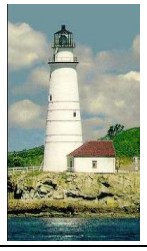

Northeast Branch Newsletter

Number 142 Winter 2018-2019

Synthetic Biology: Miracle or Monster?

The annual joint meeting co-sponsored by the Northeast Branch-ASM and the American Society for Clinical Laboratory Science of Central New England was held at Rachel's Lakeside in Dartmouth, MA, on April 18, 2018. James T. Griffith. PhD presented a fascinating talk entitled *Synthetic Biology; Miracle or Monster? Redesigning Micro-organisms into Life Forms That Make Rubber, Saffron, Vanillin, Rice, Fuels and Much More*. Dr. Griffith recently retired as Chancellor Professor and long-time chair of the Department of Medical Laboratory Science at the University of Massachusetts Dartmouth and is currently Managing Partner at Forensic DNA Associates. He has published extensively over his distinguished career and his areas of interest span a wide variety of topics.

Synthetic biology (synbio) involves a separation of evolved life from the kind of life that is currently being developed. In this area are "orthogonal" living things, that are complete organisms designed and developed in such a way that they should be kept completely artificial and (Continued on page 4)

University of MA Dartmouth Students with Speaker James T. Griffith, PhD (C) and Frank Scarano, PhD (R)

How Inkjet Printing and Artificial Intelligence Can Defeat Multidrug-Resistant Pathogens

The first fall dinner-meeting was held on September 12, 2018 at the Forefront Center for Meetings and Conferences in Waltham, MA. James E. Kirby MD, D(ABMM) presented a most interesting talk on *How Inkjet Printing and Artificial Intelligence can Defeat Multidrug-Resistant Pathogens.* Dr. Kirby is an NIH-funded Principal Investigator in the Experimental Pathology Division of the Beth Israel Deaconess Medical Center in Boston, Director of the Clinical Microbiology Laboratory at BIDMC, Program Director of the Medical Microbiology Fellowships at BIDMC; and an Associate Professor of Pathology at Harvard Medical School. The broad goals of his research laboratory are to advance the fight against infectious diseases through development of novel antimicrobials, elucidation of how bacterial pathogens cause disease, and development of next generation diagnostics. More information on his research efforts can be found at https://www.kirbylab.org. (Continued on page 7)

Inside This Issue - Programs in Review 2018

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NEB Council Meetings

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

Membership Notes

Dues reminders for 2019 will be sent to our membership via 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 membership information.

Although membership in a national organization 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.* Many Northeast Branch are also national ASM members.

Council Election Results

Congratulations to the following NEB members whose terms as Branch Officers began July 2018. President, Gregory Reppucci; President-Elect, Stefan Riedel, and Local Councilor, Carol Finn. Thank you for another great year of programs and we are looking forward to planning a busy 2019!

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Student Chapters

The NEB is associated with three active student chapters. The Boston-Area Student Chapter, the University of New Hampshire Chapter in Durham, NH, and the Maine Society of Microbiology, Orono, ME. We look forward to collaborating with them again!

FUTURE PROGRAMS

***Local Programs**:*

Announcements of Local Meetings and registration materials are posted on our website:
http://northeastbranchasm.org

April 8-9, 2019. NEB-ASM and NACMID Fourth Joint 2-day Meeting
Location: Sheraton Portsmouth Harborside Hotel, 250 Market St., Portsmouth, NH
Sponsored by: Northeast Association for Clinical Microbiology and Infectious Diseases and Northeast Branch-ASM

Preliminary Program

April 8: Three workshops: (1) Parasitology, (2) Antibiotics-Back to the Basics, and (3) Epidemics Within the Opioid Epidemic-Infectious Diseases Consequences. Also: Wine & Cheese reception and keynote speaker.

April 9: Parasitology/DPDx, Next Generation Antibiotics and their Next Generation Challenges, New Methods Impacting Bioterrorism Algorithms, Fecal Transplants, Global TB Updates Relevant to US Microbiologists. Also: student presentations, vendor exhibits, posters and prizes; CME and CMLE available.

On-line registration will be available at www.nacmid.org.

Contacts: Kristin_Palladino@uml.edu or Irene George, NEBranch-ASM@comcast.net

http://www.nacmid.org
http://www.northeastbranchasm.org

March 21, 2019. Dinner-Meeting. Speaker: Isabella Martin MD, PhD, Director, Dartmouth Hitchcock Medical Center Microbiology Laboratory. Location: Forefront Center for Meetings and Conferences, 404 Wyman Street, Waltham, MA. Register at www.northeastbranchasm.org

May 1, 2019. New England Microbiology Laboratory Directors Spring Meeting
Location: Publick House, Sturbridge, MA, 12:30 PM.

November 7-8, 2019. Region I ASM Branch Meeting. The Lantana, Randolph, MA.

National Meetings:

June 20-24, 2019 *ASM Microbe, San Francisco, CA www.asm.org/microbe*

August 1-4, 2019 *26th Annual ASM Conference for Undergraduate Educators (ASMCUE),* Tysons, VA *www.asmcue.org*

completely separate from evolved life. These are different from xenobiological life, which are organisms that are derived in such a way that they cannot interact with life as we understand it.

Evolutionary biology clearly has created a phenomenal diversity of life on the planet Earth. Dr. Griffith showed an evolutionary map showing all lifeforms existing on the planet to date and commented that we are about to add Synthetica to the Eucarya, Bacteria, and Archaea. The National Academy of Sciences defines synthetic biology as "the application of engineering principles in order to design and construct new biological parts, devices and systems and to re-design existing natural biological systems for useful purposes". Synthetic evolution/biology is exactly the same as evolutionary biology except that humans choose what the genes are and what gets expressed. These are inserted into a "microbial chassis" such as a microorganism; most of its genetic material will be removed, and functionality will be added. Eventually it may be difficult to tell them apart as synthetic life interacts with the evolved biology.

Much has happened in the past 40 years. We are combining biology, engineering, dramatic amounts of computational modeling, and computer-aided design systems and mechanisms to reprogram cellular systems. This all started in the 1960s when we were just understanding the genetic code, the central dogma of molecular biology. We had, in the 1960's, recombinant DNA ligation, restriction endonucleases, and the PCR reaction (which to date is still not very good). Automatic DNA construction followed, and we are now in the world of synthetic biology, developing standardization techniques and the abstraction of what is life, what is biological capability.

By the 1970's, we had the manipulation, transfer and cloning of DNA; a tremendous amount of manipulation and development of those subjects began after that, until we reached the 2000's and the era of the human genome project. The human genome was sequenced by 2015, much sooner than anticipated. Currently, the DNA of every major group of living organisms on the earth has been sequenced. Today, we can literally "shuffle the deck of genomic cards". We have genetic sequencing and engineering, we can write/program new DNA, and we can create new genetic machines from scratch, which is what synthetic biology is about.

Various financial forecasters have suggested that by 2020 synthetic biology will be a sixteen-billion-dollar industry, and will multiply greatly in the future. Pharmaceuticals, diagnostic tools, chemicals and energy production are a few of the areas involved.

There are many and varied incitements as well as impediments and consequences for synthetic biology in our complex world. We have energy constraints, for example, such as in India. It is not uncommon to have ordinary homes get electricity through "parasite lines". A copper wire is attached to the street electric line and then to a battery or line in someone's home; both illegal and dangerous, as regional transformers often catch on fire.

Another constraint is the cost of health care. One-third of the global population lived in extreme poverty in 1990, described by the World Health Organization (WHO) as less than a dollar a day; by 2017 it was one of ten people. However, more people worldwide are going to want more materials things. The cost of health care, a medical consequence, will rise; WHO estimates that cost for heart disease between 2013-2030 is going to double. The US spends much money on this disease and healthcare; if you go into this medical system, you stay there for life.

The availability of water is also a constraint. Tokyo, Yokohama in Japan is listed as the largest of 12 megacities in the world with 37.8 million people; listed 9th is NY City having 20.6 million people; 12th is Mexico City. However, Mexico City consumes more water than flows into the valley in which the city is located. Two billion of seven billion humans worldwide do not have enough clean/drinking water; in poor, water-strapped countries, 80% of all disease is waterborne.

There is also a squeeze on cultivable land. The University of Minnesota Global Landscape Initiative says that agriculture takes up 40% of the ice-free land on the earth and accounts for 70% of human water use. How many more crops or cows can we grow in the areas from which we get our

food? There will be two billion additional "eaters" on the planet by 2050 and climate change is predicted to decrease the crop yield by 10 to 40%. All these factors are going to play a part in how we proceed with synthetic biology.

However, food (GMO) is not like insulin, said Dr. Griffith. We've had synthetic insulin since 1968 or so; if a person has to choose between death or artificial synthetic insulin, of course they choose the synthetic insulin. Attitudes toward food, for some reason, are different. American grocery stores are full of packages labeled "no hormones, no GMO's, xx-free" etc. There are many soft spots food-wise around the world, cultural barriers also exist. We have food riots on our planet now and it's not 2050 yet. Philosophical papers from WHO suggest that hesitation towards synbio foods might fade when people are starving.

He then gave examples of several currently available foods and beverages that are fermented with synbio yeast, i.e. an organism that used to be a yeast, but was converted into a "factory". One startup company Muufri, makes 100% "animal-free" milk using synthetic microorganisms. A crowd-funded entity called the Bay Area Biohackers makes and produces vegan cheese. Evolva, a Swiss company, makes saffron, vanillin and stevia. Solazyme, makes microalgae butter, protein rich flour and vegan protein.

Additional obstacles to synbio include groups such as Friends of the Earth, who claim synbio is an extreme form of genetic engineering. The Woodrow Wilson Center suggests that requisite testing of synbio organisms must involve an environmental release, and therefore there will be a change in the species diversity and density in the microbial world. USDA authority is limited regarding different synbio applications, such as glowing plants, genetically engineered mosquitoes, biopesticides, and biomining chemicals; this is illegal in other countries.

Synbio technology involves using an existing microorganism. Frequently used are the well-known and most-studied *E. coli, Saccharomyces cerevisiae,* and mycoplasma. Mycoplasma have been among the most useful so far because they are small, accommodating, and are used to being near other cells, yeasts, parasites and plants.

Researchers removed most of the genetic material of a *M. mycoides* in 2007 and put in the genes of another mycoplasma species; the resulting *M. mycoides* had the surface markers of *M. mycoides* but acted like the other mycoplasma. Three years later, they removed the genes from the same mycoplasma, and replaced them with a synthetic group of DNA pieces called "biobricks". Involved in synbio technology are all the techniques used since the 1960's, such as PCR, restriction enzymes, gel electrophoresis, sequencing, Southern Blot, real time PCR, Northern Blot, and microarrays. The one different and key new item is biobricks, these are small packaged pieces of DNA that have defined functionality. They can be incorporated, for example, into living cells such as bacteria, to construct new biological systems. Catalogs of biobricks are available, and these, along with billions of computational efforts let you to figure out exactly what you want to do.

A kickstart project done by a group of US researchers used such biobricks and data. They ground up fireflies in a blender and fused them with mustard plants, laser printed the combined DNA onto very tiny metal entities, and gene-shot these into mustard seeds. In the first run of this process they made 600,000 seeds, and they successfully made plants that glow-in-the-dark! The idea was to have sustainable artificial light in places like parks!

The synbio design cycle uses engineering principles and employs a process called computer-aided design, in which you think of a problem, put the desired characteristics of your final product into a computer and you imagine what the endpoint might be. The computer then produces an algorithm that aids in reaching your end point. There is also CADSYNBIS, a computer information system that is connected to computer aided-design. This process involves massive computer data, management, and mangling that an individual human could never sort out. The bioengineering program used by Evolva to produce vanillin ran billions of scenarios to determine that if you started with a sugar, electricity, water, and GMO yeast, vanillin could be made that would be indistinguishable in every way from a vanilla bean extract (note: most vanilla sold in the US is made from petroleum, not vanilla bean).

Synthetic Biology (continued)

Another example of synbio design described by Dr. Griffith is *Mycoplasma genitalium* that has had most of its genetic material removed except for functions such as replication and division. This is called the *minimal organism/ cell*, which for synbio purposes, needs 151 genes. Biobricks were added, and this is now an organism known as *Mycoplasma laboratorium*. Another minimal organism, *E. coli K-12*, has about 280 genes and after adding biobricks, is now able to dissuade *V. cholerae* from making toxin. The idea is to have a "minimal cell" with the least amount of its own genetic materials remaining.

One example of synbio in use is rice. India and China are neighboring countries separated by the Himalayas, which supply water to both. The two countries represent three of five living humans and they both use rice. In a perfect rice paddy, rice grows well but feeds few people; rice is also neither drought nor sun tolerant. However, we want to grow rice everywhere, such as on tiered mountain sides, etc. GMO rice has been available and used worldwide by farmers for decades. There is also a synbio rice that was developed about six years ago that is drought sun wind and climate tolerant in other ways. This rice has been grown by farmers in India, Bangladesh and Pakistan for six years now, has a heavy yield and virtually never fails.

There is estimated to be a staggering benefit for synbio in the area of food production. The planet now has more people than we are able to feed and provide fresh potable water as well. By 2050 we will add an additional 2 billion people, and climate change will bring about an estimated decrease of 10 to 40% in crop production. At what point will the United States, England, France, etc. be negatively impacted by food and water riots as other places in the world?

Additional applications in the synthetic biology pipeline include new materials, such as spider silk, Teflon and Kevlar. Isoprene (a by-product of processing rubber) is needed to make rubber for automobile tires and other purposes, but now there is a synbio called BioIsoprene made by microorganisms. Dupont and Goodyear have invested heavily in this, although rubber made this way is not available yet. Certain coffee production is moving northward out of Costa Rica due to the unfavorable climate; however, synbio coffee plants will be going into production and should replace those lost.

Another product that has been developed but as yet is unavailable, is one that when swallowed, microorganisms would enter the GI tract and be protective, such as when synbio *E. coli K-12* prevents *Vibrio cholera* toxin production. The synbio organisms may inhibit production of products or alter products that make people ill. There are reports of an Asian package and South American package that protect against bacterial toxins and an African package that protects against parasites. There are also phages that can eliminate toxigenic *E. coli* in a few days.

Some African economies are compromised because usable farmland can't be farmed due to severe problems with parasites. In the past eight-nine years climate change has resulted in ambient weather conditions that have not permitted snow accumulation on mountains such as Mt. Kilimanjaro. This allows the overwintering of malaria mosquitoes and malaria rates in these areas/countries are increasing due to the increasing range of the mosquitoes. Artemisinin, 100% of which was previously obtained from the *Artemisia annua* plant, is now a synbio pharmaceutical made with the help of a genetically engineered yeast.

Another product being developed by two different countries can be rubbed into your skin or sprayed on after bathing. The synbio organisms will dissolve all dead skin cells on your body including whiskers; shaving will become obsolete. A related product promises to keep your skin sufficiently hydrated so that wrinkles will never form, and there is an oral rinse that will eliminate tartar, yellowing, and the need for brushing teeth. Cheese and milk production will occur without the need for cows!

On the cautionary side, there are ongoing worldwide chat rooms and discussions about synthetic biology. Clinical laboratory people pose the question of what happens when your synbio *E. coli K-12* that was doing wonderful things at a clinic appears in a patient specimen and micro ID system? By what name do we call it now? Can any of these become disease producers? The way in which they are made precludes synbio organisms from causing disease, However, when

Synthetic Biology (continued)

they get out into the world, unintended consequences can occur and we can be sure they will not stay where placed. One experiment, for example, showed that synbio corn plants were pollinated by bees, and the bees spread the pollen into neighboring fields.

There are immense diagnostic capabilities for synbio organisms, such as in infectious disease, crop production, and many other areas said Dr. Griffith, but there will be diagnostic challenges and other unintended problems that we will have to deal with.

How Inkjet Printing & AI Can Defeat MDR Pathogens (continued)

In his lecture, Dr. Kirby described his research group's efforts to accelerate and improve the accuracy, precision, and flexibility of antimicrobial susceptibility testing using inkjet printing, advanced microscopy and artificial intelligence. These tools are being combined to rapidly identify new therapeutic options for multidrug-resistant pathogens as well as to improve upon existing ones.

Dr. Kirby remarked that since we currently have "bad bugs", poor diagnostics and not enough antibiotics, multidrug resistance is a major problem. Among the ESKAPE pathogens *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) of particular interest are the gram-negative organisms. Organisms such as urinary *E. coli* have gradually become resistant to the old standby drugs such as ciprofloxacin and bactrim, rendering them unreliable for use. Drug resistance has become extremely acute in some parts of the world. For example, in Asia, the frequency of extended spectrum beta-lactamase organisms and carbapenemase resistant organisms is climbing to 60 to 70%. The impact is increased mortality, many infections that can't be treated, and an estimated $40,000 increased cost of multidrug-resistant infection.

James E. Kirby, PhD

There are a number of drivers for antimicrobial resistance such as inappropriate utilization and population growth that can easily spread resistance from one area to another. Antibiotic consumption in livestock selects for resistant organisms and a shared ecosystem allows this drug resistance to pass easily from animals to humans and spread rapidly worldwide. Medical advances such as bone marrow transplants have increased and such patients need to be put through periods of immunosuppression, making them vulnerable to infections with resistant organisms. When such an infection is treated, the normal flora is eliminated, setting up a vicious cycle. Patients then become colonized with organisms resistant to the antibiotics just used to treat them. The next infection the patient gets will be with that resistant organism, and when treated again, the organism will become even more resistant. Dr. Kirby believes that drug resistance will continue to escalate and resistance will need to be addressed from a diagnostic and therapeutic perspective.

In terms of therapeutics, we have a problem. Drug resistance inevitably appears a few years after a new drug is introduced. There has also been an antimicrobial discovery void; it is not profitable to invest in the development of new antibiotics and there are not adequate incentives. An average antibiotic development effort costs $100 million dollars and no new antibiotics have

been developed for about the past three decades. This discovery deficit occurs because antibiotics that were easily found have already been discovered; it's estimated that you have to screen about 10^7 different organisms to find a novel antibiotic, and many companies don't want to make this effort. Microorganisms are very difficult targets and getting antibiotics to penetrate them is difficult; antimicrobial compounds are very complex and are difficult to synthesize. The compound libraries that companies produce for screening are also very complex. The consequence of this is that there are antibiotic shortages in hospitals every week, thus compounding hospital therapeutic issues.

There has been some response at the federal level. The government is trying to give incentives to companies by providing five additional years of patent protection and priority or expedited reviews. Some companies are therefore investing in this area and there are some "new" drugs in the pipeline; however, many of them are not novel, they are simply derivatives of the drugs we already have, and not many are effective against ESKAPE pathogens. This is a very complicated landscape; although the number of available therapies will increase, the number of active available therapies for any given organism will decrease.

Antibiotics save lives and patients need to be given the correct drugs quickly. Theoretically, the likelihood of picking the correct drugs will decrease over time as organisms become resistant. We have laboratory testing to guide treatment decisions and we have wonderful new techniques, such as MALDI-TOF, to speciate organisms quickly. However, for some organisms such as *Pseudomonas aeruginosa*, we still don't know which of several drugs will be the appropriate therapy.

The question is what can we do in the diagnostic realm? One way to predict pathogen response to therapy is the minimum inhibitory concentration (MIC). This value predicts the efficacy of the drug. Twofold dilutions of an antimicrobial are made in a growth medium, the organism is added, and this is incubated overnight. The MIC is the lowest concentration of antibiotic that inhibits visible growth of the organism. This broth microdilution method can be done in different formats, and is typically done in 96 well plates. There is a correlation between MIC and treatment efficacy, and the MIC is important in predicting drug efficacy.

The MIC is the gold standard reference method and is complex to perform. There are actually 40 separate steps in the method because of all the dilutions that need to be made. This is time consuming, and is not practical to do in the laboratory. In clinical practice, alternate methods are used. Automated methods that extrapolate the MIC include the Vitek 2, and commercial lyophilized panels with fixed combinations of drugs. ETest (BioMerieux) has fixed combinations of drugs on a plastic strip. These work for some organisms, but not for all, and does not work for the new drug resistant organisms.

All commercial methods have their limitations. Some antimicrobials like colistin, a drug of last resort, can only be reliably tested by reference broth microdilution methods. Colistin cannot be tested at Dr. Kirby's hospital therefore they would have to wait for results from a reference laboratory. It requires about a million dollars and about four years to get a new antimicrobial onto an automated panel, therefore although new drugs are available, they cannot be evaluated.

There is much discussion about the ability to sequence organisms "on the fly" and potentially predict resistance. With HIV that has only nine genes, phenotype-based predictions for resistance work well. However, a carbapenem-resistant Enterobacteriaceae has a median of about 5100 genes but this can vary widely. Gram negatives are very complicated and to date their sequencing has not been worked out. Dr. Kirby showed a slide listing their series of genome sequenced KPC organisms. It is believed that the MIC predicts efficacy. However, some organisms that have a KPC enzyme just don't express it well and it is believed that those are treatable. By phenotyping alone, you would say that a substantial fraction of these are untreatable, but a substantial fraction can potentially be treated by increasing the dose of meripenem.

We know that delayed proper treatment leads to increased mortality. When an infected patient

enters the hospital, a sample is taken and cultured; colonies are isolated and put into an automated identification and susceptibility system. There is about a 48-hour delay before susceptibility results are obtained. Sometimes organisms are resistant to all antimicrobials tested, or a request for an additional drug to be tested is received. Cultures may need to be sent to a reference laboratory and it may be about 7 days before the patient can be treated. Patients are therefore often treated empirically, with a best guess at which antimicrobials might be effective.

Dr. Kirby's laboratory therefore sought to address this treatment delay, and first looked at eliminating the need for reference laboratory testing. They had been working with a modified inkjet printer since 2016; instead of using ink, an antibiotic stock solution is put into one of the wells; and instead of printing on paper, the printing is done in 96 or 384 well dishes. One wonderful feature of this "at-will broth microdilution susceptibility testing platform" is that various droplet sizes can be printed out, with a million-fold dynamic range. A doubling dilution panel can therefore be set up in seconds and novel combinations of antimicrobials can be tested. The 384 well plate can be filled in approximately one minute. Performance was verified by testing Enterobacteriaceae for susceptibility to numerous drugs in comparison to a broth microdilution standard. Precision and accuracy studies were also carried out. Compared to the reference microdilution method, it is actually more precise and just as accurate Dr. Kirby said. He believes this type of technology will allow clinical labs to eliminate treatment delays and the use of reference laboratories.

Another question in this area was whether we can we restore the capabilities of existing drugs. Efficacy is determined by antibiotics, i.e., the balance of antibiotic exposure and MIC. A certain dose may be sufficient to kill an organism that has a low MIC. Therefore, an elevated MIC could potentially be overcome by increasing the dose or increasing exposure (treating more frequently), as long as the pitfalls of toxicity are avoided. The Clinical Laboratory and Standards Institute (CSLI) recognized this potential and established a new category for antibacterials called the susceptible dose-dependent (SSD) category; in this category we now have semi-resistant organisms. The laboratory tested several commercial methods to see if they accurately determined if an organism falls into this SSD category and they were totally inadequate. The Vitek 2 had a 50% categorical agreement with the reference method, disk diffusion was not much better and neither was Microscan. The inkjet printer however gave very accurate results. There were some interesting issues when doing this study. The lowest concentrations in the panel with Vitek are 1-2 µg, but the quality control range at which you are supposed to test this panel with is 0.015-0.012 µg, about 10-fold too low, so you have no idea whether your panel is working or not. The inkjet on the other hand can do whatever dilutions are needed and not necessarily two-fold dilutions; quality control dilutions can be made to cover whatever range you need for the organism being tested.

Another way to rescue antibiotic effect is synergy. Synergy occurs when you combine two drugs that separately have high MIC's and the combined MIC drops into the susceptible range. Typically, synergy can be done in a two-dimensional array; two-fold dilutions of each drug are made, and you can see where the computorial MIC lies. The test can be set up manually in about 45 minutes, while using the inkjet printer, it takes a few seconds. The laboratory did, in about a few weeks, the largest examination of synergy against CRE that had been reported in the world's literature until then.

Some interesting things were discovered. The activity of any specific combination in general was unpredictable, but in 90% of the strains there was at least one combination where there was clinically relevant synergy and you could predict efficacy with normal dosing. The laboratory was interested in synergy with colistin, especially with the emergence of colistin resistance. They tested colistin against many drugs, against colistin-resistant carbapenemase producers, including NDM1 strains and NCR1-plasmid mediated colistin-resistant strains originally found in China, and now of concern in the US. What they found was that over 90% of the strains had synergy with minocycline and rifampin, and if colistin is combined with some gram-positive

agents, for example linezolide, these would be active against gram negatives. Some of these gram-positive agents could be considered potential therapeutics. Further research will be done in this area.

The next question was whether a variant of this technology can be used to reduce the time for susceptibility testing to less than the usual 48 hours. The quickest way to see if an organism is susceptible to an antibiotic would be to somehow observe individual bacteria to see whether they are dividing in the presence of an antibiotic. The concept of "microscopy antimicrobial susceptibility testing" was thus created. Organisms were immobilized onto a solid growth surface, a solidified Mueller-Hinton broth in a 364 well plate. Bacterial suspensions were placed into the printer ink wells and printed out onto the centers of solid target areas on the plate wells. A certain number of organisms per field were observed with a 40X lens after about two hours incubation; at their MIC, they did not grow. In the presence of antibiotics some organisms form filaments, others ball up, and others simply "fry and die". This method also proved to be accurate and reliable.

The next step was to see if images of the organisms could be automatically classified by looking into the center of the well instead of having someone manually read each well. The lab turned to "Deep Learning", a type of artificial intelligence programming modeled on the optical cortex, which is excellent for image analysis. The same type of artificial intelligence is used in self driving cars and face recognition programs. Investigators then trained the neural network to look for growth versus no growth and they now had automated image analysis; the program identified MIC's after 2 hours incubation. Dr. Kirby's laboratory also has applied artificial intelligence, to interpreting Gram stains (published in J Clin Micro).

Thus, the laboratory now uses an inkjet printer to print out antibiotics of various concentrations and various dilutions of organisms, an automated microscope to perform automated imaging analysis, and an artificial intelligence program to classify each well as to growth or no growth. There was an agreement of 95% with the standard methodologies. The HP D300 inkjet technology is simple enough to be based in a hospital clinical laboratory, it is flexible and, eliminates reference testing. Any new antimicrobial can be tested at will with a true MIC-based inexpensive technology; it can be performed easily in 384 well plates.

In order to improve further upon these technologies, the laboratory has currently started to work with "printing out" positive blood culture broths and the results look very promising; they will next work with urine cultures. The idea is to eventually develop an instrument with an inkjet printer, a 40x lens, and a computer with trained artificial intelligence that will provide very rapid answers.

While looking to improve the accuracy of their results, a concept called "the inoculum effect" and its impact on clinical laboratory measurements came to mind. When susceptibility tests are set up, a precise number of organisms are used, and when you increase the concentration by 100-fold, MIC's are much higher for certain drugs. Thus, people just tested one-half the target inoculum and 100x above that. CSLI allows an inoculum ranging from 2-8 x 10^5 cells per ml when testing. The laboratory performed synergy experiments to determine whether the inoculation range makes a difference. A number of different organisms in different categories were used and they found that the inoculum size of some drugs has no effect on MIC, while the MIC of others can shift from resistant to a susceptible range. Dr. Kirby mentioned that there should be an awareness of this when performing susceptibility testing.

Dr. Kirby lastly spoke of another laboratory project, the contributions of the desert pack rat to the understanding of chronic bacterial infection. *Brucella* are gram-negative organisms that naturally infect domesticated farm animals such as goats, sheep and pigs. They are transmitted to humans by drinking unpasteurized milk and exposure to the organisms during birthing of an infected cow; they are also an aerosol risk to laboratory technologists. *Brucella* causes the zoonotic disease brucellosis, symptoms of which are a chronic relapsing (undulant) fever that can last for decades, osteomyelitis, meningitis, etc.

How Inkjet Printing & AI Can Defeat MDR Pathogens (continued)

and *Brucella* is on the bioterrorism Select Agents list. The laboratory found and worked with a less infectious *Brucella*, *Brucella neatomae,* that has a natural rodent host, the desert pack rat, *Neatoma lepida*, that resides in the western states. A pack rat nest (midden) consists of many articles collected by pack rats upon which they urinate; the urine preserves the articles and bacterial flora for centuries.

Organisms causing chronic infections often grow inside human cells and in order to do that they co-opt the cell biology of their host cells. They do that by producing a "molecular syringe", which injects virulence factors into the cytoplasm of the host cell and in case of *Brucella*, alters the phagosomes that the organisms will live in to make them a hospitable place.

The laboratory created luminescent *N. lepida* organisms and as they grew, knocked out the molecular syringe, causing them to stop growing. They then added *Legionella*, that produces a different molecular syringe; *Brucella* then grew very rapidly. However, if *Legionella* was missing their molecular syringe, neither organism grew. This does not work in reverse.

Both wild type *Brucella* and *Legionella* will grow when combined, but when syringe deficient *Legionella* and wild type *Brucella* are combined, *Brucella* will grow and *Legionella* will not. There appears to be some type of crosstalk here and there is much to investigate.

A mouse model was then used to study the disease and organisms were observed growing slowly in the liver and spleen after 56 days, which is what is also seen in humans and other animals. This suggests that the syringe was important in the spread of organisms throughout the animal.

In *Brucella*-infected humans, the spleen and liver are enlarged due to the multiplication of organisms there. In mice, the spleen and liver are not enlarged if the syringe is missing from the organisms. To characterize the infection further they used luminescent organisms, and via whole-body imaging, realized most of the injected organisms were congregating and replicating around the injection site; only some were going to the liver and spleen. In the mutant, there was much less replication, and no signal in the liver and spleen. By day 7, there was much less signal at the injection site, and more signal in the liver and spleen as well as lymph nodes and thymus. No lymph node distribution was seen in syringeless mice. It seems that the syringe is helping in both colonization and replication of different body sites. The organism causes granulomatous inflammation which is characteristic of *Brucella* seen in humans. This system appears to be giving *Brucella* it's remarkable staying power and the laboratory is exploring the cell biology and genetics behind this.

Integrating Genomics and Epidemiology: Examples of Antibiotic Resistant Gonorrhea and the Resurgence of Mumps.

A dinner-meeting jointly sponsored by the Northeast Branch-ASM and the Northeast Section of the American Association for Clinical Chemistry was held on March 29, 2018 at the Forefront Center for Meetings & Conferences in Waltham, MA. Yonatan Grad, MD, PhD, Assistant Prof. of Immunology and Infectious Diseases at the Harvard T. H. Chan School of Public Health in Boston spoke on *Integrating Genomics and Epidemiology: Examples of Antibiotic Resistant Gonorrhea and the Resurgence of Mumps.*

Dr. Grad began by saying that we are now at the cutting edge of this new technology and are trying to determine how to utilize all the data generated by molecular sequencing. A variety of methods are used to observe how pathogens spread through the populations, including genomics, epidemiological tools, mathematical modeling, and microbiology. The Grad laboratory attempts to define the dynamics of spread and characterize both the genotypic and phenotypic diversity of the pathogens. The laboratory uses collections of organisms from laboratory freezers that are donated by hospitals and other facilities to study how pathogens evolve and spread. Dr. Grad always welcomes donations!

Dr. Grad first discussed influenza which seems to be in the forefront. There are two major

hypotheses as to why influenza vaccine effectiveness is modest at best. One thought is that a mismatched dominant strain is present. The mismatch can be exacerbated by viral mutations occurring during replication in chicken eggs, resulting in an unintended vaccine as occurred in 2012-13. (Sara Coby, CID, 2018, showed mutations in hemagglutinin in three spots in the 2012-13 vaccine strain). The virus may also simply mutate. The second thought is that there may be heterogeneity in the response of patients to the vaccine.

To answer the question whether vaccinated and unvaccinated people get infected by different viruses, hundreds of blood samples from around the country were sequenced. The samples had been collected before and after vaccination therefore patient response to a vaccine could to be determined. Patient clinical records were also reviewed. Is there actually a protective benefit if you are vaccinated? Even if you are protected against what you were vaccinated with, you may also get infected with a different influenza strain, one that you were not vaccinated against.

Looking at (2013) pre-vaccination titers, the laboratory found that people responded in the same way whether they were vaccinated or not. Vaccinated and unvaccinated people seemed to be getting infected with similar viruses. If they were boosted, the same reaction was seen. Only about 1/3 of people (>30%) showed a response 4 times greater than pre-vaccination levels. Basically, most of the people tested did not have a response to the vaccine. The conclusion was that the organism in 2013 was poorly immunogenic and produced a bad immune response, and that this was not due to a mismatch.

We really need to look at the way in which we make new flu vaccines said Dr. Grad, and to explore strategies to increase the immune response, such as adjuvants. We need new types of vaccines and one answer to this may be to create a universal flu vaccine against all strains. There is currently work occurring in that field.

Dr. Grad then addressed mumps and the increased outbreaks in colleges. He became interested in the disease when the outbreak at Harvard occurred. This was in a highly immunized population and these students had had two doses of MMR vaccine. Mumps is an acute viral infection spread through respiratory droplets, which can result in parotitis, orchitis, meningitis, encephalitis, and deafness. The peak incidence of the disease usually occurs in children ages 5-9 but currently, in these outbreaks, the average age of infection was 22 years.

The mumps vaccine is a live attenuated vaccine. It was developed by the prolific vaccine researcher Maurice Hilleman, using mumps virus that he isolated from his daughter, Jeryl Lynn, when she had mumps at age five. The vaccine virus strain is referred to as the "Jeryl Lynn strain." Hilleman's mumps vaccine was then used in the combined measles-mumps-rubella (MMR) vaccine.

Although the mumps vaccine was licensed in 1967; the first dose wasn't used until 1977 and subsequently the incidence of disease dropped by nearly 99%. In the late 1980s, outbreaks started to occur in adolescents 10 to 15 years old. A second dose was then recommended, not because of mumps, but because of measles outbreaks were occurring at the same time. Thereafter, approximately two hundred cases occurred annually, but in 2006 we began to see outbreaks in young adults. In 2016 there were over 6000 cases reported and in 2017 there were more than 5600 cases. Currently, the average age of infection of mumps infection is 22 years. As previously, transmission is occurring in vaccinated populations.

What is happening? What would explain the resurgence? asked Dr. Grad. There were basically two hypotheses. The first is waning protection after vaccination, such as occurs with tetanus, where a booster is recommended every 10 years. A booster here might be the solution. However, if a new strain has evolved, one that escapes immune pressure from the current vaccine, a new vaccine would be needed quickly.

The Grad laboratory studied this through mathematical modeling. They took all the vaccine effectiveness studies that had been done using the Jeryl Lynn vaccine, and using a meta-regression model asked the question "given the time since the last dose of vaccine how does immunity change over time?" Their data showed that there is indeed a waning of immunity and from this they estimated the duration of immunity.

Integrating Genomics (continued)

They had evidence that although vaccine protection lasts up to 27 years, an estimated 25% of people would lose protection after 8 years, and about 50% would lose protection after 19 years. They now had an age structured model where they could look at the shifting age distribution of cases and predict what will happen in each age group. The model indicated that peak susceptibility would occur in ages 10-19, which aligns with outbreaks occurring in that age group, and second peak in susceptibility would occur in the 20-29 age group, which aligns with what we are seeing today. Therefore, the predicted waning of immunity is consistent with what we observe and explains the shifting age distribution of cases.

This model was also used to predict what would happen if there was a "vaccine escape strain". If this was the case, you would expect to see the majority of cases occurring primarily in young children. It therefore appears that waning is the cause and a mumps booster may be the cure. These models however suggest that we actually need a trial to access the outbreaks. For example, we don't know if a vaccine given to an 18-year-old is going to behave the same way as a vaccine given to a 12-15-month-old child or a 4-5-year-old child. We don't know if it would have the same duration of protection.

Dr. Grad was also interested in other mumps outbreaks occurring throughout the country; one in northwest Arkansas was of particular interest. Twelve elementary and middle school students in Springdale, AK had mumps. Dr. Grad thought the data must be wrong as it did not fit his model of waning immunity and contacted the AK state epidemiologist, who believed it was a new strain. They worked together to see what was actually occurring.

The first case was in August 2016 in a 25-29-year-old of Marshallese descent. The story behind this was that after the United States (US) had used the Marshall Islands as a nuclear testing site it allowed the islanders to freely come and work in the US. The Marshall Islands are about 7 feet above sea level and due to climate change and rising sea levels, they are predicted to be uninhabitable in the next 20 to 30 years. People from the Islands therefore have been steadily moving to the US, and commonly moved to Springdale, where a Tyson plant is located. The city has a large closely knit, but impoverished population of about 12,000 Marshallese.

Alfred DeMaria, Jr. MD, Yonatan Grad, MD, Harvey George. PhD, President, NEB, and Nicholas Heger, Program Chair, NEAACC

The outbreak was primarily in the Marshallese and a relevant item is how tightly knit the community is. Vaccination records show that Marshallese children had been vaccinated but records for adults, particularly those from the Islands are not as good. Sequencing showed that the strains seen in Springdale were the same strains as we had in Massachusetts! If this had been a new strain, there would be many more cases and would primarily have occurred in young children. In 2017, the outbreak was declared over after 2950 cases were tracked, the entire community had been affected. It started with a few cases in the Marshallese that spilled over into the local community. This type of outbreak was also seen in Brooklyn, New York in 2009-10. It was again focused in young men 10-15 years old, in a tightly knit community where many people spend time together.

This implies that vaccination does not confer absolute protection. People are exposed to more virus in a densely knit community. It suggests that there is a relationship between the amount of virus received as an "inoculum" and the probability of being infected. Studies are being done now to explain these epidemiological curves. After the first dose of vaccine waning occurs, the second dose gives a boost, and a second waning occurs.

Integrating Genomics (continued)

The vaccine seems to confers "protection" rather than "immunity". We don't know what the mechanisms of waning immunity or protection are or what cells are involved said Dr. Grad. The vaccine came out in 1967 and he believes the outbreaks in the late 1980's, when a second dose of vaccine was recommended, reflected waning. It may be a matter of the "quantity" of virus to which you are exposed and your "level" of protection at the time.

What can be done about this? The Advisory Committee on Immunization Practices, in October 2017, suggested in that there should be a third dose of MMR as part of an outbreak response. The high-risk populations on college campuses should be vaccinated. However, there are questions as to how to implement this. How many cases constitute an outbreak, one or five or more? What does "high risk" mean, if on a sports team, do we vaccine the whole team? There is also the issue of cost effectiveness. Additional work should be done on this recommendation.

We currently dose MMR at 10-15 months, and 4-5 years. When the second dose of MMR was first introduced, it was given at either 4-5 years or 11-12 years. CDC then standardized the age to 4-5 years. What would happen if we now decided to vaccinate at 11 to 12 years? Would that provide sufficient protection that extended into college years? A new vaccine that might confer longer lasting immunity could also be produced; however, this could not be tested in the US as all children receive MMR vaccine.

Dr. Grad also commented that immunity from the measles and rubella vaccine, and via natural infections, appears to last longer than with mumps vaccine. There is no specific antibody titer above which it can be said that immunity occurs. The original Jeryl Lynn vaccine was genotype A; genotype G that is now being used, is immunologically similar. Several years ago, a study showed that genotype G can be neutralized by serum from people vaccinated with genotype A. The virus is evolving, but it it's not clear that viral evolution is leading to changes in how our immune system recognizes the difference between these two vaccines.

Dr. Grad then spoke of work being done in his laboratory with antibiotic resistance. Multidrug resistance is now widespread and this is very worrisome. What's going on and what can we do about it?

We generally think that antibiotic use correlates with resistance and resistance is expected to increase over time. This is seen with the incidence of MRSA, VRE, fluoroquinolone resistance, etc. except that over time, we have also seen that resistances in some bacterial lineages has been declining. MRSA has steadily been declining over the past 10 years or so. What is happening here?

They looked at the Brigham & Women's Hospital (B&W) and Massachusetts General Hospital and saw basically the same thing, a decline in MRSA. However, at the B&W, they were seeing more and more penicillin susceptible *Staphylococcus aureus* (Kanjilal JCM, 2017). This is strange, but it suggests that if resistance is in fact not increasing, perhaps we can do something from a prescriber perspective that might help address this. We should look at antibiotics from a view other than how they are used and the need to create new ones. Antibiotics are like gas in your gas tank said Dr. Grad. Every time you prescribe (press on the accelerator) you use gas (you drive resistance).

Clinical laboratories might envision not only a list of susceptible organisms. Perhaps there could be a list of antibiotics to recommend based on what we know about the organisms and what their likelihood is of acquiring and maintaining resistance. This is one of the themes on which he spoke.

Dr. Grad used *Neisseria gonorrhoeae* (GC) as a model organism, as there is a high burden of disease and the imminent threat of treatment resistance. CDC in 2017 reported about 400,000 cases of disease, but the true incidence is probably double that due to underreporting and the probability of asymptomatic gonorrhea circulating. GC was previously susceptible to all antibiotics but the organism is now resistant to all first line drugs used. The treatment currently recommended for gonorrhea is ceftriaxone plus azithromycin, but decreased susceptibility to these has also been reported. A report from England cited failure with ceftriaxone plus azithromycin, and spectinomycin; thus, as a last resort ertapenem is being used. If this is the only drug that can be used to treat about 400,000 cases

of GC in the future, it has huge implications for gut flora and resistance developing in other organisms that are "bystanders".

In the pre-antibiotic era, gonorrhea was treated with a mixture of cocaine and alcohol, along with caustic compounds like potassium permanganate that were used for urethral irrigation. The JAMA, in 1935, published a paper on fever therapy, hyperthermia. GC did not grow at 40°C, therefore why not heat up the patient, or part of the patient? This is why we need new antibiotics and new approaches to treatment!

Actually, there are opportunities for improved diagnostics and public health intervention. Diagnosis is by nucleic acid amplification testing; few laboratories perform culture today. However, antibiotic susceptibility necessitates culture. Both patient and contacts are treated empirically, and interestingly, although we are seeing cases of very resistant organisms, the majority of gonorrhea in the US is still totally drug susceptible (55.9%). There are other clones that are resistant only to penicillin (4.3%), or only to tetracycline. If we could identify these, rather than treating everybody with ertapenem, the susceptible organisms might perhaps be treated with penicillin, a drug which has been around for a long time. What we might need to slow the spread are rapid diagnostics for gonorrhea resistance, which leads to the question, "what is the genetic basis for resistance"? How do we best implement such tests, are there other strategies we could use to make these effective?

The Grad laboratory worked with CDC and has used genomics and modeling to answer these questions. They looked at whether resistance is emerging de novo, or whether there was a resistant clone spreading. They found for example, that ciprofloxacin resistance is reliably explained by a single mutation, while azithromycin resistance can be explained by multiple mechanisms, some of which have not yet been described. The genetic basis for mutations known to confer resistance only explains about 65% of the resistance the laboratory saw. They also found that gonococcus has been exchanging DNA with other *Neisseria*, and has picked up from these commensals in the pharynx, genes that help achieve resistance to azithromycin. Most cephalosporin resistance is explained by an alternate penicillin-binding protein. The laboratory is currently studying other mechanisms of drug resistance that might explain the other 35% of resistance.

There are a variety of methods being developed for sequencing susceptibilities, but there will always be the issue of positive and negative predictive values. This can be done for quinolones with gonorrhea, but not for other drugs as yet.

This might work with a single strain, but what if there is a mixed infection? Is the sequencing going to be accurate, because there will be mixed resistant phenotypes and genotypes? What percentage of the isolates have a resistant mutation and can you use one drug to treat this? There are still many interesting questions that are pertinent to the application of this type of technology clinically.

Dr. Grad advocates using caution in GC susceptibility testing. Our knowledge is incomplete! What do we need to address before this becomes a reality? We have undetermined mechanisms of resistance, how frequently do novel mechanisms arise? How often do we see mixed infections? What fraction of cases needs to be sequenced? There will be new antibiotics, how do we develop molecular new assays for these when we don't know what the resistance mechanisms are yet?

The relationship between the diagnostic use and the clinical use of antibiotics is important; diagnostics alone will not solve the problem. Answers here can be used for a variety of questions related to epidemiology, that helps us track how the organisms spread and identify outbreaks. This might be useful from the perspective of prevention. The Grad laboratory looked at the spread of GC resistance in US. Others are working on reconstructing transmission networks; colleagues in England sequenced >1400 isolates.

How do you turn genomic based epidemiology into actual information and clinical interventions that could be employed?. First, do we believe the networks we reconstructed? And how accurately do these networks reflect the total story occurring? If we base interventions on what we reconstruct, how do we access them? What kind of sampling do we need? There are a variety

Integrating Genomics (continued)

of questions like these that investigators are trying to solve.

The last point Dr. Grad wanted to make is democratization. These are sequencing-based tools that need to be freely available globally, since most of our gonorrhea resistance probably originates in East Asia and spreads from there around the world.

Dr. Grad lastly reminded the audience that his laboratory welcomes donations of organisms from laboratory freezers and he is always free to answer questions.

NACMID - NEBASM
Third Annual Meeting

The third annual Joint Meeting of the Northeast Association for Clinical Microbiology and Infectious Disease and the Northeast Branch, American Society for Microbiology was held on April 23-24, 2018 at the Sheraton Four Points, in Wakefield, MA. There were about 200 attendees and 36 exhibitor booths at the two-day meeting entitled *Microbiology: Going Forward*. The meeting included student posters and student oral presentations; awards for posters were supported by the ASM Branch Funding program. CMLE was available to participants through the American College of Clinical Pathology.

Four full-day workshops were held on Monday. *A Guide to Creating a Culture of Safety and Security in the Laboratory* was presented by Shoolah Escott. MS, MT(ASCP), Biosafety Manager, MA State Public Health Laboratory. The workshop focused on identifying key concerns of laboratory biosafety and of laboratory security principles, such as conducting vulnerability assessments and developing a good biosecurity plan. Case studies were included.

Antimicrobial Susceptibility Testing for Today's Microbiologist was presented by April Bobenchik, PhD, D(ABMM), MT(ASCP), Associate Director of Microbiology, Lifespan Academic Medical Center, Providence, RI and covered current topics in antimicrobial susceptibility testing for major aerobic Gram-positive and Gram-negative organisms. Topics included updates to the CLSI M100 2018 document, identifying strategies for testing new antimicrobials and new methods for susceptibility testing.

A *Clinical Mycology Workshop* was presented by Sarah K. Zimmerman, MEd, MT(ASCP) SM, Technical Supervisor Mycobacteriology, Parasitology, Mycology, Lahey Hospital & Medical Center, Burlington, MA. She emphasized the importance of performing direct examinations of fungi, discussed the salient characteristics of fungi found in clinical samples, and reviewed the updated susceptibility testing guidelines for *Candida sp*.

Culture-Independent Testing included several presentations. Rick Danforth, SM(ASCP), President, Tops Club Inc., Chelsea, ME, opened the session with *Culture Wars: A Plate Awakens.* He discussed various types of culture-independent diagnostic tests, how to identify and understand them, how they are interpreted in the laboratory and by epidemiologists, and of options for enhanced communication between clinical laboratories and public health departments.

Nancy S. Miller, MD, Medical Director, Clinical Microbiology and Molecular Diagnostics, Boston Medical Center, spoke on *An Infectious Disease Viewpoint*, and discussed culture-independent testing and automation, and the impact on infectious disease diagnostics.

Emily Harvey, BS, Epidemiologist, and Johanna Vostok, MPH. both from the MA Department of Public Health spoke from the *Epidemiologist's Viewpoint*. They discussed the impact and serious challenges of culture independent testing in public health investigations and the implications it has for public health surveillance and population health.

Joseph Rubino, PhD, from Cepheid, spoke on *Compliance Issues PAMA Updates and Opportunity.* He provided an overview on PAMA (Protecting Access to Medicare Act of 2014), its effect on clinical laboratory fee schedules and how this will affect Medicare reimbursement over the next 3 years.

The session concluded with a user panel and discussion of how culture-independent testing works in a variety of hospital settings. Panelists

NACMID-NEBASM Annual Mtg (continued)

included Cynthia Astolfi, SM(ASCP)M, Microbiology Manager, Steward Holy Family Hospital, Methuen, MA; Beverley Orr, MT(ASCP); Technical Supervisor, Clinical Microbiology and Molecular Diagnostics, Boston Medical Center; Bernadette Chirokas, SM(ASCP)M, Microbiology Manager, Tufts Medical Center, Boston, MA; Stacy Hebb, BS, MT(ASCP), Clinical Laboratory Manager, Berkshire Medical Center Laboratories, Pittsfield, MA and Valerie Whitehead, MT(ASCP)CLS, Microbiology Manager, Lifespan/Road Island Hospital, Providence, RI..

A wine and cheese reception with the vendors was held in the exhibit hall late Monday afternoon. This was followed by the keynote address, *Five Percent of the World: The 1918 Spanish Influenza Pandemic,* which was presented by Harold Sanchez, MD, FACP, Associate Chief of Pathology and Medical Director of Microbiology at the Hospital of Central Connecticut in New Britain, CT. [He is Assistant Clinical Professor of Laboratory Medicine at the Yale School of Medicine, and Associate Clinical Professor of Pathology at the Frank Netter School of Medicine at Quinnipiac University.] Dr. Sanchez is also an enthusiastic fan of medical history and President of the Beaumont Medical Club, a medical historical society in New Haven. He is particularly interested in autopsy pathology, microbiology, and the interaction between medicine and societal forces. Dr. Sanchez spoke on the history of influenza and described in detail the role social forces and the war effort played in the Spanish Influenza pandemic. The search for an explanation as to why this pandemic was so bad is still underway. The entire 1918 influenza genome has been sequenced and is being studied to determine what made this an unusual organism. Can it happen again? At least we now have a better coordinated response, coordinated reporting and archiving materials, and information can readily be disseminated worldwide.

Following the Keynote Address, Kimberlee Musser, PhD, Chief, Bacterial Diseases at Wadsworth Center, New York State

Keynote Speaker Harold Sanchez, MD

Department of Health, briefly spoke of the Centers for Disease Control and Prevention's Antibiotic Resistance Lab Network (ARLN). The network supports nationwide lab capacity to rapidly detect antibiotic resistance and inform local responses to prevent spread and protect people. She described the goals of the ARLN, how to access their testing services, and spoke of the critical role that clinical lab partners play in the ARLN.

Harvey George, President. NEB-ASM;
and
Cynthia Astolfi, President, NACMID

NACMID-NEBASM Annual Meeting (continued)

General Sessions were held on Tuesday and the morning began with the President's welcome and business meeting. This was followed by a morning presentation on *Fecal Transplants* presented by Elizabeth Hohmann, MD, Chair and Physician Director, Partners IRB, MA General Hospital, Boston, MA. She spoke of MA General's oral capsule Fecal Microbiota Transplant (FMT) program and discussed *C. difficile* colitis as an example of how the human microbiome can be beneficially manipulated. FMT probably treats *C. difficile* by restoring the normal balance of intestinal microbes. Dr. Hohmann also mentioned other interesting illness targets for microbial modulation.

April Bobenchik, PhD, then spoke on *Don't Be Resistant to AST: An Introduction to Susceptibility Testing* and covered the basic principles of antimicrobial susceptibility testing, focusing on routinely tested and reported antimicrobials. Case studies were presented as examples of both common and unusual susceptibility patterns encountered.

Sanjat Kanjilal, MD, MPH, Instructor, Harvard Medical School, Brigham & Women's Hospital, Boston, MA presented *Case Studies*, and discussed cases of actual patients who specimens were processed at the Brigham & Women's microbiology laboratory over the past year. He spoke of challenges in the identification and testing of the organisms, how information was conveyed to the clinician and how it changed patient management. He also reviewed newer methods rapid molecular diagnostic techniques that are changing clinical practice.

Michael Mina, MD, PhD, MPH, Physician Scientist, Brigham and Women's Hospital, Boston, MA, presented a *Vaccine Update.* He spoke of the benefits of vaccination and how hesitating to vaccinate threatens to undermine progress already made. He spoke of annual influenza vaccines, how strains are chosen, and the intended immunological and ecological benefits and consequences of influenza and measles vaccines.

A panel discussion on CREs, entitled *CP-CRE*: *Not Your Average Bugs,* was presented by Stephen Brecher, PhD, VA Boston Healthcare System, Boston, and Tracy Stiles, MS, M (ASCP), Director, Microbiology Division, MA Department of Public Health (MDPH). The session covered the importance of the laboratory and accurately identifying and confirming CP – CRE, and the role of infection control, nursing and the pharmacy. The MDPH requirements for organism submission, testing methods and antibiotic resistance trends in MA to date were discussed, as well as the MA laboratory role in the Antibiotic Resistance Lab Network.

The Public Health Response to Food Safety Issues was presented by Kathleen Gensheimer, MD, MPH, from the Food and Drug Administration (FDA), Hyattsville, MD. She spoke of the role of the FDA in foodborne disease surveillance and told how a public health problem is defined. She described the changing landscape of food safety and how food outbreak investigations are conducted. She emphasized that partnerships are key to achieving successful public health outcomes and necessitate collaboration and coordination of numerous players such as medical providers environmental health specialists, agriculturalists, epidemiologists, laboratorians, regulators, and industry, all working across the local state and federal level. She described challenges in both domestic and global food safety, as well as challenges arising due to evolving laboratory technologies such as culture independent diagnostic testing and whole genome sequencing.

Kathleen Gensheimer, MD, MPH and
Alfred DeMaria, Jr., MD

NEBASM-NACMID Annual Meeting (continued)

Harvey George, PhD, DABCC, FAACC, Trimark Publications, Dover, MA, spoke on *The Surveyors Are Coming! Are You Ready? Learn How to Pass Your Clinical Lab Inspection.* His presentation provided timely and highly relevant information for both senior laboratorians as well as "newcomers". He described in detail the records and documentation that must be prepared, maintained, and available for surveyor and licensing personnel review if their laboratory is to obtain and maintain their certification. The presentation included a question and answer period plus a brief attendee quiz.

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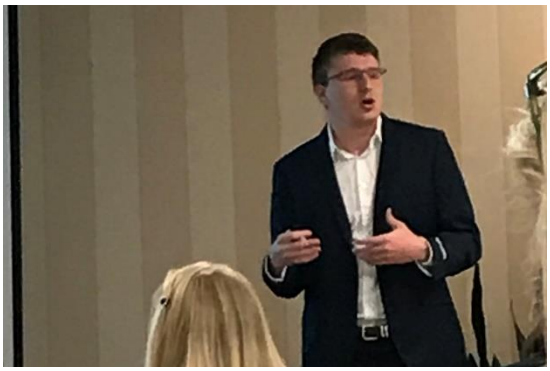
The inclusion of students is a high priority for NACMID and the NEB-ASM in order to advance student professional development and provide an opportunity to further encourage student interest in microbiology. Over 47 students attended the meeting this year.

The Tuesday poster session gave students an opportunity to present their research in a professional environment, and the three best student poster presentations received cash awards. In 1st place: were Molica Pen and Anthony Napolitano with *Screening Drug Derivatives as Antimicrobial Compounds for Disease Treatment*. In 2nd place was William Rodriguez with *Determination of Microbial Diversity and Biomolecule Extraction from Antarctic Soil* and in 3rd place was Aradhna Rana with "*Detection of Antimicrobial Activity in Sarracinea purpurea*". All three students were from Worcester State University

In addition, two student abstracts were selected for oral presentations based on the quality of the work. The first presentation was *Screening Drug Derivatives as Antimicrobial Compounds for Disease Treatment,* by Molica Pen and Anthony Napolitano, of Worcester State University, presented by Anthony Napolitano. He described the development of antimicrobial drug screening assay to test drug analogs against pathogenic bacteria.

William Rodriguez, also from Worcester State University presented *The Determination of Microbial Diversity and Biomolecule Extraction from Antarctic Soil*, in which he described the investigation of the microbiota of a soil sample from a pristine site in Antarctica from which 15 microbial specimens were isolated and characterized and genomic and meta-genomic DNA was extracted.



Anthony Napolitano, Worcester State University

Poster Session

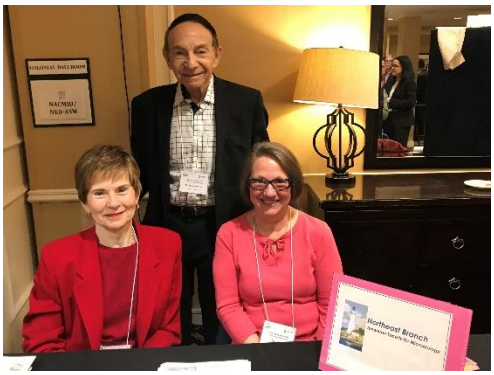
William Rodriguez, Worcester State University

Presentations of speakers who have authorized them to be posted are available at: https://nacmid.org/2018-conference

NEBASM-NACMID Annual Meeting (continued)

We would especially like to thank all the exhibitors for their support, without which this meeting would not have been possible!

Public Health Museum Table: (L-R) Ashley Gasinowski, Emy Thomas, NEB Archive Chairperson and Diana Drouiillard O'Brien



Northeast Branch Table: (L-R) Irene George, NEB Secretary; Harvey George NEB President; and Carol Finn, NEB Local Councilor

24th Boston Bacterial Meeting

The NEB was again one of the sponsors of the annual Boston Bacterial Meeting which was held at the Harvard University Science Center, Cambridge, MA on 5/31/18-6/1/18. The meeting attracts Boston-area researchers who are studying the biology of microorganisms in either academic or industrial settings. Attendance currently includes over 500 researchers from academic and biotech companies.

Science Fairs

The NEB annually donates an award of $100 to each of five MA regional fairs and the Vermont Science Fair, and also $200 to the MA Science Fair. Following are this year's winners of the NEB awards and their projects. Congratulations again to the students for their outstanding work.

Region I. Massachusetts State Science Fair: Names of winners were not available.

Region II. Worcester Regional Science and Engineering Fair. Marzuq Iqbal from the Advanced Math and Science Academy, Worcester, MA. *How Bacteria Can Help Us to Treat Diabetes.*

Region III: Bristol Community College-Rensselaer Polytechnic Institute Regional Science Fair: Alice Wong. Taunton High School. *Environmental Factors on Cellular Respiration Rates.*

Region IV. Angela Jin, Junior at Acton-Boxborough Regional High School. *Effects of Arbuscular Mycorrhizal Fungi on Brassica Growth.*

Region V. South Shore Regional Science Fair: Rebecca Cox and Sarah Lott, Grade 11, Falmouth Academy, Falmouth, MA. *"Exploring the Lower Ocean Crust. One of the Last Frontiers on Earth.*

Region VI. Daisy Wang. Grade 10, Boston Latin School, Boston, MA. *A Comparison of Meat Freshness Upon Different Thawing Methods.*

70th ASCLS-CNE Annual Convention

The 70th American Society for Clinical Laboratory Science-Central New England Annual Convention was held at the Rhode Island Convention Center in Providence, RI on April 24-26, 2018. It was jointly sponsored with the Board of Rhode Island Schools of Allied Health (BRISAH), Northeast Branch, American Society for Microbiology (NEB-ASM), the Rhode Island Cytology Association (RICA) and the-Rhode Island Society for Histology (RISH).

New England Microbiology Laboratory Directors Meetings

The New England Microbiology Laboratory Directors group has been meeting at the Publick House in Sturbridge twice a year for the past thirty years in order to share information and their experiences in the laboratory. The informal half-day agenda consists of presentations by attendees. The meetings this year were held on April 25 and October 30, and are attended by physicians, laboratory directors, epidemiologists and laboratorians from New England. Meetings are supported in part by the NEB.

Please contact Alfred.DeMaria@state.ma.us if you would like to receive meeting information. The next meeting will be held on May 1, 2019.

Northeast Branch of the American Society for Microbiology

MEMBERSHIP RENEWAL FORM

January 1, 2019 – December 31, 2019

Please check personal information.

Name: _____ ASM Member? _____ ASM Membership No _____

Preferred Mailing Address _____

Home/ Business Address _____

Phone (Day) _____ Preferred Email: _____

Phone (Other) _____ Other Email: _____

Professional Position: _____ Specialty: _____

Primary Area of interest: __Biotechnology __Education __Marketing/Sales
__Clinical/Public Health __Industrial Other: _____

Are you interested in any of the following Branch activities? __Working on Committees __Running for Office

MEMBERSHIP OPTIONS:

__Individual ($ 15.00 annually) __Individual ($ 40.00 / 3 years) __Student ($ 10.00 annually)

__Emeritus* (No Charge) *Emeritus membership is defined as a member who is in good standing for 20 consecutive years, and who is retired from their profession.

__ UPDATE ONLY ENCLOSED (changes can be emailed to NEBranch-ASM@comcast.net)

Renewals postmarked after September 1, 2018 will be effective 9/1/18-12/31/19.

Please renew either with your annual ASM membership or mail this form and dues check (payable to NORTHEAST BRANCH-ASM) to:

Patricia E. Kludt
6 Abigail Drive
Hudson, MA 01749

Date Dues Received: _____

Check No.:_____

Sept 2018

3rd Joint Annual Meeting
Northeast Branch-ASM and NACMID
April 23-24, 2018

We would like to thank the following for their continued support!

Abbott Rapid Diagnostics
Accelerate Diagnostics, Inc.
Advanced Instruments
Allergan Pharmaceuticals
American Society for Microbiology
Astellas Pharma
Beckman Coulter MicroScan
Becton Dickinson
Biofire
Biomerieux, Inc
Bruker Daltonics
Cepheid
Copan
Curetis USA
Diasorin Molecular
GenMark Diagnostics
Gold Standard Diagnostics
Hardy Diagnostics
Healthcare Tech
Hologic (CYTYC Corporation)
Liofilchem
Luminex Corporation
Medical Chemical Corporation
Merck
MetaSystems
Microbiologics, Inc.
Meridian BioScience, Inc
Oxford Immunotec
Oxyrase, Inc.
Quidel
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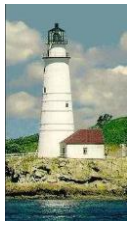
Streck
Thermo Fisher Scientific
Zeptometrix Corporation

Educational Organizations

Antibiotic Resistance Lab Network (CDC)
NACMID
NEB-ASM
The Public Health Museum



NORTHEAST BRANCH
AMERICAN SOCIETY FOR MICROBIOLOGY

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