why there were there, as the bacterium presumably was in no danger of losing its chromosome and would be dead without it anyway! However, an alternative theory now emerges for the elusive chromosomal elements. Namely, that they coordinate entry into a persister state, not necessarily killing the bacteria but making them quiescent relative to their peers, and preserving them during times when an active metabolic state would prove deleterious.

An example of one of these toxins is RelE which cleaves mRNA and inhibits translation in the cell chromosome. *E. coli* cells over-expressing RelE toxin allows the cells to become multidrug tolerant to high concentrations of antibiotics such as ofloxacin, cefotaxime and tobramycin. Therefore by turning on the toxin, artificial persisters can be created. Overexpression of another toxin, the protein kinase, which phosphorylates elongation

Persisters (continued)

factor Ef-Tu, inhibits cell growth, producing high drug tolerance and dormancy. Therefore, several different *E. coli* toxins are therefore able to inhibit protein synthesis and send cells into dormancy; indeed15 such modules have been discovered in *E. coli,* and 80 similar ones in *M. tuberculosis*.

The first clue to the involvement of an upstream "regulator" of a persister mechanism was when ciprofloxacin was used to induce the SOS response in *E. coli*. Upstream from the SOS region is a LexA repressor which is normally part of the cell canonical SOS response. If cell DNA damage occurs, as with ciprofloxacin, the LexA repressor is cleaved and triggers DNA repair enzymes and protective proteins. Interestingly, LexA also controls the TisB toxin/antitoxin module. In collaboration with NIH scientists, TisB was found to be a primarily a chloride ion channel; TisB binds to the cell membrane, opens the channel by forming a membrane pore, and depletes ATP, causing system shutdown and cell dormancy. If TisB is knocked out persisters are not formed during ciprofloxacin treatment. An unanticipated "side effect" of fluoroquinolone treatment in these experiments in cells overexpressing TisB was the occurrence of multidrug-tolerance, to both fluoroquinolones and unrelated antibiotics. Persisters appear to form randomly, but there is a possibility that other stress factors in the environment such as temperature or pH can trigger persister formation in similar ways, for example by inducing the SOS or similar regulated responses.

The diverse mechanisms by which persisters can form in *E. coli* confirm the redundancy which was thought to occur in previous experiments. A plausible hypothesis therefore was that persister antibiotic tolerance would explain why chronic diseases are recalcitrant to treatment. But there is a difference between a plausible hypothesis and causality. Koch's postulates cannot be applied to prove causality, as once persisters are introduced into an animal, they will start to grow and will no longer be persisters. Although you can theorize how persisters are related to recurrent chronic infections it will be difficult to prove they are the cause said Dr. Lewis.

A clue to solve that problem came from other independent experiments being done in search of persister genes in which they were looking for mutants which made more persisters, so that each mutation could be cataloged. A population of growing cells was treated with a high concentration of bactericidal antibiotic. Most of the population collapsed, and the surviving bacteria were harvested. This was repeated a number of times and finally a high persister mutant was obtained, which gives rise to a greater percentage of persistence on a reproducible basis. This was done with *E. coli* and the organisms were sent to the Broad Institute for genomic sequencing. Interestingly, the high persister mutants showed a high frequency of gain-of-function mutations in the HipA toxin previously mentioned. These mutations showed decreased binding of antitoxin. At the same time, they demonstrated a level of persister formation 100 times greater than wild type bacteria. Presumably, the exact same thing happens when people take high doses of antibiotics; this selects for high persister mutants, and therefore persisters may be an important part of recalcitrant infections.

To examine this hypothesis, one experiment involved a large number of *E. coli* isolates primarily from patients with urinary tract infections which were scanned by PCR amplification for hipA mutations. Fascinatingly, about half of the organisms had the hipA mutation. This supports persister formation as a causal mechanism in chronic infection, as the very mechanism associated with hyperproduction of persisters appears to be selected for and presumably provides a survival advantage during extensively treated chronic urinary tract infection. Interestingly, the same thing occurs with ofloxacin and *P. aeruginosa* in patients with cystic fibrosis; persister levels from the same patient increase over time. Many of these organisms remain completely susceptible to antibiotics by conventional means. The main culprit in the long run that correlates with the demise of these patients is the emergence of high persister mutants.

Dr. Lewis explained two facets of persister threat. Acute infections are those in which there is an intrinsic resistance related to the potential to form persisters at a low frequency. These are often cured. However, chronic infections are

those in which persister tolerance permits formation of *hip* (high persister) mutants, especially in the context of biofilms and with organisms such as tuberculosis which causes chronic infections by its nature and is primed for high persister mutations. He showed a diagram of his drug discovery project and the various pathways found that may be involved in persister formation in *E. coli.* The problem is that this indicates that there are too many redundant pathways leading to persister formation to inhibit and therefore there are no realistic targets for drug discovery.

The golden age of drug discovery was from 1940 to 1960 after which research slowed greatly. Only three new classes of antibiotics have been developed over the past thirty years and act against gram-positive organisms. There are no new drugs in sight and multidrug resistant organisms such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are becoming major problems. Synthetic drugs against genomes and proteomes were developed. However, synthetic compounds were running into a barrier; gram negatives have a cells membranes that are difficult to penetrate and efflux pumps that easily remove synthetic drugs from the cells when they do penetrate. These frustrating difficulties have led to the closing of antinfectives divisions in the Big Pharma.

Access to the target is not the issue with persisters; persisters have no specific target for a drug to reach. Ideally you want to introduce highly reactive molecules inside the dormant persister, but practically do not want not kill the patient with such a non-specific therapeutic strategy. Notably, however, there is a group of antibiotics that are different from conventional target-specific drugs called prodrugs that are primarily used to treat tuberculosis. Prodrugs such as isoniazid (1981), PZA (1952), ethionamide (1956) and metronidazole (1959) are activated by specific bacterial enzymes into reactive compounds. Therefore, the host cells are spared. The concept is that an activated, reactive prodrug will disrupt cellular components even in persisters. Thus prodrugs offer hope of developing compounds that may ultimately kill persisters.

Supporting this idea, it was possible to create an *E. coli* strain overexpressing the nfnB gene which actives the pro-drug metronidazole. When challenged with metronidazole, these cultures were "completely" sterilized. No persisters and absolutely no survivors were found. Based on this theoretical construct, Dr. Lewis' laboratory screened 10,000 compounds from 1981-89 for new prodrugs that would kill persisters in the presence of overexpressed potential prodrug activating enzymes. Using this strategy, four prodrugs were found. Dr. Lewis has begun a new screen for prodrugs using *E. coli and B. anthracis.* In pilot experiments, they have screened 50,000 compounds, identifying 48 prodrug hits and have two leads. Based on the success of this validation set, a large high throughput study is being undertaken (Lewis, K. 2007. Persister cells. Dormancy and infectious disease. Nature Rev. Microbiol. 5:48-56).

Persisters were first recognized by Joseph Bigger in 1944. There are many other unsolved puzzles in microbiology that may be related to persister (dormancy) biology such as biofilms, antibiotic tolerance, tuberculosis latency, and unculturable bacteria. Shifting to another avenue of research in his laboratory, Dr. Lewis described the discovery of a general method to grow "unculturable" bacteria. Here an environmental sample (marine sediment) is taken, diluted, and plated onto a Petri dish. Interestingly, the microscopic count of organisms is hundreds of times more than actually grow on the Petri dish; this is known as the "great plate count anomaly". He therefore decided to try to grow some of these unculturables in pure culture through use of a new strategy: by reconstituting the native environment of these organisms. Specifically, they took marine sediment, diluted it, placed it between two semi permeable membranes, and put it back into the environment from which the sample was taken. Now, there was up to 40% recovery of viable organisms. The question now was why organisms grew in the "marine" environment but not in the Petri dish. To study this, they looked at sand particles and the tight community of anaerobic bacteria living on them. They found that unculturables do not grow in unfamiliar environments. More specifically they were able to demonstrate in a bioassay that unculturable bacteria from marine sediments rely heavily on siderophores, iron chelators, produced by neighboring bacteria for their

replication. Dr. Lewis showed examples of how unculturables may be screened for novel antibiotic production using these insights in the same way; and furthermore how some can be "domesticated" in the laboratory and eventually grow on a Petri dish.

Dr. Lewis lastly spoke of exciting research being done with human microbiome manipulation. A large number of diseases are linked, by correlation at least, to particular intestinal bacteria, but the problem is that most of these organisms are unculturable and require growth factors from cultivable species. He showed a slide of a growth factor isolated by his laboratory, using factors normally produced by other commensal bacteria to allow isolation of non-culturable bacteria from the human microbiome. These studies open the door to study more than the genomes of these organisms and to begin to investigate how they may affect human health and disease. Dr. Lewis believes that understanding the nature of these growth factors for unculturables will provide useful tools that will allow screening of uncultured bacteria for drug discovery and for manipulating the microbiome.

Dengue- Something Old, Something New!

Nancy Miller, Boston Medical Center and Speaker Allan Rothman, PhD

The first dinner-meeting of 2011was held on March 2, 2011, with Alan L. Rothman, MD, formerly Professor of Medicine at the Center for Infectious Disease and Vaccine Research at the University of Massachusetts Medical School in Worcester speaking on *Dengue- Something Old, Something New!* In April 2011 Dr. Rothman joined the staff of the Institute for Immunology and Informatics, which is a research component of the University of Rhode Island's biotechnology program, as a medical researcher. His research includes the immunology and pathogenesis of viral diseases, particularly dengue, with which he started to work some twenty years ago. The disease returned to Florida after an absence of about fifty years and appeared in Palm Beach; Brazil was overwhelmed with hundreds of thousands of cases in 2008.

During his lecture Dr. Rothman touched on the history, epidemiology, immunology, and management and prevention of the disease, which has been around for a long time. In fact, a dengue-like syndrome was described in China in 265-992 AD. The first reports of an epidemic and a dengue-like illness were given by Benjamin Rush, a Philadelphia physician, in 1779-80. Epidemics of fever were reported in major port cities in the 1800's. In Charleston, NC, high fever with severe bone pain was reported, prompting the name "break-bone fever"; hemorrhage was seen but no deaths occurred. The population of Charleston was about 50,000, and 15,000 cases of a massive benign disease occurred. In the 1940's the United States (U.S.) Army identified the mosquito vector and viral etiology; massive eradication efforts were undertaken. During World War II, ecologic changes led to hyperendemic circulation and intense research efforts by both the US and Japan; however few deaths were seen. The recognition of dengue hemorrhagic fever (DHF) came as a surprise in the 1950's when epidemics occurred in Thailand and the Philippines. Increased numbers of DHF were seen in Southeast Asia in the 1960's-1970's, and in the 1980's the first reports of DHF occurred in the U.S.

Dengue was an epidemic disease in early 20th century. Disease in the U.S. was previously imported and under-diagnosed, with passive reporting. There was sporadic local transmission in Texas in the1990's, and nine cases (1 DHF) in 2005. Hawaii had 119 cases in

Dengue (continued)

2001. Overall, there was an average of 244 cases/year from 2006-08. Dengue became a notifiable disease in 2010 when we had 487 cases (5 DHF): Florida 182, New York City 115, and Puerto Rico 9955 (59 DHF). Florida, in 2009-10 had 65 cases (Key West, 63 cases; Broward County, 1 case; Miami-Dade, 1 case. There was a 5% seroprevalence in the outbreak area, about 1000 were really infected.

Syndromes of dengue viral infection include undifferentiated fever, classical dengue fever, dengue fever with hemorrhage, and dengue hemorrhagic fever, which is a distinct dengue shock syndrome. The World Health Organization (WHO) definition of DHF includes fever and plasma leakage, with a hemoconcentration ≧ 20% in the pleural effusion or ascites as the most specific symptom. Other symptoms include thrombocytopenia (<100,000 cells/µL), bleeding, diathesis, a positive tourniquet test or spontaneous bleeding, rashes and petechiae. There is currently a movement in dengue research to recategorize dengue versus dengue shock syndrome. Most of the severe dengue cases have hemorrhagic fever.

Dengue in the tropics as well as in the Caribbean, Central and South America and Asia is transmitted by *Aedes aegyptii*. The public health impact is enormous. There are 73 billion persons at risk, with 100 million infections per year; 500,000 people are hospitalized annually with 90% of these being children. There are 21,000 deaths per year which is a relatively low number considering the number of infections. However, the size of the population with illness has a great impact on the quality of life of the infected persons and the economy of the area. The Centers for Disease Control in 2002 reported a major increase in cases of dengue in the affected countries. Reemergence is occurring due to a number of reasons including population growth, poor or no planned urban expansion (overcrowding, poor sanitation), changing lifestyles (water-filled plastic containers and tires lying around), transportation (planes, trains, etc.), lack of effective mosquito control efforts, and perhaps even climate change.

Aedes aegypti, the dengue source, has a wide tropical and subtropical distribution. The mosquito is highly domesticated and prefers to live in and around homes, not in the forest; it multiplies in man-made and natural water containers. Predominantly a day feeder, it prefers humans, and transmission occurs directly from man to mosquito to man. It is easily interrupted during feeding and therefore can have many blood meals in a day. A second species of mosquito, *Aedes albopictus,* can also carry dengue viruses and it is widely distributed in the U.S., being found in more than twenty states. However, it appears less efficient than *Aedes aegypti,* as seen in the 2001 Hawaiian outbreak for which it was responsible. Climate changes appear to be factors influencing its spread, by allowing mosquitoes harboring the virus to live longer.

Dengue is caused by a group of closely related small enveloped RNA called flaviviruses. Infection with one of the four serotypes (DENV-1, DENV-2, DENV-3, DENV-4) protects against that particular serotype for a number of years and against all four serotypes for several months. Immunity then wanes and you can be infected with dengue multiple times; all serotypes cause the same syndrome. The four dengue viruses have been around for a long time, having jumped from primates to humans several hundred years ago. There seems to be a forest/enzootic, rural/epidemic, and an urban/endemic/epidemic type of virus. The life cycle of the virus includes attachment to the receptor, which is not well characterized, and fusion with the cell membrane, causing cell membrane rearrangements as occurs with all flaviviruses. The mature single-stranded RNA virus produces ten individual proteins in infected cells which Dr. Rothman described. He added that dengue is not a destructive or lytic virus; progeny are secreted from the cell without destroying it.

Stages of dengue virus infection include inoculation of the virus with local replication in dendritic cells or fibroblasts, then dissemination and viremia, with the virus being found in local/regional lymph nodes, spleen and liver twenty-four to forty-eight hours post inoculation. This occurs eighteen to twenty-four hours before symptoms occur. The patient now has a temperature and there is risk for plasma leakage and shock, which occurs three to four days after a fever, when the fever is subsiding. It does not occur at the beginning of a fever.

Dengue (continued)

Vector- host interactions, viral determinants, nutrition, host genetic factors, and innate immunity all seem to play a role in DHF. The reasons why a small number of people have severe symptoms and others mild symptoms is somewhat related to viral factors such as virulence. Some genotypes/strains are more capable of causing the syndrome, such as subtype 2. Host factors may also play a role; a modified immune response is seen in the first infection with dengue. Genes in the human leukocyte antigen system (HLA alleles which are related to immune system function) seem to be associated with dengue disease severity and hemorrhagic fever. Certain HLA alleles are associated negatively and others, positively in their interaction with the virus. Additional research is needed in this area. It has been questioned whether resistance genes play a role, as only a few cases of DHF have been seen in Africa, Cuba and Haiti. African ancestry seems to afford a lower risk and is being studied. Malnourished people may be at less risk for DHF and prior immunity may be present due to antibody and T-cells. All serotypes of dengue that are "Asian" genotypes and DENV-2 seem to cause more disease. "American" genotypes are never associated with DHF (those seen in the Americas in the 1950s).

There is not a perfect relationship between viremia and DHF explained Dr. Rothman. High viremia is necessary, but not sufficient to cause plasma leakage. However, with low viremia there is no plasma leakage. High cytokines levels point to DHF being immunologically mediated in which increased vascular permeability occurs. In most cases, DHF occurs during infection by a secondary dengue virus. A study in Thai children showed that the first time they had the disease DHF was rare, but the second time around the risk of DHF occurring was 15 to 100 times greater.

Dengue virus antibody both reacts with the infecting serotype and cross reacts with the other serotypes. Antibody-dependent enhancement of dengue virus infection (ADE) appears to occur. When serum with a high antibody titer and virus are added to cells; the serum neutralizes the infection. But when a low titer of antibody is present, it all binds to the virus leaving no antibody to neutralize the remaining virus. Antibody now enhances the infection and actually allows the virus to bind to the receptor cells. The combination of viral infection (by a second serotype) and this immune enhancement is believed to cause plasma leakage. T lymphocytes have also been implicated in DHF and appear to be cross reactive when the second exposure to the virus is to a different epitype. The dominant response therefore is not always to homologous peptides and response patterns differ for each epitype.

Evaluation and management of DHF consist primarily of general medical support; there are no specific antivirals. Leukopenia, thrombocytopenia, and elevated AST (liver function) tests are seen. One-third of illnesses have a warning sign of abdominal pain and require hospitalization and treatment. There are plasma leaks for a day or so but the disease can be successfully managed by oral fluids if detected early. Increased IgM is seen by the fifth day following an initial infection; but may not develop in subsequent infections. PCR detects the presence of viremia (proteins in blood) by the 5th day following infection.

Which patient should you be the most worried about asked Dr. Rothman? Scientists are working on algorithms to identify these and are looking at immunologic factors.

There is currently no vaccine against dengue he added. Preventive measures consist of reducing exposure to the mosquito by wearing proper clothing in endemic areas, using insect repellents, vector control and elimination, environmental management, chemical, biological and physical factors and education. Markets for a dengue vaccine in endemic regions include the general population in which you hope to interrupt dengue transmission and prevent DHF. A vaccine would be important in non-endemic areas for travelers and the military who may be visiting or serving in endemic areas. The hope is to prevent dengue fever in areas of the world visited.

In the area of drug development there is currently a phase 2 trial in Vietnam using protease/polymerase inhibitors. In 1945 Albert Sabin noted that there is evidence in support of the feasibility of a protective dengue vaccine, a long-term, homotypic immunity. Ongoing studies are attempting to see which antibodies are protective and can be used for a vaccine; for

example, neutralizing antibodies in infants are correlated with dengue virus infection in the mother. Some people having high antibody titers still were infected, while DENV-3 patients have low titers and severe disease.

The theoretical optimal vaccine would have a low potential for immunopathology and durable protective immunity. It would be non-reactogenic, protect against all four serotypes, require one-two doses and be easily stored. A problem is that there is no reliable animal model in which to test such a vaccine. Creation of a live attenuated vaccine was abandoned and vaccine made by recombinant DNA technology is in early phase studies. Chimeric flavivirus vaccine, a live virus vaccine which can be produced quickly against all four strains is in phase 3 trials now. There is an immune response to structural proteins, however a good regimen needs to be developed that will provide full immunity with the first dose. Other vaccines candidates use inactivated virus or recombinant subunits.

Greater Boston Microbiology Supervisors Group Meets Again

Nancy Miller and Beverly Orr, both of Boston Medical Center and Emy Thomas, MetroWest Medical Center

A group of Boston microbiology supervisors met on a regular basis from the late 1970s to late 1980s to discuss topics of mutual interest. It disbanded at about the time everyone was knee-deep into mergers of one kind or another. Several current and previous microbiology supervisors had expressed an interest in possibly getting together again.

Last fall, Emy Thomas, previously supervisor at MetroWest Medical Center in Natick, collected names and email addresses of microbiology supervisors who were interested in meeting again. A meeting was planned and promoted at the Region I ASM Meeting in October. The meeting and reunion dinner was held at Boston Medical Center on November 9, 2011. Nancy Miller, M.D., Director of Clinical Microbiology and Molecular Diagnostics and Beverley Orr, Microbiology Supervisor, hosted the meeting and provided a tour of the Boston Medical Center Microbiology Laboratory which contains state of the art equipment including a specimen processor. About 18 people enjoyed reconnecting at the meeting and the dinner afterwards.

The next meeting will be on February 15 at the Bunker Hill Community College, and will be hosted by Betsy Szymczak. The topics will be "Competency Testing and The Future Workforce" conducted by Beverley Orr and Clinical/Medical Laboratory Science educators. A tour of the Medical Laboratory Science Laboratories and dinner afterwards in the Culinary Arts Dining Room is included.

Emy Thomas

Boston Area Student Chapter Program

An interactive workshop, "*Teaching Strategies and Effective Presentation Styles",* was held for the ASM Boston Area Student Chapter on March 8, 2011 at Tufts Medical Center. It was presented by Gail S. Begley, PhD, Associate Academic Specialist in Biology and Director, University PreHealth Program at Northeastern University. Gail is also Local Councilor of the Northeast Branch. Students brainstormed best and worst practices for giving a research talk or a class lecture. The most important theme was respect for the audience, which includes everything from correct targeting of the level of the talk, to making eye contact and gauging ongoing audience interest and understanding throughout

the presentation. Gail also shared tips and tricks from many years of teaching and presentation experience and addressed student concerns on a range of issues from losing your train of thought to argumentative questions from the audience. Two students gave mini-presentations followed by constructive feedback from the whole group.

Gail Begley

The Following Programs Were Jointly Sponsored with Other Professional Organizations

* *The 63nd American Society for Clinical Laboratory Science Central New England (ASCLS:CNE) Annual Convention

The ASCLS:CNE Annual Convention was held at the Rhode Island Convention Center in Providence, RI on May 3-5, 2010. It was jointly sponsored with the Board of Rhode Island Schools of Allied Health (BRISAH), Bay State Chapter CLMA (CLMA); Rhode Island Cytology Association (RICA); and the Northeast Branch, American Society for Microbiology (NEB-ASM).

**Hospital Response to Chemical Emergencies

This program was designed for emergency room specialists and laboratory staff who may provide patient care during a public health emergency, and is held several times annually. It was held at the University of Massachusetts Memorial Hospital in Worcester, MA in April, Emerson Hospital, Concord, MA in May, South Shore Hospital in Weymouth, MA in late May, Norwood Hospital in Norwood, MA in October and at the State Laboratory Institute, Jamaica Plain, MA in November.

Faculty included Gloria Cheng, MS, Assistant Coordinator, Chemical Threat Response Laboratory, William A. Hinton State Laboratory Institute, MDPH; Michael Feeney, RPh, JD, CHO, Director, Indoor Air Quality Program, Bureau of Environmental Health, MDPH; and Jennifer Jenner, PhD, Coordinator, Chemical Threat Response Laboratory, William A. Hinton State Laboratory Institute, MDPH.

The programs were sponsored at no charge by the Massachusetts Department of Public Health, (MDPH) and the Northeast Branch-ASM.

**They're Out There…Are You Prepared? Agents of Bioterrorism: Sentinel Laboratory Training

This training program was designed to provide timely information to help clinical laboratorians understand their role in the Laboratory Response Network as they rule-out organisms and serve as sentinels for persons who may fall ill due to a bioterrorist event. It provided an overview of the clinical laboratory's role in the presumptive identification of primary agents of bioterrorism using laboratory demonstrations and hands-on learning exercises; safety implications were emphasized. The program was held in June, September, October and November at the State Laboratory Institute at no charge.

Faculty included Deborah Carter, MT(ASCP),LRN Laboratory Coordinator, Bioterrorism Response Laboratory; Cheryl Gauthier, MT(ASCP), Director, Bioterrorism Response Laboratory; Sandra Smole, PhD, Director, Division of Molecular Diagnostics and Virology; and Tanya Swanson, BS, MT, Supervisor, Bioterrorism Response Laboratory. All are from the William Hinton State Laboratory Institute, MDPH, Jamaica Plain, MA.

**Other Activities

The NEB also annually supports five Massachusetts regional fairs, the Massachusetts State Science Fair, and this year contributed to the Darwin Festival held at Salem State College.

Northeast Branch of the American Society for Microbiology

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