



Northeast Branch Newsletter

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Serotherapy Before COVID: Pneumonia Before Serotherapy

Scott Podolsky MD, is a primary care physician at Massachusetts General Hospital, Professor of Global Health and Social Medicine at Harvard Medical School, and Director of the Center for the History of Medicine at the Countway Medical Library. He is the author of such books as *Pneumonia before Antibiotics: Therapeutic Evolution and Evaluation in Twentieth-Century America* (2006) and *The Antibiotic Era: Reform, Resistance, and the Pursuit of a Rational Therapeutics* (2015). Dr. Podolsky spoke on *Serotherapy Before COVID: Pneumonia Before Antibiotics* and took us back to the days when pneumonia was treated with type-specific antisera (1892-1945). The program on October 18, 2022 was co-sponsored by the Northeast Branch and the Massachusetts Public Health Museum.



Many classic infectious diseases were discovered in the Golden Age of microbiology (1870's-early 1880's). Pneumonia was deadly and was believed to be untreatable (William Osler, 1892). Immunology emerged, and antiserum was used for diphtheria and tetanus; anti-pneumococcal serotherapy was unsuccessfully tried in 1891. Neufeld, in Germany, later discovered pneumococcal serotypes, and observed that only type-specific antiserum was effective. There were only 4 serotypes then vs the 100 we have today.

Rufus Cole was the first Director of a group created at the Hospital of the Rockefeller Institute in New York City to define therapies for the treatment of pneumonia (1910). (Cont. on page 4)

Harnessing Plant Microbiomes for Sustainable Agriculture

Currently Director of the Microbial Sourcing and Evaluation team at Indigo Ag in Boston, MA, Sarah Seaton, PhD, leads a team of researchers responsible for the isolation and characterization of beneficial plant-associated microbes that increase yield and decrease the need for chemical inputs in agricultural crops. She spoke on *Harnessing Plant Microbiomes for Sustainable Agriculture* on September 29, 2022. Ultimately, the goal is to harness nature to help farmers sustainably feed the planet.

Indigo was founded in Boston in 2013 as Symbiota. There are currently about 1000 employees, with headquarters and Research & Development in Boston, commercial offices in Memphis, TN, greenhouse operations in research Triangle Park, North Carolina, and global offices in other countries. Indigo has several biological products as well as technology-based products that center around their overall mission, which is to improve sustainability of agriculture, improve farmer profitability, and better align agricultural practices with consumer health. They are

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NEB Council Meetings

Council Meetings this year will continue to be held virtually until further notice. 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 2023 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 2022. President, Stefan Riedel; President-Elect, Ramy Arnaout; Treasurer, Patricia Kludt; Branch Councilor Roger S. Greenwell, Jr., and Local Councilor, Frank Scarano. Thank you for another great year of programs and we are looking forward to planning a busy 2023!

Student Chapters

The NEB is associated with two active student chapters. The Boston-Area Student Chapter, and the Maine Society of Microbiology, Orono, ME.

FUTURE PROGRAMS

Local Programs:

Announcements of Local Meetings and registration materials are posted on our website:

<http://northeastbranchasm.org>

February 16, 2023 via Zoom.

Topic: *Black Spot, Black Death, Black Pearl: Tales of Bacterial Effectors.*

Speaker: ASMDL Lecturer Kim Orth, PhD. Howard Hughes Medical Institute and University of Texas Southwestern Medical Center, Molecular Biology.

March 16, 2023.

In Person Dinner- Meeting.

Topic: *Wastewater Intelligence as Public Health Infrastructure.*

Speaker: *Mariana Matus, PhD, CEO and Co-Founder at Biobot Analytics, Cambridge, MA.*

Location: *Holiday Inn Boston-Dedham Hotel & Conference Center, Dedham, MA,*

New England Microbiology Laboratory Directors Spring Meeting

Location: *Publick House, Sturbridge, MA. Date/time to be announced.*

National Meetings:

June 15-19, 2023. ASM Microbe, Houston, TX. <https://asm.org>> Events > ASM-Microbe

2023 Travel Awards Available for Early Career Northeast Branch Members

The 2023 ASM Peggy Cotter Travel Award Program for Early Career Branch Members provides funds for outstanding early career Branch members to attend ASM Microbe 2023 to be held on June 15-19, 2023 in Houston, TX. Three \$1,650 cash awards are available to pay for registration, accommodation and travel costs associated with Microbe 2023.

All submissions must be dated no later than February 22, 2023. The Selection Committee will announce the winners by February 27, 2023.

Contact Frank J. Scarano, PhD, at fscarano@umassd.edu for program details.

ethically untenable for anyone not to receive therapy, as there was a 25% death rate with Type 1 pneumococcal pneumonia. The identification of pneumococcal types was laborious; a sputum sample needed to be grown in mouse peritoneum, and a type specific swollen capsule reaction (Neufeld Quellung reaction) observed. There were also anaphylactic reactions from the serum.

Cole thought true efficacy of the method could be demonstrated, when in 1918 the massive influenza pandemic occurred. Type-specific serum, polyvalent serum, and other treatments were used, and there was no time to perform careful studies. When all the data was assembled, they never really knew if type-specific serum worked or not. The MetLife Insurance Company lost much money in death benefits during the pandemic and agreed to fund controlled studies in major hospitals such as Bellevue Hospital, Harlem Hospital, and Boston City Hospital, which eventually involved Max Finland. They outlined the conditions to be met (Archives of Internal Medicine, 1925), that from today's

Serotherapy Before COVID (continued)

He elaborated on Neufeld type-specific therapy, and it was so efficacious that he thought that even in a clinically controlled series, it would be

Serotherapy Before COVID (continued)

perspective, included rather sophisticated guidelines. Data (1928) showed that with Type 1 pneumonia, the serum seemed to work, but it was most beneficial administered in the first few days of illness. However, patients at that time might not arrive at a hospital until the 7th day or longer.

Thus arose the question of drug delivery: how to get patients to the hospital earlier or how to treat them at home upon diagnosis. This was originally studied in Massachusetts. The Massachusetts Pneumonia Study and Service (1931-1935) was funded by the Commonwealth. Roderick Heffron headed the study and traveled statewide lecturing on how to administer serum. The state was divided into districts; each district had its own combination typing and diagnostic center. A physician would diagnose pneumonia at a patient's home, bring sputum to a diagnostic center for serotyping, and then return with serum to the patient's home and administer it. This was remarkably successful although it was not a controlled study and there was a low mortality rate. By the 1930s about 30 types pneumococci had been identified, and type-specific serum was being produced for all. The Massachusetts Department of Public Health was a pioneer in this and had a wide range of antiserum at this time.

The medical profession was asked to take the leadership in public health activities regarding pneumonia control during the New York pneumonia control program (1936). The New York State Medical Society agreed to work with the public health department but they wanted to retain ownership and not lose any of their autonomy.

In 1937, The Surgeon General of the United States Public Health Service (USPHS) initiated a national campaign to control pneumonia, and federal funds were made available; he framed public health as a fundamental human right. At that time (1930) about two thirds of the states already had pneumonia control programs. The USPHS teamed up with the Metropolitan Life Insurance Company, that played a great role in marketing and disseminating information about pneumonia using films, books, and other media. The national campaign was out to redefine pneumonia as a dangerous emergency that demanded cooperation of the state, national, public health systems, and local physicians, and

required cooperation between laboratories and clinics.

Sulfanilamide came out in 1935, but was not a good treatment for pneumonia, then sulfapyridine, and by 1945 the sulfa drugs had totally replaced antiserum. The sulfa drugs were then replaced by penicillin and other drugs. There was now a redefinition of pneumonia, and a change in medication administration and in the type of oversight. Pneumonia had reverted from being a public health crisis to being in the domain of providers and became defined as a non-emergency illness. This illustrates how a disease category can be defined and redefined in a relatively short time.

Dr. Podolsky next spoke of antimicrobials and the advent of the controlled clinical trial stemming from the antiserum story. One of the foremost antimicrobial investigators was Maxwell Finland, at Boston City Hospital (1902-1987). He participated in the first controlled studies on pneumonia and showed that, when done perfectly and statistically, type-specific serum works. However, he wrote (1942) that well-meaning clinician researchers could cheat the system by not doing alternate allocation. Sir Austin Bradford Hill, in England, published the same thoughts. When Hill was designing the Medical Research Council study of using streptomycin treatment for tuberculosis, he replaced alternate allocation with concealed randomized allocation (1948), considered to be the first randomized controlled trial. However, even by ~1951, not many studies were being done in a well-controlled fashion, which raises the question of why. The pharmaceutical industry did not perform such studies.

Dr. Podolsky then digressed into the timeline of Food and Drug Administration regulation. The 1906 Food and Drug Act dealt with purity, mandating that drugs contain what their labels say they contain. This was in an era where people were using alcohol, cocaine, and heroin for treatments and there were food concerns. Nothing was said about efficacy or safety.

The 1938 Food, Drug and Cosmetic Act was passed following a 1937 tragedy, and required proof of safety before the release of a new drug. Sulfa drugs were introduced in the 1930s, but sulfanilamide was unpalatable to children. A new liquified form, sweetened with diethylene glycol was released, resulting in over 100 deaths; it had

Serotherapy Before COVID (continued)

not been tested for safety. Efficacy was implicitly considered here but was not explicit at that time.

The 1951 Durham-Humphrey Amendment defined two specific categories for medications, prescription and over-the counter drugs. There was a postwar expansion of pharmaceuticals: steroids, antibiotics, antipsychotics, antihypertensives etc. Much antimicrobial drug resistance was now seen to sulfa drugs, penicillin and tetracycline.

Max Finland, in 1957, was upset by the way drugs were being approved and began a campaign that manufacturer's claims should not be accepted unless confirmed by reliable and unbiased reports from other laboratories and supported by controlled clinical trials rather than by testimonials and advertising. In 1959, the Saturday Review published an article exposing these concerns about testimonials, false advertising, and the inability of the FDA to adjudicate drug efficacy.

Senator Estes Kefauver in 1959, took on the pharmaceutical industry, focused mostly on prices and patent concern, but during the course of the investigation, he became concerned about drug marketing and the FDA inability to adjudicate drug efficacy. These concerns took a back seat with the advent of thalidomide, and led to the Kefauver-Harris Drug Amendments requiring that manufacturers prove the effectiveness of drug products before they go on the market, and afterwards report any serious side effects. There were a number of court cases by the late 1960's in which drugs were taken off the market, and the FDA was forced to define what they mean by well-controlled investigations, which are randomized, double-blinded control trials.

However, this only pertains to drugs going into the marketplace but says little about how physicians can use drugs already in the marketplace. Therapeutic rationalists, as far back as 1957 believed that over 95% of antibiotics were being administered inappropriately. We saw the word "superbug" by 1966, and by the early 1970s, years after the Kefauver-Harris Drug Amendment was passed, there was a 30% increase in antibiotic usage. The question again was whether physicians were using drugs rationally. Concerns persist about this today, with current microbial resistance.

We also saw the administration of therapeutics and general neglect of the public health at a national level. This certainly led to the inequitable distribution of vaccines and treatment that still we see today, leading to racial and ethnic disparities. How do you access complex therapies, whether they be monoclonal antibodies today, or pneumococcal antiserum in the 1930's?

Dr. Podolsky commented on the determination of drug efficacy today. Those who questioned the efficacy of the malaria drug hydroxychloroquine for COVID, for example, were said to be total denial. Misinformation about this and other drugs was rampant, and there are still challenges with this. The Commonwealth of Massachusetts bulletin in June 2022 stated that "the United States lacks a national public health system capable of protecting and improving health, and advancing health equity every day, and responding effectively to emergencies".

Dr. Podolsky concluded by saying that these long-standing historical tensions and concerns are still being played out today. There is a need for cooperation between national, state and local entities, cooperation between the laboratory and the clinician. This all needs to be tied to health equity, that has been a concern for decades.

Harnessing Plant Microbiomes

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essentially incentivizing farmers to grow their crops in a more sustainable way and simultaneously improving the bottom line for farmers.

Indigo has three business units, Biologics, Carbon and Marketplace. Dr. Seaton works in the Biological Unit, where develop microbial seed treatments meant to be applied to crop seeds to reduce chemical input and improve crop health under stress. Essentially, Indigo is marketing microbes that can replace chemical fertilizers, pesticides, and insecticides, all centered around sustainable agriculture and increasing productivity. The Biologics Unit is closely tied with the Carbon Business Unit that incentivizes farmers to use microbial treatments in order to reduce modern farming inputs, and to change agricultural practices in a more "planet positive" way. These include planting cover crops, not tilling at the end of the season, etc., practices that can increase the amount of carbon that is

Harnessing Plant Microbiomes

(continued)

sequestered in soil. Indigo incentivizes farmers to adopt those practices by paying them, monitoring the carbon in their soil, and paying for that increased carbon sequestration.

Agricultural products are commodity products, and the only way to ensure that the grain from one farmer, who grows his grain and



Sarah Seaton, PhD

fiber in a more sustainable way, does not get combined with that from another whose agricultural practices are different, Indigo has

developed a grain and carbon marketplace that actually tracks and transports the final grain products so that they can be traced. Farmers can be connected directly to consumers who are interested in having products that were grown in a healthy and sustainable way.

Indigo marketed only biological products from about 2013-2017 and has since expanded into technology platforms. The plant microbiome has evolved alongside its host and is critical for plant health as the human microbiome is for human health; any dysbiosis in the community will have a great phenotypic impact. There is evidence that modern agricultural practices have stripped away much of the native microbial community that is present in undisturbed soils, and modern cultivars have a far less diverse microbial community compared to their wild counterparts. Indigo tries to add back those beneficial microbes that were removed.

Dr. Seaton's team has identified microbes that they believe can impact plant health under nearly any agricultural stress that a farmer is likely to face. Microbes can produce insecticides or fungicides, eliminating the need for chemicals. They can fix nitrogen from the air, eliminating chemical fertilizers, and they can sequester micronutrients like iron.

Indigo has seed treatments for all of the core crops, corn, soybeans, wheat, cotton, and rice, that are applied on the farm prior to planting, then colonize the plants. Dr. Seaton described

how her team works to discover the best microbial candidates for commercialization. Instead of searching in soil, they choose plants in agricultural fields clearly impacted by some type of stress (control plant) and neighboring plants doing well under the same stress (superior plant). Drones are also used to collect arial data about plant health in the fields. Community analysis from each plant is done by sequencing, searching for microbes which are enriched in phenotypically healthy plants versus the unhealthy controls. They search for exclusively for endophytes that live within plant tissues, and identify bacterial and fungal communities that are enriched in the healthy plant.

Indigo has one of the largest most diverse collections of microbial endophytes in the world with about 40,000 microbes, all of which are plant associated endophytes, about half being isolated from crop samples using the approach discussed. Sequencing data is available up front, and cultivation techniques can be modified to capture fairly rare or fastidious organisms. Nearly 20,000 microbial endophytes have been isolated and identified to date, including over 200 bacteria and 300 fungal genera. Their libraries include filamentous fungi, yeast, bacteria, algae, and hundreds of novel species. All samples are sequenced, cataloged and cryopreserved.

How is a microbe assessed to be the best candidate for a commercial product? Simple high throughput assays are first used and a plant enters the laboratory associated with its genome. The first screen is a non-soil-based seedling assay, done in the Boston lab, in which the microbe is placed onto a seed and its impact on plant health studied. These are usually short-term assays of 10 to 15 days, followed by imaging to assess plant health. The best performers here go to more complex soil greenhouse assays in the Triangle Park facility. The best performers from greenhouse trials go into field trials to obtain high quality data; these are run through contract research organizations across the U.S. and globally. Microbial seed treatments are sent to them, the seeds are planted in the geographical area, and Indigo monitors the fields with high-resolution imagery as well as by plant metrics throughout the season. A variety of different models are used that take into account weather and numerous other variables to see which of the microbes perform best across different environments. This not only identifies the best

Harnessing Plant Microbiomes (continued)

microbes for a specific crop and stress but allows pinpointing individual environments where the microbe is most effective. Microbial seed treatments are thus tailored to specific crops, specific geographies, and specific stresses that an individual farmer might expect in their own growing operation based on their unique environment, crop cultivar, and other specifics.

The Ethics of CRISPR Editing

The Ethics of CRISPR Editing: Out in the World and in the Classroom was presented virtually on April 7, 2022 by Natalie Kofler, MS, PhD, Founder, Editing Nature, Senior Advisor, Scientific Citizenship Initiative, Harvard Medical School, Boston, MA. Editing Nature is a global initiative to steer responsible development and deployment of genetic technologies. Dr. Kofler is a leading voice in CRISPR and synthetic biology ethics and governance, authoring numerous publications on the topic, serving on expert panels, and contributing to UN mandated documents. CRISPR gene editing holds great promise to solve some of our world's greatest challenges, from medicine and public health to food security and climate change. CRISPR technologies also carry unintended consequences and risks to social equity. How do we ensure this technology is steered ethically?

It was announced at the 2nd International Summit of Human Gene Editing in 2018, that Dr. He Jiankui, a Chinese biophysicist had germ line gene edited two embryos that were implanted into a surrogate, resulting in the birth of twin girls. They were the first humans to be germ-line gene-edited, meaning that every cell in their body, including their eggs, had been edited and will be passed on to future generations.

The scientific community was outraged and shocked, primarily because they thought rules relating to such research were already in place. In the 1st International Summit several years prior, it was made very clear that it would be irresponsible to proceed with any clinical use of germline gene editing unless (1) relevant safety and efficacy issues had been resolved and (2) that there was broad societal consensus about the

appropriateness of the proposed application. But here is where things fell apart.

People have different ideas about safety, efficacy and broad societal consensus. Dr. He said he had polled the



Chinese community, where he worked, and received support for his idea. Such matters become very complicated, when scientists, like ourselves, can completely “govern” without having other things in place, such as having objectivity and particularly other points of view. Also concerning was that even several hi-level researchers in the United States (U.S.) knew about his intentions and did not report them.

Dr. Kofler described what should be in place so that these technologies move ahead appropriately. The real issue raised is who decides what needs to be corrected, why, and how it should be done. People whose lives are impacted by these technologies should have a say in how they are used and there should be representatives at the decision-making table who have experienced the disabilities/diseases to be corrected. A patient's perspective is important and the research community needs insight into a patient's experience.

There has been much discussion in the disability rights and disability ethics communities, with concern that people's entire identities will be eliminated; i.e., deafness. Looking at two “expert” panels on the subject that have been assembled worldwide, Dr. Kofler found that not one person on these panels had a disability. Sandy Sufian and Rosemarie Garland-Thomson, in 2021, wrote of the dark side of CRISPR: “Its potential to “fix” people at the genetic level is a threat to those who are judged by society to be biologically inferior”.

Along the same line, consent, though important, is not enough. Historically, a person's consent was often not requested. There are currently better consent processes in the U.S. A University of California research project is developing sustained partnerships with the impacted communities in surrounding areas,

The Ethics of CRISPR Editing

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which is good first step.

Past work with CRISPR can also be used to make decisions differently. CRISPR technology is now focused on sickle cell disease treatment/cure, and is showing much promise. However, there is a funding disparity compared to the impact it has. A study showed that cystic fibrosis, affecting 30,000 in the US, receives 27x more funding than sickle cell disease, that affects 100,000 people. In her opinion, this shows systemic racism in medical research and is unacceptable.

CRISPR gene editing is also being explored in numerous ways in the environment and theoretically we can choose desired traits in organisms. We are trying to suppress species such as weeds, eliminate or suppress mosquitos, give species resiliency in the face of changing climate (as coral), and even thinking of resurrecting extinct or threatened species, such as the woolly mammoth or American chestnut tree. These are complex ideas, and Dr. Kofler thinks we don't have the decision-making processes in place to honor this complexity.

What makes CRISPR so important/influential in the environment is that it allows for the production of CRISPR based gene drives. Under normal conditions, a genetically modified organism released into the wild would mate with a wild counterpart, and eventually the modification would be pushed out of the population because of natural selection (50% inheritance rate).

With a CRISPR-based gene drive, the organism will express the CRISPR gene edit desired, and also express the genetic component to create the CRISPR Cas-9 system in its offspring. Mating with a wild counterpart, produces offspring that inherit the CRISPR-edited gene and have the ability to correct the wild-type gene inherited from the wild-parent. Therefore 100% of its offspring inherit the genetic trait.

Humans can now change wild species as they never have been able to do before, and it raises many questions. One is, what happens to the shared environment, the food supply, where the species lived, do others depend on it? Many times, we don't have the answers. Promising work with gene drives is being investigated for

malaria control in Africa and in reducing Lyme disease transmission, in which there is lots of community involvement. The question is, is it okay for public engagement to be run by the technologists themselves? Is there a conflict of interest and at what point may 3rd parties need to be involved to be a neutral information source and build connections across differences?

How does one make decisions in shared environments where a variety of people live? Communities, particularly local communities, are at the center of such decisions and they must have a say in the decisions. Dr. Kofler and a group of interdisciplinary experts, in 2018 called for an overhaul of regulations. They stated that instead of decision-making being from the top down, environmental gene editing must have collective oversight. Local communities should be empowered and be in the center of how these decisions are made regarding the environment they depend on, along with global coordination and support.

She believes our lines of justice must be expanded to make these decisions "more appropriately". It must include historically marginalized human communities and groups, whether it be women, children, people of color, indigenous communities. But if we stop at humans, we won't see the entire picture. We also need to look at nonhuman ecosystems and entities when making decisions about gene editing. For example, nonhuman entities have already been granted human personhood around the world. The Whanganui River, a natural resource in New Zealand was the first to be granted legal personhood in New Zealand in 2017 and was appointed two guardians. The question now is can humans speak for nature in unbiased ways? Who will be granted representation to speak for the voiceless? Video and audio mechanisms might be created for this purpose.

Throughout her talk, Dr. Kofler emphasized that ethical use of CRISPR requires robust relationships between species and society, and CRISPR must be guided collectively. History must be reckoned with or it will repeat. Communities must be empowered in decision-making, and justice must expand beyond humans. She concluded by saying that it is going to take many people and a coalition to solve all these questions. The Scientific Citizenship Initiative at Harvard "trains scientists to become responsible participants in their communities, and strives to

The Ethics of CRISPR Editing

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create a culture that supports inclusivity, equity, cooperation and service”.

Multifunctional Syndromic Testing on a Digital Microfluidic Platform



The virtual presentation *Multifunctional Syndromic Testing to Maximize Diagnostic Yield on a Digital Microfluidic Platform* was presented by Vamsee K. Pamula, PhD, Founder and President of Baebies, at a meeting co-sponsored

on March 17, 2022 by the Northeast Branch and the Northeast Section of the American Association for Clinical Chemistry.

Baebies was founded with the mission to ensure a healthy start for everyone and is currently developing inexpensive and accessible diagnostics products. The company was started in 1914 with digital microfluidic technology that was developed at Duke University and currently has 150 employees.

Dr. Pamula used photos and videos to describe the core technology used in digital microfluidics. Digital microfluidics is a method to manipulate droplets of liquid using electric fields to change its surface tension. Complex assay protocols such as magnetic bead concentration, washing, and elution for immunoassays and sample prep for molecular assays can all be performed on a disposable plastic cartridge that is a closed system. A simple droplet operation such as transport can be built into a complex droplet operation such as thermocycling, which can then be employed in a PCR assay in a system that is designed for a specific panel of diagnostic tests. PCR is performed within minutes by simply shuttling droplets between two thermal zones. These complex droplet operations have been integrated to perform chemistry, molecular, hematology, microbiology, and immunoassays.

Further combinations of these types of assays can be designed for specific syndromic testing.

Due to the small sample volume requirements in digital microfluidics, its initial applications are in neonatal and pediatric diagnostics, where it offers obvious benefits in reducing iatrogenic anemia by maximizing the diagnostic yield from a small volume sample. Babies born in the U.S. are currently only screened for about 30 conditions while many more rare disorders can be effectively treated if identified at birth. SEEKER addresses early detection of rare diseases in babies, and is an FDA authorized Newborn Screening solution for MPS I, Pompe, Fabry, and Gaucher. Over 12 million tests, mostly newborn screening have been performed, and every 7th baby born in the US gets tested on the SEEKER platform for congenital diseases. Multiple assays are performed at the same time using one punch from a newborn dried blood spot specimen.

The Baebies FINDER platform addresses blood disorders, infectious diseases and other indications. Dr. Pamula showed slides of and described the FINDER multifunctional diagnostics platform, which was designed ground-up for neonatal diagnostics and utilizes known chemistry in droplet format. Assay panels are available for respiratory infection, sepsis blood culture, thrombophilia, acute kidney injury and numerous other clinical indications. For example, a positive COVID test result is available in 3 minutes, negatives take up to 17 minutes; there is limited detection of 100 copies/ml. A positive blood culture can be detected in half the time of the commercial incubator, in approximately 5 hours. Dr. Pamula showed additional videos of sample preparation and ultra-rapid PCR for viral targets and other assay panels. Baebies has numerous collaborators and is currently working on developing additional assay panels, such as new chemistries, and respiratory panels utilizing the same cartridge and instrument.



Diet as Microbiome-Centered Therapy

Diet as Microbiome-Centered Therapy for Chronic Inflammatory Diseases, was presented virtually on November 16, 2021 by Ana Maldonado-Contreras, PhD. She is Assistant Professor, Microbiology and Physiological Systems, and Co-Founder and Assistant Director of Operations at Center for Microbiome Research University of Massachusetts Medical School, Worcester, MA. About half of all-American adults have one or more preventable chronic illnesses related to poor diet. Severe inflammation is at the heart of these chronic diet-related illnesses and is linked to perturbation of the gut microbiome.

Diet is a modifiable non-invasive, inexpensive lifestyle change that demonstrably and rapidly shapes the microbiome. Dr. Maldonado-Contreras is interested in translating work at the bench to clinical settings, and feels that science has the capacity to change the composition of the microbiota, and to be useful as therapy for chronic inflammatory diseases. Her laboratory investigates how manipulation of the gut microbiota through diet can improve patient outcomes by developing dietary interventions targeting microbiome-immune interactions to reduce inflammation.

Inflammatory bowel disease (IBD) is an immune, chronic inflammation affecting the intestinal tract and can be categorized into Crohn's Disease, and ulcerative colitis, which is a more superficial inflammation, affecting only the colon. Both are very debilitating and are a financial burden; an estimated 1% of the entire population will have some type of IBD by 2030. There is no cure, only about 30% respond to medications and 75% stop responding to treatment.

The pathogenesis of IBD is multifactorial and includes genetic susceptibility, however, not everyone with these genetics develops the disease; a secondary trigger may be the gut environment. The implicated genes are associated with dysregulating the immune response or with barrier dysfunction, and also with the response of microbes in the gut. The role of microbiota in the pathology needs additional study.

Patients with IBD have a dysbiotic microflora, i.e., a reduction of overall bacterial

They have an increased abundance of *Proteobacteria* and *Actinobacteria* that have been associated with inflammation and reduced abundance of *Bacteroides* and *Firmicutes*,



Ana Maldonado-Contreras, PhD

especially *Clostridium*, that have been linked to nutrient production. The laboratory hypothesis is that with diet we can “feed” the “good” microbiota somehow, and go to a more balanced commensal anti-inflammatory microbiota as seen in healthy patients.

Therefore, the IBD-Anti-Inflammatory Diet (IBD-AID™) was designed in collaboration with the Center for Applied Nutrition; the diet was tested in the clinic, with a good patient response. The Medical School has a patient kitchen, provides dietary guidance, and hires chefs to actually teach patients how to cook. A very rich biobank of recipes for patients has also been developed.

A proof-of-concept study done from 2017-19 tested the hypothesis that diet can be beneficial to change the microbiota of IBD patients to normal. Results showed that the participants dramatically changed their diet and readily adopted the IBD-AID diet. Changes were seen in the consumption of prebiotic, probiotic, and beneficial foods, with a dramatic reduction in adverse foods consumption. Patients also exhibited dramatic microbiome changes; an increase was seen in the beneficial bacteria that appeared to be completely depleted in patients with IBD, which are the hallmark of dysbiosis. These bacteria are also associated with production of butyrate, a short chain fatty acid, that is involved in maintaining barrier function. Genes involved in butyrate production also seemed to be enriched during the intervention.

Foods in the IBD diet were shown to uniquely affect a variety of individual bacterial species. Bacterial enrichment could be correlated adversely, for example, with foods high in sugar such as fruits or positively with use of prebiotics/probiotics. IBD symptoms also showed a downward trend, as shown by

Diet as Microbiome-Centered Therapy (continued)

chemokine analysis. Therefore, a patient's immune profile can be modulated by this diet. The knowledge from this research may also be applied to other chronic illnesses where diet-microbiome-inflammation interactions are involved, such as obesity, type 2 diabetes and cardiovascular diseases.

Dr. Maldonado-Contreras described additional studies underway to study barrier dysfunction, which precedes IBD. The question is whether the microbiome emerging after the IBD-AID can restore the intestinal barrier. This involves measuring the electrical resistance of a monolayer of disrupted human epithelial cells studied when they are exposed to microbiota from patients on the diet, both at baseline and after intervention. High-resolution microscopy is used to investigate tight junction dynamics. Results to date show a greater resistance when intervention is used, indicating a healthier barrier. Future testing is planned using human-derived enteroids and animal models.

She then presented preliminary results of an ongoing project done with the Icahn School of Medicine at Mount Sinai, The MELODY Trial (Modulating Early Life micrObiome through DietarY) intervention on Crohn's Disease. They are studying whether the early microbiome can be modulated to reduce elevated inflammation with diet. Mothers with Crohn's disease or ulcerative colitis and their babies were followed for one-year post-partem.

The microbiome of pregnant women with IBD has been shown to differ from that of healthy pregnant women. Babies born from mothers with Crohn's disease exhibit dysbiosis and inflammation up to 3 months and elevated fecal calprotectin, a protein biomarker for IBD, up to 3 years of life. This theoretically occurs because they receive dysbiotic microbiota from the mother at this critical time of immune development. Specific bacteria were also seen to be positively or negatively associated with high levels of fecal calprotectin.

Conclusions of this study were that mothers on the IBD diet and intervention, and their babies exhibited lower levels of fecal calprotectin up to 1 year of birth, and that bacteria associated with decreased level of fecal calprotectin in infants are

missing in the vaginal microbiome of pregnant mothers with IBD.

Dr. Maldonado-Contreras and her team are currently still recruiting mothers with Crohn's Disease and ulcerative colitis to participate in the MELODY Trial and can be reached at: <https://www.umassmed.edu > melody-trial-info>

Penikese Island Leprosarium

To commemorate the centennial of the closing of the Penikese Island Leprosarium in Massachusetts in 1921, the Massachusetts Public Health Museum hosted an exhibition in Tewksbury, that focused on the lives of patients and their caregivers. The exhibit began with a panel discussion, *Penikese Island Leprosarium and the Real Story of Hansen's Disease*, via Zoom on October 21, 2021. The program was co-sponsored with the Northeast Branch.

The discussion was introduced by Alfred DeMaria, Jr., MD, former Medical Director and State Epidemiologist with the Massachusetts Bureau of Infectious Disease and Laboratory Sciences and board member of the Public Health Museum. The moderator was Paul Mange Johansen, MA, who studied and wrote about the history of Hansen's Disease for over 3 decades.

The first distinguished panelist was José Ramirez, Jr., LCSW-S, who was diagnosed with Hansen's Disease in 1968 and is now an international advocate for people with the disease. He spent his early life at the National Hansen's Disease Program in Carville, Louisiana, an experience he wrote about in his autobiography, *Squint: My Journey with Leprosy* (2009). He gave a first-hand account of life at the US Public Health Hospital, the fear, isolation and stigma, even currently, associated with it.

The second distinguished panelist, Pathologist and Immunologist David Scollard, MD, PhD spent 25 years at the National Hansen's Disease Program in Carville, LA, starting as a research scientist, later as Chief of the Clinical Branch, and finally as Director of the Program. He served as editor of the *International Journal of Leprosy* for 6 years, edited and contributed two chapters to the *International Textbook of Leprosy*, and retired to Massachusetts. He still attends monthly clinics at the Lahey Clinic. He gave an overview

Penikese Island Leprosarium (continued)

of the history of what we know about this disease today compared to what was known, and misunderstood, when Penikese Island began.

Although the World Health Assembly declared leprosy eliminated as a public health problem in 2000, many countries still have a problem. The *M. leprae* genome was sequenced in 2001, and it was found that the microbe cannot be cultured because of missing genes/enzymes for key metabolic pathways; it is dependent upon its host's cell for aid and survival. After the human genome was sequenced, a single gene was found to be responsible for human overall susceptibility or resistance to leprosy in 2004; there are now 3 other genes in this category. Dr. Scollard emphasized that the disease is curable!

The third distinguished panelist, Journalist Ken Hartnett, produced the 1994 WGBH documentary the "Lepers of Buzzards Bay", a subject that has remained fascinating to him. He recently co-founded The New Bedford Light, a free, nonprofit, nonpartisan digital news outlet dedicated to community-based coverage of important local issues. He described how Penikese Island came exist, and hopes that we never repeat the mistakes leading to its creation and continuation, calling it a "well-intentioned atrocity". We need to look with trepidation at the next disease that will cause such public fear. Public health can become politicized, and societal attitudes such as fear and contempt due to ignorance, abetted by the political and economic sides, along with false information, can distort reality as we have seen in the recent pandemic.

Additional Programs Held



The NEB virtual program held on May 4, 2021 featured Jason M. Peters, PhD, Assistant Professor, Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI, who spoke on ***Fighting Antibiotic Resistance and Climate Change with Bacterial Genetics***. The

Peters laboratory uses bacterial genetics to address the critical issues of air pollution/climate change and antimicrobial resistance, which have been identified by the WHO as the first and fifth on the list of top threats to global health. CRISPR-based functional genomics approaches are used to define the roles of gene networks in the process of using microbes to convert plant material into valuable biofuels and bioproducts as well as in antibiotic targeting and resistance. A system called CRISPRi is used to modulate essential gene function in bacteria to discover how antibiotics work, and is focused on the ESKAPE pathogens that exhibit multidrug resistance and virulence (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*). The sensitized strains can then be used for antibiotic mode of action studies. An essential gene network is able to be constructed that shows how related essential genes function together. The Laboratory is also part of the Great Lakes Bioenergy Research Center and addresses climate change by trying to engineer better biofuel-producing bacterial strains, such as *Zymomonas mobilis*, which will be of benefit over fossil fuels currently used and will produce more sustainable energy.

American Society for Microbiology Distinguished Lecturer, Bettina Fries, MD, spoke on ***Infections with Multidrug Resistant Enterobacteriaceae in the U.S.*** on April 20, 2021. Dr. Fries is Professor of Medicine and Molecular Genetics and Microbiology, and Chief of the Infectious Disease Division at Stony Brook University, Stony Brook, NY. She discussed the importance and epidemiology of multidrug resistant Enterobacteriaceae in the clinical setting and challenges that need to be overcome to develop novel treatments. The lecture was cosponsored by the Northeast Branch-ASM, the American Society for Clinical Laboratory Science of Central New England and Department of Medical Laboratory Science University of Massachusetts Dartmouth.

Dr. Fries reminded us that Alexander Fleming was the first to develop antibiotics, his paper on penicillin being published in 1929. Since then, antibiotics have saved many lives and now there are sophisticated protocols as to how to use them,

Additional Programs Held (continued)



ASMDL Lecturer Bettina Fries, MD

such as the sepsis protocols. The negative side of antibiotics however, is the global problem of multidrug resistance.

New antibiotic development has slowed dramatically during the last decades as new antibiotics are often not approved, and sales of new antibiotics are low as compared to that of drugs being used. Every current “new” antibiotic being put on the market is basically a derivative from old antibiotic classes. Also, drug resistance to a new antibiotic will most likely occur in a few years or less. ASMDL

The Center for Disease Control recently published several reports describing antibiotic resistant threats in United States (U.S.), dividing them into urgent threats, threats requiring urgent aggressive action, and pathogens of concern. The ESKAPE pathogens, (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are most troublesome. Urgent threats are carbapenem-resistant *Acinetobacter*, *Candida auris*, *Clostridium difficile*, drug-resistant *Neisseria gonorrhoea*, and carbapenem-resistant Enterobacteriaceae (CRE). Dr. Fries is primarily interested in CRE and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, from which the number of cases and deaths increased significantly from 2013-2019. ESBLs are enzymes that can break down commonly used antibiotics such as penicillin, making them ineffective. ESBL-producing Enterobacteriaceae can cause infections in otherwise healthy people, even those with no prior history of antibiotics or underlying conditions, and cause community derived infections. There are options to treating with

other antibiotics and intravenous carbapenem is often chosen. There are different types of ESBL, depending on the bacteria, that can be mediated by different genes. CTX-M, of which there are 5 subclasses, is chromosomally located and is associated with poor patient outcomes. ESBL clones of Enterobacteriaceae however, are not necessarily the same clones that transition to becoming carbapenem resistant.

Patients who require devices such as catheters, and those on long courses of antibiotics are most risk for CRE infections. CRE can carry mobile genetic elements that are easily shared between bacteria, and about 30% of CRE carry a mobile genetic element that makes an enzyme, carbapenemase, that destroys drugs. The prevention of CRE infection is usually done by combining strict antibiotic stewardship, i.e., aggressively controlling what types of antibiotics patients are given, and at the same time developing new approaches. These strategies have prevented further spread cases of some types of CRE in the United States but there still is no decrease.

Data from Stony Brook Hospital (a tertiary academic hospital in Suffolk County), about 50 miles from New York City that serves 1.5 million people showed that the most dominant multidrug-resistant Enterobacteriaceae was *Klebsiella pneumoniae*, followed by *Enterobacter*, *E. coli*, then others. Epidemiology here was done as part of the NIH-funded Crackle-2 Study, that included 80 U.S. and international hospitals. Specimens from the first thousand forty patients presenting with carbapenem resistance were analyzed. The majority of the multidrug-resistant Enterobacteriaceae were *Klebsiella*, followed by *Enterobacter*, and then *E. coli*. Genomic sequencing was done on all the isolates, and 59% of the isolates that were carbapenem resistant in laboratory testing also had a gene mediating the resistance. The ST258 clone of *Klebsiella* was important one, and had a KPC2 or KPC3 plasmid found in 54% of the resistant *Klebsiella*.

Carbapenemases are classified according to the Ambler Class, A, B, and D. The majority of them are encoded on KPC plasmids, but there are others, depending on the organism. Carbapenemase in *Klebsiella* in the U.S. is usually found encoded on the KPC plasmid. She explained that there are specific clones that dominate when a drug-resistant species is emerging, and in the U.S., for *Klebsiella*, this is

Additional Programs Held (continued)

the ST258 clone together with ST307. She showed a similar graph for *E. coli*, that shows 50% of the clones belong to ST131, the dominant and multidrug-resistance clone that is emerging. Interesting here is that the ST131 clone is also the ESBL clone in *E. coli*. Among the ESBL Enterobacteriaceae, *E. coli* and *Klebsiella* are the most dominant, but in *Klebsiella* the ESBL clone is not clonal group ST258.

Dr. Fries showed a scheme of how drug resistance can be spread. Bacteria, by conjugation can take up resistance plasmids and they can be transferred by transduction with a viral delivery system. Also, when bacteria die or undergo apoptosis, bacterial DNA becomes basically free, and it can be taken up and transferred through the process of transformation. Plasmid insertion sequences, transposons, and integrons are also important. Plasmids cannot only be shuttled between bacteria, but resistance genes can also be transferred between different genomic structures within the same bacteria. Transposons can hop onto plasmids, chromosomal resistance genes can be transferred to transposons, and then from transposons can hop onto plasmids. Much multidrug resistance is basically the result of the so-called mobile genomic elements that have many ways of hopping between the core genome of bacteria and the mobile genome. Most disturbing is that resistance is seen in strains from people never exposed to an antibiotic.

She showed a map highlighting that carbapenem-resistant *Klebsiella* is a global problem. KPC is endemic and predominant in the U.S., NDM-1 is predominant in India and Pakistan. This is because India produces an oral carbapenem, faropenem, sold over the counter, also many other countries also sell various medications and antibiotics over-the-counter. Carbapenem-resistant *Klebsiella* and KPC plasmids have been detected in all states in the U.S. Dr. Fries also showed an example of KPC-3 plasmid that can carry multiple resistance genes, and described the “new plasmid on the block”, the New Delhi metallo-beta-lactamase, a zinc-containing Class B carbapenemase, which is encoded on the NDM-1 plasmid. The NDM plasmid was not found in all states in 2016 but is currently spreading.

Dr. Fries mentioned the difficulty of doing studies with patients who are infected with

multidrug-resistant bacteria because few patients of this type are available, a multicenter study needs to be done, and the efficacy of novel antibiotics and novel approaches will need to be compared. Therefore, recently, a different way of investigating the efficacy of novel antibiotics was implemented, the Desirability of Outcome Ranking (DOOR). Instead of looking at clear-cut mortality, patients are categorized according to desirable outcomes, and thus determine whether a new antibiotic may be beneficial or not. She explained this ranking, how it works for *Klebsiella*, and explained other difficulties with such investigations.

Dr. Fries next described in detail her research with monoclonal antibodies. Antibodies against Staph enterotoxin B were made years ago and that knowledge was applied to making antibodies against the polysaccharide capsule of carbapenem-resistant *Klebsiella* (CR *Klebsiella*), as they also wanted to make a vaccine against multidrug-resistant *Klebsiella*. They succeeded in producing antibodies that bind the polysaccharide capsule of CR *Klebsiella* strains, including those with a hypermucoid capsule type. Intestinal dissemination models in mice showed that infection with CR *Klebsiella*, followed by systemic treatment with monoclonal antibodies to prevent dissemination from the gut, did not change the amount of colonization in the gut, but successfully changed dissemination from the gut systemically. Other studies found that both colonized and infected patients produce antibodies, and that some *Klebsiella* polysaccharides elicit a stronger humoral response and also elicit a cross reactive immune response.

Dr. Fries lastly spoke of the drivers of resistance. The majority of antibiotics in this world are used in animals; 75% of all antibiotics prescribed are given to livestock to increase the productivity. It is therefore very challenging to get various industries worldwide to refrain from these practices. Multidrug resistance is a global problem and requires global collaboration for control.



Additional Programs Held (continued)

Testing Strategies and Practices for Reopening Businesses in the SARS-CoV-2 Pandemic was presented virtually on April 6, 2021. The program included a discussion of perspectives and activities in the area of reopening businesses which was followed by a panel discussion with questions from the audience.



Stefan Riedel, MD



Perna Sekhri, MS



James E. Kirby, MD

The speakers were Stefan Riedel, MD, PhD, D(ABMM), Associate Medical Director of the Clinical Microbiology Laboratories at Beth Israel Deaconess Medical Center in Boston, MA and Associate Professor of Pathology, Harvard Medical School, Boston, MA, and

Perna Sekhri, MS, MBA, Project Manager for Product, at Concentric by Ginkgo Bioworks. The program moderator was James E. Kirby, MD, D(ABMM), who is an NIH-funded Principal Investigator in the Experimental Pathology Division of the Beth Israel Deaconess Medical Center, Boston, MA, Director of the Clinical Microbiology Laboratory at BIDMC and an Associate Professor of Pathology at Harvard Medical School, Boston, MA.

Dr. Riedel spoke on

testing strategies and

described several scenarios

in which he was involved in the reopening of businesses, which is a complex issue and there may not have a “best approved” answer for opening businesses. Perna Sekhri works at Concentric, whose overall mission is biosecurity but they are currently focused on providing suitable COVID testing for every school in America, primarily because parents cannot return to work until children return to school. Dr. Kirby sees vaccination as a strategy, because testing is very expensive and may not be readily available other than at well-resourced businesses. He

asked if vaccine mandates might come into play in businesses with which they Dr. Riedel and Ms. Sekhri interact. Current vaccines are less effective against some of the variants and there is a question as to who has the authority to mandate vaccination.

The virtual presentation ***The Continuous Evolution of SARS-CoV-2 Testing in Clinical Laboratories. One year on: what have we learned after the news of COVID-19 first broke?*** was held March 18th, 2021 and was co-sponsored by the Northeast Branch-ASM and Northeast Section of the



American Association for Clinical Chemistry. Stefan Riedel, MD, PhD, D(ABMM), Associate Medical Director, Clinical Microbiology Laboratories, Beth Israel Deaconess Medical Center and Associate Professor of Pathology, Harvard Medical School, Boston, MA, discussed the clinical laboratory response to the COVID-19 pandemic. He spoke of the continued challenges of the evolving laboratory testing for COVID-19, including regulatory aspects, the approach to selection and implementation of appropriate laboratory tests, and described performance characteristics for some select laboratory test methods commonly used in U.S. clinical laboratories for diagnosis of SARS-CoV-2. He raised the question of how are we planning to counter the next pandemic? And stressed the need to understand ecological, climate and human factors contributing to the occurrence of zoonoses. Dr. Riedel also is the current President of the Northeast Branch, ASM.



Additional Programs Held (continued)

The first NEB virtual program was co-sponsored with the University of MA, Dartmouth, and featured Benjamin Neuman, PhD, Associate Professor, and Head of Biology at Texas A&M University-Texarkana, who is recognized as one of the world's preeminent coronavirus researchers and sat on the international committee that named SARS-CoV-2, the virus behind the COVID-19 pandemic. His presentation on October 8, 2020, *SARS-CoV-2, COVID-19, and You (but hopefully not you)*, covered the diversity of corona-like viruses, the basic molecular machinery found in coronaviruses, how this virus changes, what it means when a virus mutates, and how this



particular virus causes disease. Dr. Neuman thought that people were probably previously infected with SARS-CoV-2 and we missed them, as the virus changes frequently as does the influenza virus. He thought we would probably need more than one inoculation per year as antibody and T cell responses don't last.

ADDITIONAL ACTIVITIES

Northeast Branch Records Available

The complete collection of Northeast Branch-ASM records at the Public Health Museum in Tewksbury, MA has been cataloged, and the catalog will be on line soon for scholars researchers, and others interested in the history of the Northeast Branch.

For information call: 978-851-7321 x 2606, or by email: info@publichealthmuseum.org

24th Boston Bacterial Meeting

The NEB was one of the sponsors of the annual Boston Bacterial Meeting that was held at the Harvard University Science Center, Cambridge, MA on June 13-14, 2022. The meeting is organized by graduate students, post-docs, and industry researchers with the shared goal of exchanging new scientific knowledge and fostering cross-institutional collaboration. The meeting attracts Boston-area scientists from industry and academia. Attendance currently includes over 500 participants.

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. A virtual meeting was held on April 11, 2022 and in-person meetings will resume in 2023. The meetings are attended by physicians, laboratory directors, epidemiologists and laboratorians from New England., and are supported in part by the NEB.

Please contact Alfred.DeMaria@state.ma.us if you would like to receive meeting information.

Science Fairs

The NEB annually donates an award of \$100 to each of five MA regional fairs and \$300 to the MA Science Fair. Congratulations again to the students for their outstanding work.



**Northeast Branch of the
American Society for Microbiology**

MEMBERSHIP RENEWAL FORM
January 1, 2023 – December 31, 2023

Please check personal information.

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

Renewals postmarked after September 1, 2022 will be effective 9/1/22-12/31/23.

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

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