ICGP

8th Meeting + October 9-12, 2022

Omaha, NE

INTERNATIONAL CONFERENCE ON GRAM-POSITIVE PATHOGENS

8th Meeting + October 9-12 2022 + Omaha, NE

WELCOME to the International Conference on Gram-Positive Pathogens (ICG^{+}P)! We are very pleased you have travelled to Omaha to join us and we hope that you have a relaxing, yet intellectually stimulating meeting. Infections caused by gram-positive pathogens, including *Staphylococcus aureus, Streptococcus pyogenes, Clostridium difficile,* and *Enterococcus faecium,* among others, are a burden on our society causing significant morbidity and mortality. This conference seeks to better understand these bacteria through fostering interactions between investigators studying multiple aspects of gram-positive pathogenesis, biology, and host defense. Another important aspect of the ICG^{+}P is the active support of pre- and post-doctoral trainees; most oral presentations are awarded to trainees or junior faculty. Ultimately, the goal of this conference is to broaden our understanding of gram-positive pathogenesis and biology through the generation of new collaborations and to gain new insights through the study of similar systems in these related pathogens.

Finally, we are very excited to have the following four keynote presentations:

Sunday, October 9th
7:15 p.m—8:15 p.m.
Jose Lemos, PhD, Associate Professor, University of Florida College of Dentistry. Gainesville, FL, USA
'Therapeutic potential of a novel P-type ATPase that protects *Streptococcus mutans* from zinc intoxication.'

Monday, October 10th
8:30 a.m.—9:30 a.m.
Shonna McBride, PhD, Associate Professor, Emory University School of Medicine. Atlanta, GA, USA
'Discovery of a mechanism linking sporulation, toxin, and motility in *Clostridioides difficile*.'

Tuesday, October 11th
8:30 a.m.—9:30 a.m.
Andreas Peschel, PhD, Professor, University of Tübingen. Tübingen, Germany
'How staphylococci balance commensal vs. pathogen lifestyles.'

Wednesday, October 12th
8:30 a.m.—9:30 a.m.
Wilmara Salgado Pabón, PhD, Assistant Professor, University of Wisconsin-Madison. Madison, WI, USA
'Anti-angiogenic effects of *Staphylococcus aureus* superantigens: targeting vascular regeneration to promote disease.'

SPONSORS

ICG+P greatly appreciates the generous support the following contributors:

ICG+P CONFERENCE SCHEDULE

Sunday, October 9th

5:00 p.m.–6:00 p.m. **Conference Check-in**
Conf. Registration Desk
Embassy Suites Hotel

6:00 p.m.–7:00 p.m. **Buffet Dinner**
Big Blue Ballroom
Embassy Suites Hotel

7:00 p.m.–7:15 p.m. **Opening Remarks**

Co-Chairs: Francis Alonzo, PhD &
Rita Tamayo, PhD

7:15 p.m.–8:15 p.m. **KEYNOTE SPEAKER 1**
Big Blue Ballroom

'Therapeutic potential of a novel P-type ATPase that protects *Streptococcus mutans* from zinc intoxication.'

Jose Lemos, PhD, Associate Professor, University of

Monday, October 10th

8:30 a.m.–9:30 a.m. **KEYNOTE SPEAKER 2**
Big Blue Ballroom

'Discovery of a mechanism linking sporulation, toxin, and motility in *Clostridioides difficile*.'

Shonna McBride, PhD, Associate Professor, Emory University School of Medicine.

Session 1: Antibiotic Resistance
Moderator: Ivan Acosta

9:30 a.m.–9:50 a.m.
Carolyn B. Ibberson, University of Oklahoma
'Precise spatial structure impacts antimicrobial susceptibility of *S. aureus* in polymicrobial wound infections.'

9:50 a.m.–10:10 a.m.
Anthony G. Pannullo, University of Iowa
'The *hexSDF* Operon Is Required for Daptomycin Resistance and Production of a Novel Glycolipid in *Clostridioides difficile*.'

10:10 a.m.–10:30 a.m.
Josué Flores Kim, UMass Chan Medical School
'Tailoring of surface polymers prevents antibiotic-induced bacteriolysis and directs cell elongation in *Streptococcus pneumoniae.'*

10:30 a.m.–10:50 a.m.
Danica Galonić Fujimori, University of California
'Revisiting mode of action of and resistance to oxazolidinone antibiotics.'

10:50 a.m.–11:10 a.m. **Break**
Courtyard

Session 2: Growth & Metabolism
Moderator: Karine Dufresne

11:10 a.m.–11:30 a.m.
Lynn Hancock, University of Kansas
'Characterization of N-linked glycoprotein utilization in *Enterococcus faecalis.'*

11:30 a.m.–11:50 a.m.
Ryan Singh, University of Nebraska Medical Center
'Staphylococcal nitric oxide synthase mitigates flavohemoglobin-mediated superoxide production to enhance fitness.'

11:50 a.m.–12:10 p.m.
Tania Wong, Columbia University
'Airway immunometabolites impact staphylococcal central metabolism and virulence via post-translational modification of targets.'

12:10 p.m.–12:30 p.m.
Diandra M. Vaval Taylor, University of Illinois at Chicago
'Two roles, one pheromone: a *Listeria mono-cytogenes* peptide pheromone that contributes to both vacuole escape and viability.'

12:30 p.m.–1:30p.m. **Lunch break**
Outside Big Blue Ballroom

1:30 p.m.–3:00 p.m. **Poster Session A**
Elkhorn Ballroom

3:00 p.m.–3:20 p.m. **Networking Break**
Courtyard

3:20 p.m.–3:50 p.m. **NIH UPDATE**
Big Blue Ballroom
Clayton Huntley – National Institutes of Health

ICG+P CONFERENCE SCHEDULE

Session 3: Regulation I
Moderator: Sasmita Panda

3:50 p.m.–4:10 p.m.
Anchal Mehra, University of North Carolina at Chapel Hill
'Establishing the regulons of CmrR and CmrT, *Clostridioides difficile* response regulators involved in motility and virulence.'

4:10 p.m.–4:30 p.m.
Karl Thompson, Howard University
'A novel SigS induced regulatory protein pair in *S. aureus*.'

4:30 p.m.–4:50 p.m.
Andrei P. Pomerantsev, NIH/NIAID
'AtxA binding is required for activation of toxin and small RNA promoters in *Bacillus anthracis*.'

4:50 p.m.–5:10 p.m.
M. Lauren Donnelly, Tufts University School of Medicine
'Development of a dual fluorescent reporter system in *Clostridioides difficile* reveals a division of labor between virulence and transmission gene expression.'

5:10 p.m.–5:30 p.m.
Ronan Carroll, Ohio University
'The small RNA Teg41 is a pleiotropic regulator of virulence in *Staphylococcus aureus*.'

8:30 a.m.–9:30 a.m. **KEYNOTE SPEAKER 3**
Big Blue Ballroom

'How staphylococci balance commensal vs. pathogen lifestyles.'

Andreas Peschel, PhD, Professor, University of Tübingen; Head of the Infection Biology Department within the Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Germany.

Tuesday, October 11th

Session 4: Host-Pathogen
Moderator: Zongsen Zou

9:30 a.m.–9:50 a.m.
Valeria M. Reyes Ruiz, Vanderbilt University Medical Center
'Defining *S. aureus* regulatory systems that respond to environmental stresses at the host-pathogen interface.'

9:50 a.m.–10:10 a.m.
Flavia Costa, University of Colorado
'Development of skin-like media for studying *Staphylococcus aureus* human skin colonization determinants.'

10:10 a.m.–10:30 a.m.
Stephen Tuffs, University of Western Ontario
'Staphylococcal superantigens can promote persistence during chronic orthopedic infection.'

10:30 a.m.–10:50 a.m. **Break**
Courtyard

Session 5: Clinical & Applied Microbiology
Moderator: Ronald Flannagan

10:50 a.m.–11:10 a.m.
Kaitlin Dailey, University of Nebraska Medical Center
'Development of CRISPR-modified *Clostridium novyi*-NT as an Intravenous, Multisite Pancreatic Cancer Therapeutic.'

11:10 a.m.–11:30 a.m.
Laura Cook, Binghamton University
'Genotypic and phenotypic characterization of *Streptococcus pyogenes* isolated from patients with acute pharyngitis and carriers.'

11:30 a.m.–11:50 a.m.
Danai Etter, University of Zurich
'*Bacillus thuringiensis* and *B. cytotoxicus* – the taxonomic collapse of the *B. cereus* group and the rise of the underdogs.'

11:50 a.m.–12:10 p.m.
Paul Planet, Children's Hospital of Philadelphia
'A phylogenomic approach to early life *Staphylococcus aureus* colonization.'

12:10 p.m.–1:10 p.m. **Lunch**
Outside Big Blue Ballroom

1:10 p.m.–2:40 p.m. **Poster Session B**
Elkhorn Ballroom

2:40 p.m.–3:20 p.m. **Networking Break**
Courtyard

Session 6: Host-Pathogen Interactions II
Moderator: Christopher Radka

3:20 p.m.–3:40 p.m.
Emilee Muster, University of South Florida
'Exploring *Staphylococcus aureus* Host-Pathogen Interaction Using Proteomic Technologies.

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3:40 p.m.–4:00 p.m.
Andrew Herr, Cincinnati Childrens Hospital Medical Center
'Structural insights into corneocyte adhesion by Staphylococci.'

4:00 p.m.–4:20 p.m.
Keenan Lacey, NYU Grossman School of Medicine
'Secreted mammalian DNases protect against *S. aureus* infection by digesting biofilms.'

4:20 p.m.–4:40 p.m.
Nathan Archer, Johns Hopkins University
'Neutrophil-intrinsic TNF receptor signaling orchestrates host defense against *Staphylococcus aureus*.'

4:40 p.m.–5:00 p.m.
Haider Manzer, University of Colorado Anschutz Medical Campus
'Epigenetic Regulation though DNA Methylation Promotes Group B Streptococcal Vaginal Colonization.'

6:00 p.m.–9:00 p.m. **Conference Banquet**
The Durham Museum

Wednesday, October 12th

8:30 a.m.–9:30 a.m. **KEYNOTE SPEAKER 4**
Big Blue Ballroom

'Anti-angiogenic effects of *Staphylococcus aureus* superantigens: targeting vascular regeneration to promote disease.'

Wilmara Salgado Pabón, PhD, Assistant Professor, Pathobiological Sciences, University of Wisconsin-Madison.

Session 7: Stress & Pathogenesis
Moderator: Chunyi Zhou

9:30 a.m.–9:50 a.m.
Kimberly Gutierrez, University of Wisconsin Madison
'Listeria monocytogenes YvcJ and GlmR contribute to muropeptide synthesis to facilitate cell wall stress response.'

9:50 a.m.–10:10 a.m.
Yi Xu, Texas A&M Health Science Center
'A novel pathogenic mechanism for *Streptococcus gallolyticus* – type VII secretion, host receptor and role in colorectal cancer.'

10:10 a.m.–10:30 a.m.
Daniel Paredes-Sabja, Texas A&M University
'Impact of *Clostridioides difficile* spore-persistence in the intestinal mucosa to disease recurrence.'

10:30 a.m.–10:50 a.m. **Break**
Courtyard

Session 8: Regulation II
Moderator: Luke Joyce

10:50 a.m.–11:10 a.m.
Madison Stock, Loyola University of Chicago
'Characterizing MroQ-dependent Regulation of *Staphylococcus aureus* Quorum Sensing.'

11:10 a.m.–11:30 a.m.
Lin Zeng, University of Florida
'Glucose PTS modulates pyruvate metabolism, bacterial fitness, and microbial ecology in oral biofilm.'

11:30 a.m.–11:50 a.m.
Wenqi Yu, University of South Florida
'Spatial regulation of surface proteins in *Staphylococcus aureus*.'

11:50 a.m.–12:00 p.m. **Closing remarks**
Big Blue Ballroom

Co-Chairs: Francis Alonzo, PhD &
Rita Tamayo, PhD

12:00 p.m.–1:00 p.m. **Lunch Break**
Outside Big Blue Ballroom

ICG+P ORAL ABSTRACTS

SESSIONS AND PRESENTERS

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Danica Fujimori

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ORAL ABSTRACTS

Monday, October 10th

Session 1: Antibiotic Resistance

9:30 a.m.—9:50 a.m.

Precise spatial structure impacts antimicrobial susceptibility of *S. aureus* in polymicrobial wound infections

Carolyn B. Ibberson[a,b,*], Juan P. Barraza[a,*], Avery L. Holmes[b], Pengbo Cao[a], and Marvin Whiteley[a,*]

[a] School of Biological Sciences and Center for Microbial Dynamics and Infection, Georgia Institute of Technology; Emory-Children's Cystic Fibrosis Center, Atlanta, GA 30310
[b] Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019
[*]These authors contributed equally to this work.

A hallmark of microbial ecology is how interactions between members of a community shape community function. This includes microbial communities in human infections, such as chronic wounds, where interactions can result in more severe disease. *Staphylococcus aureus* is the most common organism isolated from chronic wound infections and has been shown to have both cooperative and competitive interactions with *Pseudomonas aeruginosa*. Still, despite considerable study, most interactions between these microbes have primarily been characterized using *in vitro* well-mixed systems, which do not recapitulate the infection environment. Here, we characterized interactions between *S. aureus* and *P. aeruginosa* in chronic murine wounds, focusing on the role that both macro- and micro-scale spatial structure plays in disease. We discovered that *S. aureus* and *P. aeruginosa* co-exist at high cell densities in murine wounds. High resolution imaging revealed that these microbes establish a patchy distribution within the wounds, only occupying 5 -25% of the wound volume. Using a quantitative framework, we identified a precise spatial structure at both the macro (mm)- and micro (μm)-scales, which was largely mediated by *P. aeruginosa* production of the antimicrobial 2-heptyl-4-hydroxyquinoline N-oxide, while the antimicrobial pyocyanin had no impact. Finally, we discovered that this precise spatial structure enhances *S. aureus* tolerance to aminoglycoside antibiotics but not vancomycin. Our results provide new, mechanistic insights into the biogeography of *S. aureus-P. aeruginosa* co-infected wounds and implicate spatial structure as a key determinant of antimicrobial tolerance in wound infections.

9:50 a.m.—10:10 a.m.

The *hexSDF* Operon Is Required for Daptomycin Resistance and Production of a Novel Glycolipid in *Clostridioides difficile*

Anthony G. Pannullo[1], Ziqiang Guan[2], Howard Goldfine[3], Craig D. Ellermeier[1]

1-University of Iowa, Iowa City, IA, USA
2-Duke University, Durham, NC, USA
3-University of Pennsylvania, Philadelphia, PA, USA

Clostridioides difficile is a Gram-positive opportunistic pathogen that results in 250,000 infections, 12,000 deaths, and $1 billion in medical costs in the US each year. There has been recent interest in using a daptomycin analog, Surotomycin, to treat *C. difficile* infections. Daptomycin interacts with both phosphatidylglycerol and Lipid II to disrupt the membrane and halt peptidoglycan synthesis. *C. difficile* has an unusual lipid membrane composition as it has no phosphatidylserine or phosphatidylethanolamine, and ~50% of its membrane is composed of glycolipids, including the unique *C. difficile* lipid aminohexosyl-hexosyldiradylglycerol (HNHDRG). We identified a two-component system (TCS) HexRK that is required for *C. difficile* resistance to daptomycin. Using RNAseq we found that HexRK regulates a three gene operon of unknown function *hexSDF*. Based on bioinformatic predictions, *hexS* encodes a monogalactosyldiacylglycerol synthase, *hexD* encodes a polysaccharide deacetylase, and *hexF* encodes an MprF-like flippase. We find that deletion of *hexRK* leads to a 4-fold decrease in daptomycin MIC, and that deletion of *hexSDF* leads to an 8-16-fold decrease in daptomycin MIC. The Δ*hexSDF* mutant is also 4-fold less resistant to bacitracin but no other cell wall active antibiotics. Our data indicate that in the absence of HexSDF the phospholipid membrane composition is altered. In WT *C. difficile* the unique glycolipid, HNHDRG makes up ~17% of the lipids in the membrane. However, in a Δ*hexSDF* mutant, HNHDRG is completely absent. While it is unclear how HNHDRG contributes daptomycin resistance, the requirement for bacitracin resistance suggests it has a general role in cell membrane biogenesis.

10:10 a.m.—10:30 a.m.

Tailoring of surface polymers prevents antibiotic-induced bacteriolysis and directs cell elongation in *Streptococcus pneumoniae*

Josué Flores Kim[1,2#], Genevieve Dobihal[2,3], Thomas Bernhardt[2,4#], and David Rudner[2#]

UMass Chan Medical School, Worcester, MA, USA
Harvard Medical School, Boston, MA, USA
Columbia University Medical Center, NY, NY, USA
Howard Hughes Medical Institute, Boston, MA, USA
#Contibuting authors

Penicillin and related antibiotics disrupt cell wall synthesis to induce bacteriolysis. Lysis in response to these drugs requires the activity of cell wall hydrolases called autolysins, but how penicillins misactivate these deadly enzymes has long remained unclear. We show that alterations in surface polymers called teichoic acids (TAs) play a key role in penicillin-induced lysis of the Gram-positive pathogen *Streptococcus pneumoniae* (*Sp*). We find that during exponential growth, *Sp* cells primarily produce lipid-anchored TAs called lipoteichoic acids (LTAs) that bind and sequester the major autolysin LytA. However, penicillin-treatment or prolonged stationary phase growth triggers the degradation of a key LTA synthase, causing a switch to the production of wall-anchored TAs (WTAs). This change allows LytA to associate with and degrade its cell wall substrate, thus promoting osmotic lysis. Additionally, we identify WhyD as a new factor that controls the level of WTAs in *Sp* cells to prevent LytA misactivation and lysis during exponential growth. We show that WhyD is a WTA hydrolase that restricts the WTA content of the wall to areas adjacent to active peptidoglycan (PG) synthesis. Our results support a model in which the WTA tailoring activity of WhyD during exponential growth directs PG remodeling activity required for proper cell elongation in addition to preventing autolysis by LytA. Similar changes in surface polymer assembly may underlie the mechanism of antibiotic- and/or growth phase-induced lysis for other important Gram-positive pathogens.

10:30 a.m.—10:50 a.m.

Revisiting mode of action of and resistance to oxazolidinone antibiotics

Danica Galonić Fujimori

Department of Cellular and Molecular Pharmacology, Department of Pharmaceutical Chemistry, Quantitative Biosciences Institute, University of California - San Francisco, San Francisco, CA, USA

Linezolid, an oxazolidinone class antibiotic used for the treatment of Gram-positive infections, inhibits translation by binding to the peptidyl transferase center of the bacterial ribosome. Previous work has shown that, despite binding to the A site of the bacterial ribosome, this antibiotic does not inhibit formation of every peptide bond, but rather acts in a nascent peptide sequence-specific manner by stalling ribosomes when an alanine is located at the penultimate position of the nascent peptide. To understand its mode of action, we investigated structural basis for nascent

peptide-specific stalling by linezolid using cryoEM. Our work revealed that linezolid's binding site within the ribosome is formed in part by the nascent peptide. Specifically, the alanine side chain fits within a small hydrophobic crevice created by oxazolidinone, resulting in improved binding of the antibiotic, and facilitating competition with incoming aminoacyl tRNAs. While the stabilizing interaction between the antibiotic and the penultimate residue of the nascent peptide cannot be achieved with Gly, steric clash prevents simultaneous accommodation of linezolid and amino acids larger than Ala, rationalizing context-specificity. We further demonstrate that the second-generation oxazolidinone radezolid also induces stalling with alanine at the penultimate position. Modification of the ribosome by the antibiotic resistance enzyme Cfr disrupts stalling due to the repositioning of the modified nucleotide. Our findings provide mechanistic understanding of the sequence-specific of mode of action of oxazolidinones, and suggest strategies for modifying this class of antibiotics to overcome resistance due to ribosome modification.

Session 2: Growth & Metabolism

11:10 a.m.—11:30 a.m.

Characterization of N-linked glycoprotein utilization in *Enterococcus faecalis*

Zakria H. Abdullahi[1], Erica C. Keffeler[1], Mary E. Galloway[2] and **Lynn E. Hancock[1]**

[1] Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045
[2] Department of Biology, University of Central Arkansas, Conway, AR 72035

Enterococcus faecalis is a commensal bacterium of most mammalian gastrointestinal tracts including humans where it is capable of obtaining nutrients from its host. We have been interested in understanding how *E. faecalis* adapts its carbon source utilization pathways in the host environment, given that glucose availability is commonly restricted in mammals. We have previously shown that the principal pathway for glucose uptake involves the Mpt PTS system (MptABCD). Expression of the *mpt* genes is dependent on the RpoN sigma factor (σ54) and the bacterial enhancer binding protein, MptR. We examined the transcriptional response of *E. faecalis* in the absence of *rpoN* and found that the most abundantly expressed transcript (*ef2223-21*) was an operon encoding a putative sugar ABC transporter. Immediately downstream of transporter encoded genes resides an additional set of genes predicted to encode a tri-component regulatory system designated *yesLMN* that likely senses the environmental availability of the sugar substrate for the EF2223-21 ABC transporter. We show that the response regulator, YesN positively regulates *ef2223-21* expression. We found that the *ef2223-21* operon shared close homology with the NgtS-P1-P2 ABC transporter system found in *Streptococcus pneumoniae* which has been characterized to import high mannose N-linked glycans. We confirmed that the EF2223-21 transporter is responsible for utilization of N-linked glycans of the high mannose form using RNase B as the sole carbon source. We also show that three additional alpha-mannosidases are required for maximal growth on the RNase B substrate, highlighting the key components of N- linked glycan utilization *in E. faecalis*.

11:30 a.m.—11:50 a.m.

Staphylococcal nitric oxide synthase mitigates flavohemoglobin-mediated superoxide production to enhance fitness

Ryan M. Singh[1], Sujata S. Chaudhari[1], Elizabeth H. Hutfless[1], Cortney E. Heim[1], Dhananjay Shinde[1], Abdulelah A. Alqarzaee[1], Sasmita Panda[1], Margaret Sladek[1], Vineet Kumar[1], Matthew C. Zimmerman[2], Paul D. Fey[1], Tammy Kielian[1], and Vinai C. Thomas[1]*

[1]Center for Staphylococcal Research, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68198-5900, United States. [2] Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska 68198-5900, USA.

Nitric oxide (NO) is an important innate immune effector molecule that targets bacterial pathogens. Most coagulase-negative staphylococci, including the human pathogen *Staphylococcus epidermidis*, are unable to maintain redox homeostasis under nitrosative stress resulting in poor growth. The growth of these organisms is restored only upon detoxification of NO by the flavohemoglobin, Hmp. Surprisingly, *S. epidermidis* not only generates endogenous NO from a genetically encoded nitric oxide synthase (seNOS) but also depends on its activity for optimal growth. Here, we demonstrate that the growth deficit of the *S. epidermidis nos* mutant results from Hmp-mediated toxicity. Even though *hmp* is crucial for the survival of *S. epidermidis* in vivo, the heme prosthetic group within Hmp generates a significant amount of superoxide ($O_2^{\bullet-}$) and adversely impacts *S. epidermidis* growth. Nitrite derived from seNOS activity regulates *hmp* transcription and limits its toxicity. These findings are conserved in *Staphylococcus aureus* and highlight a previously unrecognized and fundamental mechanism by which the NOS-Hmp axis promotes staphylococcal fitness.

11:50 a.m.—12:10 p.m.

Airway immunometabolites impact staphylococcal central metabolism and virulence via post-translational modification of targets

Tania Wong[1], Yanling Zhang[2], Zihua Liu[2], Dario Fucich[1], Ian R. Monk[3], Shivang S. Shah[1], Shwetha H. Sridhar[4], Sebastian Riquelme[1], Robert Sebra[4], Benjamin P. Howden[3], Chu Wang[2] and Alice Prince[1].

[1]Department of Pediatrics, Columbia University, New York, NY 10032, USA [2]Synthetic and Functional Biomolecules Center, Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China [3]Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC 3000, Australia
[4]Department of Genetics and Genomic Sciences, Mt. Sinai Icahn School of Medicine, New York, NY 10029, USA

S. aureus is a major cause of healthcare-associated infections, which are difficult to eradicate, including pneumonia. *S. aureus* produces an arsenal of virulence factors to establish infection. The Type VII Secretion System (T7SS) contributes to acute virulence *in vivo* albeit with variation across strains. Interestingly, T7SS genes are highly upregulated during long term persistence as is the case in Cystic Fibrosis (CF). Given that the CF airway is replete with metabolites generated by immune cells, we hypothesized that immunometabolites induce bacterial adaptation to the airways by upregulating T7SS gene expression. Using chemoproteomic profiling, we show that the mitochondrial metabolites, itaconate and fumarate, which accumulate in the airway during infection, post-translationally modify staphylococcal metabolic enzymes and transcriptional regulators. Accordingly, this was accompanied by global transcriptional changes, notably an increase in the expression of genes involved in the T7SS. We compared the airway bacterial burden in WT BL/6 mice and *Ptenl*$^{-/-}$ mice, which were previously shown to mimic the CF airway with increased inflammation and oxidative stress. The T7SS-deficient mutant (Δ*essC*) was attenuated in the *Ptenl*$^{-/-}$ but not the WT BL/6 mice, suggesting that the T7SS is crucial for bacterial adaptation to the airway. The T7SS locus of *S. aureus* has been shown to display genetic and transcriptional heterogeneity, which may explain the variable contribution of the T7SS in virulence across strains. Here,

we show a role for the T7SS in bacterial adaptation to the host immunometabolic milieu for survival, rather than for initial colonization.

12:10 p.m.—12:30 p.m.
Two roles, one pheromone: a *Listeria monocytogenes* peptide pheromone that contributes to both vacuole escape and viability

Diandra M. Vaval Taylor[1]*, Bobbi Xayarath[1], and Nancy E. Freitag[1]

[1]University of Illinois at Chicago, Department of Pharmaceutical Sciences, Chicago, IL; * dvaval2@uic.edu

The bacterium, *Listeria monocytogenes (Lm)*, transitions from an environmental bacterium to an intracellular pathogen following entry into mammalian cells, where infections can lead to serious invasive disease and even death. It has been previously shown that pPplA, a small peptide secreted by *Lm*, contributes to bacterial virulence by enhancing escape of *Lm* from the vacuoles of non-professional phagocytic cells. Peptide pheromones are generally associated with the coordination of multicellular activities including biofilm formation, however pPplA is required within the cell vacuole without bacterial neighbors. Evidence suggests that the secretion and re-uptake of the pPplA pheromone within the vacuole signals a confined space to *Lm* and leads to specific changes in bacterial gene expression that promote vacuole disruption. As genetic mutations were constructed to facilitate receptor identification, it became apparent that bacteria were negatively affected by the complete loss of pPplA. Transduction of a complete deletion of *pplA* into a WT *Lm* strain resulted in low recovery of transductants that only appeared after several days of incubation. Whole genome sequencing of independent transductants revealed mutations in genes encoding enzymes associated with RNA metabolism; subsequent RNA transcript analysis has identified several pheromone-regulated gene products that could potentially negatively impact *Lm* fitness. Overall these results strongly suggest that second site suppressor mutations are required to restore full viability to *Lm* mutants lacking the pPplA pheromone. It is surprising and novel that a secreted pheromone contributes to bacterial viability in broth culture, suggesting that these peptide signals play a critical role in bacterial survival.

Session 3: Regulation I

3:50 p.m.—4:10 p.m.
Establishing the regulons of CmrR and CmrT, *Clostridioides difficile* response regulators involved in motility and virulence

Anchal Mehra, Elizabeth M. Garrett, PhD, and Rita Tamayo, PhD

University of North Carolina at Chapel Hill (Chapel Hill, NC, U.S.A.)

Clostridioides difficile is an urgent public health threat, causing symptoms ranging from mild diarrhea to potentially fatal pseudomembranous colitis in ~500,000 people annually in the U.S. Many bacteria can overcome external stressors via phase variation, a mechanism for introduction of phenotypic diversity within a population. In *C. difficile*, phase variation occurs through site-specific recombination in which a DNA recombinase mediates inversion of an intergenic DNA sequence containing a regulatory feature, here termed a 'switch'. Switch inversion modulates expression of the adjacent gene(s) in a reversible, ON/OFF manner. The *cmr* switch lies upstream of the *cmrRST* operon, which encodes the putative histidine kinase CmrS and response regulators CmrR and CmrT. Inversion of the *cmr* switch leads to phase variation of several phenotypes including cell and colony morphology, cell chaining, surface migration, swimming motility, biofilm formation, and virulence. We hypothesize that one or more of the genes regulated by CmrR or CmrT confer the observed phase variable phenotypes. RNA-Seq revealed fewer than 20 genes that are differentially expressed ($Log_2 > 2$, $p < 0.05$) by one or both response regulators. Gene annotations show diverse predicted functions including cell wall proteins, riboflavin biosynthesis, osmoprotection, and hypothetical proteins, most of which are not well-studied. Using gene deletions and overexpression, we are studying the roles of these genes in *cmr*--associated phenotypes and have linked some of these genes to surface migration and biofilm formation. Ultimately, we aim to understand the importance of *cmr* switch phase variation in *C. difficile* virulence.

4:10 p.m.—4:30 p.m.
A novel SigS induced regulatory protein pair in *S. aureus*

Amer Al Ali[1,4], Jamilah Alsulami[1], Joseph I Aubee[1,5], Ayotimofe Idowu[2], Brooke R. Tomlinson[3], Emily A. Felton[3], Jessica K. Jackson[3], Lindsey Shaw[3], and **Karl M Thompson**[1]

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Staphylococcus aureus is formidable human pathogen capable of causing skin and soft tissue infections (SSTIs) as well as septicemia. In 2017, there were approximately 120,000 cases of *S. aureus* induced sepsis resulting in 20,000 fatalities. The ability of *S. aureus* to cause disease comes down to its ability to rapidly adapt to environmental stressors and the host immune response. This is done through a plethora of well-known virulence genes and virulence regulators. SigS is the sole extracytoplasmic function (ECF) function sigma factor in *S. aureus*. SigS promotes the ability of *S. aureus* to survive various stressors, such as exposure to chemicals, mutagens, and extreme temperatures. In addition, SigS is necessary for virulence and immune evasion. The mechanism whereby SigS promotes these adaptive and virulence phenotypes is unknown. We set out to characterize the SigS regulon by analyzing the *S. aureus* transcriptome following transient over-expression of SigS. We identified a 500-nucleotide transcript encoding two tandemly encoded proteins with Domains of Unknown Function (DUFs). We subsequently renamed these two genes *sroAB* (Sigma S regulated *orfA* and *orfB*). Over-expression of SroA, but not SroB or SroAB together, stimulates the accumulation of SigS mRNA. Using a bacterial two hybrid approach, we demonstrated that a direct interaction occurs between SroA and SroB. We postulate that this interaction controls SroA activity and indirectly influences tightly controlled SigS levels in *S. aureus*. Taken together, SroA and SroB act as novel downstream effectors and feedback regulators of SigS in *S. aureus*.

4:30 p.m.—4:50 p.m.
AtxA binding is required for activation of toxin and small RNA promoters in *Bacillus anthracis*

Andrei P. Pomerantsev, Val Jackson-Hundley, Mary E. Sievers, Ankur Bothra and Stephen H. Leppla

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ORAL ABSTRACTS

It has been previously demonstrated that anthrax toxin activator (AtxA) binds directly to the σ^A- like promoter region of *pagA* (encoding protective antigen, PA) immediately upstream of the RNA polymerase binding site. In this study, using electrophoretic mobility shift assays and *in vivo* analyses, we confirmed AtxA-binding sites in the promoter regions of the *lef* and *cya* genes (encoding lethal and edema factors, respectively) and of two *Bacillus anthracis* small RNAs (XrrA and XrrB). Activities of all four newly studied promoters were enhanced in the presence of CO_2/bicarbonate and AtxA, as previously seen for the *pagA* promoter. Notably, the *cya* promoter was less activated by AtxA and CO_2/bicarbonate conditions. The putative promoter of a recently described third small RNA, XrrC, showed a negligible response to AtxA and CO_2/bicarbonate. RNA polymerase binding sites of the newly studied promoters show no consensus and differ from the σ^A-like promoter region of *pagA*. In silico analysis of the probable AtxA binding sites in the studied promoters revealed several palindromes. Sequence alignment of these palindromes showed some similarity (69%) between *lef* and *cya* promoters and greater similarity between the sRNA promoters (82%), excluding XrrC. All the analyzed palindromes showed very little overlap with the σ^A-like *pagA* promoter. It remains unclear as to how AtxA and DNA-dependent RNA- polymerase identify such diverse DNA-sequences and differentially regulate promoter activation of the studied genes.

4:50 p.m.—5:10 p.m.
Characterizing MroQ-dependent Regulation of *Staphylococcus aureus* Quorum Sensing

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Gram-positive bacteria produce small autoinducing peptides (AIPs), which act to regulate expression of genes that promote adaptive traits including virulence. The Gram-positive pathogen *Staphylococcus aureus* generates a cyclic AIP that controls expression of virulence factors via the accessory gene regulatory (Agr) system. *S. aureus* strains belong to one of four Agr groups (I, II, III, and IV); and each group harbors allelic variants of AgrD, the precursor of AIP. In a prior screen for *S. aureus* virulence factors, we identified MroQ, a putative peptidase. A Δ*mroQ* mutant closely resembled a Δ*agr* mutant and had significant defects in AIP production in an Agr-I strain. We show that expression of AgrD-I in a Δ*mroQ* mutant leads to accumulation of an AIP processing intermediate at the membrane that coincides with a loss of secreted mature AIP, indicating MroQ promotes maturation of AgrD -I. MroQ is conserved in all Agr sequence variants, suggesting either identical function amongst all Agr types or activity specific to Agr-I strains. Our data indicate that MroQ is required for AIP maturation and activity in Agr-I, -II, and -IV strains irrespective of background. However, MroQ is not required for Agr-III activity despite an identifiable role in peptide maturation. Isogenic Δ*agr* and Δ*agr* Δ*mroQ* strains complemented with Agr-I-IV validated the critical role for MroQ in the generation of active AIP-I, -II, and -IV, but not AIP-III. These findings were reinforced by skin infection studies in mice. Our data substantiate the prevailing model that MroQ is a mediator of cyclic peptide maturation.

5:10 p.m.—5:30 p.m.
The small RNA Teg41 is a pleiotropic regulator of virulence in *Staphylococcus aureus*

Paul Briaud[1], Rachel L. Zapf[1], Ariana D. Mayher[1,2], Aubrey K. G. McReynolds[3], Andrew Frey[4], Richard E. Wiemels[1], Rebecca A. Keogh[1], Lindsey N. Shaw[4], Jeffrey L. Bose[3], and **Ronan K. Carroll**[1,2]

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Previously our group demonstrated a role for the sRNA Teg41 in regulating production of the alpha Phenol Soluble Modulin toxins (αPSMs) in *S. aureus*. Overexpressing Teg41 increased αPSM production while deleting the 3' end of Teg41 (Teg41Δ3′ strain) resulted in a decrease in αPSM production, reduced hemolytic activity of *S. aureus* culture supernatants, and attenuated virulence in a murine abscess model of infection. In this study we further explore the attenuation of virulence in the Teg41Δ3′ strain. Using both localized and systemic models of infection we demonstrate that the Teg41Δ3′ strain is more severely attenuated than a ΔαPSM mutant, strongly suggesting that Teg41 influences more than the αPSMs. Proteomic and transcriptomic analysis of the wild type and Teg41Δ3′ strain reveals widespread alterations in transcript abundance and protein production in the absence of Teg41, confirming that Teg41 has pleiotropic effects in the cell. We go on to investigate the molecular mechanism underlying Teg41-mediated gene regulation. Surprisingly results demonstrate that certain Teg41-target genes, including the αPSMs and bPSMs, are transcriptionally altered in the Teg41Δ3′ strain, while other targets, specifically *spa* (encoding surface protein A), are regulated at the level of transcript stability. Collectively these data demonstrate that Teg41 is a pleiotropic RNA regulator in *S. aureus* that influences expression of a variety of genes using multiple different mechanisms.

Tuesday, October 11th

Session 4: Host-Pathogen Interactions I

9:30 a.m.—9:50 a.m.
Defining *S. aureus* regulatory systems that respond to environmental stresses at the host-pathogen interface

Valeria M. Reyes Ruiz[a], Catherine Leasure[a], Anderson Miller[a], Sydney Drury[a], and Eric P. Skaar[a]

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Staphylococcus aureus is one of the most frequent causes of bacterial infections. While *S. aureus* is mainly considered an extracellular pathogen, recent studies describe an intracellular reservoir of *S. aureus,* which is poorly accessed by antibiotics. Moreover, *S. aureus* that grows and persists inside macrophages can disseminate and cause disease in mouse models of infection. Studies examining the interaction of intracellular *S. aureus* with macrophages would provide insight into the development of therapeutics to treat this bacterial reservoir, which circumvents antibiotic efficacy. *S. aureus* has evolved intricate regulatory networks that allow the bacterium to produce a diverse array of virulence factors and defense mechanisms within the host environment. These include two-component signal transduction systems (TCSs), in which a histidine kinase (HK) responds to extracellular stimuli and transfers a signal to a response regulator (RR) that mediates regulation of gene expression. *S. aureus* contains 16 TCSs that respond to a diverse array of environmental signals. We have engineered reporter constructs for each of these sensing systems in *S. aureus*. Through the application of advanced imaging modalities, we will determine the *S. aureus* regulatory systems that are activated and are essential to survive the host-imposed stresses inside macrophages. Additionally, we aim to discover host factors responsible for the activation of these regulatory systems intracellularly through the use of an arrayed CRISPR screen.

These studies will elucidate novel innate immune factors required for the control of bacterial infections, as well as revealing the bacterial signaling pathways essential for overcoming host-imposed stress.

9:50 a.m.—10:10 a.m.
Development of skin-like media for studying *Staphylococcus aureus* human skin colonization determinants

Flavia G. Costa[1] and Alexander R. Horswill[1]

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The human skin, characterized by its acidity, salinity, and high concentrations of metabolic byproducts such as lactate and urea, is densely populated with bacteria that interact synergistically and competitively for survival. The dominant skin bacterial pathogen methicillin-resistant *Staphylococcus aureus* (MRSA) must adjust its metabolism and physiology in this environment and outcompete commensals for the opportunity to establish an infection. Understanding these early stages of human skin colonization by MRSA is limited by the uniqueness of human skin composition, as well as lack of laboratory media that simulates this environment. We formulated a skin-like media (SLM) to simulate the composition of healthy human skin in the laboratory. Transcriptional changes of MRSA strain LAC grown in this media were compared to growth in the commonly used media tryptic soy broth (TSB) with RNA-seq. From these data, fatty acid degradation, biotin biosynthesis, BCAA biosynthesis, purine biosynthesis, histidine degradation, and urea degradation were identified as key upregulated metabolic pathways in SLM compared to TSB. Urease activity has been shown to promote MRSA colonization in the murine kidney and here we investigated its role in skin colonization. Deletion of the operon encoding urease (Δ*ure*) resulted in a growth defect in SLM, and RNA-seq comparing the WT LAC to Δ*ure* showed transcriptional changes in metabolic pathways involved in amino acid biosynthesis, nitrogen assimilation, and stress response in the Δ*ure* strain. The role of urease in *in vivo* and *ex vivo* models, and the inhibition of urease activity using the competitive inhibitor flurofamide, are currently under investigation.

10:10 a.m.—10:30 a.m.
Staphylococcal superantigens can promote persistence during chronic orthopedic infection

Stephen W. Tuffs[1], Katherine Kasper[1], Ivor Mohorovic[1], Karine Dufresne[1], Nicholas Walton[1], Tracey Bentall[2], Johan Delport[2,3], Tina Mele[1,2,4,5], and John K. McCormick[1,6]

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A 73-year-old orthopedic surgery patient presented to London Health Science centre (LHSC) with *Staphylococcus aureus* infection complicating their recent knee arthroplasty, patellaplasty and bone graft. The bacteria were isolated prior to antibiotic treatment, but the patient reported 2 additional recurrences following first admission and isolates were taken. We performed comprehensive molecular characterisation on these isolates to understand how this patent suffered this recurrent infection. We found that the first isolate (LHSC-429) was from the CC5 clonal lineage and an MRSA, which is expected in a patient undergoing prolonged antimicrobial therapy. Surprisingly, the second two isolates (LHSC-485 and LHSC-495), despite being the same clonal linage, were MSSA and further analysis indicated these strains were carrying the pIB485-like plasmid know for carrying the superantigen genes *sed, sej* and *ser*. Further characterisation of these three isolates revealed that 485 and 495 had similar protein profiles, similar growth profile and supernatants from these isolates were able to activate T cells much more effectively than the 429 isolate. Mass spectrometry analysis reveled that the superantigen SER was produced in large quantities by the 485 and 495 strains, which suggested this factor may have contributed to the persistence of these two strains. Indeed, when we analysed these three strains in the superantigen sensitive DR4-tg mouse model of bloodstream infection we found that the 485 and 495 strains had a significant increase in liver persistence confirming the importance of the superantigen and likely allowing these MSSA to continue to persist despite antibiotic pressure in this patient.

Session 5: Clinical & Applied Microbiology

10:50 a.m.—11:10 a.m.
Development of CRISPR-modified *Clostridium novyi*-NT as an Intravenous, Multisite Pancreatic Cancer Therapeutic

Kaitlin M. Dailey [1,2], James R. Small[3], Jessica E. Pullan[4], Krysten E. Vance[1], Kyle L. McAndrews[1], William R. Miklavcic[1], Sanku Mallik[2,4], Amanda E. Brooks[2,4,5], Kenneth W. Bayles[6], Michael A. Hollingsworth[1,7]

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Pancreatic cancer has a five-year survival rate of 10% with >80% of tumors metastasizing prior to clinical detection. Current therapeutics are largely unable to deliver drugs to avascular areas. Alternatively, oncolytic bacteria, *e.g.*, *Clostridium novyi*-NT, are attracted to hypoxic/necrotic niches in solid tumor cores. While intratumoral injections of *C. novyi*-NT are promising, many tumors are inaccessible. Intravenous injections have encountered rapid clearance of *C. novyi*-NT without sepsis by the macrophage phagocytosis system. Nanoparticle drug delivery improved tumor localization by integrating the RGD motif (Arg-Gly-Asp) with a well-characterized affinity for the $\alpha_v\beta_3$ integrin overexpressed on tumor cells and tumor-associated epithelium. CRISPR/Cas9n was used to genetically modify *C. novyi*-NT to express the RGD motif in the spore surface. Spores with enhanced affinity to a surface coated with the targeted binding partner of RGD, $\alpha_v\beta_3$ integrin, have been generated. Preliminary efforts have been made to evaluate biodistribution of RGD-modified spores in an immunocompetent, syngeneic pancreatic cancer murine model. Differential PCR for *C. novyi* species after intravenous administration revealed altered biodistribution compared to non-modified counterparts, with an increase in localization to the pancreas and tumor. A clinical pathologist blinded to the treatment groups found evidence of elevated inflammation specific to tumors of the RGD-modified spore treated cohort. Further, laser microdissection of the central necrotic cores indicated presence of *C. novyi* DNA in the RGD-modified spore treated cohort. Thus, CRISPR/Cas modification of

Clostridium novyi-NT is a viable and promising avenue to develop novel IV cancer therapeutics capable of targeting both primary tumors and metastases.

11:10 a.m.—11:30 a.m.
Genotypic and phenotypic characterization of *Streptococcus pyogenes* isolated from patients with acute pharyngitis and carriers

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Group A streptococcus (GAS) is the most frequent bacterial cause of pharyngitis in children and causes both suppurative (acute otitis media, acute sinusitis, mastoiditis) and non-suppurative (post-streptococcal glomerulonephritis and acute rheumatic fever) sequelae. The carrier state of GAS is of particular significance to public health as carriers serve as a reservoir for spread of the pathogen to others in the population. No study has demonstrated the physiological characteristics of GAS as it exists in the carrier state in human patients. We have conducted preliminary experiments on carrier and acute GAS strains obtained from human specimens including transcriptomic assays on samples collected directly from patient swabs. Using the bacterial patient samples, we have also undertaken both short and long-read whole genome sequencing to compare acute and carrier strains in these patient populations and compared the cohort of strains using *in vitro* assays including biofilm formation, virulence factor production, and antimicrobial susceptibility. Our preliminary data show that GAS transcriptional profiles obtained from individuals experiencing an acute infection are generally indistinguishable from one another but are distinguishable from profiles of isogenic strains grown in laboratory medium in planktonic culture or on abiotic surfaces as biofilms. Whole genome sequencing indicates that genetic variation does not explain the acute versus carrier phenotypes, indicating other factors at play. Overall, our data indicate that transcriptomic changes or host physiology rather than genomic or phenotypic changes in bacterial strains may drive GAS carriage.

11:30 a.m.—11:50 a.m.
***Bacillus thuringiensis* and *B. cytotoxicus* – the taxonomic collapse of the *B. cereus* group and the rise of the underdogs**

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While the relevance of *Bacillus cereus* as a major cause of gastroenteritis and *Bacillus anthracis* as the cause for anthrax is undisputed, our perception of closely related members of the *B. cereus* group is rapidly changing. *Bacillus thuringiensis* has been regarded as safe biopesticide, whereas the handful of *Bacillus cytotoxicus* strains known to date gained notoriety for leading to fatal cases of diarrheal disease. A recent EFSA scientific opinion stresses the urgent need for further data allowing for improved risk assessment. We aimed to gain further insights into the ecology and hazardous potential of *B. thuringiensis* and *B. cytotoxicus* by investigating outbreak cases, using Vero cell cytotoxicity assays, and whole genome sequencing. Our results show that the relevance of these two species in foodborne disease needs to be reassessed. We were able to show that *B. thuringiensis* isolates from human outbreak cases and foods genomically matched with one of six biopesticide strains, suggesting biopesticide products as their source. Biopesticide strains can therefore present a food safety risk, underpinning the importance of assessing the hazardous potential of each strain used. Furthermore, our studies revealed that only a small number of *B. cytotoxicus* strains, which is regarded a fatal foodborne pathogen, is highly enterocytotoxic while the large majority of *B. cytotoxicus* strains do not produce detectable levels of enterotoxins. In summary, our work suggests that a paradigm shift in the perception of the *Bacillus cereus* group species in the field of food safety will be necessary.

11:50 a.m.—12:10 a.m.
A phylogenomic approach to early life *Staphylococcus aureus* colonization

Paul J. Planet[1,2,3], Alex G. Arvanitis[1], Andries Feder[1], Qianxuan She[2], Krisha Patel[1], Swetha Rajagopal[1], Lidiya Denu[1], Kelsey O'Brien[1], Jessica J. Gunoskey[2], Michael Z. David[2], Colleen Bianco[1], Ahmed M. Moustafa[2], Jeffrey S. Gerber[1,2]

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Staphylococcus aureus colonizes skin and mucous membranes across the human lifespan. Colonization is not only a risk factor for invasive disease, but also for chronic diseases such as atopic dermatitis. It is likely that early interactions between the host and colonizing bacteria have an impact on life-long health outcomes. Over a 3-year period we collected colonization swabs from a cohort of 156 healthy infants and toddlers over their first 2 years of life at 13 possible time points. We recorded clinical metadata for each patient including the mode of delivery, diet and breast-feeding status, growth curves, antibiotic use, infections, and household composition. Altogether we collected 3,747 swabs from nares, axilla, and groin. After culture, we obtained 295 *S. aureus* isolates from 83 subjects, and 73 subjects were never positive for *S. aureus*. Of the positive patients, 44 subjects were persistently colonized, 10 had intermittent colonization, and 29 had only one positive timepoint. Phylogenomic analysis of 130 genomes from 65 subjects showed that most persistent and intermittent subjects had nearly identical strains at every time point. We characterized each isolate for growth rate and hemolysis. We identified genes and alleles strongly associated with persistent colonization. Remarkably, many of these genes were involved in basic metabolic processes (eg., *atpA*, *atpB*, *gapA1*). We also used the longitudinally collected genomes to identify genomic changes that may represent adaptive in-host evolution. Ongoing work is aimed at identifying genomic characteristics associated with various clinical and microbiological variables.

Session 6: Host-Pathogen Interactions II

3:20 p.m.—3:40 p.m.
Exploring *Staphylococcus aureus* Host-Pathogen Interaction Using Proteomic Technologies

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Staphylococcus aureus is Gram-positive bacterium that colonizes every ecological niche of the human host using an army of virulence determinants. Currently, the dynamics of how *S. aureus* generates such a wide diversity of infections is poorly understood. Herein, we use proteomic and degradomic techniques to uncover how this organism adapts to diverse host niches. We first performed the most extensive characterization to date of the *S. aureus* proteome through the generation of a DIA ion library, totaling 2,184 unique protein-IDs. Using this library, we profiled *S. aureus* infection in different organs during murine sepsis. Our work revealed that the kidneys serve as a hub for virulence factors, with numerous toxins detected. Outside of the kidneys, we noted a

significant number of overlapping proteins in lung and spleen environments, with the majority being involved in catalytic activities. Next, we used N-terminomic methodologies to explore targets of *S. aureus* proteases within human lungs and neutrophils. For the former, we saw cleavage of 268 unique proteins including components of the immune system such as Complement C3, C4, immunoglobulins, as well as extracellular protein EMILIN-1 that facilitates lung development. Within neutrophils, we discovered 47 targets and of note here was Myeloperoxidase, an enzyme that influences the antimicrobial response. Additionally, we found that G-protein coupled receptors, which mediate leukocyte navigation during infection, are also cleaved by *S. aureus* proteases. Collectively, this work enhances our understanding of the pathogenic mechanisms used by *S. aureus* to engender survival and dissemination within the host.

3:40 p.m.—4:00 p.m.
Structural insights into corneocyte adhesion by Staphylococci

Joseph J. Maciag[1], Krista B. Mills[2], Paroma Roy[3], Paul D. Fey[3], Alexander R. Horswill[2], and **Andrew B. Herr**[1,4,5]

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Staphylococci use a variety of cell-surface proteins to mediate attachment to host tissue and abiotic surfaces. Recent work has shown that the lectin domain of the accumulation-associated protein (Aap) from *S. epidermidis* is essential for adhesion to host corneocytes. We report the high-resolution crystal structures of the lectin domains from Aap and its close ortholog SasG from *S. aureus*. Both lectins adopt the same fold, which resembles that of legume lectins, although Aap and SasG adopt distinct architectures around their glycan-binding sites. Glycan array screening with the Aap lectin domain revealed specificity for a number of weak ligands that all share repeating N-acetyl-lactosamine disaccharide units (i.e., galactose followed by N-acetyl-glucosamine). Isothermal titration calorimetry confirmed that N-acetyl-lactosamine binds to Aap and SasG, and mutation of aromatic residues Tyr580 or Trp392 in the putative glycan-binding pockets of Aap or SasG, respectively, abrogated binding. Soluble recombinant Aap (or SasG) lectin domains were able to inhibit *S. epidermidis* (or *S. aureus*) adhesion to corneocytes, but the inhibitory effect was lost when adding Aap-Y580A or SasG-W392A mutant proteins. Likewise, N-acetyl-lactosamine was also able to inhibit adhesion of *S. epidermidis* and *S. aureus* to corneocytes. These data help to elucidate the mechanism of staphylococcal adhesion to healthy skin and reveal overlapping specificity between Aap and SasG lectin domains.

4:00 p.m.—4:20 p.m.
Secreted mammalian DNases protect against *S. aureus* infection by digesting biofilms

Keenan A. Lacey[1], Lee Serpas[2], Sohei Makita[2], Victor J. Torres[1], Boris Reizis[2]

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There are two major secreted mammalian DNases, DNASE1 and its homolog DNASE1L3. Extracellular DNASE1L3 maintains tolerance to self-DNA in humans and mice, whereas the role of its homolog DNASE1 remains controversial, and the overall function of secreted DNases in immunity is unclear. We found that deletion of murine DNASE1 neither caused autoreactivity in isolation nor exacerbated lupus-like disease in DNASE1L3-deficient mice. However, the combined deletion of DNASE1 and DNASE1L3 (but not of either enzyme alone) rendered mice susceptible to systemic infection with *Staphylococcus aureus* by promoting severe kidney pathology. The phenotype was not associated with aberrant NETosis or defective innate immune response. Using novel immunohistochemistry techniques to identify and measure *in vivo* biofilms, we found that DNASE1/DNASE1L3 double-deficient mice had increased bacterial burden and biofilm formation in the kidney. The resulting biofilms were enriched for bacterial extracellular DNA (eDNA) and promoted the exclusion of neutrophils from bacterial abscesses, suggesting that DNases are required to digest bacterial biofilms *in vivo*. Both DNASE1 and DNASE1L3 digested DNA-containing *S. aureus* biofilms *in vitro*, and the administration of DNASE1 ameliorated *S. aureus*-induced disease in wild-type mice. Thus, DNASE1 and DNASE1L3 jointly facilitate the control of bacterial infection by digesting extracellular microbial DNA in biofilms, suggesting the original evolutionary function of secreted DNases as antimicrobial agents.

4:20 p.m.—4:40 p.m.
Neutrophil-intrinsic TNF receptor signaling orchestrates host defense against *Staphylococcus aureus*

Christine Youn[1], Yu Wang[1], Dustin A. Dikeman[1], Martin P. Alphonse[1], Sabrina J. Nolan[1], Daniel P. Joyce[1], Cristina Pontaza[1], Michael Ahmadi[1], Aron Tocaj[1], Lloyd S. Miller[2], **Nathan K. Archer**[1]

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Staphylococcus aureus is the leading cause of skin and soft tissue infections and has become a major health burden due to the emergence of antibiotic-resistant strains. Tumor necrosis factor (TNF) is a proinflammatory cytokine that is induced upon *S. aureus* exposure and whose inhibition is associated with increased risk of *S. aureus* infections in humans. However, the contribution of TNF and cognate receptors, TNFR1 and TNFR2, to host defense against *S. aureus* skin infections is unclear. Therefore, we used an *in vivo* mouse model of *S. aureus* skin infection whereby TNF, TNFR1, or TNFR2 deficient mice and wildtype (wt) mice were intradermally injected with bioluminescent *S. aureus* and monitored for 14 days. TNF, TNFR1, and TNFR2 deficient mice exhibited significantly increased bacterial burdens and skin lesions compared to wt mice. Furthermore, we identified neutrophils (PMNs) as the predominant TNFR1 and TNFR2 expressing cells. To determine the importance of PMN-intrinsic TNFR signaling, we adoptively transferred wt PMNs into TNFR1 and TNFR2 deficient mice, which significantly reduced bacterial burdens and skin lesion sizes in the mice. We discovered that TNFR1 was crucial for PMN recruitment and skin abscess formation, whereas TNFR2 was critical for NOX2 activation, ROS production, and PAD4-dependent neutrophil extracellular trap formation in PMNs. Taken together, these findings indicated that TNF orchestrated immunity against *S. aureus* via PMN-intrinsic TNFR1 and TNFR2 signaling, which has implications in the development of novel immune-based therapies against *S. aureus* and potentially other bacterial infections.

4:40 p.m.—5:00 p.m.
Epigenetic Regulation though DNA Methylation Promotes Group B Streptococcal Vaginal Colonization

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Streptococcus agalactiae (Group B *Streptococcus*; GBS), colonizes approximately one third of healthy women in the gastrointestinal and reproductive tract. GBS colonization is a risk factor for many adverse pregnancy outcomes, including stillbirths, preterm premature rupture of membranes, chorioamnionitis, as well as newborn infection. Despite the numerous and serious complications associated with maternal GBS colonization, mechanisms allowing GBS to colonize the vaginal tract or cause ascending infection are not well understood. Previously, transposon mutagenesis sequencing was conducted to identify genes that are important for GBS vaginal colonization *in vivo*, and the primary underrepresented gene found during the screen was annotated as a DNA Cytosine Methyltransferase (*dcm*). DNA methylation is an important epigenetic modification that regulates gene expression without changing the DNA sequence. Therefore, we hypothesized that GBS Dcm globally alters transcription to promote vaginal colonization. To test this hypothesis, we constructed a GBS Δ*dcm* mutant and determined that its DNA has significantly less 5mC DNA methylation as compared to DNA from the WT strain. The Δ*dcm* mutant was outcompeted by WT GBS within a murine model for vaginal colonization, which also resulted in decreased ascending infection of the mutant to the cervix and uterus. Using Nanopore sequencing we identified Dcm-dependent methylation across the genome. We paired this with transcriptome analysis of the Δ*dcm* mutant compared to WT GBS at mid-log and early stationary growth phases to identify genes that are differentially expressed due to the altered DNA methylation. Our results suggest that Dcm-mediated regulation of the GBS transcriptome promotes vaginal colonization.

Wednesday, October 12th

Session 7: Stress & Pathogensis

9:30 a.m.—9:50 a.m.

***Listeria monocytogenes* YvcJ and GlmR contribute to muropeptide synthesis to facilitate cell wall stress response**

Kimberly V. Gutierrez, and John Demian Sauer

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Pathogenic intracellular bacteria encounter a variety of stresses during infection including in the eukaryotic cytosol. Professional cytosolic pathogens including *Listeria monocytogenes*, *Shigella flexneria* and *Francisella tularensis,* among others, have adapted to survive and replicate in the hostile eukaryotic cytosol, however the mechanisms cytosolic bacteria have evolved to survive in the cytosol are not well understood. Identification and characterization of these adaptations could facilitate the development of novel therapeutics against these pathogens. We utilized *Listeria monocytogenes*, a model cytosolic pathogen, as a tool to further understand the mechanisms cytosolic bacteria use to survive in the cytosol. Previous studies demonstrated that *L. monocytogenes* requires GlmR, a conserved accessory uridyltransferase, for resistance to cell wall stress, cytosolic survival inflammasome avoidance and virulence. YvcJ, a conserved protein of unknown function, lies in the same operon as GlmR and we found that similar to GlmR, YvcJ is required for *L. monocytogenes* cytosolic survival. YvcJ is also required for inflammasome evasion, intracellular growth, and virulence. We demonstrate that Δ*yvcJ* mutants are susceptible to cell wall stress (lysozyme and β-lactam antibiotics) and untargeted metabolomics demonstrated that Δ*yvcJ* mutants have increased pools of muropeptide precursors essential for peptidoglycan production, most notably uridine diphosphate N- acetylglucosamine (UDP-GlcNAc). Previous studies in *B. subtilis* demonstrated a UDP-GlcNAc dependent interaction between YvcJ and GlmR. Ongoing studies are focused on characterizing the interaction between GlmR and YvcJ in *L. monocytogenes,* how this interaction alters GlmR enzymatic activity and finally how this interaction might impact cell wall stress responses, cytosolic survival and virulence.

9:50 a.m.—10:10 a.m.

A novel pathogenic mechanism for *Streptococcus gallolyticus* – type VII secretion, host receptor and role in colorectal cancer

John C Taylor[1], Michael McGill[2], David Threadgill[2], Kelly F. Churion[1], Magnus Höök[1], and **Yi Xu[1]**

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Streptococcus gallolyticus subsp. *gallolyticus* (*Sgg*) has a strong association with colorectal cancer (CRC), supported by numerous case reports and epidemiological surveys over the past several decades. Recent studies further highlighted a functional role of *Sgg* in the development of colon tumors, *i.e.*, *Sgg* induces CRC cell hyperproliferation and promotes tumor growth in vivo. However, the specific Sgg factors and host targets important for the pro-proliferative and pro-tumor activities of *Sgg* were unknown. We demonstrated that a type VII secretion system (T7SS) of *Sgg* strain TX20005 (designated as $T7SS^{T05}$) is important for *Sgg* to stimulate CRC cell proliferation and to promote the development of colon tumors in a pre-clinical model of CRC. We have now pinpointed the specific T7SS effector responsible for the pro-proliferative activity and identified the epidermal growth factor receptor (EGFR) as the host cell surface receptor targeted by the T7SS effector. More importantly, inhibition of EGFR signaling in vivo abolished the pro-tumor effect of *Sgg*, suggesting that this is a critical host signaling pathway targeted by *Sgg* to promote tumor growth. To the best of our knowledge, this is the first evidence linking CRC-associated microbes, and T7SS, to EGFR, which is a critically important signaling molecule in normal homeostasis and cancer. Studies are currently underway to further investigate the interaction between the *Sgg* T7SS effector and EGFR, and the biological outcomes of the interaction in more detail.

10:10 a.m.—10:30 a.m.

Impact of *Clostridioides difficile* spore-persistence in the intestinal mucosa to disease recurrence

Daniel Paredes-Sabja

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Clostridioides difficile is a Gram positive, strictly anaerobic, and spore-forming bacterium that causes diarrhea and death. During *C. difficile* infection, *C. difficile* secretes toxins that cause massive epithelium damage, contributing to disease recurrence. *C. difficile* also initiates a sporulation pathway that culminates with the formation of newly dormant spores which allow *C. difficile* to persist in the host and cause disease recurrence. Therefore, understanding how and where *C. difficile* spores persist in the gastrointestinal tract is imperative for the development of therapeutic strategies. The surface of *C. difficile* spores, the exosporium layer, serves as the primary site of interaction with the host during disease. Here, we identified a mechanism through

which *C. difficile* spores gain intracellular access in the intestinal mucosa, contributing to disease recurrence. *C. difficile* spores interact, in a concentration dependent manner, with the extracellular matrix proteins fibronectin (Fn) and vitronectin (Vn), and uses them as molecular bridges to gain entry into intestinal epithelial cells (IECs) in an $a_5\beta_1$ and $a_v\beta_1$ integrin-dependent manner. The spore exosporium surface protein, BclA3, is essential for spore entry into IECs via the Fn/Vn- integrin pathway. BclA3 contributes to disease recurrence, apparently by enhancing spore adherence to the intestinal mucosa. Inhibition of *in vivo* spore entry via Fn/Vn-integrins, strongly attenuated spore entry into IECs and diminished disease recurrence in mice. Overall, our data indicates that adhered and internalized *C. difficile* spores have a sustained impact in disease recurrence.

Session 8: Regulation II

10:50 a.m.—11:10 a.m.

Development of a dual fluorescent reporter system in *Clostridioides difficile* reveals a division of labor between virulence and transmission gene expression

M. Lauren Donnelly,[1,2] Shailab Shrestha,[1,2] John W. Ribis,[1,2] Pola Kuhn,[1,2] Maria Krasilnikov,[1,2] Carolina Alves Feliciano,[1,a] and Aimee Shen[1#]

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The bacterial pathogen *Clostridioides difficile* causes gastroenteritis by producing toxins and transmits disease by making resistant spores. Toxin and spore production are energy-expensive processes that are regulated by multiple transcription factors in response to many environmental inputs. While toxin and sporulation genes are both induced in only a subset of *C. difficile* cells, the relationship between these two sub-populations remains unclear. To address whether *C. difficile* coordinates the generation of these sub-populations, we developed a dual transcriptional reporter system that allows toxin and sporulation gene expression to be simultaneously visualized at the single-cell level using chromosomally-encoded mScarlet and mNeonGreen fluorescent transcriptional reporters. We then adapted an automated image analysis pipeline to quantify toxin and sporulation gene expression in thousands of individual cells in different media conditions and genetic backgrounds. These analyses revealed that toxin and sporulation gene expression rarely overlap during growth on agar plates, whereas broth culture increases this overlap. Our results suggest that certain growth conditions promote a “division of labor” between transmission and virulence gene expression, highlighting how environmental inputs influence these subpopulations. Our data further suggest that the RstA transcriptional regulator skews the population to activate sporulation genes rather than toxin genes. Given that recent work has revealed population-wide heterogeneity for numerous cellular processes in *C. difficile*, we anticipate that our dual reporter system will be broadly useful for determining the overlap between these subpopulations.

11:10 a.m.—11:30 a.m.

Glucose PTS modulates pyruvate metabolism, bacterial fitness, and microbial ecology in oral biofilm

Lin Zeng, Zachary Taylor, Robert A. Burne, and Alejandro R. Walker.

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Recently identified spontaneous mutants of major glucose-PTS (*manLMNO*) in stocks of *Streptococcus sanguinis* SK36 showed enhanced fitness in low-pH environment. Transcriptomic and metabolomic analyses of the *manL* mutant (SK36/*manL*) revealed redirection of pyruvate from production of lactate to acetate for extra energy extraction, resulting in excretion of greater amounts of pyruvate and H_2O_2, and increased expression of multiple alkali-generating activities. Genes showing increased expression in SK36/*manL* also included several carbohydrate transporters, putative extracellular glycosidases, intracellular polysaccharide (IPS) locus, pathways for catabolism of acetoin, ethanolamine, ascorbate, and formate, genes required for membrane biosynthesis, and those for motility and attachment. When co-cultured with *Streptococcus mutans* UA159 as a biofilm, strain SK36/*manL* significantly increased the persistence of UA159 relative to the wild-type SK36, an effect that was enhanced by the removal of oxygen and dampened by arginine. We posited that SK36/*manL* benefited UA159 partly by excretion of pyruvate, as addition of pyruvate to a SK36:UA159 biofilm similarly improved the competitiveness of UA159. Mimicking cariogenic biofilms, a reduction in medium buffer capacity (from 72 mM to 10 mM phosphate) or increase in glucose concentrations (from 18 mM to 55 mM) clearly benefited UA159, however SK36/*manL* largely abolished that advantage; likely due to enhanced aciduricity of the mutant. When the glucose-PTS was deleted in *S. mutans* and *S. gordonii,* each mutant presented altered fitness characteristics. Our study demonstrated that PTS-dependent modulation of central metabolism profoundly affects streptococcal fitness and metabolic interactions, revealing another dimension in the commensal-pathogen relationship that is central to caries etiology.

11:30 a.m.—11:50 a.m.

Spatial regulation of surface proteins in *Staphylococcus aureus*

Ran Zhang[1], Mac A. Shebes[1], Kelvin Kho[2], Salvatore J. Scaffidi[1], Timothy C. Meredith[2], **Wenqi Yu[1]**

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Surface proteins of *Staphylococcus aureus* play vital roles in bacterial physiology and pathogenesis. Recent work suggests that surface proteins are spatially regulated by a YSIRK/GXXS signal peptide that promotes cross-wall targeting at the mid-cell, though the mechanisms remain unclear. We previously showed that protein A (SpA), a YSIRK/GXXS protein and key staphylococcal virulence factor, mis-localizes in a *ltaS* mutant deficient in lipoteichoic acid (LTA) production. Here, we identified that SpA contains another cross-wall targeting signal, the LysM domain, which, in addition to the YSIRK/GXXS signal peptide, significantly enhances SpA cross-wall targeting. We show that LTA synthesis, but not LtaS, is required for SpA septal anchoring and cross-wall deposition. Interestingly, LTA is predominantly found at the peripheral cell membrane and is diminished at the septum of dividing staphylococcal cells, suggesting a restriction mechanism for SpA septal localization. Finally, we show that D-alanylation of LTA abolishes SpA cross-wall deposition by disrupting SpA distribution in the peptidoglycan layer without altering SpA septal anchoring. Our study reveals that multiple factors contribute to the spatial regulation and cross-wall targeting of SpA via different mechanisms, which coordinately ensures efficient incorporation of surface proteins into the growing peptidoglycan during the cell cycle.
kingdoms of life. Our understanding of cell division in bacteria activity of FtsZ to catalyze FtsZ depolymerization. Thus, in *S. aureus*, GpsB, an essential protein, orchestrates both FtsZ assembly and disassembly to remodel the cell division machinery during cytokinesis.

ICG+P POSTER SESSION "A" ABSTRACTS

MONDAY, OCTOBER 10, 2022
1:30 P.M.-3:30 P.M.

POSTER NUMBERS AND PRESENTERS

A01 Raeven Bastock
A02 Blake Bertrand
A03 Kevin Bollinger
A04 Camryn Bonn
A05 Jeff Boyd
A06 Mara Campbell
A07 Liana Chan
A08 Samuel Eallonardo
A09 Nicholas Faiola
A10 Jeffrey Freiberg
A11 Neal Hammer
A12 Andrea Herrera
A13 Jessica Jackson
A14 Luke Joyce
A15 Gunjan Kak
A16 Rebecca Keogh
A17 Lee Korshoj
A18 Robert Kuiack
A19 Laurie Lyon
A20 Dominic McGrosso
A21 Ute Müh
A22 Taylor Nye
A23 Sasmita Panda
A24 Alexandra Peterson
A25 Magdalena Podkowik
A26 Grishma Prabhukhot
A27 Emily Pruitt
A28 Fareha Razvi
A29 Nicole Schwardt
A30 Ryan Steere
A31 Robert Ulrich
A32 Zachary Van Roy
A33 Shaohui Wang
A34 Gabrielle Watson
A35 Jerry Woo
A36 Won-Sik Yeo
A37 Chunyi Zhou
A38 Christopher Healy

POSTER SESSION "A" ABSTRACTS

Poster # A01
Small-RNA abundance in *Staphylococcus aureus* is impacted by physiologically relevant temperatures

Raeven A. Bastock[1,2], Emily Sudnick[1,3], Emily C. Marino[1,3], Paul Briaud[1], Erin R. Murphy[2,4,5,6], and Ronan K. Carroll[1,2,4]

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[2]Molecular and Cellular Biology Program, Ohio University.
[3]Honors Tutorial College, Ohio University.
[4]Infectious and Tropical Disease Institute, Ohio University.
[5]Heritage College of Osteopathic Medicine, Department of Biomedical Sciences, Ohio University.
[6]Department of Biomedical Sciences, Ohio University.

Approximately 30% of the global population are colonized in the anterior nares by *Staphylococcus aureus*, a Gram-positive opportunistic bacterium. Although colonization is frequently asymptomatic, the bacterium's progression from the nares to the body's deeper tissues and blood can cause fatal illnesses. During the transition, the bacterium experiences a slight, but definite, temperature shift. In a healthy person, the anterior nares are 34° C, whereas the body is 37°C. Previously, we explored the effects of culturing *S. aureus* at three physiologically relevant temperatures (34°C, nares; 37°C, body; and 40°C, pyrexia), and observed changes in gene expression, protein synthesis, and virulence. Results demonstrated (i) *S. aureus* exhibits distinct transcript and proteome profiles at different temperatures, and (ii) there was poor correlation between transcript and corresponding protein levels at different temperatures. We also identified multiple temperature-responsive sRNAs. In total, 15 sRNAs displayed a >2 -fold change in abundance between 34°C and 37°C and 37°C and 40°C. These data together suggest sRNAs may function to mediate temperature-responsive gene expression in *S. aureus*. In the current study we utilize sRNA promoter activity assays and rifampicin RNA stability assays to explore the impact of temperature on sRNA production (transcription) and stability (degradation). Furthermore, we examine the rate of transcriptional changes that occur during a temperature shift from 34°C to 37°C. These data further our understanding of the transition of *S. aureus* from a colonizing commensal to invasive pathogen.

Poster # A02
Human Macrophage Polarization Status Dictates *S. aureus* Intracellular Survival

Blake P. Bertrand[a], Carolyn B. Ibberson[b], Anthony R. Richardson[c], Marvin Whiteley[d], and Tammy Kielian[a]

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S. aureus is a leading cause of medical device-associated biofilm infections that cause high morbidity and economic impact. This is due to ability of *S. aureus* biofilm to evade the host immune response driven by the anti-inflammatory cytokine IL-10. Resident tissue macrophages likely play an important role as a bacterial reservoir during the transition from planktonic to biofilm growth since *S. aureus* has been shown to survive and replicate within the macrophage phagolysosome. *S. aureus* expansion leads to macrophage lysis, releasing bacteria that may serve as a nucleation point for biofilm development. To identify *S. aureus* genes that are essential for macrophage intracellular survival and how this is affected by IL-10, primary human monocyte-derived macrophages were infected with a *S. aureus* transposon (Tn) library in strain USA300 LAC. Transposon-sequencing (Tn-seq) was performed on intracellular *S. aureus* recovered from unstimulated or IL-10 treated macrophages and compared to the Tn library inoculum. Sequencing data were analyzed using a simulation-based Monte Carlo method to identify *S. aureus* genes that were essential for macrophage intracellular survival. The essential genome of *S. aureus* was similar in unstimulated macrophages and the Tn library inoculum (18.5% vs. 18.4%, respectively), but increased to 38.6% in IL-10 treated macrophages. These Tn-seq results reveal that macrophage polarization status exerts differential pressure on *S. aureus*. Future studies will examine how IL-10 polarization influences nutrient availability within human macrophages to force the essentiality of these pathways in *S. aureus*. Understanding this host-pathogen crosstalk may identify new therapeutic targets to thwart biofilm infections.

Poster # A03
Annotated L,D-transpeptidases are not required for survival of *Clostridioides difficile*

Kevin Bollinger[1], Ute Müh[1], Craig Ellermeier[1], David Weiss[1]

[1]University of Iowa

Clostridioides difficile is a gram-positive anaerobic spore forming opportunistic pathogen and a leading cause of nosocomial infections. The cell wall is an essential structure for bacteria and contains strands of peptidoglycan (PG) which are formed by a repeating disaccharide subunit of N-acetylglucosamine and N-acetylmuramic acid. The N-acetylmuramic acid residues have a peptide sidechain that is crosslinked to provide rigidity to the cell. There are two main forms of PG crosslinks in bacteria, 4-3 crosslinks produced by penicillin binding proteins (PBPs) and 3-3 crosslinks formed by L,D-transpeptidases (Ldts). The cell wall in most well studied bacteria has mostly 4-3 crosslinks with a small proportion of 3-3 crosslinks. In contrast, over 70% of the dipeptide crosslinks in *C. difficile* are 3-3. *C. difficile* has three annotated *ldt*s. Peltier et al. (2011) showed that neither *ldt1* nor *ldt2* are essential, although when both are deleted there is a reduction in 3 -3 crosslinks. Here we used CRISPR to delete all three *ldt*s both alone and in combination. All the mutants were viable and were confirmed by PCR, western blot, and whole genome sequencing. Surprisingly, even the triple *ldt* deletion has no obvious phenotypic defect in growth rate, morphology, or resistance to cell wall targeting antibiotics. This raises the question of whether 3-3 crosslinks are dispensable in *C. difficile* or whether there is one or more unidentified *ldt*s. This question will be addressed by doing muropeptide analysis of PG from the triple deletion mutant.

Poster # A04
Repeated emergence of variant TetR family regulator FarR and increased resistance to antimicrobial unsaturated fatty acid among clonal complex 5 methicillin-resistant *Staphylococcus aureus*

Camryn M. Bonn[1], Iftekhar M. Rafiqullah[2], Yi Meng Qian[1], Jennifer Guthrie[1,3], Marta Matuszewska[4], D. Ashley Robinson[2], Peter B. Stathopulos[5], and Martin J. McGavin[1,3]

[1]Western University Department of Microbiology and Immunology, London, Ontario, Canada, [2]University of Mississippi Medical Center Department of Microbiology and Immunology, Jackson, Mississippi, United States, [3]Schulich School of Medicine and Dentistry, London, Ontario, Canada, [4]University of Cambridge Department of Medicine, Cambridge, United Kingdom, and [5]Western University Department of Biochemistry, London, Ontario, Canada

POSTER SESSION "A" ABSTRACTS

Resistance-nodulation-division (RND) superfamily efflux pumps promote antimicrobial resistance and colonization but are poorly characterized in Gram-positive species. In previous work, *in vitro* selection for enhanced linoleic acid resistance in *S. aureus* USA300 promoted recovery of strain FAR7, where a H121Y substitution in the TetR family regulator FarR promoted increased expression of the RND pump FarE. Hypothesizing that similar amino acid substitutions could emerge *in vivo,* we searched >20,000 *S. aureus* genomes for variant FarR proteins. *S. aureus* clonal complex CC5 uniquely exhibited $FarR^{C116Y}$, $FarR^{E160G}$, $FarR^{E93EE}$, $FarR^{P165L}$, and $FarR^{E160G}$ variant strains, most of which were represented in the USA100 lineage of hospital-associated (HA-MRSA). Of these, $FarR^{C116Y}$ and $FarR^{E160G}$ were sufficient to confer increased resistance to uFFA. Strains with $FarR^{E93EE}$ were more resistant to linoleic acid through a FarR-independent mechanism, while strains with $FarR^{P165L}$ did not exhibit increased resistance. A predicted model of the FarR dimer was generated and *in silico* techniques identified acyl-phosphates as a potential activating ligand of FarR. A predicted binding pocket at H356 and K357 in the final helix of the C- terminal ligand-binding domain was also identified. Success of *S. aureus* colonization can be attributed to the diverse array of resistance mechanisms it possesses, including circumvention of toxicity caused by uFFA. Our data support the emergence of FarR amino acid substitution variants as a means of promoting increased resistance to uFFA in the USA100 lineage of HA-MRSA that is endemic in the Western hemisphere, representing a potential mechanism of adaptive evolution to human hosts in a clinical setting.

Poster # A05

Triclosan activates SaeRS-dependent virulence factor expression in *Staphylococcus aureus*

Erin E. Price[1], Javiera Norambuena[1], Kylie Ryan Kaler[1], Natalie Burchat[2], Ashley DuMont[3], Victor Torres[3], Harini Sampath[2], and **Jeffrey M. Boyd***

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3 Department of Microbiology, New York University, New York, NY 10016, USA

In the human pathogen *Staphylococcus aureus*, the two-component system regulatory system SaeRS is responsible for the regulation of virulence factors production and is essential for successful pathogenesis. SaeRS can be stimulated by neutrophil-derived signals and cellular respiratory status. It has also recently been shown to be stimulated by the presence of fatty acids. In this study, we demonstrate that triclosan, an anti-bacterial drug that targets fatty acid synthesis, functions as an activator of *saeRS*. Treatment of *S. aureus* with triclosan decreased overall fatty acid production. However, triclosan treatment increased the accumulation of the fatty acid arachinic acid which increased SaeRS output. These findings present implications for the widespread use of triclosan as an antimicrobial agent in household products, as well as a persistent environmental pollutant.

Poster # A06

The predominant role of SarA as a repressor of *Staphylococcus aureus* protease production is correlated with relative virulence in a murine osteomyelitis model.

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We previously demonstrated that MgrA, SarA, SarR, SarS, SarZ, and Rot bind at least three of four promoters associated with genes for the extracellular proteases aureolysin, ScpA, SspA/B, and SplA-F. We also demonstrated that mutation of *sarA* results in a greater increase in protease activity than mutation of the genes for any of the other loci. However, mutations of these regulatory loci have previously been shown to alter potentially relevant *in vitro* phenotypes as a result of changes in protease production. Thus, the goal of these experiments was to compare the relative impact of the regulatory loci *in vivo* and *in vitro* in order to determine which loci has the most impact on virulence through protease regulation. To this end, we compared the virulence of *mgrA, sarA, sarR, sarS, sarZ*, and *rot* mutants in a murine osteomyelitis model. Experiments were completed with mutants generated in the methicillin-resistant USA300 strain LAC and the methicillin-sensitive USA200 strain UAMS-1. In both strains, mutation of *sarA* had a greater effect than mutation of any other loci, resulting in greater reductions in bacterial burdens in the bone, cortical bone destruction, and reactive bone formation. This result was correlated with reduced cytotoxicity and reduced biofilm formation, which was directly attributable to increased protease production. Mutation of *sarA* also increased the survival of LAC and UAMS-1 in whole human blood to a greater extent than mutation of any of the other regulatory loci, but this effect was not correlated with increased protease production.

Poster # A07

Long-Term Durability of Protective Immune Memory Against MRSA Skin Infection

Liana C. Chan[1], Hong K. Lee[1], Ling Wang[1] and Michael R. Yeaman[1,2]

[1]The Lundquist Institute at Harbor-UCLA, Torrance, CA; David Geffen SOM, University of California, Los Angeles, Los Angeles, CA; [3]Johns Hopkins University, Baltimore, MD;

Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Our prior studies discovered a role for innate immune memory in protection against recurrent methicillin-resistant *S. aureus* (MRSA) SSSI. Priming infection 8 weeks earlier resulted in protective memory upon recurrent infection in wild-type and *rag1*$^{-/-}$ backgrounds. To test durability of this memory, we infected wild-type mice one year prior to secondary challenge. Briefly, 8-week old mice were infected with MRSA subcutaneously, allowed to resolve for one year, and infected again with the identical MRSA strain. Lesion sizes were measured on days 1, 3, 5 & 7 post-secondary challenge. On day 7 post-challenge, MRSA were enumerated in skin abscesses, kidney, spleen and liver. Remarkably, primed mice exhibited protective memory in skin, manifest as reduced lesion sizes and MRSA burden in abscesses during secondary MRSA challenge. However, there was no protection against disseminated infection, as similar MRSA burden was observed in the kidney, spleen and liver of naïve versus primed mice. Cellular signatures of protection were represented by increased numbers of monocytes and NK cells in abscesses, and monocyte intensification in draining inguinal lymph nodes. As expected, cell populations in the spleen were not different. In summary, present findings indicate that protective immunity to *S. aureus* infection is tissue-targeted, involves monocytes and NK cells, and durable. These insights enhance understanding of mechanisms of immune protection vs. *S. aureus*, and may hold novel targets for vaccine and immunotherapeutic development against MRSA.

POSTER SESSION "A" ABSTRACTS

Poster # A08
Post-transcriptional regulation of virulence factor InlB leads to increased vertical transmission of *Listeria monocytogenes*

Samuel J. Eallonardo[1] and Nancy E. Freitag[1,2]

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2-Department of Pharmaceutical Sciences, University of Illinois Chicago, Chicago, IL, USA

Listeria monocytogenes (Lm) is a common environmental bacterium that can also act as a foodborne pathogen. Susceptible individuals such as pregnant women are more likely to be infected and develop severe invasive disease. Epidemiologic data has long supported the observation that certain clades of *Lm* are much more likely to be associated with epidemics and severe disease, however the reasons for this within species variation are not fully understood. We have previously published the observation that high InlB expression in certain *Lm* strains confers enhanced vertical transmission in animal models. InlB is a virulence factor that is non-covalently bound to the cell surface and promotes bacterial uptake into host cells. One highly invasive strain, 07PF0776, has approximately 15-fold more surface InlB compared to reference strain 10403S. This difference persists when prfA, a virulence regulator that controls *inlB* transcription, is activated. Despite the difference in protein abundance, there is no difference in *inlB* transcript between the two strains. Similarly, there is no difference in InlB protein stability or cell wall affinity. Switching *inlB* alleles between strains does not impact surface InlB levels. However, expression of *inlB* from a plasmid abolishes the difference in expression. These findings indicate that some *Lm* strains exhibit increased InlB abundance due to alterations in pre- or co-translational regulation of *inlB.* These data also suggest that expression of *inlB* from its native locus is necessary for the observed regulation.

Poster # A09
Elucidating the role of the Group B Streptococcus two component system SaeRS in vaginal colonization

Nicholas Faiola, Laura Cook

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Group B *Streptococcus* (GBS) is an opportunistic pathogen known for causing sepsis and meningitis in newborns. GBS infections of neonates are strongly correlated with the vaginal colonization state of the mother. Treating mothers with antibiotics during labor is currently the only preventative measure against GBS disease but comes with pitfalls as it's use can alter the newborn gut microbiota and increase the risk of disease by other organisms. We aim to examine new treatment targets in GBS regulated by the two-component system SaeRS. In a mouse model of GBS vaginal colonization, SaeRS was shown to be the most highly upregulated two component system compared to *in vitro* growth. SaeR directly regulates two GBS genes important for mucosal colonization, *pbsP* and *bvaP,* both of which were also highly upregulated in vaginal growth. While data suggest that a signal is detected by SaeRS in the vaginal tract to elicit transcriptional changes important for vaginal colonization, the signal sensed is currently unknown. This project aims to identify the vaginal signal(s) sensed by SaeRS using a transcriptional reporter of the *pbsP* promoter region. We will also characterize SaeRS signaling on a molecular level including investigating the SaeS protein domains required for signaling and interaction with potential partners. Successful completion of these goals will provide an in-depth understanding of the two-component system SaeRS in GBS and its regulation of genes important for vaginal colonization.

Poster # A10
Elucidating a novel mechanism of antibiotic tolerance in *Staphylococcus aureus* biofilms

Jeffrey A. Freiberg[1], Valeria M. Reyes Ruiz[2], Kyle T. Enriquez[3], and Eric P. Skaar[2]

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[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN USA,
[3]Vanderbilt University School of Medicine, Nashville, TN, USA

Staphylococcus aureus is a leading cause of infections in the United States and is associated with high rates of morbidity and mortality even when treated with appropriate antibiotics. The ability to form biofilms, microbially derived communities where cells grow attached to a surface surrounded by a complex extracellular matrix, has been associated with persistent *S. aureus* infections. A defining feature of *S. aureus* biofilms is a high tolerance to antibiotics. Despite the clinical significance of the antibiotic tolerance seen in biofilm-mediated infections, the mechanism by which this occurs is poorly understood. To identify staphylococcal proteins responsible for antibiotic tolerance during biofilm growth, mature biofilms were grown using a methicillin-resistant *S. aureus* (MRSA) strain representative of the predominant clinical isolate. These biofilms were subsequently exposed to cell wall targeting antibiotics for 48 hours and then subjected to whole proteome analysis using LC-MS/MS. SAUSA300_2378, a previously uncharacterized protein, was identified as having the greatest relative increase in abundance. Although there was no significant difference in vancomycin susceptibility between a strain containing a transposon insertion in SAUSA300_2378 and its isogenic parental strain during planktonic growth, the mutant showed a significant increase in vancomycin susceptibility during biofilm growth. SAUSA300_2378 is a protein highly conserved among sequenced *S. aureus* strains with no discernable sequence or structural homology to any known protein outside of the *Staphylococcus* genus. Therefore, this protein is potentially responsible for a novel mechanism by which *S. aureus* avoids antibiotic-mediated killing. This may present an exciting new target for specific anti-MRSA therapy.

Poster # A11
A crucial role for lipoteichoic acid assembly in the metabolic versatility and antibiotic resistance of *Staphylococcus aureus*

Troy A. Burtchett, John C. Shook, Laura E. Hesse, Philip C. Delekta, Robert S. Brzozowski, Alhakam Nouri, Alexa J. Calas, Catherine M. Spanoudis, Prahathees J. Eswara, and **Neal D. Hammer**

Staphylococcus aureus transitions between aerobic respiration and fermentation to generate energy needed to proliferate within distinct host tissues. Previous studies revealed that reducing metabolic potential and perturbing either lipoteichoic acid (LTA) or wall teichoic acid (WTA) production decreases viability. However, the mechanism(s) by which teichoic acids support *S. aureus* metabolic versatility are unknown. LTA is an essential component of the Gram-positive cell envelope that maintains ion homeostasis, regulates autolytic activity, and acts as an osmoprotectant. Structurally, LTA is a glycerolphosphate polymer that is attached to the cell via a diglucosyl-diacylglycerol lipid anchor. Synthesis of the glycolipid anchor is dependent on YpfP. Consequently, in *ypfP* mutants the glycerolphosphate chain is bound to diacylglycerol and cells contain less cell-associated LTA. To assess how LTA impacts *S. aureus* metabolic versatility, we generated *ypfP* mutant strains and monitored proliferation in respiration-arresting conditions. Aerobically, the mutants demonstrate wild type growth; however, inactivation of *ypfP* causes varying degrees of proliferation defects upon induction of respiration arrest, including

exposure to the aminoglycosides gentamicin and tobramycin. LTA production in *ypfP* mutants cultured anaerobically is further altered and the membrane potential is decreased. However, we find that fermentative growth is restored upon supplementation with cations. We also observe that in strain Newman, the *ypfP* mutant displays an anaerobic viability defect caused by induction of prophages. In total, this work demonstrates that the LTA glycolipid anchor plays a critical role in *S. aureus* metabolic versatility by maintaining ion homeostasis and the membrane potential in respiration-arresting growth conditions.

Poster # A12
Contributions of the Signaling Peptide SpoV to *Streptococcus pyogenes* Virulence

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We used peptidomics to identify the signaling peptide SpoV (streptococcal peptide controlling virulence) from culture supernatants of *Streptococcus pyogenes* (Group A Streptococcus, GAS). The expression of *spoV* was more abundant in isolate MGAS315 (serotype M3) compared to isolate NZ131 (serotype M49) which was attributed to naturally occurring mutations in the CovRS/RocA (control of virulence / regulator of *cov*) regulatory system among M3 isolates. Deletion of *spoV* altered the expression of several GAS virulence factors including streptolysin O (*slo*) and streptolysin S (*sagA*). The addition of synthetic SpoV peptide derivatives, but not control peptides, to *spoV* mutant cultures restored *slo* expression. Complementation of the *spoV* mutant with plasmids encoding *spoV* with (51 aa) or without (21 aa) the signal peptide sequence also restored *slo* expression. The results indicate that intracellular interactions of SpoV are important in the control of *slo* expression. We are currently investigating if SpoV requires a functional Opp (oligopeptide permease) system for reimportation to the cytoplasm to alter the expression of virulence factor transcripts. Because peptide signaling plays an important regulatory role during disease progression and SpoV affects gene expression, we used a *spoV* mutant in isolate MGAS315 to assess the contribution of SpoV to GAS virulence by using a murine model of invasive disease and an ex vivo human model (Lancefield assay). We then used antibodies to SpoV to evaluate their ability to decrease morbidity and mortality in mice. Results showed that SpoV is essential for GAS virulence, and targeting the peptide has therapeutic potential.

Poster # A13
Elucidating the regulatory basis for enhanced proteolysis in a clinical isolate of *Staphylococcus aureus*

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Staphylococcus aureus boasts an arsenal of virulence determinants, including 10 secreted proteases that play a dual role in infection: first, by modulating the stability and abundance of other *S. aureus* virulence factors, and secondly, by cleaving host factors to facilitate infection. Given their importance, we set out to explore natural variability in protease production in a collection of clinical isolates. Our initial screen revealed most isolates demonstrated limited proteolysis, however strain SA-337 from the USA300 lineage exhibited hyperproteolysis on casein (7.7-fold) and gelatin (~2-fold) zymography. This strain also exhibited decreased hemolysis (17-fold) and survival (4% vs 61%) within human blood. To explore the basis for these phenotypes, we performed RNA sequencing, revealing increased expression of Aureolysin (68.7-fold), Staphopain B (4.9-fold), and V8 (4.1-fold), and decreased expression of PSMα (313-fold), PSMβ (30-40-fold), and α-hemolysin (3.6-fold) in SA-337. The expression of classical virulence factor regulators was largely unchanged in SA-337, however some less well studied elements (SarV, SarZ and ArgR) were increased in expression (range: 2.9-5.5-fold). Surprisingly, upon disruption of these regulators in SA-337, we observed markedly decreased proteolysis and/or increased hemolysis. Genome sequencing of SA-337 also revealed nonsynonymous SNPs in the DNA binding domains of Rot and CcpA. Importantly, we observed decreased proteolysis upon *rot* and *ccpA* distruption in SA-337, which is counter to that observed in laboratory strains. Collectively, this work shines new light on virulence factor control and suggests that regulatory circuits may be strikingly different in contemporary strains isolated from infected patients.

Poster # A14
The glycolipid, Glc-DAG, contributes to the pathogenesis of Group B streptococcal meningitis

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Group B *Streptococcus* (GBS) is a leading cause of neonatal meningitis, which requires traversal of the endothelial blood-brain barrier (BBB), resulting in inflammation and tissue damage. A critical site for host-pathogen interaction is the bacterial cellular membrane. Membrane lipids and the membrane linked polymer, lipoteichoic acid (LTA), are important for, cell division, protein localization, stress responses, and pathogenesis. GBS encodes two glycosyltransferases responsible for synthesis of Glucosyl-Diacylglycerol (Glc-DAG), by IagB, and LTA lipid anchor, Glc2-DAG by IagA. Previous studies have demonstrated the importance of IagA to GBS BBB penetration, but the contribution of IagB and Glc-DAG during meningitis progression is unknown. To evaluate the role of IagB, we performed allelic exchange of *iagB* and determined that the resulting GBSΔ*iagB* mutant does not synthesize Glc-DAG. GBSΔ*iagB* membranes are devoid of both Lys-Glc-DAG and Glc2-DAG. We investigated the interaction of GBSΔ*iagB* with human brain microvascular endothelial cells (hCMEC) and found the Δ*iagB* mutant exhibited significantly reduced ability to attach and invade hCMECs compared to WT. Using an *in vivo* model of hematogenous meningitis, mice infected with GBSΔ*iagB* exhibited increased survival and significantly less bacterial burden in the brain and blood compared to WT infected mice. These data suggest that GBS Glc-DAG contributes to bloodstream survival and the development of meningitis. Furthermore, we have derived membrane vesicles (MVs) from GBS and have begun to characterize the lipidome. We have found that MVs contain glycolipids and LTA precursors, and ongoing studies aim to characterize the role of MV lipids during GBS pathogenesis.

Poster # A15
Th1 and Th17 cells are critical for bacterial containment during *Staphylococcus aureus* craniotomy infection

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A craniotomy is performed to access the brain for tumor resection, localization and resection of epileptogenic foci, and aneurysm clipping. Despite prophylaxis, infectious complications after craniotomy range from 1-3%, with approximately half caused by

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Staphylococcus aureus (*S. aureus*), which forms a biofilm on the bone flap that is recalcitrant to antibiotics. Using our novel mouse model of *S. aureus* craniotomy infection, Th1 and Th17 cells were found to preferentially infiltrate the brain but not the subcutaneous galea; however, their functional importance is unknown. RAG1 knockout (KO) mice displayed significant increases in bacterial burden in the brain, galea, and bone flap at days 3, 7, and 14 post -infection, which was negated following the adoptive transfer of either *in vitro* skewed Th1 or Th17 cells. Interestingly, *in vitro* skewed Th17 cells acquired Th1 characteristics upon migrating into the brain *in vivo* as revealed by the production of Th1-associated cytokines such as IFN-γ and TNF-α with minimal IL-17A. We established that the window of T cell protection was during acute infection, since delaying Th1 or Th17 adoptive transfer until day 7 post-infection was no longer able to attenuate *S. aureus* burden in RAG1 KO mice compared to T cell transfers at day -1 or 3 after infection that were effective. *In vitro* studies established that both Th1 and Th17 cells augmented microglial and macrophage *S. aureus* bactericidal activity, whereas only Th1 cells increased IL-12p70 production. Collectively, these findings highlight the importance of an early adaptive immune response for bacterial containment during *S. aureus* craniotomy infection by promoting the antimicrobial activity of infiltrating leukocytes and resident microglia. This work was supported by NIAID R01 AI169788.

Poster # A16

Group B Streptococcus Adapts to Hyper-Inflammation in Diabetic Wounds by Increasing Nuclease Activity

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Diabetic wound infections have poor healing outcomes due to the presence of numerous pathogens and a dysregulated immune response. The prevalence of Group B *Streptococcus* (GBS) in diabetic wounds is as high as 25% of individuals with a diabetic foot infection and are one of the most commonly isolated organisms from polymicrobial diabetic wound infections, with *Staphylococcus aureus* being the most frequently isolated co-microorganism. Here, we utilize a murine model of GBS wound infection and determine via dual RNAseq that GBS promotes the upregulation of inflammatory pathways and neutrophil recruitment in the diabetic wound. Further, we find that GBS triggers neutrophil activation, elastase production, and neutrophil extracellular trap (NET) formation. Bacterial RNAseq demonstrates that GBS upregulates the nuclease *nucA* 83-fold in diabetic infection in comparison to an input control. In addition, GBS colonies recovered from diabetic wound homogenates contain mutations in the two-component system *covRS.* These environmentally acquired *covR* mutations exhibit increased hemolysis of sheep's blood and increased nuclease activity in a FRET assay. Additionally, we demonstrate that co-infection with *S. aureus* results in significantly greater GBS recovery in diabetic wound tissues, possibly due to the contribution of *S. aureus nuc* in NET degradation. Collectively, these data suggest that GBS adapts in diabetic wound infection to survive hyper-inflammation, and that co-infection with bacteria such as *S. aureus* may assist GBS survival. Future studies are ongoing to determine the role of GBS and *S. aureus* nucleases in polymicrobial diabetic wound infection.

Poster # A17

TREM2 senses pathogen- and damage-associated molecular patterns to promote *Staphylococcus aureus* craniotomy infection with sex-dependence

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Craniotomy is a neurosurgical procedure involving removal and replacement of a skull fragment (bone flap) to access the brain. Despite precautions, infection occurs at 1-3%, with half due to *Staphylococcus aureus* forming a biofilm on the bone flap. Infections carry significant morbidity as the bone flap is often discarded alongside long-term antibiotic therapy. During infection, immune cells encounter both damage- and pathogen-associated molecular patterns (DAMPs and PAMPs) from the surgical procedure and bacteria, respectively. Triggering receptor expressed on myeloid cells-2 (TREM2) is a major pathology-induced signaling receptor in immune remodeling known to bind many anionic ligands encompassing both DAMPs and PAMPs. These studies used a mouse craniotomy model to assess the role of TREM2 in promoting infection. Data from scRNA-seq confirmed *trem2* upregulation in brain-resident microglia at day 7 post-infection. Comparing spatiotemporal attributes after sterile sham surgery vs. *S. aureus* infection via flow cytometry revealed monocytes were the major leukocytes recruited to the brain, whereas granulocytic myeloid-derived suppressor cells (G-MDSCs) and neutrophils were most numerous in the galea and bone flap. Patterns were similar for sham and infected mice at day 3 post-surgery despite substantial bacterial burden (10^5-10^6 CFU), implicating surgical damage as a driver of the acute immune response. Further, TREM2 loss exhibited sex-dependent effects, as bacterial burden in the brain and galea decreased in female but not male TREM2 KO mice at days 7-14 post-infection compared to WT. These results suggest TREM2 elicits anti-inflammatory responses to DAMPs and PAMPs following craniotomy to mask infection and promote biofilm formation.

Poster # A18

From survive to thrive: Evaluating the role of GraS and novel fatty acid metabolic pathways in promoting *Staphylococcus aureus* adaptation to antimicrobial free fatty acids of human skin

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Staphylococcus aureus is a Gram-positive microbe that asymptomatically colonizes 30% of humans, where it is well adapted to survive on the skin in the presence of innate defense mechanisms, including unsaturated host-derived free fatty acids (HDFFA). Previously, we found conditions that would be encountered on human skin, including acidic pH and cationic antimicrobial peptides, conferred increased resistance of *S. aureus* USA300 to unsaturated HDFFA through a mechanism that was dependent on the GraS sensor kinase and GraS-regulated gene, MprF. Although potentially toxic, HDFFA also represent a valuable source of lipids for membrane synthesis and energy production. Therefore, we investigated the function of the unstudied genes *fadXDEBA* in *S. aureus*, which are hypothesized to play a role in metabolism of fatty acids through ß-oxidation. Measuring expression of the first gene in the locus, *fadX,* we found expression was upregulated by exogenous fatty acids in a concentration dependant manner, and repressed by glucose. Furthermore, acidic pH further enhanced *fadX* expression, indicating acidic pH activates both resistance and metabolic pathways against HDFFA. Growth of *S. aureus* in media supplemented with exogenous fatty acids resulted in improved growth, viability, and protease expression, as well as alterations to phospholipid membrane composition, in a *fadXEDBA*-dependant manner. Finally, deletion of either GraS or FadXEDBA resulted in reduced virulence in a murine abscess model, an infection model where bacteria encounter high levels of HDFFA. While HDFFA can function to inhibit bacterial growth, *S. aureus* has evolved to thrive in this environmental niche through GraS, MprF, and FadXEDBA.

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Poster # A19
Methicillin-resistant *S. aureus* fibronectin binding proteins contribute to female reproductive tract colonization

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic pathogen that is a frequent colonizer of human skin and mucosal membranes, including the vagina, with vaginal colonization reaching nearly 25% in some populations. MRSA vaginal colonization can lead to transmission to newborns and aerobic vaginitis (AV), and bacterial ascension into the upper reproductive tract can lead to infertility and adverse birth outcomes. USA300, the most prominent MRSA clone to colonize pregnant individuals, is known to be a robust biofilm former and causative agent of invasive infections; however, little is known about how it colonizes and ascends in the female reproductive tract (FRT). Using *in vitro* cell culture models, I have determined that the MRSA fibronectin binding proteins (FnBPs) are critical for association with human vaginal epithelial cells (hVECs) and that MRSA invades hVECs through fibronectin-bridging with human $\alpha_5\beta_1$ integrin. By growing MRSA expressing fluorescent proteins on hVECs and imaging after 18 hours, I have shown that both FnBPs are important for biofilm formation on hVECs. FnBPs were also shown to be sufficient for these phenotypes by heterologous expression of each FnBP in an adhesin-deficient strain of *Staphylococcus carnosus*. Additionally, a MRSA strain lacking both FnBPs is attenuated in its ability to ascend into the uterus in our murine vaginal colonization model compared to the WT. Better understanding of MRSA colonization and ascension in the FRT can ultimately inform drug design and treatment strategies to limit its presence in the vagina or prevent ascension, especially during pregnancy and in those prone to AV.

Poster # A20
Peptidogenomic discovery of *Staphylococcus aureus* microproteins with cytolytic and keratin perturbing activities

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Microbial pathogenesis is a complex process involving crosstalk between a myriad of host and pathogen factors. Of these factors, our understanding of bacterial microproteins and their contributions to virulence is limited relative to larger proteins. The human pathogen *Staphylococcus aureus* is known to produce a diverse array of virulence factors that contribute to its pathogenesis; however, microproteins as *S. aureus* virulence factors remain an understudied aspect of its virulence. To address this knowledge gap, a peptidogenomic workflow was developed and applied to define the secreted microproteins of *S. aureus*. Using this approach, we identified fifty-seven non-redundant microproteins detected as 120 peptido-forms with high-confidence. Within this group two microproteins, termed *S. aureus* microprotein 1 (SAM1) and *S. aureus* microprotein 2 (SAM2), were found to have bioactivity and to be regulated by the classical *S. aureus* accessory gene regulator (*agr*) system. Further investigation has shown that SAM1 appears to act in an analogous manner as established phenol-soluble modulins (PSMs), though our data indicate SAM1's cytolytic effects are a result of unique pathway interactions not yet ascribed to any PSM class. SAM2 possesses unique bioactivity perturbing cell-to-cell contacts reminiscent of much larger enzymatic exfoliative toxins. Using biochemical and genetic approaches, we show that both SAMs can enhance *S. aureus* pathogenesis *in vitro* and *in vivo* through distinct mechanisms. This work highlights the value of studying the microproteome and its contribution to bacterial virulence.

Poster # A21
CRISPRi Phenotyping of Essential Cell Wall Genes in *Clostridioides difficile*

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Clostridioides difficile is a Gram positive, anaerobic, spore forming pathogen that is the leading cause of hospital acquired diarrhea, resulting in about 12,000 deaths per year in the United States. *C. difficile* infections (CDI) are often triggered by broad-spectrum antibiotics that disrupt the normal gut microbiota, and treating CDI with such antibiotics, while initially effective, often leads to relapse. Antibiotics that inhibit *C. difficile* selectively are likely to improve outcomes. One promising target for such drugs is the *C. difficile* cell envelope, as it has several unusual features. These include a proteinaceous S-layer and atypical peptidoglycan with primarily glucosamine instead of *N*-acetylglucosamine and primarily 3-3 peptide crosslinks rather than the 4-3 crosslinks that predominate in most bacteria. Here we describe a CRISPRi-based screen of cell wall-associated genes identified as essential in a previous large-scale Tn mutagenesis of strain R20291 (Dembek et al., 2015, mBio). Our screen involves silencing expression of these genes with 2 independent sgRNAs (for reproducibility) and evaluating effects on viability and cell morphology. To date we have tested 48 genes. 35 of these were confirmed as essential and knockdown often resulted in expected phenotypes, such as filamentation in the case of *ftsZ*. We have also found some nonessential genes, presumably false positives in the Tn-seq screen. Finally, we have found several unexpected phenotypes, including elongated cells for *cdr1170* (putative RNase), *cdr2029* (putative cation transport) and *cdr_3519* (putative membrane protein), or severely curled cells for genes *cdr1124* and *cdr1044* encoding hypothetical membrane proteins.

Poster # A22
GmPcides: New class of antibiotics to kill multidrug-resistant Gram-positive pathogens

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Healthcare-associated infections (HAIs) affect 1 in 31 patients in hospitals across the US and result in an additional $28.4 billion in annual healthcare expense. Gram-positive (GmP) pathogens that frequently cause HAIs, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE), are becoming increasingly difficult to treat with the current arsenal of antibiotics. To address the lack of therapeutics available to treat these highly resistant infections we have developed GmPcides, a new class of antibiotic based on a bicyclic 2-pyridone scaffold that are broadly efficacious against GmP pathogens, including multidrug-resistant staphylococcal and enterococcal species, as well as streptococci. We show that GmPcides have bacteriostatic activity against actively dividing exponential phase enterococcal cells and unusual bactericidal activity against non-dividing stationary phase cells. Moreover, we show that the mechanism of GmPcide-mediated stationary phase cell death is dependent on the Atn autolysin and the stationary phase protease GelE. We demonstrate that in addition to their stand-alone antibiotic activities, sublethal concentrations of GmPcides have synergistic killing effects when combined with sublethal levels of standard-of-care antibiotics such as vancomycin, gentamicin, and ciprofloxacin. Finally, we show that these combinatorial effects can be explained, in part, by altered transmembrane influx and efflux activities in the presence of GmPcides. Taken together, the unusual killing activity of GmPcides against non- dividing cells coupled with their synergistic effects when combined with several different classes of standard-of-care antibiotics make them attractive alternatives to expand our arsenal of treatments for multidrug-resistant GmP pathogens.

Poster # A23

Alanine racemase counters organic acid anion intoxication in *Staphylococcus aureus*

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Organic weak acids excreted as byproducts of host and pathogen metabolism are significant determinants of infection outcome. Due to their lipid permeable nature, weak acids can accumulate in the cytoplasm as anions to alter cellular homeostasis and inhibit growth. Intriguingly, the gram-positive pathogen *Staphylococcus aureus* can produce, tolerate, and grow in millimolar amounts of the weak acid, acetate. Here we explore the basis of staphylococcal tolerance to acetate intoxication. We demonstrate that the acetate anion inhibits D-alanyl-D-alanine ligase (Ddl) activity and perturbs intracellular D-ala-D-ala pools to compromise peptidoglycan crosslinking. However, the acetate-mediated inhibition of Ddl negatively impacted staphylococcal growth only when alanine racemase (*alr*) was inactivated, and the intracellular D-ala pools were substantially lowered. In the absence of *alr*, the native levels of D-amino transferase (Dat) activity could not sufficiently replete intracellular D-ala pools to restore growth. Furthermore, the *alr* mutant was sensitive to different biologically relevant weak acids indicating a broad conservation of inhibitory mechanism. Our findings suggest that Alr activity may have evolved as a mechanism to maintain high intracellular D-ala concentrations and counter weak acid anion intoxication.

Poster # A24

Two are Better Than One: ZccR and Smu1790c Control the Expression of the ZccE Metal Exporter in *Streptococcus mutans*

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A resident of dental biofilms, *Streptococcus mutans* is a keystone pathogen of dental caries, one of the most prevalent and overlooked diseases globally. Zinc is an essential trace metal to all forms of life that becomes poisonous at high concentrations. Because of its antimicrobial and anti- inflammatory properties and relatively low toxicity to mammalian cells, zinc is used therapeutically for various infectious and noninfectious conditions, including through incorporation in toothpastes and mouthwashes. Zinc efficacy in preventing dental caries remains controversial and the mechanisms allowing zinc survival in oral bacteria like *S. mutans* are largely unknown. Recently, we discovered *S. mutans* tolerates higher zinc compared to other streptococci because of a metal- translocating P1B-type ATPase (termed ZccE) unique to the species. Additionally, *zccE* is positively regulated by a MerR-type regulator immediately upstream, named ZccR (*zccE* regulator). Gel mobility shift assays revealed that ZccR directly and specifically binds the *zccE- zccR* intergenic region (IGR) in a zinc-dependent manner. While D*zccE* and D*zccR* strains are hyper-sensitive to zinc salts, stable suppressor strains can arise in the D*zccR* background. qRT- PCR analysis revealed basal *zccE* transcription is much higher in the D*zccR* suppressors than the parent strain. Whole genome sequencing identified a second MerR -type regulator, *smu1790*, with an early truncation all suppressor strains shared. Deletion or truncation of *smu1790* partially restored zinc sensitivity in D*zccR*. Thus, *zccE* is regulated by two MerR regulators that likely interfere with each other's capacity to bind the *zccE-zccR* IGR. Studies investigating this and defining the scope of each regulon are ongoing.

Poster # A25

The quorum-sensing *agr* system of *Staphylococcus aureus* primes tolerance to lethal oxidative stress

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The *agr* quorum-sensing system is known to link *Staphylococcus aureus* metabolism to virulence, but how *agr* affects cell survival during severe stress is poorly understood. We recently reported that *agr* activity increases bacterial survival during treatment with

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lethal concentrations of H_2O_2, a crucial host defense against *S. aureus*. Here we report that protection by *agr* surprisingly extends beyond post-exponential growth to the exit from stationary phase and to subsequent cell proliferation. Protection against killing by H_2O_2 depended on the *agr* effector molecule RNAIII and Rot, a transcription factor whose translation is inhibited by RNAIII. Expression data revealed that deletion of *agr* shifts metabolism to a fermentive mode, indicating that wild-type *agr* promotes aerobic respiration. Moreover, epistasis between deletion of *agr* and sortase, which anchors surface proteins to the cell wall, suggested that the reduced killing by H_2O_2 seen with wild-type *agr* strains occurs through down-regulation of surface proteins that perturb respiration. Respiration generates reactive oxygen species (ROS), moderate amounts of which can protect from subsequent challenge by lethal concentrations, thereby explaining *agr*-mediated protection from subsequent lethal H_2O_2 doses. SodA, which dismutates O_2^- to H_2O_2 , helps induce the low, protective levels of ROS triggered by *agr*, because increased survival of wild -type *agr* cells in response to H_2O_2 required *sodA*. Thus, homeostatic regulation of ROS levels facilitates the protective effect of *agr*. Selective enrichment and preferential multiorgan dissemination of *agr* wild-type strains during intraperitoneal infection in mice was uncoupled from *agr* activation kinetics, demonstrating the importance of *agr*-mediated priming *in vivo*.

Poster # A26

Effect of surface topography and shear stress on biofilm formation by *Listeria monocytogenes* in presence of promotor bacteria

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Pathogens can form biofilm on equipment surfaces and contaminate foods during processing. Promotor bacteria *Ralstonia insidiosa* may facilitate stronger biofilm formation of pathogens. Effects of hydrodynamic shear stresses and abiotic surface topography on single and dual species biofilm formation of *L. monocytogenes* was investigated. CDC bioreactor was inoculated with *L. monocytogenes* alone or with *R. insidiosa* for 48 h to grow biofilms on stainless steel (SS), PTFE, polycarbonate (PC) and EPDM coupons. *L. monocytogenes* and *R. insidiosa* populations in biofilms from surface coupons (n=144) were determined by spiral plating on MOX and TSA, respectively. The log CFU/cm^2 from three individual replicates were analyzed. *L. monocytogenes* recovered from dual-species biofilms from SS, PTFE and PC surfaces were statistically higher ($p < 0.05$) at the lowest shear stress of 0.013 N/m^2 compared to 0.043 and 0.088 N/m^2. At the highest shear stress of 0.088 N/m^2, *L. monocytogenes* populations recovered from dual-species biofilms were higher ($p < 0.05$) on PTFE (8.17 log CFU/cm^2) and EPDM (7.93 log CFU/cm^2) compared to SS 316L (7.38 log CFU/cm^2) and PC (7.57 log CFU/cm^2). *L. monocytogenes* recovered from dual-species environment were significantly higher than single-species biofilms under all shear stresses. PTFE and PC surface had higher surface roughness (3.17 and 2.36 µm, respectively) compared to EPDM (1.11 µm) and SS 316L (0.71 µm). Surface topography and shear stress both, impact *L. monocytogenes* biofilm formation in a dual species environment. *R. insidiosa* promotes *L. monocytogenes* biofilm formation on all materials and under all shear stresses.

Poster # A27

Elucidating factors affecting the utilization of exogenous fatty acids by *Staphylococcus aureus* using comprehensive lipidomics

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Staphylococcus aureus is a major human pathogen that is highly adaptive to the host environment. *S. aureus* only synthesizes straight-chain or branched-chain fatty acids via the type II fatty acid synthesis (FASII) pathway but can also utilize host-derived straight-chain fatty acids and unsaturated fatty acids (UFAs). To facilitate the incorporation of exogenous fatty acids (eFAs) into its membrane, *S. aureus* secretes two main bacterial lipases, Geh and Sal1, along with an esterase, 0641, to release fatty acids from host lipids. Once released by the lipases, fatty acids can be taken up by the bacteria, phosphorylated by the fatty acid kinase (FakA), and incorporated into the bacterial lipids. Incorporated serum UFAs can alter lipid packing in the membrane, affecting the susceptibility of *S. aureus* to fatty acid synthesis inhibitors and cell -membrane targeting antibiotics. Here, we aim to determine the substrate specificity of *S. aureus* secreted lipases and the effect of human serum albumin (HSA) and AFN-1252 on eFA incorporation using hydrophilic interaction liquid chromatography coupled with ion mobility-mass spectrometry. We also aim to characterize changes in membrane fluidity and the formation of reactive oxygen species (ROS) resulting from incorporated eFAs. Our work shows that a) Geh is the primary lipase responsible for hydrolyzing cholesteryl esters, b) all three lipases hydrolyze triglycerides, c) HSA serves as a source of eFAs for *S. aureus,* d) AFN-1252 leads to an increase of UFAs in the membrane with or without eFAs, and e) incorporated unsaturated eFAs leads to decreased membrane fluidity and increased ROS formation.

Poster # A28

Growth of *Staphylococcus aureus* in a defined medium

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When *S. aureus* JE2 is grown in chemically defined medium containing 14mM glucose (CDMG14), acetate is excreted concomitantly with catabolism of glucose during exponential phase. However, following depletion of glucose, acetate is not consumed nor are amino acids that fuel glutamate synthesis (proline and arginine). Therefore, post-exponential growth is not observed. This observation is in contrast to medium lacking glucose (CDM) and, in addition, the commonly utilized complex medium tryptic soy broth, where acetate is utilized in the post-exponential phase to drive the TCA cycle thus generating reducing power and ATP synthesis via respiration. First, these studies determined that acetate catabolism during growth in CDM was dependent upon *acsA* encoding acetyl-CoA synthetase. Inactivation of *acuC,* encoding a deacetylase, resulted in abrogated acetate catabolism whereas introduction of *acuA* (encoding an acetylase) into an *acuC* background phenocopied WT JE2 acetate consumption. These data suggest that AcuA functions to acetylate AcsA inactivating enzymatic activity whereas AcuC deacetylates AcsA thus activating activity. Further, it was determined that lowering the concentration of glucose to 3.5 mM (CDMG3.5) resulted in acetate consumption and arginine and proline catabolism. In addition, we found that addition of excess amino acids or a non-PTS carbon source such as gluconic acid (11.5mM) to CDM resulted in abrogated acetate catabolism.

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However, in both media arginine and proline were catabolized documenting that acetate catabolism and CcpA-dependent amino acid catabolism are not linked. Although *acsA, acuA,* and *acuC* transcription are upregulated in CDM in comparison to CDMG, transcriptional data comparing growth in CDMG14 and CDMG3.5 were not different suggesting that transcriptional regulation of *acsA* does not facilitate acetate catabolism in CDMG(3.5). Indeed, metabolomic analyses found that growth in CDMG14 resulted in enhanced intracellular concentration of Acetyl-CoA suggesting that growth in medium with excess carbon source facilitates a block in acetyl-CoA consumption, potentially via inactivation of TCA cycle activity, In conclusion, growth of *S. aureus* in defined medium should use low concentrations of the primary carbon source to ensure subsequent activation of the global transcriptional response linked to activation of the TCA cycle, post-exponential growth and respiration.

Poster # A29
Investigating *Listeria monocytogenes* infection of the gallbladder

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Listeriosis, caused by the bacterium *Listeria monocytogenes* (*Lm*), is the third leading cause of death among foodborne illnesses in the United States. Following oral inoculation of the host, *Lm* disseminates from the gastrointestinal tract to peripheral organs, from which *Lm* colonizes the gallbladder and replicates to high densities. The gallbladder then becomes the primary bacterial reservoir and source of fecally excreted bacteria. Despite its importance in *Lm* pathogenesis, little is known about how *Lm* survives and replicates in the gallbladder. The gallbladder lumen stores concentrated bile, which is antimicrobial, but *Lm* is resistant to bile stress *in vitro*. Moreover, we recently characterized the *Lm* Δ*rex* mutant that is hyper-resistant to bile stress, but attenuated in the gallbladder during murine infection. These data indicate that bile is not the primary impediment to bacterial infection of the gallbladder. Furthermore, we observed that Δ*rex* disseminated to the gallbladder and replicated similarly to wild type (WT) *Lm* in the first 24 hours post-infection, but was cleared from this organ after four days. This led to our working hypothesis that Δ*rex* induces a more robust immune response than WT, leading to increased clearance at later stages of infection. Ongoing work is aimed at characterizing *Lm* replication in the murine gallbladder during infection and the host immune response in this organ.

Poster # A30
Investigating the global impact of DNA supercoiling on *Staphylococcus aureus* gene expression

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Staphylococcus aureus is both a commensal and opportunistic human pathogen. *S. aureus* causes disease with a wide spectrum of severity, ranging from boils and soft tissue infections to life-threatening septicemia, meningitis, and endocarditis. To cause such diseases, *S. aureus* has adapted to reside in a variety of human tissues, including the heart, bones, joints, and various bodily fluids. For *S. aureus* to cause infection in these different niches, it must deploy an assortment of regulatory and pathogenic processes. One understudied mechanism of gene regulation in *S. aureus* is DNA supercoiling. Supercoiling is a dynamic structural property of DNA, known to modulate virulence gene expression in several bacterial pathogens, however its role in *S. aureus* is not well understood. Here we investigate how alterations in DNA supercoiling influence gene expression in *S. aureus*. Sub-inhibitory concentrations of novobiocin were added to *S. aureus* cultures to inhibit DNA gyrase activity causing global relaxation of DNA supercoiling. The relaxation of supercoiling was monitored by chloroquine gels and the impact of this relaxation on gene expression was monitored by RNA-seq. Results demonstrate widespread changes in gene expression in response to relaxation of supercoiling. Using supercoiling responsive genes identified by RNA-seq, we are investigating whether supercoiling directly or indirectly influences the activity of *S. aureus* promoters, as has been described in other organisms. Finally, to further understand the role of supercoiling regulated gene expression in vivo, we have examined the global supercoiling response of *S. aureus* during growth in a variety of human bodily fluids.

Poster # A31
Prophage-encoded methyltransferase increases virulence in an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*

Robert J. Ulrich[1], Magdalena Podkowik[1], Rebecca Tierce[2], Irnov Irnov[1], Keenan Lacey[1], Daiane Boff[1], Chunyi Zhou[1], Gregory Putzel[1], Nora Samhadaneh[1], Francois-Xavier Stubbe[3], Erin E. Zwack[1], Sabrina M. Morales[1], Alejandro Pironti[1], Victor J. Torres[1,*] and Bo Shopsin[1,*]

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We recently identified a clonal variant of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 causing a cluster of severe skin and soft tissue infections in children from Brooklyn, New York (USA300-BKV). The primary event associated with emergence of USA300-BKV was acquisition of a mosaic version of phage Φ11 (mΦ11). We introduced mΦ11 and wild-type Φ11 into the prototype CA-MRSA strain USA300-LAC and showed that strains containing mΦ11 produced significantly larger skin abscesses in mice than USA300-LAC or USA300-LAC containing wild-type Φ11. However, like many temperate bacteriophages in *S. aureus*, mΦ11 does not contain any apparent virulence factor, so the gene(s) responsible for increased virulence were unknown. Here we show that a phage-encoded adenine methyltransferase (MTase) increased virulence and biofilm formation in USA300. Specifically, we performed *en bloc* deletions of mΦ11 in USA300-LAC and localized the virulence gene(s) to a block containing MTase. Deletion and complementation studies showed that MTase was necessary for the skin abscess phenotype, and expression of MTase in wild-type USA300-LAC was sufficient to increase murine skin abscess size. Additionally, we discovered that expression of MTase in wild-type USA300-LAC promoted biofilm formation *in vitro*. Notably, MTase lacks a partner restriction endonuclease, suggesting it may be involved in transcriptional regulation, as has been found for other 'orphan' methyltransferases. Collectively, these data suggest that a phage-encoded methyltransferase increases USA300 virulence and biofilm formation through epigenetic changes. More generally, the results support the idea that the role of phages in virulence extends beyond acquisition of toxin genes.

Poster # A32
Histone Deacetylase and BET Proteins Regulate Inflammatory Mediator Production in Response to *Staphylococcus aureus*

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A craniotomy is a neurosurgical procedure performed to access the intracranial compartment. Despite advancements in surgical technique, infections occur in 1-3% of cases, half of which are caused by *Staphylococcus aureus* (*S. aureus*). This pathogen

forms a biofilm on the bone flap surface. Once mature, it seeds the brain parenchyma with planktonic bacteria, eliciting microglial activation and leukocyte recruitment into the central nervous system (CNS). This is accompanied by unique transcriptional signatures which support cellular programming and coincides with the induction of inflammatory mediators including tumor necrosis factor-α (TNF-α), CCL2, and IL-6. Epigenetic mechanisms are partially responsible for immune cell activation, allowing rapid response to noxious stimuli. We hypothesized that *S. aureus* drives epigenetic modifications in CNS immune cells to regulate their effector function. We designed a high-throughput *in vitro* screen of an epigenetic compound library using primary mouse microglia, which identified Bromodomain and Extraterminal domain (BET) family members and various histone deacetylases (HDACs) as critical for regulating TNF, IL-6, and IL-10 production by microglia in response to *S. aureus*. Similar trends were observed in macrophages, neutrophils, and granulocytic myeloid-derived suppressor cells (G-MDSCs), all key components of the immune response elicited by craniotomy infection. Expression of various Class I HDACs were altered in G-MDSCs, neutrophils, macrophages, and microglia following *in vitro* and *in vivo S. aureus* exposure. Our data suggest that inflammatory mediator production is under the control of the acetylation system during CNS craniotomy infection and aberrant epigenetic regulation and environmental signals may be important in dictating bacterial persistence.

Poster # A33

The nontoxigenic *Clostridioides difficile* CCUG37785 protects mice against infection with *C. difficile* R20291

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Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL 33612

Symptoms of *C. difficile* infection (CDI) are attributed largely to two *C. difficile* toxins, TcdA and TcdB. In this study, we aimed to determine whether the non-toxigenic strain *C. difficile* CCUG37785 can protect mice against *C. difficile* infection. Firstly, we compared *C. difficile* CCUG37785 with *C. difficile* R20291 in sporulation, germination, adhesion to human intestinal epithelial cells, motility and biofilm formation. We found that *C. difficile* CCUG37785 exhibited significantly higher adhesion and sporulation rates, significantly lower spore germination rates and biofilm formation, while comparable motility in comparison with *C. difficile* R20291. Secondly, we evaluated the prevention and treatment efficacy of *C. difficile* CCUG37785 spores in mice against *C. difficile* infection. Our data showed that pre-oral inoculation of *C. difficile* CCUG37785 spores prior to infection with strain R20291 provided almost full protection in mice against developing CDI symptoms. However, oral inoculation of *C. difficile* CCUG37785 spores after infection with strain R20291 only provided minor protection in mice against developing CDI symptoms. Further analysis showed that mice pretreated with *C. difficile* CCUG37785 spores secreted significantly less amount of *C. difficile* R20291 spores, while mice treated with *C. difficile* CCUG37785 spores after infection with R20291 secreted similar amount of R20291 spores in comparison with mice infected with R20291 spores only. Our data not only highlight the potential use of *C. difficile* CCUG37785 as a therapeutic strain for the prevention of primary and recurrent CDI in humans and but also further support its use as an oral mucosal vaccine carrier against CDI.

Poster # A34

Identification of Arginine Transporters in *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive bacterium that colonizes approximately 30% of the population and causes infection in various host niches including the skin and soft tissue. Glycolytic activity is required to initiate a skin and soft tissue infection indicating that carbon catabolite repression (CCR) mediated by CcpA likely represses secondary carbon source metabolism, including that of many amino acids. Due to CcpA-mediated repression, *S. aureus* is an arginine and proline auxotroph in chemically defined medium containing glucose (CDMG). Thus, we hypothesize that arginine transport is required for skin and soft tissue infection. Within *S. aureus* USA300, two known arginine/ornithine antiporters are encoded on the two copies of the ADI operon, ArcD1 and ArcD2. However, when the two antiporters are inactivated, *S. aureus* grows in the presence of canavanine, a toxic arginine analog, indicating there are other unidentified arginine transporters importing the toxic analog. Therefore, to identify essential arginine transporters, we utilized Nebraska Transposon Mutant Library (NTML) and suppressor screens using canavanine. Following the screening of amino acid permease mutants from the NTML, SAUSA300_1231 and SAUSA300_2383 displayed reduced growth yields in CDM. In addition, thirteen of the fourteen suppressor mutants resistant to canavanine displayed a single nucleotide variation in the uncharacterized amino acid permease SAUSA300_2383. In conclusion, the protein encoded by SAUSA300_2383 was identified as a potential arginine transporter and provides insight into proteins that are potentially required during infection.

Poster # A35

A peptide motif in two distinct Gram-positive virulence regulators links carbon catabolite repression to virulence gene expression

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Carbon catabolite repression (CCR) is a conserved phenomenon in bacterial species, where genes involved in the transport and catabolism of less preferred sugars are repressed in the presence of a preferred sugar. In some pathogens, such as *Streptococcus pyogenes* and *Listeria monocytogenes*, it was observed that the master transcriptional regulators of virulence are also subject to CCR. In *S. pyogenes*, Mga is a unique transcription factor that interacts with Phosphotransferase System (PTS) sugar transport proteins, which via phosphorylation events, exert CCR on Mga regulation of virulence and quorum-sensing (QS) genes. Previous studies suggested that the Mga C-terminus $EIIB^{Gat}$ domain is only involved in Mga oligomerization; however, our results indicate this domain also plays a role in CCR of the Rgg2/3 QS system. A single amino acid substitution of a cysteine residue within the $EIIB^{Gat}$ domain suppressed induction of Rgg2/3 QS in a carbohydrate dependent manner. The cysteine residue is flanked by three aromatic amino acids, and this 4-amino acid sequence is similar to a motif within glycerol kinase that is phosphorylated by PTS components. The master virulence regulator PrfA of *Listeria monocytogenes* contains a similar motif, and substitution of the cysteine residue within this motif resulted in alteration of virulence traits in a carbohydrate dependent manner. These results strongly suggest that this novel conserved motif is central to the regulation of virulence gene expression by CCR in at least two important Gram-positive pathogens.

Poster # A36

Putative oligoendopeptidase PepF proteins control the SaeRS two-component system in *Staphylococcus aureus*

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POSTER SESSION "A" ABSTRACTS

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The SaeRS two-component system (TCS) controls the production of key virulence factors in *Staphylococcus aureus*. The system is activated via phosphorylation during infection in response to neutrophil-produced factors including human neutrophil peptides. During a screen for factors required for full Sae function, we identified genes coding for two putative oligoendopeptidase enzymes that we have named PepF1 and PepF2. Bioinformatics analysis indicates PepF1 and PepF2 belong to the M3 family of zinc-dependent peptidases that have been extensively studied in eukaryotes. However, the biological roles of M3 peptidases in prokaryotes remain enigmatic. Herein, we show that disruption of *pepF1* or *pepF2* results in reduced basal Sae activity and HNP-mediated induction. Sae activity was further reduced in the *pepF1 pepF2* double mutant. Sae activity was increased in the mutants when wild-type *pepF* alleles were ectopically expressed but not when alleles coding for putative active site mutants were expressed, suggesting oligoendopeptidase activity is important for Sae function. Lack of PepFs reduced coagulase activity and altered the abundance of secreted proteins in a Sae-dependent and Sae-independent manner. We also found that PepF proteins are required for full virulence in a mouse model of systemic infection and for survival in human blood. These findings suggest that *S. aureus* PepFs are important for virulence by regulating Sae TCS activity. Current work is focused on identifying substrates for *S. aureus* PepF enzymes and identifying factors that control *pepF* expression. In doing so we will gain understanding of *pepF* regulation and how this relates to control of Sae activity.

Poster # A37

Experimental Evolution of *Staphylococcus aureus* Identifies Pathways That Promote Intestinal Colonization

Chunyi Zhou[1], Miranda B. Pawline[1], Alejandro Pironti[1,2], Magdalena Podkowik[2,3], Robert J. Ulrich[3], Alannah Lejeune[1], Sabrina Morales[3], François-Xavier Stubbe [4], Anthony R. Richardson[5], Paul D. Fey[6], Ken Cadwell[1,2,7], Victor J. Torres[1,2], and Bo Shopsin[1,2,3]

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Depletion of commensal microbiota increases susceptibility to gastrointestinal colonization and subsequent infection by antibiotic -resistant opportunistic pathogens such as *Staphylococcus aureus*. Here we colonized germ-free mice to investigate how gut lacking microbiota shapes the evolution of *S. aureus*. Genetic analyses and animal models revealed that fitness (manifested as emergence of a dominant clone) changed concurrently with the emergence of mutations in two mutually exclusive genetic pathways involving either (*i*) *walKR* that increases autolysis and biofilm formation, or (*ii*) *ahrC*/*arcR* that are predicted to increase arginine synthesis and consumption. Additionally, mutation in either pathway tended to be preceded by mutations predicted to increase glucose uptake, thereby priming the clones for success. The *walKR* and *ahrC/arcR* mutants co- existed and did not rise to fixation, perhaps owing to spatial partitioning. In contrast to these observations, if an intact murine microbiota was present, only wild-type *S. aureus* was recovered, indicating that evolution is shaped by the microbiota. Moreover, we found that colonization- adaptive mutations often attenuated virulence and antimicrobial resistance, indicating that the microbiota maintains these hallmark traits of *S. aureus*. Collectively, these data provide mechanistic insight into the ways that *S. aureus* diverges genetically and phenotypically in the absence of microbiota, pointing to metabolic genes and pathways that fine-tune cell proliferation and fitness in the gastrointestinal tract. Our results also uncovered a previously unknown metabolic interplay between colonization, virulence, and antimicrobial resistance that may constrain *S. aureus* evolution, providing a potential explanation to the extensive species-wide diversity in these important characteristics.

Poster # A38

The adjacent Pi ATP-binding protein-encoding genes *pstB1* and *pstB2* of *Enterococcus faecalis* have different cellular functions

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Enterococcus faecalis is a gram-positive gastrointestinal commensal that has emerged as a leading healthcare-associated pathogen. Clinical isolates are often antibacterial resistant, form biofilms, and are adaptable to many environments, making treatment difficult. Understanding genetic mechanisms responsible for *E. faecalis* virulence is critical identifying novel methods for treatment.

Phosphate homeostasis contributes to virulence and biofilm formation in many bacterial species. The high-affinity importer called the phosphate specific transport (Pst) operon is one mechanism by which bacteria acquire Pi. *E. faecalis* contains both *pstB1* and *pstB2* as a part of this operon. The functions of the proteins encoded by these genes have never been addressed in any of the species that contain both. We have generated in-frame deletions of *pstB1* and *pstB2* in *E. faecalis* in order to characterize the roles of these genes.

Δ*pstB1* and Δ*pstB2* exhibited increased susceptibility to bile salts and SDS on solid medium. Δ*pstB1* growth lagged compared to WT, and Δ*pstB2* was unable to be grown in chemically defined medium (CDM). The Δ*pstB1* strain exhibited lower Pi uptake, which was rescued by ectopic expression of *pstB1* and over-expression of *pstB2*. Interestingly, the Δ*pstB2* strain appeared to lose Pi into the medium. Finally, in biofilms grown for 6 hours in CDM, Δ*pstB1* had less stainable biofilm biomass compared to WT. Both complemented and cross-complemented mutants showed restoration to WT. Biofilm formation of Δ*pstB2* could not be measured since the strain did not grow in CDM unless the mutant was complemented with ectopic expression of WT *pstB2*.

ICG+P POSTER SESSION "B" ABSTRACTS

TUESDAY, OCTOBER 11, 2022
1:10 P.M.—2:40 P.M.

POSTER NUMBERS AND PRESENTERS

B01 Ivan Acosta
B02 Daiane Boff
B03 Nick Carpino
B04 Soumyadeep Chakraborty
B05 Ayan Chatterjee
B06 Karine Dufresne
B07 Ashley DuMont
B08 Timothy Enroth
B09 Liwei Fang
B10 Ronald Flannagan
B11 Vijay Gondil
B12 Luke Handke
B13 Saika Hossain
B14 Mary-Elizabeth Jobson
B15 Theodora Karagounis
B16 Callahan Katrak
B17 Pola Kuhn
B18 Kaylie Lam
B19 Catherine Leasure
B20 Cheyenne Lee
B21 Abraham Mechesso
B22 Krista Mills
B23 Adriana Morales Rivera
B24 Amber Nguyen
B25 Dane Parker
B26 Christopher Radka
B27 Zachary Resko
B28 Itidal Reslane
B29 Concepcion Sanchez
B30 Cleofes Sarmiento
B31 Gwenn Skar
B32 Qing Tang
B33 Nathanial Torres
B34 Nicholas Walton
B35 Marcus Wittekind
B36 Brianne Zbylicki
B37 Zongsen Zou

POSTER SESSION "B" ABSTRACTS

Poster # B01
Lipoic acid mediates oxidative stress tolerance in *Staphylococcus aureus*

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Lipoic acid (LA) is a metabolic cofactor present in all domains of life, playing an essential role in the activity of several multienzyme complexes involved in the central carbon metabolism, such as pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (OGDH), branched-chain 2-oxoacid dehydrogenase (BCODH) complexes, and the glycine cleavage system. Besides its function as an enzymatic cofactor, LA is considered a broad spectrum antioxidant, scavenging free radicals and recycling low molecular weight antioxidants. However, little is known about how the bacterial cell rewires LA metabolism to respond to oxidative stress. Using the human pathogen *Staphylococcus aureus* as a model, we demonstrated that the LA attachment to the glycine cleavage system H-like protein (GcvH-L) is required to tolerate reactive chlorine species (RCS). GcvH-L is part of an unusual operon, *sirTM-lplA2,* that encodes the lipoyl ligase, LplA2, which transfers LA to major metabolic complexes that require the cofactor for function. The *sirTM-lplA2* locus also contains open reading frames encoding Macro, an ADP-hydrolase, and an ADP-ribosylating sirtuin (SirTM) predicted to be involved in oxidative stress homeostasis. We found that Δ*sirTM* and Δ*gcvH-L* mutants dramatically impaired the ability to cope with oxidative stress and had an attenuated phenotype during systemic infection, including reduced bacterial loads from the peritoneal cavity and kidneys. Our data suggest the interplay between posttranslational modifications on GcvH-L regulates oxidative stress resistance in *S. aureus,* where SirTM senses the oxidative stress and regulates LA attachment to GcvH-L to alleviate RCS-mediated cell death.

Poster # B02
Understanding the pathogenesis of *Staphylococcus aureus* skin and soft tissue infections by using a mouse-adapted strain

Daiane Boff[1], Kody Mansfield[2], Xuhui Zheng[1], Keenan Lacey[1], Piotr Konieczny[2], Silva Holtfreter[3], Shruti Naik[2], Victor J. Torres[1]

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Staphylococcus aureus is a Gram-positive bacterium that is responsible for a large number of skin and soft tissue infections (SSTIs). While mouse models have been extensively used to study host-pathogen interactions during SSTIs, these models have many limitations. One of these limitations is the use of human-adapted *S. aureus* strains and the requirement of high doses of bacteria to cause disease. A second limitation is that many virulence factors produced by *S. aureus* isolates form human infections are not active in mice. In an attempt to overcome these limitations, we are studying SSTIs using a natural mouse-adapted *S. aureus* strain (strain DIP). In our preliminary studies, we have observed that DIP is notably more virulent than the human-adapted SSTI strain USA300-LAC in SSTI models of subcutaneous and intradermal infection. Specifically, the strain DIP induced more severe dermonecrotic lesions even at lower inocula compared to strain LAC. Surprisingly, DIP was also readily able to disseminate form the localized skin infection into the bloodstream inducing sepsis. To identify potential virulence factors that are driving the increased virulence of DIP we are combining comparative genomics, proteomics and mutagenesis. Taken together, we hope that these models will enable us to gain much needed insight into how *S. aureus* thrives as a skin pathogen.

Poster # B03
Protection from systemic *S. aureus* infection by inactivation of the Sts phosphatases

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The Suppressor of TCR signaling proteins (Sts-1 and Sts-2) are two homologous phosphatases that negatively regulate a number of hematopoietic cell signaling pathways, including in T cells, bone marrow monocytes and other phagocytes, mast cells, and platelets. They have a unique multi-domain structure, with an N-terminal ubiquitin-association (UBA) domain, a central Src-homology 3 (SH3) domain, and a C-terminal histidine-phosphatase (HP) domain. Located between the UBA and SH3 domains is a recently discovered 2H-phosphoesterase domain with associated phosphodiesterase (PDE) activity. Here, we demonstrate that mice lacking expression of both Sts proteins (*Sts* -/-) are profoundly resistant to infection by the Gram positive bacterium *Staphylococcus aureus*, with resistance characterized by enhanced survival following high dose intravenous infection. In addition, *Sts*-/- animals display accelerated bacterial clearance in peripheral organs, including liver, spleen, and kidney. The accelerated bacterial clearance observed in *Sts*-/- tissues occurs in the absence of any overt increases in the expression of key pro-inflammatory cytokines. We are currently investigating cell types involved in mediating the phenotype of enhanced resistance to *S. aureus* and characterizing relevant anti-bacterial effector responses. To that end, we have observed that *Sts*-/- BM-derived macrophages exhibit markedly enhanced restriction of intracellular CFUs. Our observations implicate the Sts proteins as important regulators of the host immune response to *S. aureus* and underscore the potential for a role for Sts in regulating functionally relevant immune signaling pathways.

Poster # B04
Functional characterization of an OmpR family two-component system from *Clostridioides difficile* R20291

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Clostridioides difficile R20291, a hypervirulent strain, has been a focal point of study for the Clostridia-associated disease for quite a long time. There are several two-component systems (histidine kinase and associated response regulator) involved in regulating various pathways within the organism, which can lead to controlling the associated disease. OmpR family proteins are mainly well established for their role as transcriptional regulators and signal receivers from its associated histidine kinase. Such a two-component system was identified in *Clostridioides difficile* R20291 strain which was strategically located upstream of another gene involved in regulating S-layer protein and flagellin family proteins. Hence the primary focus of this project is to investigate the transcriptional regulation of the two-component system on cell surface protein leading to virulence control. CRISPR-Cpf1 one-step gene knockout strategy was employed to create a strain in which both the histidine kinase and the response regulator of the two-

component system were disrupted. Various functional parameters were investigated that showed marked differences among the wild type and the knockout stain. RNA sequencing was further employed to understand the disruption in transcriptional equilibrium among various genes regulated by the two-component system under investigation.

Poster # B05

The Role And Dynamics Of Ethanolamine-Utilizing Bacterial Microcompartments

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Bacterial pathogens face many challenges within the host environment including nutrient starvation. Therefore, finding alternative nutrient and fuel sources is of key importance. Ethanolamine (EA) is a potential source of carbon, nitrogen and/or energy in host environments for many bacteria. These bacteria have a *eut* (ethanolamine utilization) locus that consists of the genes required for EA metabolism. Catabolism of EA takes place inside special microcompartments known as bacterial microcompartments (BMC), which are viral capsid-like proteinaceous icosahedral structures. In this study using *Listeria monocytogenes* as a model organism, the importance and dynamics of BMC formation was examined with the hypothesis that it is a nutrient source that contributes to intracellular replication. *L. monocytogenes* was able to grow on a nitrogen-free minimal media (ACMM) only when EA or glutamine was added as a nitrogen source. On the other hand, *eutV* and *eutB* knockout strains were not able to utilize EA as a nitrogen source and did not grow. Addition of glutamine as a nitrogen source rescued the growth defect in the knockout strains. qRT PCR analysis showed that the *eut* genes were upregulated upon addition of EA to the ACMM media. Confocal imaging of the recombinant bacteria expressing EutK-mNG showed the formation of BMC like structures in presence of EA, which was greatly reduced in the *eutV* knockout strain. The *eutV* and *eutB* knockout strains were defective of intracellular replication in BMDM. The study confirms the hypothesis that in the model organism *L. monocytogenes*, EA can be used as a nutrient source, specifically of nitrogen, and that this metabolism contributes to optimal intracellular replication.

Poster # B06

TSST-1 promotes the early stage of *Staphylococcus aureus* vaginal colonization

Dufresne, K., Coleman, C.E.M., Kasper, K.J., McCormick, J.K.

Staphylococcus aureus became infamous in the early 1980s for causing menstrual toxic shock syndrome (mTSS) that was associated with the use of high-absorbency tampons. Today, mTSS remains a rare but life-threatening disease that is a significant risk to women's health. Although mTSS is a dangerous disease, it is also vital to understand the mechanisms allowing *S. aureus* to colonize the vaginal tract as this is a key step for mTSS pathogenesis. Previously, Chiaruzzi *et al*. (2000) identified the presence of *S. aureus* on tampons from 27% of healthy women and moreover, Gajer *et al*. (2012) followed the temporal dynamics of the vaginal microbiota from 32 women and observed presence of *Staphylococcus* species in the majority of the volunteers. The key determinant of mTSS is the superantigen TSST-1, that forces the activation of T cells and can lead to a cytokine storm. Thus, the aim of this study is to determine if TSST-1 contributes to vaginal colonization by *S. aureus*.
To evaluate how TSST-1 may contribute to vaginal colonization, we inoculated the vaginal canal of BALB/c mice with *S. aureus* MN8, or MN8 D*tst*, and measured the colony-forming units (CFU) at various timepoints. The menstrual cycle of mice was synchronized by beta-estradiol 24 hours prior to bacterial inoculation. After 3 days of colonization, there was a significant decrease in colonization in MN8 D*tst* compared with wildtype MN8. However, by day 7, the MN8 D*tst* strain reached similar levels to wildtype MN8. The difference in colonization could be caused by immune response manipulation alone or by the fact that TSST-1 may result in niche remodelling that inhibited other members of the vaginal microbiota. When we evaluated the vaginal production of inflammatory cytokines between 2 hours and 3 days, we noted an increase in IL-1b and MIP-1b in wildtype MN8 at 24 hours, but with an increase in pro-inflammatory cytokines by MN8 D*tst* at 12 and 48 hours. To evaluate if TSST-1 conferred an advantage in niche remodelling, we added chloramphenicol to water while the *S. aureus* strains harboured the chloramphenicol resistant pCM29 plasmid. Three days post-inoculation, the antibiotic-treated mice had no difference between the wild-type and D*tst* groups in term of CFU and both strains recovered CFU similar to wild-type strain without antibiotics treatment. This project suggests that the production of the superantigen TSST-1 may influence colonization of the vaginal tract by competing against the vaginal microbiota.

Poster # B07

The murine host does not promote restoration of capsule production by USA300 *Staphylococcus aureus*

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Capsular polysaccharide (CP)-based vaccines have been successful against bacterial pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*. There is still no approved vaccine to prevent *Staphylococcus aureus* infections, but CP5 and CP8, the predominate capsule types produced by *S. aureus*, have long been considered viable vaccine antigens. After showing promise in preclinical vaccination models, two CP-based *S. aureus* vaccines were tested in clinical trials. However, these vaccines failed in late-stage trials due to lack of efficacy to prevent *S. aureus* infection, despite inducing strong anti-capsule antibody responses in patients. The failure of these CP-based vaccines may be partly explained by the observation that most contemporary USA300 *S. aureus* isolates do not produce capsule. Genomic studies have uncovered that USA300 strains contain up to four mutations in the capsule locus abolishing capsule production. Despite these findings, a recent study reported that USA300 isolates can revert to produce capsule *in vivo*, reinvigorating the discussion of including CP in a *S. aureus* vaccine. To test the possibility that host-mediated pressure can revert the mutations found in USA300, we engineered isogenic capsule-positive (Cap^+) and capsule-negative (Cap^-) USA300 strains, and utilized naturally occurring Cap^+ and Cap^- clinical isolates. Our study revealed no host-mediated reversion by USA300 strains. Moreover, we observed no impact of capsule on bacterial burden of USA300 in a murine bloodstream-infection model, suggesting that capsule is dispensable for USA300 infection. Our data further support the idea that targeting capsule might be unnecessary for the development of an effective *S. aureus* vaccine.

Poster # B08

***Staphylococcus aureus* Virulence Determinants During the Transition from Skin Colonization to Infection**

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POSTER SESSION "B" ABSTRACTS

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Staphylococcus aureus and its antibiotic-resistant derivative, methicillin-resistant *Staphylococcus aureus* (MRSA), are the top causative agent of skin and soft tissue infections globally. In recent years, much work has been conducted in trying to elucidate specific factors that help MRSA transition from healthy skin colonization to an active infection, yet many of the specific virulence determinants and skin-relevant mechanisms remain largely uncharacterized. We developed a new model of asymptomatic colonization of mouse skin and used this model to determine the MRSA transcriptional profile by RNA-seq at 5 and 24 hr post-colonization. At 5 hr post-colonization, using a fold-change cutoff of 2 ($p < 0.05$), we found that 512 genes were upregulated whereas 403 were downregulated. Using the same criteria at 24 hr post-colonization, 544 genes were upregulated and 340 were downregulated. Virulence genes encoding for numerous toxins (e.g. PVL, Hlg), proteases (Spls, Ssp) and adhesins (e.g. SasF, SraP, ClfA, FnbA), and many genes involved in amino acid and fatty acid metabolism were significantly upregulated during healthy skin colonization. To follow-up on these RNA-seq data, MRSA mutants lacking surface proteins SasF and SraP were tested in a murine infection model and found to be important for skin abscess development. Elucidating and confirming the mechanisms by which MRSA transitions from skin colonization to infection is an important step that needs to be understood in order to effectively prevent and treat MRSA infections.

Poster # B09
Study of MroQ-mediated post-translational modification of the quorum sensing peptide AgrD

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The accessory gene regulatory (Agr) system of *Staphylococcus aureus* is essential for infection. It controls expression of several virulence factors via a small cyclic peptide pheromone known as an autoinducing peptide (AIP). The Agr system encodes a precursor peptide, AgrD, that consists of an α-helical leader, AIP, and charged C-terminal tail. The AgrD precursor peptide is matured to AIP by the action of two enzymes, AgrB and MroQ. AgrB cleaves the C-terminal tail and promotes formation of a thiolactone-containing Leader-AIP peptide intermediate. MroQ, a recently characterized protease, is responsible for cleaving the N-terminal α-helix and assists in transport of mature AIP across the lipid bilayer. However, neither the regulation of MroQ nor the physical attributes of AgrD that dictate maturation and transport are fully defined. We found the mroQ gene expression is not regulated by Agr and expression levels appear to remain consistent throughout growth. In addition, several engineered peptide intermediates were sufficient to drive MroQ-dependent N-terminal cleavage, AIP maturation, or peptide export. We found the MroQ efficiently removes the N-terminal leader form both linear Leader-AIP and Leader-AIP-thiolactone, suggesting that proteolytic activity does not depend on cyclization of the peptide. Nevertheless, thiolactone ring formation remains essential to the generation of active AIP. Thus, we propose either a sequential or tandem peptide processing event involving both MroQ and AgrB. Furthermore, expression of Leader-AIP-thiolactone in ΔagrB mutant led to reduced Agr activation, suggesting that AgrB and MroQ may act together to facilitate peptide transport in addition to peptide cleavage.

Poster # B10
Elucidating the iron acquisition mechanisms that enable *Staphylococcus aureus* growth within macrophages

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The macrophage phagolysosome represents an antimicrobial niche that is nutrient deplete. Despite this, *Staphylococcus aureus* can replicate within the mature phagolysosome which necessitates that *S. aureus* acquire nutrients, such as iron, for growth. To understand which iron acquisition loci *S. aureus* strain USA300 uses to replicate within macrophages a mutant, D4, was created that lacked the *sfa*, *sbn, cntKLM and feoAB* loci encoding staphyloferrin A/B, staphylopine and the ferrous iron transporter FeoAB, respectively. Growth analysis under iron restricted conditions using mock macrophage infection assays revealed that D4 cannot grow and in fact loses viability. In contrast, upon macrophage infection and gentamicin protection, D4 can replicate indicating the bacteria derive iron from within the macrophage. Fluorescence-based bacterial proliferation assays in conjunction with confocal microscopy confirmed that D4 commences growing inside LAMP-1-positive phagosomes akin to wild-type *S. aureus*. Using D4, we sequentially inactivated the *sst* and *fhuCBG* loci, that encode catechol and hydroxamate siderophore utilization, giving rise to the strains D5 and D6. Remarkably, these strains, despite failing to grow *in vitro* under conditions of iron restriction in the presence of norepinephrine or deferoxamine, retain the ability to proliferate upon macrophage infection. Finally, using a systemic murine model of infection we find that wild-type USA300 and D6 display similar bacterial burden in the liver and kidney at 96h post-infection. Taken together these data indicate that *S. aureus* is not reliant on known siderophore acquisition systems for growth within macrophages and to colonize visceral organs *in vivo* under the conditions employed here.

Poster # B11
Penicillin binding protein-4: a novel target for the therapeutic intervention of *Staphylococcus aureus* infections

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Peptidoglycan (PG) biosynthesis is required for bacterial viability and is mediated, in part, by a set of penicillin binding proteins (PBPs), which catalyze PG crosslinking and are the cellular targets of β-lactam antibiotics. Recent studies indicate that *S. aureus* PBP4 mediates resistance to fifth-generation cephalosporins and that the protein may be a previously unappreciated virulence factor that modulates cortical bone osteocyte lacuno-canalicular network (OLCN) invasion, providing a niche for re-occurring bone infection. Consequently, small molecule inhibitors of *S. aureus* PBP4 may have therapeutic promise as agents that: 1. Reverse antibiotic resistance, 2. Reduce re-occurring osteomyelitis, or 3. Both. We developed a high throughput screen and secondary assays to identify putative PBP4 inhibitors. Fractional inhibitory concentration testing revealed a subset of compounds that re-

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duced *S. aureus* PBP4 mediated cephalosporin resistance in several strain backgrounds and had no impact on human cell viability. Hits could be further distinguished as agents that reduce *S. aureus* PBP4 transcription or those that are likely to bind the protein directly, as judged by fluorescent b-lactam studies. The front runner compound, 9314848, is a relatively strong PBP4 inhibitor (apparent IC_{50} 13.4 μM), reduced PBP4-mediated ceftibiprole resistance 16-fold and also eliminated (EC_{50} 6.25 μM; 2.5 μg/ml) PBP4-mediated transmigration through *in vitro* μSiM-CA channel pores engineered to mimic OLCNs, suggesting it is a promising scaffold for translational development. Computational modeling and follow-on chemical design have allowed a workable structure activity relationship and synthesis of analogs with improved PBP4 inhibitory activities that are now the subject of lead generation.

Poster # B12

Regulation of arginine biosynthesis in *S. aureus* by ARG family transcriptional regulators

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S. aureus encodes two pathways for the synthesis of the amino acid arginine from other amino acid precursors: the well-conserved glutamate conversion pathway and a novel proline conversion pathway. Irrespective of these biosynthetic pathways, *S. aureus* is generally regarded as an arginine auxotroph. Arginine synthesis in bacteria is frequently governed by members of the ARG family of transcriptional regulatory proteins, which form oligomers and repress this process in the presence of arginine. *S. aureus* USA300 strains encode three ARG family homologs: *ahrC*, *argR1*, and *argR2*. It has been demonstrated recently that mutations in *ahrC* restore arginine prototrophy via upregulation of *arcA1B1D1C1* and *argGH*. To more fully characterize the regulation of arginine biosynthesis by *ahrC*, recombinant AhrC protein was expressed in *E. coli* and purified. Electrophoretic mobility shift assays were performed with the purified protein and promoters of *arcA1B1D1C1* and *argGH*. These assays demonstrated that AhrC bound to both of these promoters with nanomolar affinity in the absence of arginine. Surprisingly, addition of arginine to the binding reaction did not enhance AhrC binding to either promoter. Bacterial adenylate cyclase-based two-hybrid assays revealed that, as expected, AhrC could oligomerize with itself, but it could also form oligomers with ArgR1 and ArgR2. In contrast, ArgR1 and ArgR2 could not form oligomers with themselves or each other. Current studies are focused on how these regulatory protein interactions precisely modulate expression of arginine biosynthetic pathways.

Poster # B13

The broad-spectrum metallophore staphylopine sensitizes *Staphylococcus aureus* to copper poisoning during infection

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During infection, *Staphylococcus aureus* and other pathogens rely on small molecules, or metallophores, to obtain the essential nutrient zinc. The *cnt* locus of *S. aureus* encodes proteins necessary for the synthesis, export, and import of the metallophore, staphylopine. While essential, transition metals are also toxic in excess, and the host harnesses the toxicity of copper to combat invaders. However, the mechanism by which copper enters bacteria remains largely unknown. Staphylopine and other metallophores can bind and import transition metals other than zinc in conjunction with their transporters. While their broad-spectrum metal-binding ability is generally considered beneficial for bacteria, the current studies tested the hypothesis that it sensitizes bacteria to host-imposed copper toxicity. Growth and elemental analyses showed that the Cnt system increases the susceptibility of *S. aureus* to copper intoxication in culture and drives cellular copper accumulation. Loss of the Cnt system nearly eliminated copper accumulation, implying that this transporter is a significant route of copper entry into *S. aureus.* Relying on the Cnt system leads to increased expression of the copper efflux pump, CopA, even at nanomolar levels of copper, indicating that its use significantly increases the threat posed by copper. A *S. aureus* skin infection model revealed that the use of the Cnt system leads to copper import during infection. Collectively, these observations demonstrate that even though metallophore use is crucial to overcome host-imposed zinc starvation, the limited metal selectivity of metallophores can adversely affect bacteria by importing non-zinc metals like copper.

Poster # B14

SSR42 is a noncoding RNA with a multifaceted role in regulating *Staphylococcus aureus* virulence

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Discovered a decade ago, SSR42 is one of, if not the, longest non-coding RNA in the *S. aureus* cell. Highly conserved across strains, the SSR42 transcript has increased stability in stationary phase and is a primary regulator of hemolytic and cytolytic behavior. Despite this, the mechanistic role of SSR42 in *S. aureus* virulence factor regulation and pathogenesis remains unclear. Herein, we demonstrate that SSR42 abundance in stationary phase is greater than almost every other transcript in the cell. We further reveal that SSR42's regulation of hemolysis occurs independently of the SaeRS system and is substrate specific. Using transcriptomic analysis, we demonstrate that SSR42 is a novel primary regulator of secreted protease activity, staphyloxanthin pigment production, and biofilm formation. Target prediction software suggests strong, direct interaction between SSR42 and virulence determinants, including *aur*, *PVL, lukAB, hlgBC* and the *spls*. Transcriptional profiling confirmed that SSR42 regulation does not occur through the promoter of these elements; instead, translational fusion studies demonstrate binding events occur post-transcriptionally, facilitating stabilization or degradation of targets. Using a novel high-throughput screen, we explored SSR42 regulation, revealing that it is primarily activated by the global regulators SarA, CodY and Rsp, whilst SarR, SarZ and Rot repress its expression. Finally, using a murine infection model, we demonstrate that an SSR42 mutant was severely abrogated in its ability to cause disease, solidifying SSR42's role as indispensable during infection. Collectively, we present the characterization of SSR42, an underestimated effector of *S. aureus* virulence that influences nearly every facet of the infectious process.

Poster # B15

***Staphylococcus aureus* gastrointestinal colonization in atopic dermatitis**

Theodora Karagounis[1], Magdalena Podkowik[2], Julia Shendero-

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vich[2], Natalia Arguelles[2], Anusha Srivastava[2], Carli Needle[5], Greg Putzel[2], Vikash Oza[1,3], Alejandro Pironti[2], Victor Torres[2], Bo Shopsin[2,4]

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Atopic dermatitis (AD) is known to be associated with increased colonization by *Staphylococcus aureus*. Although the nares are considered the *primary site* of *S. aureus* colonization, an underappreciated observation is that gastrointestinal (GI) colonization is common in infants, in whom the age-distributed incidence of AD mirrors the known incidence of *S. aureus* GI colonization. To estimate the extent of GI colonization in children with AD, the prevalence of *S. aureus* rectal carriage in 33 children with AD flare was determined. The prevalence of *S. aureus* colonization in the nares, lesional site, and rectum were 39%, 43%, and 57% respectively. Genomic comparisons of *S. aureus* isolates revealed that AD subjects are primarily colonized with one clone across all body sites, indicating potential transmission between sites. Screening isolates for *agr* functionality, which is associated with AD flare and thought to be essential for GI colonization, identified mixed populations of *agr*-defective and wild-type *S. aureus* in lesional and rectal sites. In contrast, the nares were solely colonized with *agr* wild-type. Longitudinal analysis of study subjects after topical treatment of AD revealed that disease remission correlated with a decrease in colonization density of lesional and rectal sites, but not the nares. Collectively, these data suggest that 1) the gut may represent a reservoir for *S. aureus* colonization in AD, and 2) *agr*-functional cells can protect *agr*-defective organisms, enabling GI tract colonization. Additionally, correlation between AD flare and *S. aureus* rectal colonization suggests previously unknown connections between skin health and *S. aureus* gut carriage.

Poster # B16

Partners in Grime: Exploring the Synergism between *Candida albicans* and *Streptococcus mutans* beyond the Biofilm Matrix

C. K. Katrak, B. A. Garcia, M. Nguyen, M. Lorenz and J. Abranches

Co-infection with *Streptococcus mutans* and *Candida albicans* is associated with dental caries. When these two microorganisms are co-cultivated, production of extracellular matrix is increased resulting in enhanced virulence and environmental stress tolerance. While *C. albicans* expresses catalase, an enzyme that breaks down H2O2 into H2O and O2, *S. mutans* is catalase-negative and highly susceptible to detrimental effects of H2O2. The purpose of this study is to investigate the role of *C. albicans'* catalase in the protection of dual *S. mutans* and *C. albicans* biofilms from H2O2. To determine whether catalase increased survival to acute oxidative stress, biofilms of *C. albicans* SC5314 (*CaWT*), its catalase mutant (*CaΔcat*), and *S. mutans* UA159 (*Smu*) were individually and co-cultivated for 48hrs. A time-kill assay using 0.25% H2O2 was completed and CFUs were quantified. Survival rate of *Smu* in a dual species biofilm in the presence of *CaWT* was greater than the survival rate when co-cultivated with *CaΔcat* or when grown as a monospecies biofilm ($p<0.0001$). To determine the importance of catalase on biofilm exposure to sublethal oxidative stress, *CaWT*, *CaΔcat* and *Smu* were individually and co-cultivated in BHI + 2mM sucrose on saliva-coated plates. After 10hrs, 0.005% H2O2 or the peroxigenic commensal *Streptococcus A12* was added to biofilms and allowed to grow for additional 10hrs. Biomass of *Smu*, *CaΔcat*, and *dual CaΔcat & Smu* biofilms was reduced by adding sublethal 0.005% H2O2 or *Streptococcus* A12 ($p< 0.05$). Catalase produced by *C. albicans* protects *S. mutans* against H2O2 and peroxigenic commensal bacteria.

Poster # B17

DNA methylation promotes *Clostridioides difficile* sporulation by enhancing transcription of a gene encoding the cell fate determinant SpoIIE

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Many bacteria use DNA methylation as a mechanism to epigenetically regulate diverse cellular processes such as chromosome replication, DNA repair, and transcriptional activation. This post-replicative modification can result in phase-variable gene expression, leading to phenotypic cell variants with distinct functions or cellular fates. While recent advances in methylome sequencing have revealed that DNA methylation is ubiquitous in bacteria, the exact mechanism by which it epigenetically regulates phenotypic heterogeneity remains unclear in most cases. We recently determined that methylation by the *Clostridioides difficile*-specific DNA methyltransferase CamA promotes spore formation. Since the formation of aerotolerant spores is critical for this enteric pathogen and obligate anaerobe to transmit disease, we sought to elucidate the mechanism by which DNA methylation enhances sporulation. Using transcriptional reporters, RNA-Seq, and qRT-PCR, we show that CamA promotes the transcription of *spoIIE*, which encodes a regulator of asymmetric division that also functions to activate the earliest acting sporulation-specific sigma factor σ^F. Notably, inactivation of a single CamA methylation site in *spoIIE*'s promoter region reduces both *spoIIE* expression and the frequency of asymmetric division. Since the methylation site overlaps with the binding site of the master transcriptional regulator Spo0A, which directly activates *spoIIE* transcription, we propose a model in which DNA methylation increases the binding affinity of Spo0A for the *spoIIE* promoter. Given that SpoIIE activation commits *B. subtilis* cells to completing sporulation, our data suggest a mechanism by which CamA-mediated methylation regulates cell fate and enhances disease transmission.

Poster # B18

Efficacy of metal-binding lipoproteins as part of a protein-based enterococcal vaccine

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The ability of pathogens to scavenge trace metals during infection has emerged as an important bacterial virulence trait, with recent studies demonstrating that surface-associated lipoproteins may be viable vaccine targets. A resident of the gastrointestinal tract, *Enterococcus faecalis* is also a prevalent opportunistic pathogen causing a variety of localized and systemic infections. Recently, our group showed that the manganese (EfaA) and zinc (AdcA and AdcAII) binding lipoproteins are essential for *E. faecalis* virulence. Here, we sought to explore the potential of these lipoproteins, alone or in combination, for the development of a protein-based enterococcal vaccine. EfaA, AdcA and AdcAII full-length proteins were produced using the XpressCF™ cell- free expression system (CFPS) and verified by mass spectrometry and gel electrophoresis. Antigen-primed (singly or in combination) polyclonal antibodies were raised and their specificity validated by ELISA. Neutrophil -mediated opsonophagocytic killing assay indicated that either single-antigen or multi-antigen (EfaA+AdcAII and EfaA+AdcA+AdcAII-specific) antisera mediate killing of *E. faecalis*. Multi-antigen but not single-antigen antisera conferred significant protection to the larvae of *Galleria mellonella* against *E. faecalis* lethal dose infection. However, passive immunization with single antigen antisera significantly reduced *E. faecalis* load in the peritoneal cavity, spleen, or kidney after intra-peritoneal infection. Animal studies to test the protective effect of multi-antigen antisera are ongoing. Collectively, our results indicate that immunization with EfaA, AdcA and AdcAII generates opsonic and neutralizing antibodies while providing proof-of- concept evidence that these metal -binding lipoproteins can be further explored for the development

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of a protein-based vaccine to prevent a broad range of enterococcal infections.

Poster # B19

Modulation of heme synthesis through post-translational regulation of glutamyl-tRNA reductase in *Staphylococcus aureus*

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Staphylococcus aureus is a formidable pathogen capable of causing disease in every tissue type within the human host. With the incidence of drug-resistant *S. aureus* strains on the rise, the development of new antimicrobials is becoming increasingly important. Heme synthesis in *S. aureus* represents an attractive therapeutic target as heme is an important cofactor for cellular redox reactions. *S. aureus* can acquire heme from the host or synthesize heme endogenously. High levels of heme are toxic so *S. aureus* has evolved mechanisms to balance heme uptake, efflux, and synthesis. One such mechanism the regulation of heme synthesis. Glutamyl-tRNA reductase (GtrR) catalyzes the first dedicated step in heme biosynthesis and is post-translationally regulated by heme and the membrane protein HemX. Alanine scanning mutagenesis identified residues on GtrR whose mutation abrogates heme binding yet does not impact heme production *in vivo*. GtrR was recently identified as a putative substrate of the kinase Stk1 leading us to hypothesize that Stk1 tunes heme synthesis through phosphorylation of GtrR. Indeed, lack of Stk1 or Stp1, Stk1's cognate phosphatase, alters the ability of *S. aureus* to adapt to heme toxicity suggesting that the kinase-phosphatase pair may function in modulating heme levels within the cell. Work investigating the specificity of Stk1 for GtrR and the mechanism through which phosphorylation impacts heme synthesis is ongoing. An improved understanding of how *S. aureus* regulates heme biosynthesis could uncover universal mechanisms employed by bacteria to adapt to various environmental niches and guide development of antimicrobials designed to perturb heme homeostasis.

Poster # B20

Kip proteins promote *Clostridioides difficile* growth in bile salts

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Clostridioides difficile is an anaerobic pathogen that commonly infects patients who have taken antibiotics. After taking antibiotics, the intestinal microbiome becomes dysbiotic, changing the bile salt profile within the colon. Some bile salts promote germination of *C. difficile* spores, while others prevent germination and growth. Although we understand how *C. difficile* spores respond to bile salts, the mechanisms cells use to cope with bile salt exposure is poorly understood. In other organisms, the Kip proteins regulate sporulation and metabolic processes in response to environmental cues, but the link between the *kip* genes in *C. difficile* and bile salts has not been explored. In my data, I demonstrate that the Kip proteins promote *C. difficile* sporulation and growth in response to bile. Exposure of *C. difficile* to the bile salts deoxycholate (DCA) and chenodeoxycholate (CDCA) induces expression of the four-gene *kipOTIA* operon. Exposure of a Δ*kipIA* mutant to DCA and CDCA results in a lower sporulation frequency and decreased growth when compared to the wild-type strain. Both phenotypes are complemented by expression of the full *kip* operon. These data suggest that the *kip* operon promotes sporulation and growth of *C. difficile* in bile salts through a currently unknown mechanism. Uncovering the Kip protein function(s) will clarify how *C. difficile* senses and responds to the host environment.

Poster # B21

How methicillin-resistant *Staphylococcus aureus* USA300 responds to database-designed antimicrobial peptides horine and verine

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Antimicrobial peptides are important candidates for developing novel antibiotics against multidrug-resistant pathogens. Here, we performed transcriptome analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 LAC in the presence of sub-lethal concentrations of two distinct database designed peptides: horine and verine. RNA samples were extracted from MRSA USA300 and subjected to sequencing analysis. Compared with the non-treated control, horine, verine, and vancomycin treatment significantly up-regulated (FC > 2, P < 0.05) the expression of 28, 18, and 2 genes, and down-regulated 46, 59 and 51 genes (Fold change, FC < -2, P < 0.05), respectively. The number of regulated genes (FC > 2 or FC < -2, and P < 0.05) shared between horine and verine (46) were higher than those shared between verine and vancomycin (33), and between horine and vancomycin (26) treatments. While 23 regulated genes were common for MRSA USA300 treated with either horine, verine or vancomycin. Up-regulated genes in horine and verine treated MRSA were related to diverse pathways, mainly cationic antimicrobial peptide resistance (*dltABCD*, *mprF, vra*, and *vraF*), two-component system (*vraE* and *vraF*), and teichoic acid biosynthesis (*dltBCD*). Genes related to citrate cycle, glycolysis, pyruvate and carbon metabolism were also up-regulated while quorum-sensing related genes such as *epiA* were down-regulated in horine and verine treated MRSA. *USA300HOU-1298*, associated with benzoate and xylene degradation pathways, was down-regulated in all treatment groups. These results shed light on the genetic basis of the response of MRSA USA300 strain to sub-lethal horine and verine.

Poster # B22

The role of surface protein G (SasG) in *Staphylococcus aureus* healthy human skin colonization

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Staphylococcus aureus is a Gram-positive opportunistic and highly virulent pathogen that causes the vast majority of skin and soft tissue infections. *S. aureus* colonizes the skin transiently and asymptomatically, posing an infection risk. Our recent published findings indicate high expression of surface protein G (SasG) enhances the ability of *S. aureus* to bind skin cells. SasG is a giant sortase-anchored protein that contains two distinct domains: the N-terminal A domain and the B repeat domain that extends the protein from the cell wall. Analysis of *S. aureus* genomes indicates there are at least three sequence variants of SasG, and we discovered that some skin-infecting strains secrete the SasG A domain. Our recent studies on SasG and the homologous *S. epidermidis* Aap protein indicate the lectin subdomain is important for binding human corneocytes. Additionally, in *S. carnosus*–a surro-

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gate model organism that does not naturally adhere to corneocytes–expression of full-length SasG promotes adhesion. Preliminarily, we have found that blocking with purified lectins from different SasG types and Aap reduces SasG-mediated adhesion, confirming the role of the lectin in binding. We have determined the unknown ligand is most likely a glycoprotein, and co-incubating SasG and Aap-expressing strains with purified glycans identified via screening reduces adhesion to corneocytes. We have discovered an additional competitive function of truncated *S. aureus* USA300 SasG, which is secreted and can interfere with *S. epidermidis* binding. These findings may provide a possible therapeutic target in preventing *S. aureus* skin colonization for those at risk for infection.

Poster # B23
Characterization of a surface-associated c-di-AMP phosphodiesterase in *Enterococcus faecalis*

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Cyclic di-AMP is a conserved signaling nucleotide in bacteria responsible for controlling important physiological functions, including osmoregulation, central metabolism, and envelope homeostasis. In addition, c-di-AMP stimulates the STING-mediated type I interferon response when found extracellularly, thereby playing an underappreciated role in the activation of host immune responses. In *Enterococcus faecalis*, intracellular levels of c-di-AMP are controlled by the diadenylate cyclase CdaA and the phosphodiesterases (PDEs) DhhP and GdpP. We recently showed that either complete loss of c-di-AMP (Δ*cdaA*) or intracellular c-di-AMP accumulation (PDE mutants) drastically impaired the general fitness and virulence of *E. faecalis*. In addition to cytosolic PDEs, an LPxTG-anchored ectonucleotidase with 5' metallophosphoesterase-5' nucleotidase domain (MP-NT) has been identified in few bacteria and shown to reduce extracellular c-di-AMP levels that dampened pro-inflammatory responses. Here, we describe the initial characterization of OG1RF_10056, a surface-anchored protein that contains two conserved MP-NT domains. Using a markerless strategy, we generated a ΔOG1RF_10056 strain and used it to determine how the loss of OG1RF_10056 affected the expression of phenotypes linked to c- di-AMP regulation. While inactivation of OG1RF_10056 did not impair *E. faecalis* growth under various stress conditions in vitro or ex vivo, the ΔOG1RF_10056 strain displayed a marked defect in biofilm formation. Importantly, loss of OG1RF_10056 led to a ~50-fold increase in extracellular c-di-AMP compared to the supernatant of the parent strain. Work is underway to characterize OG1RF_10056 biochemically and to determine whether its activity can be associated with the generally mild inflammatory responses typically observed in enterococcal infections.

Poster # B24
Characterization of a novel lantibiotic produced by a skin commensal *Staphylococcus hominis*

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Coagulase-negative staphylococci (CoNS) are the dominant human skin colonizers and are known to produce diverse antimicrobials that directly impair the growth of pathogenic competitors. *Staphylococcus hominis* is the second most frequently isolated CoNS from healthy human skin. To date, bactericidal molecules, hominicin and hogocidin, derived from specific strains of *S. hominis* have been described and shown to selectively kill drug-resistant pathogenic *Staphylococcus aureus*. **The contribution of *S. hominis* produced antimicrobials to modulate the skin homeostasis by shaping the resident microbiota through kin selection and niche specialization is currently unknown.** Here, we report a *S. hominis* strain D11 isolated from atopic dermatitis skin, exhibiting broad antimicrobial activity against species of CoNS (e.g., *S. hominis*, *S. epidermidis,* and *S. haemolyticus*). BAGEL4, a prediction tool for gene clusters involved in the biosynthesis of bacteriocins, identified an antimicrobial peptide cluster belonging to a class of lantibiotics on D11's 19.8 kb plasmid. Lantibiotics are ribosomally synthesized, post- translationally modified secreted antimicrobial agents. Preliminary biochemical characterization revealed D11 lantibiotic to be of low -molecular weight (~10 kDa), heat stable, and susceptible to proteolytic digestion. Given the ubiquity of *S. hominis* on healthy skin and its capability to produce antimicrobials, **I hypothesize that skin commensal *S. hominis* produce antimicrobial lantibiotics as a competitive strategy to shape the composition of the skin microbiota.** The implication of this study will better our understanding on the role of plasmid-borne lantibiotics and the prevalence of bacteriocin-producing CoNS strains in the microbial ecology of human skin in health and disease.

Poster # B25
Impact of the pentose phosphate pathway on *Staphylococcus aureus* metabolism and pathogenesis

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Staphylococcus aureus is an important pathogen that leads to significant disease through multiple routes of infection. We recently published a Tn-seq screen in a mouse acute pneumonia model and identified a hypothetical gene (SAUSA300_1902) with similarity to a lactonase of *Escherichia coli* involved in the pentose phosphate pathway (PPP) that was conditionally essential. Transposon mutant libraries of *S. aureus* as well as targeted attempts have been able to generate mutations in several genes involved in the PPP. We show here that mutation of 1902 has significant impacts on ATP output and respiration. RNA-seq analysis identified compensatory changes in gene expression for glucose and gluconate as well as reductions in the pyrimidine biosynthesis locus. These differences were also evident through unbiased metabolomics studies and 13C labeling experiments that showed mutation of 1902 led to increases in glucose and 6-phosphogluconate and reductions in ribose-5P, UMP, GMP and pyruvate. These nucleotide reductions also impacted the amount of extracellular DNA in biofilms and reduced biofilm formation. Mutation also limited the capacity of the strain to resist oxidant damage induced by hydrogen peroxide and paraquat. We demonstrated the importance of these changes on virulence in three different models of infection, covering respiratory, skin and septicemia, demonstrating significant attenuation in all of these models. This work demonstrates the multifaceted role metabolism can play in multiple aspects of *S. aureus* pathogenesis.

Poster # B26
Structure, function, and mechanism of oleate hydratase - a virulence factor for pathogens and commensals

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Gut commensal bacteria hydrate dietary unsaturated fatty acids using the flavoenzyme oleate hydratase (OhyA). These hydroxylated fatty acids (*h*FA) are thought to communicate with the immune system to create a more tolerant environment for the commensal bacteria. The pathogen *Staphylococcus aureus* also contains an OhyA. OhyA is required for *S. aureus* to grow in the pres-

ence of palmitoleate, a major skin antimicrobial fatty acid for innate immunity, but the *h*FA it produces are not used for lipid synthesis. Instead, they are released into the extracellular environment and may function with the immune system, but mechanistic details are lacking. Employing X-ray crystallography, we captured multiple substrate and product complexes revealing how OhyA accomplishes the difficult catalytic task of regio- and stereospecific hydration of isolated carbon-carbon double bonds. We show flavin adenine dinucleotide is a structural cofactor that organizes the active site and deduce the catalytic mechanism from the crystal structures and biochemical analyses of relevant mutants. We use a lipidomics pipeline and infection models to measure the *h*FA composition at a *S. aureus* skin infection site and show the profile mirrors the commensal *h*FA composition in stool. A *S. aureus ohyA* knockout strain has a three-log reduction in bacterial burden after 24 hours compared to parent and complement strains indicating OhyA expression is required for *S. aureus* virulence. Taken together, these results suggest *h*FA are critical to immune evasion and OhyA is a unifying strategy to survive in the host.

Poster # B27

Elucidating the role of a Staphylococcal Glucosaminidase in Host Innate Immune Responses

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The cell wall of *Staphylococcus aureus* is a critical cell envelope constituent and virulence factor that subverts host immune defenses and provides protection against environmental stressors. Peptidoglycan chains of the *S. aureus* cell wall are processed to characteristically short lengths by the glucosaminidase, SagB. It is well established that peptidoglycan is an important pathogen-associated molecular pattern (PAMP) recognized by the host innate immune system which leads to production of the pro-inflammatory cytokine, IL-1β. However, how bacterial processing of peptidoglycan drives IL-1β production is comparatively unexplored. Here, we tested the involvement of staphylococcal glucosaminidases in shaping innate immune responses and identified SagB as a mediator of IL-1β production. We found that a Δ*sagB* mutant fails to activate IL-1β production by macrophages and dendritic cells and that processing of peptidoglycan by SagB is essential for this response. Our data indicate that SagB-dependent IL-1β production occurs independently of canonical PAMP receptor engagement and NLRP3 inflammasome-mediated caspase activity. Rather, the Δ*sagB* mutant has reduced caspase-independent cleavage of pro-IL-1β resulting in intracellular accumulation of the pro- form in macrophages. Furthermore, SagB is required for virulence in systemic infection and promotes IL-1β production in skin and soft tissue infection models. Taken together, our results suggest that the length of *S. aureus* cell wall glycan chains drives optimal IL-1β production by innate immune cells through a previously undescribed mechanism related to IL-1β maturation.

Poster # B28

Interrogation of genes required for arginine metabolism in a murine model of infection

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Staphylococcus aureus encodes biosynthetic pathways to synthesize arginine, however, it remains a functional auxotroph when grown in a chemically defined medium lacking arginine (CDM-R). We recently reported that 54% of *S. aureus* clinical isolates collected from diverse sites of infection can grow in CDM-R (prototroph). Whole-genome sequencing of 40 isolates identified several mutations in loci known to regulate arginine biosynthesis including *ccpA*, *ahrC*, the *arcABDC* regulatory region, *proC,* and *rocA*. We found that these mutations either increase the transcription of ornithine carbamoyl transferase (*arcB1*) or increase the intracellular ornithine pool facilitating the conversion of ornithine into citrulline and subsequently arginine. These observations suggest that mutations are selected during *S. aureus* infection that enhances arginine biosynthesis facilitating proliferation and persistence within the host. To confirm these data, mice were retro-orbitally challenged with *S. aureus* JE2 and kidneys were excised at 20 days post-infection. It was determined that a significant proportion of the bacterial burden had the ability to grow on CDM-R suggesting that mutations that facilitate arginine biosynthesis are selected within the kidney. In addition, preliminary data suggest that genes which function in arginine catabolism (*rocD*) are also important in kidney persistence. However, it is unclear what aspect of arginine catabolism is important within the kidney niche. Additional experiments will be performed to assess the importance of mutations within arginine catabolic genes such as *rocF, proC, rocA, gudB,* and *arcA* in the 20-day kidney abscess model.

Poster # B29

Uncovering the functional differences of *Staphylococcus aureus* microproteins and their role in host-pathogen interactions

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Historically, *S. aureus* is a commensal organism that persistently colonizes the skin and upper respiratory tract of humans but can readily cause severe disease ranging from skin lesions to invasive endocarditis, particularly during times of immunodeficiency. Recently, there has been an emergence and steady rise of CA-MRSA. Thus, substantial effort has been placed upon understanding the basis of CA-MRSA pathogenicity. Microproteins are peptide effector molecules originating from small open reading frames (smORFs). In bacteria, the functions of microproteins range from signaling molecules to potent toxins. However, smORFs that encode for microproteins are often overlooked due to their size and the fact that they may be embedded within larger ORFs. Our lab identified 57 microproteins, encompassed by 115 peptido-forms in *S.aureus* (USA 300 TCH1516). Within this cohort, our lab characterized two *S. aureus* microproteins 1 (SAM1) and 2 (SAM2), that enhanced CA-MRSA pathogenesis *in vitro* and *in vivo* through distinct mechanisms of action. In addition to SAM 1 & 2, our lab has identified two additional microproteins in *S.aureus,* SAM3a and SAM3b, which have yet to be fully characterized. Interestingly, these microproteins are found in two distinct gene loci, and possess differing chemical characteristics, despite sharing 78% similarity in their amino acid sequences. We hypothesize SAM3a and SAM3b are important in pathogenesis of CA-MRSA. We aim to test our hypothesis by assessing changes in *S.aureus* virulence after genetic manipulation of SAM3a & b, both *in vitro* and *in vivo.*

Poster # B30

Interplay of CodY and CcpA in regulating central metabolism and biofilm formation in *Staphylococcus aureus*

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Staphylococcus aureus is a medically important pathogen with high metabolic versatility allowing it to infect various niches within a host. *S. aureus* utilizes two major transcriptional regulators, CodY and CcpA, to remodel metabolic and virulence gene expression in response to changing environmental conditions. Previous studies revealed that inactivation of either *codY* or *ccpA* has a pronounced impact on different aspects of staphylococcal physiology and pathogenesis. To determine the contribution and interplay of these two regulators in modulating central metabolism, virulence, and biofilm development we constructed and characterized the *codY ccpA* double mutant in *S. aureus* UAMS-1. In line with previous studies, we found that CcpA and CodY control the cellular metabolic status by altering carbon flux through the central and overflow metabolic pathways. Our results demonstrate that *ccpA* inactivation impairs biofilm formation and decreases incorporation of eDNA into the biofilm matrix whereas disrupting *codY* resulted in a robust structured biofilm tethered together with eDNA and PIA. Interestingly, inactivation of both *codY* and *ccpA* decreases biofilm biomass and reduces eDNA release in the double mutant. Compared to inactivation of *codY*, the *codY ccpA* mutant did not overexpress toxins but maintained overexpression of amino acid metabolism pathways. Furthermore, the *codY ccpA* mutant produced high amounts of PIA, in contrast to the wild-type strain and *ccpA* mutant. Combined, the results of this study suggest that the coordinated action of CodY and CcpA modulate central metabolism, virulence gene expression, and biofilm-associated genes to optimize growth on preferred carbon sources until starvation sets in.

Poster # B31

CSF proteome differences in an animal model of gram-positive versus gram-negative CSF shunt infection

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Cerebrospinal fluid (CSF) shunt infection is a common and devastating complication of the treatment of hydrocephalus. Timely and accurate diagnosis is essential as these infections can lead to long term neurologic consequences like seizures, decreased IQ and impaired school performance. Currently the diagnosis of shunt infection relies on bacterial culture, however, culture is is not always accurate especially as these infections are frequently caused by bacteria capable of forming biofilms like *Staphylococcus epidermidis*, *Cutibacterium acnes*, and *Pseudomonas aeruginosa* and may have very few planktonic bacteria in the CSF to be picked up on culture. Using our novel rat model of CSF shunt infection, we aimed to discover CSF biomarkers that would differentiate *S. epidermidis*, *C. acnes*, and *P. aeruginosa* infection from sterile shunt placement. *P. aeruginosa* demonstrated a far great number of differentially expressed proteins when compared to sterile animals or the gram-positives *S. epidermidis* or *C. acnes* and these changes persisted throughout the 56-day time course. *S. epidermidis* demonstrated an intermediate number of differentially expressed proteins especially at early time points but over the course of infection this dissipated. *C. acnes* infection induced the least amount of change in the CSF proteome when compared to sterile infection at early time points and again this dissipated over the course of infection. Despite the differences in the proteome of each of these bacteria when compared to sterile, there are proteins that are common to all types of infection especially at early time points which are candidate diagnostic biomarkers.

Poster # B32

Regulation of glutamine metabolism in Staphylococcus aureus by the PII signaling family protein PstA

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How bacteria sense nitrogen levels and regulate nitrogen metabolism are central questions in microbial physiology. In *Staphylococcus aureus*, glutamine is synthesized by the glutamine synthetase GlnA using glutamate and ammonia. Beyond its enzymatic activity, GlnA also acts as a chaperone of the transcriptional repressor GlnR to stabilize the binding of GlnR to DNA. When there is enough nitrogen source, GlnA is feedback inhibited (FBI) by glutamine. Along with FBI-GlnA, GlnR strongly inhibits the transcription of the *glnR-glnA* operon. How *S. aureus* modulates its nitrogen metabolism in response to nitrogen limitation, however, is unknown. In this study, we found that the PII signaling family protein PstA is essential in a nitrogen limiting environment, and that it regulates glutamine metabolism by protein-protein interactions. These data thus unveil the missing puzzle piece of the nitrogen regulatory circuitry in *S. aureus*.

Poster # B33

SarU is a novel and important regulator of proteases and toxin production in *Staphylococcus aureus*

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Recently, our group demonstrated that an understudied member of the SarA family of global transcription factors, SarU, is a positive effector of secreted protease activity in the human pathogen *Staphylococcus aureus*. Herein, we reveal that in addition to controlling the production of major proteases, SarU also strongly influences production of alpha-hemolysin, the bipartite toxins HlgBCA and LukED, and the adherence factor ClfA. To explore the impact of these SarU regulatory events, we assessed the activity of key virulence factors in our mutant strain. Here, we observed that a *sarU* mutant displayed limited killing of human neutrophils and was profoundly less hemolytic towards sheep, bovine, and human blood in comparison to the wild-type strain. Of note, we found that the induction of *hla* restored hemolytic activity back to wild-type levels in the *sarU* mutant. Interestingly, using a murine model of pneumonia, we observed no variation in colonization between the WT and mutant strain in female mice, however there was ~1-log reduction in viability of the *sarU* mutant in male mice. As seen previously for skin and soft tissue infections, this discrepancy appears to be mediated by impaired production of alpha-toxin in the *sarU* mutant. However, despite decreased production of virulence products, we observed that the *sarU* mutant colonized the spleen better than the wild-type strain. Collectively, our data indicates that SarU is a major regulator of virulence factor gene expression in *S. aureus*; furthering our understanding of the highly complex network governing disease progression within this important human pathogen.

Poster # B34

Effect of prior nasal colonization by *S. aureus* on downstream sepsis with the colonizing strain

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Infection from *Staphylococcus aureus* can lead to diseases ranging from mild skin and soft tissue infections to life threatening endocarditis, pneumonia, and bacteremia. It is known that approximately 50% of the human population is at least transiently colonized by *S. aureus*, and prior colonization has been found to be a large risk factor for invasive disease. On the other hand, it has also been shown that people who have previously been colonized have better prognosis when encountering invasive disease from an endogenous (colonizing) source. *S. aureus* expresses small, robust proteins called superantigens (SAgs) that function to mass activate T cells by cross-linking MHC II with the TCR in the absence of antigen specificity. Staphylococcal SAgs have been shown to be important factors during invasive disease. This leads to the hypothesis that protection against invasive disease from prior colonization is due to humoral immunity built against the specific SAgs of the colonizing strain. To test this, we will use transgenic mice expressing HLA-DR4 to ensure proper sensitivity to SAgs. Over the course of four weeks, the transgenic mice will be inoculated intranasally with specific *S. aureus* MW2 strains (wild-type, deleted staphylococcal enterotoxin C or sham) at two week intervals to ensure consistent colonization. At four weeks, sepsis will be inflicted with the wild-type MW2 strain and then organs will be harvested, and bacterial burdens analyzed. This research will give insight into how prior colonization gives protection against invasive infections and could potentially open a new avenue of vaccine research regarding SAgs.

Poster # B35

The novel protein ScrA is an essential regulator of *Staphylococcus aureus* virulence acting through the SaeS two-component system

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Staphylococcus aureus is a Gram-positive commensal that can also cause a variety of infections in humans. *S. aureus* virulence factor gene expression is under tight control by a complex regulatory network, which includes sigma factors, sRNAs, and two-component systems (TCS). Previous work in our laboratory demonstrated that overexpression of a previously unannotated small protein, ScrA, leads to changes in virulence gene expression consistent with SaeRS activation, including increased expression of intracellular adhesins, hemolysins, and a decrease in protease expression. This resulted in a distinct clumping phenotype, increased biofilm formation, and increased hemolytic activity against human erythrocytes. Here we further explore the mechanism of action of ScrA and demonstrate that ScrA-associated phenotypes are mediated by the SaeRS system. RNA-seq analysis revealed that overexpression of ScrA in a Δ*saePQRS* strain did not result in any significant changes in gene expression. In contrast, overexpression of ScrA in wild-type *S. aureus* leads to widespread changes in gene expression. We demonstrate that increases in human erythrocyte lysis, previously observed upon overexpression of ScrA, are HlgA-dependent, while aPSM expression is unaltered. To explore the impact of ScrA on pathogenesis we perform a murine systemic model of infection and reveal that an *scrA* mutant strain is attenuated for virulence compared to the wild type. Finally, we show that *scrA* expression is dependent on the ArlRS TCS, suggesting that ScrA may act as a link between the ArlRS and SaeRS two-component systems, with ArlRS stimulating *scrA* expression, which in turn stimulates the SaeRS system.

Poster # B36

Defining the Role of FtsH2 in Daptomycin Resistance in *Clostridioides difficile*

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Clostridioides difficile is a Gram-positive, spore-forming, anaerobic, opportunistic pathogen, and is the most common cause of healthcare-associated diarrhea. The cell envelope of Gram-positive bacteria is often a target for antibiotics, so understanding the biogenesis of the atypical *C. difficile* cell envelope provides potential for the discovery of novel drug targets. The lipopeptide antibiotic daptomycin binds the peptidoglycan precursor, lipid II, in the presence of phosphatidylglycerol. Understanding the mechanisms of daptomycin resistance may provide insight into the biogenesis of the *C. difficile* cell envelope. We performed a selection for spontaneous daptomycin resistant mutants and determined that loss of the membrane bound AAA+ protease, FtsH2, increases daptomycin resistance. *C. difficile* encodes two FtsH homologs, FtsH1 and FtsH2, and we found that loss of FtsH1 does not impact daptomycin resistance, suggesting that the proteases have distinct targets. We hypothesized targets of FtsH2 accumulate in the absence of FtsH2, contributing to increased daptomycin resistance. To identify FtsH2 targets, we used label-free quantitative proteomics to identify proteins that are more abundant in the absence of FtsH2. We identified ~13 proteins to be more abundant in the absence of FtsH2. We are using CRISPRi to knock down the expression of the corresponding genes in an *ΔftsH2* mutant and overexpressing the genes in wildtype and to determine the effect on daptomycin resistance.

Poster # B37

Novel Dihydrothiazolo Ring-fused 2-Pyridone Antimicrobial Compounds Treat *Streptococcus pyogenes* Skin Infection

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Gram-positive pathogens represent a significant source of healthcare-associated infections, which cause an additional $28.4 billion in healthcare expenses annually and are increasingly antibiotic resistant. To develop new therapeutics for treating diseases caused by Gram-positive pathogens, we have developed GmPcides, a novel family of small-molecule antimicrobials based on a peptidomimetic dihydrothiazolo ring-fused 2-pyridone scaffold, that are highly active against Gram-positive pathogens. The objective of this study was to evaluate the efficacy of GmPcides for treating skin and soft tissue infections (SSTI) caused by *Streptococcus pyogenes,* a common cause of SSTI in both community and hospital settings. Screening our compound library for minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations identified GmPcide PS757 as highly active against *S. pyogenes*. Further, treatment of *S. pyogenes* biofilm with PS757 revealed that it could effectively: i) prevent initial biofilm development; ii) cease maturation of developing biofilms; and iii) eradicate mature biofilms, demonstrating that PS757 was efficacious against all phases of biofilm formation. In a murine model of subcutaneous infection, compared to untreated animals, subcutaneous delivery of PS757 to *S. pyogenes* infected mice resulted in: i) reduction of cutaneous lesion area, ii) attenuation of bacterial burdens; iii) less weight loss during the infection; iv) more rapid clearance of the infection; and v) faster time to healing of the cutaneous wound. Taken together, these data demonstrate that GmPcides-based antimicrobial therapy shows considerable promise for development as an effective treatment for SSTI caused by *S. pyogenes* and other Gram-positive bacterial pathogens.

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