APHL 2020 Virtual Conference
Poster Abstract List

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Poster #1

Providing Resources to Enhance Collaboration in the Detection of Public Health Threats and Effective Response

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The Association of Public Health Laboratories (APHL) provides key resources to public health laboratories (PHLs) through the Sentinel Laboratory Partnerships and Outreach Subcommittee (SLPOS) to assist in their ability to effectively communicate with and provide guidance to sentinel clinical laboratories. In fiscal year 2020, along with input from the American Society for Microbiology (ASM) and the Centers for Disease Control and Prevention (CDC), SLPOS developed and updated essential resources that focus on enhancing the relationships between public and private laboratories.

SLPOS members began the process of updating the ASM Laboratory Response Network (LRN) Sentinel Level Clinical Laboratory Guidelines, which provide standardized, practical methods and techniques to rule-out or refer suspected bioterrorism agents. These guidelines provide clinical laboratorians with information on safety, testing materials, specimen collection and processing, rule-out testing flowcharts, etc. They are a critical resource utilized by both clinical and public health laboratorians.

Due to changes in the ASM guidelines and to ensure harmonization of reference materials, SLPOS is also updating the Clinical Laboratory Preparedness and Response Guide, a companion critical resource for clinical laboratorians. Other resources, such as the APHL Biothreat Agents Identification Bench Cards and poster, will also be updated as necessary, to ensure resource materials remain consistent.

SLPOS has developed other valuable resources for both clinical and public health laboratorians. The Biothreat Organism Evaluation tool assists PHLs to evaluate the ability of their Sentinel Clinical Laboratories to use the rule-out procedures according to the ASM Guidelines. The Clinical Laboratory Exposure Assessment and Monitoring tool assists PHLs with assessing whether or not there have been any exposures within the clinical laboratory to a bioterrorism agent during culture work-up and rule-out testing. It also provides guidance on follow-up prophylaxis and treatment if exposures are identified. Another resource, the Packaging and Shipping Evaluation Tool, provides a standardized resource for PHLs to assess the proper packaging and shipping of biological threat agent rule-out samples sent to them by clinical sentinel laboratories.

The resources developed by the members of SLPOS are vital tools that support the mission of collaboration within the LRN to enable a quick and accurate detection of public health threats and provide a timely, effective response.

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Poster #2

Molecular Paradigm Shifts in Newborn Screening Systems

H. Desai, O. Akinsola and G. Zarbalian, Association of Public Health Laboratories, Silver Spring, MD

Newborn screening is known to be one of the most effective public health programs. With the rapid expansion of conditions that can be detected and the advent of next-generation sequencing (NGS), newborn screening systems must leverage relevance of new screening technologies to augment current methods for complex multigene disorders and coordinate the results with short- and long-term follow up. The implementation period for conditions added to the recommended uniform screening panel (RUSP) vary across regions, and newborn screening public health laboratories prepare by addressing the social, ethical, financial, and legal questions of adopting new methodologies. For this poster we showcase different screening methodologies and workflows for cystic fibrosis (CF) and severe combined immunodeficiency (SCID) to demonstrate the paradigm shift in adopting new technologies to complement current methods and the impact they have on follow-up procedures. We also demonstrate that there is no "one size fits all" or "cookie cutter" approach to newborn screening and each program must tailor their practices to best meet the needs of newborns in their state and detect rare disorders in a timely manner.

Presenter: Hiral Desai, Association of Public Health Laboratories, Silver Spring, MD, Email: hiral.desai@aphl.org

Poster #3

Communication: A Cornerstone for the Laboratory Response Network

J. Diethelm, Association of Public Health Laboratories, Silver Spring, MD

A robust laboratory network is critical to effectively respond to known and emerging public health threats. To address this need within the United States, the Laboratory Response Network (LRN) was founded in 1999 through a collaboration between the Centers for Disease Control and Prevention (CDC), the Federal Bureau of Investigation (FBI) and the Association of Public Health Laboratories (APHL). At its inception the primary objective was to create a system for responding to biological threats. Today the LRN has expanded to form a network of state and local public health, federal, military, and international laboratories capable of responding to biological, chemical, radiological and other public health threats, such as emerging infectious diseases.

The LRN supports its members through the following core functions: ensuring rapid deployment of resources, strengthening partnerships, developing and improving diagnostics, and continuous training. Using these tools and systems the LRN enables front-line sentinel clinical laboratories to reliably rule-out and/or refer potential threats to LRN Reference Laboratories that are capable of providing specialized testing for unknown agents in high containment facilities.

Over the past two decades the LRN has focused on building its capacity for preparedness and response within laboratories across the United States. However, as the world becomes more interconnected, programs like the LRN may soon become an international need. Despite the advances of international public health systems, the ability to communicate efficiently amongst public health laboratories and larger agencies continues to challenge many. This poster highlights the communication capabilities of the LRN, which should be leveraged by countries needing improved systems as it is a program founded on collaboration and continuously balances connections between local, state, federal and private stakeholders.

Presenter: Jennifer Diethelm, Association of Public Health Laboratories, Silver Spring, MD, Email: jennifer.diethelm@aphl.org

Poster #4

Clinical Laboratory Biosafety: Understanding their Needs

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Since 2015, APHL has worked to enhance and improve biosafety and biosecurity capacity in our nation's laboratories. Through the efforts of both the APHL Biosafety and Biosecurity Committee (BBC) and Biosafety and Biosecurity Partners Forum, APHL has developed numerous tools, trainings and other resources to support biosafety and biosecurity practices at both Public Health Laboratories along with sentinel clinical laboratories. Recently through the efforts of the BBC and Partners Forum, in 2018 APHL developed the "Biosafety Practices and Needs in Clinical Laboratories Survey" intended for sentinel clinical laboratories across the United States to comprehensively assess their laboratory biosafety practices, APHL collected information on (1) institutional biosafety practices; (2) linkages with public health laboratories and (3) unmet biosafety needs. After reviewing the aggregate survey data, APHL continued their efforts to better understand the sentinel clinical laboratories needs by convening four in person forums in 2019 "Biosafety Forum: Public Health Laboratory Outreach, Clinical Laboratory Engagement and Needs" across the country where biosafety professionals and laboratory leadership discussed the current and unmet biosafety and biosecurity needs and challenges of both public health laboratories and clinical laboratories. Currently APHL has collected the top needs addressed from the forums and survey and are addressing them through the BBC, Partners Forum other external partners. This poster will showcase the needs identified from the clinical laboratories and how APHL has and plans to assist these laboratories across the country to better enhance their biosafety and biosecurity practices.

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Poster #5

Informatics Curriculum Framework

R. Shepherd, Association of Public Health Laboratories, Silver Spring, MD

Over the past several years, APHL, in cooperation with CDC, has developed materials in support of a public health informatics curriculum, as well as a series of web-based introductory courses to the topic of laboratory informatics. Since February of 2019, approximately 20 subject matter experts from the public health community, CDC, and APHL have been working across workgroups to develop a robust curriculum framework, currently in the final stages of development.

This poster will serve as an opportunity to share the completed framework, which is intended to serve as a visual map, cataloging the necessary skills and competencies required to advance in the field of laboratory informatics by defining objectives and goals in the following categories ranging from beginner to expert levels:

- Data Concepts
- Data Systems
- Interoperability
- IT Infrastructure
- Laboratory Activities
- Standards and Regulations

The viewer will be able to examine layers within the framework as well as develop an understanding of the competencies needed to excel in a lab informatics role, and how this fits into an overall public health curriculum.

Presenter: Rachel Shepherd, Association of Public Health Laboratories, Silver Spring, MD, Email: rachel.shepherd@aphl.org

Poster #6

Smooth Sailing on the Data Lake

D. Shirazi, D. Sanderson, R. Merrick and M. Sibley, Association of Public Health Laboratories, Silver Spring, MD

This poster will examine one of the more recent innovations on the AIMS Platform--the data lake. The data lake is a new technological capability that will revolutionize the ways we can store, access, and utilize data. As opposed to the traditional data warehouse that stores data categorically according to purpose, a data lake serves as a single repository for enterprise-wide raw data, meaning that all structured and unstructured data from a variety of sources is dumped in a single pool (or lake). When raw data is accessible from a variety of sources and it can be repurposed to meet multiple data needs and can be more easily used for reporting, visualization, analytics and machine learning.

Benefits of this solution include:

- Ability to import different formats (As long as you can validate the upload content, the data lake will accept the data format (HL7, CSV, excel file… etc.)
- Access to the original/raw data
- Validation (structure and content)
- Data field Error handling
- Lake is searchable
- Fast data loads
- Supports updating of tests/value sets without the assistance of a developer
- Reduced cost, liability and maintenance (Product ≈70% done; 30% to get ARLN data linked)
- Will be able to bring new programs on much faster

This poster will look at the process and implications of the data lake for several of the programs beginning to utilize the data lake solution, such as ARLN.

Presenter: Dari Shirazi, Association of Public Health Laboratories, Silver Spring, MD, Email: dari.shirazi@aphl.org

Poster #7

Findings from an APHL-sponsored Quality Manager Focus Group Discussion

B. Su, Association of Public Health Laboratories, Silver Spring, MD

Introduction: In December 2019, APHL hosted an in-person focus group discussion on quality and safety issues and gathering information about the gaps and needs of quality managers and quality management programs, including general safety, within public health laboratories. Participants ranged from laboratory directors who did not have designated quality manager position to public health laboratory quality managers who manage multiple quality staff. APHL collected in-depth qualitative information that will help guide the Association in the development of new resources, including training. The poster outlines some of the important findings.

Methods: During the one-day meeting, the 10 quality managers and laboratory directors answered qualitative questions regarding quality and safety, specifically focused on the following topics:

- challenges related to quality and safety experienced at different organizational levels at the laboratory;
- strategies for tackling the challenges identified; and
- skills, competencies and trainings needed for quality and safety staff

APHL staff captured important discussion points on each topic using white flip chart paper. Participants ranked discussion points to prioritize need and future projects.

Results: The focus group participants stressed the importance of all levels of public health laboratory staff understanding the importance and value of quality and safety, not just when audits are occurring. Two important jobs aids were identified as a priority need- a standardized position description and a learning map. Position descriptions not only help justify the position, but also reinforces the need of quality and safety at the laboratory and clarifies roles and responsibilities. The participants emphasized the value of being empowered and having support from laboratory management to move forward with implementing quality system improvements on a staff-wide level. Additionally, they prioritized the impact of culture, and the need to hold hands-on trainings and drills on a regular basis to ensure that staff know the proper protocols when issues arise, e.g. non-conforming events, spills, emergency situations.

Conclusions: Building and nurturing a culture of quality and safety ultimately improves the quality of the data that the laboratory produces and creates a safe working environment for the staff. APHL will work with the participants to address the gaps that were identified during the focus group discussion. Next steps include creating a position description for quality managers that aligns to public health laboratory competencies, a learning map for quality managers that outlines what trainings new quality managers should take and resources they should be aware of; and collection model practices of hands-on exercises that can be adopted for trainings and drills."

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Poster #8

New Online Course on Analytical Validation of a Biochemical Genetic Test Using Liquid Chromatography - Tandem Mass Spectrometry

B. Su[1], B. Chen[2]; [1]Association of Public Health Laboratories, Silver Spring, MD, [2]Centers for Disease Control and Prevention, Atlanta, GA

Introduction: Biochemical genetic tests (BGTs) are highly specialized laboratory procedures for the evaluation, diagnosis, and clinical management of inherited metabolic diseases. Over the last two decades, the advent of expanded newborn screening (NBS) for inherited metabolic disorders using tandem mass spectrometry (MS/MS) and other technologies has presented an increasing demand for biochemical genetic testing in diagnosing presumptive cases detected through NBS. However, BGTs are highly complex procedures performed on a wide variety of patient specimen types. Biochemical genetics laboratories need easily accessible training materials in developing and implementing new tests using the MS/MS technologies.

Method: Since 2013, CDC has been collaborating with the Association of Public Health Laboratories (APHL) to help genetic and NBS laboratories in their quality improvement activities. Findings from two discussion groups held in 2013 identified topic areas in which training resources are desired, including user-friendly online courses on developing and validating new test procedures. (https://www.aphl.org/aboutAPHL/publications/Documents/CDCRecommendationsGoodLabPractices_OCT2014.pdf). To meet this need, experts in biochemical genetic testing and MS/MS technologies were enlisted to develop a new online course titled "Validating a New Test Using Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS) in a Biochemical Genetics Laboratory." Pilot testing was conducted by laboratory professionals to ensure its usefulness for the target audience.

Results: This intermediate-level multimedia online course discusses a stepwise plan for setting up and validating a new LC-MS/MS based assay for application in a biochemical genetics laboratory. A specific example of quantifying methylmalonic acid in human plasma is used to describe each of the steps required to develop and validate the method. An outline of the validation report is provided that includes how to review, interpret and document validation tests, quality control results, and proficiency tests. Continuing education credits are available from this course free of charge, including 1.5 hours of the ASCLS P.A.C.E. credit. The course is expected to be publicly available on CDC TRAIN website at https://www.train.org/cdctrain/home in Summer 2020.

Conclusions: This online training module is intended to help laboratory professionals understand the regulatory environment of a biochemical genetics laboratory, define the steps required to develop and validate an assay based on LC-MS/MS, and describe the contents of the validation report. Course evaluation results and feedback from the participants will be closely monitored to assess the utility and learning outcomes.

Presenter: Bin Chen, Centers for Disease Control and Prevention, Atlanta, GA, Email: bkc1@cdc.gov

Poster #9

Rebuilding Response Capacity to Natural Disasters in Puerto Rico, US Virgin Islands and Houston

J. Sutton, Association of Public Health Laboratories, Silver Spring, MD

In recent years, natural disasters have intensified and become increasingly more frequent, placing a tremendous amount of pressure on the public health systems that respond. The Association of Public Health Laboratories' Public Health Preparedness and Response (PHPR) program works collaboratively across the association, with CDC and other external partners to improve the effectiveness and capabilities of public health systems to prepare for, respond to, and recover from public health crises, such as hurricanes. Following the 2017 hurricanes, APHL was awarded a $15.1 million, two-year cooperative agreement by the US Centers for Disease Control and Prevention (CDC) Center for State, Tribal, Local and Territorial Support (CSTLTS) to help strengthen the public health system's response and recovery in three jurisdictions - Houston, Puerto Rico and the US Virgin Islands.

To support the response and recovery from Hurricanes Harvey, Irma and Maria, APHL's PHPR program coordinates with health departments and CDC to increase their staffing capacity, provide technical training and support, and rapidly procure the supplies, equipment and services needed to restore and maintain essential public health activities. This poster will highlight ongoing recovery efforts, lessons learned and considerations for preparing and responding to future threat events.

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Poster #10

Tracking Antimicrobial Resistance with the Oxford Nanopore Technologies MinION Sequencer

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Tracking the spread of antimicrobial resistance genes is critical in the context of preventing and controlling healthcare-associated infections, including those caused by carbapenemase-producing organisms. However, the short reads generated by commonly-used sequencing platforms limit interpretation because they are not able to completely resolve features such as the location of resistance genes and the organization of resistance-bearing plasmids. The Oxford Nanopore Technologies MinION sequencer provides long reads which overcome these limitations and additionally promises rapid sequencing library preparation and faster turnaround times with little capital investment. Together, this suggests that it could be useful either as a supplement or an alternative to short-read sequencing platforms. As part of a feasibility study to determine the utility of the MinION in healthcare-associated outbreak detection and characterization, carbapenemase-producing organisms were sequenced on both the Illumina and MinION platforms. Inclusion of long-read data provided additional information that may be useful for inferring relatedness and transmission dynamics, and MinION data alone was sufficient for antimicrobial resistance gene localization and preliminary characterization.

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Poster #11

An Automated Whole Genome Sequence Analysis and Data Visualization Pipeline Used to Investigate Antimicrobial Resistance Patterns in Cases of Shigellosis in California

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The California Department of Public Health's Infectious Disease Laboratory Branch has significantly increased its phylogenetic analysis of bacterial and viral isolates for epidemiology and other public health applications. Manual sequence analysis methods that were satisfactory on small data sets did not scale well for larger work volumes due to the increased complexity of integration and visualization of phylogeny data with clinical/epidemiological information, manual data entry and report generation, and dependence on costly proprietary software. To solve this, we developed an automated pipeline that streamlines de novo assembly, phylogenic tree building, and data visualization of outbreak whole genome samples using a series of open source command line tools and custom scripts. The pipeline takes in raw whole genome sequencing (WGS) data and completes quality control, de novo assembly, phage masking, single nucleotide polymorphism (SNP) calling, plasmid assembly and characterization, and antimicrobial resistance (AMR) gene detection. SNP data is used to create a phylogenetic tree that is overlaid with plasmid, AMR gene, phenotypic lab testing, and other epidemiological data. In addition to the pipeline, we created a custom AMR gene database using multiple sources. The AMR database is normalized to allow for consistent annotations across sources that can be automatically applied to data. Here, we use our pipeline to look at emerging AMR patterns in *Shigella* cases in California. As certain resistance genes and plasmids become more common among *Shigella*, their impact on drug susceptibility must be systematically studied in order to inform public health recommendations on the treatment and prevention of *Shigella* infections. In this investigation, WGS analysis allows us to discover AMR genes and plasmids in our isolates. We then gain a phenotypic insight into how these features affect drug susceptibility through antimicrobial susceptibility testing (AST). Phylogenetic analysis allows us to compare emergent lineages of *Shigella* with archival isolates to gain a more detailed understanding of evolutionary AMR changes currently underway. Our automated pipeline eases the analysis and visualization of data from this multivariate population study.

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Poster #12

Prevalence of Ciprofloxacin and Azithromycin Resistance in California *Shigella* species

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The Centers for Disease Control and Prevention (CDC) have declared antibiotic-resistant *Shigella* a serious threat, and it is estimated that 27,000 drug-resistant *Shigella* infections occur in the United States each year. According to 2016 CDC National Enteric Disease Surveillance data, most shigellosis cases in California are caused by *Shigella sonnei* (65%) followed by *Shigella flexneri* (33%). Infection with *Shigella* can cause diarrhea, fever and stomach cramps. Symptoms typically resolve within seven days; however, in some cases treatment with antibiotics may be necessary to decrease illness severity and reduce bacterial shedding. Ciprofloxacin and azithromycin are the primary antibiotics used to treat shigellosis. The California Department of Public Health - Microbial Diseases Laboratory (CDPH-MDL) performed Sensititre microbroth dilution testing on 1,137 *Shigella* isolates received from November 2018 through July 2020 to detect resistance to ciprofloxacin and azithromycin. A total of 566 *S. sonnei* and 571 *S. flexneri* were examined. Resistance to ciprofloxacin was evaluated based on the Clinical and Laboratory Standards Institute (CLSI) M100 30th Edition (2020) breakpoint of ≥1 ug/mL. Ciprofloxacin resistance was detected in 65 (11%) of the *S. flexneri* and 224 (40%) of the *S. sonnei* tested. Azithromycin epidemiological cut off (ECV) values for *S. flexneri* and *S. sonnei* of ≥16 and ≥32 ug/mL respectively were used to determine non-wild-type strains based on guidelines from the CLSI M100 30th Edition (2020). Azithromycin non-wild-type was detected in 300 (53%) of the *S. flexneri* and 209 (37%) of *S. sonnei* tested. Dual resistance to azithromycin and ciprofloxacin was detected in 191 (17%) of the *Shigella* isolates. Rates of resistance observed in California are higher than those reported in national surveillance and were found in isolates throughout the state.

In 2017, the CDC reported an increase in *Shigella* isolates with decreased susceptibility to ciprofloxacin (DSC), defined as having a minimum inhibitory concentration (MIC) range of 0.12-1.0 ug/mL. DSC *Shigella* are of concern because even though they were not considered resistant by the 2017 CLSI guidance, they often encode a quinolone resistance gene. It has been suggested that treatment of DSC with ciprofloxacin could lead to further reduction in ciprofloxacin sensitivity and treatment failure. In this study, 102 (18%) *S. flexneri* and 203 (36%) *S. sonnei* had MIC within the DSC range. The high proportion of both resistant and DSC isolates found by CDPH-MDL highlights the growing problem of drug-resistant *Shigella*.

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Poster #13

Antimicrobial Susceptibility of Carbapenem-resistant Pseudomonas aeruginosa Isolates Tested at CDC, 2018-2019

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Introduction: The emergence of carbapenem-resistant Pseudomonas aeruginosa (CRPA) is a major public health threat as treatment options are limited. Carbapenemase-producing CRPA is particularly concerning as resistance genes are sometimes carried on mobile genetic elements. We describe the antimicrobial susceptibility of CRPA isolates tested at the Centers for Disease Control and Prevention (CDC).

Methods: We analyzed suspect carbapenemase-producing CRPA submitted by the Antibiotic Resistance Laboratory Network (AR Lab Network) and a sample of CRPA from the Emerging Infections Program (EIP)'s laboratory- and population-based surveillance sent to CDC between 1/1/2018 and 10/31/2019. Antimicrobial susceptibility testing was performed by reference broth microdilution against 15 drugs according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Interpretive criteria were applied using CLSI's M100 29th ed. We confirmed carbapenemase production in all isolates using the modified carbapenem inactivation method (mCIM). Real-time PCR was performed for up to four carbapenemase genes: blaKPC, blaNDM, blaIMP and blaVIM. Isolates were categorized as carbapenemase gene-positive (CP-CRPA), mCIM positive/PCR negative (mCIM+/PCR-), mCIM indeterminate/PCR negative (mCIM IND), mCIM negative (non-CP). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) were defined using standard definitions.

Results: Of 903 confirmed CRPA (resistant to imipenem, meropenem or doripenem), 575 (63.7%) were non-CP, 223 (24.7%) were CP-CRPA (76 blaVIM, 74 blaKPC, 34 blaIMP, 29 blaNDM, 10 with two carbapenemase genes), 94 (10.4%) were mCIM+/PCR-, and 11 (1.2%) were mCIM IND. EIP contributed 509 (88.5%) of the non-CP, 5 (2.2%) of the CP-CRPA, 7 (7.4%) of the mCIM+/PCR-, and 0 mCIM IND isolates. Among non-CP and CP-CRPA, 77% and 99.6% were MDR, respectively; 47.7% and 94.6% were XDR, respectively. Susceptibility to ceftolozane-tazobactam was 90.1% among non-CP CRPA but was not observed among CP-CRPA. The respective percent susceptible for non-CP versus CP-CRPA was 55.3% and 1.8% for ceftazidime, 51.7% and 4.9% for cefepime, and 85% and 24.2% for ceftazidime-avibactam.

Conclusion: Carbapenemase-producing P. aeruginosa tested at CDC displayed a high frequency of the XDR phenotype. In 2019, CDC shared guidance to help public health laboratories identify CP-CRPA more efficiently by focusing mechanism testing on isolates that are nonsusceptible to ceftazidime or cefepime. This study's findings further suggest that nonsusceptibility to ceftolozane-tazobactam among CRPA may serve as a good indicator of CP. Public health and clinical laboratories should continue efforts to detect CP-CRPA to help reduce transmission and improve patient safety.

Presenter: Amelia Bhatnagar, Centers for Disease Control and Prevention, Atlanta, GA, Email: wmt7@cdc.go

Poster #14

Rapid, Sensitive and Cost-effective Diagnosis of Pathogens from Fixed Tissue Specimens Using Syndrome-based Pyrosequencing Panels

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Background: Rapid identification of pathogens is crucial for patient management and appropriate public health response. For investigations of infectious diseases of unknown etiologies, outbreaks and unexplained deaths, evaluation of a wide-range of pathogens is usually necessary. Culture-based identification of pathogens is tedious and time-consuming. Also, often specimens are not processed for culture, particularly in cases of sudden fatality, and fixed tissues are the only specimens available. The main objective of this work was to develop syndrome-based pyrosequencing (PSQ) assays/panels for rapid and specific diagnosis of pathogens from fixed tissues.

Methods: PSQ assays were developed for pathogens associated with encephalitis (West Nile virus, eastern equine encephalitis virus, La Crosse virus), myocarditis (enterovirus, parvovirus B19, adenovirus, CMV), Zika-Dengue like illness (Zika virus, chikungunya virus, dengue virus, Leptospira) and granulomatous infections (Mycobacterium spp.). Nucleic acids extracted from formalin-fixed, paraffin-embedded (FFPE) cell cultures and tissues from the PCR-confirmed cases of targeted pathogens were evaluated by the PSQ assays. Suspect Zika virus, chikungunya virus and *Mycobacterium tuberculosis* complex (MTBC) cases that were negative by conventional tissue-PCR were also tested. Pathogen-specific in-situ hybridization (ISH) was performed on selected PSQ-positive cases.

Results: PSQ assays correctly identified tested pathogens in diverse tissue types and culture controls, and showed 100% specificity and ≥ 90% sensitivity for all targeted pathogens. In addition, the assays were able to detect targeted pathogens from quite fragmented nucleic acids and identified Zika virus, chikungunya virus and MTBC species in 15%, 21% and 24% of previously tissue-PCR negative cases, respectively. Pathogens identified by PSQ assays were also directly localized in the same tissues by pathogen-specific ISH assay. Data will be presented.

Conclusions: Syndrome-based PSQ assays are cost-effective diagnostic tools for rapid, sensitive and specific identification of pathogens, using a limited amount of tissue samples. The assays will enhance the prompt public health response to outbreaks and unexplained deaths, and particularly valuable for the cases in which conventional specimens are unavailable for testing.

Presenter: Julu Bhatnagar, Infectious Diseases Pathology Branch, Division of High-Consequence Pathogens and Pathology, NCEZID, Centers for Disease Control and Prevention, Atlanta, GA, Email: zrn1@cdc.gov

Poster #15

Evaluating Data Quality in Antimicrobial Resistance Surveillance Laboratories: A Model from Ethiopia

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Background: With support from CDC, the Ethiopian Public Health Institute launched an Antimicrobial Resistance (AMR) Surveillance Network in July 2017, including three sentinel laboratories (SLs) and the National Clinical Bacteriology and Mycology Reference Laboratory (NRL). To monitor data quality, NRL performs confirmatory identification (ID) and antibiotic susceptibility testing (AST) on a subset of isolates, but lacked a standardized method for quantifying discordance and summarizing the findings.

Methods: To define AST error types, we adapted the categorical agreement classification scheme (with NRL confirmatory testing results as the gold standard): minor error (SL-intermediate {I} / NRL-susceptible {S} or resistant {R}, or vice versa), major error (SL-R / NRL-S), and very major error (SL-S / NRL-R). We added an "interpretation" error type to capture incidents of SLs interpreting zone sizes as the wrong SIR category. As an interpretation error may generate a minor, major, or very major error, each isolate-antibiotic combination may have up to 2 AST errors. ID errors were classified as major error (species incorrect) and very major error (genus and species incorrect). We developed a tool to automatically calculate error types and frequencies and analyzed retrospective laboratory data.

Results: The SLs submitted a total of 99 isolates to NRL for confirmatory testing from January 1 to September 30, 2019: 36 *Escherichia coli*, 25 Staphylococcus aureus, 21 *Klebsiella pneumoniae*, 13 *Acinetobacter* baumannii, and 4 of other genera. This represents approximately 28% of the AMR isolate data submitted to the network during the same time frame. Five (5%) isolates had ID errors: 1 major and 4 very major. Thirty-six isolates had a total of 62 AST errors: 8 (13%) interpretation errors, 35 (56%) minor errors, 5 (8%) major errors (ME), and 14 (23%) very major errors (VME). The 5 ME and 14 VME were distributed among 4 and 11 isolates, respectively. AST errors were found across multiple genera: 18 (29%) *E. coli*; 15 (24%) S. aureus; 23 (37%) *K. pneumoniae*; and 6 (10%) other genera. The three SLs each submitted 22, 32, and 45 isolates. The per-lab percent of isolates with ID errors was 0%, 3%, and 9%; the per-lab percent of isolates with major AST errors was 0%, 0%, and 11%; VME were 5%, 3%, and 22%.

Conclusions: In this study 11% of isolates undergoing AST at SLs had one or more VME, results that under-estimated antibiotic resistance, a critical problem for both clinical and surveillance purposes. Applying categorical agreement criteria to proficiency testing results provides a novel framework to systematically evaluate the quality of AMR data. This can be a valuable tool to provide quantitative feedback and guide laboratory improvements in order to improve overall data quality of the AMR surveillance system.

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Poster #16

Evaluating and Standardizing the CDC Infectious Diseases Laboratory Test Directory to Improve Specimen Submission Timeliness and Pre-analytical Quality

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Background: The Centers for Disease Control and Prevention (CDC) Infectious Diseases Laboratory Test Directory (TD) is the externally facing list of test orders that public health labs and other submitters use to submit specimens to CDC for diagnostic and non-diagnostic testing. The purpose of the TD is to provide clear and uniform pre-analytical specifications that expedite specimen submission transit time and ensure high specimen quality. However, per a customer feedback survey, TD content has not always been understood and at times has challenged specimen submission timeliness and pre-analytical quality.

Methods: To address customers' concerns, CDC's Infectious Disease Specimen Submission Change Control Board (IDSS CCB) conducted an in-depth evaluation of the TD content over the span of 20 meetings in four months, identified areas for improvement, and developed standardization guidance for each test order field. Since the TD was developed before the standardization guidance, implementation efforts were scaled to engage associated laboratories for every test order. The guidance was rolled out using a series of educational training sessions that were aligned with two previously scheduled TD update cycles. This was followed by eight months of strategic dialogue (e.g., broadcast communications, direct emails, and phone calls) with laboratory points of contact regarding necessary revisions to TD content to ensure every test order complied with the guidance.

Results: As a result of these efforts, a total of 3,622 changes were reviewed and implemented in the TD during 2019 compared to routine 806 changes in 2018. Of the 3,622 changes, a total of 38 test orders were deleted, 8 added, and 81 test order names revised. The other 3,495 changes were specific content revisions to test order fields such as point of contact, specimen labeling, acceptable sample/specimen type, CLIA regulatory status, shipping and specimen handling instructions, among others. The culmination and implementation of these changes ensured all 316 test orders within the TD were compliant with the standardization guidance by the TD's annual publication date of December 6, 2019. Preliminary evidence also suggests that this effort has helped to better define internal CDC workflows and improve compliance with internal policies and federal regulations.

Conclusions: The purpose of this standardization effort was to improve the content within the TD to better enable customers to submit higher pre-analytic quality specimens to CDC for testing and reduce turn-around-times for submission. The IDSS CCB plans to examine anecdotal data over the next year to track its success in achieving these goals. Moving forward, CDC also plans to share information learned about further impacts of this effort as this process could be replicated by other laboratories to improve their customers' specimen submission quality and processes.

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Poster #18

S-Typeline: A Rapid, Flexible Strain Characterization Service for *Streptococcus pneumonia*

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CDC's Active Bacterial Core surveillance (ABC) activities include "the determination of the incidence and epidemiological features of invasive pneumococcal disease." The description of serotypes and AntiMicrobial Resistance (AMR) phenotypes of invasive pneumococcal disease (IPD) isolates is a critical part of epidemiological surveillance and vaccine formulation.

 Currently, surveillance and serotyping of ABC reported cases are handled internally by CDC streptococcus laboratory workers, using a highly adapted automated approach tailored to our instruments and resources.

To provide this expertise to a national audience we have derived from this a more flexible, faster, serotyping, MLST and AMR description service: StrepTypeLine available through the CDC's OAMD portal.
S-typeline utilizes mostly k-mer and machine learning based tools and is thus not reliant on full assembly to generate accurate MLST, serotype and antibiotic resistance data. This allows for a uniquely high degree of flexibility in NGS inputs, allowing for a wider range of sequencing data to be processed accurately.

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Poster #19

Tinsel – An RShiny Application for Annotating Outbreak Trees

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Across the United States, PulseNet member public health laboratories now perform isolate whole genome sequencing for foodborne pathogen surveillance, a milestone for protecting our food supply, by providing higher resolution genomic data for identifying common sources of contamination. This genomic data is analyzed to determine relationships between pathogen isolates, and the analysis endpoint is often a phylogenetic ("outbreak") tree, which allow scientists to visualize the evolutionary relationships of organisms. During a foodborne outbreak, additional sample and epidemiology data can be represented on trees to help identify potential sources. The importance of visualization and annotation cannot be understated, as these trees are often the basis for discussions between the lab scientists, epidemiologists, and bioinformaticians investigating known and potential outbreaks. However, there is no widely available, user-friendly tool available to create these visualizations. Our goal is the development of an open-source phylogenetic tree visualization and annotation tool, which will be available online or hosted locally. To accomplish this goal, we built Tinsel, an interactive web application, using the R programming language and the R package Shiny. In addition to the free and open source nature of R, R has powerful graphic capabilities along with additional phylogenetic packages that are the gold standard for phylogenetic applications. The input requirements for Tinsel include a tab-delimited genetic distance matrix file based on high quality single nucleotide polymorphism (SNP) or allele counts, a Newick formatted tree file generated from the matrix data, and a tab-delimited file containing sample metadata. Once the base tree image has been displayed, visualization changes can be performed via user inputs. One of the main annotation features is the ability to easily display the genetic distance between isolates or clades on the phylogenetic tree. This annotated phylogenetic tree can then be downloaded in various formats for presentations or publications. Broadly, these images are helpful for epidemiologists, laboratorians, public health officials, and stakeholders when discussing the genetic differences and evolutionary relatedness between isolates and/or clades in response to foodborne outbreaks. A completed version of Tinsel will be available through the CDC OAMD Portal and also as a R package via the Comprehensive R Archive Network (CRAN) with documentation that describes basic usage and sample datasets for labs that require local data analysis. Currently the Tinsel source code, documentation, and datasets are available here: https://github.com/jennahamlin/Tinsel.

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Poster #20

Creation of an Online Newborn Screening Educational Program Using Adaptive Learning

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Newborn screening is a public health program that identifies conditions that can affect a child's long-term health or survival. Early detection, diagnosis, and intervention can prevent death or disability and enable children to reach their full potential.

A well-trained workforce is essential to ensuring that public health laboratories have the capacity to conduct the critical activities of newborn screening to ensure timely detection of affected newborns. High turnover of staff, emerging technologies, implementation of new complex conditions, and policy changes that impact newborn screening practice all point to the need for a comprehensive educational program. Such a program should be targeted at the needs of the evolving newborn screening community to deliver consistent communication of best practices and new content.

One of the challenges in designing educational resources for a community with diverse educational and technical capabilities is how to present content that engages both the novice and the expert learner. To address these needs, CDC's Newborn Screening and Molecular Biology Branch has created an online educational product. CDC has contracted with an educational vendor to develop the product. The educational product uses an adaptive learning platform that adjusts in real-time to the needs of each learner and constantly tailors the lesson content and teaching approach based on the responses of each individual learner. As such, both the novice and the expert can take the same course and have different learning experiences, with the aim of moving both learners towards greater mastery of the course material. The initial release of the educational product includes three modules that cover: 1) Amino Acid Disorders, 2) Severe Combined Immunodeficiency (SCID), and 3) Molecular Methods. These modules will provide Continuing Education credits and will be available free of cost through the CDC TRAIN Learning Network, a comprehensive catalog of online public health training opportunities.

The online newborn screening product will educate public health professionals from US newborn screening programs about the newborn screening system, current and emerging technologies, current and anticipated screened conditions, and policies and issues that impact practice. The product will be on-demand and free with material curated by the CDC. An adaptive learning platform has been shown to increase learner motivation and competency in other biomedical and clinical fields. It is anticipated that this online educational product will reinforce learning newborn screening professionals, incorporate learner self-evaluation, be responsive to variations in learner response, and provide real-time adaptive response to individual learners.

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Poster #21

Hepatitis A Virus Survival on Drug Paraphernalia

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Background: The ongoing hepatitis A outbreak in the United States has concerned public health authorities since March 2017. The outbreak has already spread throughout 30 states and includes primarily persons who use drugs, including persons who inject drugs (PWID) and homeless individuals. Contaminated drug injection paraphernalia and sharing of these items are suspected to be one of multiple causes of hepatitis A virus (HAV) transmission in those populations.

Method: We used a standard plaque assay to investigate HAV infectivity. Liquid suspensions of HAV were tested to examine the effects of time and temperature on viral infectivity. We also examined HAV survival on commonly used drug paraphernalia, such as needles, syringes, cookers, tourniquets, and cotton balls/filters frequently shared among PWIDs. We investigated the effect of low pH on HAV survival using citric acid, which is frequently used by PWIDs during dose preparation. We also compared the plaque assay results with those concurrently obtained by RT-PCR to establish whether viral HAV RNA levels could be used as surrogates for plaque assay results.

Result: We found that HAV suspended in PBS at room temperature was able to infect FRhk4 cells for more than 17 weeks. HAV remained viable in syringes/needles (semi-dry conditions) for up to 10 weeks depending on the size of the needles and the syringe dead-volumes. HAV survival in dry conditions on cooker, tourniquet and cotton balls/filter surfaces did not exceed 4 weeks. HAV retained its infectivity for more than 10 weeks at pH as low as 2. PCR results suggest that RNA is amplified from both infectious and non-infectious HAV.

Conclusion: Our findings show that HAV can survive and remain infective in the PWID setting for 4 to 10 weeks depending on the type of paraphernalia examined. These findings suggest that sharing of drug paraphernalia by PWIDs can potentially facilitate the transmission of HAV within these populations. Moreover, our results confirm that the plaque assay is currently the only reliable method to determine the infectivity of HAV in vitro.

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Poster #22

Establishing a CDC Standardized Approach to Test Deployment

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Public health laboratories conduct diagnostic and surveillance testing using CDC-developed tests under applicable regulations, standards, and quality system requirements. Recent recommendations from the Government Accountability Office (GAO) and the Association of Public Health Laboratories (APHL) have highlighted the need for a standardized process to improve CDC's test deployment practices. To meet these recommendations, CDC convened a chartered working group comprised of internal program subject matter experts with broad experience in laboratory preparedness, test deployment, procedure implementation for diagnostic use or surveillance, collaboration with external partners, and regulatory affairs.

This working group has reviewed elements important to the overall standardization process. Such elements include consideration of the laboratory equipment used by partner laboratories, the availability of reference materials for test validation, the importance of providing procedural details to meet regulatory requirements, and the need for guidance on result interpretation to implement a sustainable and robust deployment process to meet public health laboratory needs.

Here, we provide 1) a brief overview of the objectives, activities, and priorities of the CDC working group to support the standardization of laboratory practices, strategic coordination, and improved communication between CDC and public health partners to streamline test deployment practices, and 2) a venue to obtain feedback on these efforts from state, local and territorial public health laboratory representatives.

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Poster #23

Next Generation Sequencing Quality Initiative

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Next Generation Sequencing (NGS) is being broadly implemented across clinical and state and local public health laboratories, with diverse applications including infectious disease diagnostic testing as well as public health surveillance and outbreak response. Although NGS assays are becoming more routinely available, the laboratory and data analysis workflows being developed across total test processes can be fragmented and complex. This leads to numerous challenges in the implementation of NGS assays in the clinical and public health laboratory settings for test validation, quality control, quality assurance, result interpretation, reporting, and compliance with regulatory requirements such as CLIA. The Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), and state public health laboratories (PHLs) are partnering to develop an NGS-focused quality management system (QMS) to address challenges CDC and state PHLs encounter when they develop and implement NGS-based tests. The initiative will create free, customizable, ready-to-implement guidance documents, standard operating procedures, and forms. This project leverages the ongoing expertise and activities of the CDC NGS Quality workgroup and review board, APHL NGS subcommittee, APHL Laboratory Systems and Standards Committee, and the ad hoc PulseNet Steering Committee. The TCC reaches across CDC laboratories and state PHLs to collect, develop, and distribute QMS tools and guidance to help build this foundational QMS for NGS testing. The TCC conducts interactive discussions to develop standardized quality practices and protocols to ensure NGS test results are consistent, accurate, and adhere to regulatory requirements, where applicable. The TCC develops SOPs, forms, and tools to aid labs implementing quality processes for NGS methods. These documents and tools are housed on a public webpage and are available for use by all laboratories. Topics addressed within these resources include Personnel Management, Equipment Management, NGS Process Management, Internal Laboratory Assessments, Facilities and Safety, and NGS Information Management.

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Poster #24

Data for Workforce Development: What do we have? What do we need?

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Public health and clinical laboratories face an array of challenges in workforce development, including recruitment, retention, and staff training and professional development. While there is an abundance of awareness and extensive, first-hand experience with these challenges within the laboratory community, it is unclear exactly what, and how much, hard data exist regarding the things we think we "know" about public health and clinical laboratory workforce development. To answer this question, we conducted an environmental scan to uncover existing data and gather new data to better understand the laboratory workforce and workforce development challenges. The scan consisted of a literature review as well as interviews, focus groups, and other discussions with CDC staff, external partners, and members of the public health and clinical laboratory communities. We present findings from the literature review, which assessed the amount, quality, and scope of available information related to the workforce and challenges associated with the education, recruitment, professional development, and retention of laboratory professionals. Out of 4,000+ data sources uncovered from searching key words and phrases within four scientific databases and the internet, 227 sources were eligible for inclusion in the literature review by meeting criteria related to topic area, audience, and publication year. Most data— and the most robust data — for both public health and clinical laboratory workforces related to the categorization of each workforce at the national level. However, data were scarce for most other topics, including the awareness and perceptions of careers in laboratory science, best practices in retention, and the root causes of the workforce shortage. We also present themes from discussions with members of the laboratory community and other stakeholders regarding workforce development needs and challenges, particularly in the training and professional development of current laboratory professionals. In total, the environmental scan demonstrates several areas for improvement in the gathering and measurement of workforce development data. This scarcity of information prevents the development of data-driven strategies and efforts that could be used to better identify and address critical needs in the creation and maintenance of a well-trained and skilled laboratory workforce. The findings from the environmental scan will help enhance CDC's engagement with partners in developing goals, programs, and resources to address laboratory workforce development needs at the national level.

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Poster #25

Enhancing National Foodborne Outbreak Response and Surveillance by Building Whole Genome Sequencing Capacity in Public Health Laboratories Through Standardized, Hands-on Training

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Eighty-three federal, state, and local public health laboratories (PHLs) from across the country make up PulseNet, the national network that conducts surveillance and detection of foodborne outbreaks by using bacterial DNA fingerprints. PulseNet detects and investigates approximately 280 foodborne illness clusters annually. In 2019, PulseNet transitioned away from using Pulsed Field Gel Electrophoresis (PFGE) and instead implemented whole genome sequencing (WGS) technology for laboratory-based surveillance. To build and implement WGS capacity effectively across this broad network, it is imperative to have a trained and competent laboratory workforce performing standardized protocols. To achieve this goal, PulseNet has been collaborating with the Association of Public Health Laboratories (APHL) to host WGS training workshops since 2014. During the week-long, hands-on workshop, public health professionals learn to perform DNA extraction, prepare genomic libraries, set up and perform sequencing runs, and perform maintenance of sequencing equipment. Attendees are trained on WGS analysis using Applied Maths BioNumerics software and participate in associated hands-on analysis exercises. This customized combination of wet and dry lab training provides the knowledge and skills required to perform and troubleshoot whole genome sequencing and analysis, to interpret data critically and then to utilize this data to detect and investigate foodborne outbreaks. Trainees return to their home laboratories prepared with the skills necessary to pass PulseNet's certification program. Subsequently they are able to generate, analyze and share sequencing data from enteric pathogens and are capable of training other public health professionals in their laboratories.

PulseNet WGS workshops are hosted at CDC up to three times per year and, while standardized, are continually improved upon to meet the needs of the PHLs as WGS technology evolves and PHLs adapt their workflows. Surveys are provided to each attendee at the end of the workshop and the feedback is used to improve subsequent trainings. Training checklists, job aids and handouts have been developed to aid in both the administration of these courses and to assist in trainee learning. To date, PulseNet has trained over four hundred laboratory scientists on WGS methods and certified 65 PulseNet labs to perform WGS. In this way, CDC and PulseNet are ensuring that public health laboratories are utilizing the latest technology and state-of-art knowledge to improve their ability to detect, investigate, and stop foodborne illness.

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Poster #26

CDC Trioplex Real-time RT-PCR Assay: Determining Reagent Stability to Maximize Use, Minimize Waste, and Maintain Preparedness

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Reagent availability is critical for public health laboratories' preparedness to respond to outbreaks. Short reagent shelf-life may limit the availability of these reagents. The CDC Trioplex Real-time RT-PCR Assay (Trioplex rRT-PCR) was authorized for Emergency Use by the Food and Drug Administration (FDA) March 17, 2016 in response to the Zika virus outbreak. Shortly thereafter, the CDC's Laboratory Preparedness and Response Branch (LPRB) began real-time classical stability studies on the assay reagents to determine the shelf-life and in-use stability. The original shelf-life of the reagents was two years from date of manufacture (DOM) at 2-8°C for the Trioplex rRT-PCR Primer and Probe Set (KT0166) and one year from DOM at -20°C for the Trioplex rRT-PCR Positive Control Set (KT0167). All shelf-life and in-use stability testing was performed in accordance with LPRB's Product Release Testing for the CDC Trioplex rRT-PCR Primer and Probe Set (KT0166), Positive Control Set (KT0167), and Verification Panel work instruction, as described in the approved stability testing study plans. For shelf-life testing, one kit from each of three lots was tested in triplicate at each time point. For in-use stability testing, one kit from one lot was tested in triplicate at each time point. The average Cycle Threshold (Ct) value at each time point was calculated and compared to the defined acceptance criteria. With the testing performed to date, the shelf life of KT0166 has been extended from 24 to 30 months, and the shelf-life of KT0167 has been extended from 12 to 24 months. In addition, KT0167 has in-use stability of 24 months when stored at 2-8°C or when rehydrated and stored at -20°C. Currently, there are thousands of primer and probe vials in inventory that have not been assembled into kits. Continued stability testing will allow us to maximize the expiration date of these reagents. Long-term stability data will support extension of expiration dates of reagents already assembled into kits to maximize use of these valuable reference reagents. At the time of this shelf-life extension, there were 156 positive control kits in inventory that were about to expire, and a production delay with the new lot of positive control. With the approval of the shelf-life extension, the reagents in inventory were able to be reworked by CDC's Division of Scientific Resources (DSR) to include the new 2-year expiry dates, thereby, preventing a lapse in availability of the positive control reagent to laboratories. Extension of the shelf-life of Trioplex rRT-PCR reagents allowed CDC to make use of available inventory reducing manufacturing cost and prevent a lapse in reagent availability that would have brought public health laboratories' ability to test for Zika virus using this assay to a stop.

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Poster #27

Skip the Isolate: Evaluating the Risks Associated with *Neisseria gonorrhoeae* During DNA Extraction From Clinical Specimens

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Neisseria gonorrhoeae, the causative agent of gonorrhea, has developed resistance to nearly every class of antibiotic recommended for treatment; thus, antibiotic resistance data are important for both diagnostics and surveillance. Since most clinical specimens do not have associated isolates, our laboratory is exploring the ability to detect antimicrobial resistance determinants directly from genital and extragenital (e.g., pharyngeal, rectal) clinical specimens. These specimens could introduce laboratory hazards associated with exposure to *N. gonorrhoeae* and in some case *N. menigitidis*. The first step to processing these clinical samples involves DNA extraction. Here, we evaluated the potential risks associated with four DNA extraction procedures available in our laboratory (QIAmp DNA mini kit-both manual and QIAcube automated platform; iPrep PureLink gDNA Blood kit-automated; and QIAsymphony DSP kit-automated). We identified a total of 34 potential hazards, and each was assessed for biological, chemical, or physical risk before and after mitigation; only hazards identified in more than one procedure were included in the analysis. Before mitigation, 62.5% of the hazards were associated with moderate to high risk. Based on mitigation measures included in the written procedures, risk was reduced for all hazards; however, residual risk did remain. Due to the number of potential hazards, we conducted a Pareto analysis to identify hazards with the highest risk, which if properly addressed, should maximize risk reduction with minimal staff effort. This analysis revealed three potential ways to mitigate risk when handling clinical specimens: 1) all work must be done in the BSC, 2) adjusting the placement of equipment during DNA extraction can prevent/minimize aerosolization of biological hazards, 3) specimen transfer should include glove changes and secondary containment. Incorporating these modifications into procedures for extracting DNA from clinical specimens can reduce the risk of laboratory-acquired infections with *N. gonorrhoeae* and many other pathogens that may be present in these specimens.

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Poster #28

Innovative Approach to Laboratory Risk Assessments

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Many laboratories use a lengthy, standardized form as the basis of their risk assessments. A new approach using a risk assessment tool utilized on construction sites would prompt laboratory scientists to incorporate the act of assessing risk present in day to day lab activities and keep those identified hazards in mind as they are working.

At first glance, a laboratory and a construction site do not have much in common. A construction site is loud, exposed to the elements, and dirty. Laboratories are clean, climate-controlled spaces with tightly controlled access. However, when you look closer, there is a great deal in common with these two work environments. Both work environments demand the use of personal protective equipment, use large instruments and equipment, and require an understanding of the hazards present to work safely. These industries both try to mitigate the hazards by performing risk assessments.

Many laboratories perform risk assessments by utilizing a form that attempts to outline a proposed laboratory activity and assign an overall assessment of the activity's risk. A risk matrix is utilized to quantify the severity and probability of a non-conforming event occurring in order to determine the overall risk of the activity or procedure. Risk assessments in laboratories are typically performed when a new assay or activity is planned and are reviewed annually for changes.

The risk assessment utilized at a local construction site is formatted differently but could be easily adapted to laboratory work. The construction site risk assessment tool asked three very simple questions to encourage workers to think about the activities being performed and what risks were associated with those activities. These questions included "What tasks are we doing today?", "How could we get hurt?", and "How can we keep from getting hurt?". A simple, three step risk assessment like the one used at the construction site could encourage laboratory scientists to think about the risk associated with their daily activities. This approach may be more useful for day to day laboratory activities and help laboratory scientists incorporate risk assessment into their daily routine.

Presenter: Shannon York, Centers for Disease Control and Prevention, Atlanta, GA, Email: phy3@cdc.gov

Poster #29

Targeted Enrichment of Pathogenic *Escherichia coli* DNA From Stool Samples for Metagenomic Subtyping Analysis

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Culture-independent diagnostic tests allow for efficient diagnoses of foodborne pathogens, but do not yield isolates critical for outbreak surveillance and investigation. Shotgun metagenomic sequencing allows pathogen subtyping directly from stool, but its practicality for routine public health surveillance activities is limited by the costs associated with deep sequencing and complex analytical pipelines required to resolve low pathogen signal to high stool background noise. For pathogens such as Shiga-toxin producing *Escherichia coli* (STEC), this is further complicated by the similarities between pathogenic STEC and commensal *E. coli* genomes. Shotgun metagenomics is further limited by challenges with phasing, or the ability to differentiate between, pathogenic and commensal *E. coli* sequences.

We tested the Roche HyperCap bait capture system to enrich for STEC DNA. The baits were designed by tiling across two target STEC O157:H7 genomes (F7353 and K4623), excluding the 16S rRNA genes. Enrichment was tested using mock disease stool, prepared by spiking health stool DNA with 2% target STEC isolate DNA. To characterize phasing issues, we applied baits to DNA samples that were equal mixes of two different *E. coli* isolates that include the target STECs, a non-target STEC, and two commensal *E. coli* strains. To distinguish the different components, DNA libraries from stool and isolates were indexed separately before mixing. Samples were sequenced before and after bait capture using an Illumina MiSeq. The sequences were aligned against the reference genome F7353 using Bowtie2 and coverage was assessed using R. High quality SNP analysis was performed using lyve-SET (https://github.com/lskatz/lyve-SET).

The proportion of STEC DNA in the mock disease sample increased from 2% to 93% after bait capture, and 97% of the enriched STEC reads mapped to the reference STEC genome, indicating high target specificity. Bait captured STEC sequences also showed high breadth of coverage across the whole genome. The baits did not preferentially enrich for any one *E. coli* strain, but bias from the bait design was observed: coverage maps show that regions in the genome encoding mobile genetic elements were over-enriched. How this regional bias contributes to subtyping strains is currently being analyzed.

The bait capture system showed promising results in enriching STEC DNA from a complex stool background. From low levels of pathogen input, the enriched DNA showed high breadth whole genome coverage that is necessary for subtyping using SNP analysis. However, because the baits cannot distinguish different strains of *E. coli*, they can also enrich for commensal *E. coli*, making subtyping analyses more difficult. Ongoing work includes performing SNP analysis on data generated from bait captured samples containing two different *E. coli* strains and understanding how bait bias may affect analysis.

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Poster #30

Resolution of Intermittent Calibrator Control Fails of a 4th Generation HIV-1/2 Ag/Ab Combo Immunoassay at a Paper-based Laboratory

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Continuous monitoring of quality control is an important part of quality assurance (QA). However in the absence of an electronic laboratory information system (LIS) or sufficient personnel, this QA practice is often difficult to maintain. The Public Health Laboratory at Dallas County Health and Human Services (DCHHS) is a 25 person laboratory that processes HIV tests using the CDC recommended algorithm, starting with a 4th generation HIV-1/2 Ag/Ab Combo immunoassay (HIV Combo). Here we present a case study of a QA investigation at DCHHS. The investigation was triggered several months after increased but intermittent calibrator control fails of two subsequent lots of HIV Combo kit with fail rates of 5% and 10%, as compared with the 0% fail rate at baseline. The increased calibrator control fails led to unexpected spending of $26,937 in the re-running of failed plates. More common causes of assay fails such as faulty instrument, improper reagent storage, operator error were ruled out. To identify the potential cause of the fails, quality control, calibrator, operator and other data from 7 months of testing, representing over 185 runs, were manually inputted into the statistical software JMP SAS from instrument print-outs of each HIV Combo run. Statistical tests identified the HIV Combo lot, Wash buffer lot, and Stop solution lot as having significant statistical correlation with the failed runs. As a corrective action, we directed the use of new lots of both the current HIV Combo and Wash buffer lots which reduced the calibrator control fail rate from 10% to 1.8%. We also changed the Stop solution lot but observed no additional decrease in calibrator control fail rates. Measurements of turn-around time between Test Order and Test Result increased from 1.4 days at baseline, to 1.6 days and 2.4 days with the defective HIV Combo kits, to 1.7 days during the corrective action. Thus while maintaining patient testing, we were able to conclude that the HIV Combo Lot or the Wash buffer lot, together or individually, contributed to the increases in failed runs and our corrective action reversed negative trends observed during testing. Lastly, the data generated from this investigation enabled us to obtain a $26,937 refund from the manufacturer. Thus this case study highlights the critical importance of continuous monitoring of quality control for quality assurance of laboratory services. Paper – based laboratories should strive to allocate resources to maintain an effective quality assurance program by reallocating duties of existing personnel to include quality assurance, seeking additional funding for new personnel, or implementation of an electronic LIS to reduce waste and improve the quality of laboratory services.

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Poster #31

Supporting Instrument Interfaces for Public Health Laboratories

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Public health laboratories (PHLs) use instruments with a diverse set of capabilities and complexity that integrate with LIMS applications. Existing instrument interfaces are rigid, often proprietary, and tightly coupled. Development and maintenance of such interfaces is complex, time consuming, and dependent on third-party software, as well as unnecessarily expensive. These factors can deter PHLs from implementing instrument interfacing. An architecture that is loosely coupled, reusable, and able to support agile implementations can be a cost-effective and sustainable alternative for any laboratory. J Michael consulting (JMC) is collaborating with the South Carolina Department of Health and Environmental Control (DHEC) and the OpenELIS Foundation to integrate DHEC's laboratory instruments with its OpenELIS application using Rhapsody, a high-performance interoperability engine that is capable of electronic transfer, conversion, storage, or display of data from devices/applications. Rhapsody has built-in standard communication adaptors to connect with instruments. The Rhapsody components are portable, modular, and reusable. These features give the flexibility of a plug-and-play model to integrate different instruments with minimal development effort. The JMC team will implement DHEC interfaces in phases. In Phase I, JMC will develop a prototype of the bi-directional interface with OpenELIS and Abbott Architect. The interface will import run setup information from OpenELIS, validate, transform, and convert the data to an instrument-readable format. Once the instrument completes its test run, the interface will read the results, validate, transform, and process the results data into the OpenELIS backend. In Phase II, JMC will decrease the development effort for the remaining 20 instrument interfaces by using the portable and reusable components developed during Phase I. Notably, the integration will be the same for each instrument type; for example if there are 5 GC/MS instruments, JMC will develop only one Rhapsody route. The interfacing for all the instruments at the DHEC laboratory with OpenELIS can be achieved by leveraging the existing Rhapsody engine, a software that DHEC already utilizes for electronic messaging with various partners. This approach will not only eliminate the upfront cost of third-party software, it will also allow DHEC to flexibly use existing communication adaptors and functions in a cost-effective manner. Moreover, it provides ownership of the interfaces to the laboratory, rather than to a vendor, and with improved performance and reliability. DHEC will be able to customize, maintain, and adapt reusable Rhapsody instrument interface templates to instruments and LIMS applications throughout the enterprise.

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Poster #32

Ongoing Disseminated Neisseria gonorrhea Outbreak Within Michigan, 2019

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Background: Neisseria gonorrhea is a prominent communicable disease in the United States resulting in 583,000 reported cases annually. Among all reported cases, approximately 0.5-3% of patients will develop a disseminated gonococcal infection (DGI), which occurs when *N. gonorrhea* enters the bloodstream and disseminates to distal sites within the body. These rare infections can present as bacteremia, septic arthritis, or in severe cases, endocarditis or meningitis. Due to increasing antibiotic resistance for *N. gonorrhea*, CDC has classified drug-resistant cases to be an urgent threat in the 2019 antibiotic resistant threats report. Currently, there is no routine surveillance for *N. gonorrhea* DGI cases in Michigan.

Methods: Since August 2019, there have been 16 DGI cases in Michigan associated with an ongoing investigation. Whole genome sequencing was performed on a total of 22 *N. gonorrhea* isolates; ten isolates from the ongoing investigation, ten additional DGI and *N. gonorrhea* cases from 2019, and two historic isolates from 2018. Bioinformatic analyses were performed to examine relatedness of isolates based on pan genome and single nucleotide polymorphism (SNP) analysis. Extraction of antimicrobial resistance genes and multi-locus sequence typing genes was performed using Abricate.

Results: Phylogenetic trees based on pan genome and SNP analysis identified two clusters of isolates. A large cluster of 11 related isolates that were 0-20 SNP different and a smaller cluster of four isolates with 0-6 SNP difference. Within the large cluster, ten isolates are part of the ongoing investigation and were typed as ST-7822. Antimicrobial profiles identified a high frequency of penicillin (100%) resistance genes, as well as, resistance genes present for tetracycline (4.5%) and ampicillin (18.2%).

Discussion: This data highlights a rare ongoing outbreak of DGI cases. The relatedness of DGI isolates and the presence of a diverse range of antimicrobial resistance genes supports the need for DGI surveillance and sequencing analysis to help identify cases that should be pursued for epidemiological linkage and monitor the presence of antibiotic resistance genes.

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Poster #33

Increased Prevalence of Bovis Bacille Calmette-Guérin Strain Isolates from Sterile Sites: A 2010-2019 Michigan Study

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Background: The attenuated *Mycobacterium bovis* strain Bacille Calmette-Guérin (BCG) is widely known as the vaccine strain used for tuberculosis (TB) prevention. BCG is also used therapeutically for the treatment of non-muscle invasive bladder cancer. While literature describes disseminated BCG infections as rare phenomenon, there has been an observable increase in isolates cultured from sterile sites such as bone and whole blood submitted to the Michigan Bureau of Laboratories, suggesting dissemination. A BCG isolate retrospective review of laboratory records from 2010 to 2019 was performed in order to determine the prevalence of isolates collected from disseminated cases.

Methods: A BCG disseminated case was defined as a positive culture originating from any site excluding urine or vaccination injection sites (i.e. arm wound). Initial identification to the *Mycobacterium tuberculosis* complex level was performed by HPLC or MALDI-TOF. *M. bovis* identification was made through biochemical testing of niacin, nitrate reduction, pyrazinamidase, and TCH growth characteristics. The designation of BCG was given when MIRU pattern 2y2324253322 and MIRU2 pattern 051352253205 was observed. Source, age at time of culture, and gender were determined using the information system STARLIMS. If duplicate cultures were submitted, only the first per source type was included.

Results: Within the nine-year retrospective study, 43 BCG isolates were identified. The patient profile was predominantly male (41 male and 2 female) with a median age of 73 (ranging from 2 to 95 years of age). Of the 43 isolates, 47% (20 of 43) were from disseminated sites such as blood, spinal tissue, and bone. A steady increase in isolates was observed, topping to a total annual number of 9 in 2019. Interestingly, half of the BCG isolates cultured from disseminated sites occurred within 2018 and 2019 alone. For both years, disseminated infections comprised of 67% of the total BCG isolates received.

Conclusion: Males are described as having a higher prevalence of non-muscle invasive bladder cancer which likely explains the disproportionately high male representation within the review. An interesting and unexpected find was the rise in disseminated infections particularly within the last two years of the study. This observed phenomenon may be due to multiple factors including an ageing population that may be receiving more bladder cancer treatment. Unfortunately, there was incomplete data as to whether the isolates captured from disseminated sites were from current or prior BCG immunotherapy patients. Other potential explanations include increased usage of immunomodulating biologics that may increase the risk of disseminated BCG in those patients receiving BCG therapeutically. Further work is currently being conducted to gather more patient history data from our retrospective investigation. Overall, our findings emphasize the importance of identifying previous BCG therapy recipients for the consideration of disseminated infection.

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Poster #34

Updated: Laboratory Response Network-Chemical (LRN-C) Level 3 Resource Handbook

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The APHL Chemical Threat Collaborative Workgroup (CT-CWG) developed the Laboratory Response Network-Chemical (LRN-C) Level 3 Resource Handbook as a guidance document for LRN-C partner members and coordinators who provide Level 3 outreach to healthcare facilities, first responders, and any other agency expected to respond to a chemical release resulting in human exposure. As defined by the Centers for Disease Control and Prevention (CDC) Level 3 responsibilities contain specific funded benchmark Level 3 capabilities. APHLs Environmental Health Committee (EHC) and the CT-CWG provided subject matter expertise for development and maintenance of a universal guidance document designed to provide practical information to be used as recognized methods to meet these benchmarks, for development of robust Level 3 outreach and training programs, and to help identify and close any gaps in their current jurisdictional programs.

Since the first version release in November 2014, updates and improvements were made in 2015 and 2016. The EHC proposed a handbook refresh in late 2016 with suggestions for short- and long-term goals that would enhance and update this resource for Level 3 outreach programs. The short-term revisions were completed in 2017 and the long-term enhancements were completed and approved in November 2019.

Production and enhancements to the Level 3 Resource Handbook were organized by volunteer efforts of the CT-CWG members, by meetings hosted through an APHL SharePoint site, and represents a collective compilation of experience-based recommendations from the APHL EHC and CT-CWG.
This poster presents the culmination of these efforts and portrays the transformation of this working document; the most recent version of the Laboratory Response Network-Chemical (LRN-C) Level 3 Resource Handbook.

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Poster #35

Molecular Characterization of *Neisseria gonorrhoeae* Strains in High Prevalence Jurisdiction Exhibits Clonal Lineage with Decreased Susceptibility to Azithromycin

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Background: *N. gonorrhoeae* (GC) has emerged as a global threat with an estimated 550,000 drug resistant Gonorrhea infections per year in the U.S. GC has developed resistance to almost all antimicrobials, including third-generation cephalosporins and azithromycin. Antibiotic resistant *N. gonorrhoeae* (ARGC) are associated with key molecular traits; e.g. accepting/integrating exogenous DNA into genome, acquisition of macrolide resistance from commensal Neisseria, low level resistance by mutation and enzymatic modification, upregulation of Efflux pumps, and high level resistance by point mutation, thus contributes to emergence of resistance. GC strains with evidence of evolution of ARGC, antimicrobial resistant variants with elevated MICs, and those collected from same patient at different body sites or repeat infection yields distinct strains were analyzed.

Methods: A subset of *N. gonorrhoeae* clinical isolates (n=97) received during the period of 2017-18 was selected based on phenotypic antibiotic susceptibility (AST) profiles, specimen sources, high risk patient cohort and whole genome sequening (WGS) performed on Illumina MiSeq platform at Dept. of State Health Services Lab, Texas. Parsnp was utilized to align GC core genomes assembly to study evolutionary hierarchy and genetic polymorphism. PubMLST databases were used for molecular typing and microbial genome diversity. Average Nucleotide Identity (ANI) allowed finding nucleotide-level genomic similarity between the coding regions of unknown strains against reference strain. NG-MAST sequence types were interpreted using CosmosID bioinformatics pipeline.

Results: Single nucleotide polymorphism (SNP)-based phylogenetic tree was generated upon core genome analysis grouped 97 isolates into 31 sequence types (STs) consisting of 3 clusters/clades with less than 10 SNPs. The most common STs were ST-9363 (n=16; 16.5%), and ST-1579 (n = 13; 13.4%). Isolates with decreased susceptibility to azithromycin were associated with ST-1579 (n=12), ST-12093 (n=2), and ST-9363 (n=1). None of the study isolates had reduced susceptibility to Cefixime or Ceftriaxone. Ciprofloxacin resistance was associated with ST-1579 and ST-7363. AMR markers penA, blaTEM, tetM, and 23S rRNA variants were also detected.

Conclusions: A wide variation in sequence types was obtained in surveillance jurisdiction, however almost all isolates with decreased susceptibility to azithromycin belonged to ST-1579 and also expressed gene markers for Ciprofloxacin resistance. This analysis demonstrates the necessity to incorporate advanced molecular methods like next generation sequencing (NGS) as a routine surveillance tool to aid in molecular epidemiology, evidence-based antibiotic treatment and better characterize sexually-transmitted disease clusters and/or public health outbreaks involving ARGC.

Presenter: Joshua Weiner, City of Milwaukee Health Department, Milwaukee, WI, Email: jweine@milwaukee.gov

Poster #36

Prevalence of *Mycoplasma genitalium* in High Risk Patients Seeking Testing at a Local Public Health Laboratory

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Introduction: *Mycoplasma genitalium* (MG) is a slow growing, self-replicating bacterium, lacking cell wall and predominantly found in urogenital tract of both sexes. The infection may persist asymptomatic for months or years in individuals, or can be found in men with non-gonococcal urethritis and women with cervicitis and pelvic inflammatory disease. Early diagnosis and effective treatment are, therefore, important in preventing sequelae and ongoing transmission, particularly the transmission of drug-resistant strains to sex partners. Current treatment guidelines are inconsistent about the need for presumptive treatment of sexual contacts of *M. genitalium*–infected patients, which further complicates as found to be co-infected with commonly known sexually transmitted pathogens. Implementation of nucleic acid amplification (NAAT) assay improves monitoring disease prevalence, co-STI infections and implement best clinical practices.

Methods: Aptima® analyte specific reagents (ASR) validation for MG NAAT involved 219 residual clinical specimens those previously tested for chlamydia, gonorrhea, trichomonas and/or herpes simplex virus were tested on the Panther® System (Hologic, Inc. Marlborough, MA). A variety of sample types (urine, urethral, vaginal, rectal) and specimen transport tubes (Aptima® Urine, Unisex and Multitest Specimen Transport Tube; MicroTest™ M4 Transport Tube) were validated in this process. Subsequent Aptima® FDA-cleared IVD assay verification involved the use of 81 pre-qualified specimens (45 clinical specimens, 36 Validation Panel samples through Hologic, Inc.).
Samples received by STD clinic laboratory were analyzed for MG surveillance, monitoring positivity rate and co-infections.

Results: MG was detected in 148 of 763 samples tested. 15% of the MG positive patients presented with at least one laboratory confirmed STI co-infection (chlamydia, gonorrhea, trichomonas, and herpes). There were more number of men tested than women and urine was the most common specimen type to be tested for MG. The sensitivity of the assay was comparable between urine and urethral swab in males. We only had one rectal swab submitted which turned out to be negative.

Conclusion: Overall prevalence of *M. genitalium* was determined to be 19%, which is significantly higher than national gonorrhea or chlamydia prevalence. Prescription practices by the STI physicians has been optimized based on this data by treating non-specific urethritis (NSU) with doxycycline; recurring infections are treated with moxifloxacin. Prior to launch of the MG assay, NSU and PID patients were empirically treated based on symptoms. Major achievement in public health practice is adequate treatment for high-risk STI public health clinic population. Our laboratory and STI clinic is exploring impact of macrolide resistance testing on jurisdictional patient cohort.

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Poster #37

Comparing Drug Profiles in MNDOSA and EVALI ED Encounters

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In 2017, the Minnesota Department of Health (MDH) created the Minnesota Drug Overdose and Substance Use Pilot Surveillance Activity (MNDOSA) to respond to the misuse of drugs epidemic. This ongoing overdose surveillance system combines the reporting of drug overdoses with enhanced toxicology testing of clinical specimens by the MDH Public Health Laboratory (PHL). The analytical testing panel includes 266 drugs or their metabolites, including novel substances. During 2019, MDH-PHL analyzed 183 MNDOSA-related clinical specimens from 97 emergency department (ED) encounters, resulting in the detection of 100 different drugs. Multiple drugs were detected in all specimens, indicating that polysubstance use was prevalent.

Beginning in August 2019, MDH began investigating the novel outbreak associated with e-cigarette, or vaping product use-associated lung injury (EVALI). This investigation involved reviewing medical records, interviewing effected individuals, and obtaining clinical specimens to be analyzed at MDH-PHL. Testing of the clinical specimens was performed utilizing the MNDOSA enhanced toxicology testing platform. MDH-PHL analyzed 73 EVALI-related clinical specimens from 64 ED encounters. Multiple substances were detected in all EVALI cases.

MDH-PHL data shows that the detection pattern between MNDOSA and EVALI encounters are vastly different. MNDOSA specimens on average had 10 drugs detected with the most prevalent being amphetamine (67%), methamphetamine (63.9%), norephedrine (54.6%), THC-COOH (36.1%), and fentanyl (28.9%). Caffeine and cotinine were also present in 96.9% and 95.9% of specimens. In comparison, an average of 5 drugs were detected in EVALI specimens, with the most prevalent being THC-COOH (81.3%), followed by acetaminophen (53%), ondansetron (39.1%), and lorazepam (26.6%). Caffeine and cotinine were present in 37.5% and 50% of specimens. It should be noted that ondansetron, acetaminophen, and lorazepam may have been administered during treatment prior to sample collection, and may not be related to substance misuse.

The MNDOSA and EVALI data sets reflect the importance of having access to broad clinical sample testing protocols. By leveraging our existing capabilities from MNDOSA, MDH-PHL was able to rapidly respond to EVALI and provide relevant information about an emerging novel outbreak. Providing chemical testing results back to medical professionals proved to be useful in characterizing and understanding the various symptoms and treatment options.

As the burden of drug overdose and substance use continues to increase nationwide, advancing our understanding of the chemicals involved is imperative. Tracking of drug overdoses, substance use clusters, and chemicals related to these incidences will give public health officials a better understanding of the changing drug landscape and allow more informed and faster responses.

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Poster #38

Molecular Epidemiology of the First NDM-1-producing *Klebsiella pneumoniae* Outbreak in Minnesota

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New Delhi-metallo-β-lactamase (NDM) is an enzyme, found in gram negative bacteria, capable of hydrolyzing penicillin, cephalosporin, monobactam, and carbapenem classes of antibiotics; which contributes to bacterial resistance to these antimicrobials. Though uncommon in the United States, NDM-producing *Enterobacteriaceae* are more common in healthcare settings in other countries and are an emerging public health threat. In Minnesota, fewer than 10 cases were reported annually from 2012-2018 most of whom received recent healthcare abroad. Between December 2018 and May 2019, the first outbreak of NDM-producing *Klebsiella pneumoniae* occurred in Minnesota among eleven cases with an epidemiologic link to one long-term care facility, and without patient history of international travel. One case also harbored an NDM-producing *Escherichia coli*.

Isolates were obtained from 9/11 cases. Whole genome sequencing (WGS), using the Illumina platform and single nucleotide polymorphism (SNP) analysis, demonstrated relatedness between all nine *K. pneumoniae* isolates with SNP differences ranging from 1-18. WGS analysis also determined that all *K. pneumoniae* isolates were multi-locus sequence type 147 and harbored the blaNDM-1 gene. Additional genes conferring resistance to β-lactams, aminoglycosides, fluoroquinolones, phenicol, sulphonamide, and trimethoprim were identified in all nine isolates. Antimicrobial susceptibility testing by broth microdilution confirmed resistance to β-lactams, fluoroquinolones, and aminoglycosides per CLSI guidelines; and found MICs ≤0.25 μg/mL for colistin, polymyxin B, and tigecycline (no available CLSI breakpoints). Bioinformatics analysis identified two IncF family plasmids (IncFIB(pKPHS1), IncFIB(pQil)) and an IncR plasmid in all the isolates. IncF family plasmids have been associated with extended-spectrum β-lactamases and genes encoding resistance to quinolones and aminoglycoside.

Detailed epidemiological and WGS data point to clonal spread of NDM-producing *K. pneumoniae* in this outbreak. However, since NDM is typically found on a plasmid, there is potential for horizontal gene transfer of antibiotic resistance between different bacterial genera. We did find two different genera of NDM-1-producing organisms (*K. pneumoniae* and *E. coli*) with a potential common plasmid. Additional analysis including long-read sequencing is required to demonstrate the presence of plasmids and potential relatedness. Genomic characterization of outbreak isolates is useful in understanding the mechanisms of transmission and the scope of spread. This can inform outbreak response and assessment of infection control interventions.

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Poster #39

Assessment of the Instant Pot® for Suitability in Sterilizing Mobile Laboratory Biohazardous Waste

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Proper disposal of biohazardous waste remains a significant challenge for mobile laboratories, particularly those performing surveillance and/or diagnostics for high consequence pathogens. The identification of portable, low cost solutions for sterilizing biohazardous waste generated by laboratory activities is therefore a priority. A recent publication determined that the commercially available 8-qt Instant Pot® pressure cooker was capable of inactivating *Geobacillus stearothermophilus* spores, a commonly used biological indicator (BI) for validating steam sterilization. The study was limited, however, in that 1) it only tested one of the available pressure cooker sizes, and 2) the conditions under which spore inactivation were tested did not accurately replicate how biohazardous waste is typically packaged for inactivation and disposal. We therefore assessed the ability of two different sizes of Instant Pot® to inactivate *G. stearothermophilus* spores packaged in different substrates as per the biohazardous waste commonly generated by surveillance and diagnostic activities conducted using mobile laboratories.

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Poster #40

A Point-of-care Device for Isothermal Amplification Detection of *Candida auris*

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Candida auris is an emerging, multidrug-resistant pathogen that has caused outbreaks in healthcare settings among patients with complex healthcare needs. This pathogenic fungus is difficult to identify by standard laboratory methods and misidentification may lead to inappropriate clinical management. Moreover, delayed identification of *C. auris* in healthcare facilities can lead to more expansive and damaging outbreaks that are difficult to bring under control. Thus, there is a significant need for a low complexity, point-of-care device that can reliably and inexpensively detect *C. auris* in skin swab samples. The T1-isothermal (T1-ISO) platform uses an integrated consumable designed for swab samples. Our goal is to adapt the T1-ISO for rapid point-of-care detection of *C. auris* using custom-designed, isothermal amplification assays. To address this, we leveraged a novel bioinformatics approach, previously developed by MRIGlobal, known as TMArC, to reveal motif DNA sequences that differentiate *C. auris* from related fungi for assay development. TMArC can be used to identify genetic markers that effectively differentiate *C. auris* from related species. This information is then directed through an automated bioinformatic pipeline which predicts oligonucleotide sequences for high efficiency LAMP assays compatible with the T1-ISO platform. Our long-term goal is to evaluate LAMP assays on the T1-ISO with clinical skin swab samples and compare performance of the T1-ISO to the established qPCR assay. We envision that our overall approach will be amenable to development of point-of-care tests for other infectious diseases.

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Poster #41

A High-Quality, Hybrid Whole Genome Sequencing and Analysis Pipeline for Characterization of Resistance Profiles in *Mycobacterium tuberculosis*

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The WHO estimates that in 2017, 3.6% of new cases of tuberculosis cases and 17% of previously treated cases were multi-drug resistant. Of those, an estimated 8.5% of cases were extensively-drug resistant. The prioritization of research that allows for characterization of resistance profiles to inform drug treatment of these cases remains crucial. Here, MRIGlobal presents a whole genome sequencing and analysis pipeline for the National Institutes of Health (NIH-DAIDS) *Mycobacterium tuberculosis* (Mtb) Quality Assessment Program (TBQA), in partnership with Johns Hopkins University (JHU) to produce high-quality MTb genomic assemblies. These data seek to connect genomic markers to drug susceptibility. To date, 51 clinical isolates have been received from JHU for analysis. We present an initial set of 11 priority isolates, selected based on drug susceptibility profiles, collection regions, and other phenotypic findings. Using carefully extracted gDNA, the pipeline utilizes both the Oxford Nanopore MinION and the Illumina MiSeq platforms to create a hybrid genome assembly of long reads and short reads. Results are analyzed for predicted markers of antibiotic resistance and cross referenced with existing AST data provided by JHU. This data will contribute to the body of research concerning identification of genetic elements potentially associated with resistance to specific classes of antimicrobial agents. These repository results will supplement ongoing NIH-DAIDS clinical trials by adding to the completeness and quality of the characterization data available for each isolate.

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Poster #42

Development and Validation of a Multiplexed, Custom AmpliSeq Panel for Antimicrobial Resistance Characterization of Isolates for the NIH *Mycobacterium tuberculosis* Quality Assurance Program

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The World Health Organization (WHO) estimates that in 2016, 490,000 people developed multidrug-resistant tuberculosis (MDR-TB). However, only an estimated 1 in 5 MDR-TB patients receive the correct antibiotic regimen and better, more cost efficient diagnostic tools are essential for detecting and treating MDR-TB patients. Here, MRIGlobal, in collaboration with Johns Hopkins University (JHU) and Illumina, Inc, presents a multiplexed amplicon-based sequencing panel to determine antimicrobial resistance (AMR) for clinical *Mycobacterium tuberculosis* (Mtb) isolates for the National Institutes of Health (NIH-DAIDS) *Mycobacterium tuberculosis* Quality Assessment Program (TBQA). The panel leverages Illumina's AmpliSeq technology and iSeq 100 and MiSeq platforms in conjunction with established single nucleotide polymorphisms (SNPs), insertions, and deletions in the Mtb genome that have previously been shown to correlate with phenotypic AMR detection using standardized culture-based detection systems. We present this panel in an effort to provide guidance and better information to help better diagnose and treat the unique AMR profile of each patient's disease.

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Poster #43

Mosquito-borne Disease Impact of Environmental and National Disasters: Lessons Learned from Brazil and Mozambique

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Natural and environmental disasters similarly cause significant changes in the environment including flooding, contamination of water supplies and displacement of human populations that can affect the ecosystem in unpredictable ways. The environmental impact of these disasters can potentially increase risk factors for mosquito-borne disease transmission that can result in serious public health problems long after the initial impact of the disaster. We aimed to investigate the infectious disease impact of two disasters of major significance in 2019: a dam break in Minas Gerais, Brazil, and the cyclones in Mozambique. We conducted an assessment of surveillance data of several mosquito-borne diseases including malaria, dengue, chikungunya and Zika from each affected region before and after each disaster and compared 2019 data to previous years during the same time period and/or transmission season. In Brazil, the disaster contaminated the Rio Paraopeba, which has an extensive river basin that encompasses 48 cities. In 2019, the river basin area reported 28.3% of the probable dengue cases in Minas Gerais. Compared to 2018, there was a substantial increase in confirmed dengue cases in the river basin area, 488 cases in 2018 vs. 46,961 in 2019. Mozambique reported decreased cases of malaria immediately after the cyclone most likely as a result of disruption of health systems in the affected areas and significant displacement of human populations. Innovative surveillance approaches in the wake of a disaster may be necessary to improve data collection in the immediate and long-term aftermath of a disaster to inform where interventions should be implemented. We aim to continue this analysis during the 2020 transmission season to determine if these disasters could affect mosquito-borne disease transmission across multiple seasons.

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Poster #44

Real-Time Metagenomics with PanGIA

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As the technology of sequencing evolves with lower costs, more affordable applications arise. Portable sequencing platforms such as the Oxford Nanopore Technologies MinION is enabling field-forward genomic or metagenomic sequencing. While targeted molecular amplification-based assays such as quantitative real-time PCR (qPCR) confirm the presence or absence of a specific and well characterized organism, these assays do not survey the myriad of flora that are present, nor are they resilient to mutations in their genomic targets. For this reason, portable technologies relevant to various sectors including those working in public health laboratory professions. The ability of Next Generation Sequencing to broadly identify as many organisms in a given sample as possible could vastly improve our health and safety standards by elucidating more of the truth of our environment. Sample preparation for MinION is typically shorter than for less mobile sequencing platforms, saving time in the field. Further, the MinION produces read data in real-time, allowing for "sequence until" style analyses with the potential to drastically reduce sample to answer timeframes. Toward this goal, we are presenting "real-time PanGIA," an algorithmic change to MRIGlobal's PanGenomics of Infectious Agents metagenomic biosurveillance program so that it takes advantage of the data streaming feature of the MinION. PanGIA was designed as the bioinformatics portion of the Defense Threat Reduction Agency's Sample to Sequence program. While PanGIA was intended for the detection of pathogens, its database can readily be customized to favor food-borne, public health concerning organisms. PanGIA bioinformatics displays a combination of taxonomic profiles and genetic markers of interest through an intuitive graphical user interface that updates as more data is produced by the MinION. This GUI reduces or removes the need for a trained bioinformatician to run sample analysis by automatically generating a dynamic and interactive report that clearly illustrates the contents of the sequencing run. We believe that technologies like real-time PanGIA can pave the way for more complete infectious disease surveillance, outbreak response, and inspection of environmental health threats.

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Poster #45

A State Population Study (NJHANES) for Surveying Environmental Contaminants in New Jersey

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From 2016-2018, the New Jersey Department of Health (NJDOH) conducted a large-scale biomonitoring study for emerging and legacy environmental contaminants throughout NJ. This study measured analytes from three groups, including toxic metals in blood (n=3000) and urine (n=1000), per- and polyfluoroalkyl substances (PFAS) in serum (n=1000), and polychlorinated biphenyls in serum (n=1000). However, since this biomonitoring study utilized convenience sampling by procuring specimens from clinical laboratories and blood banks in NJ, the obtained data are limited in generalizing study results for direct comparison with other population-based studies, such as the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers of Disease Control and Prevention (CDC).

To fill in knowledge gaps identified in the large-scale biomonitoring study, NJDOH is conducting a population-based surveillance for study-selected environmental contaminants. This new study, called NJ Health and Nutrition Examination Survey (NJHANES), will use a multistage, cluster, and probability-based random sampling approach to recruit ~500 NJ residents over two years. NJHANES will expand upon the previous study to survey additional analytes, including: 1) newly emerging PFAS (e.g., GenX) in serum, 2) polybrominated diphenyl ethers (PBDEs) in serum, 3) organochlorine pesticides (OCPs) in serum, 4) speciated mercury in blood, 5) mercury in urine, 6) speciated arsenic in urine, 7) volatile organic compound (VOC) metabolites in urine, 8) polycyclic aromatic hydrocarbon (PAH) metabolites in urine, and 9) nicotine metabolites in serum. NJHANES will collect health and nutrition-related individual and household information during the subject recruitment period to differentiate exposure routes and to further link them with measured environmental contaminant levels. Multiple approaches, such as collecting samples from home visits and utilizing local medical facilities, will be considered for specimen collection.

Even though each state has different situations and challenges, the NJHANES-presented approach is another example of how a population-based surveillance study can be designed to achieve the following objectives: 1) provide data representative of the statewide population's exposure to state-specific environmental contaminants and 2) establish baseline data for the state. In addition, these state-level data will help in assessing current body burden(s), identifying additional at-risk subpopulations, addressing disparities within the population, and possibly, disclosing currently unrecognized contaminations and exposure routes within a state.

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Poster #46

Method Development for PCBs, OCPs and Toxic Metals in Dried Blood Spots – Potential Indicators for Early Detection of Autism Spectrum Disorder

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New Jersey has the highest rate of autism spectrum disorder (ASD) in the nation and the discrepancy continues to grow. In utero exposure to multiple environmental contaminants has been linked to pathogenesis. Early detection of these risk factors and early diagnosis are keys to maximizing the effects of life-altering therapeutic treatment and emotional management.

The New Jersey Department of Health (NJDOH)-Environmental and Chemical Laboratory Services (ECLS) was awarded a competitive state biomonitoring grant in the period of 2019-2024 from the U.S. Centers for Disease Control and Prevention (US CDC) to conduct a research project for exploring whether the Identification of prenatal environmental stressors linked to ASD such as toxic metals and persistent organic pollutants (POPs) can influence on potential autism-indicative biomarker data from newborn dried blood spot (DBS) cards. To test this research question, the NJDOH will leverage the untested DBS cards stored in NJ Newborn Screening (NJ NBS) program. These residual specimens will be used to establish new testing methods for 1) toxic metals such as lead, cadmium and mercury; 2) POPs such as organochlorine pesticides (OCPs); and polychlorinated biphenyls (PCBs). Analytical challenges are relatively low blood volume, volume variability, and background present from DBS. Sensitivity and precision will be optimized and the potential interferences will be evaluated.

These challenges will be circumvented by 1) optimizing sample preparation procedures, 2) incorporating/analyzing a blank circle for each individual DBS card, and 3) utilizing the most sensitive instruments. We are evaluating our approach in terms of maximizing extraction, lowering interfering background levels, as well as enhancing method sensitivity/precision. Briefly, a half-inch DBS circle (estimating equivalent blood volume ~75 µL specimen) and a half-inch blank circle (filter paper only) will be cut from the same DBS card. The samples will be extracted using or modified from extraction steps described in reference methods (CDC 3016.8-03 or EPA 200.8 for metals and CDC 6502.02 for POPs) and subsequently analyzed by highly-sensitive instruments (ICP-QQQ for metals and HRGC-HRMS for POPs) at NJDOH-ECLS labs.

The newly developed new methods will be utilized to determine select environmental contaminant levels in DBS cards for metals (n=500-1000) and OCPs/PCBs (n=250-500) and obtained data will be associated with ASD-related biomarker levels previously determined by NJ NBS program in the same sample to establish if there is an influence on potential indicative biomarker data.

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Poster #47

Standard-of-Care Prenatal Screening for Lead and Mercury: A Case for Early Intervention

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"Lead and mercury are very harmful to growing babies since exposed mothers can pass these metals on to their babies through the umbilical cord. Lead and mercury can affect the development of the brain and body, change behavior, and cause many other problems. Developing babies are the most vulnerable to these effects. Taking steps early to reduce mothers' risks of exposure will help protect babies from these harmful effects.

The NJ Biomonitoring Program partnered with University Hospital (UH) in Newark, NJ to offer prenatal lead and mercury testing as standard-of-care to the UH patient population. All patients receive educational materials that explain the importance of screening for lead and mercury and how to minimize exposure and protect their families. An extra tube of blood is collected at the expectant mother's first prenatal visit and cord blood representing the baby's exposure is collected at birth. These samples are sent to the New Jersey Department of Health Metals Laboratory for testing and the results are sent back to the providers to help guide them in making any necessary medical decisions regarding their patients' care. These results become part of the children's medical records to help ensure proper medical care as necessary throughout their lives.

Patients with elevated lead ($\geq$ 5 µg/dL) or mercury ($\geq$ 5 µg/L) receive medical treatment provided by UH staff as part of normal patient care protocols. NJ Poison Control is available to provide educational assistance to the patients and medical staff. Mothers' blood is tested throughout pregnancy to ensure that interventions are successful, and the results are compared to the results of a cord blood at sample collected birth. Patients with elevated lead levels receive case management and home inspections and, where appropriate, remediations and abatements from local departments of health. Elevated mercury samples are speciated to determine what species of mercury is present to help elucidate the exposure source of the mercury.

To date, we have analyzed samples from over 2000 individual patients. This program has helped identify several extremely high lead and mercury cases and has shown that there is a surprising percentage of patients with elevated mercury levels. Most of the elevated mercury samples are predominantly or completely methylmercury, indicating consumption of large fish as a likely source. Providing this testing at this stage of pregnancy is allowing medical staff and patients to address these exposures early and improve health outcomes for their babies.

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Poster #48

Analysis of Genetic Variation of Carbapenemase-producing Carbapenem-resistant Organisms Within Individual Patients: A Clinical Workflow Revisited

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According to the 2019 Antibiotic Resistance Threats Report issued by the Centers for Disease Control and Prevention, carbapenem-resistant *Acinetobacter* species and carbapenem-resistant *Enterobacteriaceae* are designated as urgent threats in the United States. The rise of carbapenem resistance in nosocomial settings is of great concern as dissemination of carbapenemase genes can occur rapidly throughout health care facilities, geographic regions, and among bacterial species. Current clinical laboratory workflows to characterize carbapenemase-producing carbapenem-resistant organisms (CP-CROs) from patient specimens often entails the analysis of a single cultured bacterial isolate. This isolate is used for all phenotypic, molecular, and genomic analyses. Yet, the genetic variability of CP-CROs within individuals has not been well described. Here, we evaluate the degree of genetic variation of CP-CROs, isolated from a subset of colonized individuals, to aid in establishing thresholds for the determination of relatedness through Whole-genome sequencing (WGS) analysis. Patient rectal swabs routinely submitted through the Northeast Antibiotic Resistance Laboratory Network are screened by molecular methods to detect colonization of CP-CROs. Swabs are tested using the Cepheid Xpert® Carba-R assay that detects the presence of beta-lactamase (bla) genes: blaKPC, blaNDM, blaVIM, blaIMP, and blaOXA-48. Positive swabs are cultured with selective media: MacConkey agar, CHROMagar™ mSuperCARBA™ agar, and MacConkey broth. Distinct colony morphologies are sub-cultured and screened for the presence of carbapenemase genes with lab-developed real time PCR assays. The PCR-positive isolates are identified with MALDI-TOF mass spectrometry. For this study, an additional 5 to 10 colonies demonstrating the same PCR-positive morphology description are cultured and confirmed for carbapenemase gene presence. This collection of intra-patient PCR-positive isolates are sent for short (Illumina® NextSeq®) and potentially long (Oxford Nanopore MinION) read sequencing and analyzed through bioinformatic pipelines to establish genetic variation among isolates. Initial work with 18 positive rectal swabs from 3 health care facilities has identified blaKPC-*Escherichia coli*, blaKPC-*Klebsiella pneumoniae*, blaKPC-Pseudomonas aeruginosa, blaKPC-Citrobacter freundii, blaNDM-*K. pneumoniae*, and blaVIM-*P. aeruginosa*. We aim to characterize the intra-patient genetic variability of CP-CROs. This data, along with additional genomic and epidemiologic data, will allow for robust characterization of the spread of carbapenem resistance and will inform the reporting of relatedness of CP-CROs in future investigations.

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Poster #49

Nanopore Sequencing Workflow for Assessing the Relatedness of Antibiotic Resistance Plasmids From Carbapenem-resistant Bacteria

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Carbapenem resistant organisms (CROs) frequently carry carbapenemase and other antibiotic resistance (AR) genes on plasmids or other mobile genetic elements, which contain repetitive elements that are difficult to assemble using short-read sequencing alone. Therefore, long-read sequencing using technologies such as the Oxford Nanopore Technologies (ONT) MinION is increasingly being used to characterize the genomic context of AR genes among CROs. At the Wadsworth Center, we have sequenced 84 CROs identified through the Antibiotic Resistance Laboratory Network (ARLN) using both MinION and Illumina MiSeq sequencing. These CROs represent eight bacterial species and include isolates from outbreak and transmission investigations during which plasmid transfer between genetically unrelated bacteria was suspected, as well as isolates with dual or unusual AR mechanisms. We have developed an analytic workflow that includes the use of two different assembly methods: 1) a long-read assembler, Flye, followed by polishing with MinION and Illumina reads; and 2) a hybrid assembler, Unicycler. For each isolate, the two assemblies are compared and evaluated by assessing the number, length, and circularity of contigs, the percentage of MinION and Illumina reads that map to the assemblies, the presence of structural variants in the MinION reads compared to the assembly, and the presence and genomic context of AR genes. If necessary, additional assemblies are created by modifying MinION read filtering cutoffs or assembler parameters. Using this workflow, a total of 33 CROs have been analyzed to date, generating assemblies that are complete or near completion to address epidemiologic questions for 31 isolates (94%). Discrepancies between assemblies produced by Flye and Unicycler were common, with neither assembler producing complete assemblies for all isolates, which emphasizes the need for multiple assembly methods and thorough post-assembly evaluation. MinION sequencing has added valuable information about the structure and relatedness of plasmids containing AR genes to epidemiologic investigations. In one case, an identical plasmid with AR genes was identified from three different bacterial species from a single healthcare facility, which may indicate transfer of this plasmid between organisms. The use of MinION sequencing for CROs at the Wadsworth Center will continue to contribute to investigations including those in which transfer of plasmids is suspected and provide a greater understanding of the diversity of resistance plasmids in the Northeast.

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Poster #50

Veterinary Laboratory High Consequence Pathogen Triage Protocol and the Laboratory Response Network

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In 2018, there was a unique case involving a human infection with *Francisella tularensis* following exposure to a squirrel. *F. tularensis* is a Tier 1 Select Agent and poses high morbidity risk. The Oregon Veterinary Diagnostic Lab (OVDL) at Oregon State University received a deceased squirrel from an OR resident who had cared for it in her home, resulting in her exposure. The OVDL quickly identified lesions of concern within tissue samples and provided them to the ODVL Bacteriology Section for culture. Utilizing the American Society for Microbiology Sentinel Clinical Laboratory Guidelines, the OVDL promptly suspected the presence of *F. tularensis* and notified the Oregon State Public Health Lab (OSPHL). Arrangements were made for shipment to OSPHL that day for confirmation via the OSPHL courier service. OSPHL expedited Laboratory Response Network reference level testing and confirmed *F. tularensis*. The results were communicated back to the exposed person, who subsequently sought further medical attention and proper antibiotic treatment. Existing relationships between OVDL and the Oregon Health Authority facilitated timely, effective communication throughout the case. The case led to collaborative discussions and strengthened working relationships between public health and veterinary lab systems in several ways and resulted in a joint risk assessment and further expansion of the High-Consequence Pathogen Triage Protocol at the OVDL. Since the OVDL accepts samples from numerous animal species across the U.S., triage of animals with potential for Tier 1 Select Agent infection is complex. Upon review, the group broadened and enhanced the existing protocol to potentially speed recognition and processing of such animals, and to reinforce use of appropriate precautions when handling these samples. The OVDL sample intake form was also modified to facilitate prompt collection of pertinent risk-based information, which helps prioritize certain sample types, provide precautionary info to minimize potential exposures to high risk samples, and is expected to lead to earlier recognition and notification to public health and to OVDL staff when such agents are present. These collaborative efforts have further strengthened existing relationships and contributed to the safety of all those who may come in contact with samples at high risk for containing transmissible select agents. These efforts put all agencies involved in a better position to protect the health of the general public. The triage protocol may be a useful template for other veterinary diagnostic laboratories and the collaborative process that produced it may be useful for addressing other types of disease conditions seen in animals that can affect the health of animal owners or forensic investigations.

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Poster #51

Clinical Laboratory Exposure Assessment and Monitoring Tool

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Laboratory acquired infections (LAIs) are a constant risk in clinical laboratories, and labs are responsible for providing protection to staff, yet exposures to biological agents still occur. Biosafety professionals from public health laboratories and from the clinical laboratory community continuously work to improve biosafety practices to reduce LAI threats. A new resource called, the Clinical Laboratory Exposure Assessment and Monitoring Tool has been developed by APHL members to assist these efforts and will be released in 2020.

Direct feedback from a Sentinel Laboratory Director of Microbiology was received by an APHL committee member who then shared the idea with the APHL Sentinel Laboratory Partnerships and Outreach Subcommittee and the APHL Biosafety and Biosecurity Committee. The original proposal was to develop an exposure monitoring resource specifically for biothreat agents. The APHL workgroup further developed and expanded this idea to also include more commonly encountered LAIs, in addition to biothreat agents. The workgroup also included other functions and resources which has evolved into the Clinical Laboratory Exposure Assessment and Monitoring Tool. In addition to the comprehensive agent specific exposure monitoring resources, the new tool is designed to assist clinical laboratories with assessing potential exposure events and making their own determinations for low, moderate or high-risk potential exposures. Clinical laboratories can jointly use the tool with their partners such as occupational health, infection control, epidemiologists, or others who may be involved with exposure determinations, symptom monitoring, or other post-exposure follow up needs. Those using the tool will be asked a series of checklist style questions (e.g., PPE use, biological safety cabinet use, any aerosol generating procedures, etc.) that will help provide the information needed to make their exposure determinations.

Information generated from the tool assists laboratories with required notifications and with performing their own biological risk assessments from the potential exposure event to aid with follow up and mitigations. The tool is offered in an electronically accessible format and a printed format. Within the tool there is an overview of all the LAI organisms and their expected symptomologies that can be printed in a single large poster size format. There are links to external resources within the tool for biosafety, biological risk assessments, organism risk grouping, as well as agent specific treatment guidelines. This new standardized information collection method for LAIs can be shared with the broader biosafety and clinical laboratory communities to help aid with enhancing laboratory systems and preventing potential exposure events.

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Poster #52

Integrating Competency Guidelines for Public Health Laboratory Professionals into a Medical Laboratory Technician Training Program

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In 2015, the Centers for Disease Control and Prevention (CDC) and the Association for Public Health Laboratories (APHL) published the Morbidity and Mortality Weekly Report (MMWR): Competency Guidelines for Public Health Laboratory Professionals. The guidelines cover a range of public health laboratory workforce competence domains grouped by four ability levels. While primarily written for public health laboratory professionals, the guidelines are also applicable to student laboratory training programs.

The Oregon State Public Health Lab (OSPHL) has a longtime partnership with the Portland Community College Medical Laboratory Technology (MLT) Department to foster a strong student training program, promote awareness of public health, and to enhance laboratory workforce initiatives. The OSPHL introduced the MMWR guidelines to the MLT Program Director (PD) with the intent to use them for curriculum enhancement and further alignment with professional practices that results in graduates with stronger employment skills. The PD worked closely with the OSPHL to evaluate the guidelines for cross-cutting and specialized applicability (sub)domains that would be most relevant and beneficial to public health and clinical laboratory fields. The competency guideline domains, subdomains, and ability levels were mapped to align with general clinical laboratory and public health professional expectations and the National Accrediting Agency for the Clinical Laboratory Sciences (NAACLS) program evaluation requirements. By focusing on measurable objectives for program assessment and continuous quality improvement, these efforts further establish a baseline standardization for students to achieve a "beginner or competent" level for the chosen (sub)domains.

This poster outlines strategies and methods for adapting and integrating the MMWR Competency Guidelines into an existing MLT program to enhance laboratory systems. An additional objective is to assess and compare student outcomes modeling academic and industry expectations and skills for professional laboratorians. This process can be modeled by other MLT programs to promote standardization and increase development for those graduates entering the clinical or public health workforce. These efforts align with best practices that target recruitment, development and retention of a new generation of laboratorians as outlined in APHL's Strategic Map for 2018-2020.

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Poster #53

Potential Transfer of a *Klebsiella pneumoniae* Carbapenemase Carrying Plasmid Between *Klebsiella pneumoniae* ST307 and *Escherichia coli* in a Single Patient

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Klebsiella pneumoniae carbapenemase (KPC) is an Ambler class A carbapenemase of critical public health importance. KPC's are typically found on plasmids of *Klebsiella pneumoniae*, but can be acquired by other *Enterobacteriaceae* via horizontal gene transfer. In 2018, as part of routine carbapenem resistant *Enterobacteriaceae* surveillance, the State Hygienic Laboratory at the University of Iowa (UI) identified an *Escherichia coli* isolate harboring a KPC. Nearly one year later, a *Klebsiella pneumoniae* isolate from the same patient was also confirmed as KPC positive. Whole-genome sequencing utilizing the Illumina MiSeq platform was performed for both isolates. Multi locus sequence typing demonstrated that the *K. pneumoniae* was ST307, a worldwide emerging clone harboring plasmid-mediated antimicrobial resistance genes. Bioinformatics analysis conducted using an in-house pipeline run on the UI Argon High Performance Computing cluster indicated that both *Klebsiella* and *E. coli* isolates were KPC-3 variants and shared identical repA plasmid replicon sequences. Further BLAST and sequence mapping analysis demonstrated high sequence similarity between the two isolates for an approximately 45,000bp region containing both the KPC-3 gene and the repA replicon. In order to demonstrate the potential transfer of plasmids between the two organisms, we intend to reconstruct and compare the entire plasmid sequences using both short- (Illumina MiSeq) and long- (Oxford Nanopore MinIon) sequences reads. Transfer of plasmids harboring genes conferring resistance to carbapenems pose a serious threat to public health. This may pose an additional challenge to effective treatment and infection control if these transfer events can occur between two common human pathogens within the same patient potentially over the course of a prolonged time period.

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Poster #54

Expanding Bioinformatics Capabilities at a State Public Health Lab through Partnership with a University

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Increased public health applications of next-generation sequencing has created new laboratory challenges including additional demands for bioinformatics expertise and computing power. During the past year, the State Hygienic Laboratory (SHL) greatly expanded its bioinformatics capability. An SHL IT application developer started pursuing a bioinformatics certificate and SHL became a host lab for an APHL Bioinformatics Fellow. Additionally, SHL is housed within the University of Iowa (UI), and as such, has access to the UI high performance computing (HPC) Argon cluster and other analytic resources. The Argon cluster consists of over 15,000 processor cores with access to more than 200 software packages. SHL was able to obtain a group account on Argon, allowing our bioinformaticians to install pipelines necessary for sequence data QC, sequence typing, antibiotic resistance and virulence profiling, and outbreak investigations. Lab analysts can access the group account via an individual login system and execute one or more pipeline(s) in parallel. Available pipelines include modified versions of the Type pipeline (Colorado Department of Public Health and Environment), reference-free pipeline (Utah Public Health Laboratory) and Lyve-SET (Centers for Disease Control and Prevention), as well as, Staphopia (Emory University). These pipelines utilize Singularity images created from Docker containers available on the State Public Health Bioinformatics (StaPH-B) Docker Hub.

SHL continues to explore new tools and analysis platforms. As SHL generates more sequence data, organizing sequence files needed for specific analysis is a growing challenge. SHL is developing a PostgreSQL database that will track the location of fastq files and pipeline outputs, as well as, pipeline versions (for reproducibility) and metadata. The database, hosted on SHL's servers, will allow us to quickly locate files according to specific parameters such as organism name, sequence type, or collection facility. The search output will be used to automatically recover files of interest and start subsequent analysis. SHL is also utilizing the UI Interactive Data Analytics Service (IDAS) resource as the front end for the files tracking database and to host one of its applications for analyzing resistance and virulence genes. Finally, SHL started to develop a workflow to automatically track Carbapenem-resistant *Enterobacteriaceae* (CRE) genes and organisms based on the combined AMR data from SHL's information system and WGS analysis output.

Local universities can be instrumental in providing the necessary computing resources to expand bioinformatics capacity at a public health laboratory. An overview of this partnership, as well as, the pipelines and tools used by SHL could provide a road map for other state public health laboratories.

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Poster #55

Measuring Success in Response to Outbreaks

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The frequency of outbreaks of novel infectious diseases continues to increase. The recent Ebola and Zika virus outbreaks demonstrated variable preparedness of many public health systems with delayed identification and confirmation of infected patients which led to higher morbidity rates in certain areas. The public health laboratory response to these outbreaks needs to move faster in the future to reduce the spread of disease. The APHL Infectious Disease Committee developed a survey to ask public health laboratories about their recent experience with the Zika virus outbreak as an indicator of a typical outbreak response. The Zika virus outbreak was chosen because it represented a new pathogen which most states had no diagnostic capabilities at the onset. Eleven U.S. public health laboratories volunteered to participate in the phone interview survey, this was determined statistical to be sufficient. Laboratories were asked about several factors, including, when they began to prepare to respond to the Zika virus outbreak, limiting factors in new test implementation, time required to bring on a new test method, and how long it took to complete each task in the process.

The time required to perform and report a Zika virus test result from the initial response to implement a Zika virus assay ranged from 7 to 90 days, with a median of 30 days. While some laboratories began preparations to develop and validate a Zika virus assay before it was identified the U.S., most laboratories waited for the release of an emergency use authorization test before starting their validation. Major limiting factors for most labs were adequate staff to perform testing, result reporting systems, financial issues, the availability of reagents and control specimens to perform validation studies. Equipment and space considerations were also notable. The most difficult tasks to bring on a new test were the validation and result reporting. Most laboratories performed multiple tasks to bring on the test simultaneously while some took a more linear approach.

Based on the survey results, we conclude that all public health laboratories are not able to respond to the challenges of bringing on new assays in the same manner. It is wise for all public health labs to work towards a best practice model for the challenges of bringing a new test on board during an outbreak.
Identifying overlaps in diagnostic development needs across different priority pathogens would allow more timely and cost-effective use of resources than a pathogen by pathogen approach.

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Poster #56

An Outbreak of *E. coli* O103 Traced to Sprouts: Implications for the FSMA Produce Safety Rule

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Sprouts are often linked to foodborne outbreaks because of the warm humid conditions necessary to produce them. In this outbreak, Iowans became ill with *E. coli* O103 after eating at a restaurant chain in central and eastern Iowa. This outbreak was identified through routine surveillance on December 18, 2019. After notification, Iowa's food retail and manufacturing agency, the Iowa Department of Inspection and Appeals (DIA), notified the franchise owners and sprout grower. Both parties voluntarily suspended the sales of clover sprouts.

A cohort study was conducted. Phone interviews were performed by Iowa Department of Public Health (DPH) epidemiologists and local public health partners using a standard *E. coli* questionnaire and an outbreak-specific supplemental questionnaire. Spent irrigation water (SIW) and 16 packages of clover sprout samples were collected and sent to Iowa's State Hygienic Laboratory (SHL) for testing.
Twenty-three illnesses were identified. Of these, 22 were laboratory confirmed and one probable. A confirmed case was defined as a person with *E. coli* 0103 infection, whose clinical isolate is related within 0-2 SNP's, and with an isolate collection date between 11/26/2019 – 12/21/2019. A total of 22 (96%) cases met this case definition from 10 Iowa counties. Of these, 14 (64%) were females. The median age among cases was 29 (range 18-50 years). One case was hospitalized. No deaths were reported.
Statistical analysis was performed to identify the specific food that caused the illness. Clover sprouts were found to be significant with 9/20 (45%) of the cases reporting consuming sprouts. This is significantly higher than the average of 4% of people that mentioned eating sprouts based on the 2006 – 2007 CDC FoodNet Population Survey.

All clinical and environmental isolates submitted to SHL were identified as carrying the stx1a toxin gene using real-time PCR, serotyped by conventional agglutination, and sequenced using PulseNet procedures. SHL identified *E. coli* O103 from 12/16 clover sprouts packages and corresponding SIW. Both the environmental samples were highly related to the 22 human clinical isolates by whole genome sequencing (0-2 SNP's).

The FSMA Produce Safety rule establishes science-based minimum standards for the safe growing, harvesting, packing and holding of fruits and vegetables grown for human consumption which includes sprout. Sprout growing operations are subject to sprout-specific requirements which include testing spent irrigation water for Salmonella and *E. coli* O157:H7. STEC testing is not included in this requirement. Given the fact that there have been other reports of non-O157 STEC outbreaks and the recent occurrence of this *E. coli* O103 outbreak associated with sprouts has major implications to the FSMA Produce Rule. STEC testing of SIW should be recommended as best practice for improving sprout safety to prevent future outbreaks of toxigenic *E. coli*.

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Poster #57

Developing a Bioinformatics Program: The Texas Experience

S. Marcellus and R. Lee, Texas Department of State Health Services Laboratory, Austin, TX

Problem/Objective: As gene therapy, genetic research, and molecular technology have expanded, newborn screening methodologies have attempted to keep pace, resulting in the need for streamlined molecular data analysis and interpretation. The Texas Newborn Screening Program has begun development of a bioinformatics program to efficiently analyze and interpret Next Generation and Sanger sequencing data to screen newborns for SCID (severe combined immunodeficiency) and other conditions. Most states with newborn screening (NBS) bioinformatics capabilities have adapted their existing infectious disease (ID) informatics infrastructure for use in newborn screening. The Texas DSHS Laboratory is building infrastructure for NBS and ID concurrently, from the ground up.

Methods: The Texas Laboratory started the information gathering process by seeking input and expertise from numerous partners, including local laboratory information technologists (IT) and departmental IT, the University of Texas Bioinformatics Consulting Group (UT-BCG), and other state public health laboratories such as the Virginia Division of Consolidated Laboratory Services (DCLS). We are collaborating with local and departmental IT to determine the options of hardware and software that are permitted by our governing bodies and are compatible with existing systems. UT-BCG and Virginia DCLS provided training, feedback on their informatics implementation processes, and will be great resources throughout our development process. Numerous pipelines and DNA analysis tools created by public health bioinformaticians are available for review and we will select code that will best fit Texas' needs. To improve the workforce, the Texas Laboratory applied and was awarded a fellowship and additional training was provided to the bioinformatics fellow and other laboratory technologists.

Results and Conclusions: The Bioinformatics Program for the Texas NBS Program and ID Program is currently in development. The needs of both the NBS and ID programs are being weighed during platform selection. During platform selection and program development, IT has had several concerns including security, access, network bandwidth, and hesitancy to implement a new system. Open and frequent communication is reducing the issues with IT. The creation and implementation of a joint NBS and ID bioinformatics pipeline will facilitate faster test times, higher accuracy and precision in results, quicker result reporting and will allow for administration by a single bioinformatician. Without a bioinformatics program, critical NBS referrals could be slowed, likely causing delays to diagnosis and treatment of time-critical diseases. Laboratory test results could also be delayed for ID patients, likely causing a delay in outbreak detection and reporting. A bioinformatics program is necessary for the timely reporting of ID and NBS.

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Poster #58

A Continuous Quality Improvement of the Texas AR Lab Network Testing Process

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Background: AR Lab Network laboratories provide critical services to rapidly detect antibiotic resistance and produce data to inform responses, prevent or contain spread of infections, and protect people. Over the past years, the Texas DSHS AR laboratory had undergone changes in testing type, technology, and workload that prompted an evaluation of the current testing process to improve efficiency and keep meeting public health needs and regulatory requirements. The purpose of this continuous quality improvement project is to review the Texas AR Lab Network testing process to assess, identify, and implement improvement opportunities.

Methods: This quality improvement project was based on the five stages of Lean Six Sigma methodologies: Define, Measure, Analyze, Improve, and Control (DMAIC). To define the current state, six months of data were analyzed, processes were observed and interviews with laboratory staff were used to define problems that resulted in inefficiency and specify goals of the project. Baseline process performance was measured by mapping and timing steps of testing processes and calculating testing completion times. Review, visualization, and analysis of data collected enabled the identification of potential causes of the problem and improvement opportunities. Potential ideas and solutions were proposed through brainstorming with laboratory staff and management. The best and most practical solution was then selected, approved, and implemented. After changes were applied, data were recollected and analyzed to measure and confirm improvements.

Results and Conclusion: The overall goal of this study was to simplify the AR laboratory work process by eliminating non-value-added steps. The pre-analytical and analytical phases of the AR testing process were both reviewed and no necessary changes were identified. The main bottleneck was identified in the post analytical testing procedure where multiple, nonessential steps extended the amount of time it took for results to be compiled and released. These redundancies were removed, and the process was automated to streamline and decrease the amount of time spent in this phase. Implementing these changes allowed for additional and unexpected improvements including adding a second day of testing which reduced batch sizes and improved turnaround time. Adoption of a quality improvement methodology has been proven to increase efficiency while empowering laboratory staff to seek opportunities for improvement, evaluate the present process, and make changes.

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Poster #59

Community Health Screening and Education through Laboratory Science Workshop: Participant Evaluation of the 2018 Pilot Program in Aco, Peru

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Public health outreach initiatives underutilize laboratory medicine students and professionals. This is a missed opportunity to engage lab science students in the health screening and education of their local communities as well as in partnerships with lab science students from other countries. This poster will discuss a novel bilingual community health screening and education (CHS & E) through laboratory science service-learning (SL) study abroad (SA) program that could be utilized to improve community engagement and understanding of public health and laboratory science.

In collaboration with The Foundation for International Medical Relief of Children (FIMRC), Clinical Associate Professor and 2019 Adult, Professional, and Community Education doctoral student, Joanna Ellis traveled with five CLS senior-level students to Huancayo, Peru in June of 2018 for a
CHS & E through laboratory science SL-SA program. Utilizing the self-powered Lab-in-a-Suitcase from International Aid, the group conducted four CHS & E through lab science events in four different communities. We conducted hemoglobin, urinalysis, glucose, cholesterol, and sexually transmitted infection screenings on more than 160 people. The CUY Project was the most involved activity with six interactive stations for the families to learn about anemia, parasites, and nutrition through lab tests. Parents and children learned more about their conditions through fun and interesting laboratory science activities and tests. The lab-science based activities illustrated the biological impact of their lifestyle and dietary choices in a new and impactful way. This poster will describe the preparation, implementation, and first stage of evaluation of the CHS & E through laboratory science workshops. One hundred percent of the parent participants surveyed stated that the workshop would help them make decisions in caring for their children and would help prevent future health problems. This poster session is an opportunity to discuss how laboratory professionals can interact with their community and showcase the value of the field in public health education initiatives.

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Poster #60

Bioinformatic Characterization of Mobile Genetic Elements Conferring Pathogen Antimicrobial Resistance

R. St. Jacques, L. Turner and K. Libuit, Virginia Division of Consolidated Laboratory Services, Richmond, VA

Each year, approximately 700,000 premature deaths worldwide are related to antimicrobial resistant (AMR) pathogens. It is estimated that by the year 2050, this number could rise as high as 10 million. Genes conferring antimicrobial resistance disseminate through both vertical and horizontal gene transfer via mobile genetic elements like plasmids and bacteriophages. The Virginia Division of Consolidated Laboratory Services (DCLS) performs susceptibility testing and targeted AMR gene profiling of carbapenem-resistant organisms (CRO) isolated from hospitalized patients in Virginia. A genomics approach to AMR genotyping provides additional insights into genes associated with resistance, prediction of drug resistance, and mobile genetic elements conferring AMR genes. To develop high-confidence predictive bioinformatic workflows for identifying resistance markers, 20 CRO isolates were sequenced at DCLS using the Illumina MiSeq and characterized for AMR determinants using traditional wet lab methods. The laboratory analyzed sequence reads for the presence of AMR determinants using Shovill v1.0.4 for genome assembly and StarAMR v0.5.1 to screen assemblies for plasmid-borne AMR determinants.

Resistance genes identified in the dataset included KPC (*Klebsiella pneumoniae* carbanepenemase), NDM (New Delhi metallo-beta-lactamase), OXA-48 (oxacillinase), and IMP (Imipenemase Metallo-beta-lactamase). Plasmids were predicted in 15 of the 20 strains tested. These results suggest that plasmid-borne AMR genes are highly mobile and are associated with drug-resistant infections in Virginia. Genes that confer antibiotic resistance were called at drug class level.

While these predictions are dependable, they utilize short-read assemblies that may miss entire genes if the locus is in a region with highly repetitive nucleotide sequences, or if the gene is split across contiguous reads. To determine the complete genome, identify additional AMR genes, and elucidate plasmids with AMR genes, Nanopore MinION long read data will be combined with the short read data produced by the Illumina MiSeq using the bioinformatic tool Unicycler. Complete genomes for strains predicted to have plasmids will be annotated to identify other potential AMR genes. Accurate plasmid prediction and mapping of AMR genes to these mobile elements is important in studying healthcare facility infectious disease outbreaks, as plasmids conferring AMR genes may be transferred among bacteria.

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Poster #61

Identifying Drivers of KPC Transmission in CRE Using Long Read Sequence Data

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Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) are an urgent threat to global public health. CRE infections are associated with high mortality rates and difficult to treat. In the United States there were an estimated 13,100 infections and 1,100 deaths due to CRE in 2017. CRE are susceptible to few, if any, antibiotics, and approximately 30% of CRE carry a carbapenemase that can spread between species and across genera via mobile elements. The most common carbapenemase in the United States is the *Klebsiella pneumoniae* carbapenemase (KPC). Understanding the spread of KPC among CRE is critical to decreasing the number of infections and deaths due to CRE. Here, we use whole genome sequencing to develop a retrospective picture of KPC diversity and transmission dynamics in Wisconsin.

Methods: In partnership with the Wisconsin Department of Public Health, we selected 147 CRE isolates collected from 2011-2018 in Wisconsin for sequencing. In order to accurately determine if carbapenem resistance was encoded on the chromosome and/or a mobile element, we used a hybrid approach and sequenced each isolate on the Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) MinION sequencing platforms. We used ONT long reads for genome assembly and highly accurate PacBio circular consensus sequences to polish the assemblies. We identified putative plasmids using Mash with PLSDB, a database of >15,000 complete bacterial plasmids. Additionally, we used Abricate to screen for plasmid replicons and KPC genes using the PlasmidFinder database and the Comprehensive Antibiotic Resistance Database, respectively.

Results: We were able circularize 99% of the chromosomal sequences in our sample of CRE. Mash matched 98% of the remaining circular sequences with at least one plasmid in PLSDB. Abricate identified plasmid replicons in 91% of these putative plasmids. These results indicate our sample of CRE has 422 putative plasmids from 12 different plasmid incompatibility groups. We found several variants of KPC in our sample, including KPC 1-5. KPC-3 was most prevalent. The majority of KPC genes present in our sample were plasmid encoded (90%). However, we also identified a transposable element (Tn4401) containing a KPC gene in 25% of the putative plasmids. Additionally, we found Tn4401 containing a KPC gene in the chromosome of six of our CRE isolates, two of which also had Tn4401 encoding a KPC gene integrated into a plasmid.

Conclusions: Our results suggest the plasmid diversity of CRE in Wisconsin is extensive and mobile elements play a major role in the spread of KPC among these isolates. While additional work is needed to elucidate the transmission method of KPC in CRE, this work provides new insights into the drivers of KPC spread in Wisconsin.

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Poster #62

Next Stop Biosafety! Engaging all Levels

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The 2015 Ebola outbreak highlighted the need for robust biorisk management programs in the public health sector and clinical laboratories as well as the importance of cooperation between various specialties and organizations. ABSA (The American Biological Safety Association) International and APHL continue to partner and provide outreach on biosafety and biosecurity topics. One of the many current challenges is the limited funding which has affected all PHL biosafety officers, both in their own biosafety and biosecurity programs and in their outreach to clinical laboratories. In 2019, clinical laboratory biosafety/biosecurity training and awareness of biosafety best practices were identified as critical needs by state public health biosafety officers. Despite very real potential risks in a clinical setting, these laboratories are typically understaffed and hampered in efforts to provide training opportunities for procedures, such as risk assessment and biosafety inspections, that are not currently mandated by law. This poster will review ABSA-provided educational opportunities, free /low-cost training links, educational/networking opportunities through ABSA regional affiliates, how to access the ABSA public health list-serve open to all, how to access the infectious agent risk group database, among others. This information will be useful to state/city BSOs as well as clinical laboratory managers and safety representatives.

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Poster #63

The Importance of Investing in Robust Chemical Testing Capabilities in the Environmental Health Laboratories

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Recent public health emergencies and community concerns have been the result of harmful chemical exposures. Building upon the expertise, experience and relationships developed through laboratory networks such as the Laboratory Response Network for Chemical Threats (LRN-C), the National Biomonitoring Network (NBN) and the nascent Opioids Biosurveillance System, public health and environmental laboratories were poised to respond efficiently and effectively to events involving electronic cigarettes and vaping (EVALI), per- and poly-fluorinated substances (PFAS), lead and opioids overdoses. Investments in specialized analytical instrumentation, technical training, quality systems and partnering with public health and clinical partners were critical in identifying sources and extent of exposures, and prevention and treatment of disease.

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