



CAFPA-ASM D.C. Branch FALL MEETING 2021-LIGHTNING TALKS
October 27th | 1:50-2:20 pm | 3-5 min/talk



1:50 pm—Evaluating the risks associated with utilization of modified washing machines in the processing of leafy greens

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The CDC reports that 46% of foodborne illness incidents are attributed to produce, of which leafy greens are responsible for most of these. Small and medium-sized leafy green growers commonly retrofit washing machines to dry triple-washed leaves, utilizing their spin cycle, effectively turning them into large salad spinners. However, the potential for this practice to cause microbial contamination and the degree to which it can pose a risk to cause foodborne illnesses has not been explored. This project aims to investigate the risk of cross-contamination associated with the common practice of drying leafy greens using washing machines to help inform best practices to mitigate risks. *Listeria innocua* was utilized as a surrogate for *L. monocytogenes*, and 10^3 CFU/ml was inoculated and dried onto 250 g of spinach per run to mimic natural contamination. Prior to the run and post the inoculation a wash step was introduced, and the inoculated spinach was made to run for 2 minutes. After the spin, three contact surfaces of the machine, loading basket, internal chamber and the water collection chamber were sampled using sterile microbial swabs to enumerate the relative levels of the bacterial transfer, with 3 swabs per region. The swabs were then plated and enumerated using 3M *Listeria* Petrifilms. A recovery range of 10^1 - 10^3 CFU/ml of from different contact surfaces was recovered from the various points, with the direct contact surface containing consistently the highest levels of *Listeria*. This suggests that use of these machines could pose a risk of contamination and validates the need for establishing cleaning and sanitation guidelines to improve the safety of processing leafy greens in this manner.

1:55 pm—Investigating the role of SepA in enteroaggregative *Escherichia coli* biofilm and virulence in *Galleria mellonella*

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Enteraggregative *E. coli* (EAEC) is associated with acute and chronic diarrhea, adheres in an aggregative pattern, and creates thick biofilms on the intestinal mucosa. Epidemiological data suggest that SepA, a serine protease autotransporter of Enterobacteriaceae (SPATE), is important for EAEC disease. The genes for SepA and other proteins important for EAEC biofilm formation and aggregative adherence are encoded on the pAA plasmid. We deleted *sepA* in clinical EAEC strains and assessed biofilm formation. We observed that 4/5 $\Delta sepA$ strains demonstrated increased biofilm staining compared to the wild-type (wt) control. However, all of the strains secreted similar amounts of SepA. We therefore hypothesize that SepA modulates biofilm formation via cleavage of a target present in only some EAEC.

We next transferred the pAA from a wt EAEC and an isogenic $\Delta sepA$ derivative into commensal *E. coli*, HS. We found that HS with pAA $\Delta sepA$ plasmid demonstrated more biofilm staining than HS with wt pAA plasmid. This result suggests that the SepA target is likely encoded on the pAA. When we infected *Galleria mellonella* with the wt or $\Delta sepA$ strain, we found that the *Galleria* infected with the wt strain exhibited increased melanization and mortality compared to the $\Delta sepA$ strain. Because melanization is part of the inflammatory immune response to pathogens in *Galleria*, we hypothesize that SepA triggers an inflammatory response in *Galleria* and is important for EAEC virulence in this model. By understanding the function of SepA in biofilm formation, we hope to better understand the role of SepA in EAEC pathogenesis and uncover therapeutic targets or vaccine candidates.



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2:00 pm—Improving produce safety via the elicitation of plant stress responses with CAP

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Cold atmospheric plasma (CAP) is a nonthermal antimicrobial treatment well suited for delicate food products such as leafy greens. CAP produces UV light, reactive oxygen and nitrogen species (RONS) that may inactivate pathogens through oxidative cell damage. Prior work with CAP and fresh produce has indicated that the treatment triggers plant stress response in leafy greens. Some antioxidants and other beneficial compounds such as flavonoids are produced in response to abiotic stress in plant tissue.

A bench-top dielectric barrier discharge (DBD) plasma device (Advanced Plasma Solutions, Malvern, PA) was used for all experiments. Conventionally grown, pre-washed, spinach was treated with CAP for 1 minute at varying power levels, with flavonoid content measured using the diphenylboric acid 2-aminoethyl ester (DPBA) fluorescence assay. The assay was selected because it could be performed immediately after treatment with minimal additional processing of spinach. Spinach was stomached with 2% DPBA in DMSO and fluorescence was measured at emission wavelength 400 nm and excitation wavelength of 465 nm. Epigallocatechin gallate (EGCG) was used as the reference flavonoid compound.

Compared to control (0.70 ± 1.40 mgM EGCG Eq /g), flavonoid content in spinach leaves treated with 50 and 120 W plasma increased significantly ($p < 0.05$) to 4.59 ± 1.32 mgM EGCG Eq /g and 3.92 ± 1.83 mgM EGCG Eq /g, respectively. No significant difference ($P > 0.05$) was observed in flavonoid concentration between these two treatments. Future work will characterize the response of the spinach phenylpropanoid pathway to CAP treatment using qRT-PCR to evaluate enzymatic activity at different power levels.

2:05 pm—Near real-time genomic surveillance of multidrug-resistant organisms and associated outbreaks across an extensive network of hospitals in the United States

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Active surveillance is critical for detecting and preventing the transmission of multidrug-resistant organisms (MDRO) in health care facilities. The Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) is the primary surveillance organization for the DoD and collects MDROs from an extensive network of US military treatment facilities (MTFs) across the Military Health System (MHS). Traditionally, investigations by the MRSN were requested by infectious disease physicians or infection control staff upon the suspicion of nosocomial transmission. Recently the MRSN developed an approach for the routine detection, in near real-time, of possible MDRO outbreaks. This new service, originally deployed for five facilities, no longer depends on human pattern detection but employs systematic genomic comparison of newly received MDROs to a repository of >85,000 historical isolates. Upon detection of highly genetically related isolates, epidemiological analyses are initiated and the MTF is immediately alerted.

During 2019-2020, for a single MTF and tracking only infections due to MDR *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, this effort resulted in the detection of 28 clusters of potential transmission involving 74 patients. Among the successful outcomes, a multi-ward outbreak involving six patients caused by a carbapenem-resistant *A. baumannii* was detected early, tracked, and successfully eradicated. The MRSN has now expanded this effort to 13 MTFs and a VA hospital, and additional species, making the MHS the largest health system in the world benefiting from routine, genome-based surveillance of bacterial outbreak and providing a generalizable blueprint for the implementation of such services at large scale.



2:10 pm—Evasion of the NLRP3 Inflammasome activation by *Mycobacterium tuberculosis* via the serine/threonine kinase PknF in infected macrophages

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Mycobacterium tuberculosis (Mtb) has evolved strategies to evade host innate immunity and persist inside host macrophages. Macrophages encode the cytosolic sensor proteins, NOD like receptor (NLRs), which recognizes pathogen/danger associated molecular patterns (P/DAMPs) and lead to assembly of the inflammasome signaling complex that activates caspase-1 and results in release of mature IL-1 β and host cell death via pyroptosis. Here we show that Mtb inhibits NLRP3 inflammasome activation and subsequent production of IL-1 β in an ESX-1 independent manner. We identified the serine/threonine kinase PknF as one protein of Mtb involved in the NLRP3 inflammasome inhibition, since the *pknF* deletion mutant of Mtb compared to Mtb-infected cells induces increased production of IL-1 β and increased pyroptosis in BMDMs. The increased production of IL-1 β was found to be dependent on the NLRP3, the adaptor protein, ASC, and the proteases caspase-1 and -11, as revealed by studies performed in BMDMs derived from the corresponding knockout mice. The IL-6 production by Mtb *pknF* mutant remained unchanged compared to Mtb-infected cells, suggesting that the mutant did not affect the priming step of inflammasome activation. In contrast, the activation step was affected since potassium efflux, chloride efflux and the generation of reactive oxygen species played a significant role in inflammasome activation but there was no role for calcium fluxes. In conclusion, we reveal a novel role for Mtb PknF in innate immune evasion through inhibition of the NLRP3 inflammasome.

2:15 pm—Using wastewater based epidemiology (WBE) to analyze SARS-CoV-2 trends at WWTPs in Southern Maryland

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Wastewater-based epidemiology has been shown an effective tool to monitor the prevalence of numerous viruses, including SARS-CoV-2. Using WBE to analyze wastewater of a large area can help to predict trends over time with respect to different populations. In this study, we analyzed the presence of the SARS-CoV-2 virus in wastewater from five wastewater treatment plants (WWTPs) in Southern Maryland. The wastewater data was compared with the number of the confirmed COVID-19 cases in the respective geographic areas during the period of the Oct. 2020 – Aug 2021. The use of the Pepper Mild Mottle Virus (PMMoV) (a fecal indicator) was also used to look at normalization effects and the impact of dilution. Many variations were seen between WWTPs and their correlations. On average, roughly 60% of the WWTPs had a good correlation between calculated wastewater values and representative clinical cases reported in the area. A specific focus looked at how correlations shift over time, including investigating the reduction and subsequent increase of Covid-19 cases following the implementation of the vaccine and the rise of the Delta variant, respectively.