American Society for Microbiology
Kentucky / Tennessee Branch
2025 Spring Meeting

Keynote Speakers

Dr. Gladys Alexandre
The University of Tennessee

Dr. Tera Levin
University of Pittsburg

April 4-5th, 2025

Clinical and Translational Research Building
University of Louisville, Health Sciences Campus

We would like to thank the following sponsors for supporting this meeting:
University of Louisville Department Of Microbiology and Immunology
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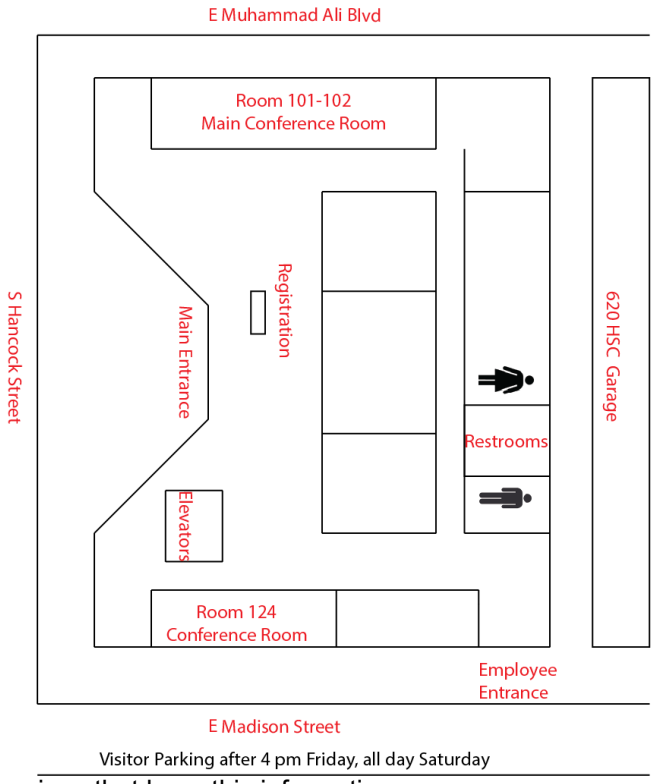
Meeting Location: The meeting will be held in the Kosair for Kids Clinical and Translational Research Building (CTRB) on the first floor. Enter through the doors facing Hancock Street. Do not enter through the doors near the 620 HSC garage on the south side of the building. Posters boards will be set up in the CTR Atrium starting Saturday morning. Water fountains are available near the restrooms. Overflow seating for the keynote sessions will be in Room 124.



Building address: 505 South Hancock Street, Louisville

Parking

There are a few options for parking on UofL's HSC campus.

1. Parking by CTRB: there are parking spots located on the south side of Madison street that can be used after 4 pm on Friday or all day Saturday.
2. Non-metered street parking (green dashed lines on map). There are no parking meters on these blocks.
3. Metered Parking on the street (red dashed lines on map):
   - Operating hours for all meters are Monday - Saturday, 7:00 a.m. to 6:00 p.m. <u>This means free parking after 6:00 p.m. Monday through Saturday and free all day Sunday!</u>
   - Parking meter rate at traditional meters is $2.25/hour for the first 2 hours and $3.35/hour for each additional hour, and are limited to 4 hours of parking. Please look for signs that have this information.
4. Garage Parking: The 620 HSC garage offers parking spaces for an hourly fee (max $12 per day). The entrance for the paid parking spaces is off Clay street (near 520 S Clay Street).

Meeting Schedule

**note all times are in EST

Overview:

Friday, April 4th

3:30 – 6:30 PM	Registration
5:00 – 6:30 PM	Dinner available
6:00 – 8:45 PM	Keynote session 1; Room 101
8:45 – 9:00 PM	Break
9:00 – 9:45 PM	Microbiology Trivia Game; Room 101-102

Saturday, April 5th

7:30 – 11:00 AM	Registration
8:00 – 9:30 AM	Breakfast available
8:30 – 9:30 AM	Concurrent Sessions 2 (Room 101) & 3 (Room 124)
9:30 – 9:45 AM	Break
9:45 – 11:15 AM	Concurrent Sessions 4 (Room 101) & 5 (Room 124)
11:15 – 12:45 PM	Poster Presentations
1:00 – 2:00 PM	Lunch Sessions Concurrent for Trainees (101) and Faculty (124)
2:00 – 4:00 PM	Keynote Session 2 (Room 101)
4:00 – 4:15 PM	Awards Presentation and Close of Meeting (Room 101)

Friday April 4th

Registration: 3:30 - 6:30
Dinner Served: 5:00 - 6:30

Session 1:
Moderator: Dr. Ryan Doster
Room 101-102

Start time	Abstract number	Speaker/Institution	Title
6:00	Dr. Ryan Doster University of Louisville		Opening Remarks
6:05	F_O1	Dr. Saurabh Chattopadhyay, University of Kentucky College of Medicine	Non-transcriptional role of IRFs in controlling viral inflammation
6:30	**KN1**	**Dr. Gladys Alexandre University of Tennessee**	**Integrating metabolism with bacterial chemotaxis signaling: the metabolism-behavior axis of soil bacteria**
7:30		Break	
7:40	PD_O1	Dr. Kendall Stocke University of Louisville	Tyrosine phosphorylation coupling of one carbon metabolism and virulence in the endogenous human oral pathogen Porpyromonas gingivalis
7:55	PD_O2	Dr. Gustavo Santiago-Collazo St. Jude Children's Research Hospital	Two-component system 11 Regulates Competence and Colonization in Streptococcus pneumoniae
8:10	PD_O3	Dr. Nagwa El-Baz University of Louisville	Lactobacillus crispatus-Loaded Electrospun Nanofibers: An Antibiotic-Free Approach to Prevent and Treat Group B Streptococcus Infection
8:25	PD_O4	Dr. Mahendar Kadari University of Louisville	Defining copper tolerance mechanisms in Yersinia and their role in virulence
Break			
9:00	Microbiology Trivia Game		Sign up at registration table by 7:00pm

Saturday April 5th

Registration table open: 7:30-11:00
Continental Breakfast Served 8:00-9:30

Session 2:
Moderator: Dr. Kathryn Ramsey
Room 101-102

Start time	Abstract Number	Speaker/Institution	Title
8:30	Gb_O1	Bibek Lamichhane University of Kentucky	Targeting quorum sensing with novel small molecules: New insights into controlling Salmonella infections.
8:42	Gb_O2	Christopher Farrell University of Louisville	Glucose-containing carbohydrates modulate Group B Streptococcus growth and biofilm
8:54	Gb_O3	Alex Labossiere University of Louisville	“What’s SUPP?” Developing an In Vitro Model for Healthy Oral Biofilms
9:06	Gb_O4	Hannah Richards Vanderbilt University	Magnetic Bead Electrochemical Sandwich Assay for Investigating Group B Streptococcus-Induced Inflammatory Responses in an Instrumented Fetal Membrane on-a-chip
9:18	Gb_O5	Subarna Roy University of Louisville	Understanding how Yersinia pestis responds to metal restriction by the host nutritional immunity protein calprotectin during plague

Session 3:
Moderator: Dr. James Collins
Room 124

Start time	Abstract Number	Speaker/Institution	Title
8:30	Ga_O1	Abigail Pyburn ETSU	Cyclic-di-GMP phosphodiesterase STM3615 regulates Salmonella physiology
8:42	Ga_O2	Avni Patel University of Tennessee, College of Veterinary Medicine	Optimization of a Leptospira growth inhibition assay to assess the antibody efficacy.
8:54	Ga_O3	Golam Mahbub Faisal University of Kentucky	The Pathogenomics of Salmonella enterica Circulating in Poultry Farms
9:06	Ga_O4	Thomas Williams ETSU	Cyclic-di-GMP Regulated Motility in Acinetobacter baumannii
9:18	Ga_O5	Ajran Kabir University of Kentucky	Genomic Insights into Antimicrobial Resistance and Virulence of Salmonella enterica Isolated from Horses and their Public Health Implications

Break 9:30 – 9:45

Session 4:
Moderator: Dr. Jala Venkatakrishna
Room 101-102

Start time	Abstract Number	Speaker/Institution	Title
9:45	PD_O5	Dr. Pracheta Sengupta University of Kentucky	Non-transcriptional function of IRF7 in antiviral immunity
10:00	PD_O6	Dr. Jordan Cannon Circular Biosciences	Engineering an enzyme additive to improve bioplastic degradation
10:15	PD_O7	Dr. Oscar Vazquez Ciros University of Kentucky	A Quorum Sensing pathway regulates virulence in Streptococcus agalactiae
10:30	PD_O8	Dr. Rachel Washburn Kentucky Geological Survey, University of Kentucky	Microbial Hazards and Health Potential: An Integrative Geohealth Framework Evolving at the Kentucky Geological Survey
10:45	PD_O9	Dr. Derica Tavares University of Louisville	Host response to pathogenic vs. endophytic fungi

11:00	PD_O10	Dr. Mohammad Rahman University of Kentucky	O-glycosylation of serine/threonine-rich intrinsically disordered regions (IDRs) of membrane proteins in Streptococcus pyogenes

Session 5:
Moderator: Dr. Donghoon Chung
Room 124

Start time	Abstract Number	Speaker/Institution	Title
9:45	Ga_O6	Casey Stophel ETSU	Isolation and characterization of siderophore(s) from Pseudomonas mosselii
9:57	Ga_O7	Anna Ayers ETSU	Investigation of Sensory Cyclic-di-GMP Phosphodiesterases in Salmonella
10:09	Gb_O6	Deepa Karki University of Louisville	Mechanistic Insights into Sindbis Virus Infection: Noncapped Genomic RNAs Enhance the Translation of Capped Genomic RNAs to Promote Viral Infectivity
10:21	Gb_O7	Katelyn Sheneman University of Louisville	Manipulation of extracellular vesicle biogenesis aids Yersinia pestis pathogenesis
10:33	Gb_O8	Cierra Isom University of Louisville	Mechanistic Insights into Sindbis Virus Infection: Noncapped Genomic RNAs Enhance the Translation of Capped Genomic RNAs to Promote Viral Infectivity
10:45	PD_O11	Dr. Santanu Das University of Kentucky	Macrophage-specific IRF3 curbs viral inflammation in the lung

Poster Presentations: 11:15-12:45

1:00 Lunch Sessions:
Trainee Session: (take your lunch)
Room 101
Moderator: Whitney Heard

1:00	Advocacy 101	Amalia Corby, ASM Director of Federal Affairs, American Society for Microbiology
1:30	ASM Young Ambassador Programming discussion	Whitney Heard, ASM Young Ambassador for the state of Kentucky University of Louisville
1:45	Attending ASM Microbe, a grad student prospective	Taylor Garrison and Denny Gao University of Louisville

Faculty Session:
Room 124: KY/TN Branch Meeting

Session 6:
Moderator: Matt Lawrenz
Room 101-102

Start time	Abstract Number	Speaker/Institution	Title
2:00	**KN2**	**Dr. Tera Levin University of Pittsburg**	**The evolution of immunity and pathogenesis within environmental battlegrounds**
3:05	F_O2	Dr. Alison J. Eastman Vanderbilt University	Modeling early events in bacterial chorioamnionitis using an organ-on-chip of the maternal-fetal interface
3:30	F_O3	Dr. James Collins University of Louisville	The Role of Diet in Asymptomatic C. difficile Carriage

4:00 Trainee Awards Presentation and Close of Meeting

U = Undergraduate or Post-baccalaureate
Ga = Graduate student year 1-2
Gb = Graduate student year 3-5
PD = Post Doc
O = Other
F = Faculty
P = poster, O = oral
Example: Ga_O1 = Grad student 1-2, Oral, abstract 1, Gb_P3 = Grad student year 3-5, Poster abstract 3

Oral Presentations (in order of presentation)

Room 101	**Session 1: Moderator Dr. Ryan Doster**
F_O1	**Non-transcriptional role of IRFs in controlling viral inflammation** Saurabh Chattopadhyay, Sukanya Chakravarty, Shumin Fan, Ritu Chakravarti University of Kentucky College of Medicine Type-I interferon (IFN) system is the first line of defense against viral infection. Virus infection is rapidly detected by cellular sensors, which activate the IFN regulatory factors (IRFs) via a series of signaling pathways. IRFs are transcription factors that are known for inducing IFNs and antiviral genes. For the first time, we uncovered a non-transcriptional function of IRF3 in inducing apoptotic cell death of virus-infected cells. Recently, we revealed a new function of IRF3, independent of transcriptional or apoptotic activities, to inhibit NF-kB-dependent inflammatory gene expression (Popli et al., PNAS, 2022). IRF3 interacts with NF-kB-p65 and sequesters it in the cytosol to inhibit its nuclear translocation. Moreover, the anti-inflammatory function of IRF3 in macrophages is critical for protection against pulmonary viral inflammation (Chakravarty et al., Sci Adv, 2024). IRF7, which also possesses an NF-kB-binding motif, suppresses inflammatory gene expression (Fan et al., JBC, 2024). The presentation will highlight how IRFs regulate inflammatory responses independent of their transcriptional activation.
KN1	**Dr. Gladys Alexandre** **University of Tennessee** **Integrating metabolism with bacterial chemotaxis signaling: the metabolism-behavior axis of soil bacteria**
PD_O1	**Tyrosine phosphorylation coupling of one carbon metabolism and virulence in the endogenous human oral pathogen Porpyromonas gingivalis** Kendall S. Stocke(1), Satya D. Pandey(1), Shunying Jin(1), John D. Perpich(1,2), Lan Yakoumatos(1), Hirotaka Kosaki(1), Daniel W. Wilkey(3), Zackary R. Fitzsimonds(1), Aruna Vashishta(1), Ian Snider(1), Mukesh K. Sriwastva(1), Hong Li(4), Jiu-Zhen Jin(1), Jixiang Ding(1), Daniel P. Miller(1,‡), Michael L. Merchant(3), Juhi Bagaitkar(5,6), Silvia M. Uriarte(1), Jan Potempa(1,7), Richard J. Lamont(1) (1) Department of Oral Immunology and Infectious Diseases, University of Louisville (2) Department of Pharmaceutical Sciences, Sullivan University College of Pharmacy and Health Sciences (3) Division of Nephrology and Hypertension, Department of Medicine, University of Louisville; (4) James Graham Brown Cancer Center, University of Louisville (5) Center for Microbe and Immunity Research, Nationwide Children's Hospital (6) Department of Pediatrics, The Ohio State College of Medicine (7) Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University; Krakow, Poland. (†) Current address: Brown Cancer Center, University of Louisville; Louisville, USA. (‡) Current address: Department of Microbiology and Immunology, Virginia Commonwealth University Background: Periodontitis is a disease where a single microbe, such as Porphyromonas gingivalis (Pg), can disrupt the oral microbiome toward a pro-inflammatory state. Typically, reduced availability of para-aminobenzoate (pABA), a precursor to folate and one-carbon metabolism (OCM), decreases microbial virulence. However, Pg behaves differently: when pABA is limited, Pg becomes more virulent. pABA inhibits the low molecular weight tyrosine phosphatase Ltp1, which normally limits the activity of the bacterial tyrosine (BY) kinase Ptk1, a key regulator of Pg's virulence. This study investigated how limited pABA availability or disruption of Ptk1 signaling affects Pg's virulence and OCM flux. Methods: We used Pg strains deficient in pABA synthesis (ΔpabC) and the BY kinase Ptk1 (Δptk1) to assess virulence factor expression and OCM flux. We tested these strains using gingipain protease activity assays, measuring folate levels, RNASeq, protein mass spectrometry, and in vivo experiments in mice.

	Results: Both mutants showed enhanced virulence, including increased alveolar bone loss and diminished neutrophil response in mice. RNASeq data indicated that regulation of virulence factors was largely posttranslational, as gene expression of known virulence factors was minimally affected. Both mutants exhibited increased membrane-bound gingipain protease activity and decreased gingipain secretion into the environment, along with impaired folate production. However, the ΔpabC mutant recovered folate production and Ptk1 phosphorylation status with pABA supplementation. Mass spectrometry confirmed interactions between Ptk1 and key enzymes involved in OCM and folate synthesis, several of which were phosphorylated by Ptk1, including GcvT, GlyA, and ALP, the latter of which had its alkaline phosphatase activity altered by phosphorylation. Conclusions: Ptk1 regulates virulence in Pg by balancing OCM flux via the modulation of gingipain protease partitioning. This study highlights how Pg can remain non-pathogenic in healthy contexts despite having potent virulence factors."
PD_O2	**Two-component system 11 Regulates Competence and Colonization in Streptococcus pneumoniae** Gustavo Santiago-Collazo, Abigail McKnight, Haley Echlin and Jason Rosch St. Jude Children's Research Hospital Streptococcus pneumoniae (pneumococcus) is a leading cause of severe infections, such as pneumonia, sepsis, and meningitis, posing a particularly high risk to young children and immunocompromised individuals. Despite the availability of vaccines and treatments, pneumococcal infections still account for approximately 11% of the global childhood mortality, underscoring the urgent need for novel therapeutic strategies to reduce this burden. The capacity of S. pneumoniae for natural transformation and DNA uptake enhances its genetic adaptability, driving antibiotic resistance and immune evasion. Although two-component systems (TCSs) are known to regulate key processes in bacterial adaptation, the specific mechanisms by which TCS11 modulates bacteriocin production and genetic exchange in S. pneumoniae remain largely unexplored. TCSs are signal transduction pathways that rely on the interaction between a histidine kinase (HK) and a response regulator (RR), which together modulate gene expression in response to environmental signals. Using a combination of genetic, molecular, and in vivo approaches, we aimed to characterize TCS11 and its role in enhancing competence and colonization. Our data showed that deletion of the tcs11 operon reduced DNA uptake in vitro and downregulated competence genes regulated by the comDE two-component system. Conversely, overexpression of the tcs11 operon increases DNA uptake and upregulates comDE-regulated competence genes. Moreover, in vivo results indicated that disrupting TCS11 leads to defects in colonization and transmission. These findings implicate TCS11 as a key contributor to pneumococcal competence and colonization.
PD_O3	**Lactobacillus crispatus-Loaded Electrospun Nanofibers: An Antibiotic-Free Approach to Prevent and Treat Group B Streptococcus Infection** Nagwa El-Baz(1), Anthony Kyser(2), Christopher Farrell(3), Hermann Frieboes(2), Ryan Doster(1,3) (1) Department of Medicine, Division of Infectious Diseases, School of Medicine, University of Louisville (2) Department of Bioengineering, Speed School of Engineering, University of Louisville (3) Department of Microbiology and immunology, School of Medicine, University of Louisville Background: Group B Streptococcus (GBS) colonization of the gravid reproductive tract is a risk factor for adverse pregnancy outcomes and severe neonatal illness and mortality. Lactobacillus dominant vaginal microbiomes provide protection against pathogens like GBS by lowering vaginal pH and secreting bacteriocins. We hypothesized that using commensal bacteria may be an antibiotic-free approach to limit GBS colonization. We investigated the ability of electrospun nanofibers (EFs) loaded with Lactobacillus crispatus (Lc) to mitigate GBS growth and modulate the immune responses from vaginal epithelial cells. Methods: Vaginal epithelial cells (VK2/E6E7) were cultured on transwells at an air-liquid interface. Electrospun nanofibers (EFs) were synthesized using polyethylene oxide (PEO), and Lc (strain MV-1A-US) cultures were incorporated into EFs. For the prevention model, VK2/E6E7 cells were pre-treated with Lc alone, nanofibers loaded with Lc (EFs-Lc), nanofibers without bacteria (EFs), or mock treatment (phosphate buffered saline (PBS)) for 24 h followed by the addition of GBS CNCTC 10/84. For the treatment model, VK2/E6E7 cells were infected with GBS for 24 h followed by treatment with Lc alone, EFs-Lc, or EFs, or PBS. Apical and basal supernatants were collected, and membranes were fixed for bacterial quantification, cytokine analyses and scanning electron microscopy (SEM), respectively. Results: EFs released viable L. crispatus, as demonstrated by quantitative culture and SEM images. Pre-treatment with EFs-Lc did not prevent GBS establishment but increased anti-inflammatory IL-1RA and decreased the pro-inflammatory IL-8 release. The treatment model showed a reduction in GBS colony-forming units (CFU) and an increase in anti-inflammatory signaling. Conclusion: In our in vitro model of the vaginal epithelium, EFs delivered viable L. crispatus, which modulated the inflammatory signaling of vaginal epithelial cells by increasing IL-1Ra production and

	decreasing IL-8 in response to GBS infection. These results suggest that electrospun nanofibers can deliver beneficial components of the vaginal microbiome.
PD_O4	**Defining copper tolerance mechanisms in Yersinia and their role in virulence** Mahendar Kadari(1), Casey Weber(1), Sarah L Price(1), Thomas E Kehl-Fie(2), Robert D Perry(3), Matthew B Lawrenz(1,4) (1) Department of Microbiology and Immunology, University of Louisville School of Medicine (2) Department of Microbiology and Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign (3) Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky School of Medicine (4) Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville Yersinia pestis is the etiological agent of plague that emerged from enteric pathogen Yersinia pseudotuberculosis around 6000 years ago. A key barrier to infection is host-imposed nutritional immunity, a mechanism by which the host sequesters trace metals like iron, zinc, and manganese to restrict bacterial colonization. While Copper (Cu) is also an essential trace element that serves as a cofactor for number of enzymes, Cu is extremely toxic if present in excess. Recent studies suggest that Cu intoxication contributes to the host immune response to kill microbes. However, the role of Cu restriction and intoxication, and how Y. pestis maintains Cu homeostasis, during plague is unknown. The main aim of this project is to identify Cu tolerance mechanisms in Y. pestis and define their contributions to virulence. For many bacteria, CopA is required to maintain Cu homeostasis and limit Cu toxicity. However, a Y. pestis copA mutant was only slightly more sensitive to Cu toxicity than the parental Y. pestis strain, suggesting additional copper tolerance mechanisms in Y. pestis. Interestingly, Y. pestis has two frameshift mutations in the gene encoding the Yersinopine (Ypn) importer. Ypn is an opine-type metallophore that can bind to Cu, and homologs of this metallophore have been linked to Cu acquisition in Staphylococcus aureus. Because Y. pestis appears unable to import Ypn, we hypothesized that Ypn may act as a secreted factor contributing to Cu resistance. Similar to the copA mutant, a cnt mutant unable to produce Ypn was not significantly more sensitive to Cu toxicity than WT Y. pestis. However, the double copA cnt mutant was highly susceptible to Cu stress. Together, these data suggest that CopA and Ypn are redundant Cu tolerance mechanisms. Interestingly, Y. pseudotuberculosis lacks the same point mutations in the Ypn importer and a Y. pseudotuberculosis copA mutant is significantly attenuated for growth under Cu stress. Taken together, our data suggest that during its evolution from Y. pseudotuberculosis, Y. pestis acquired mutations in the Ypn importer that increased its resistance to Cu stress.
Room 101	**Session 2 – Moderator Dr. Kathryn Ramsey**
Gb_O1	**Targeting quorum sensing with novel small molecules: New insights into controlling Salmonella infections.** Bibek Lamichhane, Khaled A. Shaaban, Larissa V. Ponomareva, Jon S. Thorson, Yosra A. Helmy; Department of Veterinary Science, Martin Gatton College of Agriculture, Food, and Environment, University of Kentucky Center for Pharmaceutical Research and Innovation, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky Background: Salmonella is a major foodborne pathogen causing severe gastroenteritis in humans and animals. It is primarily transmitted to humans through consumption of contaminated poultry and poultry products. While antibiotics like ciprofloxacin and enrofloxacin are common treatment methods, rise in multidrug resistant Salmonella has necessitated the development of alternatives therapeutics. This study aims to evaluate the effect of novel small molecule (SMs) inhibitors of quorum sensing to control Salmonella infections in vitro. Materials and Methods: We screened about 2000 SMs for their effect on autoinducer-2 (AI-2) production in Salmonella. Overnight cultures were grown in the presence of 1 µL of each molecule for 6 hours, and the cell-free supernatants from treatments without growth inhibition were incubated with Vibrio harveyi BB170. The AI-2 production was assessed using a bioluminescence assay. Molecules that inhibited QS/AI-2 without affecting growth were further tested for their effect on biofilm formation, expression of virulence-associated genes, intracellular survival in human intestinal cells, cytotoxicity in colorectal carcinoma cells, and hemolysis of sheep blood. Results: We selected 10 SMs that inhibited AI-2 activity with no growth inhibition for further development. The selected SMs demonstrated better efficacy compared to benchmark QSIs of Salmonella and inhibited biofilms by up to 95-100%. RT-PCR analysis demonstrated the downregulation of genes associated with virulence, quorum sensing, biofilm formation, and motility. Additionally, 3 SMs completely cleared

	intracellular Salmonella in human intestinal cells, while 4 showed no cytotoxicity at 20 μM. None of the selected compounds showed hemolysis in sheep RBC at 1 μM. In the future, we will continue in vitro evaluation and evaluate the effect of the top two SMs in Salmonella colonization in chickens. Conclusion: Our small molecules effectively inhibited quorum sensing in Salmonella, highlighting their potential as alternative therapeutics to control Salmonella virulence.
Gb_O2	**Glucose-containing carbohydrates modulate Group B Streptococcus growth and biofilm** Christopher Farrell, Ryan S Doster University of Louisville Background: Group B Streptococcus (GBS) is a gram-positive opportunistic pathogen that causes perinatal infections, but GBS infections in non-pregnant adults with comorbidities such as diabetes mellitus are increasing. Diabetes mellitus is a metabolic disorder that results in elevated glucose concentrations in the blood and tissues. Outside of glucose, other dietary sugars, such as maltose, have also been linked to insulin resistance and elevated glucose levels. As the prevalence of diabetes is increasing worldwide, a pressing need exists to understand the mechanisms of GBS virulence in response to elevated glucose levels. We hypothesize that glucose is a major driver of GBS virulence and sought to investigate its effect on GBS growth and biofilm formation, two factors that affect GBS virulence. Methods: To identify which carbohydrates GBS can use to grow, a carbon utilization assay was performed to screen 190 carbon sources. To understand how different carbohydrates affect GBS physiology, we used a chemically defined media containing glucose or other carbohydrates to examine GBS growth and biofilms under biologically relevant concentrations (90 mg/dL, 180 mg/dL, 360 mg/dL). A crystal violet plate assay and scanning electron microscopy were used to examine biofilm formation. Results: Growing GBS in increasing glucose concentrations resulted in increased growth and biofilm formation. Carbon utilization screening identified 10 additional carbon sources that GBS could utilize for growth, and 7 were chosen for further testing: maltose, maltotriose, sucrose, mannose, galactose, glucosamine, and fructose. Glucose-containing carbohydrates (maltose, maltotriose, and sucrose) significantly increased GBS growth and biofilm formation compared to structurally different carbohydrates such as mannose, fructose, galactose, and glucosamine. Conclusions: These data indicate that glucose-containing carbohydrates play an essential role in GBS metabolism and influence biofilm formation, which may be relevant in patients with diabetes. Further investigation is needed into the pathways by which GBS utilizes carbohydrates to enhance biofilm production.
Gb_O3	**“What’s SUPP?” Developing an In Vitro Model for Healthy Oral Biofilms** Alex Labossiere, Matthew Ramsey University of Louisville, School of Dentistry A large number of adults in the US are affected by periodontal disease. A major feature of periodontal disease is a dysbiotic shift within the oral microbiota leading to lower species diversity and an increase of oral pathogens. However, it's common to detect these same pathogens in the healthy oral cavity in low abundance. This suggests oral commensals play a preventive role in oral disease. Understanding the healthy microbiota may explain how disease prevention occurs. To study healthy organisms it's beneficial to study them in their healthy host context. Current models do not well encapsulate the supragingival plaque (SUPP) environment and population diversity. Our goal is to replicate biofilms of healthy SUPP in vitro that mimic in vivo composition. To achieve this goal, we are developing an in vitro SUPP model for healthy oral biofilms. Ex vivo SUPP is inoculated on saliva-coated hydroxyapatite discs in media with boiled or filtered human saliva and human serum. After lysing samples, species are identified via V1-V2 16s rRNA microbiome characterization. Changes in α and β diversity over the course of a week are then evaluated. Currently, our model improves representation of healthy plaque commensals better than prior models by promoting a diverse SUPP population of aerobes and anaerobes. Such plaque commensals include Corynebacterium matruchotii (CM) that stably attaches with oral streptococci including Streptococcus mitis which we found induced upregulation of CM lactate catabolism genes. To validate our model, we will assess fitness of CM mutants unable to utilize lactate, already shown to be growth deficient in Sm coculture. We will measure fluorescence intensity of our wildtype and mutant CM in our model to evaluate CM fitness within a competitive polymicrobial community. When completed, this model will provide a healthy framework for further testing of mechanistic assumptions of bacterial interaction within SUPP biofilms.
Gb_O4	**Magnetic Bead Electrochemical Sandwich Assay for Investigating Group B Streptococcus-Induced Inflammatory Responses in an Instrumented Fetal Membrane on-a-chip** Hannah A. Richards(1), Alison J. Eastman(2), David E. Cliffel(1) (1) Vanderbilt University

	(2) Vanderbilt University Medical Center Problem: Preterm birth (PTB), often precipitated by infection-associated or sterile inflammation of the fetal membranes (chorioamnionitis), remains a leading cause of neonatal mortality. The mechanisms underlying infection-driven inflammation and preterm premature rupture of membranes (PPROM) are not fully deduced, and current diagnostic tools lack sensitivity for early detection. A key challenge is the absence of physiologically relevant models that accurately mimic microbial interactions and inflammation in gestational membranes. Method of Study: To explore this, we developed an instrumented fetal membrane on-a-chip (IFMOC), a microfluidic platform replicating the choriodecidual layers of gestational membranes, paired with a magnetic bead electrochemical sandwich assay (MBESA) for real-time cytokine quantification. The MBESA consisted of magnetic Dynabeads functionalized with interleukin-1β (IL-1β) antibodies, horseradish peroxidase (HRP), and 3,3',5,5'-tetramethylbenzidine (TMB) to generate an electrochemical signal. IL-1β expression was measured with the MBESA-IFMOC system following exposure to lipopolysaccharides (LPS), a potent inducer of inflammation, and Group B Streptococcus (GBS), a bacterial pathogen associated with chorioamnionitis. Results: The MBESA demonstrated high sensitivity and reproducibility in detecting IL-1β within IFMOC cultures, with a dynamic range of 10–600 pg/mL. Significant increases in IL-1β levels were observed in response to LPS and GBS, confirming the system's ability to model chorioamnionitis-driven inflammation. No statistically significant differences were found between MBESA and ELISA methods, validating its accuracy and reproducibility. Conclusions: This IFMOC-MBESA platform provides a physiologically relevant in vitro system for studying PTB-associated inflammation, enabling real-time detection of cytokine responses to infection. Future work will integrate the MBESA with IFMOC outflow ports for continuous monitoring and expand its application to additional inflammatory mediators. This approach offers a versatile tool for advancing therapeutic development in maternal-fetal health and for investigating host-pathogen interactions in additional organ on-a-chip models."
Gb_O5	**Understanding how Yersinia pestis responds to metal restriction by the host nutritional immunity protein calprotectin during plague** Subarna Roy(1), Sarah L. Price(1), Taylor M. Garrison(1), Sabine Waigel(2), Thomas E. Kehl-Fie(3), Matthew B. Lawrenz(1,4). (1) Department of Microbiology and Immunology, University of Louisville School of Medicine, (2) Brown Cancer Center, University of Louisville, (3) Department of Microbiology and Immunology, University of Iowa and (4) Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville Nutritional immunity includes the active sequestration of key biometals such as iron, zinc, and manganese from invading bacteria. We have shown that calprotectin (CP), a metal-binding nutritional immunity protein produced mainly by neutrophils, is the primary barrier for zinc acquisition by Yersinia pestis during infection. Furthermore, we have shown that Y. pestis uses the zinc transporter ZnuABC, the metallophore yersiniabactin (Ybt), and an inner membrane protein YbtX to acquire zinc during infection. However, beyond the expression of these metal acquisition systems, how Y. pestis responds to overcome CP-mediated metal restriction is still unknown. To investigate this question, we analyzed the Y. pestis transcriptome during interactions with CP to identify changes in the expression of genes and pathways that may improve the fitness of the bacterium under CP-dependent metal restriction. RNA was extracted from Y. pestis incubated with recombinant forms of CP that can sequester (a) zinc, manganese, and iron, (b) only zinc, or (c) unable to sequester metals. RNAseq analysis identified 302 genes that were significantly dysregulated at least 2.7-fold (169 genes upregulated; 133 genes downregulated) under CP-mediated sequestration of zinc, manganese, and iron. Of these genes, 140 appear to be specifically regulated in response to zinc sequestration (72 genes upregulated; 68 genes down regulated), as they were also dysregulated in Y. pestis incubated with CP only able to sequester zinc. In addition to systems required for metal acquisition, several genes encoding ribosomal proteins were significantly dysregulated in Y. pestis experiencing metal restriction, suggesting dynamic changes in ribosome content that possibly impacts translation. Specifically, ykgM and ykgO, which encode the zinc independent paralogs of the ribosomal proteins L31 and L36 respectively, were the most upregulated genes under CP-induced zinc restriction. We further showed that a ΔykgMO mutant has a growth defect under CP-induced zinc restriction compared to wild-type Y. pestis. Moving forward, we will define the contribution of these zinc independent L31 and L36 ribosomal proteins in translational activity and virulence of Y. pestis.

Room 124	**Session 3: Moderator Dr. James Collins**
Ga_O1	**Cyclic-di-GMP phosphodiesterase STM3615 regulates Salmonella physiology** Abigail Pyburn, Alexandra Pulliam , Erik Petersen Department of Biomedical Health Sciences, College of Public Health, East Tennessee State University Foodborne-related diseases pose a global health threat, with Salmonella being a leading cause worldwide. To develop better prevention strategies against Salmonella-related food poisoning, we need a deeper understanding of how Salmonella senses its environment to adjust its behavior and enhance its chances of survival. One way bacteria achieve this is through second messengers, molecular signals that help relay this type of information. A key second messenger of interest is cyclic-di-GMP that bacteria use to regulate genes that enhance survival and infectious potential by influencing processes such as biofilm formation, flagellar motility, and virulence. Previous studies identified the cyclic-di-GMP phosphodiesterase STM3615 as important for Salmonella survival inside macrophages and virulence in a mouse model. Here, we investigated STM3615's role in Salmonella physiology. Using a dye-based agar assay, we found that deleting STM3615 reduced survival in the stationary phase. Microscopy revealed that the mutant also exhibited a shorter bacterial morphology. Given that both phenotypes relate to bacterial division, we tested its susceptibility to A22, an antimicrobial that disrupts bacterial replication machinery, and observed significantly reduced survival. STM3615 contains multiple domains, including transmembrane, periplasmic, HAMP, and phosphodiesterase (PDE) domains. Surprisingly, the periplasmic domain, rather than the PDE domain responsible for breaking down cyclic-di-GMP, emerged as the key regulator of bacterial morphology and division. A protein fold prediction algorithm suggested STM3615 interacts with a periplasmic protein partner to mediate this response. Using random transposon mutagenesis, we identified mutations in the Rcs pathway—linked to envelope stress and morphology regulation—that restored wild-type phenotypes. Future research will investigate STM3615's interactions with a periplasmic binding partner to further define its role in cell division. Understanding this mechanism could provide new insights into bacterial growth regulation, with implications for therapeutic strategies and infection control.
Ga_O2	**Optimization of a Leptospira growth inhibition assay to assess the antibody efficacy.** Avni Patel, Sreekumari Rajeev Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee Background: Leptospirosis caused by the spirochete bacteria Leptospira, is a worldwide neglected zoonotic disease with global human and animal health impact. Anti-Leptospira antibody plays a significant role in protecting by binding to the lipopolysaccharide on the outer membrane of the bacteria. Antibody-mediated protective response is commonly evaluated using animal models. So, considering the 3Rs of animal research, it is ideal to develop an in vitro Leptospira growth inhibition assay for the initial evaluation of antibodies that can potentially protect the animals before moving to the in vivo models. In this study, we attempted to optimize an in vitro growth inhibition assay to conduct a preliminary evaluation of antibody efficacy. Methods: We incubated Leptospira interrogans serovar Manilae in a dose range from 10^3 to 10^7 per mL with anti-Leptospira polyclonal antibodies generated in rabbits against this serovar. We compared the Leptospira growth using commercially available EMJH (Ellinghausen-McCullough-Johnson-Harris) and in-house prepared EMJH media. Leptospira treated with PBS and heat inactivated rabbit serum were used as negative controls. Cultures were examined for the presence of Leptospira growth using darkfield microscopy on days 0,2,4,7,14 and 28. Results: The bacterial growth was inhibited until day 14 compared to the controls. We observed that 10^4 bacteria, 1:10 antibody, and a 14-day incubation would be an optimal combination to conduct this assay, and these conditions effectively inhibit the growth of Leptospira in the presence of antibodies. Conclusion: In conclusion, this assay can be used as a preliminary step in evaluating the presence of potentially protective antibodies in vaccinated animals and to assess the efficacy of vaccine candidates. In the future, we will evaluate different Leptospira serovars and clinical serum samples for further optimization."
Ga_O3	**The Pathogenomics of Salmonella enterica Circulating in Poultry Farms** Golam M. Faisal, Yosra A. Helmy Department of Veterinary Science, Martin-Gatton College of Agriculture, Food and Environment, University of Kentucky Background: Salmonella enterica remains a significant concern for public health. Understanding its genomic diversity, virulence potential, and resistance mechanisms is critical for effective control strategies. This study aimed to characterize S. enterica isolated from poultry farms in Kentucky, focusing on phylogenomic

	relationships, sequence type diversity, and the distribution of Antimicrobial Resistance (AMR) and virulence-associated genes. Methods: Whole genome sequencing (WGS) was performed on 16 S. enterica isolates obtained via drag swabs from poultry farms in Kentucky using the Illumina MiSeq platform. Multilocus sequence typing (MLST) determined sequence types (STs), and serotyping was performed using SeqSero2 and SISTR. To enhance comparative analysis, 190 publicly available S. enterica genomes from poultry in Kentucky (2014–2025) with matching STs were retrieved from GenBank and Enterobase, resulting in a dataset of 206 genomes. Core genome single nucleotide polymorphism (SNP) analysis was conducted using Snippy, followed by phylogenomic analysis. AMR genes, virulence factors, and plasmid replicons were identified using ABRicate. Results: Our findings indicate considerable genetic diversity among S. enterica isolates, despite their shared geographical origin. WGS analysis identified multiple S. enterica serovars, with S. Agona as the most prevalent, while ST13 was the predominant sequence type. A total of 40 unique AMR genes were detected, including blaCTX, sul2, and tetA, conferring resistance to β-lactams, sulfonamides, and tetracyclines. Virulence gene analysis identified 117 distinct genes, including invA, spvB, sopE, fimH, and lpfA, associated with invasion, immune evasion, and adherence. Plasmid analysis revealed 15 replicons across 7 isolates. Conclusions: This study highlights the genomic diversity, AMR burden, and virulence potential of S. enterica in poultry farms in Kentucky. The presence of key resistance and virulence genes underscores the need for enhanced biosecurity and antimicrobial stewardship to mitigate Salmonella transmission risks."
Ga_O4	**Cyclic-di-GMP Regulated Motility in Acinetobacter baumannii** Thomas Williams, Gabe Smith, Garrett Reynolds, Erik Petersen East Tennessee State University Hospital-acquired infections from multi-drug resistant pathogens is an emerging and dangerous issue that threatens the current state of healthcare environments. Acinetobacter baumannii is known for causing opportunistic infections in susceptible patients partially because it is able to survive for extended periods of time on inanimate objects such as hospital medical equipment. Motility mechanisms in A. baumannii permit this colonization and involve a structure known as a type IV pilus. This structure comprises a complex of proteins that work together to produce bacterial attachment and movement. One pilus regulatory factor is a bacterial secondary messenger known as cyclic-di-GMP, and previous research has identified a role in A. baumannii type IV pilus motility for cyclic-di-GMP-related proteins. Using models from other organisms, our current research has focused on using a Bacterial Two-Hybrid system assay to identify cyclic-di-GMP-related proteins that interact with the pilus machinery. We have discovered that the cyclic-di-GMP phosphodiesterase 1138 encoded in A. baumannii dimerizes with itself – supporting its case for having enzymatic activity – though it does not interact with the pilus machinery. Protein 2255, which contains a PilZ superfamily domain, binds to the ATPase PilB that is a part of the protein complex involved in the type IV pilus. This suggests the idea that 2255 could function as an adaptor protein that allows other proteins/molecules to regulate Type IV pilus function. I am currently investigating further proteins to determine their interaction with pilus machinery. In conclusion, further investigation into this signaling mechanism and how it relates to type IV pilus regulation in A. baumannii could provide vital information which could lead to novel treatments towards this dangerous pathogen.
Ga_O5	**Genomic Insights into Antimicrobial Resistance and Virulence of Salmonella enterica Isolated from Horses and their Public Health Implications** Ajran Kabir, Erdal Erol, Yosra A. Helmy, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky Veterinary Diagnostic Laboratory, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky INTRODUCTION: Salmonella is a foodborne pathogen that represents a serious risk to global public health with a wide host range including horses. In horses, Salmonella infections can range from being asymptomatic to causing severe clinical disease. OBJECTIVES: Our study focused on investigating the virulence, antibiotic resistance and whole genome sequencing analysis for in-depth genotypic study. METHODS: A total of 2,182 samples were tested for the presence of Salmonella infection. Samples were enriched in tetrathionate broth and then cultured on XLT4 agar plates. Serotyping was performed according to the Kauffmann-White-Le Minor scheme followed by biofilm formation screening using crystal violet assay. The resistance profile of the isolates was determined by broth microdilution assay using the Sensititre™ Vet. Whole genome sequencing was conducted using the Illumina MiSeq platform, followed by genome assembly and annotation, which led to the identification of additional genomic features.

	RESULTS: The overall prevalence of Salmonella was 1.2% with 11 different serotypes identified. Salmonella Typhimurium was the most prevalent serotype with a 19.2% prevalence. All of the isolates were biofilm producers and motile. An overall 11.4% of the isolates were identified as multidrug-resistant (MDR). Whole genome sequence of these isolates revealed that all of our isolates contained more than 100 virulence genes and more than 30 AMR genes. Multi locus sequence typing (MLST) revealed that our isolates have 11 different sequence type and ST-19 was the most prevalent. Phylogenetic analysis found that these isolates are closely related to other Salmonella species isolated from several sources. CONCLUSIONS: The presence of MDR pathogenic Salmonella in cattle and horses is alarming for both human and animal health. It is important to perform more monitoring and surveillance studies to track the source of infection of Salmonella and develop preventive measures. Keywords: Salmonella, biofilm formation, antimicrobial resistance, MDR, resistant genes, horses."
Room 101	**Session 4: Moderator Dr. Jala Venkatakrishna**
PD_O5	**Non-transcriptional function of IRF7 in antiviral immunity** Pracheta Sengupta(1), Shumin Fan(2), Ritu Chakravarti(1,3), Saurabh Chattopadhyay(1,2) (1) Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine (2) Medical Microbiology and Immunology, University of Toledo, Medical Center (3) Internal Medicine (Rheumatology), University of Kentucky College of Medicine Interferon regulatory factor 7 (IRF7) is a master regulator of type-I interferon (IFN) in innate immunity. Virus infection causes phosphorylation of IRF7 activating its transcriptional role by translocating into the nucleus to induce the expression of antiviral genes. Virus infection also leads to activation of NF-κB, another key transcription factor that induces pro-inflammatory cytokines. Inflammation is required during the clearance of virus infection, but excessive inflammation is detrimental to the host. IRF7 and NF-κB are key factors in orchestrating efficacious anti-viral responses, so we studied the crosstalk between the two and its influence on the inflammatory and anti-viral responses. The results revealed the interaction of IRF7 with the p65 subunit of NF-κB dampens the NF-κB-induced inflammation. The results suggest the anti-inflammatory function of IRF7 is independent of its transcriptional activity. Moreover, IRF7 is involved in other non-infectious diseases, such as autoimmunity, in which inflammation drives pathogenesis. So far, studies of IRF7's involvement in these diseases have been limited to its transcriptional activity. Thus, our study not only, for the first time, uncovered a non-transcriptional role of IRF7 but also provided new aspects in exploring IRF7's role in pathogenesis associated inflammation, such as in virus infection and autoimmune diseases.
PD_O6	**Engineering an enzyme additive to improve bioplastic degradation** Jordan A. Cannon(1), Todd B. Reynolds(2) (1) Circular Biosciences LLC (2) University of Tennessee Knoxville Poly-L-lactic acid (PLLA) is the most abundant bioplastic, with production expected to grow significantly over the next decade. While PLLA is biobased and industrially compostable, it can persist in the natural environment for decades. Enhancing its biodegradability is essential to ensuring it does not contribute to environmental plastic pollution. Current state-of-the-art technology revolves around incorporate biodegrading enzymes into PLLA, allowing for faster degradation in environments other than industrial compost. For these technologies to be viable, highly active PLLA-degrading enzymes are essential. We have engineered a catalog of potent PLLA-degrading enzymes, derived from wildtype subtilisins (serine proteases) from Bacillus species. Our initial studies identified BpAprE from Bacillus pumilus as an enzyme capable of degrading high-molecular-weight PLLA, while a homologous enzyme, BsAprE from Bacillus subtilis, had no ability to degrade PLLA. Using a comparative, mutational analysis, we modified key binding pocket residues in these enzymes, leading to a significant increase in PLLA degradation by both enzymes. Structural modeling revealed that these changes expanded the binding pocket and increased its hydrophobicity, improving enzyme-polymer interactions. Further optimization targeted surface-associated residues hypothesized to be involved in enzyme adsorption to the polymer. Key surface-associated amino acids in BpAprE were introduced into BsAprE, significantly enhancing the polymer binding and degradation activity of the enzyme. This resulted in an engineered BsAprE variant with a 30,000-fold increase in PLLA degradation activity relative to the wildtype enzyme. With these advances, we are developing an enzyme-based additive to be incorporated directly into PLLA products. This additive will enable rapid biodegradation outside of industrial composting, reducing environmental persistence and supporting sustainable bioplastic use."
PD_O7	**A Quorum Sensing pathway regulates virulence in Streptococcus agalactiae**

	Oscar J. Vazquez-Ciros(1), Marilia M. Manta(1), Svetlana Zamakhaeva(1), Konstantin V. Korotkov(2), Natalia A. Korotkova(1) (1) Microbiology, Immunology and Molecular Genetics, College of Medicine, University of Kentucky (2) Molecular and Cellular Biochemistry, College of Medicine, University of Kentucky Quorum sensing (QS) is a communication system employed by bacteria, allowing them to coordinate their behavior based on population density. Bacteria produce and release signaling molecules called autoinducers into the environment. As the bacterial population grows, the concentration of these autoinducers increases. Once a critical threshold is reached, the autoinducers will modulate various physiological activities, including motility, virulence or biofilm formation. In the present work, using a series of biochemicals, bioinformatics and genetic manipulation techniques, we propose that Streptococcus agalactiae [Group B Streptococcus [GBS)] may use a QS regulatory pathway to modulate the expression of virulence factors such as β-hemolysin/cytolysin (β-H/C) and other secreted proteins. In addition, we identified that overstimulation of this QS pathway induces a temperature-dependent lysis of GBS. In this study we used a peptide-free chemically defined medium to investigate a possible QS pathway involved in the regulation of GBS virulence. Our findings revealed that the chemical nature of the autoinducer triggering the activation of this QS pathway is a small peptide that requires Opp system for its translocation into the cell. Once inside the cell, this small peptide interacts with and activates the transcriptional regulator RovS. After Rovs is activated, it leads to the regulation of its targeted genes, like the cyl operon, which encodes for the genes involved in the production of β-H/C. Additionally, we identified two other dysregulated genes: a putative secreted transglutaminase and another small peptide. In other organisms, similar genes have been reported to play a crucial role in biofilm formation. Therefore, here we present a novel mechanism in GBS that employs a QS pathway that regulates the expression of different virulence factors."
PD_O8	**Microbial Hazards and Health Potential: An Integrative Geohealth Framework Evolving at the Kentucky Geological Survey** Rachel L. Washburn(1), Kevin Tidgewell(2), Mike M. McGlue(1) (1) Kentucky Geological Survey, University of Kentucky (2) College of Pharmacy, University of Kentucky As part of an expanding geohealth initiative, the Kentucky Geological Survey (KGS) has established microbial hazards and resource potential as an emerging research theme, alongside investigations into geologic drivers of radon exposure and the long-term health impacts of natural hazards like floods, landslides, and extreme weather. While geohealth efforts have traditionally emphasized hazards, we extend this scope to explore the biomedical and environmental health relevance of geological-microbial interactions. Kentucky's unique geologic settings—karst landscapes, nutrient-poor cave systems, critical mineral mines, and deep boreholes—harbor potentially diverse but mostly uncharacterized microbial communities, including extremophiles with biotechnological and therapeutic potential. Active projects include the spatial mapping of microbial populations throughout the state, starting in oligotrophic cave environments. KGS is developing a long-term strategy to integrate microbial abundance, diversity, and metabolite data with geospatial and environmental datasets, taking advantage of robust physical sample archives, statewide map products, and extensive cyberinfrastructure for web-based data serving. These integrated datasets are foundational for the construction of microbiome mapping products aimed at characterizing microbial baselines and forecasting infection risk during extreme events. Leveraging the interdisciplinary framework of geohealth and spatial infrastructure of the KGS enables the development of microbial forecasting models and targeted surveillance systems to strengthen public health preparedness by identifying regions of heightened virulence, pathogenicity, and antimicrobial resistance during disasters.
PD_O9	**Host response to pathogenic vs. endophytic fungi** Derica G Tavares, Baruri, S., Onyiri, K.C., Shamsudeen N., Perlin, M.H. University of Louisville Members of the Microbotryum species complex of fungal pathogens infect wildflower species in the Carnation family, causing anther-smut disease, and the complex serves as a model for emerging infectious diseases. During infection of Dianthus seguieri plants by the fungus Microbotryum superbum some plants show infection in the floral tissue (symptomatic plants), whereas others fail to do so (asymptomatic plants). Here, we study M. superbum as both a pathogen and as an endophyte using RNA-seq analysis. Buds and meristematic tissue were collected from symptomatic and asymptomatic D. seguieri plants infected by M. superbum. Confocal fluorescence microscopy, scanning and transmission electron microscopy (SEM, TEM) were also used, to observe the early infection process. Meristem tissue of asymptomatic plants showed more differentially expressed genes (DEGs) (1,672) than symptomatic plants (449). Meanwhile, buds of

	symptomatic plants showed more DEGs (1,572) than buds of asymptomatic plants (138). Gene Ontology (GO) enrichment analysis showed more enriched pathways for the meristem stage than for the bud stage. The bud stage of asymptomatic and symptomatic plants showed 7 and 14 enriched pathways, respectively. The common enriched pathways in asymptomatic and symptomatic buds were “cellular process” and “biosynthetic process”. The meristem stage of asymptomatic and symptomatic plants showed 18 and 19 enriched pathways, respectively. The “defense response” pathway was enriched only in symptomatic plants. However, the “response to other organisms”, the “biological process involved in the interspecific interaction between organisms”, and the “response to biotic stimulus” pathways were enriched in both symptomatic and asymptomatic plants. GO analysis showed no activation of the host defense response in asymptomatic plants, which was shown to be critical at the meristem stage for symptomatic plants. This suggests that M. superbum as an endophyte can deactivate and avoid plant defenses, while not being eliminated from its host.
PD_O10	**O-glycosylation of serine/threonine-rich intrinsically disordered regions (IDRs) of membrane proteins in Streptococcus pyogenes** Mohammad Mizanur Rahman(1), Svetlana Zamakhaeva(1), Jeffrey S. Rush(2), Catherine Chaton(2), Cameron Kenner(1), Konstantin V. Korotkov(2), Natalia Korotkova(1,2) (1) Department of Microbiology, Immunology & Molecular Genetics, University of Kentucky (2) Department of Molecular and Cellular Biochemistry, University of Kentucky Background: Intrinsically disordered regions (IDRs) of proteins play critical roles in various cellular functions including post-translational modification and cell signaling in eukaryotes. However, their roles in bacteria are poorly understood. Methods: In this study, a deep neural network method was used to predict extracytoplasmic IDRs in the proteome of Streptococcus pyogenes (group A streptococcus or GAS). Lectin affinity chromatography followed by proteomic analysis, mutagenesis, immunoblotting, and in vitro assays were utilized to identify the role of IDRs in GAS biology. Results: We identify that serine/threonine-rich C-terminal IDRs of four membrane proteins are glycosylated with α-glucose, indicating the presence of O-glycosylation pathway in GAS. The identified glycoproteins participate in protein folding and peptidoglycan synthesis. Mutagenesis approach reveals that two glycosyltransferases, GtrBSpy and PgtC2Spy, are involved in protein glycosylation. Kinetic analysis demonstrates that GtrBSpy possesses a micromolar affinity for UDP-glucose and undecaprenyl phosphate (Und-P), suggesting that GtrBSpy catalyses the first step of protein glycosylation in the cytosol transferring UDP-glucose to the lipid career, Und-P. We suggest that PgtC2Spy transfer a glucose moiety from a glycolipid to the IDRs of membrane-proteins. Finally, we identify that GtrBSpy and PgtC2Spy are crucial for GAS protection from antimicrobial proteins and peptides, cathepsin G and LL-37. Conclusion: These data suggest that O-linked IDR-glycosylation might contribute to GAS evasion of innate immune defences."
Room 124	**Session 5: Moderator Dr. Donghoon Chung**
Ga_O6	**Isolation and characterization of siderophore(s) from Pseudomonas mosselii** Casey Stophel, Dr. Ranjan Chakraborty College of Public Health, East Tennessee State University Iron is essential for growth of most microorganisms with a few exceptions. It functions as an important cofactor in many important biological processes. However, abundant iron found in soil is rendered insoluble and is unavailable to microorganisms for use. To overcome this restriction, many bacterial species produce siderophores, a small secondary metabolite that has high affinity for iron. Siderophores allow for bacterial cells to obtain otherwise insoluble iron. Pseudomonas mosselii, a gram negative, aerobic and motile bacterium was isolated from a local soil sample. P. mosselii is known to be an opportunistic pathogen & has been reported to have antimicrobial activities against plant pathogens. P. mosselii was found to produce siderophore(s) under an iron restricted environment, however, they have not been structurally characterized. Therefore, it was of our interest to isolate, purify and structurally characterize the siderophore produced by P, mosselii. Our preliminary chemical characterization indicates a catechol type fluorescent compound most likely belonging to pyoverdine family. It was also found to have a strong antimicrobial activity against gram positive bacterium. At present, further purification and structural characterizations are underway in our laboratory.
Ga_O7	**Investigation of Sensory Cyclic-di-GMP Phosphodiesterases in Salmonella** Anna Ayers and Erik Petersen

	East Tennessee State University Salmonella enterica serovar Typhimurium infects humans and animals via consumption of contaminated food or water. Human infections with Salmonella typically lead to gastrointestinal disease but may also cause a systemic infection if it reaches the bloodstream where Salmonella is taken up by macrophages. After undergoing phagocytosis, Salmonella creates a vacuole inside of the macrophage allowing Salmonella to survive and continue to infect healthy cells. We want to study Salmonella's adaptation inside macrophages to find out what signals are being used to trigger intracellular survival. Cyclic-di-GMP is one signaling system used by Salmonella to respond to its environment. External stimuli activate phosphodiesterases (PDEs) and diguanylate cyclases (DGCs) to degrade and synthesize cyclic-di-GMP respectively. Three PDEs were identified that work to degrade cyclic-di-GMP during intracellular survival and are required for full virulence. Two of these PDEs (STM2215 and STM2503) contain sensory domains that stimulate the dimerization required for activity. To monitor the response of these sensory domains to potential signals, we have modified a Bacterial Two-Hybrid system and Miller Assay to measure dimerization of PDEs when exposed to specific signals found inside of a macrophage. We are testing a wide range of potential signals that could be the source of intracellular stimuli to these PDEs. Our end goal is to see what type of signals STM2215 and STM2503 respond to once inside of a macrophage that promotes Salmonella survival in the midst of phagocytosis.
Gb_O6	**Mechanistic Insights into Sindbis Virus Infection: Noncapped Genomic RNAs Enhance the Translation of Capped Genomic RNAs to Promote Viral Infectivity** Deepa Karki(1), Autumn T. LaPointe(1,3), Cierra Isom(1), Milton Thomas(1), Kevin J Sokoloski(1,2) (1)Department of Microbiology and Immunology, University of Louisville, School of Medicine, (2) Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville (3) Department of Molecular Genetics and Microbiology, University of New Mexico Alphaviruses are globally distributed, vector-borne RNA viruses with high outbreak potential and no clinical interventions, posing a significant global health threat. Previously, the production and packaging of both capped and noncapped viral genomic RNAs (cgRNA and ncgRNA) during infection was reported. Studies have linked ncgRNA production to viral infectivity and pathogenesis, but its precise role remains unclear. As we recently published in Karki et. al. 2025, Nucleic Acids Research, to define the benefits of ncgRNAs, pure populations of capped and noncapped Sindbis virus (SINV) gRNAs were synthesized and transfected into host cells. The data showed that mixtures of cgRNAs and ncgRNAs had higher infectivity compared to pure cgRNAs, with mixtures containing low cgRNA proportions exceeding linear infectivity expectations. This enhancement depended on co-delivery of cgRNAs and ncgRNAs to the same cell and required the noncapped RNAs to be viral in origin. Contrary to the initial hypothesis that the ncgRNAs serve as replication templates, the cgRNAs were preferentially replicated. Subsequent analyses to understand the impact at the molecular level revealed the influence of the ncgRNAs on viral translation and vRNA synthesis / accumulation. Thus, the ncgRNAs contribute meaningfully to vRNA infectivity despite being largely inert. Using a replication-deficient system, the ncgRNAs were found to exert a controlling influence on early replication events by directly enhancing the translation of incoming cgRNA. The enhancement afforded by the ncgRNAs is dependent on their translational inactivity, as modestly restoring the translational capacity of the ncgRNAs abrogated the enhancement of cgRNA function. Further analysis revealed that the non-structural genomic sequence and non-translatable sub-genomic sequence function in trans to promote efficient translation of cgRNA. Collectively, these data provided evidence that the ncgRNAs enhance the translational efficiency of incoming cgRNAs, producing a cumulative effect by increasing vRNA replication and facilitating the production of infectious virus particles.
Gb_O7	**Manipulation of extracellular vesicle biogenesis aids Yersinia pestis pathogenesis** Katelyn R. Sheneman, Timothy D. Cummins, Michael L. Merchant, Joshua L. Hood, Silvia M. Uriarte, Matthew B. Lawrenz University of Louisville Extracellular vesicles (EVs) are key mediators of intercellular communication between immune cells. These lipid-bound vesicles contain proteins, lipids, and nucleic acids that represent the immunologic state of a given cell. Upon release, these EVs can fuse with other immune cells, establishing biochemical communication between cells. Yersinia pestis is the etiologic agent of the disease known as plague. A hallmark of plague is the subversion of the host immune response by disrupting host signaling pathways required for inflammation. This manipulation promotes maintenance of a non-inflammatory environment for the first 36hpi, allowing the bacteria to proliferate unhindered from host intervention. This delay in inflammation and immune signaling is essential for disease manifestation. Previous work has elucidated the role of the Y. pestis type 3 secretion system (T3SS) and Yop effectors as essential virulence factors for

	dysregulation of immune signaling. Despite their importance regarding cellular signaling and communication, the role of EVs during plague has yet to be defined. We have previously shown that Y. pestis manipulates EV biogenesis and inhibits the production of EVs by human neutrophils. Here, we expanded on these in vitro studies to show that Y. pestis manipulates EV production during pneumonic plague. Using nanoparticle tracking and proteomics of EVs isolated from the bronchial alveolar fluid during pneumonic plague, we discovered that both EV release and cargo packaging changes over the course of infection. Stark contrasts of the EV population exist at 24hpi (during the non-inflammatory phase) and 48hpi (during the pro-inflammatory phase) suggest significant changes in EV synthesis during the course of infection. Moreover, infection with a Y. pestis mutant lacking the Yop effectors demonstrated active inhibition of EV production by Y. pestis, with significant changes in EV cargo selection in mutant infected animals. Together, these data suggest that manipulation of EV biogenesis is another mechanism used by Y. pestis to suppress inflammation during the early stages of infection.
Gb_O8	**A Molecular Switch in the Alphavirus Capsid: The Novel Role of Threonine 87** Cierra Isom(1), V. Landers(1,3), and Kevin J. Sokoloski(1,2) (1) Department of Microbiology and Immunology, University of Louisville School of Medicine (2) Center for Predictive Medicine, Biodefense, and Emerging Infectious Diseases, University of Louisville (3) University of Massachusetts Chandler Medical School Alphaviruses are of significant public health concern due to their capacity to cause severe arthritis or encephalitis in otherwise healthy individuals. To address the current lack of therapeutics, our lab focuses on characterizing the molecular host/pathogen interactions underlying pathogenesis. Recently, we identified an interaction between the alphavirus capsid (CP) protein and the host innate immune response component, Interleukin-1 Receptor Associated Kinase 1 (IRAK1) that resulted in decreased IRAK1 signaling. Molecular and bioinformatic assessments have indicated that the CP protein is a likely target for IRAK1 phosphorylation; as such we hypothesize that the phosphorylation of the CP protein by IRAK1 is critical for alphaviral infection. Bioinformatic efforts informed our investigations on the impact of IRAK1 kinase activity on viral biology, allowing us to create phosphorylation-site specific, Sindbis virus (SINV) mutant strains. To this end phosphoablative and phosphomimetic SINV CP protein mutants were developed. We found that the phosphorylation of CP contributes to the regulation of cargo selection and binding, and nucleocapsid core formation. These data were further informed by the identification of a second site revertant mutant that restores a WT phenotype in the phosphoablative background via the introduction of new sites for phosphorylation. Furthermore, we extended our investigations to the biological effects of co-expressing the phosphomutant CP proteins alongside WT CP proteins to determine if any effects were dominant negative. Together these data suggest that the conserved threonine motif within the CP mediates a crucial event during the viral lifecycle and that the CP protein event(s) and interactions required for completion of the viral lifecycle are modulated by phosphorylation. In conclusion, the alphavirus CP/IRAK1 interaction is critical for viral biology and results in reciprocal functional effects to host and viral proteins.
PD_O11	**Macrophage-specific IRF3 curbs viral inflammation in the lung** Santanu Das(1), Sukanya Chakravarty(1,2), Ritu Chakravarti(1,3), Saurabh Chattopadhyay(1,2) (1) Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine (2) Medical Microbiology and Immunology, University of Toledo, Medical Center (3) Internal Medicine (Rheumatology), University of Kentucky College of Medicine Viral infection elicits inflammation leading to pathogenesis and mortality. IRF3, a critical component of innate antiviral immune responses, interacts directly with p65 subunit of the pro-inflammatory transcription factor NF-κB, and inhibits its activity thus suppressing inflammatory gene expression in virus-infected cells and mice. We evaluated the cell type-specific function of IRF3 responsible for suppressing respiratory viral inflammation using newly engineered conditional Irf3 knockout (KO) mice. Irf3 KO mice, upon respiratory virus infection, showed increased susceptibility and mortality. Irf3 deficiency aggravated viral infection causing enhanced inflammatory gene expression, lung inflammation, immunopathology, and damage, accompanied by increased infiltration of pro-inflammatory macrophages. Specific deletion of Irf3 in macrophages (Irf3MKO) displayed, like Irf3 KO mice, increased inflammatory responses, macrophage infiltration, lung damage, and lethality, indicating that anti-inflammatory role of IRF3 in these cells is sufficient to suppress lung inflammation. Bulk RNA-seq analyses revealed enhanced NF-κB–dependent pro-inflammatory gene expression along with activation of inflammatory signaling pathways in infected Irf3MKO lungs. The presentation will highlight how IRF3 inhibits inflammatory signaling pathways in macrophages to prevent viral inflammation and pathogenesis.

Room 101	**Session 6**: **Moderator Dr. Matt Lawrenz**
KN2	**Dr. Tera Levin** **University of Pittsburg** **The evolution of immunity and pathogenesis within environmental battlegrounds**
F_O2	**Modeling early events in bacterial chorioamnionitis using an organ-on-chip of the maternal-fetal interface** Alison J. Eastman(1), Hannah Richards(2), Brian O'Grady(3) David Cliffel(2), Jennifer A. Gaddy(4) (1) Department of Obstetrics and Gynecology, Vanderbilt University Medical Center; (2) Department of Chemistry, Vanderbilt University; (3) Department of Ophthalmology, Vanderbilt University Medical Center; (4) Division of Infectious Disease, Vanderbilt University Medical Center Inflammation in the gestational membranes (chorioamnionitis or CAM) is often clinically silent until an adverse outcome occurs, such as preterm prelabor rupture of membranes and preterm birth. One of the most prevalent causes of CAM is infection with the bacterium Group B Streptococcus (GBS). We built an organ-on-chip model of the maternal-fetal interface in the gestational membranes comprised of fetal cytotrophoblasts (CTBs, or fetal chamber) in one chamber, maternal decidual stromal cells (DSCs, or maternal chamber) in the other chamber, with Type IV collagen and a hydrogel separating the chambers. Both chambers are subjected to microfluidic flow across the chambers to mimic sheer stress and nutrient and waste exchange. We model acute GBS CAM by infecting the maternal chamber with GBS and comparing it to uninfected state or to a disseminated GBS infection where both maternal and fetal chambers are infected. In subsequent experiments, we seed both chambers with macrophages to address how cells of the innate immune system affect the whole tissue response to GBS infection. We hypothesize that acute CAM will have different immune mediators elevated than disseminated, and that macrophages will augment the response magnitude. We found that infection does not spread between chambers, and macrophages do not migrate across the hydrogel. MMP9 is induced from the CTBs of the fetal side only when DSCs of the maternal side are infected. IL-1 and TNF are induced exclusively in a disseminated infection and exclusively from the CTBs. IL-6 is significantly induced from both DSCs and CTBs during disseminated infection. Macrophages increased IL-1 from the fetal chamber only, and suppressed MMP9 until disseminated infection. This suggests cells of the fetal membranes are capable of responding to acute CAM distinctly from disseminated CAM, and some microenvironments in the gestational membranes help suppress inflammation locally while augmenting it in neighboring microenvironments.
F_O3	**The Role of Diet in Asymptomatic C. difficile Carriage** James Collins, Daniel Erickson, Michelle Chua, Katelyn Sheneman, Lily Hernandez Department of Microbiology & Immunology, University of Louisville Background: Clostridioides difficile (C. difficile) is a gram-positive, spore-forming bacterium that is a major cause of healthcare-associated infections. While symptomatic C. difficile infection (CDI) can lead to severe gastrointestinal issues, asymptomatic C. difficile carriage is also prevalent and poses significant challenges for infection control. Recent studies suggest that diet plays a crucial role in modulating the gut microbiome, which, in turn, can affect C. difficile colonization and carriage. High-carbohydrate diets have been shown to prolong dysbiosis and persistent C. difficile carriage, highlighting the importance of dietary composition in managing asymptomatic colonization of the gut. Understanding the dietary factors that influence C. difficile carriage could provide new insights into the prevention of CDI and improvement of gut health. Methods: Using preclinical mouse models, we investigated the effects of diet on asymptomatic C. difficile carriage and subsequent CDI. Mice were fed a control, high-sucrose (chow), high-sucrose (water), or Western diet. We monitored C. difficile carriage (CFUs in stool) and subsequent disease following antibiotic administration. Stool samples were analyzed using shotgun metagenomics and untargeted metabolomics. Results: High-sucrose and Western diets increased susceptibility to C. difficile carriage, leading to active disease after antibiotic use. Mice on a control diet remained undetectable for C. difficile and did not develop CDI after antibiotic use. Metabolomics revealed that a high-sucrose chow altered the gut metabolic environment before infection, and this distinct environment persisted post-CDI. The microbiome of high-sucrose chow-fed mice was significantly altered, with one week of sucrose chow consumption shifting the gut community structure to one more susceptible to C. difficile carriage. Conclusions: Both high-sugar and Western diets create a niche favorable for C. difficile spore germination and low-level carriage. These findings may explain the ~15% of healthy adults who are asymptomatic carriers."

Poster presentations (in alphabetical order by presentor)

UP1	**The Inhibitory Effect of Two Unknown Appalachian Microbes on Bacillus Species.** Chukwufumnanya Aninyei, Dr. Sean Fox Department of Health Sciences at East Tennessee State University Antibiotic resistance (ABR) occurs when microbes modify themselves in order to defend against drugs that were previously successful in eradicating their kind. The number of ABR cases has continued to rise requiring the discovery of new bacterial mechanisms capable of identifying new or novel means of therapeutics. To achieve this, soil samples were gathered at 36°17'59" N 82°21'55" W near the water tower at East Tennessee State University. The bacterium were extracted and monitored for any zones of inhibition against competing strains of common human opportunistic pathogens. Those displaying any such properties were initially tested against E. coli, S. aureus, and B. subtills. Of the six that were tested, two produced inhibiting properties, against B. subtilis. They were then tested against other Bacillus species and successfully created zones of inhibition against B. megaterium and B. licheniformis. Upon gram staining, it was discovered that both organisms are Gram positive. DNA was then extracted from the two unknown specimens and identified by their 16S rRNA gene sequence. Further characterization will determine the contact-dependent or independent mechanism of inhibition upon both planktonic and biofilm modes of growth.
UP2	**Organismal Diversity during the Establishment of a Sourdough Culture** Zoe DeBolt*, Kelsea Richmer*, Clara Henderson*, Margaret Wallen Indiana University Southeast *co-presenting Background: Any professional baker will describe the dynamic nature of a sourdough culture in the kitchen, with qualities related to breadmaking changing daily. To a microbiologist, a sourdough culture represents an ecosystem, teeming with multiple microbial species, all competing to carve out their ecological niche. Sourdough cultures are established by mixing nutrient sources, like flour and sugar, and waiting for environmental microbes to colonize. In this study, we followed a sourdough culture from establishment to maintenance, identifying microbial occupants along the way. Methods: Total DNA extractions were produced from daily samples taken over the course of establishment of the culture, which took 16 days. Briefly, the sourdough culture was diluted in sterile water and treated with lysozyme to break bacterial cell walls or lithium acetate to disrupt fungal cell walls. Cell lysates were treated with Proteinase K and DNA precipitated with alcohol. Degenerate primers for bacterial and fungal ribosomal RNA sequences were used to amplify these regions. Amplicons were inserted into a cloning vector and sequenced to identify species present in the culture. Results: The microbes initially detected likely were introduced as contaminants in some of the initial food products used to establish the culture. Bacterial species diversity varied much more than that of the fungal inhabitants. While the fungal species remained relatively stable (Saccharomyces species were the only ones detected), the bacterial inhabitants, particularly fermenters, in the culture changed dramatically over the course of sample collection. Conclusions: The dynamic nature of sourdough cultures observed by both chefs and microbiologists is likely the result of the changing bacterial population over the lifetime of a culture. While the fungal populations remain relatively stable, the identity of bacterial inhabitants changes considerably. The source of bacteria may be the food products used to feed the culture or environmental microbes introduced during handling."
UP3	**Novel Antivirulence Drugs Against the Bubonic Plague** Briana Harness, Subarna Roy, Mahendar Kadari, Nishad Chandrika, Oleg Tsodikov, Sylvie Garneanu-Tsodikova, Matthew B. Lawrenz, University of Louisville The causative agent of the bubonic plague, Yersinia pestis, is responsible for the deaths of millions of people over the course of multiple pandemics. While the prevalence of the plague has decreased overtime, Y. pestis still remains endemic in the western United States. In addition to the persistence of the plague, concern for plague outbreaks has increased due to climate change and its potential as a potent bioweapon. Y. pestis requires biometals such as zinc and iron to survive, and hosts restrict bacterial growth through the sequestration of these metals, referred to as nutritional immunity. One host protein directly responsible for metal restriction is calprotectin. To colonize the host, Y. pestis must circumvent calprotectin-mediated nutritional immunity. To achieve this, Y. pestis utilizes the siderophore, yersiniabactin, to scavenge iron and zinc. Yersiniabactin is essential for virulence, and thus, strategies that inhibit the ability of Y. pestis to use yersiniabactin could have potential therapeutic advantages. Recently, it was discovered that an RND-efflux system is the primary export mechanism for yersiniabactin. This discovery suggests that targeting RND-efflux

	pharmacologically could limit the virulence potential of Y. pestis and other bacteria that use yersiniabactin to overcome nutritional immunity. Our goal here is to identify compounds that can inhibit RND-efflux systems and yersiniabactin export. Because RND-efflux systems also increase antibiotic resistance by exporting antibiotics, we first tested a panel of potential RND-efflux inhibitors for the ability to increase Y. pestis antibiotic susceptibility. Antibiotic susceptibility was determined by defining changes in the minimum inhibitory concentration (MIC) of antibiotics in the presence of the compounds. We showed that compounds NTC-11-48 and NTC-11-147 increased sensitivity to both kanamycin and tetracycline, indicating that it inhibits RND-efflux in Y. pestis, and is a novel candidate to inhibit the efflux of yersiniabactin. We are currently determining the direct impact of NTC-11-148 and NTC-11-147 on the export of yersiniabactin and on the ability of Y. pestis to grow under metal-limited conditions.
UP4	**Development of Biotinylated probes to Increase RNA Sequencing Specificity for Clostridioides difficile** Lily Hernandez, Michelle Chua, James Collins University of Louisville Clostrioides difficile is an opportunistic nosocomial pathogen which inflames the lining of the colon leading to symptoms from moderate to severe diarrhea, abdominal pain, and death. Even during severe infection, C. difficile only makes up about 1-2% of the microbiota. There has been a rise in asymptomatic carriage with up to 15% of healthy adults found to be carriers. In these cases, the abundance of C. difficile is even lower ~ 0.1 %. It is unknown what enables asymptomatic C. difficile carriage. A clear picture can be formed through RNA sequencing by studying the transcriptome of C. difficile within a complex microbiota, however, it is limited by the read depth required to obtain sufficient reads. To overcome this limitation, we will design biotinylated probes which will extract C. difficile cDNA from a complex pool enabling enrichment prior to sequencing which makes it so that this technique is more efficient in sequencing the target. Probe libraries designed to specifically bind to C. difficile were synthesized. PCR and subsequent transcription with Biotin-16-UTP was used to generate biotinylated probes. To test the probes, several groups of differing concentrations of C. difficile RNA will be mixed with mouse stool RNA to create a library to generate cDNA. The cDNA and biotinylated RNA probes will be incubated together and then streptavidin beads will be used to pull out the biotinylated probes that have bound to the cDNA. The bound cDNA will then be sequenced using the Illumina platform and analyzed. The sensitivity of the probes will be tested against traditional RNA sequencing without the biotinylated probes. Successful development of this platform will enable us to probe the RNA expression levels of C. difficile within a complex gut microbiota more sensitively.
UP5	**Potential Antimicrobial Properties of Rhus typhina tannins on Candida albicans** Kelsey Lawson, Tasha Phillips, Alex Hopke, Allison Scherer University of Virginia's College at Wise This research looks at the antimicrobial properties of tannins found in Rhus typhina specific to the Appalachia region and how effectively they inhibit the growth of Candida albicans in vitro. Candida is a type of yeast that commonly causes fungal infections in immunocompromised individuals. The most common species is C. albicans which is responsible for infections such as vaginal yeast infections, oral thrush, and disseminated candidiasis. Candida auris is an emerging multi-drug resistant (MDR) species that has proven to be resistant to all three main classes of antifungal medicines and has a mortality rate of 30-60%. MDR fungal species are expected to continue to rise, and the search for alternative antimicrobials will benefit patients at risk for debilitating fungal infection. We performed a plate-based killing assay, and inhibition of C. albicans growth in the presence of tannins found in Rhus typhina was evaluated by spectrophotometer reading. The tannins PGG and gallic acid effectively inhibited C. albicans' growth. However, another tannin, 4-methoxy-3,5-dihydroxybenzoic acid, appears to promote C. albicans' growth in vitro. Plants, including Rhus typhina, utilize a variety of chemical means to combat microbes in nature. We hope that by examining natural tannins from the Appalachia region, we might gain insight into more of these natural compounds in fighting fungal infection. Future research will include examining the neutrophil—Candida interactions with the addition of these tannins.
UP6	**The Presence of Pseudomonas aeruginosa in Sink Spouts and Drains** Makayla McDowell and Cynthia Ryder Midway University Pseudomonas aeruginosa is a widespread bacterium that infects immunocompromised individuals. It typically infects individuals with chronic infections, burn wounds, and, most commonly, those with cystic fibrosis. The bacterium can be found almost anywhere but primarily in water sources. This study aimed to determine if water sources such as sink spouts and drains could host P. aeruginosa strains or other species of

	Pseudomonas. The women's public restrooms of ten locations were sampled, one sample for the spout and one for the drain. Pseudomonas bacteria were detected in most of the samples collected. More specifically, P. aeruginosa was found in both the sink and drain for some of the restrooms. Though, there was more evidence found in the drain than the sink. Out of 37 sink samples, 2 spout and 11 drain samples collected were visually identified as P. aeruginosa. For future studies, the suspected P. aeruginosa samples should be confirmed through DNA analysis or genetic sequencing to ensure their accuracy.
UP7	**Microbial Signatures in the Lungs of Preterm Infants: Insights from Metagenomic Sequencing** Johnny Mendel, Kori Williams, Hong Huang, Minjae Kim University of Kentucky Preterm birth (born at less than 37 weeks gestational age) occurs in the United States in 10% of total births per year. These preterm infants have under-developed biological systems and, as a result, are much more vulnerable to the effects of contracted infection. The goal of this project was to develop a pipeline that can be used to analyze the sequenced genomes of DNA collected on cheek swab samples, ultimately allowing us to determine key components of the microbiome in the lungs of newborn infants. Analysis revealed distinct microbial profiles, with an enrichment of opportunistic pathogens such as K. pneumonia, S. epidermidis, and L. rhamnosus, all of which have the potential to cause illness in infants, including sepsis. Additionally, PERMANOVA analysis demonstrated that sex and gentamicin exposure were significant factors influencing beta-diversity, indicating potential sex-based and antibiotic-driven shifts in the lung microbiome composition. The results of this analysis should be further studied and utilized to provide insight for focused treatments on preterm infants.
UP8	**Clinical Pseudomonas aeruginosa Isolates in Competition with E. Coli, S. aureus, and K. Pneumoniae** Ceaver Perry and Cynthia Ryder Midway University Pseudomonas aeruginosa is an opportunistic gram-negative bacterium that primarily infects immunocompromised patients. Previous studies at Midway University have identified low competitive ability in the environment however, with antibacterial resistance increasing, it is important to understand the competitive nature of clinical isolates. This study will focus on the competitive nature of clinically isolated strains of P. aeruginosa compared to Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus. By conducting competition assays between these common infectious bacteria and strains of P. aeruginosa found in similar infection types and environments, observations can be made about these strain's potential competitive ability. Results showed that many of the strains had the ability to outcompete S. aureus, while K. pneumoniae co-existed with many of the strains tested. The clinical strains also proved able to establish themselves upon a lawn in delayed competition assays."
UP9	**Prevalence and Antimicrobial Resistance Patterns of Methicillin-Resistant Staphylococcus aureus in Animal, Food, and Environmental Samples** Rosbelly Rios, Ajran Kabir, and Yosra A. Helmy Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky Background: Methicillin-resistant Staphylococcus aureus (MRSA) represents a global public health challenge, recognized for its multidrug resistance and ability to cause severe infections in humans and animals. Its adaptability and persistence pose significant threats to both clinical and agricultural settings. Objective: This study aimed to assess the prevalence of MRSA in various animals, food, and environmental samples and evaluate the phenotypic and genotypic antimicrobial resistance (AMR) and virulence in isolates. Methods: 551 samples were collected from diverse sources, including animal fecal samples, meat samples from supermarkets, and water samples from wastewater sources. All samples were enriched overnight in nutrient broth and cultured on Mannitol Salt Agar plates, followed by overnight incubation. Confirmation of S. aureus and Methicillin-Resistant S. aureus was performed using a polymerase chain reaction. The confirmed isolates underwent antibiotic susceptibility testing using broth microdilution against eleven antibiotics from eight different classes, biofilm formation assay, hemolysis assay, and catalase and coagulase activity. Results: The prevalence of S. aureus in the tested samples was 19.1% in dogs, 7.7% in water, 33.3% in horses, 11.1% in beef, and 54.6% in chicken. The prevalence of MRSA was 94%. All the isolates were 100% resistant to azithromycin, with 93.8% showing resistance to chloramphenicol, clindamycin, gentamycin, and tetracycline. Additionally, 93.8% of the isolates exhibited multidrug resistance to more than three antibiotic classes. All the isolates produced beta hemolysis and biofilm. Catalase and coagulase activity were positive in all isolates, confirming their enzymatic capabilities.

	Conclusion: The extensive multidrug resistance observed in MRSA isolates highlights the critical need for improved antimicrobial stewardship and innovative strategies to combat this pathogen. We can safeguard public health by addressing MRSA's prevalence and resistance patterns through targeted research and enhanced infection control measures. Understanding MRSA resistance will aid in creating new treatments and improving outcomes for humans and animals."
UP10	**The Effect of Different Nutrient Classes on Growth and Biofilm Formation of Streptococcus agalactiae** Shree Satyavolu(1), Christopher Farrell(2), Ryan Doster(2,3) (1) University of Louisville (2) Department of Microbiology and Immunology, University of Louisville (3) Department of Medicine, University of Louisville School of Medicine Background: Streptococcus agalactiae, also known as Group B Streptococcus (GBS), is a Gram-positive bacterium that colonizes the genitourinary tract of 15-30% of pregnant women during late pregnancy. GBS rectovaginal colonization is a major risk factor for invasive GBS infections leading to adverse pregnancy outcomes and neonatal mortality. Biofilms are thought to promote GBS colonization as biofilms provide protection against host immune responses. Little is known regarding how different nutrients influence GBS biofilm formation. We sought to understand how different nutrient class including amino acids, lipids, nucleotides, and carbohydrates influence GBS biofilm formation. Methods: To assess GBS growth and biofilm formation, a chemically defined medium (CDM) was prepared. CDM contains essential macromolecules including nucleotides, amino acids, lipids, and carbohydrates in defined concentrations that allow for evaluation of their effects on GBS physiology. To determine if adding higher concentrations of individual nutrient classes would impact GBS growth and biofilm formation, 5 fold increases of the following groups were added to the base CDM recipe containing 20 mM glucose as the sole carbohydrate: 5X Nucleotides, 5X Inorganics, 5X Amino Acids, and 5X other. 2 GBS strains were used to examine growth by measuring culture turbidity (OD600) and a crystal violet assay was used to measure biofilm formation. Results: GBS growth was highest in the 5X Amino Acids group, suggesting amino acids play a critical role in bacterial proliferation. Biofilm formation was strain-dependent, with significant biofilm production observed in the 5X Nucleotides group. Conclusion: The data suggest that additional nutrients beyond glucose enhance GBS growth and biofilm formation. While amino acids contribute to bacterial growth, nucleotides appear to promote biofilm production. Further investigation is needed to determine optimal nutrient conditions for GBS biofilm formation."
UP11	**Investigating the antagonistic relationship between B. cenocepacia and S. aureus through a metabolomics-based approach** Hayden Skaggs, Deborah R. Yoder-Himes University of Louisville Department of Biology Staphylococcus aureus asymptomatically colonizes the nasal cavity and pharynx of up to 60% of the human population and, as an opportunistic pathogen, can breach its normal habitat, resulting in life-threatening infections. In this work, we show that another CF pathogen, Burkholderia cenocepacia, produces one or more secreted compounds that can prevent S. aureus biofilm formation and inhibit existing S. aureus biofilms. This study aims to explore the mechanism of biofilm inhibition exhibited by B. cenocepacia on S. aureus through the lens of metabolomics. To accomplish this, biofilm inhibition assays were run using supernatant from biofilms generated by a B. cenocepacia clinical isolate. The supernatant was analyzed using metabolomics, with chief metabolites being further investigated through biochemical inhibition assays. Genetic mutants of B. cenocepacia clinical isolates were also employed in further biofilm inhibition assays. We found that the inhibitory activity exhibited by B. cenocepacia results in the death of S. aureus through a contact-independent mechanism, potentially mediated through the siderophore pyochelin, extracellular iron concentration, and perhaps additional compounds. Future efforts remain dedicated to further understanding the exact mechanism of the antagonistic relationship between B. cenocepacia H111 and S. aureus NRS77.
UP12	**Co-culture of P. aeruginosa and S. aureus in Planktonic Growth Conditions** Trinity Smith and Cynthia Ryder Midway University Pseudomonas aeruginosa and Staphylococcus aureus are opportunistic pathogens that cause chronic infections. When co-existing, they create more damage than mono-species infections. The purpose of this study is to determine the ability of clinical strains of P. aeruginosa to grow in planktonic co-culture with S. aureus. To do this, a coculture assay was performed to find the ratio of surviving cells of both organisms after

	overnight broth co-culture from inoculation ratios of 1:1 or 1:2 P. aeruginosa :S. aureus. The results demonstrated that P. aeruginosa still overpowers S. aureus in its number even when the number of Staphylococcus aureus cells are increased, which indicates that P. aeruginosa has a growth advantage over S. aureus in planktonic co-culture.
GaP1	**Antimicrobial Activity of Probiotic E. coli Nissle 1917 Supernatant Against Campylobacter jejuni** Nada A. Fahmy, Bibek Lamichhane, Yosra A. Helmy Department of Veterinary Science, University of Kentucky, Lexington, Kentucky, USA Background: Campylobacter jejuni is a leading cause of foodborne gastroenteritis, primarily transmitted through contaminated poultry and poultry products. It can cause severe complications, including Guillain-Barré Syndrome. The probiotic E. coli Nissle 1917 (EcN) exhibits a protective effect against C. jejuni by modulating the host immune response, competitively excluding pathogens, and preserving gut barrier integrity. This study evaluates the impact of E. coli Nissle 1917 cell-free supernatant (EcN-CFS) on the growth and virulence factors of C. jejuni in vitro. Methods: The effect of EcN-CFS on C. jejuni growth was assessed using co-culture and agar-well diffusion assays. The effect of EcN-CFS on biofilm formation and pre-formed biofilms of C. jejuni was assessed using a crystal violet staining assay. Furthermore, the effect of EcN-CFS on key colonization processes, adhesion, invasion, and intracellular survival was evaluated using human colorectal adenocarcinoma (HT-29 MTX) cells. Additionally, the effect of EcN-CFS on the expression of virulence-associated genes was investigated to gain deeper insights into its role in modulating C. jejuni pathogenicity. Results: Our results showed that EcN-CFS significantly inhibited C. jejuni growth, reducing bacterial counts in liquid media by approximately 2.5 logs after 24 hours, and achieved complete inhibition of the bacteria within 48 hours. The largest inhibition zone was observed after 3 hours of incubation (21 ± 0.6 mm). Additionally, EcN-CFS reduced pre-formed C. jejuni biofilm by over 80% and inhibited biofilm formation by more than 70%. Pretreatment of polarized HT-29 MTX cells with EcN-CFS significantly ($P < 0.05$) reduced C. jejuni invasion and intracellular survival. EcN-CFS significantly downregulated the expression of genes involved in motility, cytotoxin production, colonization, and quorum sensing. Conclusion: This study highlights the potential of EcN as a promising antibiotic alternative for controlling C. jejuni infection. Additionally, EcN-CFS might secrete bioactive molecule(s) that is responsible for its antimicrobial activity."
GaP2	**Metagenomic Insights into the Adaptation and Selection of Nitrosomonas spp. for Partial Nitritation Under Saline Conditions** Sareh Aghababababaee(1), Suin Parka (2), Kyungjin Cho, Taeho Lee(2), Eunsu Lee(5), Hyokwan Bae(2,6), Minjae Kim(1) (1) Department of Civil Engineering, University of Kentucky (2) Department of Civil and Environmental Engineering, Pusan National University, Republic of Korea (3) Center for Water Cycle Research, Korea Institute of Science and Technology, Republic of Korea (4) Division of Energy & Environment Technology, KIST school, Korea University of Science and Technology Republic of Korea (5) R&DCenter, POSCO E&C Republic of Korea (6) Institute for Environment and Energy, Pusan National University, Republic of Korea Partial nitrification (PN) is a key biological process for cost-effective nitrogen removal from saline wastewater, as it reduces the costs associated with aeration and external carbon supplementation. An efficient PN system can be established by simultaneously suppressing the activity of nitrite-oxidizing bacteria (NOB) and promoting the activation of ammonia-oxidizing bacteria (AOB) in nitrifying systems. In our study, we implemented various operational strategies, including high free ammonia (FA), low-dissolved oxygen (DO), and high temperature, to establish an efficient PN system. Our previous 16S rRNA sequencing study identified Nitrosomonas europaea, Nitrosomonas nitrosa, and Nitrosomonas mobilis as core ammonia-oxidizing bacteria (AOB) contributing to PN under salt stress. However, the underlying functional adaptations and genetic mechanisms driving the selection of these species remain unclear. To further investigate these microbial adaptations, we are conducting a shotgun metagenomic analysis on biomass samples collected from the same PN system. This approach will allow us to examine the genomic potential of nitrifying communities, identify functional genes linked to ammonia oxidation and salt tolerance, and explore microbial interactions shaping PN performance under saline conditions. Our analysis aims to determine key metabolic pathways involved in nitrogen cycling and assess potential genetic adaptations that enable AOB persistence in high-salinity environments. By integrating metagenomic data with process performance metrics, we seek to enhance our understanding of microbial selection and adaptation in saline PN systems. These insights

	could contribute to optimizing wastewater treatment strategies for nitrogen removal under challenging environmental conditions. Preliminary findings and ongoing analyses will be presented at the conference.
GaP3	**Exploring Environmental Microbiomes: A Multi-Omics Approach Comparing Healthcare Related and Non-Healthcare Settings** Ka'ili Cruz(1), Tariq Chaudry(2), John Bauer(2), Hong Huang(3), Brandon Schanbacher(3), Carrie Hobbs(3), Benjamin Robinson(3), Minjae Kim(1), John Mendel(4), Sareh Aghababaee(1) (1) Department of Civil Engineering, Stanley and Karen Pigman College of Engineering, University of Kentucky (2) Department of Pediatrics, College of Medicine, University of Kentucky (3) Kentucky Children's Hospital Office of Pediatric Research, College of Medicine, University of Kentucky, Lexington (4) Department of Chemical Engineering, Stanley and Karen Pigman College of Engineering, University of Kentucky Healthcare associated infections (HAIs) occur within 1.7 million hospitalized patients in the U.S. each year, causing more than 98,000 (one in 17) deaths, and imposing an estimated economic burden of $28–45 billion. Roughly 21.6% of HAIs are linked to Opportunistic Premise Plumbing Pathogens (OPPPs), such as Legionella spp., which thrive in premise plumbing biofilms and exhibit resistance to disinfection. Beyond their direct impact, OPPPs can also serve as reservoirs for pathogens from the human microbiome, highlighting the need to study the microbiome of built environments. To investigate this issue, our team has been collecting swab and water samples from water-related devices within healthcare-associated and high-foot-traffic, non-healthcare buildings on the University of Kentucky campus. Using shotgun metagenomic sequencing, we aim to characterize the microbial communities in these environments. Additionally, we have been monitoring various disinfection byproducts (DBPs) in our water samples monthly, including bromochloroacetonitrile, bromodichloromethane, bromoform, chloroform, dibromoacetonitrile, dibromochloromethane, dichloroacetonitrile, and trichloroacetonitrile. Preliminary results indicate the presence of each compound, except for bromoform, which was below the detection limit. Further correlation analyses will assess potential DBP-related effects on microbial diversity in drinking water.
GaP4	**Emerging Threats of Multidrug-Resistant Salmonella in Companion Animals** Golam M. Faisal, Ajran Kabir, Bibek Lamichhane, Rosbelly Rios, Tasmia Habib, Yosra A. Helmy Department of Veterinary Science, Martin-Gatton College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY Background: Companion animals can act as reservoirs for Salmonella, shedding the pathogen into the environment and increasing zoonotic transmission risks. Many infected pets remain asymptomatic, making detection and control challenging. The emergence of multidrug-resistant (MDR) Salmonella in companion animals further increases public health concerns. This study aimed to investigate the prevalence, serotypes, virulence traits, and antimicrobial resistance (AMR) profiles of Salmonella isolates from healthy dogs and cats in Central Kentucky. Methods: Fecal samples (n=233) from cats (n=40) and dogs (n=193) were collected from veterinary clinics and animal shelters. Samples were enriched and plated on XLT4 agar, with Salmonella confirmation performed via PCR targeting the invA gene. Traditional serotyping was conducted to determine the distribution of Salmonella isolates. Bacterial motility was assessed through swarming and swimming assays, and biofilm formation was evaluated using the crystal violet assay. AMR and virulence genes were identified by PCR, while antimicrobial susceptibility testing was conducted using the broth microdilution method. Results: Salmonella was detected in 8.1% (19/233) of samples. The most prevalent serotype was Salmonella Catumagos (n=13), followed by S. Infantis (n=3) and S. Mbandaka (n=3). Among isolates, 84.2% exhibited strong biofilm formation, while 31.6% and 84.2% demonstrated high swarming and swimming motility, respectively. The most frequently detected virulence genes included invA (100%), hilA (100%), siiA (100%), siiC (100%), and adrA (100%). AMR genes blaTEM (68.4%), blaCTX (94.7%), strA (63.1%), and sul2 (68.4%) were predominant. All isolates exhibited MDR, with the highest resistance observed against macrolides and trimethoprim-sulfamethoxazole. Conclusions: Asymptomatic Salmonella shedding by companion animals poses a significant public health concern, as direct and indirect transmission to humans can occur through close contact, contaminated environments, or pet food. The high prevalence of MDR strains underscores the need for enhanced veterinary surveillance, responsible pet handling, and antimicrobial stewardship to mitigate zoonotic risks."

GaP5	**Group B Streptococcus growth under acid stress** Sierra Ginocchio, Ryan Doster Department of Microbiology and Immunology, University of Louisville School of Medicine Background/Intro: Streptococcus agalactiae, also known as Group B Streptococcus (GBS) is an opportunistic pathogen that colonizes the gastrointestinal and female reproductive tract of 15-30% of healthy individuals. However, GBS rectovaginal colonization during pregnancy increases risk for ascending infection resulting in neonatal infections and mortality. Lactobacillus dominant vaginal microbiomes provide protection against urogenital pathogens, such as GBS, through secretion of lactic acid, which lowers the vaginal pH. We sought to understand how lactobacilli may provide protection against GBS and investigate GBS responses to acid stress. Hypothesis/Objective: To understand the effect of Lactobacillus lactic acid production on GBS survival, we examined GBS growth under acid-stress conditions. To allow for future study of GBS-lactobacilli interactions, we investigated several different medias for the ability to grow both organisms to find a common growth broth for co-culture experiments. Methods: To understand how acid stress conditions affect GBS growth, we completed growth curves with bacterial culture media adjusted to various pHs with either the inorganic hydrochloric acid (HCl) or L-lactic acid, an organic acid made by some Lactobacillus species. To find a bacterial growth media that would support growth of both GBS and Lactobacillus, growth curves were completed with L. crispatus VPI 3199 using several different bacterial and cell culture medias. Results: GBS growth was impaired at acidic pHs with minimal growth at pH 4.5 and no growth at pH 3.5. GBS growth was similar between media conditioned with lactic acid or HCl. While GBS grows in several bacterial growth broths, it does not grow well in MRS, the typical growth broth for lactobacilli species. L. crispatus did not grow in any traditional GBS growth broths. A comparison of bacterial broth ingredients suggested additional components including magnesium, manganese, tween80, and glucose that when added to Todd Hewitt's broth might allow for Lactobacillus growth with GBS, but this strategy did not produce consistent growth. Conclusion: Acidic pH limits GBS growth, and no difference was seen in GBS growth between media conditioned with organic or inorganic acids. L. crispatus does not grow under aerobic conditions in any common bacterial growth broth even when additional nutritional components were added. Additional strategies are needed to examine the relationship between lactobacilli and GBS to better understand microbe-microbe interactions that influence the vaginal microbiome and GBS rectovaginal colonization."
GaP6	**Targeting Rhodococcus equi: In Vitro Assessment of Next-Generation Probiotics as a Therapeutic Strategy** Ajran Kabir, Bibek Lamichhane, Yosra A. Helmy Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky Introduction: Rhodococcus equi is a severe pneumonia-causing pathogen in foals responsible for opportunistic infections in immunocompromised humans. Antibiotics such as rifampin and macrolides are considered the first line of treatment against R. equi infection. However, the development of antimicrobial resistance in R. equi poses a challenge to conventional treatments which necessitate the development of alternative therapeutics. Objectives: This study evaluates the in vitro efficacy of selected next-generation probiotics (NGP) strains against R. equi to explore their potential as biotherapeutic agents. Methods: A total of 38 probiotic strains were screened for antagonistic activity against R. equi isolates using agar well diffusion assays. Strains with high inhibitory zones (mm) were further subjected to co-culture inhibition assays using cell-free supernatants (CFSs). The crystal violet assay was conducted to evaluate the impact of specific probiotic treatment on both the formation of R. equi biofilms and the disruption of preformed biofilms. Cell culture assays were conducted to assess the effects of probiotic whole cultures, and CFSs on the adhesion, invasion, and intracellular survival of R. equi in murine macrophages. Results: The top six probiotics were selected based on their zones of inhibition observed in the agar-well diffusion assay. These selected strains demonstrated significant inhibition of R. equi growth after 12 hours ($P < 0.05$) and achieved complete bacterial clearance by 120 hours when co-cultured with R. equi. Furthermore, the selected probiotics inhibited the multi-drug resistant R. equi strains. The CFSs of five probiotic strains exhibited over 90% inhibition of both biofilm formation and preformed R. equi biofilms. Additionally, treatment with probiotic CFSs led to a significant reduction in intracellular R. equi survival after 24 hours ($P < 0.05$) in murine macrophages. Conclusion: These findings highlight the potential of probiotics as promising alternative therapeutics for controlling R. equi infections, offering a novel strategy to mitigate antibiotic resistance and enhance host immune defense.

	Key words: R. equi, Foals, Probiotics, Antimicrobial resistance
GaP7	**NAD+ cleavage by a cell-wall attached protein in Streptococcus agalactiae.** Marilia Manta, Oscar Ciros, Cameron Kenner, Rokon Karim, Jon Thorson, Konstantin Korotkov, Natalia Korotkova University of Kentucky BACKGROUND: Streptococcus agalactiae, or Group B Streptococcus (GBS), is a Gram-positive pathogen part of the gastrointestinal and vaginal tract of humans. Although present in healthy adults, it can develop from asymptomatic to invasive state. It is particularly dangerous for newborns, pregnant and immunocompromised people. Nicotinamide adenine dinucleotide (NAD+) have a fundamental role in redox reactions, signaling, immune response and stress resistance. NAD+ homeostasis is fundamental to keep a balanced metabolism in humans. NAD+ is also important for proper bacterial metabolism. Hence, some bacteria can modulate NAD+ levels according to their metabolic requirements by expressing NAD-utilizing toxins, NADases, or promote NAD+ production by host cells. It was previously reported that GBS can secrete NAD+ into the growth medium. Our previous analysis of GBS membrane proteins revealed NudP, a cell-wall attached protein that have homology to enzymes that can cleave NAD+. Moreover, AlphaFold3 analysis predicted that NudP can bind NAD+. Thus, we hypothesize that GBS can modify host NAD+ using NudP, modulating pathogenesis and host immune response. METHODS: Obtained purified NudP and did analysis with LCMS and malachite green after incubation with NAD+. We created isogenic ΔnudP and measured NAD+ concentration in medium using a kit and analyzed strains growth in a chemically defined medium containing NAD+ or NAD+ precursors. RESULTS: NudP can cleave NAD+ and produce adenosine, nicotinamide mononucleotide and phosphate. Reaction can be inhibited by adenosine. As WT, ΔnudP can grow in the presence of NAD+ and still consumes NAD+ from medium, but less than WT. CONCLUSIONS: This suggests that there is a second enzyme able to cleave NAD+ in GBS. We can speculate that GBS can modulate host NAD+ levels using NudP and a second unknown enzyme. This mechanism could have impact in host-pathogen interaction, suggesting a new strategy used by GBS to increase pathogenesis.
GaP8	**Unraveling the Dance Between and the Alphavirus Capsid protein and Pelle During Antiviral Evasion** Sayra Moni, Kevin J Sokoloski University of Louisville The Toll pathway is a critical part of the antiviral response, functioning as an early detection system against pathogens. In insects, the Pelle protein plays a role similar to that of human IRAK1, acting as a signal transducer that connects the Toll receptor to the transcriptional response. However, in humans, alphavirus capsid (CP) proteins can disrupt IRAK1 signaling, weakening the host's defenses against viral infections. Mosquitoes, which transmit alphaviruses like Chikungunya and Sindbis—linked to severe diseases such as intense musculoskeletal pain and encephalitis—also rely on the Toll pathway for their immune response. Nonetheless, the impact of the alphaviral capsid protein to Pelle signaling remains unknown. We hypothesize that alphavirus capsid proteins interact with Pelle in a way that mirrors their interaction with IRAK1. To test this, we conducted NanoLuc-based BiMolecular Complementation assays to quantitatively assess the capacity of the CP protein to interact with the Pelle proteins of Aedes spp. mosquitos. In addition, we examined different domains of the CP proteins to pinpoint potential interaction sites and identify the necessary and sufficient interaction determinants. Our comparisons have revealed that Pelle interacts with the CP protein similarly to IRAK1 and utilizes the same interaction determinants. Further analysis using different alphaviruses CP proteins confirmed that the interaction was evolutionarily conserved. Interestingly, extending these observations to Pelle proteins from various mosquito species reveals biases regarding the strength of the CP/Pelle interaction suggesting a potential role during viral transmission and vector host competence. Statistical analysis of the underlying quantitative data using Student's t-test demonstrates significant results. Together, these findings provide insights into viral strategies for evading host defenses. Future research will explore this interaction to assess its impact on mosquito Toll signaling. By uncovering viral-host interactions, this research could identify therapeutic targets to combat alphavirus infections and improve immune response strategies."
GaP9	**Polymicrobial interactions of Raoultella planticola and Chromobacterium violaceum** Katelyn Miller, Dr. Sean Fox East Tennessee State University Chromobacterium violaceum and Raoultella planticola are two soil bacteria with co-growth tendencies that have potential implications in human health. Raoultella planticola, formerly known as Klebsiella planticola, is a bacterium with a near identical genotype to the well-known and highly antibiotic-resistant pathogen

	Klebsiella pneumoniae. While Klebsiella is best known for its role in nosocomial infections, Raoultella has recently been increasingly found in nosocomial infections, although its pathogenicity and prevalence has not been thoroughly investigated. Chromobacterium violaceum produces the secondary metabolite violacein, which has antimicrobial properties and potential influence on microbial environments. This research aims to find the potential synergetic or inhibitory interactions of R. planticola and C. violaceum, particularly in the role of antibiotic resistance and pathogenicity. Characterization of Raoultella resistance mechanisms could provide crucial insight into the similar antibiotic resistance mechanisms of Klebsiella pneumoniae, while also creating a benchmark of comprehension for the potential rising pathogenicity of Raoultella planticola itself. Isolates of both R. planticola and C. violaceum were collected from Blue Hole Falls in East Tennessee on four separate occasions and were grown in both mono- and co-cultures. Preliminary data suggests potential enhanced Raoultella planticola growth and antibiotic resistance in co-cultures via growth curves, spectrometry, Kirby Bauer, biofilm assays, and violacein activity. Further experiments will continue to explore inhibitory or synergetic interactions and continued exploration of antibiotic resistance mechanisms of R. planticola and its persistence in polymicrobial environments. Understanding these interactions could provide insight into R. planticola's clinical and ecological significance.
GaP10	**Investigating the role of Il-22 in Urolithin A-mediated protection against recurrent C. difficile Infection** Fariha Nasme, Sweta Ghosh, Daniel Erickson, James Collins, Venkatakrishna Rao Jala, Department of Microbiology and Immunology, University of Louisville UofL-Brown Cancer Center Center for Predictive Medicine, University of Louisville Center for Microbiomics, Inflammation and Pathogenicity, University of Louisville Center for Integrative Environmental Health Sciences, University of Louisville Clostridioides difficile infection (CDI) is a major healthcare-associated disease, leads to severe intestinal inflammation and colitis. Recurrent CDI (rCDI), relapse of disease within 8-10 weeks is a major problem with no known treatments. Interleukin-22 (Il-22) is a well-established cytokine known to enhance gut barrier functions and protects against chemical-induced colitis models. However, its role in C. difficile (CD)-induced colitis is poorly understood. Previously, our laboratory demonstrated that microbial metabolite Urolithin A (UroA) protects against C. difficile-induced colitis in acute pre-clinical mouse model. Further, it was shown that UroA treatment increased Il-22 levels. Based on these findings, we hypothesize that UroA protects against rCDI in IL-22-deppendent manner. To test this hypothesis, wild type and Il-22-/- mice are subjected recurrent CDI mouse model. Briefly, mice were treated with clindamycin(10mg/kg) 1-day prior to CD infection and followed by clindamycin injection at day 14 and 28 to induce rCDI. Mice were treated 3-times a week with UroA (20 mg/kg) or vehicle. Mice were euthanized on day 39 and characterized the colitis phenotype. Our results suggest that UroA treatment protected wild type mice from CDI-induced loss of body weight change, shortening of colon length and colonic inflammation. On the other hand, UroA failed to protect Il-22-/- mice against CDI-induced body weight loss, colon inflammation (colon length and histology). Currently, we are evaluating the molecular mechanisms underlying Il-22-dependent UroA's protective activities against CDI, which potentially unveil the novel therapeutic strategies for combating recurrent CDI.
GaP11	**Appalachian Assassin: Pseudomonas koreensis contact dependent killing of Salmonella** Braxton E. Stout, Sean J. Fox Department of Biomedical Health Sciences, College of Public Health, East Tennessee State University Pseudomonas, commonly known as a pathogen in various illnesses, has an extraordinary ability to target other harmful pathogens that are often overlooked. This project focuses on the antimicrobial properties of polymicrobial interactions and their theraputic potential. Soil samples from the Appalachian region were collected and screened for their ability to inhibit pathogens commonly associated with hospital-acquired infections. Two standout strains, Pseudomonas baetica (S1S3-2) and Pseudomonas koreensis (S1S3-13), exhibited the capacity to hinder thirteen different pathogens, including Salmonella arizoniae. To investigate this inhbition, we are employing transposon-based mutagenesis using the plasmid 'pRL27' to transform wild-type (WT) Pseudomonas species. Our goal is to phenotypically screen for mutants that lose the ability to form a zone of inhibition (ZOI) against the pathogens they once inhibited. The implications of this research are significant. By unravelling the antimicrobial mechanisms of these soil microbes, we could develop a better understanding of polymicrobial interactions while also identifying new targets to fight pathogenic bacteria.
GaP12	**Isolation and Characterization of Siderophores from Soil Isolates** Kemper Rasnake, Dr. Ranjan Chakraborty East Tennessee State University

	Bacteria employ various mechanisms to outcompete rivals in both host environments and natural ecosystems. One such mechanism is the production of siderophores, which sequester insoluble iron (Fe3+) from their surroundings. Many species can produce multiple types of these siderophores namely, Pseudomonas aeruginosa, which produces pyoverdine and pyochelin, both of which are essential for iron acquisition and contribute to virulence. Numerous siderophores have been structurally characterized, enhancing our understanding of their interactions with bacteria and hosts. Soil samples were collected from areas in northeastern Tennessee and southwest Virginia and screened for siderophore-producing colonies on Chrome Azul S (CAS) agar. Colonies exhibiting siderophore production were selected for sequencing to identify bacterial species. If siderophore had not been previously characterized from these species, they were further investigated to structurally characterize the siderophores. The characterization was accomplished after the siderophores were isolated and purified using conventional methodology using column chromatography. Structure was determined using mass spectrophotometry and NMR spectroscopy. Characterization of novel siderophores will provide insights into their potential application in combating antibiotic resistance. Specifically, siderophore-antibiotic conjugates which could facilitate drug entry into bacterial cells, offering an innovative approach to treat nosocomial infections. This research may contribute to the development of more effective therapeutic strategies and improved clinical outcomes.
GbP1	**CHARACTERIZING THE ROLE OF PHYTOHORMONES ON NITROGEN FIXATION IN AZOSPIRILLUM BRASILENSE** Ishita Banerjee, Dr. Gladys Alexandre University of Tennessee Background: The rhizosphere, the soil surrounding plant roots, is influenced by root exudates and microorganisms. Conditions in the rhizosphere in turn affect microbial activities. Phytohormones and microbially produced hormones, that can alter plant physiology, further modulate rhizosphere interactions. The rhizosphere is thus a competitive environment in which microbes and plant roots compete for resources. Bacteria have evolved strategies to adapt to the competitive environment of the rhizosphere including through sensing of phytohormones. An ethylene receptor (AzoEtr1) was recently characterized in the Alphaproteobacterium Azospirillum brasilense with this receptor regulating the colonization of plant root surfaces by the bacterium. A. brasilense is a soil bacterium able to promote plant growth through a variety of mechanisms including the production of plant growth-promoting hormone auxin (Indole 3 acetic acid (IAA)) and nitrogen fixation. Exposure of A. brailsense to ethylene induces a metabolic response that suggests nitrogen starvation, however, the exact mechanism is unknown Given the impact of ethylene on nitrogen metabolism in A. brasilense and the likely presence of both auxin and ethylene in the rhizosphere, we aimed to characterize how ethylene and auxin affect nitrogen fixation in A. brasilense. Methods: Using transcriptional reporter assays, we compared the promoter activity of selected genes necessary for nitrogen fixation in the presence of ethylene and auxin in WT and the etr1mutant background. We are complementing this analysis with physiological assays to test hypotheses generated by promoter activity analyses. Results: In nitrogen-fixing conditions, ethylene suppressed promoter activity of glnB, rpoN, and nifH, whereas auxin caused no change. Promoter activity of nifH and glnB were abolished in the etr1 mutant background as compared to WT. Ethylene increased ipdC (encodes a key enzyme for auxin production) promoter activity in etr1-independent manner. Conclusion: Our results suggest that ethylene negatively regulates nitrogen fixation via Etr1 and glnB while auxin does not directly affect nitrogen fixation gene expression in A. brasilense. As ipdC promoter activity is increased upon ethylene treatment, we speculate that ethylene induces auxin production."
GbP2	**Dengue virus modulates critical cell cycle regulatory proteins in human megakaryocyte cells** Swarnendu Basak(1), Shovan Dutta(2), Supreet Khanal(3), Girish Neelakanta(1), Hameeda Sultana(1) 1 Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville 2 Cleveland Clinic, Center for Immunotherapy and Precision Immuno-Oncology (CITI), Lerner Research Institute, Cleveland, OH 3 Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, CBER, U.S. Food and Drug Administration, Silver Spring, MD Dengue virus (DENV) infection suppresses human megakaryocytes, leading to reduced platelet production, thrombocytopenia, dengue haemorrhagic fever (DHF), dengue shock syndrome (DSS), and ultimate death. Previous studies have shown that DENV (serotype 2) reduces polyploidy in human megakaryocytes, a condition linked to decreased platelet production. To investigate the mechanism underlying DENV-mediated polyploidy reduction in megakaryocytes, we examined cell cycle modulation in MEG-01 cells, an in vitro

	leukemic human megakaryocytic cell line. Our study demonstrates that DENV2 significantly modulates cell cycle signaling in megakaryocytes. Protein profile microarray analysis revealed the upregulation of key cell cycle regulators, including CDK4, CDK1, and Cyclin B1, alongside the downregulation of Chk1, GSK3-beta, CUL-3, and E2F-3. These results were further validated by quantitative real-time PCR and immunoblotting, which confirmed the upregulation of CDK4, CDK1, and Cyclin B1 upon DENV2 infection. Silencing CDK4, CDK1, and Cyclin B1 significantly reduced DENV2 replication, emphasizing their role in viral replication. Immunoprecipitation assay revealed enhanced interactions between Cyclin B1 and CDK1 during infection, suggesting substantial changes in cell cycle regulation and the endomitosis process. In conclusion, our findings provide molecular insights into DENV2-mediated cell cycle modulation in megakaryocytes, shedding light on its role in thrombocytopenia and related complications.
GbP2	**Investigating the role of bS21 in vancomycin resistance in Staphylococcus aureus** Kira Bernabe(1), Kathryn Ramsey (2) (1) University of Rhode Island, (2) University of Louisville Staphylococcus aureus (S. aureus) is a Gram-positive coccus that can cause serious disease and is also part of human normal flora. S. aureus is a growing public health threat, particularly in healthcare settings, because of its success in developing or acquiring resistance to commonly used antibiotics. Vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA) are of elevated concern because vancomycin is considered a drug of “last resort”. Vancomycin is a glycopeptide antibiotic that targets the cell wall by interfering with peptidoglycan synthesis. In multiple instances, clinical isolates of VISA have been isolated with mutations in the gene rpsU. This gene encodes the ribosomal protein bS21, which plays a role in initiation of translation. The reported rpsU mutations in VISA strains are predicted to lead to loss of functional bS21. Furthermore, a screen for genes that impact vancomycin susceptibility in S. aureus identified that loss of rpsU is sufficient to lead to increased vancomycin resistance. The link between loss of a ribosomal protein and resistance to a cell-wall targeting antibiotic in S. aureus remains unknown. Our goal is to elucidate the molecular mechanism that leads cells without bS21 to have increased vancomycin resistance. We have generated S. aureus cells with a clean deletion of rpsU and validated that they have increased resistance to vancomycin compared to wild type cells. Future work will focus on understanding how this change in ribosome composition may impact gene expression, possibly by altering translation initiation, and resistance to vancomycin. We expect that this work will elucidate a novel link between the ribosome and vancomycin resistance in S. aureus.
GbP3	**Signal integration between PtsN1 and core chemotaxis signaling proteins affect metabolic regulation in Azospirillum brasilense** Tasneem Ehsan and Gladys Alexandre Biochemistry & Cellular and Molecular Biology Department, The University of Tennessee, Knoxville Background: Azospirillum brasilense are motile and chemotactic soil bacteria that colonize a wide array of plant species. A. brasilense utilizes two chemotaxis systems, named Che1 and Che4 to direct flagellar-dependent chemotaxis. Mutations that abolish these chemotaxis systems affect non-chemotaxis phenotypes such as nitrogen fixation and flocculation through unknown mechanisms. Recent pulldown assays to identify non-chemotaxis interactors of CheA1 and CheA4 identified a PtsN1 (phosphoenolpyruvate; sugar phosphotransferase system (PTS) nitrogen, PtsNtr) homolog which is a carbon and nitrogen global regulator conserved in Bacteria. The goal of this study is to confirm PtsN1 interactions with CheA1 and CheA4 and any other chemotaxis proteins and to characterize the role of PtsN1 in regulating non-chemotaxis phenotypes in a Che1- and Che4-dependent manner. Methods: We used Bacterial two-hybrid assay (BacTH) to characterize the interaction between chemotaxis proteins and PtsN1. We also used overexpression of PtsN1 in wild type and chemotaxis mutant backgrounds, and a deletion strain lacking PtsN1 to examine the functional link between PtsN1 and chemotaxis systems. We also used a RpoN promoter reporter assay to track the role of PtsN1 on nitrogen fixation. Additional BacTH assays were used to test interactions between PtsN1 and global regulators of carbon and nitrogen metabolism. Results: BacTH assays showed interaction of PtsN1 with CheA1 and CheA4 but not with histidine kinases encoded in chemosensory pathways that do not function in chemotaxis. Deletion of the P5 domain of CheA1 (CheA1P5) or CheA4 (CheA4P5) abolished interactions between PtsN1 and CheA1 or CheA4. PtsN1 also interacted with CheW1 and CheW4. These results suggest PtsN1 interacts with chemotaxis signaling arrays base plate proteins. A ΔptsN1 strain had a delayed growth in presence of potassium nitrate similar to observed previously in a ΔcheA4 strain and consistent with interactions between these proteins. Our preliminary results indicate that overexpression of PtsN1 in the Δche1, ΔcheA1, Δche1Δche4 and ΔcheA1ΔcheA4 mutant backgrounds restores growth defects relative to wild type. We are currently analyzing how PtsN1 overexpression affects chemotaxis mutants under conditions of nitrogen fixation. PtsN1

	overexpression further increased the flocculation rate of the Δche1 and Δche1Δche4 mutant backgrounds. Given that a ΔptsN1 flocculates slower than WT, we hypothesize that the flocculation phenotype of chemotaxis mutants in A. brasilense is related to their interaction with PtsN1. Other experiments regarding promoter assays and additional BacTH are ongoing. Conclusion: Our data highlights an integrated metabolism with chemotaxis signaling through interaction with the PtsN1 global regulator in A. brasilense."
GbP5	**Revealing novel functions of putative cytotoxins in Chlamydia** Gracie Eicher, Kenneth A. Fields University of Kentucky College of Medicine Chlamydial species are important pathogens that represent a paradigm for understanding successful obligate intracellular parasitism. C. trachomatis is a prevalent human pathogen, whereas C. muridarum is a murine-specific pathogen often used to study chlamydial disease in a small animal model. Although their genomes are largely conserved, various Chlamydial species exhibit distinct tissue and host tropisms. It is postulated that the so-called plasticity zone (PZ), a region of the chromosome with a high number of polymorphisms, could be cause of species-specific variability in infection biology. The presence/absence of genes encoding glycosyltransferases designated as putative cytotoxins represents an intriguing example of this diversity. C. muridarum express multiple homologous proteins (TC0437, TC0438, and TC0439) that show similarity to a family of larger cytotoxins. The PZ of common urogenital C. trachomatis serovars express a truncated protein (CT166) while ocular serovars of members of the lymphogranuloma venereum (LGV) biovar lack the toxin genes completely. The putative chlamydial cytotoxins have been proposed to mediate immediate toxicity in highly infected epithelial cells by interfering with actin polymerization. We used FRAEM mutagenesis to delete tc0437-0439 in a single C. muridarum strain. This multi-gene mutation did not interfere with immediate toxicity but did show reduced invasion efficiency. Immunolocalization and trypsin degradation assays provided evidence supporting localization of CT166 to the surface of EBs and RBs. Although additional work is required, these data suggest that one function of the putative cytotoxins is to promote infection directly as attachment or adhesin factors. These functions could contribute to species-specific infection biology.
GbP6	**Dietary Sucrose Indirectly Enhances C. difficile Pathogenesis** Daniel Erickson, Katelyn Sheneman, Michelle Chua, James Collins University of Louisville Added sugars constitute about 13% of daily caloric intake in an average American diet, with 30% of Americans consuming excessive amounts of sugar. It is unknown how diets rich in simple sugars effect the pathogenesis of C. difficile, the most common hospital-acquired infection in the United States. Our study utilized a diet rich in sucrose, the most widely consumed added sugar, to investigate the effects of dietary sugar on C. difficile infection (CDI). Despite sucrose being non-metabolizable by C. difficile, the dietary sugar significantly enhanced C. difficile pathogenesis. Following antibiotic pre-treatment and C. difficile challenge, we monitored mice for factors such as colonization, bacterial and toxin burden, and clinical signs of disease. Mice fed the sucrose-rich diet experienced significantly worse CDI symptoms, potentially explained by an elevated toxin burden and exacerbated gut inflammation. The sucrose-rich diet was associated with a prolonged carrier phenotype, where mice consuming the diet were colonized by C. difficile at higher levels initially and were unable to clear the infection up to seventy-two days post infection. Mice fed the sucrose-rich diet experienced a shift in their gut microbiota favoring a profile that was depleted in taxa antagonistic to C. difficile and enriched in taxa capable of cross-feeding C. difficile with nutrients. Similarly, the sucrose-rich diet was associated with a shift in the gut metabolome, potentially supporting more efficient C. difficile Stickland Metabolism. We hypothesize that these shifts in the gut microbiome and metabolome are driving the enhanced disease severity and prolonged bacterial burden observed in mice fed the sucrose-rich diet.
GbP7	**hTERT immortalized mesenchymal stem cell-derived EVs treatment reduces ZIKV-induced cortical neuronal death, infection, and exosome-mediated transmission** Kehinde Fasae, Ana Melentijevic Eckert, Girish Neelakanta, and Hameeda Sultana The University of Tennessee, Knoxville Background: Mesenchymal stem cells (MSC) derived-extracellular vesicles (MSC-EVs) are paracrine effectors of MSCs, which play strategic roles in mediating intercellular communication between MSCs and target cells. Since MSC-EVs take on the function and properties of their mother cells and have lower immunogenicity, they have demonstrated beneficial effects in several preclinical and clinical disease models including viral infections and neurological disorders. In this study, we examined the neuroprotective effects of MSC-EVs on primary cortical neurons infected with Zika Virus (ZIKV).

	Methods: Neuronal cell death has been observed following ZIKV infection. The American Culture Type Collection (ATCC) 2022 challenge championing the use of the product “ATCC® SCRC-4000-EXM ™ in innovative and new applications, allowed us to evaluate the effects of hTERT immortalized MSC-EVs on ZIKV-infected neurons. The neurons were pre-treated with the MSC-EVs or GW4869, and thereafter exposed to ZIKV infection for 72 hours. Results: Our results show increased cell viability of hTERT MSC-EVs-treated ZIKV-infected neurons compared to the untreated infected neurons, and a concomitant decrease in the markers of apoptosis including Caspase 3/9, Bax, and Bcl-2). Further, ZIKV transcripts were reduced in neurons treated with hTERT MSC-EVs, increased Interferon-beta (IFN-B) and decreased TNF-alpha expression. Inhibition of EV production and/or release by GW4869 treatment followed by hTERT-MSC-EVs incubation affected exosome-mediated ZIKV transmission in primary cortical neurons. Conclusion: Our findings suggest a potential neuroprotective, pro-survival, and antiviral effect of hTERT MSC-EVs treatment on neurological dysfunction associated with ZIKV infection. This study sheds light on the promising therapeutic potential of hTERT MSC-EVs in ZIKV-mediated complications such as microcephaly in newborns and Guilian barre syndrome in adult humans."
GbP8	**Active Vitamin D Reduces SARS-CoV-2 Infection Through Modulation of LL-37 and ACE2 Levels** Denny Gao, Erika L. Figgins, Donghoon Chung, Gill Diamond University of Louisville SARS-CoV-2 leads to COVID-19, which has infected over 750 million people and taken over 7 million lives. SARS-CoV-2 rapidly mutates and can cause breakthrough infections, making the development of additional treatments a pressing concern. Vitamin D deficiency is associated with worse outcomes in respiratory diseases including SARS-CoV-2. We have been studying the mechanisms through which vitamin D affects SARS-CoV-2 infection in the context of the human lung adenocarcinoma cell line Calu-3, which is commonly used in SARS-CoV-2 research. The hormonally active form of vitamin D, 1,25(OH)2D3, regulates genes associated with immune function and inflammation, such as through increasing mRNA levels of CAMP, which leads to increased production of the antimicrobial peptide LL-37. We have observed that active vitamin D significantly reduces mRNA levels of a replication-deficient strain of SARS-CoV-2 (ΔORF3-E SARS-CoV-2). Pre-incubation of LL-37 with ΔORF3-E SARS-CoV-2 before infection also significantly decreases mRNA levels of ΔORF3-E SARS-CoV-2, and pre-treatment with a CAMP-specific siRNA also significantly reduces ΔORF3-E SARS-CoV-2 mRNA, indicating the importance of LL-37 in combating SARS-CoV-2 infection. Interestingly, pre-treatment with active vitamin D still significantly decreases mRNA levels of ΔORF3-E SARS-CoV-2 when co-treated with CAMP siRNA, suggesting additional mechanisms are at play. We determined that active vitamin D significantly reduces ACE2 protein levels intracellularly and on the cell surface, and does so in a time-dependent and dose-dependent manner. However, while vitamin D primarily acts through regulating gene expression, pre-treatment of active vitamin D did not affect mRNA levels of ACE2; vitamin D instead acts by increasing degradation of ACE2. These findings support the use of vitamin D as a potential therapeutic against SARS-CoV-2 infection.
GbP9	**CRISPR-Cas12a is an efficient method to genetically alter Klebsiella pneumoniae** Taylor M. Garrison(1), Phoenix Gray(1), Amanda Brady(1), James Collins(1), Matthew B. Lawrenz(1,2) (1) Department of Microbiology and Immunology, University of Louisville (2) Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases, University of Louisville Klebsiella pneumoniae is a serious public health threat and major cause of hospital acquired infections. The rising incidence of multidrug resistance among K. pneumoniae strains has resulted in difficulty treating infections. Therefore, there is an urgent need to better understand K. pneumoniae’s pathogenesis to help develop new therapeutic approaches. However, traditional methods to generate site-specific mutations in K. pneumoniae have proven to be difficult and time consuming. Thus, we sought to develop a more efficient and rapid method to generate deletions in K. pneumoniaeutilizing a CRISPR-Cas12a system. To achieve this, we generated a plasmid-based system containing the Cas12a gene from Acidaminococcus sp. controlled by a tetracycline inducible promoter. We also integrated a cloning region downstream of a small RNA promoter to allow for quick engineering of the plasmid to contain a protospacer to target specific genes and gene-specific flanking regions for repair/resolution. To test the efficacy of this single plasmid-based system, we engineered plasmids to target genes related to metal-acquisition in K. pneumoniae (e.g., the ZnuABC ABC transporter and the yersiniabactin siderophore). We transformed the plasmid into K. pneumoniae KPPR1 and induced the Cas12a system using anhydrous tetracycline. Cultures were diluted into fresh media containing inducer every 24 h. At each passage, the bacterial culture was plated, and clones were screened for loss of the CRISPR-Cas12a plasmid and deletion of the targeted genes. Via this system, we were able to rapidly recover in frame deletions within 72 hrs, with 90% recovery by passage four. Whole genome sequencing confirmed the deletion and no off-target mutations were recovered. Together these data indicate that this

	CRISPR-Cas12-mediated mutagenesis system can quickly and reproducibly generate in frame deletions in K. pneumoniae.
GbP10	**The effects of sugar phosphate toxicity on growth and competitive fitness of Enterococcus faecium and Enterococcus faecalis** Michelle Hallenbeck, James Collins University of Louisville Vancomycin-resistant Enterococci (VRE) pose a serious public health risk. Their multidrug resistance necessitates the search for alternative treatment methods. One option lies in targeting their sugar metabolism genes, which VRE possess more of compared to commensal strains. One way to do this is by blocking a particular step in sugar metabolism to cause a buildup of toxic phosphorylated intermediates, a phenomenon known as sugar phosphate toxicity. We have generated mutants in several different sugar metabolism pathways in E. faecium and E. faecalis to examine the possibility of leveraging this phenomenon in the development of novel treatment options. We generated clean deletion mutants in manA, galT, and galE in E. faecium and E. faecalis. We conducted in vitro growth curves, dose curves, and co-culture competitions in the presence vs absence of the toxic sugar for each mutant (mannose for manA, galactose for galT and galE). We found that all three mutants in E. faecium demonstrated reduced fitness when competed against the wild-type strain in M1 minimal media alone, M1 with glucose, or BHI; however, in M1 with mannose, the fitness defect of the mutant was significantly exacerbated. For both E. faecium manA and E. faecalis manA, we observed a dose response, with increasing concentrations of mannose leading to a reduction in growth. Curiously, the EC50 for E. faecalis manA is 13.17 mM, while the EC50 for E. faecium manA is only 61 M. E. faecium galT and galE are sensitive to galactose in the micromolar range, with an EC50 of 63.69 M for galE and 166.6 M for galT. Interestingly, knocking galK out of the E. faecium galT strain negated the toxic effect. Sugar phosphate toxicity resulted in reduced growth and competitive fitness for E. faecium and E. faecalis mutants in the presence of the specific sugar for which the metabolism pathway was interrupted. Interestingly, E. faecium appears to be more sensitive to these effects than E. faecalis, with growth inhibition occurring at a much lower sugar concentration. We also demonstrated that the effects of sugar phosphate toxicity can be negated in E. faecium galT by preventing the formation of the toxic phosphorylated product. Further investigation is necessary to understand how to develop viable treatment plans to target this phenomenon.
GbP11	**A platform supporting generation and isolation of random transposon mutants in Chlamydia trachomatis** Caroline Hawk(1), Nur Hamdzah(1), Zoe Dimond (2,3), Kenneth A. Fields(1) (1) Department of Microbiology, Immunology, & Molecular Genetics, University of Kentucky College of Medicine, Lexington, KY (2) Host-Parasite Interactions Section, Laboratory of Bacteriology, Rocky Mountain Laboratories, NIAID/NIH, Hamilton MT (3) Current address, Yecuris Corp., Tualatin, OR 97062 Chlamydia species represent a paradigm for understanding successful obligate intracellular parasitism. C. trachomatis is a prevalent human pathogen exerting a tremendous negative impact on reproductive fitness, particularly in females. A complete understanding is lacking regarding how these bacteria create and maintain an intracellular niche, avoid/subvert host defense mechanisms, and cause disease. Forward genetic approaches provide one of the most powerful strategies available to accomplish untargeted elucidation of gene function. The utility of transposon-mediated random mutagenesis in supporting forward genetics is well established in a multitude of genetically tractable systems. Although initial progress has been made in Chlamydia, significant barriers remain that preclude leveraging the full benefit of transposon mutagenesis. We have engineered a platform that couples conditional plasmid maintenance with a previously described, riboswitch-regulated expression of C9 transposase. Transposition is accompanied by plasmid loss, thereby allowing selection of mutants. We provide evidence that this new system allows isolation of specific mutants from a complex pool. This step is an important advance in providing a mechanism capable of supporting down-stream studies interrogating chlamydial biology.
GbP12	**A Meal in Disguise: Uptake of Filifactor alocis-laden Neutrophils Trick Macrophages and Fuel Inflammation.** Ruth A. Idowu(1), Aruna Vashista(2), Richard J. Lamont(2), Silvia M. Uriarte(1, 2) (1) Department of Microbiology and Immunology, School of Medicine, University of Louisville, (2) Department of Oral Immunology and Infectious Diseases, School of Dentistry, University of Louisville

	Background: Filifactor alocis is a Gram-positive, obligate anaerobe that has emerged as a diagnostic marker of periodontitis. Neutrophils, key players in host defense, undergo constitutive apoptosis. This process can be delayed or accelerated by the neutrophil interactions with bacteria. Macrophages clear apoptotic neutrophils via “efferocytosis,” a process that relies on the upregulation of “eat-me” signals, such as phosphatidylserine (PS), and the downregulation of “don’t-eat-me” signals, including cluster of differentiation (CD) 31 and CD47, on neutrophils. We previously showed that F. alocis delays neutrophil apoptosis, but whether macrophages effectively degrade infected neutrophils remained unknown. We hypothesized that F. alocis manipulates neutrophil surface markers to gain access to macrophages, hijacking efferocytic clearance and fueling inflammation. Methods: Human neutrophils were stimulated with F. alocis for 0, 3, 6, and 18 hours, and the expression of PS, CD31, and CD47 was analyzed by flow cytometry. At 18 hours, neutrophils were co-incubated with human monocyte-derived macrophages (HMDMs) for 2 hours to assess their engulfment. HMDMs were then stimulated with LPS for 3, 6, and overnight to measure IL-6, IL-23, and IL-1β expression by qPCR. Results: F. alocis-infected neutrophils exhibited reduced PS at all time points and decreased CD31 by 3 hours, while CD47 remained unchanged until 18 hours. Despite these altered signals, macrophages still engulfed F. alocis-laden neutrophils at rates comparable to apoptotic neutrophils. However, unlike apoptotic neutrophils, the engulfment of F. alocis-infected neutrophils triggered a significant increase in IL-6, IL-23, and IL-1β mRNA expression upon LPS stimulation, thereby promoting sustained inflammation. Conclusions: As expected, ingesting apoptotic neutrophils by macrophages produces a pro-resolution phenotype. In contrast, ingesting F. alocis-laden neutrophils promotes a pro-inflammatory phenotype. This meal in disguise fuels a persistent inflammatory response. This study highlights a novel immune evasion strategy by which F. alocis manipulates neutrophil-macrophage interactions to survive in the inflamed oral mucosa."
GbP13	**Beyond antibiotics: Using probiotics to control Salmonella infections in humans and animals.** Bibek Lamichhane, Yosra A. Helmy; Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky Background: Salmonella is a one of the most common cause of foodborne gastroenteritis in animals and humans of all age groups. The infection is treated using antibiotics like cephalosporins and fluoroquinolones. However, the increase in antimicrobial resistance has necessitated the development of alternative therapeutics. Next-generation probiotics (NGPs) are derived from the normal microbiota and have not been used against any pathogen. This study aims to identify NGPs with high effectiveness against Salmonella infections in vitro. Materials and Methods: We screened 38 probiotics strains for their effect on Salmonella growth using agar well diffusion assay and selected NGPs with high growth inhibition for further development. Selected probiotics were co-cultured with Salmonella in liquid media. We also evaluated their effect on biofilm formation and preformed biofilms. Similarly, the ability of the probiotics to inhibit Salmonella adhesion, invasion and survival in intestinal cells were evaluated. Additionally, we examined NGPs' effect on Salmonella virulence genes using RT-PCR. The experiments were replicated at least twice, and results were statistically analyzed using two-way ANOVA with Tukey analysis. Results: All probiotics effectively inhibited Salmonella growth, and the top 7 probiotics were selected for further development. All the selected candidates significantly inhibited Salmonella growth in liquid media and had high inhibition (>95%) of biofilm formation and preformed biofilms. They significantly($p<0.05$) inhibited Salmonella adhesion, invasion, and intracellular survival in human intestinal cells. RT-PCR analysis revealed a significant downregulation of genes associated with virulence factors, colonization, motility, and quorum sensing of Salmonella. Conclusion: Our findings highlight NGPs as promising antibiotics alternatives for combating Salmonella infections in horses. Future research will focus on continued in vitro evaluation and assessing the efficacy of the top two candidates in vivo in foals. Keywords: Salmonella, Antibiotic resistance, NGP, alternatives"
GbP14	**A Novel Mechanism of Group B Streptococcal Resistance to Human Phospholipase A2** Nicholas R. Murner(1), Marieke M. Kuijk(3), Oscar J. Vazquez-Ciros(1), Mohammad M. Rahman(1), Tanmoy Mukherjee(1), Cameron W. Kenner(1), Kevin S. Mciver(5), Konstantin V Korotkov(2), Nina M. van Sorge(3,4), Natalia Korotkova(1,2) (1) University of Kentucky department of microbiology, immunology, and molecular genetics (2) University of Kentucky Department of Biochemistry (3) Department of Medical Microbiology and Infection Prevention, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands

	(4) Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam University Medical Center location AMC, Amsterdam, the Netherlands (5) Cell Biology and Molecular Genetics, Maryland Pathogen Research Institute, University of Maryland, College Park, Maryland, USA5 Group B Streptococcus (GBS) is a leading cause of severe infection in neonates. Due to immature immune systems, neonates rely heavily on cationic antimicrobial proteins secreted by innate immune cells to fend off bacterial infections. Of these proteins, the most effective at preventing GBS colonization is Human Group IIA Phospholipase A2 (HGIIa). HGIIa lyses bacteria by directly attacking the phospholipid membrane, generating toxic lysolipids from normal membrane phospholipids. Using transposon sequencing, we identified an operon in GBS that allows the bacteria to resist the action of HGIIa. The operon is comprised of three genes- degV, SGNH, ypmS. The degV gene makes a known FakB like protein that assist in the creation of new membrane phospholipids from free fatty acids. Using structural predictions, we predicted the activities of the last two proteins in the operon. The protein SGNH was modeled as a lipid hydrolase, and the final protein YpmS was predicted to be a lipid binding protein. We verified our original predictions by demonstrating SGNH cleaves lipids, and YpmS binds specific lipids. Together, we hypothesize that these three genes create a novel system for recycling lipids in GBS. The SGNH/YpmS proteins bind and cleave lipids in the membrane, allowing for DegV to assist in generating new, unaffected phospholipids. This lipid recycling pathway allows for the bacteria to resist the antimicrobial activity of HGIIa, by ameliorating the effects of the toxic lyso-phospholipids generated by the enzyme.
GbP15	**Mosquito Exosomal tetraspanin CD151 regulates ZIKA and DENV2 infection** Durga Neupane, Girish Neelakanta, Hameeda Sultana University of Tennessee Mosquito-borne flaviviruses such as Zika virus (ZIKV), and dengue virus (DENV) infections have a significant impact worldwide. These viruses cause asymptomatic or mild fever to hemorrhagic disease, multiple organ failure, microcephaly, and death. Apart from the geographical spread of their primary vectors contact with the human population, a major factor contributing to the global threat of flaviviruses is the lack of treatment strategies and effective vaccine(s). Recent studies have highlighted the importance of tetraspanins for the development of novel therapies. Despite their importance in numerous diseases, it is still unknown how tetraspanin proteins play roles in arthropod-borne flaviviral infections. In this study, we used two important human pathogens ZIKV and DENV2 (serotype 2) to study the role of mosquito tetraspanins combed from the Aedes aegypti genome. Among the seven tetraspanins selected, CD151 showed significant upregulation upon both ZIKV and DENV2 infection in mosquito cells and in EVs-derived from these cells. To further understand the role of CD151, we used RNAi-mediated silencing that significantly reduced the viral burden. Co-immunoprecipitation and immunofluorescence analyses also showed direct interaction and co-association of CD151 with ZIKV and DENV2 viral proteins. Furthermore, the inhibition of exosome release by GW4869 (inhibitor of exosome biogenesis) significantly reduced the viral burden and transmission via EVs. Overall, our study suggested that tetraspanin CD151 acts as a potential therapeutic to block ZIKV and DENV2 infections
GbP16	**Type 1 Conventional Dendritic Cells are inefficient at internalizing Listeria monocytogenes** Joshua Nowacki, Sarah E.F. D'Orazio University of Kentucky Department of Microbiology, Immunology, and Molecular Genetics Type 1 conventional dendritic cells (cDC1s) are BATF3-dependent, FLt3 ligand-induced migratory cells that present antigen to naive CD8+ T cells. During Listeria monocytogenes (Lm) infection, inflammation can also drive the differentiation of monocytes into CD11c+ cells that phenotypically share properties with both macrophages and dendritic cells (mDC). We previously showed that Lm could replicate exponentially inside mDCs generated from murine bone marrow cultured with GM-CSF. In this study, we show that cDC1s generated by culturing bone marrow with Flt3L are inefficient at phagocytosing live Lm. Based on the results of a gentamicin protection invasion assay, only 0.2% of the inoculum was internalized by 2 hours post infection. We also used flow cytometry to compare the internalization of GFP-expressing Lm by our cDC1 and mDC, and verified that the cDC1 had an unactivated phenotype prior to infection and 5.7 fold fewer cDC1 internalized Lm compared to mDC. The cDC1 did express low levels of E-cadherin, the receptor for Lm internalin A-mediated endocytosis, but infection of the cDC1 with mutant Lm lacking internalin A had no effect on bacterial internalization, suggesting that phagocytosis was the primary mechanism of bacterial uptake. cDC1 also inefficiently internalized GFP-expressing E. coli which indicate that this may be a generalized property of conventional dendritic. These results suggest that cDC1 may not present antigen to T cells in vivo primarily as a result of direct infection by Lm, but instead via antigen cross-presentation following

	internalization of cellular debris resulting from another cell type that more readily supports intracellular growth of Lm.
GbP17	**Architecture of Chemotaxis Chemoreceptor Arrays in Rhizobium leguminosarum bv. viciae** Elaine Nunan, Chris Tinkey, Gladys Alexandre University of Tennessee Knoxville The soil is a harsh environment with wide fluctuations in many factors such as temperature, pH, salt concentrations, and moisture. To survive in such an environment, motile bacteria can sense physicochemical conditions in their surroundings and move towards conditions that promote their survival and away from deleterious ones, using chemotaxis. During chemotaxis, bacteria use a large array of allosterically coupled chemoreceptors to sense and relay information to cytoplasmic chemotaxis signal transduction proteins (CheA, CheW, CheY) to alter direction of flagellar rotation. The chemotaxis genes in Rhizobium leguminosarum bv. viciae, a plant growth promoting bacteria, are organized as two distinct chemotaxis operons (che1 and che2), each consisting of the genes required to produce a functional chemotaxis system. Two additional chemotaxis genes encoding for CheW homologs are also found outside of the operons, which we refer to as orphan CheWs. Previous work showed that che1 is essential for chemotaxis toward pea plants and for nodulation while che2 appears to contribute to chemotaxis in a minor role. Here we aim to characterize the subcellular organization of chemoreceptors arrays in R. leguminosarum using fluorescence microscopy to visualize the subcellular localization of fluorescently labelled chemotaxis proteins in the parent and chemotaxis mutant derivatives background as well as by characterizing chemotaxis protein interactions using BacTH assays. Results indicate that CheA1 interacts with CheW1 and both orphan CheWs. Whereas CheA2 interacts with CheW2 and one of the orphan CheWs (CheW4). Data indicate that CheA1-YFP localizes at the cell poles, as expected. However, deletion of che1 or che2 operons or both has marginal effect on CheA1-YFP polar localization, suggesting that proteins outside of che1 and che2 contribute to the location of CheA1-YFP. We are currently testing the role of the orphan CheW homologs. Collectively ongoing and current data will provide a working model for the organization of chemotaxis proteins in R. leguminosarum that will provide molecular mechanisms for chemotaxis signal transduction in this species.
GbP18	**Prenylated Effectors PelC and PelE are Required for Biogenesis of the Legionella pneumophila-containing Vacuole** Cheon Jee Shin, Christopher T. D. Price, Yousef Abu Kwaik Department of Microbiology and Immunology, University of Louisville Background: Legionella pneumophila is an intracellular pathogen that establishes a replicative niche within host cells by subverting vesicular trafficking through its Type IV Secretion System (T4SS). L. pneumophila injects over 350 effector proteins into eukaryotic host cells that manipulate cellular processes. Many of these effectors mimic eukaryotic proteins by harboring domains and motifs, such as the prenylation motif (-CaaX). Previous studies identified 12 L. pneumophila genes encoding C-terminal -CaaX motif-containing proteins, with strain AA100/130b possessing seven, designated as prenylated effectors of Legionella (Pels). Ectopic expression of Pels revealed membrane localization, yet their subcellular localization and function during infection remain unknown. Methods: Subcellular localization of the Pels were determined by generating L. pneumophila strains that express 4HA-tagged Pels at the N-terminus and visualized under confocal microscopy. To assess if the Pel effectors were required for intracellular replication in host cells the pel genes were knocked out, and the resulting mutants were analyzed for replication within amoebae and human monocyte-derived macrophages (hMDMs). To determine the role of Pels in the trafficking of the Legionella-containing vacuole (LCV), localization of endoplasmic reticulum (ER) and lysosomal markers to the LCV was examined using confocal microscopy. Results: Pel proteins spatially localize to the LCV in a T4SS-dependent manner and was significantly reduced upon substitution of the conserved cysteine residue in the -CaaX motif, indicating the necessity of host-mediated prenylation. Deletion of pelC and pelE resulted in a significant reduction in intracellular replication in hMDMs and Acanthamoeba polyphaga, but not Vermamoeba vermiformis. The ΔpelC and ΔpelE mutants exhibited impaired remodeling of the LCV by the ER, and their vacuoles were trafficked through the endosomal-lysosomal degradation pathway. Conclusions: Pel effectors are prenylated proteins that localize to the LCV during infection. PelC and PelE contribute to intracellular replication and LCV biogenesis by promoting ER-derived vacuole formation and lysosomal evasion."
GbP19	**Mouse hepatitis virus infection mitigates experimental autoimmune encephalitis development** Kelly Walter(1), Divyasha Saxena(2), Stanley Perlman(3,4), Jian Zheng(1,2) (1) Department of Microbiology and Immunology, University of Louisville

	(2) Center for Predictive Medicine, University of Louisville (3) Department of Microbiology and Immunology, University of Iowa; (4) Pediatrics, University of Iowa Background: Viral infections have been found to affect the central nervous system (CNS), causing damaging neuroinflammation. The mechanism underlying viral infection-caused neuroinflammation is only vaguely understood. Methods: This mechanism is investigated by infecting mice with a sublethal dose of mouse hepatitis virus (MHV) i.c. and subsequently inducing experimental autoimmune encephalitis (EAE). Results: Viral infection was found to ameliorate myelin oligodendrocyte glycoprotein 35-55 (MOG35-55) peptide-induced EAE development and symptoms. The mitigation of EAE development in MHV infections was found to be mediated by regulatory T cells, Foxp3 CD8 T cells, that persist past infection. These regulatory T cells were found to suppress infiltration of inflammatory CD4 T cells into the CNS and inhibit local microglia functionality. More interestingly, i.n. SARS-CoV-2 infection was also found to suppress the subsequent EAE immunization, although no active virus replication could be identified in CNS. Conclusions: This suggests that multiple viruses can mediate EAE-specific neuroinflammation within the CNS, independent of infection method. Identifying the mechanisms contributing to the impact of prior infection on the development of neuroinflammation will allow for new potential therapeutic targets against both neuroinflammation damage and viral infections."
GbP20	**Glycerol phosphate modification on Streptococcus mutant cell wall plays a key role in pathogenicity of S. mutans.** Svetlana Zamakhaeva, Jeffrey Rush, Robert Danaher, Konstantin Korotkov, Natalia Korotkova, University of Kentucky Streptococcus mutans is a gram-positive pathogenic bacterium, causing biofilm-associated diseases such as caries (oral cavities) and infective endocarditis. Cell surface of S. mutans include serotype specific rhamno-polysaccharides (RPS) covalently attached to peptidoglycan layers. Depending on serotype – c, e, f or k, polyrhamnose backbone of RPS is decorated with -1,2-, ß-1,2-, -1,3-glucose (Glc) or -1,3-galactose correspondingly. Recently we discovered additional RPS modification – glycerol phosphate (GroP), that is attached to some of the Glc moieties of serotype c specific carbohydrates (SCC) by enzyme SccH. Here we showed that GroP is a universal modification for all serotype-specific carbohydrates in S. mutans and homologs of SccH are present in genomes of each serotype. To understand the role of GroP modification in pathogenicity of S. mutans, we tested biofilm formation of WT and GroP-less mutants in media with sucrose (to mimic oral pathogenicity rout). Using crystal violet assay, we identified that both WT and sccH mutants in serotypes c and e produced robust sucrose-dependent biofilms. In serotype k and f deletion of sccH enhanced the biofilm formation, while wild types produced less biofilm. Water-insoluble glucans produced by secreted glycosyltransferases GtfB and GtfC are the main component of extracellular sucrose-dependent biofilm matrix. Anthrone assay revealed that ΔsccH strains across all serotypes produced significantly less water-insoluble glucans during growth on sucrose compared to the WT. Decreased glucan production in ΔsccH mutant of serotype c correlated with decreased level of secreted GtfB and GtfC compared to WT as was shown by immunoblotting. Further, by infecting oral cavities with serotype c WT and ΔsccH in rat caries model we revealed there is no difference in colonization rate on 44 dpi between these two strains. But the WT caused more severe enamel damage. Therefore, GroP cell wall modification plays a key role in virulence of S. mutans."
PDP1	**UROLITHIN A TREATMENT REDUCED CLOSTRIDIOIDES DIFFICILE INDUCED COLITIS AND TOXIN PRODUCTION** Sweta Ghosh(1,2), Daniel Erickson(1), Michelle J Chua(1), James Collins(1,3,4), Venkatakrishna Rao Jala (1,2,4,5) (1) Department of Microbiology & Immunology, University of Louisville (2) UofL-Brown Cancer Center, Louisville, KY (3) Center for Predictive Medicine, University of Louisville (4) Center for Microbiomics, Inflammation and Pathogenicity, University of Louisville (5) Center for Integrative Environmental Health Sciences, University of Louisville Clostridioides difficile causes significant damage to the gut epithelium leading to increased permeability, severe diarrhea, and colitis. Toxins like TcdA and TcdB produced by C. difficile contribute to the epithelial damage and increase the severity of colitis. In this study, we explored the effects of microbial metabolite, Urolithin A (UroA) on C. difficile infection (CDI) and toxin production. Our studies revealed that UroA treatment improved the C. difficile -induced pathogenesis by improving overall clinical scores, protecting from

	a shortening of colons, and restoring tight junctional proteins in the preclinical model. Additionally, UroA treatment upregulated the expression of tight junctional proteins (TJPs) and mucin production in mouse intestines and in cultured intestinal organoids. Importantly, culture supernatants of C. difficile grown with UroA showed a significant reduction in rounding of Vero-2 cells compared to supernatants of C. difficile grown in the presence of Vehicle, suggesting that UroA have an impact on C. difficile toxin production. Further, RNA-seq analysis of C. difficile cultures grown in the presence of UroA suggested that UroA directly downregulates the expression of several genes located in the pathogenicity locus (PaLoc). These include tcdA, tcdB, tcdE, encoding a holin that mediates toxin release from C. difficile cells, and tcdR, which is an alternate sigma factor that directs transcription by recruiting RNA polymerase to the toxin gene promoters and its own promoter. Overall, our findings reveal a novel aspect of UroA activity, as it appears to act at both the bacterial and host levels to protect against CDI-induced colitis pathogenesis.
PDP2	**A Computational Framework to Evaluate Microbial Epoxide Hydrolase Expression in Healthy Individuals, Heavy Drinkers, and Patients with Alcohol-Associated Liver Disease** Hannah E Hanford, Jeffrey B Warner, Josiah E Hardesty, Rui Treves, Jingzhi Wang, Jane Frimodig, Dennis R Warner, Craig J McClain, Irina A Kirpich University of Louisville Background: Alcohol-associated liver disease (ALD) is a highly prevalent condition resulting from excessive alcohol consumption. Our previous studies suggested that elevated soluble epoxide hydrolase (EH) plays a pathogenic role in ALD. EHs are widely expressed by various mammalian cells/tissues, and also by the gut microbiota. The current study aims to test the hypothesis that microbial EH gene expression is increased in human ALD. Methodology: FASTQ files from fecal shotgun metagenomic sequencing for healthy controls (HC, n=12), heavy-drinking individuals (HD, n=23), and patients with alcohol-associated hepatitis (AH, n=59) were processed through a bioinformatic pipeline. The ShortBRED Quantify pipeline (with ~110,000 putative EH marker peptide sequences) was used to obtain EH gene counts (gene abundance), that were tabulated for each subject and averaged within each group. Significant differences in gene counts in HD and AH compared to HC were determined by Student's t-test. Results: 7,001 EH markers representing 1,419 proteins with putative EH activity were detected across all subjects. To increase the odds of identifying true EH proteins, we filtered for those containing 75% total markers per protein, resulting in 259 candidates. Among them, 128 EHs were shared across all three groups, 11 were unique to HC, 14 to HD, 38 to AH. Total EH gene abundance was significantly increased in HD and AH compared to HC. Among the top microbial species that express EH proteins, Alistipes, Clostridium, Limosilactobacillus, and other gut pathogens had increased relative abundance in HD and AH compared to HC. Conclusions: Individuals consuming alcohol had increased gut microbial EH expression, which may contribute to ALD pathogenesis and represent a novel feature of alcohol-associated gut dysbiosis. Future work will further investigate the mechanistic role of EHs and their metabolites in ALD to elucidate the role of microbial vs. host EHs for the development of novel therapeutic strategies.
PDP3	**Phagocytosis and Lysosomal Evasion by Amoebae Harboring Legionella in Macrophages** Christopher T. D. Price and Yousef Abu Kwaik, Department of Microbiology and Immunology, University of Louisville, KY Background: A major knowledge gap in innate immunity is the long-standing dogma that macrophages are unable to phagocytose eukaryotic amoebae due to their relatively large size. In addition to inflicting various infectious diseases, amoebae species graze on other microorganisms, but many pathogenic microbes, such as Chlamydia, Francisella, and Legionella have evolved to evade amoebae predation and replicate inside these cells. Results: Here we show that macrophages efficiently phagocytose non-pathogenic and pathogenic amoebae and amoebae harboring intracellular Legionella. Phagosomes containing amoebae trophozoites evade the endosomal-lysosomal degradation pathway and acquire endoplasmic reticulum proteins, while amoebae cysts are trafficked into degradative lysosomes. Infection of human macrophages or mice with amoebae harboring intracellular Legionella is followed by robust bacterial proliferation and a pro-inflammatory response. Conclusions: We conclude that amoebae are efficiently phagocytosed by macrophages where they evade the endosomal-lysosomal degradation pathway, and amoebae harboring pathogenic bacteria are a carrier vehicle and a Trojan Horse that is phagocytosed by macrophages leading to a productive bacterial infection.
PDP4	Mohamed Salah(1), Bibek Lamichhane(1), Khaled A. Shaaban(2), Larissa V. Ponomareva(2), Jon Thorson(2), and Yosra A. Helmy(1)

	(1) Maxwell H.Gluck Equine Research Center, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky (2) Center for Pharmaceutical Research and Innovation, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky Introduction: Rhodococcus equi is one of the significant equine pathogens responsible for purulent bronchopneumonia and mortality in foals less than 6 months of age. Foals can acquire infection by inhaling airborne dust particles contaminated with R. equi from the environment, particularly soil and manure. The infection is controlled using antibiotics such as rifampin and macrolides. However, rising antibiotic resistance has necessitated the development of alternative therapies. Small molecules (SMs) are low molecular weight compounds that can be targeted to specific bacterial cellular processes and can exhibit narrow to broad-spectrum activities. Objectives: Our study aims to screen and evaluate novel SMs with high efficacy against R. equi in vitro. Methods: We screened approximately 2,000 small molecules (SMs) to assess their impact on the growth of R equi. SMs that exhibited 100% growth inhibition were further evaluated to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), their effect on R. equi-preformed biofilms and on the intracellular survival of R. equi in murine macrophage cell lines. Results: Ten SMs exhibited strong activity against multidrug-resistant R. equi strains, with MIC and MBC values as low as 0.078 μM and 0.156 μM, respectively. Five out of 10 SMs completely inhibited biofilms of R. equi at 10 μM. All the selected SMs significantly reduced intracellular R. equi survival within macrophages ($p < 0.05$) at 5 μM. In the future we will focus on assessing the long-term stability and safety of these SMs. We will work on understanding how they modulate their action on phagocytic cells and evaluate their effect in inhibiting the colonization of R. equi in foals. Conclusion: Our findings suggest that these novel SMs could serve as potential alternatives to traditional antibiotics for controlling R. equi infections. Keywords: R. equi, antibiotic resistance, alternatives, small molecules"
PDP5	**PMAxx dye-assisted colorimetric dual DNAzyme-LAMP (PD-cDDLAMP) for rapid molecular phenotypic antimicrobial susceptibility testing of E. coli** Alaa H. Sewid(1), Joseph H. Ramos(1), Benti D. Gelalcha(2), Yuri Matsuoka(1), Oudessa Kerro Dego(3) and Shigetoshi Eda(1) (1) School of Natural Resources, The University of Tennessee (2) Department of Biomedical and Diagnostic Sciences College of Veterinary Medicine, The University of Tennessee (3) Departments of Animal Science, The University of Tennessee Institute of Agriculture Rapid antimicrobial susceptibility testing (AST) is crucial for combating antimicrobial resistance and guiding effective therapy. Conventional phenotypic AST methods are often time-consuming, requiring 18–72 hours for results, while genotypic approaches often depend on pre-existing knowledge of resistance markers. To address these gaps, we developed PMAxx dye-assisted colorimetric Dual DNAzyme-LAMP (PD-cDDLAMP), a rapid nucleic acid -based phenotypic AST method that differentiates antibiotic-susceptible “signal-off” and resistant bacteria “signal-on”. This approach leverages the viability selective binding of DNA-cross linker dye (PMAxx) to DNA from membrane-compromised cells, combined with a user-friendly colorimetric DNAzyme-LAMP readout system. PD-cDDLAMP achieved 91.67% sensitivity, 83.33% specificity, and 87.5% accuracy in detecting E.coli exposed to ampicillin and tetracycline, outperforming current methods by reducing antibiotic exposure time to just 30 minutes. The method was further validated in spiked milk samples, demonstrating its potential for on-site AST in dairy farming. PD-cDDLAMP’s simplicity, speed, and point-of-care compatibility make it a promising tool for rapid AST."
OP1	**n3 PUFA-enriched diet modulated gut microbiota in male and female ethanol-fed mice** Yasmeen Abdelfadil, Hannah Hanford, Rui Treves, Alice Rodgerson, Jo Goykhberg, Jingzhi Wang, Urelys Casiano-Esquilin, Dennis R. Warner, Irina A. Kirpich University of Louisville Introduction/Purpose: Alcohol consumption is often associated with gut microbiota dysbiosis, which plays a pathogenic role in alcohol-associated multiorgan pathology. Previous studies demonstrated that n3-PUFA dietary supplementation exert health benefits in humans and animal models. This study aimed to test the hypothesis that n3-PUFA dietary supplementation attenuates EtOH-induced alterations in gut microbiota. Methods: C57BL/6J WT 10-12 week old male and female mice were fed standard or modified (n3 PUFA-enriched) Lieber-deCarli diets for 5 weeks with or without EtOH (EtOH-fed or control mice, respectively).

	Shotgun metagenomic analysis was performed on freshly collected fecal samples. Raw FASTQ files were processed using a metagenomic bioinformatic pipeline, including Fast QC, Trimmomatic, Bowtie2, Megahit, Kaiju, and data were visualized in R software. Results: The most abundant microbial phyla across all groups included Bacillota, Bacteroidota, Thermodesulphobacteriota, Campylobacterota, Actinomycetota, Deferribacteriota ,Pseudomonodata, and Verrucomicrobiota. In female vs male control mice, the percentages of Bacteroidota and Verrucomicrobiota phyla were higher, while Bacillota and Campylobacteriota were lower. Thermodesulfobacteriota was represented to a greater extent in EtOH-fed male and female vs the control group. EtOH feeding decreased levels of Bacillota in male mice, which was prevented by the n3-PUFA diet. In females, EtOH resulted in an increase in Bacillota regardless of the diets. Phylum Bacteriodota was decreased, while Verrucomicrobiota was expanded in EtOH-fed male but not female mice with no effect of n3-PUFA supplementation. Conclusions: There were sex-specific differences in the gut microbiota between male and female mice. Chronic EtOH consumption induced alterations in the gut microbiota, which were modulated by a diet enriched in n3-PUFAs. Further studies will identify the effect and underlying mechanisms mediated by n3-PUFAs on overall gut health in the context of chronic alcohol consumption."
OP2	**Development of Chikungunya Virus Resistant to the RNA Mutagen β-d-N4-hydroxycytidine** Koji Barnaby, Deepak Singh, Brian Alejandro, Donghoon Chung University of Louisville The broad-spectrum RNA mutagen β-d-N4-hydroxycytidine (NHC) has been shown to have antiviral activity against many viruses including coronaviruses and alphaviruses through an accumulation of mutations seemingly randomly across the entire viral genome, yet the specific mechanism of action linking this increase in mutations to antiviral activity still remains poorly understood. In utilizing a robust alphavirus infection model of the positive-sense, single-stranded RNA virus Chikungunya (CHIKV), the mechanism of action of NHC can be elucidated. In serially passaging CHIKV 181/25 in the presence of set concentrations of NHC, there was a collapse in the viral populations at higher NHC concentrations suggesting the quality or quantity of the mutations led to this crash out, and there was an increase in viral titers after a sudden decrease suggesting resistant mutations. An additional serial passaging of CHIKV 181/25 in the presence of increasing NHC concentration as passages increase was conducted to determine if mutations in nsp4 conferred resistance to NHC. This serial passaging was done by a process deemed as “Cacheless Cloning” where in the nsp4 from each passaged virus was introduced into a clean CHIKV 181/25 backbone for the subsequent passage. These viral populations were sequenced to determine the mutational landscape of the non-structural proteins for each passage.
OP3	**The structure and function of TbTim50, a PAP enzyme, and an important virulence factor for Trypanosoma brucei infection** Hira Karim(1), Pankaj Sharma(2), Jamaine Davis(3), Tina M Iverson(2), Minu Chaudhuri(1) (1) Department of Microbiology, Immunology, and Physiology, School of Medicine, Meharry Medical College (2) Department of Pharmacology and Biochemistry, School of Medicine, Vanderbilt University, (3) Department of Biochemistry, Cancer Biology, Neuroscience, and Pharmacology, School of Medicine, Meharry Medical College Trypanosoma brucei is a parasitic protozoan and the causative agent for African trypanosomiasis, a fatal disease in humans and ruminants. T. brucei possesses a single reticular mitochondrion. The translocase of the mitochondrial inner membrane in T. brucei (TbTIM) imports most of the mitochondrial proteins from the cytosol and is structurally and functionally distinct from its counterparts in fungi and humans. TbTIM consists of a few conserved Tim subunits and several other trypanosome-specific proteins. We identified a Tim50 homologue in T. brucei (TbTim50). Unlike Tim50s in other eukaryotes, TbTim50 lacks a transmembrane domain within the N-terminal half but possesses a conserved C-terminal domain phosphatase motif with a perfect signature sequence, DXDX(T/V). Previously, we showed that the recombinant TbTim50 has phosphatidic acid phosphatase (PAP) activity, mutation of the Ds in the active site abolished this activity, and it binds specifically with PA. A global lipidomic analysis revealed a significant down regulation in the levels of triacyl- and diacylglycerol (TAG and DAG), as well as the levels of the phosphatidylcholine (PE) and phosphatidylethanolamine (PE) in TbTim50 knockdown vs control parasite, indicating that TbTim50 is indeed a PAP and plays role in membrane lipid biogenesis. Structural modeling of TbTim50 revealed that the C-terminal domain (200-423 residues), is organized around a Rosmann-like fold, but the N-terminal domain (1-199 residues), is an intrinsically disordered region (IDR). We hypothesize that this IDR is responsible for TbTim50 membrane association and regulation of its activity. We have purified the recombinant full length TbTim50, and the individual N- and C-terminal domains. Applying the structure-function approach, we will determine the experimental structure of this protein and investigate the role of intrinsic disorder domain in the mechanism of action of TbTim50, which is an important virulence factor for T. brucei infection.

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OP4	**Select point mutations in Zika virus confer resistance to MWAC-3475** Reece Tomlinson(1), Brian Alejandro(1, 2), Koji Barnaby(1), Eunjung Kim(1), Donghoon Chung(1) (1) University of Louisville, Department of Microbiology and Immunology (2) Eurofins Genomics" Background: Serial passaging of a virus in the presence of an antiviral compound can be utilized to select for potentially drug-resistant mutants. Sequencing of the virus can identify the mutations present before further investigating if the mutations confer true resistance via reverse genetics. After serial passaging of Zika virus in the presence of a compound exhibiting antiviral properties (MWAC-3475), four mutations in the NS4B protein emerged: V241L, A245S, A245T, and V248G. Following identification of the mutations, a reverse genetics approach was initiated to investigate the potential of drug-resistance by introducing the appropriate mutation in an otherwise clean viral genome with a reporter gene (Renilla luciferase). Methods: The mutation of interest was introduced utilizing whole plasmid mutagenesis. Following identification of a successful viral genome construct, viral RNA was transcribed and then chemically transfected into Vero76 cells. The initial transfection was collected on day post-infection (DPI) 7 while the virus amplification was collected on DPI 11. Mutant viruses were then screened against antiviral compounds under the same conditions as the wild-type virus utilizing luminescence-based assays (Renilla luciferase). Results: Three mutants were successfully recovered (V241L, A245T, and V248G). When compared to the wild-type EC50 for the original hit compound, there was a significant increase in the EC50s for mutant viruses. This trend was also seen for a compound analogous in structure to MWAC-3475. When compared to control compounds for Zika virus (NITD-008 and NHC), the wild-type virus and mutants viruses showed similar EC50s. Conclusions: The NS4B mutations discovered from serial passaging of Zika virus in the presence of MWAC-3475 appear to confer resistance to the compound (and analogous compounds) as shown by the increase in EC50s for the mutant viruses when compared to the wild-type virus. Further testing could be done to better understand the mechanism of action of the select antiviral compound.