Control Number: 2022-A-24-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Next-generation sequencing and machine learning-based approaches for precise diagnosis of infections

Author Block: **V. Servellita**, A. Sotomayor-Gonzalez, J. Nguyen, P. Saldhi, K. Foresythe, N. Brazer, J. Streithorst, P. Oluniyi, D. Stryke, C. Y. Chiu;
University of California San Francisco, SAN FRANCISCO, CA.

Abstract Body: As artificial intelligence continues to revolutionize health care ushering in a new era of precision medicine, more accurate diagnostic tools are urgently needed to complement emerging advances in therapeutics. Genomic-based diagnostic tests have been successfully implemented in areas such as clinical oncology and the genetics of rare diseases, yet applications for infectious disease diagnosis remain limited. New diagnostic tools are necessary as many infectious diseases remain difficult to diagnose using conventional tests given overlapping clinical manifestations. Timely diagnosis of an infection with an unknown causative agent during outbreaks can also inform public health interventions to curtail the spread of disease. We have established a biorepository of well-curated clinical samples (whole blood, plasma, nasopharyngeal/oropharyngeal swab samples, respiratory fluids) collected as part of the standard of care or through prospective enrollment. By combining a clinically validated metagenomics bioinformatics pipeline, host response transcriptomic RNA sequencing (RNA-Seq) analysis and machine learning (ML) based algorithms, we have developed an automated computational pipeline for pathogen detection, immune profiling and the development of classifier models to diagnose and predict causes of infections. From whole blood, we have identified candidate gene classifier panels that differentiate Lyme disease from healthy controls and other tickborne infections, as well as discriminate MIS-C from COVID-19 and controls with high sensitivity and specificity. We have also developed machine learning-based models that can differentiate severe from mild COVID-19 disease from respiratory swab samples and blood, or discriminate severe clinical manifestations of COVID-19 disease such as multisystem inflammatory syndrome in children (MIS-C) from other acute infectious syndromes. Our findings demonstrate that coupling next-generation sequencing methods with machine learning-based approaches has the potential to accelerate and improve clinical diagnosis of infectious diseases, thus impacting both public health and patient care outcomes.

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Control Number: 2022-A-79-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Metagenomic Profiling of Gut Microbiome of Diarrheic and Healthy Children in Nigeria

Author Block: **H. Ugboko**, O. Nwinyi, O. Onile-ere;
Covenant University, Ota, NIGERIA.

Abstract Body: Globally, an estimated 1. 2 million children die from diarrheal diseases annually. Eighty percent (80%) of them are within their first two years of life. The gold standard for identification relies on traditional techniques, which take a long time and process to identify difficult to culture bacteria. Here is a metagenomic profile of diarrheic and healthy children aged between 0-5 years in Southwest, Nigeria. Bacterial genomic DNA was extracted from

stool samples obtained from consenting children. The V3-V4 regions of the 16s rRNA gene were amplified and sequenced on the Illumina Miseq platform. Phylogenetic diversity, species richness, and relative abundance of bacterial taxa were determined using the CLC Genomics Workbench v12.0. The data obtained contains untrimmed and demultiplexed paired end sequencing reads for the gut microbiome profile of eight children, 4 diarrheic children and 4 healthy children. The total number of reads in the dataset are 135,252 ranging from 35 to 301bp. Six bacterial phyla comprising 78 genera were identified. The gut microbiome profile revealed Firmicutes (61%), Bacteroidetes (17%), Proteobacteria (15%), Actinobacteria (5%), Fusobacteria (1%), and Verrucomicrobia (1%). In the diarrheic group, the relative abundance of phyla showed a decreasing order of Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Fusobacteria, except phylum Verrucomicrobia which was not identified. There was a remarkable decrease in the abundance of Proteobacteria (7%) in the healthy group compared to the Diarrheic group (38%). *Escherichia coli*, *Shigella*, *Staphylococcus,* and *Klebsiella* had increased species richness in the diarrheic group, whereas, *Bifidobacterium*, *Faecalibacterium*, *Lactobacillus*, *Clostridium* (sensu stricto), and *Bacteroides* were significantly increased in the healthy group.This study revealed a higher alpha diversity index among healthy children than those with diarrhea. This suggests that diarrhea harms the beneficial microbes in the gut of children.

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Control Number: 2022-A-85-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Real-time bacterial whole genome sequencing surveillance for hospital outbreak detection and investigation

Author Block: **A. J. Sundermann**, M. P. Griffith, V. Rangachar Srinivasa, K. Waggle, A. Ayres, G. M. Snyder, D. Van Tyne, J. W. Marsh, L. H. Harrison;
University of Pittsburgh, Pittsburgh, PA.

Abstract Body: **Background**: Real-time application of whole genome sequencing (WGS) surveillance has the ability to detect healthcare outbreaks more completely and faster than traditional infection prevention methods. We sought to analyze the impact of a real-time WGS surveillance program and infection prevention (IP) interventions to reduce healthcare-associated transmission at our hospital.
Methods: We analyzed clinical bacterial isolates collected between October 1, 2021 and June 1, 2022. Select bacterial pathogens from patients who were hospitalized for ≥3 days or had a recent healthcare exposure in the prior 30-days at our hospital system were collected and sequenced. Isolates were considered related for those with ≤15 single-nucleotide polymorphism (SNP) differences for all organisms except *Clostridioides difficile* (≤2 SNPs). IP interventions were implemented by the hospital IP team and clusters were monitored to assess for continuation or resolution of the outbreak.
Results: 1,195 isolates collected from 1,066 unique patients were sequenced. There were 85 (7.9%) patients involved in 30 clusters. The average cluster size was 2.8 patients (median 2, range 2-9) with larger clusters consisting of VRE and *K. pneumoniae*. The average time from patient culture date to isolate sequencing was 15.5 days (median 14, range 4-48) and average time from IP notification to intervention was 13 days (median 10, range 0-55). 58 (68.2%) of clustered patient isolates had an identified epidemiological link. Notable clusters include two endoscope outbreaks and multiple unit-based transmissions. There were 19

infections that occurred after an initial cluster detection, of which 5 occurred on the same epidemiological route before the IP intervention was implemented, 0 on the same route after the IP intervention, 9 by a different route, and 5 by an unknown route.
Conclusions: Real-time WGS surveillance for healthcare-associated transmission is feasible, and detects outbreaks with intervenable transmission routes. Interventions to date appear to have been successful at halting subsequent transmission via the same route. Application of real-time WGS is likely to significantly reduce healthcare-associated transmission and improve patient safety.

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Control Number: 2022-A-87-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Complete Genome Sequencing of Crimean-Congo Haemorrhagic Fever Virus (CCHFV) directly from clinical samples: a comparative study between targeted enrichment and metagenomic approaches.

Author Block: **J. D'Addiego**, N. Wand, E. Kennedy, R. Hewson;
UK Health Security Agency, Salisbury, UNITED KINGDOM.

Abstract Body:
Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is an orthonairovirus associated with outbreaks across Europe, Middle East, Asia and Africa. Whilst most CCHF cases are subclinical, 12% are severe and sometimes fatal. There are currently five genotypes of CCHFV, while Europe 1/ clade V seems to predominate in eastern Europe, Africa 3 (genotype III) and Africa 4 (genotype IV) have been identified in Spain. Next-generation sequencing is poised to play a crucial role in the study of viral genetic determinants which may increase virulence and transmission. Low titre clinical samples present a challenge for the recovery of complete genome sequences, for which metagenomic sequencing approaches such as Sequence-Independent Single-Primer Amplification (SISPA) lack the required sensitivity. The aim of this study was to develop novel methodologies for direct sequencing of CCHFV genomes from clinical samples, to enable research into genetic determinants associated with increased virulence and disease severity.
Sets of primers targeting 500bp fragments of CCHFV genomes were designed against Europe 1 sequences utilizing the Primal Scheme website. RNA was extracted from samples utilizing QIAamp viral RNA kit. cDNA was made with random hexamers. PCR amplification was performed in 25µl reactions. Sequencing libraries were prepared following manufacturers' instructions. Reads were mapped utilizing mimimap2 or BWA-MEM. Sequencing depth was determined utilizing SAMTOOLS.
Near complete genome sequences (>99% coverage) were recovered from tested samples utilizing the targeted enrichment protocol, whilst genome coverage following SISPA enrichment dropped significantly for samples with lower virus titre.
We have developed a novel, PCR-based enrichment protocol for sequencing complete Europe 1 CCHFV genomes which can be adapted to different sequencing platforms. Near complete genome sequences were recovered with the targeted approach from all tested samples including those with low viral loads for which only partial genome recovery was possible with SISPA enrichment.
We conclude that our enrichment method outperforms metagenomic enrichment protocols in terms of genome sequences recovery for samples with low virus titre and offers a tool to further study genetic determinants of virulence and disease severity in the Europe 1 clade of

CCHFV, which could lead to better understanding of CCHFV pathogenesis. Our targeted approach can be easily adapted to other CCHFV clades.

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Control Number: 2022-A-94-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Molecular characterization of Staphylococcus aureus in Benin: what about the clones identified with patients and healthcare workers?

Author Block: **C. YEHOUENOU**;
University of Abomey Calavi, Cotonou, BENIN.

Abstract Body:

ABSTRACT

Background : Methicillin-resistant *Staphylococcus aureus* (MRSA) constitutes a serious public health, with considerable impact on patients and important health care costs. Asymptomatic carriers, both patients and healthcare workers, constitute important MRSA reservoirs in the hospitals. The aim of this study was to describe the molecular characteristics and clonal diversity of MRSA strains isolated on pus samples and with healthcare workers in public hospitals.

Methods: In this prospective study conducted in six public hospitals and two wards between January 2019 and January 2020, a total of 304 patients and 70 HCWs were enrolled. On admission, wound swab was analyzed for patients with SSIs and nasal swab for HCWs. Identification and antimicrobial susceptibility testing were done respectively by MALDI-TOF mass spectrometry, and Phoenix Beckton Dickinson automated. The whole genome sequencing was performed to describe the antimicrobial resistance genes, the virulence factors (PVL, TSST) as well as typing methods (MLST) and the phylogenetic relationships to study the relatedness between samples.

ResultsOf the 27 confirmed MRSA isolates, 100% were susceptible to vancomycin, teicoplanin, cefazoline, linezolid and fusidic acid. 100% were resistant to all beta-lactams antibiotics. Resistance rates to other non-beta lactam classes were as the following: 74% were resistant to trimethoprim/sulfamethoxazole, 66.7% were resistant to ciprofloxacin, gentamicin, and amikacin, and 29.7% resistance to erythromycin. Molecular typing revealed five sequence types among which the most common were ST8 (55.5%, n=15), ST152 (18.5%, n=5) and ST121(18.5%, n=5). Genes for PVL (lukF-PV) were detected in all of isolates and the toxic syndrome toxin 1 (TSST-1, tst) genes in 29.6% (n=8) of isolates. The virulence genes and antimicrobial resistance were found for both healthcare workers and patients.

Conclusion: This study provides valuable information for understanding the resistance mechanisms of S. aureus collected in hospitals in Benin. The detection of MRSA in HCWs associated to markers of antibiotic resistance and virulence factors is of a major concern. In this setting where we suffer from a lack of infrastructures and resources for correct hand hygiene practices, the
implementation of multimodal WHO strategy remains the first steps to reduces cross transmission of MRSA through hospitals.

Keywords : MRSA, healthcare workers, sequence type and PVL.

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Control Number: 2022-A-95-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Next-Generation Sequencing Identifies Genomic Clusters of Invasive Group A Streptococci Contributing to Antimicrobial Resistance

Author Block: **Y. Li**, L. McGee;
Centers for Disease Control and Prevention, Atlanta, GA.

Abstract Body: **Background** Next-generation sequencing technology has been increasingly used to identify clinical isolates that form genomically closely related clusters (genomic clusters), which indicate close temporal relatedness during infection transmission. Here we investigate the role of genomic clusters in antimicrobial resistance among invasive infections caused by Group A Streptococcus (GAS). **Methods** We analyzed invasive GAS (iGAS) isolates identified through the Active Bacterial Core surveillance (ABCs), a laboratory- and population-based surveillance for severe bacterial infections currently implemented in 10 U.S. states, in 2015-2019. Whole genome sequencing (WGS) of iGAS isolates was performed on an Illumina Miseq platform. The WGS reads were processed by a bioinformatics pipeline to infer isolate *emm* type, antimicrobial susceptibility, and draft whole genome assembly. Whole genome assemblies that contained <1.5M total bases or >150 contigs were excluded from analysis as a sequencing quality control measure. Pair-wise single-nucleotide polymorphism (SNP) distances among all iGAS isolates belonging to the same *emm* type were calculated using the MUMmer package. An isolate was defined as clustered if it differed from another isolate by ≤10 SNPs per 1.5Mb of the two aligned genomes. The chi-squared test for trend in proportions (trend test) was used to evaluate proportion trends over time. Association between genomic cluster and antimicrobial resistance were assessed by Fisher's exact test. **Results** A total of 4369 iGAS isolates identified in 2018-2019 and 5261 isolates identified in 2015-2017 were included in this study, accounting for approximately 85% of all iGAS infection cases reported to the ABCs in the five years. The WGS-based cluster detection identified 5576 (59.9%) clustered isolates. Belonging to a genomic cluster was significantly associated with erythromycin non-susceptible (EryNS) (OR=1.22; p<0.001) and clindamycin non-susceptible (CliNS) (OR=1.32; p<0.001) strains. The proportion of clustered EryNS isolates among all isolates increased significantly from 10.5% (553/5261) in 2015-2017 to 17.1% (747/4369) in 2018-2019 (p<0.001). In contrast, the proportion of non-clustered EryNS isolates remained comparable between the two periods (7.6% vs. 7.8%; p=0.65). The trend test for proportion of clustered EryNS isolates from 2015 to 2019 showed a significant increase (p<0.001) while no significant trend was found for proportion of non-clustered EryNS isolates (p=0.60). A similar pattern of expansion in the clustered, but not the non-clustered, CliNS isolates was also observed for CliNS isolates in 2015-2019. **Conclusions** From 2015 to 2019, nearly all the increase of EryNS and CliNS among iGAS isolates in ABCs could be accounted for by genomic clusters of resistant isolates, suggesting a prominent role of temporally and genomically related iGAS infections in facilitating the spread of antimicrobial resistance.

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Control Number: 2022-A-145-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Broad Surveillance of Circulating Respiratory Infections: Implementation, Scalability, and Data Sharing Strategies for Clinical and Public Health Laboratories

Author Block: **C. Clark**, H. Houdeshell, A. Vest, E. Arnold, M. Hardison;
Aegis Sciences Corporation, Nashville, TN.

Abstract Body: The COVID-19 global pandemic underscored the importance of both broad community surveillance and detailed analysis of pathogens. As the world moves from a pandemic to endemic state, the scientific and medical community must recognize the value of monitoring non-CoV2 respiratory pathogens. This study aims demonstrates the benefit of surveillance at scale and establishes platforms for data sharing to support public health decisions in response to emerging infectious diseases. Through a collaboration with Illumina, Walgreens, and Aegis Sciences Corporation, residual respiratory specimens submitted for COVID-19 testing from patients across the United States were sequenced using the Illumina® Respiratory Pathogen ID/AMR Target Enrichment Panel (RPIP), a next-generation sequencing assay capable of simultaneously detecting 280 viral, bacterial, and fungal respiratory pathogens. Specimens were selected for sequencing based on their COVID-19 results and the number and type of symptoms reported, and results were analyzed using the IDbyDNA® Explify RPIP Analysis Application on Illumina® BaseSpace Sequence Hub. Sequencing and demographic data were parsed and analyzed to create a surveillance dashboard of respiratory pathogens found. Initial studies demonstrated 54% of samples were positive for one or more viral pathogens, including 7.5% viral co-infections, and 38% co-positives for a bacterial target, many of which appeared to be normal flora. 44% of COVID-19 negative samples were infected with another respiratory virus, the most common of which was *Influenza* (12%, 25% NAI resistant), *Rhinovirus* (7%), and *Human Parainfluenza Virus* (3%). Bacteria were detected in 26% of all samples, mostly commensal, with the notable exception of *M. catarrhalis* (6.1%). Thirteen AMR alleles that influence efficacy of eight different drug classes were identified. No fungal organisms were present. Respiratory infections were present in all age groups and in 18 of 42 states analyzed. Risk factors, such as asthma and chronic lung or heart disease, were prevalent in 50% of individuals who tested positive for non-CoV2 respiratory infections. Next-generation sequencing provides a cost effective, high-throughput option for broad pathogen tracking and surveillance. This study demonstrates how laboratories can leverage collaborative relationships with public and private sector entities to expand resources, innovation, and capabilities to support these efforts and offers a framework for laboratories to quickly respond to biological threats and support public health preparedness and decisions aimed at limiting the spread of health risks within the community.

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Control Number: 2022-A-151-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Genomic epidemiology and population structure of *Salmonella enterica* in Peru over the last 20 years

Author Block: J. Caro-Castro[1], F. Guzman[2], W. Quino[1], D. Flores-Leon[1], **R. G. Gavilan**[1];
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Abstract Body: *Salmonella enterica* is the main cause of salmonellosis in humans and is considered one of the most important etiologies due to its impact on public and animal health. There are more than 2,500 serovars of *S. enterica*; however, some serovars such as *S.* Enteritidis

and *S.* Typhimurium are more prevalent globally. In recent years, the appearance of serotypes such as S*. infantis* with a phenotype resistant to multiple antimicrobials has increased interest in studying these microorganisms using whole genome sequencing (WGS) to understand the evolutionary history of *S. enterica*. The objective of the present study was to analyze the population structure and phylogenetic relationship of *S. enterica* strains isolated in Peru from 1999 to 2017. A total of 1,000 strains were characterized by microbiological and PCR methods as *S. enterica*, and sequenced using the Illumina Miseq platform. Of the 1,000 sequenced genomes, 903 presented good quality values, so they were used in subsequent analyses. In silico serotyping classified 845 strains into 41 serotypes, while 58 strains were not classified into any serotype due to they did not fit the Kauffman-White scheme. On the other hand, the MLST classified 795 strains into 39 different STs, while the remaining 108 strains could not be assigned to a previously described ST. The most frequent genotypes were ST-11 (*S.* Enteritidis, n=221), ST-32 (*S.* Infantis, n=196) and ST-19 (*S.* Typhimurium, n=125). A total of 367,960 SNPs were determined; however, only 4,337 belonged to the core genome. The phylogenetic structure revealed a composition according to the distribution of genotypes by MLST. A group of genes which confers antibiotic resistance to beta-lactams, quinolones and tetracyclines were detected in different STs, having different resistance profiles. Using the annotation of the 903 genomes of *S. enterica*, a pan-genome composed of 19,030 gene clusters was obtained, while the analysis of the variability between *S. enterica* strains determined by principal component analysis (PCA), using the matrix of presence/absence of clusters of accessory genes (15,803 clusters), determined that there is a grouping of strains of the same ST or serotype. In conclusion, the presence of a large number of circulating serotypes and genotypes in Peru was determined. In conclusion, the presence of a large number of circulating serotypes in Peru was determined by WGS, being the most frequent *S.* Enteritidis, *S.* Infantis and *S.* Typhimurium. In addition, there is great variability among all the genotypes detected according to the phylogeny, pan-genome and PCA analyses, showing the necessary to continue with molecular epidemiological surveillance of the different *S. enterica* genotypes to prevent future outbreaks and infections by highly virulent and antimicrobial resistant strains.

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Control Number: 2022-A-186-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: Genomic analysis of West Nile Virus in Maricopa County and the Greater Southwest Region

Author Block: **C. M. Hepp**[1], D. E. Erickson[1], Z. Barrand[1], C. L. Ridenour[1], D. Lemmer[1], K. Simmons[1], P. Hawkinson[1], B. N. Brock[1], K. Sheridan[1], M. Valentine[1], H. Centner[1], J. Townsend[2], J. Will[2], N. Busser[2], D. M. Engelthaler[1];
[1]Translational Genomics Research Institute - Pathogen and Microbiome Division, Flagstaff, AZ, [2]Vector Control Division of Maricopa County Environmental Services Department, Phoenix, AZ.

Abstract Body: **Since the first detection of West Nile virus (WNV) in the US in 1999, nearly 55,000 people have tested positive and more than 2,700 have died. Over the past decade, Arizona has ranked 3rd for highest total and neuroinvasive disease cases, with the majority occurring in Maricopa County. While 2021 was an average year for WNV cases across much of the US**

(n=2,695), AZ faced its highest number of cases ever (n=1,693), with Maricopa County residents comprising ~86% of those cases. At the onset of this study in 2016, we hypothesized that the county likely experienced repeated introductions year after year, and that given the distance between several southwestern counties that frequently report human cases (eg. Riverside, Clark, Washington, and Yuma counties), outbreaks were geographically distinct. To address our hypotheses, we've partnered with vector control agencies throughout the southwest, who have provided more than 800 WNV positive samples that we sequenced using a novel tiled amplicon sequencing approach. The resulting genomes and associated spatiotemporal metadata were analyzed within Bayesian phylogenetic and Maximum Likelihood-based phylogeographic frameworks. Our study reveals novel insights regarding West Nile virus in Maricopa County and the greater southwest: 1) WNV is endemic in Maricopa County, overwintering and reemerging annually, with a limited number of new and short-lived importations, 2) This endemic WNV population is the longest known in any US county, persisting over the past decade, 3) Preliminarily, that genomically-derived effective population size estimates are strong predictors of spillover risk, and 4) WNV in Maricopa County repeatedly spills over into other southwestern counties, indicating that this viral population is not only important for the public health of Maricopa County residents, but the rest of the region as well. Genomic results have been made publicly available, to provide situational awareness to our partners and the public:
https://nextstrain.org/community/HeppLab/WestNileVirus@main/NorthAmerica?c=county

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Control Number: 2022-A-187-NGS

Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology

Publishing Title: After the data: Navigating the utilization of next generation sequencing data for informed public health

Author Block: **H. Blankenship**;
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Abstract Body: Over the past two years with the SARS-CoV-2 pandemic, we have seen an exponential growth in the number and diversity of sequences that are generated from various platforms to different organisms, allowing for more information to be available without a plan on data utilization. Public health is now faced with the challenges on how to use and communicate the data, how to identify what data is considered quality, and how to leverage partnerships to assist with bandwidth as pandemic response is not over. This presentation would navigate the challenges that are faced with communication to state and local epidemiologists and integration of data types into aging disease surveillance systems. Sequencing data does not fit within the black/white, yes/no, and positive/negative that are used for other data types, opening instead a realm of opportunities depending on the question that need to be answered. As more laboratories gain the capacity and funding to perform routine sequencing, standardization of data types is required to ensure that statewide surveillance does not develop any gaps. Additionally, identification of quality data metrics for internal and external sequencing data becomes critical for documentation and policy development to ensure that surveillance and outbreak detection are confident in analyses. As response to the pandemic continues, public health relies on the utilization of

academic partners to bridge the needs for public health response. This ranges from sample acquisition and sequencing platform development to advanced genomic epidemiology modeling and transmission dynamics for organisms of importance. Elucidation of these key dynamics will help to inform public health response while not stressing the bandwidth of public health staff. Overall, this talk aims to present the key areas that need growth and development within public health to ensure that the data being generated is used in real time response for public health.

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