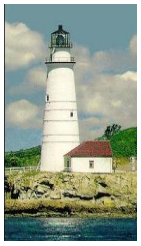

Northeast Branch Newsletter

Number 140 Winter 2016 - 2017

Pediatric Clinical Microbiology

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 24, 2016 at the Forefront Center for Meetings & Conferences in Waltham, MA. Alexander McAdam, MD, PhD, Medical Director of the Infectious Diseases Diagnostic Laboratory at Boston Children's Hospital spoke on *Pediatric Clinical Microbiology: Little Things Mean a Lot.*

Dr. McAdam began by saying that there are a number of ways in which adults and children differ that affect the diagnostic testing for infectious disease. For example, children are more vulnerable immunologically than adults; maternal immuno-globulin crosses the placenta and protects newborns for only the first 6 months. Immunity then wanes, resulting in frequent infection upon first contact with organisms causing meningitis or septic arthritis, or with respiratory viruses, such as respiratory syncytial virus.

Children also have different bacterial flora than adults and this affects the utility of some diagnostic tests in children. Since they are much smaller than adults, they can't spare much blood; therefore the volume of the sample that can be collected is less

(Continued on page 3)

(L to R) Roy Peake, NEAACC President, Speaker. Alexander McAdam, MD, and Joel Lefferts, NEAACC

NACMID- NEBASM
First Annual Meeting

The first Joint Meeting of the Northeast Association for Clinical Microbiology and Infectious Disease and the Northeast Branch, American Society for Microbiology took place on April 11-12, 2016 at the Holiday Inn Boxborough in Boxborough, MA. There were over 100 attendees and 31 exhibitor booths at the two day meeting entitled *"Hot Topics in*

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Inside This Issue

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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. 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 2017 have been sent to our membership via e-mail. Members who did not provide an e-mail address were 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.* The Northeast Branch currently has 169 members of which 123 are national ASM members.

Council Election Results

Congratulations to the following NEB members whose terms as Branch Officers began July 2016. Harvey George; President; Gregory Reppucci, President-Elect; Pat Kludt, Treasurer and Roger Greenwell, Local Councilor. Thank you for your contributions this year and we are looking forward to working with everyone in planning a busy 2017!

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!

than for adults, which presents some problems when testing.

Children, by their behavior, are also exposed to organisms adults are less likely to be exposed to. Congenital infections around at the time of delivery, such as syphilis and HIV are of concern; about 80% of the cases of rabies worldwide occur in children less than 5 years old. Children can be exposed to uncommon pathogens, such as the roundworm *Baylisascaris procyonis*, that can cause eosinophilia and meningoencephalitis. Raccoons carry the worm, and children playing in contaminated soil ingest infective eggs; most cases occur in 1-2 year old children.

Children's bacterial flora begins to change with age at two weeks; a high *S. aureus* level decreases to adult level by age 4. *S. pneumoniae* increases from age 1-4 then drops, and *N. meningitidis* increases at ages 8-22 then also drops; these two organisms are likely to cause invasive infections in children. Meningitis is often seen in adolescence.

Such bacterial microbiota can cause positive test results for some assays in children, but are not associated with illness. Binax, for example, has a new urine test for *S. pneumoniae* invasive disease in adults; the antigen test shows >95% specificity in adults, but though it has 100% sensitivity in children, its specificity is only 55.9%, because children are colonized with a high burden of *S. pneumoniae*. Likewise, 25% of children less than 6 months are colonized with *C. difficile* which causes symptomatic infection in adults but it is challenging to diagnose infection in children because of the colonization.

Dr. McAdam gave several examples of infections requiring special testing in children. Causes of meningitis can be bacterial, viral, and some drugs such an anti-inflammatories. Pre-vaccine data show that young children are more likely to develop meningitis than older children or young adults. Children in the developed world are now immunized against *Hemophilus influenza*, *Streptoccus pnemoniae* and *Neisseria meningitides*, which could otherwise cause childhood infection. Steps are also taken to reduce the transmission of group B streptococcus from mothers to children during childbirth.

The primary cause of viral meningitis in children is enterovirus. Treatment and hospitalization are usually not required and there is a low mortality rate. In contrast, bacterial meningitis has a 1-5% mortality and 10-30% of cases have sequellae such as hearing loss and seizures; antibiotic treatment and inpatient care are required. Therefore rapid differentiation between bacterial and viral meningitis is both useful and important as it affects patient care and its cost.

Bacteria in spinal fluid can be rapidly detected by Gram stain, which has an overall sensitivity of about 60%, is inexpensive and worth doing. Sensitivity varies with antigen tests and is comparable to Gram stain, i.e. 5*9% for S. pneumoniae* and 44% for *N. meningitidis*, but antigen tests rarely detect organisms in addition to those detected by Gram stain and don't provide additional information in pretreated patients.

Enteroviruses cause about 85-90% of the cases of viral meningitis seen and RT-PCR is very useful here. Both user-developed and an FDA approved assays (such as Cepheid Xpert EV) are available. The sensitivity of PCR assays is higher (>95%) than culture (50-75%). Statistics at Boston Children's Hospital show that the volume and numbers of CSF samples tested peak in the summer and fall; about 18% of those tested are positive for enterovirus by RT-PCR assay. The laboratory looked at the clinical utility of enterovirus PCR in-house, and found that in-house assay reduces turnaround time associated with both hospital stays, the duration of antibiotic treatment, and is therefore cost-effective.

An FDA approved assay for a number of pathogens causing meningitis and encephalitis that uses cerebrospinal fluid was developed in 2015, the Biofire FilmArray Meningitis /Encephalitis System. This multiplex PCR assay is able to detect 14 bacteria, viruses and fungi in one assay, i.e. organisms from different kingdoms, which is unique. The test requires about 2 minutes hands-on time and has turn-around time of about 2 hours. Dr. McAdam then described the FilmArray system in detail and spoke of the sensitivity and specificity for each

FUTURE PROGRAMS

Local Programs:

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

April 3 & 4, 2017
NACMID & Northeast Branch-ASM
2nd Annual Meeting!

Featuring

Evolving topics in Microbiology

Preliminary Program

Keynote Speaker Dr. David Nicolau (*sponsored by Cepheid)*

Four full-day workshops:
Medical Parasitology with Lynne Garcia (*sponsored by Medical Chemical*), Medical Mycology with Nancy Miller, Anaerobic Microbiology with Gloria Petruzziello, and Antimicrobial Stewardship

Day Two General Sessions: The Gut Microbiome, Selected Parasitic Protozoa, MALDI-TOF, Student Presentations, Microbiology Career Panel, Genetic Engineering

Vendor Exhibits! Posters & Prizes! Wine & Cheese!
CMLE Available through the ASCP, CME through the AMA

Location: The Sheraton Four Points, Rte 128, Wakefield, MA

http://www.nacmid.org
http://www.northeastbranchasm.org
Contact: Kristin Palladino <Kristin_Palladino@uml.org> or Irene George <NEBranch-ASM@comcast.net>

March 16, 2017. ***Clinical and Laboratory Optimization of a Hepatitis C Testing Strategy.***
Speakers: Camilla S. Graham, MD, MPH, Assistant Professor of Medicine, Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston and Nicole V. Tolan, PhD, DABCC Associate Director of Clinical Chemistry, Medical Director of Point of Care Testing , Beth Israel Deaconess Medical Center, Instructor in Pathology at Harvard Medical School.
Location: Forefront Center for Meetings and Conferences, 404 Wyman Street, Waltham, MA
Sponsored by: NEB-ASM and the Northeast Section American Association for Clinical Chemistry

Week of April 23, 2017. Dinner Meeting
Location: Rachel's Harborside, Dartmouth, MA
Sponsored by: Northeast Branch-ASM and ASCLS-CNE

Local Meetings: (continued)

April 26, 2017. New England Microbiology Laboratory Directors Spring Meeting
Location: Publick House, Sturbridge, MA

May 1, 2017. Dinner Meeting with **ASMDL Speaker Steven C. Ricke*, Foodborne Salmonella spp. in Food Production Systems: How Do They Get There and How to Keep Them Out.***
Location: Forefront Center for Meetings and Conferences, 404 Wyman Street, Waltham, MA

National Meetings:

June 1-5, 2017
ASM Microbe**,** New Orleans, LA. www.asm.org/microbe

July 27-30, 2017
24th Annual ASM Conference for Undergraduate Educators (ASMCUE)**,** Sheraton Denver Downtown Hotel, Denver, Colorado. See: www.asmcue.org

For additional information on ASM Meetings/Conferences see:http://conferences.asm.org/

Pediatric Clinical Microbiology (continued)

organism. He mentioned that there are few publications on this assay; information can be obtained from the package insert. *Cryptococcus neoformans* is the primary cause of fungal meningitis and there is a publication on the use of this test in a study of HIV–infected men in Uganda who presented with meningitis. The test was highly specific and detected 100% of the cases of *Cryptococcus* upon admission. As the patients were treated, the numbers of yeast in their CSF fell and the assay became negative.

Regarding the use of multiplex PCR for the detection of meningitis and encephalitis, Dr. McAdam noted that in the developed world there would be a low rate of positive results for most pathogens because we have effective vaccines against them. As a result of the low prevalence of these diseases and a high assay specificity, some (false) positive results would occur, therefore the test has a low predictive value for disease in the developed world.

Gram stain and culture, which remain the gold standard tests for bacterial meningitis in children, are recommended for diagnostic testing of CSF for meningitis; rapid RT-PCR for enterovirus should also be done, while bacterial antigen tests are not recommended.

Acute bacterial arthritis, in which joints turn red from bacterial infection of the joint spaces, is another problem seen in children ages <2 and falls off at ages 2-5. Since pathogens causing septic arthritis differ somewhat, pathogen identification is very important.

S. aureus is the pathogen most frequently found in adults while some studies show that *Kingella kingae* is most commonly found in children. The best detection methods are culture of joint fluid onto solid media, with added blood culture, which can dramatically increase bacterial detection. Other diagnostic approaches include either universal PCR, in which the 16S ribosomal RNA gene is the target, or pathogen-specific PCR can be used.

Kingella are small gram negative rods occurring in pairs or short chains that are found primarily in the oropharyngeal flora of children 1-2 years old. Joint and bone infections, and rarely, endocarditis, occur in colonized children. The organisms are usually susceptible to beta-lactam antibiotics and resistant to trimethoprim.

Recommendations made by Dr. McAdam for bacterial testing of joint fluid from children are to perform a Gram stain and add the remainder to a blood culture bottle if a small sample only is available; to add culture media if an ample

sample is available, as this may speed up bacterial detection, and to use PCR if available as it significantly increases the detection of *Kingella.*

Bacteremia can be diagnosed by blood culture but compared to an adult, small people have little blood and phlebotomy was shown to be the primary cause of anemia in very-low birth weight children (<1.5 kg). An old misconception regarding the volume needed for culture was that collecting 1-5 mL of blood per culture is adequate in infants and children. This is not true, as one study showed that low concentrations of bacteria are common in pediatric bacteremia, with 1 to 1-10 CFU most often found with *S. aureus, S. pneumoniae and E. coli.* Increasing the blood volume from 2 mL to 6 mL has been shown to increase the yield by 33% but the patient's size and hemodynamic status must also be considered. Recommend-ations therefore are to use age/weight-specific guidelines for blood culture volume.

Another study looked at volumes actually submitted for blood culture and showed that many children have insufficient amounts collected; this was remedied by distributing educational posters. Pediatric blood culture bottles are available, but are probably not needed. Advantages of these are the use of smaller volumes, therefore smaller blood volumes are needed, and the reduced amount of anticoagulant might increase the growth of some pathogens. Unfortunately there are no good studies of direct comparisons of yields using adult and pediatric blood culture bottles.

Blood cultures in adults include an aerobic and anaerobic blood culture, but are anaerobic blood cultures useful in children? Obligate anaerobes are very infrequent causes of sepsis in children (only 0.8-2.1% are positive) and a second aerobic culture is preferable to anaerobic culture. Anaerobic cultures should be used with children with risk factors such as neutropenia, and patients with infections associated with anaerobes (abdominal, decubitus ulcers head-neck injuries, crushing trauma, bites,).

Dr. McAdam lastly spoke about respiratory syncytial virus (RSV) and bronchiolitis, an inflammation of the bronchioles. There are about 60 million episodes seen annually, and in the United States, 75-125,000 children are hospitalized annually for RSV. RSV is so common that most children are infected by the virus by the age of 2. Most healthy children recover in 1-2 weeks while others, such as premature infants, can develop apnea or cyanosis and require hospitalization. Bronchiolitis is caused by RSV about 80% of the time in children 6 months to 2 years old. Symptoms of RSV mimic the common cold, with fever, cough, and rhinorrhea, but distinctive of bronchiolitis is wheezing, usually heard on exhaling, as in asthmatic disease.

Dr. McAdam described bronchioles as tiny tubes that are the last passage of oxygen into the lungs; they connect to the alveoli (air sacs) where oxygen exchange occurs. RSV causes death of bronchiole epithelial cells, leading to mucus formation, thickening bronchiole walls and consequently, narrowing of the bronchioles. Upon inhaling, the bronchioles are pulled open, but upon exhaling, the bronchioles close and sometimes remain stuck shut, thus causing the wheezing.

Choices for testing for RSV are rapid antigen testing by immunofluorescence and viral culture, both of which are not very sensitive, and a number of molecular methods. The rapid antigen test had a bad reputation because detection of influenza is very poor, having a reported sensitivity of about 30% to 50% or lower. These tests, however, are not that bad for RSV detection. They are FDA approved only for use in young children, and have a sensitivity of about 80% in that population.

In general, the current consensus is that RT-PCR is the gold standard for detection of RSV in children with reported sensitivities as high as 98%. Large multiplex PCR assays are also available for respiratory pathogens, such as for meningitis. Manufacturers state that some of these tests can distinguish between RSV-A and RSV-B, but the clinical utility of this is unknown. When choosing a test for RSV detection, Dr. McAdam recommends using approved and validated molecular testing that has the best sensitivity for the specific pathogen you are looking for (RSV in this case) and also noted that physicians should be educated about the use of such tests.

NACMID- NEBASM 1st Annual Meeting (cont.)

in Microbiology: Challenges & Solutions". The meeting included student posters and student oral presentations; awards for posters were supported by ASM Regional Funding program. CMLE was available to participants through the American Society for Clinical Pathology.

Nancy Miller, President, NEB-ASM (L) and
Cynthia Astolfi, President, NACMID

The meeting began on Monday with two hand-on and two managerial workshops. *Gram Stains – Morphology Still Matters* was presented by Linda Zuchowski, BS, MT(ASCP)SM, Microbiology Manager, Midwest Region, Quest Diagnostics, Lenexa, KS and focused on improving Gram stain expertise, particularly on the recognition and management of poor quality smears, on improving stain technique, and on how to enumerate and characterize cellular elements on Gram stained smears accurately. The Monday afternoon hands-on workshop, *Good Practices and Tabletop Demonstrations*, was presented by Emelie Villapa, MT (AMT), MAEd, Assistant Supervisor, and Lewis Curtis, MT(ASCP), both from the Boston Medical Center, Microbiology and Molecular Diagnostics Laboratory, Boston, MA. The workshop provided hands on experience with table top molecular instrument-ation to demonstrate best practices relevant to molecular techniques in the microbiology and molecular diagnostics laboratory. The management workshop, *A Tale of Two Labs: Is That Instrument the Right Fit for Your Lab and How to Sell it to the C Suites* was presented by Beverley L. Orr, MT(ASCP), Microbiology Technical Supervisor, Boston Medical Center, Boston, MA. This workshop instructed managers on how to analyze instrumentation, how to create a return on investment with which the administration would be pleased, and addressed most of the pitfalls associated with the smooth implementation of a new instrument.

Stephen M. Brecher, PhD, VA Boston Health Care System, West Roxbury, MA and CS, BU School of Medicine, University of MA / Dartmouth, presented the Monday afternoon management workshop entitled *From Tragedy to Triumph to Trepidation: Antibiotics at Age 75. A workshop on Tackling Resistance Issues*. He reviewed the history of antibiotic resistance, presented an update on gram-positive resistance issues, and focused on a number of current antibiotic resistance issues.

A wine and cheese reception with the exhibitors was held in the exhibit hall late Monday afternoon, followed by the keynote address *Zombies and Infectious Diseases in Popular Culture*, which was given by ASM Distinguished Lecturer Tara C. Smith, PhD, Associate Professor, Kent State University College of Public Health who spoke on zombies/zombie media as an excellent way to teach students and the public about the spread of infectious diseases but at the same time it can teach inaccuracies and wrongly alarm the public about infectious diseases.

Linda M. Mann and Stephen M. Brecher

General Sessions were held on Tuesday and included a morning presentation by Linda M. Mann, PhD, D(ABMM), Sacramento, CA, on *Finding the Balance in AST Verification; Recommendations for CLSI M52*, who reviewed the recommendations for verification of commercial AST systems from the new CLSI

NACMID- NEBASM 1st Annual Meeting (cont.)

M52 document; Dr. Mann co-chaired the CLSI M52 document development committee.

Alfred DeMaria, Jr and Michael Pentella

Michael A. Pentella, PhD, D(ABMM), Director, Massachusetts Department of Public Health State Public Health Laboratory, Jamaica Plain, MA presented two lectures on biosafety. *"Improving Biosafety in our Nation's Laboratories"* described the components of a strong biosafety program and how to recognize the best practices in biosafety and biosecurity. "*Implementing an Effective Biosafety Program"* described the necessary tools to build an effective prevention program. He also presented a third session on *Laboratory Decontamination and Waste Management* in which the basic principles of handling waste and decontamination of the environment in regard to safety were reviewed.

ASM Distinguished Lecturer (ASMDL) Tara Smith presented a lecture, *Science Denial and the Internet*, on Tuesday morning, in which she described the internet as the new wild west with few rules applying. Dr. Smith discussed the state of science communication online, and what scientists can do to improve available information and to counter scientific misinformation.

Student oral presentations included *Vibrio Research*, by Joseph Park, HHM I Research Fellow, Boston University School of Medicine; who described the CRISPR/Cas system and its utility in engineering genome-wide knockout libraries in human cultured cell lines and showed how such studies demonstrate the power and generalizability of our the CRISPR screening approach in uncovering the host genetics that underlie host-pathogen interactions.

Student Speakers Joseph Park and Feng Xu

Feng Xu, PhD Candidate from University of New Hampshire *spoke on Dynamics of Emergent and Invasive Pathogen Lineages of Vibrio parahaemolyticus (Vp) in the Northeast*, and described the population structure of *Vibrio parahaemolyticus(Vp)* in Northeast region and the development of tools to enhance specific surveillance of strains of most threat.

Validation of a Method for Isolation of Clostridium difficile from Environmental Samples was presented by Christian Beato-Melendez, PhD Candidate, UMass Lowell; while most isolation protocols are designed for use with stool specimens, he described different culture-based methods for isolating and detecting C. difficile from the environment and their challenges and advantages.

Three cash awards were presented for outstanding posters which were: *Spectroscopic Characterization of a Novel Nitric Oxide Sensing Protein* by Bezalel Bacon, Stony Brook University, New York; *Functional Analysis of Soil Microbes* by Zachary Window and Kyle S. MacLea, University of New Hampshire -Manchester Biological Sciences; and *Testing Statistical Equivalence of Varying Methods* by Lindsey Dyer, Rapid Micro Biosystems. ASMDL speaker Tara Smith (photo L) and Nancy Miller, Northeast Branch President, discussed the presentations with the students over lunch.

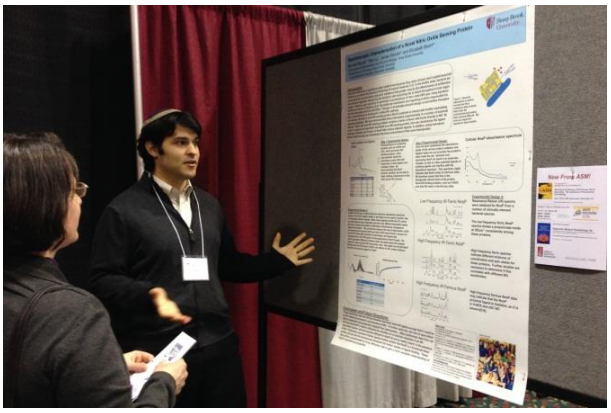
NACMID- NEBASM 1st Annual Meeting (cont.)

Stephen M. Brecher briefly spoke on how antibiotic use disrupts the human gastro-intestinal tract and results in disease such as that caused by *Clostridium difficile*. This was followed by a most informative video entitled *Are You Ready for the Next Hollywood Disease?,* which was written, produced, and directed by Richard Danforth, SM(ASCP), Laboratory Program Advisor, Department of Health and Human Services, Health and Environmental Testing Laboratory, Augusta, ME. The film depicted the spread of organisms from the reservoir of transmission, which can be located both outside and inside the laboratory, and methods of control. Among the numerous items discussed were risk assessment, laboratory practices and equipment, personal protective equipment, immunization and surveillance, hazard identification, decontamination and waste disposal.

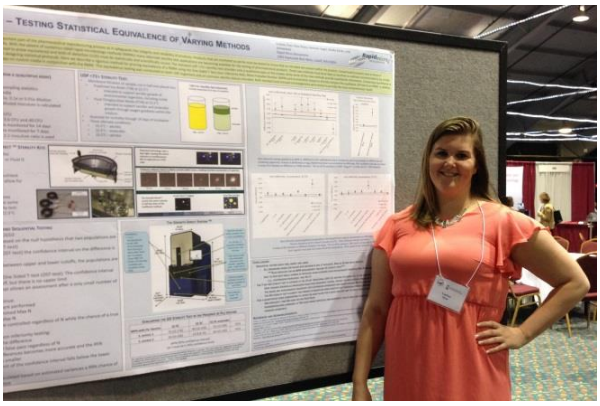
Juan M. Perez Velazquez, MD, Assistant Professor, University of Massachusetts Medical School, Worcester, MA spoke on *Lyme Disease-Diagnostic Challenges and Clinical Controversies* in which he presented an overview of the current guidelines, diagnostic tools and clinical controversies related to Lyme disease.

The concluding session of the meeting, *Update on Zika Virus*, included Sandra Smole, Division Director, MA Dept of Public Health who spoke on available testing methods and their limitations, sample types, and presented an overview of the current diagnostic response efforts during this current public health emergency. Alfred DeMaria, Jr., Medical Director and State Epidemiologist at the MA Department of Public Health, then spoke on the emergence and clinical manifestation of Zika, and reviewed the virology and diagnostic approach to this viral infection.

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



Bezalel Bacon, Stony Brook University, New York



Lindsey Dyer, Rapid Micro Biosystems

Zachary Window, University of New Hampshire

NACMID- NEBASM 1st Annual Meeting (cont.)

The Public Health Museum, Tewksbury, MA Exhibit
(L to R) Volunteers Jocelyn Isadore and Mary Ferguson

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

Microbial Metabolism and Antibiotic Efficacy

The second program of the year was presented twice before two different audiences. The first time, co-sponsored with the American Society for Clinical Laboratory Science of Central New England (ASCLS:CNE), it was held on April 20, 2016 at Rachel's Lakeside in Dartmouth, MA before primarily a student audience. The program was repeated with updated data for the NEB membership on September 15, 2016 at the Forefront Center for Meetings and Conferences in Waltham, MA

Peter Belenky, PhD, Assistant Professor at Brown University Department of Molecular Microbiology and Immunology, joined the Department in 2014 where he studies the responses of microbial communities and isolated microbes to external stress such as antimicrobial agents.

Dr. Belenky's lecture, *Microbial Metabolism and Antibiotic Efficacy* described his recent work relating bacterial metabolism to antibiotic activity. His work demonstrates that bactericidal antibiotics induce a metabolic burst that is linked to and required for bactericidal antibiotic efficacy. Blocking this metabolic response inhibits antibiotic activity, whereas upregulating this activity leads to antibiotic potentiation. This work also identified that many currently utilized empirical therapeutic drug combinations are actually antagonistic to the activity of bactericidal drugs because they lead to inhibition of essential metabolic activity.

Dr. Belenky first spoke of the importance of antibiotics, giving as an example of how precarious life was without them. William Hewson, the English surgeon, born in London in 1739. Hewson, sometimes referred to as the "father of hematology", was also one of the first early immunologists. He did much work and research with cadavers and by the age of 29 had written several papers on coagulation, identification of red blood cells, clotting factors, the lymphatic system etc. and was also a friend of Benjamin Franklin. He died at age 35 of sepsis three days after nicking his finger while dissecting a cadaver. This is a sad example of life before antibiotics but antibiotics have changed life expectancy and are critical to our existence today. They are amazing, very effective, and easy to prescribe, but resistance to them is making them less amazing as well as less effective. The time lag between the introduction of new antibiotics and the development of resistance is significant. We may someday get to the point where antibiotic resistance may preclude most types of surgery because of the risk of infection. Antibiotic resistant infections are responsible for about 25,000 deaths annually in the U.S. and close to 10 million deaths worldwide. CDC recognized the problem and in 2013 proposed several core actions to combat this. One of these ways was

Microbial Metabolism (continued)

to improve the usage of today's antibiotics. The goal of Dr. Belenky's research is to extend the useful lifespan of today's antibiotics by using simple ways to modify antibiotic therapy. He went on to describe how basic research in microbiology research can lead to changes in clinical therapy.

Dr. Belenky's work focuses on metabolism because metabolic activity has been shown to be an important modulator of antibiotic susceptibility. For example, antibiotic tolerant biofilms have a reduced metabolic activity and *Mycobacterium tuberculosis* can become antibiotic tolerant by going into a metabolically inactive slow-growing state. There is evidence that this quiescent slow-growing state is related to the tricarboxylic acid (TCA) cycle. Numerous investigators have described metabolically deficient clinical isolates from patients that had antibiotic-tolerant infections. One way to measure TCA cycle activity and bacterial respiration in particular, is to look at oxygen consumption rates since in respiration glucose and oxygen are consumed.

For the purposes of this talk Dr. Belenky grouped antibiotics into two categories, bactericidal (drugs that kill bacteria), and bacteriostatic (drugs that only inhibit growth and the bacteria recover once the drug is removed). Bacteriostatic drugs he used are chloram-phenicol (Cam), erythromycin (Erm), spectinomycin (Spc) and tetracycline (Tet). The bactericidal drugs he used are ampicillin (Amp), gentamicin (Gen), and norfloxin (Nor), daptamycin (Dap), and levofloxacin (Levo). Rifampicin (Rif) is neither bacteriostatic nor bactericidal; it seems be slightly bactericidal for gram positives, and mostly bacteriostatic in gram negative organisms. *E. coli* and *S. aureus* were the test organisms used.

In the first part of the study a Sea Horse analyzer with a 96-well plate format was used. The instrument measures, in real time, oxygen levels in media (oxygen consumption rates, OCR) and pH (extra-cellular acidification rates, ECAR). Therefore we are most likely detecting fermentation and respiration, and drugs can be injected at any point

(L to R) Frank Scarano, PhD, NEB National Councilor; Speaker Peter Belenky, PhD, and Harvey George, PhD, NEB President

E. coli was treated with various concentrations of bactericidal drugs, and plotting OCR vs time, a robust burst in respiration was observed with Amp, Gen, and Nor. A most interesting observation was that the bacteriostatic Cam continuously inhibited respiratory activity until the drug was removed. The cells never recovered, did not respire, but still grew. Much of the subsequent work in the laboratory was built from this initial observation. Adding Cam to *E. coli* at concentrations below the MIC dramatically inhibits respiration, but adding Cam in concentrations significantly above the MIC still shows a significant reduction in respiratory activity. Adding Nor does the opposite and is the most robust at elevating respiratory activity, but does this in concentrations that are significantly above the MIC; and there is only a slight boost when concentrations below the MIC are used.

Dr. Belenky's favorite data was observing *E. coli* cells grow normally when no drug is given; giving Cam results in nearly no respiration; adding Nor to the same cells results in a burst of respiration, but giving Cam at any point after giving Nor still results in reduction. Therefore the bacteriostatic suppression of respiration by Cam is dominant over the elevation seen with Nor. He was able to demonstrate that the same thing happens with Amp, Gen, and Nor. Similar activity occurs in *S. aureus*, with wide concentrations of Cam, even those below the MIC. Therefore we have suppression in both gram-positive and gram-negative bacteria.

Microbial Metabolism (continued)

Dr. Belenky, in his post-doc years, became acquainted with an infectious diseases fellow from MGH named Michael Lobritz, who is co-author of the paper from which the data in this talk were taken (Lobritz, PNAS, 2015). Dr. Belenky showed Dr. Lobriz the aforementioned data, and in turn was shown a paper, written in 1951, entitled *Treatment of Pneumococcic Meningitis with Penicillin Compared with Penicillin plus Aureomycin, Studies including Observations on an Apparent Antagonism Between Penicillin and Aureomycin* (a bacteriostatic and bactericidal antibiotic together). Patients treated with penicillin had about a 30% death rate, combination therapy had about a 79% death rate. A dramatic reduction in efficacy occurred with combination therapy. Today we use many drug "cocktails" and drug combinations, and much of our treatments are empirical, we estimate what should be the best therapy. Many times also there is insufficient data demonstrating the effectiveness of such combinations. Even at that early date, problems with combination therapy were observed.

A larger experiment was subsequently conducted in which *E. coli* and *S. aureus* were treated with bacteriostatic drugs (Tet, Cam, Erm, Spc); cells did not die, there was 100% viability. A dramatic reduction in cell number occurred when a bactericidal drug was used (Nor, Amp, Gen, Rif). However, pre- treatment of cells with bacteriostatics before giving bactericidals, or treatment with bacteriostatic drugs 30 minutes after giving bactericidal drugs, produces a dramatic reduction in bactericidal drug efficacy. A similar effect occurs with *S. aureus* using Levo, Gen and Dap. Thus the data demonstrates that bacteriostatic antibiotics inhibit bactericidal toxicity; when a bacteriostatic drug is given at any point, essentially the cells stop dying. They also tested the drug 2-4 dinitrophenol, an early weight-loss drug recently associated with a death in Rhode Island and still used by some body builders to burn fat quickly. It inhibits respiratory activity rapidly in *E. coli* as do the other bacteriostatic drugs.

This data was submitted for publication but they were asked by reviewers if inhibiting respiratory activity in mutants would have the same effects. They then knocked out the three key terminal cytochrome oxidases in the *E. coli* respiratory chain; so these organisms could not respire. When given Nor, the wild type gets a boost, does not respire, but does not die. The same happens with Amp, Gen, and Nor, using a range of high concentrations. Therefore having this terminal cytochrome oxidase pathway intact is critical to antibiotic activity; we usually think of the antibiotic as having a target; if the target is inhibited, the cells don't die.

To show exactly the opposite they knocked out ATP synthase; these cells can respire but can't make ATP and as a result start respiring more without receiving any benefit, therefore respire more, trying unsuccessfully to make more ATP, etc. Their respiratory rates become extremely high and they also ferment, acidifying the media. The mutant cells now become more susceptible to Amp, Gen, Nor. This data showed that if you essentially inhibit respiratory activity, the cells don't die; if you upregulate respiration, they die more quickly.

Conclusions resulting from these experiments were that bactericidal antibiotics elevate respiration, while bacteriostatic antibiotics inhibit respiration, and inhibiting respiratory activity blocks antibiotic toxicity, and that cellular respiration is linked to antibiotic toxicity

Interesting studies that came out at the same time demonstrated that antibiotics inhibit many of what are called "futile cycles "in bacteria. For example, the glycosides will induce protein synthesis, mistranslation, aggregation, breakdown of those aggregates, and the cycle keeps repeating. Such cycles consume a lot of ATP and a very costly to the cell.

The second part of the talk covered metabolism and looking to see if these metabolic pathways are actually activated, and if there is any cellular damage. We don't know why cell metabolism leads to bacterial death but Dr. Belenky believes it is due to production of reactive oxygen species (ROS).

The hypothesis is that antibiotics induce target specific processes that threaten or can alter metabolism, respiration and iron homeostasis in the cell, which produces ROS and other damaging molecules in the cell. They treated *E. coli* with Amp, Gen, and Nor at

various concentrations, and looked for production of various oxidative damaging molecules, such as hydroxyl radicals, hydrogen peroxide, etc., using fluorescent-reactive dyes. They found that these antibiotics induce multiple forms of such molecules. An increase in ROS no longer occurs when gyrase-A in Nor is knocked out, therefore this pathway is directly linked to ROS production.

They then did a metabolomic study with *E. coli* and a similar one with *S. aureus*. *E. coli* was treated with Amp, Kan, and Nor at a concentration allowing cells to die slowly so that investigators could collect cells in various stages of death. Cells, exposed to drugs for 30, 60 and 90 minutes, were compared to untreated *E. coli* cells. A company called Metabolon was used, that does LC/MS/MS and GC/MS/MS, and claims they can identify thousands of molecules. Dr. Belenky found about 266 differentially quantitated metabolites.

He showed a graph of the data obtained. Amp and Kan metabolites clustered so closely together that at 30 minutes they were virtually indistinguishable. Nor more closely resembled the drug-treated cells than the untreated cells. In the beginning the initial metabolic response created by the bactericidal drugs was difficult to differentiate. Specific sets of metabolites such as nucleotides, lipids, etc. were studied further. Looking at TCA cycle intermediates, there was a dramatic change, an increase of nearly 15%, in succinate and citrate levels. Increases in key metabolic co-factors such as nicotinamide adenine dinucleotide (NAD) occurred, while acetyl-CoA increased dramatically. This all is evidence of extremely high cell metabolic activity. Nucleotides however, decreased as was seen in the guanosine nucleotides degradation pathway. This, with evidence of damaged lipids, led him to think there might be some type of oxidative damaging process occurring. That was confirmed by looking at glutathione metabolism. Glutathione is an important antioxidant used for protection of proteins by both us and bacteria. The entire metabolic pathway was found to be elevated; not only do bacterial cells preferentially oxidize glutathione to prevent themselves from oxidative damage but they also make more glutathione to provide the protection. *S. aureus* showed similar data.

The laboratory was trying to show that cellular respiration is linked to antibiotic toxicity. The conclusions of the metabolomics work were that bactericidal antibiotics induce similar metabolic responses in cells, there are changes in the TCA cycle, increases in energy metabolism, and decreases in nucleotide and lipid metabolism.

The laboratory still needed to answer the question why cells die. They believed it was due to ROS responses that they had previously detected. There was strong evidence of some type of oxidation occurring which was leading to cell death. Large important molecules like proteins, lipids and nucleic acids were most likely involved and hence were studied.

They then looked at what happens when an antioxidant enzyme is overexpressed. Wild type cells and a catalase mutant not totally active, were treated with Amp, Kan, and Nor. When extra-cellular glutathione was added on top of these cells, there was less oxidation and less cell death, indicating that oxidation is occurring and probably provides an enzyme or molecule that protects cells from the oxidative damage hence producing antibiotic resistance.

They next looked at three biomarkers for oxidative stress and damage to proteins. They first used an ELISA assay to measure the amount of protein carbonylation, where proteins are converted to aldehydes and ketones in cells treated with Amp, Kan and Nor vs untreated cells. Little protein carbonylation occurred in untreated cells. When cells were given a lethal concentration of hydrogen peroxide (which also acts as the control) there is a great deal of oxidation, therefore protein carbonylation. Two of the drugs, Kan and Nor produced more oxidation than with hydrogen peroxide alone. Therefore significant elevation in protein carbonylation occurs when bactericidal drugs are used.

Another ELISA assay was used to detect levels of malondialdehyde (MDA), an important indicator of cell lipid oxidation. This same assay is used by the food industry to detect rancidity in fat. The laboratory found increased levels of MDA with Amp, Kan, Nor and hydrogen peroxide, indicating lipid

peroxidation. Cell carbonylation and liver peroxidation do not occur with bacteriostatic drugs.

The presence of 8-oxoguanine on DNA and RNA as a result of possible guanine oxidation is also an indicator of oxidative stress and can be determined by ELISA assay. When cells were treated with hydrogen peroxide, Kan and Nor, the 8-oxoguanine levels on DNA and RNA were elevated somewhat. The reactions were fairly stable and therefore confusing but this is most likely due to specific known repair enzymes in bacteria that act as a protective mechanism. The enzymes clean up the 8-oxoguanine on DNA and when they are knocked out, much higher levels of 8-oxoguanine in DNA are seen. RNA does not have such enzymes and therefore significantly higher levels of oxidation are seen than in DNA. The accumulation of damaged DNA and RNA therefore affect DNA stability.

When guanine on DNA is oxidized to 8-oxoguanine, if this occurs in two nearby areas, mispairing leading to mutations occurs, and more importantly, sometimes nucleotide excision repair occurs. Nucleotide excision repair with two closely situated 8-oxoguanine produces double-stranded DNA breaks (DSB). Two such breaks in the bacterial chromosome are sufficient to induce death; therefore a little damage is sufficient to kill bacteria.

At this point they wanted a good way to measure the DSB in the bacterial chromosome where the 8-oxoguanine is located. At this time a paper came out from Texas linking a phage protein called Gam to DSB; Gam protects phage DNA from bacterial degradation by binding tightly to double stranded phage DNA. When Gam is overexpressed in bacterial cells, every point where a DSB break occurs will be labeled by the fluorescent protein-based probe Gam-GFP. Fluorescent foci, where DSBs occur, were detected in *E. coli* cells treated with hydrogen peroxide, Amp, Kan and Nor.

Therefore Dr. Belenky's laboratory was therefore able to show that there is a metabolic consequence as a result of being treated by antibiotics. Bacterial respiration is linked to antibiotic toxicity and cells undergo significant oxidative stress as a result of elevated metabolic activity. Bactericidal antibiotics were shown to induce similar metabolic responses; and antibiotic toxicity was shown to lead to oxidative damage of key cellular components. There are primary targets and secondary pathways which lead to elevated respiratory metabolism, production of ROS, and DNA, protein and lipid damage, all of which contribute to cell death.

What are the direct clinical implications of this? Much of this work was done in collaboration with MA General Hospital clinician Michael Lobritz, who treats patients with serious drug-resistant infections. One of the patients with *S aureus* endocarditis was not doing well with standard treatment. The organism was resistant to most antibiotics with only some susceptibility to daptomycin and chloramphenicol and the idea was to treat with both drugs. However, since Dr. Belenky's laboratory data shows low susceptibility when *S aureus* is treated with daptomycin and chloramphenicol, on that basis it was decided to treat with daptomycin alone, and the patient recovered. This was an example of how a simple laboratory observation can change the course of therapy because in such rare cases therapy is simply determined empirically by a physician. We don't have to find new drugs said Dr. Belenky, we just need to find more specific ways to use them.

There is also the idea of metabolic potentiation; work from Dr. Belenky's post-doctoral laboratory studies showed that if you trick bacteria to be more metabolically active, and respire more, they will be more susceptible to certain antibiotics. In some cases you can start with persister bacteria that lead to lone-term tolerant infections, you can actually jumpstart their metabolism by giving them specific sugars that bypass the normal metabolic blocks in order to increase their normal respiratory activity. A company that came out of this work is now looking at these potentiation strategies.

The final thing that Dr. Belenky is most enthusiastic about is the development of a possible diagnostic tool for antibiotic resistance. If an ampicillin-susceptible isolate is treated with ampicillin and the oxygen consumption rate measured, the treatment, depending on the concentration used, results in either a burst of

Microbial Metabolism (continued)

growth, death, or we don't have a chance to detect the respiratory burst. When an ampicillin-resistant isolate is used and treated with ampicillin, there is no respiratory burst or death; the organisms grow steadily. This assay to determine resistance takes about 60 minutes, with no need to grow the cells overnight. They are designing a microfluidic device that includes a probe for oxygen and pH in which the test can be done more rapidly.
Reference:https://www.brown.edu/research/labs/belenky/home,

Ruth B. Kundsin Luncheon

A luncheon celebrating the 100th birthday of a rare and unique individual, Ruth B. Kundsin, Sc.D., was held on August 24, 2016 at Captain Fishbones in Marina May, Quincy, MA

(L to R) Ruth Kundsin and Emy Thomas, NEB Archivist

. Ruth is a long time and honored member of the Northeast Branch and was a pioneer in the field of microbiology. As a "housewife" in the 1940's she decided to pursue a career in microbiology, going on from working the clinical microbiology bench to a master's degree in 1949 (BU School of Medicine) to a doctorate in microbiology, epidemiology and biostatistics in 1958 (Harvard School of Public Health) and then 51 years at the Peter Bent Brigham/ Brigham and Women's Hospital running a laboratory and being hospital epidemiologist for 12 of those years.

Over lunch, the razor sharp and vivacious Dr. Kundsin told stories of her years of productive work in the laboratory, often dealing head on, especially in the early days, with male colleagues who did not see a leadership role for women in science to be proper. She told luncheon attendees of attending rounds and meetings at which she would sit alone as the only woman, with colleagues avoiding sitting next to her. These experiences led her to be a feminist spokesperson and role model, successfully challenging barriers to women scientific researchers throughout her career. She spoke of colleagues and friends over the years (with a number attending the lunch), sharing stories of a rich, creative and challenging life in science.

Retired at age 84, Ruth remains active with a personal trainer, her family, a busy social life and competitive swimming (52 medals and counting). For all of her contributions to microbiology and the local microbiology community, we salute Ruth Kundsin on her 100th birthday, and look forward for her continuing active participation in that community for years to come.

Tracing the Enterococci from the Paleozoic Era to Hospital Pathogen

A fall dinner-meeting sponsored by the NEB was held on November 2, 2016 at the Forefront Center for Meetings & Conferences in Waltham, MA. Michael S. Gilmore, PhD, currently the Sir William Osler Professor of Ophthalmology, and Microbiology and Immunobiology, Harvard Medical School, spoke on *Tracing the Enterococci from the Paleozoic Era to Hospital Pathogen.* Dr. Gilmore is also Principal Investigator of the Harvard-wide Program on Antibiotic Resistance and his research focuses on the evolution and development of multidrug-resistant strains of enterococci, staphylococci, and streptococci, and the development of new therapeutic approaches.

Tracing the Enterococci (continued)

He spoke of his research on the evolution of enterococci and explained why they are called "the cockroach of bacteria".

Michael S. Gilmore, PhD

Dr. Gilmore first referred to a current article in *Nature* about the collision between a planet-like object called Thea and Earth 4.57 billion years ago, giving rise to our moon. The powerful collision obliterated life and whatever else there may have been on Earth. Therefore we can date the first life on Earth at some point after that. The earliest evidence of life that we have is from fossils and other bits and pieces of information, such as that found in the Australian Shark Bay stromatolites; these are fossilized layers of bacteria that have been dated as 3.8 billion years old. If fossilized life was so abundant that we can still find it, then the origin of life must have been much earlier-perhaps somewhere between 3.8 - 4.5 billion years ago, and it was most likely single cell microbial life. Our best guess is that there are now about 10^{29} or 10^{30} bacteria on the planet. Today, it would take *E. coli* in lysogeny broth (LB Broth) about 55 hours to get to that number. The earliest microbes obviously couldn't replicate as efficiently as laboratory organisms; perhaps they took a year, 1000 years or even a million years to divide. Even if it took a million years, it means that in 400 million years there would be today's number of 4.6×10^{30} organisms.

The point was that long ago the earth became saturated with bacteria, but they didn't continue to multiply because of limited resources, specifically, they were energy limited. Energy came mainly from the sun, but the amount of energy the earth receives from the sun has not changed profoundly in the last 2 billion years. Why then is the amount of biomass on earth still relatively constant? This is because life is a zero-sum game added Dr. Gilmore - if a new type of life such as humans arises, it has to squeeze its way in and take the place of something else. And humans exist because they are predators, they eat something else. Therefore life is saturated, but, fundamental to the discussion tonight, was why new species evolve.

Development of a new life strategy, predation for example, can lead to the evolution of new species. The first microbes used whatever simple nutrients were available, then more complex organisms such as the parasitic *Bdellovibrio* evolved and utilized other more complex nutrients; humans are simply a refinement of these. New species also develop when a new ecological niche opens up. For example, if the planetary temperature increases, killing some life form, then something that is heat-adaptable can move in.

Dr. Gilmore then spoke of the genus *Enterococcus*, as his research interests lie in discovering where enterococci came from. He showed a phylogenetic dendrogram of microbes, a family tree, of many genera. He then posed the question as to what type of event occurred, or life strategy evolved, that led to the development of a new genus like *Enterococcus*. They are members not only of the human microbiome, but are shared by mammals, birds, reptiles, and even insects. One of the reasons they are of particular interest is that in the 1970's they evolved as a leading cause of multi-drug-resistant hospital infections, such as

Tracing the Enterococci (continued)

bloodstream, surgical site, abdominal, and urinary tract infections. They are extremely difficult to treat and kill and have developed nearly every type of antibiotic resistance possible. Enterococci have been called "the cockroach of bacteria" by one of his friends.

Roger S. Greenwell, Jr., PhD (L), and students from the Biology Dept., Worcester State University

Looking at the *Enterococcus* family tree, the grandparents were Gram positive bacteria called *Carnobacterium.* These organisms are mainly associated with causing spoilage of frozen meat and fish. They are psychrophyles, non-spore formers, live in cold temperatures and are therefore very durable. Living carnobacteria can be grown today from ice cores in Alaska and similar places. *Carnobacterium* gave rise to *Vagococcus*, a gram positive coccus associated with fish that lives in the fish gut and sometimes cause infections. A genome for *Vagococcus* had never been sequenced therefore Dr. Gilmore's laboratory obtained a sample to sequence from bass intestine. *Vagococcus* gave rise to *Enterococcus,* and two genera branched off from *Enterococcus.* The genus *Tetragenococcus* on which literature is sparse, occurs somewhere in nature, is halophilic and is found in fermented food such as soy sauce The bee pathogen *Melissococcus*, infects the midgut of bee larvae, causing European foulbrood. *Enterococcus* gave rise to *Streptococcus* and *Lactococcus*. What kind of events occurred or what kind of niches opened up that allowed V*agococcus* and E*nterococcus* to emerge and exist?

Dr. Gilmore showed a diagram of the human gut microbiome and the relative numbers of each microbe within it. The most abundant species are those such as *Bifidobacterium* and *Bacteroides*, and on the other end of the diagram, among the least common that are still considered part of the core intestinal flora, are two species of *Enterococcus, E. faecium* and *E. faecalis*. These two are the main causes of multidrug-resistant hospital infections among enterococci.

Hospital infections occur when a patient is hospitalized and treated with antibiotics to prevent or treat an infection, but at the same time they destabilize the microbiome. "Colonization resistance" is defined as the stable relationship, homeostasis, or balance that we currently have with our microbes. If we are exposed to a few pathogenic organisms for example, we may not become ill as there are no niches or opportunity for them to take hold and cause infection, i.e. we are "pre-colonized". The enterococci we currently have in our guts are not highly drug-resistant; treatment of hospitalized patients with antibiotics creates a chaos in our normal flora and facilitates colonization and drug-resistance. We know that hard surfaces in hospital rooms are a main source of drug-resistant *Enterococcus*. Patients may acquire a few enterococci from these hard surfaces orally, the organisms join the 10^{13}-10^{14} we already have in our gut and multiply. Some patients on many

Carolina Munoz-Agudelo.-Worcester State College.-Winner of ASM T-Shirt Raffle.

antibiotics can develop almost a monoculture of enterococci in their gut. Enterococci are then likely to get into a surgical wound, enter the urinary tract via catheter, or even translocate

from the gut to the bloodstream. The sick patient also spreads them throughout the room and sets up an opportunity for the next patient to get infected.

Dr. Gilmore's laboratory is seeking the answers to many questions regarding enterococci. Among these are why, among all the organisms in the gut microbiome, have enterococci emerged as a problem, as there are so few of them in the gut; why not *Bifidobacterium* or other microbes? What predisposed them to emerge as leading causes of multidrug-resistant hospital infections, not once but twice, with the species *faecalis* and *faecium*, as these two species are not that close on the family tree? The *Enterococcus* family tree branched off from *Vagococcus* and currently contains about 50 different species. Why are there so many different species instead of just one? How is one species different from the rest? What are the properties that are special to enterococci?

To answer some of these questions, the laboratory first sequenced the genomes of a number of different species in the *Enterococcus* family tree to learn about the genes that they contain. The average bacterium contains about 4000 genes, each having about 1000 bases, so the entire circular genome would be 4 million nucleotides long. On the other hand, the average genome of an enterococcus contains only about 3000 genes.

The *Enterococcus* genomes were compared to those of their relatives, *Carnobacterium, Vagococcus, Lactococcus, Melissococcus* and *Tetragenococcus.* The first lesson learned was that the enterococcal species vary widely in their genomes. The average was about 3 million bases, the largest was 5.4 million bases. The next question to answer was whether there was an advantage to having either a large genome or a small genome. Looking at *E. coli* that has a genome of about 4.5 million bases (4500 genes), we know it will grow on minimal media. This means that it has genes allowing it to synthesize just about everything it needs to survive. Genome size then appears to be a measure of the variability in the ecology that the different enterococci would have evolved into. The enterococcal genomes sequenced were smaller than that of *E. coli*, meaning that those enterococci cannot synthesize all the nutrients needed and have to consistently import them from other sources. They depend on their host or on other microbes in the community where they live, and hence, are specific to certain hosts. Species with the largest genomes have genes for many contingencies, can synthesize more of their required nutrients and can live in many places. Smaller genomes therefore indicate host dependence and specificity while large genomes indicate greater independence.

A family tree of relatedness was then constructed based on the genome sequences; genomes closely related were placed on branches close to each other, more distant genomes were on farther branches. The family tree started with *Vagococcus*, then split into a group with large genomes, next to this was a group with small genomes; *E. faecalis* and *E. faecium* were not close to each other on the genomic tree. They also found that enterococci are generally associated with land animals. A few are associated with marine animals, but these eat fish and insects, and fish in turn eat insects; it is believed that land enterococci enter marine animals this way. One thing observed was that the sequences of the bee pathogen *Melissococcus*, and of *Tetragenococcus* were well imbedded within this phylogeny, i.e. they don't connect to the genus *Enterococcus* from the "outside", as *Carnobacterium* and Vagococcus do. Neither should be a separate genus and both should be called *Enterococcus*.

What distinguishes the enterococci? The laboratory found that all enterococci have 1037 of their genes in common; the others all vary. Looking at where else in nature any of these 1037 genes occur, about 400 genes are common to nearly all other bacteria, such as genes for DNA polymerase and ribosomes. There was also another group of about 247 (rare) genes that occur in between 1-20% of other bacteria. However, 10 genes are unique to enterococci and could not be found anywhere else in nature.

Thus *Enterococcus* has a combination of 10 unique genes that don't occur anywhere else, and a small group of genes that occur rarely outside of *Enterococcus* but occur in a special combination in the genus. The laboratory was

Tracing the Enterococci (continued)

particularly interested in these two groups of genes in order to find out what makes enterococci different from *Carnobacterium* and *Vagococcus*; why did these two genera change? Therefore they closely looked at what kinds of genes comprised the 10 genes unique to enterococcus and the group of 247 genes that are found in its ancestors but rarely occur in other bacteria. Bioinformatics tools helped with this by classifying genes into those used for energy production, amino acid metabolism, etc. They found that most of the 257 genes analyzed were classified as S or R, meaning "we don't know". They are hypothetical genes, genes for which we have no ascribable function. Therefore we just don't know what makes enterococci special said Dr. Gilmore. Additional work is needed here.

The laboratory took another approach and looked at where else the genes that rarely occur outside of *Enterococcus* were found, and they occurred in *Carnobacterium* and Vagococcus, *Streptococcus, Lactococcus, Pediococcus, Listeria, Clostridium,* etc., mostly in gram-positive bacteria that are host-associated. These appear to be genes that are important for gram positive organisms to live in the gut or to be host-associated. In general, where genes occur tells us of their function.

Dr. Gilmore added that in clinical microbiology, enterococci can be found in both hospital blood samples and in beach samples, where they would be an indicator of fecal contamination. Samples can be plated on either Chrome Agar or Bile Esculin Azide Agar to isolate enterococci, but *Vagococcus* also grows on these media and looks just like an enterococcus. This indicates that *Vagococcus* already acquired the ability to resist bile and azide that are used in media to kill off all organisms except enterococci. However, *Carnobacterium* cannot tolerate high levels of bile. Therefore in branching off from *Carnobacterium to Vagococcus, Vagococcus* learned how to tolerate/resist bile (fish have bile as do larger animals).

Vagococcus shares many of the genes that define *Enterococcus,* but since branching from *Vagococcus*, enterococci acquired 126 genes that *Vagococcus* does not have. Therefore another way to uncover what is unique about enterococci is to ask what these 126 genes do that distinguish it from *Vagococcus*. The function of most of those genes has to do with cell wall metabolism, i.e., mainly with reinforcing the cell wall, making it less penetrable and with cell wall stress responses. Therefore something occurred that caused *Enterococcus* to adapt and tolerate stress that *Vagococcus* did not experience.

(L-R) Harvey George, PhD, NEB President, Janira Prichula, Michael Gilmore PhD, and Gabriella Dacuncha

Another approach taken was to measure phenotypes. A test panel called the Biolog Phenotype Assay has an integrated system of cellular assays, instrumentation, and bio-informatics software, and employs microtiter plates. When a culture is added, if your bacterium is resistant to what is in the well, growth will occur; if sensitive to what is in the well, there is no growth. The Assay can test 1344 behaviors, and when that was done, there were only 45 behaviors that separated *Enterococcus* from its parents and offspring. He showed a map of this.

Things that separate *Enterococcus* from its ancestors include: growth in a high pH and urea which are hydrogen bond solvents, resistance to chloroxylenol (a disinfectant called Dettol), to hi levels of salt and borate which are otherwise toxic, and resistance to a variety of beta-lactams. All enterococci, even environmental species are resistant to all these agents. The hardened cell wall allows them to also withstand desiccation nearly 10 times better than their parents, and they are more resistant to starvation. This is

why they are called the "cockroach of bacteria"; it is difficult to dry them, starve them, etc., even though they are non-spore-formers.

The laboratory was then interested in when *Enterococcus* branched from *Vagococcus,* and what caused enterococci to become stressed, salt, bile, desiccation resistant, etc.? A good estimate would be when animals in which they occur became more prevalent. To find what this meant in terms of years, they compared the genome of one enterococcus to another to see how far they drifted apart. The more identical species are, the more recently they have separated; when less identical it means they separated a longer time ago. Therefore time can be connected with sequence divergence.

Each enterococcus pair was measured and the results plotted. Results ranged from 90% identical (would be next to each other on the family tree) to 65% identical (these species would be far apart on the tree). Ancestors like *Carnebacterium* and *Vagococcus* were much farther apart and in separate groups. They found the "boundary" for the genus and saw where the genus started. Now they needed to connect time with the distance on the map. They next plotted the percentage of genes that two species share against the measurement of time (how much the sequences have drifted apart) and measured and plotted the similarity in the ecologies they inhabit. Theoretically, said Dr. Gilmore, the plot ought to look like a triangle, and it does. However, one noticeable variation, a gap in the middle of the *Enterococcus* family tree occurs, because for some reason in the distant past, new species of enterococci were not being formed for a while. They therefore looked at the amount of drift between the genomes to find a common ancestor for each of *Enterococcus* pairs.

When determining a date, molecular clocks are used to give a good first approximation; refinement is then needed for concordance with the fossil record to zero in on a specific time. They applied molecular clocks that other people had developed to these cases. It had been shown that *E. coli* branched from *Salmonella* about 140 million years ago, *E. coli* branched from *Vibrio* about 400 million years ago, and divisions occurred in the genus *Aeromonas* about 184 million years ago (based on comparison of 16S RNA sequences). *E. coli* and *Salmonella* have genomes that are 82% identical, *Aeromonas* genomes have about 78% genetic identity and *E.coli* and *Vibrio* share about 68% genetic identity. Plotting time vs sequence divergence in this manner gave a straight line. They then applied their *Enterococcus* amounts of divergence and found that *Enterococcus* diverged from *Vagococcus* longer ago than *E. coli* diverged from *Vibrio*, suggesting an old genus with a date of about 550 million years ago.

However, there were no animals around 550 million years ago. The Cambrian explosion didn't happen until 542 million years ago; therefore their data needed to be refined with the fossil record. They estimated that *Enterococcus* arose about 500 million years ago but not before the animals appeared. The animals in the Cambrian explosion arose in the ocean, and since enterococci are more common in land animals this suggests that enterococci likely arose when the animals crawled ashore. The original date based on the molecular clock suggests about the same time as Cambrian explosion, but that's not consistent with the fossil records. The fossil record, with enterococcus being found mainly inland animals, shifts the time that enterococci most likely branched from *Vagococcus* to about 425 million years ago, and they have been dividing ever since.

Therefore "terrestrialization" occurred when animals first crawled ashore and the first to crawl ashore were arthropods. Replotting those dates on a geologic time scale, with the Cambrian explosion about 542 million years ago started in the sea, and animals crawled ashore about 425 million years ago. Also, about 251 million years ago the largest mass extinction on earth, the Permian Extinction, occurred, which sits right in the middle of the gap in the *Enterococcu*s family tree. Moreover, if you superimpose the development of all animals (land and sea), the diversification of *Enterococcus* largely parallels the diversification of animals; where there is no diversification of animals, there is no diversification in *Enterococcus*, which seems to go along with its host. When the host develops a new diet or a

Tracing the Enterococci (continued)

new animal species, a new species of *Enterococcus* evolves; and the cycle repeats.

Dr. Gilmore summarized for us the evolution of enterococci. There have been at least five Snowball Earth events where the Earth has been frozen almost solid, killing off much of life, but something like *Carnobacterium* that lives in ice would persist. The most recent global freeze was about 650 million years ago, leaving a few cracks in the ice and perhaps a little water around deep sea vents where hot geothermal energy exists; we think at that point, *Carnobacterium* thrived. The Cambrian explosion then occurred in the ocean 541 million years ago and *Vagococcus* appeared in the guts of fish. Note that the gut flora, the microbiomes of fish are dropped into a friendly watery environment and sediments loaded with bacteria can recirculate into fish. However, when animals started to crawl ashore, feces dropped on land, a non-hospitable environment, that was isolated, gets desiccated, and microbes would either die or reenter the food chain by being eaten by something as a land animal. It was terrestrialization and the necessity to survive on land in isolation that most likely selected for the origin of the genus *Enterococcus;* as new land animals evolved so did new species of enterococci.

Looking at the original question of why *Enterococcus* emerged as a leading hospital pathogen, the hospital environment selects for traits that allow colonization and transmission; enterococci need to withstand starvation, dessication on surfaces, to resist disinfectants and antibiotics, and ultimately transfer from host to host. Dr. Gilmore would argue that all these traits were learned when *Enterococcus* first emerged from the sea 425 million years ago and that is why it emerged, twice, as a leading hospital pathogen.

Science Fairs

The NEB annually donates an award of $100 to each of five MA regional fairs and the VT science fair, and $300 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 unavailable.

Region II. Worcester Regional Science and Engineering Fair. Desmond Goodwin from St. Marks School, Southborough, MA. *Testing the Stability of Orbit in Mycobacterium smegmatis.*

Region III. Bristol Community College-Rensselaer Polytechnic Institute Regional Science Fair. Katherine R. Boadle, 10th grade, 16 years old, Bishop Feehan High School, Attleboro, MA. *Lipid Content in Algae, the Foundation of Biofuel Production*.

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

Region V. The South Shore Regional Science Fair had two winners. Ariana Riske and Ayssa McGillicuddy, Both Grade 11, 16 years old, Stoughton High School. *The Effectiveness of Third World Refrigeration Methods* and
John Cox, Grade 11, 16 years old, North Quincy High School. *The Effect of Wing Anatomy on the Speed of Birds.*

Region VI. Sophia Tang, Grade 11, Boston Latin School. *Identification of Borrelia burgdorferi Associated Protein Biomarkers.*

68th ASCLS-CNE Annual Convention

The 68th American Society for Clinical Laboratory Science-Central New England Annual Convention was held at the Rhode Island Convention Center in Providence, RI on April 26-28, 2015. It was jointly sponsored with the Bay State Chapter CLMA (CLMA), and Board of Rhode Island Schools of Allied Health (BRISAH), Northeast Branch, American Society for Microbiology (NEB-ASM), Rhode Island Cytology Association (RICA), and Rhode Island Society of 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, which are usually held in April and October, are attended by physicians, laboratory directors, epidemiologists and laboratorians from New England.

Please contact Alfred.DeMaria@state.ma.us if you would like to receive meeting information. Meetings are supported in part by the NEB.

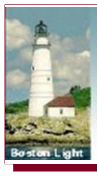
Biosafety and Biosecurity: Minimizing the Risks in the Laboratory

The sessions of this September training program were designed to identify key concerns of laboratory biosafety, focusing on biosafety level 3 (BSL3) practices in a BSL2 environment, proper use of personal protection equipment (PPE), biological safety cabinets (BSCs), and conducting risk assessments. The biosecurity session presented laboratory security principles described in the 5th edition of Biosafety in Microbiological Laboratories (BMBL). Participants also learned how to conduct vulnerability assessments and develop a good biosecurity plan. Faculty included Tanya Swanson, BS, MT, Supervisor, Bioterrorism Response Laboratory, Shoolah Escott, MS, MT(ASCP) Biosafety Manager and Cynthia Condon, BS, M(ASCP). All are from the William Hinton State Laboratory Institute, MDPH, Jamaica Plain, MA.

Agents of Bioterrorism: Sentinel Laboratory Training

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

Faculty included Cynthia Condon, BS, M (ASCP), LRN Coordinator, Bioterrorism Response Laboratory, Cheryl Gauthier, BS, MT (ASCP), Director, Bioterrorism Response Laboratory; Scott Hennigan, Supervisor, Molecular Diagnostics Laboratory; Sandra Smole, PhD, Director, Division of Molecular Diagnostics & Virology; and Tanya Swanson, BS, MT, Supervisor, Bioterrorism Response Laboratory. All are from the William A. Hinton State Laboratory Institute, MDPH.



Northeast Branch of the American Society for Microbiology

DUES STATEMENT - RENEWAL
January 1, 2017 - December 31, 2017

Please check personal information.

Your dues are paid thru _____

Name: _____ ASM member? ____ ASM Membership No: _____

Preferred Mailing Address: _____

Home/ Business Address: _____

Phone (daytime): _____ Preferred email: _____

Phone: _____ Member type: _____

Professional position: _____ Degree(s)/Year: _____

Specialty: _____ Institution: _____

The above information is correct? _____

Please indicate below updates to the contact information currently listed in our database.

( ) Business phone: _____ Business email: _____

( ) Home phone: _____ Home email: _____

Primary area of interest:

__Biotechnology
__Clinical/Public Health
__Education
__Industrial
__Marketing/Sales
__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)
.....
__N/A -- UPDATE ONLY

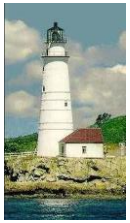
*Emeritus membership is defined as a member who is in good standing for 20 consecutive years, and who is retired from their profession.

Renewals postmarked after September 1, 2016 will be effective January 1, 2017. Please make checks payable to: NORTHEAST BRANCH_ASM and send with this form to:

Patricia E. Kludt
6 Abigail Drive
Hudson, MA 01749

Date dues received: _____
Check No: _____

September 2016



NORTHEAST BRANCH
AMERICAN SOCIETY FOR MICROBIOLOGY

Irene H. George, Secretary
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Dover, MA 02030

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Has your membership expired?