INTERNATIONAL CONFERENCE ON GRAM-POSITIVE PATHOGENS

6th Meeting + October 9-12 2016 + 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
7:15-8:15 pm
Dr. Ken Bayles, Professor and Associate Vice Chancellor for Basic Science Research in the Department of Pathology & Microbiology at the University of Nebraska Medical Center in Omaha, NE
"*Staphylococcus aureus* biofilm development and the origins of multicellularity."

Monday October 10th
8:00-9:00 am
Dr. Gary Dunny, Professor in the Department of Microbiology and Immunology at the University of Minnesota in St. Paul, MN
"Sensing, adaptation and competitive fitness in *E. faecalis*."

Tuesday October 11th
11:10 am-12:10 pm
Dr. Borden Lacy, Associate Professor of Pathology, Microbiology and Immunology, Associate Professor of Biochemistry at Vanderbilt University School of Medicine in Nashville TN
"Structures and functions of the *Clostridium difficile* toxins, TcdA and TcdB."

Wednesday October 12th
8:30-9:30 am
Dr. Shiranee Sriskandan, Professor and Hon. Consultant of Infectious Diseases at the Imperial College Faculty of Medicine in Hammersmith Hospital in London England
"Explaining disease phenotype: lessons from *Streptococcus pyogenes."*

SPONSORS

ICG+P greatly appreciate the generous support of Burroughs Wellcome Fund and 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**
Riverfront Ballroom
Embassy Suites Hotel

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

Co-Chairs: Melody Neely and Craig Ellermeier

7:15 p.m.-8:15 p.m. **KEYNOTE SPEAKER 1**
Riverfront Ballroom

'*Staphylococcus aureus* biofilm development and the origins of multicellularity.'

Dr. Ken Bayles, Professor and Associate Vice Chancellor for Basic Science Research in the Department of Pathology & Microbiology at the University of Nebraska Medical Center

Monday, October 10th

8:00 a.m.–9:00 a.m. **KEYNOTE SPEAKER 2**
Riverfront Ballroom

'Sensing, adaptation and competitive fitness in *E. faecalis*.'

Dr. Gary Dunny, Professor in the Department of Microbiology and Immunology at the University of Minnesota

Session 1: Metabolism
Moderators: Jeff Bose & McKenzie Lehman

9:00 a.m.- 9:20 a.m.
Francis Alonzo, Loyola University Chicago
'*Staphylococcus aureus* Tissue Infection During Sepsis is Supported by Differential Use of Bacterial or Host-Derived Lipoic Acid.'

9:20 a.m.– 9:40 a.m.
Neal Hammer, Michigan State University
'CtaM is required for menaquinol oxidase aa3 function in *Staphylococcus aureus*.'

9:40 a.m.– 10:00 a.m.
Austin Mogen, University of Florida
'Staphylococcus aureus nitric oxide synthase protects against endogenous reactive oxygen species by contributing to aerobic respiratory metabolism.'

10:00 a.m.-10:20 a.m. **Break**
Outside Riverfront Ballroom

Session 2: Antibiotic Development
Moderators: Pam Hall & Katy Patras

10:20 a.m.-10:40 a.m.
Jennifer Colquhoun, University of Rochester
'RnpA, characterization of a novel *Staphylococcus aureus* antimicrobial target.'

10:40 a.m.-11:00 a.m.
Prabagaran Narayanasamy, University of Nebraska Medical Center
'Novel MenA inhibitor against MRSA and its biofilm.'

11:00 a.m.-11:20 a.m.
Wilhelm Oosthuysen, University of Iowa
'Inhibition of Staphylococcal growth and colonization by species of Corynebacterium.'

11:20 a.m.-11:40 a.m.
Corey Parlet, University of Iowa
'Apicidin mediated attenuation of MRSA virulence corresponds with quorum sensing inhibition and enhanced immune effector responses.'

11:40 a.m.-12:00 p.m.
Nabil Abraham, Yale University School of Medicine
'Divide & Conquer: Synergy and mechanistic underpinnings of tick-borne antivirulent protein.'

12:00 p.m.-12:30p.m. **Lunch break**
Outside Riverfront Ballroom

12:30 p.m.-2:30 p.m. **Poster Session A**
Riverfront Ballroom

Session 3: Sporulation
Moderators: Rita Tamayo & Tea Ho

2:30 p.m.-2:50 p.m.
George Stewart, University of Missouri
'Assembly of the Exosporium Layer of the *Bacillus anthracis* Spore.'

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2:50 p.m.-3:10 p.m.
Joseph Sorg, Texas A&M University
'Dissecting the mechanism of DPA release during *Clostridium difficile* spore germination.'

3:10 p.m.-3:40 p.m. **Break**
Outside Riverfront Ballroom

Session 4: Secretion
Moderators: Francis Alonzo & Karan Gautam Kaval

3:40 p.m.-4:00 p.m.
Laty Cahoon, University of Illinois at Chicago
'A structural comparison of *Listeria monocytogenes* protein chaperones PrsA1 and PrsA2 reveals molecular features required for virulence.'

4:00 p.m.-4:20 p.m.
Ronan Carroll, Ohio University
'An intracellular peptidyl-prolyl cis/trans isomerase (PPIase) is required for folding and activity of the *Staphylococcus aureus* secreted virulence factor nuclease.'

4:20 p.m.-4:40 p.m.
Kimberly Kline, Nanyang Technological University Singapore
'HtrA Monitors Sortase-Assembled Pilus Biogenesis in *Enterococcus faecalis*.'

4:40 p.m.-5:00 p.m.
Thanh Truc Luong, University of Texas Health Science Center at Houston
'Recycling of the thiol-disulfide oxidoreductase MdbA by VKOR in the biofilm-forming actinobacterium *Actinomyces oris*.'

5:00 p.m.-5:20 p.m.
Justin Thornton, Mississippi State University
'Factors affecting the release and activity of pneumolysin.'

6:00 p.m.-9:30 p.m. **Conference Banquet**
The Durham Museum

Tuesday, October 11th

8:00 a.m.–8:30 a.m. **NIH UPDATE**
Riverfront Ballroom
Clayton Huntley National Institutes of Health

Session 5: Virulence Factor ID
Moderators: Thomas Kehl-Fie & Jason Rosch

8:30 a.m.-8:50 a.m.
Yoann LeBreton, University of Maryland
'Using Tn-Seq to Explore Group A Streptococcal Fitness During Soft-Tissue Infection .'

8:50 a.m.-9:10 a.m.
Hannah Rowe, St. Jude Children's Research Hospital,
'Modeling Nasopharyngeal Fitness of *Streptococcus pneumoniae* in Sickle Cell Disease.'

9:10 a.m.-9:30 a.m.
Luis Alberto Vega, University of Maryland College Park
'The Transcriptional Regulator CpsY is Necessary for Innate Immune Evasion in *Streptococcus pyogenes*.'

9:30 a.m.-9:50 a.m.
Aimee Wilde, Vanderbilt University
'The Skeletal Environment Dictates *S. aureus* Virulence Responses and Mediates Metabolic Processes in Vivo.'

9:50 a.m.-10:10 a.m. **Break**
Outside Riverfront Ballroom

Session 6: Biofilms
Moderators: Vinai Thomas & Lorne Jordan

10:10 a.m.-10:30 a.m.
Casey Gries, University of Nebraska Medical Center
'Cyclic di-AMP released from *Staphylococcus aureus* biofilm induces a macrophage type I interferon response.'

10:30 a.m.-10:50 a.m.
Kristi Frank, Uniformed Services University
'Genetic and transcriptomic approaches reveal transcriptional reprogramming of *Enterococcus faecalis* during biofilm-associated infection.'

10:50 a.m.-11:10 a.m.
Logan Bulock, University of Nebraska Medical Center
'The metabolic control of programmed cell death during biofilm development and zebrafish infection.'

ICG+P CONFERENCE SCHEDULE

11:10 a.m.-12:10 p.m. **KEYNOTE SPEAKER 3**
Riverfront Ballroom

'Structures and functions of the *Clostridium difficile* toxins, TcdA and TcdB.'

Dr. Borden Lacy, Associate Professor of Pathology, Microbiology and Immunology at Vanderbilt University School of Medicine

12:10 p.m.-12:40 p.m. **Lunch Break**
Outside Riverfront Ballroom

Session 7: Metals
Moderators: Neal Hammer & Bindu Nanduri

12:40 p.m.-1:00 p.m.
Lindsey Brown, Mississippi State University
'Zinc availability alters *Streptococcus pneumoniae* biofilm formation.'

1:00 p.m.-1:20 p.m.
Andrew Herr, Cincinnati Children's Hospital Medical Center
'Intercellular adhesion mediated by Aap: deciphering the assembly code.'

1:20 p.m.-1:40 p.m.
Thomas Kehl-Fie, University of Illinois Urbana-Champaign
'Cambialism enhances the ability of *Staphylococcus aureus* to resist calprotectin and nutritional immunity.'

Session 8: Multi-Species Interactions
Moderators: Kim Jefferson & Jessica Kubicek-Sutherland

1:40 p.m.-2:00 p.m.
Carrie Graham, University of Texas Health Science Center at Houston
'*Enterococcus faecalis* produces a bacteriocin that inhibits *Candida albicans* virulence and biofilm formation.'

2:00 p.m.-2:20 p.m.
Andrea Herrera, University of South Dakota
'The PrtF.2 fibronectin-binding protein of *Streptococcus pyogenes* promotes specific attachment to influenza infected epithelial cells.'

2:20 p.m.-2:40 p.m.
Kimberly McCullor, The University of Oklahoma Health Sciences Center
'*Streptococcus pyogenes* Bacteriophage A25: the Genomic Tale of Escaped Lysogeny in a High Efficiency Transducing Phage.'

2:40 p.m.-3:00 p.m.
Matthew Thoendel, Mayo Clinic
'Identification of Gram-Positive Pathogens in Synovial Fluid Using a Metagenomic Shotgun Sequencing Approach.'

3:00 p.m.-3:20 p.m. **Break**
Outside Riverfront Ballroom

Session 9: Regulation
Moderators: Kristi Frank & Revathi Govind

3:20 p.m.-3:40 p.m.
Prahathees Eswara, University of South Florida
'Regulation of cell division in *Staphylococcus aureus.'*

3:40 p.m.-4:00 p.m.
Heba Alnaseri, Western University
'Identification of FarR binding site responsible for auto-repression and efflux-mediated resistance to antimicrobial fatty acids.'

4:00 p.m.-4:20 p.m.
Bettina Buttaro, LKSOM Temple University
'Redox regulation of plasmid copy number and transfer of the pheromone inducible conjugative plasmid pCF10.'

4:20 p.m.-4:40 p.m.
Shaun Brinsmade, Georgetown University
'CodY directly represses the Sae two-component system in *Staphylococcus aureus*.'

4:40 p.m.-5:00 p.m.
Christina Krute, University of Kansas Medical Center
'VfrB is required for the activation of the *Staphylococcus aureus* SaeRS two-component system.'

5:00 p.m.-5:20 p.m.
Reid Wilkening, University of Illinois at Chicago
'Coordinating group behavior for successful host colonization: Quorum sensing in *Streptococcus pyogenes*.'

7:30 p.m.-9:30 p.m.
Poster Session B and Networking Session
Riverfront Ballroom with Cash Bar

ICG+P CONFERENCE SCHEDULE

Wednesday, October 12th

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

'Explaining disease phenotype: lessons from *Streptococcus pyogenes*.'

Dr. Shiranee Sriskandan, Professor and Hon. Consultant of Infectious Diseases at the Imperial College Faculty of Medicine in Hammersmith Hospital in London England

9:30 a.m.–10:00 a.m. **Break**
Outside Riverfront Ballroom

Session 10: Host Pathogen Interactions
Moderators: Kimberly Kline & Cory Parlet

10:00 a.m.–10:20 a.m.
Dudley McNitt, West Virginia University
'Scl1 structural element mediates wound colonization by group A *Streptococcus* and disrupts healing response.'

10:20 a.m.-10:40 a.m.
Jakub Kwiecinski, University of Iowa
'ArlRS – MgrA signaling cascade is the central regulator of adhesion and clumping in *Staphylococcus aureus* intravascular infections.'

10:40 a.m.-11:00 a.m.
Liwen Deng, San Diego State University
'The contribution of the LytR transcriptional regulator on Group B Streptococcal colonization and disease .'

11:00 a.m.-11:20 a.m.
Daniel Kennedy II, Mississippi State University
'BH3-only Protein PUMA and Innate Immunity in Pneumococcal and Staphylococcal Infections.'

11:20 a.m.-11:40 a.m.
Naren Gajenthra Kumar, Virginia Commonwealth University
'Role of Lipids in Cross-talk between S.aureus and Host.'

11:40 a.m.-12:00 p.m.
Wei Xu, Washington University in St. Louis
'Host and Bacterial Proteases Influence Virulence in a Murine Model of Enterococcal Catheter-Associated Urinary Tract Infection.'

12:00 p.m. Closing remarks
Riverfront Ballroom

Co-Chairs: Melody Neely and Craig Ellermeier

12:10 p.m. **Lunch Break**
Outside Riverfront Ballroom

ICG+P ORAL ABSTRACTS

SESSIONS AND PRESENTERS

Session 1: Metabolism

Francis Alonzo

Neal Hammer

Austin Mogen

Session 2: Antibiotic Development

Jennifer Colquhoun

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Wilhelm Oosthuysen

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Session 3: Sporulation

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Christina Krute

Reid Wilkening

Session 10: Host Pathogen Interactions

Dudley McNitt

Jakub Kwiecinski

Liwen Deng

Daniel Kennedy II

Naren Gajenthra Kumar

Wei Xu

ORAL ABSTRACTS

Session 1: Metabolism

9:00 a.m.-9:20 a.m.

Staphylococcus aureus Tissue Infection During Sepsis is Supported by Differential Use of Bacterial or Host-Derived Lipoate

Azul Zorzoli, James P. Grayczyk and **Francis Alonzo III**

Loyola University Chicago – Stritch School of Medicine Department of Microbiology and Immunology, Maywood, IL 60153

Staphylococcus aureus is a pathogen that causes a range of mild to severe infections. Central to *S. aureus* survival during infection is its adaptability when faced with nutrient restriction. Of interest to our laboratory is *S. aureus* acquisition of trace nutrients including the metabolic cofactor lipoate. Lipoate is required for oxidative and one-carbon metabolism in all forms of life therefore its acquisition is essential for survival in most species. Little is known about the acquisition and synthesis of lipoate in *S. aureus* or its roles in facilitating metabolic homeostasis during infection. Furthermore, bioinformatic analyses allude to a complex biosynthesis and salvage pathway in *S. aureus* that implies unique adaptive traits. Therefore, we hypothesize that lipoate biosynthesis and/or salvage pathways of *S. aureus* dictates optimal infection of the lipoate-deficient host. We used a genetic approach to decipher the pathways of lipoate biosynthesis and salvage. Our results suggest that S. aureus encodes enzymes with functions that resemble *Bacillus subtilis* lipoate biosynthesis enzymes: a lipoate synthetase, an octanoyl transferase, and a lipoyl transferase. In contrast, *S. aureus* encodes two lipoate protein ligases involved in salvage, a feature not seen for most pathogenic Gram-positive bacteria that maintain intact biosynthesis pathways. These ligases facilitate lipoate scavenging from certain *S. aureus*-infected tissues, whereas de novo biosynthesis is favored in others. Together our results define the pathways of lipoate metabolism in *S. aureus* and indicate that the composite functions of biosynthesis and salvage promote optimal metabolic flux within varied ecological niches.

9:20 a.m.-9:40 a.m.

CtaM is required for menaquinol oxidase aa3 function in *Staphylococcus aureus*

Neal D. Hammer[a*†], Lici A. Schurig-Briccio[b*], Svetlana Y. Gerdes[c], Robert B. Gennis[b], and Eric P. Skaar[d†]

[a]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824 [b]Department of Biochemistry, University of Illinois, Urbana, IL 61801; [c]Fellowship for Interpretation of Genomes, Burr Ridge, IL 60527; [d]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232.

Staphylococcus aureus is the leading cause of skin and soft tissue infections, bacteremia, osteomyelitis, and endocarditis in the developed world. The ability of *S. aureus* to cause substantial disease in distinct host environments is supported by a flexible metabolism that allows this pathogen to overcome challenges unique to each host organ. One feature of staphylococcal metabolic flexibility is a branched aerobic respiratory chain composed of multiple terminal oxidases. Whereas previous biochemical and spectroscopic studies reported the presence of three different respiratory oxygen reductases (*o*-type, *bd*-type and aa_3-type), the genome encodes only two respiratory oxygen reductases, *cydAB* and *qoxABCD.* Previous investigation showed that *cydAB* and *qoxABCD* are required to colonize specific host organs, the murine heart and liver, respectively. This work seeks to clarify the relationship between the genetic studies showing the unique roles of the *cydAB* and *qoxABCD* in virulence, and the respiratory reductases reported in the literature. We establish that *QoxABCD* is an aa_3-type menaquinol oxidase, but this enzyme is promiscuous in that it can assemble as a bo_3-type menaquinol oxidase. However, the bo_3-type form of *QoxABCD* restricts the carbon sources that can support the growth of *S. aureus.* In addition, *QoxABCD* function is supported by a previously uncharacterized protein we have named CtaM that is conserved in aerobically respiring Firmicutes. In total, these studies establish the heme A biosynthesis pathway in *S. aureus*, determine that QoxABCD is a type-aa_3 menaquinol oxidase, and reveal CtaM as a new protein required for type-aa_3 menaquinol oxidase function in multiple bacterial genera.

9:40 a.m.-10:00 a.m.

Staphylococcus aureus nitric oxide synthase protects against endogenous reactive oxygen species by contributing to aerobic respiratory metabolism

Austin B. Mogen[1], Ronan K. Carroll[2], Jeffrey Culver[3], Christopher Petucci[3], Lindsey N. Shaw[4], and Kelly C. Rice[1*]

[1]Department of Microbiology and Cell Science, IFAS, University of Florida, Gainesville, FL, USA; [2]Department of Biological Sciences, Ohio University, Athens, Ohio, USA; [3]Metabolomics Core, Sanford Burnham Prebys Medical Discovery Institute at Lake Nona, Orlando, FL, USA [4]Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, FL, USA

S. aureus is notorious for being resistant to multiple antibiotics. A promising target for drug development is the *S. aureus* nitric oxide synthase (saNOS) protein. Although the exact mechanism is unknown, saNOS contributes to virulence and protection against oxidative stress and antimicrobials. Here we describe a previously -unknown contribution of saNOS to aerobic respiratory metabolism. When grown aerobically, reactive oxygen species (ROS) and superoxide levels were elevated in a *S. aureus nos* mutant, independent of catalase activity. Inhibition of NADH dehydrogenase (NDH) limited ROS production in the nos mutant, suggesting that elevated ROS is due to altered NDH activity. NO contributes to respiration in various organisms, and likewise, our results suggest that NOS-derived NO alters *S. aureus* membrane potential and CTC reduction. Multiple transcriptional and metabolic changes were also observed in a *S. aureus nos* mutant, as assessed by RNAseq and targeted metabolomics analyses, respectively. Specifically, expression of genes associated with stress response, anaerobic/lactate metabolism, and cytochrome biosynthesis were increased in the *nos* mutant relative to wild-type. Metabolites utilized to produce reducing equivalents by the right arm of the TCA cycle were depleted in a nos mutant (citrate and α-ketoglutarate), whereas fumarate and malate levels were increased relative to wild-type. A significant reduction in lactate levels was also observed in the nos mutant. Collectively, these results support an important role for saNOS in limiting ROS accumulation during aerobic respiration, the loss of which may signal multiple transcriptional and metabolic adaptations.

Session 2: Antibiotic Development

10:20 a.m.-10:40 a.m.

Staphylococcus aureus RnpA: Investigation of essential function and identification of novel inhibitors

Jennifer M Colquhoun[1], Tess M Eidem[1], Andrew M Beckley[1] and Paul M Dunnman[1].

[1]Department of Microbiology and Immunology, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA

Staphylococcus aureus is a predominant cause of nosocomial and community acquired bacterial infections and has developed resistance to front-line antibiotics. Simply put, new agents are needed for the therapeutic intervention of *S. aureus* disease. The ribonuclease (RNase) RnpA represents a unique target for antibiotic development since it is hypothesized to catalyze two essential cellular processes, mRNA turnover and tRNA processing. Consequently, corresponding inhibitors may represent

dual-threat antimicrobial agents that limit two required biological functions and for which resistance may be slow to develop. Using a high-throughput screen, we previously identified novel chemical classes of RnpA inhibitors, including RNPA2000, which is a small molecule inhibitor of RnpA-mediated mRNA turnover and tRNA processing but suffers from a limited in vivo half-life that precludes its therapeutic development. Using RNPA2000 as a chemical probe, we have developed novel cell-based assays to efficiently query larger chemical space to identify additional chemical classes of RnpA inhibitors. This method has identified several structurally distinct RnpA inhibitors and we are currently employing a medicinal chemistry-based optimization program. Concurrently, as a means to both improve our understanding of the enzyme's cellular functions and allow rational design/ optimization of RnpA inhibitors, we are also defining the enzyme's active sites. Screening of an inducible RnpA alanine scanning mutant library has identified several mutants that display growth defects, which are currently being interrogated for altered mRNA degradation and/or tRNA processing function(s) to determine the enzyme's amino acid residues that are indispensable for function and *S. aureus* viability.

10:40 a.m.-11:00 a.m.

A novel MenA inhibitor against MRSA and its biofilm formation

Seoung-ryoung Choi, Joel Frandsen, Madeline Cloonan and **Prabagaran Narayanasamy**

Department of Pathology and Microbiology, University of Nebraska Medical center, Omaha, NE 68158

Given the growing prevalence of increased resistance to currently available antibiotics among strains of *S. aureus,* new treatment options and counter-approaches to target this lethal bacterial pathogen is needed. Our long term goal is to develop a novel and effective drug against Gram-positive bacteria, including drug resistant bacteria. In many microorganisms, menaquinone is an essential component and an obligatory component of the electron transfer pathway. Menaquinone is used by Gram positive bacteria in both aerobic and anaerobic conditions and its biosynthetic pathway is not present in human. Hence, MenA an enzyme involved in biosynthesis of menaquinone is a leading drug target. Here, we have designed, synthesized and tested a novel non-traditional inhibitor against MenA. Our drug like compound is very specific against MenA and highly active at low µmolar concentration. The new compound also inhibited the growth of MRSA including 10 clinical strains and also inhibited the biofilm formation by MRSA at low µmolar concentration. The discovered compound significantly reduced the bacterial load and the levels of the pro-inflammatory cytokines tumor necrosis factor-α from the host cells. This study provides evidence that our bicyclic compound has great potential for treatment of MRSA and lays foundation for further analysis and development towards translational research.

11:00 a.m.-11:20 a.m.

Inhibition of Staphylococcal growth and colonisation by species of Corynebacterium.

Wilhelm F. Oosthuysen, Jeffrey S. Kavanaugh and Alexander R. Horswill

Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA

Human mucosal surfaces and the skin are colonized with a myriad of bacteria that includes opportunistic pathogens, such as *Staphylococcus aureus* and *Streptococcus pneumonia*, and some non-pathogens, such as *Corynebacterium* sp. and *Propionibacterium* sp. Research has identified molecular interactions between colonizing bacteria, and it has been shown in several recent examples that the presence of a particular colonizing bacterial species can result in the abolishment of other bacteria. Utilizing an in vitro spot assay, we screened 95 *Corynebacterium* isolates for their ability to impair the growth of *S. aureus* strains LAC (USA300 MRSA) and RN4220 (MSSA), and we identified 16 isolates capable of inhibiting the growth of these *S. aureus* strains. In follow-up studies utilizing *Corynebacterium* spent media, we identified 3 isolates that were able to strongly inhibit the growth of *S. aureus*. Treatment of the spent media from these three *Corynebacterium* isolates with Proteinase K or heat-inactivation disrupted the inhibitory function to varying degrees, suggesting it is proteinaceous in nature. Size fractionation of the protein(s) using molecular weight cut-off columns indicated the activity is between 10 – 50 kDa. Current research is focused on isolating the bioactive protein(s) using cation/anion exchange and size exclusion chromatography, followed by identifying the target through proteomic approaches. In vitro co-culture experiments using human nasal and skin cells exposed to mixtures of MRSA and *Corynebacterium* species are in progress to further investigate the interaction. The identification and characterization of the *Corynebacterium* bioactive protein(s) could result in alterative treatments for people at risk of developing severe staphylococcal disease.

11:20 a.m.-11:40 a.m.

Apicidin mediated attenuation of MRSA virulence corresponds with quorum sensing inhibition and enhanced immune effector responses.

Corey P. Parlet[1], Jeffrey S. Kavanaugh[1], Heidi A. Crosby[1], Nadja B. Cech[2], Nicholas H. Oberlies[2] and Alexander R. Horswill[1]

[1]University of Iowa, Department of Microbiology, Iowa City, IA
[2]University of North Carolina Greensboro, Department of Chemistry and Biochemistry, Greensboro, NC

As the leading cause of infectious mortality in the United States, *S. aureus*-induced disease represents a major healthcare problem. The alarming rise of infections caused by virulent, antibiotic resistant strains, such as emerging methicillin-resistant *S. aureus* (MRSA) isolates, highlight the need for new interventions that inhibit MRSA pathogenicity and potentiate host defense responses. The expression of MRSA virulence factors is a function of *agr*-driven quorum sensing, making this system a promising target for anti-MRSA therapies. Through *agr*-reporter-based screens, a class of compounds, called apicidins, were found to inhibit quorum-sensing activity across MRSA isolates. To test the efficacy of apicidin *in vivo*, mice were challenged intradermally with 2x107 MRSA (+/-) 5 µg apicidin. The abatement MRSA virulence in the apicidin-treated group was demonstrated by reduced: weight loss, dermonecrosis and cutaneous bacterial burden. By challenging mice with an *agr*-reporter strain, we also found that the apicidin-mediated attenuation of MRSA pathogenesis corresponded with reduced quorum sensing activity *in vivo*. To evaluate apicidin's impact upon anti-MRSA effector responses, we assessed polymorphonuclear neutrophil (PMN) accumulation and function at cutaneous sites of infection. Flow cytometric analysis revealed that apicidin increased the density of PMNs within infected wounds 24 hours post infection. In addition, we found that the number of PMNs that phagocytosed MRSA organisms *in vivo* was increased in lesional skin preparations from apicidin treated mice. Together, these results indicate that apicidin -mediated quorum quenching represents a novel strategy to limit MRSA virulence and promote host defense.

11:40 a.m.-12:00 p.m.

Divide & Conquer: Synergy and mechanistic underpinnings of tick-borne antivirulent protein

Nabil M. Abraham [1,2], Lei Liu [1], Brandon L. Jutras [2,3], Akhilesh K. Yadav [4], Sukanya Narasimhan [1], Kimberly K. Jefferson [5], Felipe Cava [4], Christine Jacobs-Wagner [2,3,6,7], Erol Fikrig [1, 2, 7]

[1] Section of Infectious Disease, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT USA

ORAL ABSTRACTS

[2] Howard Hughes Medical Institute, Chevy Chase, MD, USA [3] Microbial Sciences Institute, Yale University, West Haven, CT USA [4] Laboratory for Molecular Infection Medicine, Department of Molecular Biology, Umea Center for Microbial Research, Umeå University, Umeå Sweden [5] Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA USA [6] Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA [7] Department of Microbial Pathogenesis, Yale University, New Haven, CT, USA

New strategies are needed to combat antibiotic resistance, especially against pathogens such as methicillin-resistant *Staphylococcus aureus*. Tick antifreeze glycoprotein, IAFGP, has the novel secondary function of inhibiting bacterial biofilm formation. We now demonstrate that IAFGP enhances the efficacy of antibiotics against *S. aureus*. Synergy with daptomycin - an antibiotic used to treat methicillin-resistant *S. aureus* - was observed *in vitro* and *in vivo* using *iafgp*-transgenic mice and fly model systems. Furthermore, synergy with ciprofloxacin or gentamicin, antibiotics not generally used to treat *S. aureus*, was also observed. The mechanism underlying this synergy was characterized whereby IAFGP binds to the terminal D-alanine residue of the pentapeptide chain of *S. aureus* peptidoglycan, altering the ability of bacteria to form biofilms and affecting antibiotic permeability. Synergy of IAFGP with antibiotics traditionally used to treat *S. aureus*, and enhancement of the potency of antibiotics not commonly used against this microbe, provide novel therapeutic strategies to combat bacterial infections.

Session 3: Sporulation

2:30 p.m.-2:50 p.m.

Assembly of the Exosporium Layer of the *Bacillus anthracis* Spore

Hsin-Yeh Hsieh, Jorge Durand-Heredia, and **George C. Stewart**

Department of Veterinary Pathobiology and Bond Life Sciences Center, University of Missouri, Columbia, Missouri, USA.

Bacillus anthracis is a zoonotic pathogen in which the spore is often the infectious entity. *B. anthracis* spores have as their outermost layer an exosporium, a balloon-like outer layer consisting of an outer nap layer comprised of a collagen-like glycoprotein, BclA and a proteinaceous basal layer. The exosporium plays important roles in persistence of the infectious spores in soil environments and on surfaces of human habitats, in the process of spore germination and subsequent outgrowth of the bacterial pathogen, and in the earliest interactions between the spores and the innate immune systems of infected hosts. The composition, assembly process, and function of the exosporium are incompletely understood. The best studied *B. anthracis* exosporium protein is BclA. It has an N-terminal domain that has been shown to be critical for positioning and stable attachment of BclA to the basal layer. This process is dependent on the basal layer protein BxpB (ExsFA) and to a lesser extent to the sequence similar ExsFB. During assembly of the exosporium, BclA is cleaved, resulting in loss of the N-terminal 19 amino acids. It has been hypothesized that this processing event is coupled to a covalent attachment of BclA to an exosporium protein or proteins, with BxpB and ExsFB being likely candidates. Our studies support the physical linkage of BclA with an exosporium basal layer protein. However, no covalent association with BxpB or ExsFB was found. Results of experiments to identify BclA-interactive partner proteins will be presented along with a model for exosporium assembly.

2:50 p.m.-3:10 p.m.

Dissecting the mechanism of DPA release during *Clostridium difficile* spore germination

Michael B. Francis and **Joseph A. Sorg**

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Clostridium difficile, a strict anaerobe, survives outside hosts in the form of a dormant spore. In a host, *C. difficile* spores germinate in response to certain bile acids and amino acids. Previously, we identified the bile acid germinant receptor as the germination-specific, pseudoprotease, CspC. In our working model, bile acids activate CspC which transmits the germination signal to the CspB protease. Activated CspB then cleaves the spore cortex lytic enzyme, pro-SleC, to the active form resulting in degradation of the spore cortex layer. In support of this model, we found that cortex degradation precedes the release of dipicolinic acid (DPA) from the core. This result is opposite to what is observed during germination by *Bacillus subtilis* and all other spore forming bacteria studied to date. In these organisms, DPA release through the SpoVAA – AF channel precedes cortex degradation. Interestingly, *C. difficile* only encodes orthologues of *spoVAC* (encoding a mechanosensing protein), *spoVAD* (encoding a DPA-binding protein) and *spoVAE* (unknown function). Here, we hypothesized that DPA release from the spore core during *C. difficile* spore germination occurs due to changes in osmolarity upon cortex degradation. In support of this hypothesis, we find that DPA release is blocked under high osmolyte concentrations but cortex degradation remains unaffected. These results support the building evidence that *C. difficile* spore germination proceeds through a novel germination pathway.

Session 4: Secretion

3:30 p.m.-3:50 p.m.

A structural comparison of *Listeria monocytogenes* protein chaperones PrsA1 and PrsA2 reveals molecular features required for virulence

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Listeria monocytogenes is a Gram-positive environmental bacterium that lives within soil but transitions into a pathogen upon contact with a mammalian host. The transition of *L. monocytogenes* from soil dweller to cytosolic pathogen is dependent upon secreted virulence factors that mediate cell invasion and intracellular growth. PrsA1 and PrsA2 are secreted bacterial lipoprotein chaperones that contribute to the folding of proteins translocated across the bacterial membrane; PrsA2 is required for *L. monocytogenes* virulence, whereas the function of PrsA1 remains to be determined. We have solved an X-ray crystal structure of PrsA1 and have used this model to guide comparison structure-based mutagenesis studies with PrsA2. Targeted mutagenesis of PrsA2 demonstrates that oligomerization of PrsA2 as well as molecular features of the foldase domain are required for protein secretion and virulence, whereas a functional role was uncovered for PrsA1 in bacterial resistance to alcohol. Interestingly, PrsA2 membrane localization is not required for all PrsA2-dependent activities, suggesting that the lipoprotein retains function when released from the bacterial cell. PrsA chaperones are thus multifaceted proteins with distinct domains adapted to accommodate the functional needs of a diverse array of secreted substrates.

3:50 p.m.-4:10 p.m.

An intracellular peptidyl-prolyl *cis/trans* isomerase (PPIase) is required for folding and activity of the *Staphylococcus aureus* secreted virulence factor nuclease

Richard E. Wiemels[1], Stephanie M. Cech[1], Nikki M. Meyer[1], Caleb A. Burke[1], Andy Weiss[2], Anastacia R. Parks[2], Lindsey N. Shaw[2] and **Ronan K. Carroll[1]**

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Staphylococcus aureus is an important human pathogen that relies on a large repertoire of secreted and cell wall-associated proteins for pathogenesis. Consequently, the ability of the organism to cause disease is absolutely dependent on its ability to synthesize and successfully secrete these proteins. In this study we investigate the role of peptidyl-prolyl *cis/trans* isomerases (PPIases) on the activity of the *S. aureus* secreted virulence factor nuclease (Nuc). We identify a staphylococcal cyclophilin-type PPIase (PpiB) that is required for optimal activity of Nuc. Disrupting *ppiB* results in decreased nuclease activity in culture supernatants, however the levels of Nuc protein are not altered, suggesting that the decrease in activity results from misfolding of Nuc in the absence of PpiB. We go on to demonstrate that PpiB exhibits PPIase activity in vitro, is localized to the bacterial cytosol, and directly interacts with Nuc in vitro to accelerate the rate of Nuc refolding. Finally, we demonstrate an additional role for PpiB in *S. aureus* hemolysis and demonstrate that the *S. aureus* parvulin-type PPIase PrsA also plays a role in the activity of secreted virulence factors. Deletion of *prsA* leads to a decrease in secreted protease and phospholipase activity, similar to that observed in other Gram-positive pathogens. Together these results demonstrate, for the first time, that PPIases play an important role in the secretion of virulence factors in *S. aureus*.

4:10 p.m.-4:30 p.m.

HtrA Monitors Sortase-Assembled Pilus Biogenesis in *Enterococcus faecalis*

Adeline Yong, **Kimberly Kline**

Enterococcus faecalis causes a wide range of infections including urinary tract infections, bacteremia, wound infections, and endocarditis. To effectively colonize and infect the host, *E. faecalis* uses several virulence factors that are translocated across the membrane and subsequently attached to the cell wall by the membrane anchored enzyme Sortase A (SrtA). In the absence of SrtA, translocated substrate proteins accumulate in the membrane instead of attaching to the cell wall resulting in membrane stress. In response to such stress, many bacteria rely on protein quality control processes that involve proteases and chaperones. Here we tested the hypothesis that the membrane-anchored HtrA (DegP) serine protease helps *E. faecalis* manage membrane- or cell wall-associated stress in response to accumulation of sortase substrates. Using the endocarditis and biofilm-associated pilus (Ebp) as a prototype sortase substrate, we have found that a strain lacking both SrtA where sortase substrates accumulate in the membrane and HtrA, displays an altered cell morphology including thickened cell wall, minicells, spherical cells, and chaining. These findings suggest that membrane sorting of sortase substrates and cell division may be tightly regulated and perturbations in sorting may affect co-localised process of cell wall synthesis and division. Transcriptomic and proteomic analyses showed down-regulation of cell wall biogenesis factors, as well as increased pilin expression in Δ*srtA*Δ*htrA* as compared to Δ*srtA* suggesting a secondary role of HtrA in regulating pilus biogenesis under membrane stress conditions. Deletion of the *ebp* operon in Δ*srtA*Δ*htrA* resulted in the reversion of the aberrant cell morphology to wildtype *E. faecalis*. Finally, we present evidence for a previously undescribed two component regulatory system in sensing membrane stress associated with aberrant sortase substrate accumulation in *E. faecalis*. Together, these findings indicate that HtrA in *E. faecalis* functions in monitoring both pilus biogenesis and cell cycle checkpoints in response to membrane stress.

4:30 p.m.-4:50 p.m.

Recycling of the thiol-disulfide oxidoreductase MdbA by VKOR in the biofilm-forming actinobacterium *Actinomyces oris*

Truc Thanh Luong, Melissa Reardon-Robinson and Hung Ton-That

Department of Microbiology & Molecular Genetics, University of Texas Health Science Center, Houston, TX, USA

Post-translocational protein folding is not well understood in Gram -positive bacteria. It has recently been shown in the high GC-content actinobacterium *Actinomyces oris* that a membrane-bound thiol-disulfide oxidoreductase named MdbA catalyzes disulfide bond formation of nascent polypeptides transported by the Sec translocon. Reactivation of MdbA requires the vitamin K epoxide reductase VKOR; however, the molecular mechanism of MdbA reactivation is not known. We present here a topological view of membrane-bound VKOR with two pairs of exoplasmic cysteine residues C93/C101 and C175/C178 that participate in disulfide relay. Like the phenotypes of the *vkor* deletion mutant, alanine substitution of these cysteine residues abrogated polymicrobial interactions and biofilm formation, concomitant of the failure to form adhesive pili on the bacterial surface in these mutants. Intriguingly, C101A mutation resulted in a high molecular weight complex positive for MdbA and VKOR by western blotting. Consistent with this observation, co-immunoprecipitation with antibodies against MdbA detected VKOR in the C101A mutant, but not in others; by alkylation with methoxypolyethylene glycol-maleimide, we demonstrated that MdbA was locked in a complex form with C101A mutation. Furthermore, ectopic expression of *Mycobacterium tuberculosis* VKOR in the *A. oris* □*vkor* mutant partially rescued its defects, whereas the *M. tuberculosis* VKOR isogenic mutants defective in disulfide relay did not. We propose here a model of C101-mediated disulfide relay required for MdbA reactivation that appears to be conserved in Actinobacteria.

4:50 p.m.-5:10 p.m.

Factors Affecting the Release and Activity of Pneumolysin

Joseph C. Bryant[1], Ridge C. Dabbs[1], Katie L. Oswalt[1], Lindsey R. Brown[1], Jason W. Rosch[2], Keun S. Seo[1], Matthew W. Brown[1], Janet R. Donaldson[3], Larry S. McDaniel[4], **Justin A. Thornton[1]**

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Streptococcus pneumoniae (pneumococcus) is an important human pathogen causing infections such as pneumonia, meningitis, and otitis media, affecting primarily young children and the elderly worldwide. Pneumococcus produces a pore-forming cytotoxin, pneumolysin (PLY) and also produces up to millimolar concentrations of hydrogen peroxide (H_2O_2) through the activity of the enzyme pyruvate oxidase (SpxB). We hypothesized that correlation exists between PLY release and the activity of SpxB. Additionally, we investigated whether proteases could potentiate the effects of PLY. When exposed to sterile wild-type supernatant, a significant loss of A549 cell viability was seen, whereas SpxB-supernatant failed to affect viability. We observed a significant reduction in the amount of PLY in the supernatant of various strains upon deletion of *spxB*. Furthermore, PLY released by strain WU2 was significantly reduced when grown in the presence of exogenous catalase. A significant correlation was observed between H_2O_2 production and PLY released in a panel of clinical isolates ($p<0.05$, $r2=0.3167$). Complementation of *spxB* significantly increased PLY release. Interestingly, trypsin exposure to epithelial cells previously exposed to sub-lytic concentrations of PLY dramatically enhanced loss of cell integrity. Therefore, we conclude an apparent connection exists between the activity of SpxB and the ability of the pneumococcus to release PLY into the extracellular space. This release could be related to endogenous H_2O_2 or to other byproducts of SpxB which could affect membrane potential. Additionally, SpxB activity could be a niche-dependent

method of modulating PLY release depending on the oxygen availability of the infection site.

Session 5: Virulence Factor ID

8:30 a.m.-8:50 a.m.

Using Tn-Seq to Explore Group A Streptococcal Fitness During Soft-Tissue Infection

Yoann Le Breton[1], Ashton T. Belew[1], Emrul Islam[1], Najib M. El-Sayed[1,2] and Kevin S. McIver[1].

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Streptococcus pyogenes (Group A Streptococcus, GAS) is a major human pathogen causing self-limiting infections of the skin and throat, as well as severe life-threatening invasive disease. GAS pathogenesis is complex; however, colonization of the epithelium followed by subepithelial tissue progression represents a common step in GAS infections. We developed a *mariner* mutagenesis system (*Krmit*) to produce highly complex mutant pools tractable for transposon sequencing (Tn-seq), which was previously used to define the set of essential genes (non-mutable) in the GAS core genome. Here, a near-saturation *Krmit* library of GAS 5448, representative of the invasive M1T1 serotype circulating worldwide, was cultivated in THY rich media and subjected to our Tn-seq pipeline. This allowed us to determine the genetic requirements for *in vitro* fitness; mutations that showed neutral, increased or decreased fitness. The same *Krmit* library of GAS 5448 was inoculated subcutaneously into immunocompetent hairless Crl:SKH1-*hr*BR mice, producing an abscess after 24 hours post infection (HPI) that progressed to an ulcerative lesion by 48 HPI. Library composition in lesions was monitored by Tn-seq, generating gene fitness indices of mutants differentially represented between input (T_0) and output pools (12, 24 and 48 HPI). After comparing to our *in vitro* Tn-seq dataset, we were able to define the GAS 5448 genetic requirements for *in vivo* fitness in lesions. Validation in selected genes was obtained by testing the fitness of defined mutants during competitive subcutaneous infection with 5448. Of particular emphasis have been genes in our dataset previously annotated as “of unknown function”.

8:50 a.m.-9:10 a.m.

Modeling Nasopharyngeal Fitness of *Streptococcus pneumoniae* in Sickle Cell Disease

Hannah M. Rowe, Aaron W. Poole, Ti-Cheng Chang, Jason W. Rosch

St Jude Children's Research Hospital, Memphis TN USA

Sickle cell disease (SCD) is caused by a mutation in human beta-hemoglobin, causing red blood cells to become rigid and bent. The resultant endothelial inflammation increases susceptibility to pathogens such as *Streptococcus pneumoniae* (pneumococcus). Even with routine vaccination and antibiotic prophylaxis SCD patients remain more susceptible to invasive pneumococcal disease than the general population. Previous work in our laboratory identified pneumococcal virulence factors essential for invasive disease in normal hosts to be dispensable in a murine model of SCD. Since nasopharyngeal colonization is a necessary prerequisite to development of invasive pneumococcal disease, we hypothesize that different colonization and mucosal virulence factors are important in a SCD host than a normal host. To test this, we performed a TnSeq screen comparing bacteria recovered from nasal lavage and lungs of normal and SCD mice. Conventional virulence factors and factors implicated in iron, arginine and purine metabolism, known to be altered in SCD patients, were identified. Targeted mutations in these genes have been generated and are being tested in murine SCD infections. On-going work is generating TnSeq libraries in clinical isolates from SCD patients to capture more of the pneumococcal pan-genome in clinically relevant strains, to better identify different colonization factors in pneumococci during infection of SCD patients.

9:10 a.m.-9:30 a.m.

The Transcriptional Regulator CpsY is Necessary for Innate Immune Evasion in *Streptococcus pyogenes*

Luis Alberto Vega, Kayla M. Valdes, Ganesh Sundar, Emrul Islam, Kevin S. McIver and Yoann Le Breton. Dept. of Cell Biology and Molecular Genetics & Maryland Pathogen

Research Institute, University of Maryland, College Park, MD 20742

Streptococcus pyogenes is an exclusively human pathogen that infects a large swath of the human population. Group A streptococcus (GAS) produces a variety of virulence factors in a highly regulated fashion to colonize multiple tissues and effectively evade host immune responses. We identified the LysR-family transcriptional regulator CpsY of GAS as necessary for innate immune evasion in a screen for immune evasion genes that combines existing human neutrophil killing assays with transposon deep sequencing (Tn-seq) of complex mutant libraries generated in a 5448 (M1T1) background. Insertional inactivation of the gene encoding CpsY reduces GAS resistance to killing by human phagocytic immune cells. Abrogation of this susceptibility by the inhibition of phagocytosis indicates the regulatory activity of CpsY is necessary for GAS to evade opsonophagocytic killing. However, the CpsY-null mutant did not display any reduction in virulence in either subcutaneous or intraperitoneal murine models of infection, nor was CpsY required for growth in mouse blood, thus suggesting that CpsY activity is specific to the human innate immune response. Furthermore, the CpsY-null mutant does not display the methionine-dependent phenotypes related to virulence observed in other streptococci. Expression of genes orthologous to those regulated by the GAS CpsY homologs MetR and MtaR in *Streptococcus mutans* and *Streptococcus agalactiae* is unchanged by loss of CpsY in GAS. RNAseq analysis of the CpsY -dependent transcriptome in M1T1 5448 suggest that CpsY influences a unique and heretofore uncharacterized regulon required for human immune cell resistance in GAS.

9:30 a.m.–9:50 a.m.

TnSeq analysis of *S. aureus* osteomyelitis reveals hypoxic responses as key determinants of the host-pathogen outcome

Aimee D. Wilde[1], Daniel J. Snyder[1], Nicole E. Putnam[1], Michael D. Valentino[2#a], Neal D. Hammer[1], Zachery R. Lonergan[1], Scott A. Hinger[3], Esar E. Aysanoa[1], Catlyn Blanchard[4], Paul M. Dunman[4], Gregory A. Wasserman[5#b], John Chen[6#c], Bo Shopsin[5], Michael S. Gilmore[2], Eric P. Skaar[1,7], and James E. Cassat[1,8]

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affiliation: Exosome Diagnostics, Inc. Riverside Technology Center; Cambridge, MA, USA; #bPresent affiliation: Department of Microbiology, Boston University School of Medicine, Boston, MA, USA; #cPresent affiliation: Yong Loo Lin School of Medicine, National University of Singapore

The pathogen *Staphylococcus aureus* is capable of infecting a range of organ systems in the human body. This flexibility requires a spectrum of metabolic and virulence programs in order to invade and replicate within diverse environments. The skeleton is an exceptionally common site of invasive staphylococcal infection, yet the pathogenesis of bone infections is poorly understood. In this study, we characterize the genetic programs required to sustain infection in a murine osteomyelitis model using transposon sequencing (TnSeq) analysis. TnSeq identified more than 200 genes important for invasive *S. aureus* osteomyelitis, two of which encode the bacterial two-component system, SrrAB. SrrAB is known to affect survival of *S. aureus* during hypoxic and nitrosative stress. Consistent with this finding, we discovered that oxygen levels in bone decrease during osteomyelitis. Moreover, an *srrAB* mutant fitness during osteomyelitis was significantly improved by neutrophil depletion, whereas respiratory burst deficiency only partially restored fitness, suggesting that neutrophils impose both hypoxic and nitrosative stress *in vivo*. Furthermore, we discovered changes in staphylococcal virulence programs when culturing bacteria under reduced gas exchange conditions to mimic the hypoxic bone environment *in vitro*. This hypoxic growth triggered significant increases in quorum-sensing-dependent toxin production and cytotoxicity towards mammalian cells. Conversely, aerobic growth limited quorum sensing and toxin production in an SrrAB-dependent manner, suggesting a mechanism for *S. aureus* sensing and response to the various host organ systems it encounters. Our data elucidate the genetic programs required for invasive osteomyelitis and demonstrate bacterial hypoxic responses as critical determinants of the host-pathogen interaction.

Session 6: Biofilms

10:10 a.m.–10:30 a.m.

Cyclic di-AMP released from *Staphylococcus aureus* biofilm induces a macrophage type I interferon response

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S. aureus is a leading cause of community- and nosocomial-acquired infections, with a propensity for biofilm formation. *S. aureus* biofilms actively skew the host immune response toward an anti-inflammatory state; however, the biofilm effector molecules and their mechanism(s) of action responsible for this phenomenon remain to be fully defined. The essential bacterial second messenger cyclic diadenylate monophosphate (c-di-AMP) is an emerging pathogen-associated molecular pattern during intracellular bacterial infections, where c-di-AMP secretion into the infected host cytosol induces a robust type I interferon (IFN) response. Type I IFNs have the potential to exacerbate infectious outcomes by promoting anti-inflammatory effects; however, the type I IFN response to *S. aureus* biofilms is unknown. Additionally, while several intracellular proteins function as c-di-AMP receptors in *S. aureus*, it has yet to be determined if any extracellular role for c-di-AMP exists and its release during biofilm formation has not yet been demonstrated. This study examined the possibility that c-di-AMP released during *S. aureus* biofilm growth polarizes macrophages towards an anti-inflammatory phenotype via type I interferon signaling. DacA, the enzyme responsible for c-di-AMP synthesis in *S. aureus*, was highly expressed during biofilm growth, and 30-50% of total c-di-AMP produced from *S. aureus* biofilm was released extracellularly due to autolytic activity. *S. aureus* biofilm c-di-AMP release induced macrophage type I IFN expression via a STING-dependent pathway and inhibited macrophage killing of intracellular *S. aureus*. These findings identify c-di-AMP as another mechanism for how *S. aureus* biofilms promote macrophage anti-inflammatory activity, which likely contributes to biofilm persistence.

10:30 a.m.–10:50 a.m.

Genetic and transcriptomic approaches reveal transcriptional reprogramming of *Enterococcus faecalis* during biofilm-associated infection

Kristi L. Frank[1], Cristina Colomer-Winter[2], Anthony O. Gaca[3], and José A. Lemos[2]

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Enterococcus faecalis is a leading cause of biofilm-associated infections, including endocarditis and implanted foreign body infections. Our goal is to understand the genetic mechanisms that enable *E. faecalis* to establish biofilm-associated infections and persist in a host. We previously paired transcriptomic and genetic approaches to characterize the *E. faecalis in vivo* transcriptome and identify *in vivo*-activated promoters in a rabbit subcutaneous foreign body (FB) abscess model. We used the same genetic screening technique to identify *in vivo*-activated promoters in a rabbit model of infective endocarditis. Analysis of these global data sets revealed two significant transcriptional reprogramming events in *E. faecalis* during mammalian infection. First, abundant genome-wide activation of antisense promoters was observed in the FB abscess and infective endocarditis genetic screens. In contrast, a very low degree of antisense promoter activation was reported when the same *E. faecalis* library used in our screens was tested in two *in vitro* conditions and in an invertebrate host. These results suggest that transcription from antisense promoters is enriched during mammalian infection and may serve as a gene regulation mechanism. Second, *E. faecalis* enter a stringent response(SR)-like state eight hours post-infection in the FB abscess model. We subsequently determined that, while the SR does not appear to be activated in *E. faecalis* endocarditis vegetations, (p)ppGpp levels affect *E. faecalis* colonization of rabbit heart valves, invasion of human coronary artery endothelial cells, and survival in human whole blood and serum. Understanding how these responses are activated may lead to new treatments for *E. faecalis* infection.

10:50 a.m.-11:10 a.m.

The metabolic control of programmed cell death during biofilm development and zebrafish infection.

Logan L. Bulock[1], Derek E. Moormeier[1], Marat R. Sadykov[1], Rebecca E. Hodges[2], Simon J. Foster[2], and Kenneth W. Bayles[1]

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Staphylococcus aureus is an important human pathogen that causes a wide array of diseases ranging from minor skin infections to life threatening diseases such as severe sepsis, necrotizing pneumonia, endocarditis, and bacteremia. The success of this pathogen in the modern era is partially attributable to its ability to form biofilm communities that are resistant to immune defenses, antibiotics, and other environmental stresses. Recent studies from our laboratory demonstrate that *S. aureus*

biofilms display differential expression of the *cid* and *lrg* genes involved in programmed cell death (PCD) during development that are associated with microniches varying in nutrient and oxygen availability. Preliminary data has shown the various microcolony structures formed display distinct metabolic states in vitro as evidenced by the differential expression of metabolic genes, altered tower type formation following metabolic disruption, and staining for respiratory activity and reactive oxygen species. Studies using a zebrafish model of infection revealed differential expression of the PCD genes suggesting that similar metabolic differentiation also occurs within an infected animal. The results of these studies provide important insight into the metabolic cues controlling PCD both in vitro and in vivo.

Session 7: Metals & Multispecies Interactions

12:40 p.m.-1:00 p.m.

Zinc availability alters the formation of *Streptococcus pneumoniae* biofilms

Lindsey R. Brown[1], Rachel Caulkins[1], Jason W. Rosch[2], and Justin A. Thornton[1]

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Streptococcus pneumoniae is a commensal of the nasopharynx; however, it is also the most common causative agent of otitis media and the leading killer of children under the age of five worldwide. Colonization of the nasopharynx largely occurs through the formation of biofilms. Bacteria found growing within biofilms have increased protection from antibiotics and the immune system; and it is within these structures that horizontal gene transfer allows for the exchange of genetic material, making biofilms an extremely important aspect of pneumococcal virulence. Therefore, understanding ques from the environment that contribute to the formation of biofilms is critical. It is already known that iron is essential for adequate formation of pneumococcal biofilms; however, little is known about the roles of other physiologically important metals such as copper, zinc, and manganese. In this study, we investigated the effect of metals on pneumococcal biofilm formation. *In vitro* biofilm assays were analyzed via crystal violet staining to detect total biofilm mass, and cell viability was assessed through plating and confocal microscopy. Our results showed that pneumococcal biofilms increase as zinc concentrations increase, in a dose dependent manner ($p<0.001$). Interestingly, this seems to be a zinc-dependent effect, as altering copper and manganese concentrations did not enhance the ability to form biofilms ($p=0.9$). Interestingly, alterations in biofilm formation do not appear to be due to changes in viability of bacteria within these biofilms. Scanning electron microscopy analysis revealed structural differences between biofilms grown in varying concentrations of zinc. Analysis of biofilm formation in mutant strains lacking the autolysin, LytA, and pyruvate oxidase, SpxB, revealed that zinc is not affecting pneumococcal autolysis or functioning as an antioxidant. The findings from this study indicate that changes in metal availability can significantly alter the ability of pneumococcus to form biofilms, and thus, the organism's ability to cause disease. Pneumococcus encounters a variety of metal concentrations as it traverses through the human body. Hindering the ability of the bacteria to acquire metals through the use of novel therapeutics could thereby affect bacterial colonization, and has the potential to inhibit progression to invasive disease.

1:00 p.m.-1:20 p.m.

Intercellular adhesion mediated by Aap: deciphering the assembly code

Andrew B. Herr

Divisions of Immunobiology & Infectious Diseases Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Staphylococcus epidermidis can form protein-based biofilms mediated by the accumulation-associated protein (Aap), even in the absence of extracellular polysaccharide. In these protein-based biofilms, the B-repeat region of Aap mediates intercellular adhesion after cleavage of the A domain. We previously demonstrated that Aap B-repeats self-assemble in the presence of Zn2+ to form rope-like structures between staphylococci. The B-repeat region contains up to 17 nearly-identical sequence repeats, assumed to be functionally equivalent. However, we now show that Aap Brepeats exist as two subtypes, defined by a cluster of consensus or variant amino acids. These variable residues are positioned near the zinc-binding (and dimerization) site and the stability determinant for the B-repeat fold. We have characterized four B-repeat constructs to assess the functional relevance of the two Aap B-repeat subtypes. Biophysical experiments demonstrated that constructs with the variant sequence show reduced or absent Zn2+-induced dimerization, and that the variant sequence can significantly stabilize the fold, depending on its location within the construct. Crystal structures revealed that side chains from the variant sequence form an extensive bonding network that can stabilize the fold. Furthermore, altered distribution of charged residues between consensus and variant sequences changes the electrostatic potential near the Zn2+ binding site, providing a mechanistic explanation for the loss of zincinduced dimerization in the variant constructs. These data suggest an assembly code that defines preferred oligomerization modes of the B-repeat region of Aap and a slipgrip model for initial contact and firm intercellular adhesion during biofilm formation.

1:20 p.m.–1:40 p.m.

Cambialism enhances the ability of *Staphylococcus aureus* to resist calprotectin and nutritional immunity

Yuritzi M. Garcia[1], Anna Barwinska-Sendra[2], Emma Tarrant[2], Eric P. Skaar[3], Kevin Waldron[2], **Thomas E. Kehl-Fie**[1]

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Staphylococcus aureus is a serious threat to human health due to the increasing prevalence of antibiotic resistance. One method that vertebrates use to combat pathogens is to limit the availability of essential metals, such as manganese (Mn), a defense known as nutritional immunity. The ability to cause devastating disease despite experiencing metal starvation during infection indicates that *S. aureus* possesses currently unknown mechanisms that enable it to overcome this defense. *S. aureus* expresses two superoxide dismutases (SODs), SodA and SodM. Our studies have revealed that SodM, which is not possessed by other staphylococci, enhances the ability of *S. aureus* to grow and resist oxidative stress when experiencing Mn starvation both in culture and during infection. Conversely, SodA does not protect *S. aureus* from Mn starvation and only promotes resistance to oxidative stress in Mn-replete environments. Analysis of the biochemical properties of both SODs revealed that SodA is Mn-dependent, but that SodM is fully active with both Mn and iron (Fe), or cambialistic. Examination of the metalation state of SodM following growth of *S. aureus* in the presence of the Mn-sequestering immune effector calprotectin revealed that this SOD is populated with Fe. Cumulatively, our results reveal that cambialism enables *S. aureus* to mitigate the impact that nutritional immunity has on SOD activity, enhancing the ability of the bacterium to resist oxidative stress. These studies provide a molecular rationale for the acquisition of SodM by *S. aureus* and to demonstrate a physiological role for cambialism during

infection.

Session 8: Multispecies Interactions

1:40 p.m.–2:00 p.m.

Enterococcus faecalis produces a bacteriocin that inhibits *Candida albicans* virulence and biofilm formation

Carrie E. Graham, Melissa R. Cruz, Michael C. Lorenz, and Danielle A. Garsin

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The human microbiota is a diverse polymicrobial population comprised of both bacteria and fungi that can be exploited to provide novel treatment and prevention strategies for infectious diseases. *Candida albicans*, a polymorphic yeast, is a constituent of the oralgastrointestinal tract of most humans where it interacts with other species, such as the Gram-positive bacterium, *Enterococcus faecalis*. We have demonstrated a specific interaction between *E. faecalis* and *C. albicans,* whereby a secreted bacterial factor inhibits hyphal growth in biofilms and in the *Caenorhabditis elegans* infection model, abrogating virulence. The inhibitor was present in wild-type *E. faecalis* supernatants, but not in mutants impaired in the Fsr quorum sensing system. Based on known targets of Fsr, we postulated that the activity could be encoded by *ef1097,* which encodes a putative secreted bacteriocin. Deletion of *ef1097* abrogated the inhibitory activity whereas addition of recombinant full-length EF1097 (rEF1097136) weakly inhibited *C. albicans* biofilm formation. In further work, we learned that EF1097 is cleaved into a smaller, more active form by the Fsr-regulated secreted proteases GelE and possibly SprE. A synthetically produced version of the mature EF1097 (sEF109768) was 10,000-fold more active and both inhibited and dismantled *in vitro* biofilm formation. Additionally, sEF109768 is protective in both a *C. elegans* and a murine infection model. Taken together these results support a model, in which the Fsr quorum sensing system regulates the expression and processing of EF1097 into an active inhibitor of *C. albicans* biofilm formation and virulence.

2:00 p.m.–2:20 p.m.

The PrtF.2 fibronectin-binding protein of *Streptococcus pyogenes* promotes specific attachment to influenza infected epithelial cells

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Influenza A virus (IAV) and *Streptococcus pyogenes* (GAS) are two important contributors to viral infections complicated by bacterial superinfection. Clinically, GAS superinfection produces severe pneumonia and is associated with increased mortality due to altered host response after IAV infection, especially within the lung. Thus, the global gene expression response of A549 lung epithelial cells infected with A/Hong Kong/1/68-H3N2 (HK68) influenza virus was characterized using microarrays to identify host genes responsive to IAV infection. Microarray and qPCR analysis revealed a global increase of transcript encoding host extracellular matrix adherence proteins (namely fibronectin and tenascin) induced during IAV infection. The success of an invasive bacterial pathogen depends on the ability to utilize host components that are readily available within the host environment. Therefore, we hypothesized that increases in expression of fibronectin and tenascin after IAV infection, may be a critical determinant to the severity and outcome of GAS superinfection. To test this hypothesis, we developed a mouse model of IAV-GAS superinfection with MGAS315 strains deficient in the fibronectin-binding protein, *prtF.2*. Inactivation of *prtF.2* significantly decreased virulence in infected mice. Additionally, we discovered that PrtF2 attaches to the extracellular matrix protein, tenascin. Our results suggest a novel GAS-host attachment mechanism, which may contribute to increased virulence after IAV infection. Furthermore, this novel interaction may be equally relevant to *S. pyogenes* pathogenesis, in the absence of influenza virus. Continued studies seek to further understand bacterial mechanisms associated with increased virulence after IAV-GAS superinfection.

2:20 p.m.–2:40 p.m.

Streptococcus pyogenes Bacteriophage A25: the Genomic Tale of Escaped Lysogeny in a High Efficiency Transducing Phage

Kimberly A. McCullor, Catherine J. King, Maliha Rahman, William M. McShan

Department of Pharmaceutical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, U.S.A.

Bacteriophage A25 has historically been used to study phage-host dynamics in *Streptococcus pyogenes.* Literature describes A25 as a highly efficient transducing lytic phage capable of transferring drug resistance genes amongst Group A Streptococci. Our goal is to gain insight into this ability by using next generation sequencing and bioinformatics. This analysis provided a better understanding of bacteriophage's role in horizontal gene transfer in *S. pyogenes* as well as the evolutionary history of A25. Genome sequencing was performed using the Illumina platform. BLASTP homology and Artemis were used for genome annotation and construction. Geneious, Clustal Omega, and Circoletto software were used for bioinformatics analysis. Analysis of the genome revealed that A25 probably originated from a lysogenic phage that lost its modules for integration and maintenance. A25 has a *cro*-like repressor, upstream operator, and downstream region that shares close homology with lysogenic phages of *S. pyogenes* strains MGAS10270, MGAS10750, and MGAS315. When screened for A25 susceptibility, these strains failed to produce plaques suggesting superinfection immunity repressing the A25 infection process. No other lysogenic regulatory genes were found within the genome, indicating escape from lysogeny. The A25 genome is mosaic in nature, sharing homology to phages from other streptococcal species such as phage MM1 of *Streptococcus pneumoniae* and phage SMP of *Streptococcus suis*, suggesting lysogeny escape was a distant event. The large subunit terminase gene of A25 has homology to phages known to use *pac*-type packaging mechanisms in comparison to *cos*-type packaging phages, which helps explain its well-known ability to mediate high-frequency transduction.

2:40 p.m.–3:00 p.m.

Identification of Gram-Positive Pathogens in Synovial Fluid Using a Metagenomic Shotgun Sequencing Approach

Matthew J. Thoendel[1], Patricio Jeraldo[2], Kerryl E. Greenwood-Quaintance[3], Janet Yao[2], Nicholas Chia[2], Arlen D. Hanssen[4], Matthew P. Abdel[4], and Robin Patel[1,3]

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Detection and identification of prosthetic joint infection (PJI)

pathogens remains challenging yet is very important for management of these difficult to treat infections. Gram-positive pathogens are responsible for the majority of these infections. Metagenomic shotgun sequencing is emerging as a powerful tool with the potential to change how many difficult to diagnose infections, including PJIs, are diagnosed. We sought to test a metagenomics approach to identify pathogens in synovial fluid collected from joints with previous total arthroplasties. Synovial fluid had been previously collected from 30 subjects with prior total knee arthroplasty and archived at -70°C. Human DNA was removed using the MolYsis basic kit prior to DNA extraction, whole genome amplification, and shotgun sequencing. Three samples were also evaluated without MolYsis pretreatment. Pathogen identification was performed using a pipeline incorporating LMAT and MetaPhlAn2 software. Using this approach, for all five culture-positive PJI cases, the pathogen was identified by metagenomic analysis. Pathogens included *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus salivarius. Acinetobacter johnsonii* was identified as a potential pathogen in one of two culture-negative PJIs. No potential pathogens were identified in 23 synovial fluid samples from uninfected joints. Comparison of two samples with and without MolYsis pretreatment revealed an increase in the number of known pathogen reads by 1,150 and 8,080-fold with pretreatment, which allowed for additional analysis, including genome assembly and annotation. These findings highlight the potential for metagenomics to identify pathogens in synovial fluid from joints suspected to be infected, which should be readily applicable to other normally sterile sources.

Session 9: Regulation

3:20 p.m.-3:40 p.m.

Regulation of cell division in *Staphylococcus aureus*

Catherine Spanoudis[1], Gianni Graham[1], Marissa G. Viola[2], Jodi L. Camberg[2], Karl M. Thompson[3], Kumaran S. Ramamurthi[4], and **Prahathees Eswara[1]**

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Cell division is a fundamental biological process that occurs in all kingdoms of life. Our understanding of cell division in bacteria stems from studies in the rod-shaped model organisms Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis*. The molecular underpinnings of cell division regulation in non-rod-shaped bacteria remain to be studied in detail. Given that essential cell division proteins act as attractive antibacterial drug targets, it is imperative for us to identify key cell division factors especially in pathogens to help counter the emergence of multi-drug resistance. In *Staphylococcus aureus*, a spherical Gram-positive opportunistic pathogen that causes a range of diseases from minor skin infections to life-threatening sepsis, we have identified the role of an essential protein GpsB in the regulation of cell division. We have discovered that GpsB, unlike its orthologs in rod-shaped organisms, interacts directly with the central cell division protein FtsZ. We report that GpsB localizes to cell division sites and constricts with the cell division machinery. Overproduction of GpsB results in cell enlargement typical of FtsZ inhibition, while depletion of GpsB results in cell lysis and nucleoid -less minicell formation. In vitro experiments show that GpsB bundles FtsZ polymers and subsequently stimulates the GTPase 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.

3:40 p.m.-4:00 p.m.

Identification of FarR binding site responsible for auto--repression and efflux--mediated resistance to antimicrobial fatty acids

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The divergent *farER* genes of *Staphylococcus aureus* encode a regulator of resistance to antimicrobial fatty acids FarR, and an efflux pump FarE. *S. aureusΔfarER* exhibited loss of inducible resistance to linoleic acid, and although FarR is a TetR family regulator which typically repress expression of a divergent gene, we found that FarR is needed to induce *farE*. Through previous *in vitro* selection for increased resistance of *S. aureus* to linoleic acid, we identified fatty acid resistant clone FAR7, where a single nucleotide polymorphism caused an H121Y substitution in FarR. Compared to wild type *S. aureus*, FAR7 exhibited increased expression of *farR* and *fare* under non--inducing conditions, and a significantly higher induced level of *farE*. Electrophoretic mobility shift assays revealed a FarR binding site in the *farER* intergenic segment, that overlaps with the +1 transcription start site of *farR* as determined by 5′′--RACE. H121YFarR produced by *S. aureus* FAR7 failed to bind to this operator site, and nucleotide substitutions within the operator abolished binding of native FarR. Conversely, FarR and H121YFarR bound equally well to a second operator site upstream of the predicted *farE* promoter. Therefore, like other TetR regulators, FarR represses its own expression. However, FarR is required for induction of *farE*, and increased resistance of *S. aureus* FAR7 to linoleic acid is due to an H121Y substitution in FarR, causing loss of auto--repression, and increased expression of both *farR* and *farE*. Our data define a new paradigm for regulation of a divergently transcribed gene by a TetR family regulator.

4:00 p.m.-4:20 p.m.

Redox regulation of plasmid copy number and transfer of the pheromone inducible conjugative plasmid pCF10

Jose Olivencia, Mauro Meloni, Bryan Utter and **Bettina Buttaro**

Lewis Katz School of Medicine, Temple University, Philadelphia, PA USA

Pheromone inducible conjugative plasmids of *Enterococcus faecalis* encode virulence factors, antibiotic resistance genes and mediate chromosomal exchanges between strains of *E. faecalis*. The protein PrgW has two roles in pCF10 biology. It is the replication initiation protein and a transcriptional activator of the *prgZ* (pheromone receptor gene). PrgW binds to direct repeats in the origin of replication and the *prgZY* promoter. PrgW contains three cysteines that are present in a majority of pheromone inducible conjugative plasmid replication proteins. When cells are grown under oxidative stress (0.5 M H_2O_2) pCF10 copy number increases from approximately 3 copies per cell to approximately 10 copies. In the current studies, we found while plasmid copy number increased in the present of H_2O_2, plasmid transfer decreased by two logs. The decrease in pCF10 transfer is accompanied by a decrease in *prgZY* transcription. EMSA assays performed the presence of the reducing agent DTT and the oxidizing agent diamide revealed oxidation of PrgW increases its binding to origin of replication. PrgW can only be detected by Western blotting when 100 OD equivalents are loaded on the gel, suggesting PrgW is present in low concentrations. Taken together these data suggest PrgW is a redox switch. Under oxidative

stress, oxidized PrgW is recruited to the origin of replication increasing plasmid replication. This reduces the available pool of PrgW so *prgZY* transcription is decreased potentially leading to a reducing the amount pheromone receptor PrgZ ultimately reducing the induction of conjugation by cCF10.

4:20 p.m.-4:40 p.m.

CodY directly represses the Sae two-component system in *Staphylococcus aureus*

Kevin D. Mlynek[1], Marat R. Sadykov[2], Derek E. Moormeier[2], Kenneth W. Bayles[2] and **Shaun R. Brinsmade[1]**

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The global regulatory protein CodY directly and indirectly controls the expression of over 300 metabolism and virulence genes in *Staphylococcus aureus* by monitoring the availability of branched-chain amino acids and GTP. We previously showed that CodY represses nuclease (*nuc*) expression via the SaePQRS two-component system; CodY likely controls *sae* indirectly by repressing the Agr quorum sensing system, promoting repressor of toxins (Rot)-mediated repression of the *sae* P1 promoter. However, *sae* induction precedes *agr* induction as CodY activity is decreased, suggesting our understanding of how CodY regulates Sae is incomplete. Using gel shift assays, we discovered that CodY binds to a DNA fragment containing the *sae* P1 promoter region, suggesting additional direct regulation by CodY at *sae*. Using quantitative realtime RT-PCR, we measured the expression of *saeP* and *nuc* during in vitro exponential growth in a suite of single and double mutant strains of two independent lineages of *S. aureus*. Consistent with a model incorporating direct CodY regulation, *saeP* and *nuc* transcript abundances increased only mildly (~2-fold) in a *rot* mutant but were significantly increased in a *rot codY* double mutant (5- and 22-fold, respectively). Interestingly, while *rot* mutant cells exhibit stochastic expression of *nuc,* lifting CodY-mediated repression abrogates the stochastic expression phenotype of *nuc.* Our results highlight a previously unrecognized role for CodY in controlling virulence gene expression in conjunction with three major virulence regulators in *S. aureus,* revealing a potential mechanism by which CodY acts as a master regulator of pathogenesis by tying nutrient availability to virulence gene expression.

4:40 p.m.-5:00 p.m.

VfrB is required for the activation of the *Staphylococcus aureus* SaeRS two-component system

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During previous studies, we identified the fatty acid kinase VfrB as a potent regulator of α-hemolysin and other virulence factors in *Staphylococcus aureus*. In this study, we have demonstrated that VfrB is a positive regulator of the SaeRS two-component regulatory system. Analysis of *vfrB* and *saeR* mutant strains revealed that VfrB is functioning in the same pathway as SaeRS. At the transcriptional level, expression of SaeRS class I (*coa*) and class II (*hla*) target genes is down-regulated in the *vfrB* mutant compared to the wild-type strain. In addition to this, expression of *saePQRS* is decreased in the *vfrB* mutant strain, demonstrating a need for the protein in the auto-regulation feedback of SaeRS. The requirement for VfrB-mediated activation is circumvented when SaeS is constitutively active due to an SaeS (L18P) substitution. Furthermore, activation of SaeS via HNP-1 overcomes the dependency of VfrB for transcription of class I promoters. Consistent with the role of VfrB in fatty acid metabolism, the expression of *hla* is decreased in the *vfrB* mutant with the addition of exogenous myristic acid, indicating a role for VfrB in stimulating SaeRS in response to saturated fatty acids. Lastly, we identified that amino acids predicted to be key to VfrB activity are required for VfrB-mediated α-hemolysin production. Collectively, this study implicates VfrB as a novel accessory protein needed for activation of SaeRS in *S. aureus*.

5:00 p.m.-5:20 p.m.

Coordinating group behavior for successful host colonization: Quorum sensing in *Streptococcus pyogenes*

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Group A Streptococcus (GAS, *Streptococcus pyogenes*) a major cause of human disease, exists in the body in at least two different lifestyles: an avirulent mode of carriage and a virulent disease-causing state. The mechanisms by which GAS maintain each lifestyle and switches between them remains unclear. In GAS, the invariable presence and conservation of quorum sensing (QS) pathways, used by bacteria to coordinate gene expression among populations, implies an important role for this human-restricted microorganism. The QS pathway comprised of the transcription factors Rgg2 and Rgg3 responds to short hydrophobic peptide (SHP) pheromones to induce gene expression leading to biofilm formation, cellular aggregation and increased resistance to lysozyme. This system is induced by environmental conditions akin to those GAS would encounter in the nasopharynx. We have determined that *spy49_0414c,* a small gene in the *shp2* operon, alters the cell wall, modifying display of surface proteins and resulting in the observed phenotypes. Preliminary data also establish that *spy49_0414c* expression alters bioavailability of the IL-8 protease SpyCEP and enhance fibronectin binding. We have also recently found that expression of genes in the *shp3* operon limit release of the hemolysin SLO. Collectively, these findings indicate the Rgg2/3 QS system plays an important role in mediating host-bacteria interactions via modifications to the cell surface as well as altering secretion of virulence factors. We are currently investigating underlying mechanisms that will help us understand these phenotypes, and have profound effects on GAS lifestyles in the host.

Session 10: Host Pathogen Interactions

10:00 a.m.–10:20 a.m.

Scl1 structural element mediates wound colonization by *group A Streptococcus and disrupts healing response*

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Group A *Streptococcus* (GAS) is a human-adapted pathogen that causes in excess of 700 million infections worldwide each year, with an estimated 517,000 deaths. It is commonly accepted that GAS infection starts with wound colonization by binding to deposited extracellular matrix, which is significantly enriched with cellular fibronectin (cFn) isoforms containing the extra domain A (EDA). During wound healing, keratinocytes express an integrin

($\alpha_9\beta_1$) that binds the EDA segment of cFn. At the same time, the streptococcal collagen-like protein 1 (Scl1), which is expressed on the GAS surface, also binds EDA, within the $\alpha_9\beta_1$ integrin binding site. We *hypothesize* that the Scl1-EDA/cFn interaction targets GAS to wound-microenvironment enriched in EDA/cFN, promoting tissue colonization and delaying wound healing. An epidermal wound was prepared in a skin equivalent model, infected with GFP-expressing GAS, and analyzed by standard histopathology and two-photon fluorescent (TPF) microscopy. Tissue infected with GAS exhibited delayed wound closure as late as day 5 post-infection. Gram stain of infected tissues showed biofilm formation on the surface of exposed dermis, as well as bacterial invasion extending deep into dermal layer. TPF imaging showed bacterial structures within tissue encased in a glycocalyx, a reminiscent of a classic biofilm. Homology modeling, protein engineering and binding assays identified the Scl1-functional element interacting with EDA. Deciphering the Scl1 binding mechanism to EDA/cFn will provide the basis for the development of inhibitors to this interaction and prevent host invasion.

10:20 a.m.-10:40 a.m.

ArlRS – MgrA signaling cascade is the central regulator of adhesion and clumping in *Staphylococcus aureus* intravascular infections

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Adhesion of *Staphylococcus aureus* to blood vessels and formation of bacterial clumps in plasma are the essential steps in pathogenesis of sepsis, endocarditis and metastatic infections. Little is known about the regulation of these processes, and most laboratory experimental systems don't capture the complex environment of the vasculature, where adhesion occurs under the constant shear stress of flowing blood. By using a flow chamber system, we simulated vasculature adhesive conditions and used it to study the role of a recently described regulatory axis, ArlRS – MgrA. Inactivation of this cascade resulted in inhibition of *S. aureus* binding to an array of ligands found in the vascular wall, such as fibrinogen, fibrin, collagen, fibronectin and von Willebrand factor. The inhibition was caused by a set of large surface proteins, called Ebh, SraP and SasG, that are repressed by ArlRS – MgrA and prevent adhesion to host molecules, most likely by shielding the functional exposure of smaller surface adhesins, such as ClfA and FnbpA. Expression and truncation studies demonstrated that the inhibition effect was dependent both on the amount of Ebh, SraP and SasG present on the surface and on the size of these proteins. In parallel studies looking at *S. aureus* clumping in the presence of plasma, the same dependence on the amounts of Ebh, SraP and SasG expressed and protein size was observed. Thus, the ArlRS – MgrA signaling cascade is an important regulator of *S. aureus* binding to host matrix molecules and a potential therapeutic target for treating vascular infections.

10:40 a.m.-11:00 a.m.

The contribution of the LytR transcriptional regulator to Group B Streptococcal colonization and disease

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Poster # A01

The ClpXP protease acts as a crucial mediator of bacterial programmed cell death

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Maturation of microcolonies during bacterial biofilm development is often characterized by cell death within clonal subpopulations, a process that is thought to be analogous to eukaryotic programmed cell death (PCD). Although the mechanisms underlying bacterial PCD remain unclear, cell death is primarily potentiated by weak acid metabolic byproducts like acetic acid that accumulate within biofilm microcolonies. Here we explore the role of the major intracellular protease, ClpP in modulating acetate dependent cell death. We demonstrate that mutation of *clpP* surprisingly limits acetate mediated cell death due to decreased production of endogenous reactive oxygen species (ROS). Similarly, inactivation of *clpX* which encodes an ATP dependent unfoldase that translocates denatured proteins to the proteolytic chamber of the ClpP protease, phenocopied the *clpP* mutant suggesting a crucial role for the ClpXP protease in mediating acetate dependent cell death. We have additionally identified the global transcriptional regulator, Spx as the downstream effector of ClpXP dependent cell death. Mutation of *spx* dramatically increased endogenous ROS even under non-acidic conditions, presumably due to disruption of intracellular thiol homeostasis. These findings collectively suggest that weak acids could trigger bacterial PCD by modulating ClpXP proteolytic activity.

Poster # A02

The role of the anti-sigma factor RsiV in lysozyme sensing and stress response in *Bacillus subtilis* and *Clostridium difficile*

***Ana N Castro**[1], Lincoln T Lewerke[1], Ben D Cortes[2], Jessica L Hastie[1], Craig D Ellermeier[1]

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ECF σ factors are alternative σ factors that allow many bacteria to sense and respond to changes in the environment. σV, an ECF σ factor, is found in low GC Grampositive bacteria, induces resistance to lysozyme and is important for virulence in pathogens. In the absence of lysozyme, σV is inhibited by the anti-σ factor RsiV. In response to lysozyme, RsiV is degraded by regulated intramembrane proteolysis (RIP). RIP is initiated by signal peptidase cleavage of RsiV at site-1 resulting in the release and activation of σV. We demonstrate *in vitro* that signal peptidase is sufficient for cleavage of RsiV only in the presence of lysozyme. By altering the signal peptide, we find that the spacing between the cleavage site and the transmembrane is critical to RsiV avoiding signal peptidase cleavage in the absence of lysozyme. The lab previously determined the X-ray crystal structure of the extracellular domain of RsiV in complex with lysozyme. This structure revealed that RsiV does not bind near the signal peptidase cleavage site. This has led to our model in which binding of lysozyme to RsiV triggers a conformational change, which allows signal peptidase to recognize the cleavage site. Currently, we are determining the structure of full-length RsiV in both the absence and presence of lysozyme. Together this will provide information on the changes that occur to RsiV upon binding lysozyme that lead to σV activation. This will broaden our knowledge on a novel role for signal peptidase and could lead to novel drug targets.

Poster # A03

SACOL1789, a putative stress response protein, is a novel protective vaccine antigen in the model of soft tissue and skin infection of *Staphylococcus aureus*

Brian L. Cheng[1], Travis Nielsen[2], Paul Pantapalangkoor[2], Susan Boyle-Vavra[1], Christopher P. Montgomery[1], Brad Spellberg[2],

the LytR gene in our murine meningitis model and histological studies reveal that it induces increased inflammation of the tissues surrounding the brain. Using RT-qPCR and ELISA, we have seen that human brain microvascular endothelial cells (hBMEC) secrete more inflammatory chemokines and cytokines, such as IL-8, CXCL1, IL-6 and IL-1β, when infected with the LytR mutant. Additionally, the LytR mutant demonstrated an increased ability to invade hBMEC as we visualized more intracellular organisms by EM analysis. Interestingly, using our mouse model of vaginal colonization we observed that the LytR mutant is cleared rapidly while wild type bacterial are able to persist in the vaginal tract. Ongoing RNAseq studies are underway to identify downstream gene targets of the LytR response regulator in order to better understand the changes in gene expression that occur during the transition from asymptomatic colonization to inflammatory disease. Ultimately, uncovering how LytR signaling affects bacterial colonization and virulence may lead to the development of more effective therapies to prevent GBS infection.

11:00 a.m.-11:20 a.m.

BH3-only Protein PUMA and Innate Immunity in Pneumococcal and Staphylococcal Infections

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The Gram-positive bacteria *Streptococcus pneumoniae* (pneumococcus) and *Staphylococcus aureus* are opportunistic pathogens capable of causing serious diseases in humans in the absence of a sufficient immune response. After previously demonstrating the pro-apoptotic, BH3-only Bcl2 family member Puma to be important for survival during pneumococcal infection in a murine model, we hypothesized that Puma functions to help regulate innate immune cell fate and that its importance would also extend to protection against staphylococcal infections. Compared to Puma+/+ mice, Puma-/- mice challenged intradermally with *S. aureus* strain USA300 demonstrated significantly more severe dermonecrotic lesions. Analyses of dermonecrotic lesions revealed Puma-/- mice contained approximately 2 log10 more CFU/g of *S. aureus* at seven days post-infection than did Puma+/+ mice. Puma-/- mice also exhibited greater than 100% more neutrophils and less than one-fourth the number of macrophages in dermonecrotic tissue samples than their Puma+/+ counterparts. Differentiated Puma-/- neutrophil progenitors were less apoptotic than Puma+/+ neutrophil progenitors after a two hour exposure to *S. pneumoniae* TIGR4 strain followed by 22 hours of incubation in growth medium (12% more death in Puma-/- v. 39% more death in Puma+/+) in the absence of bacteria. These findings suggest that Puma plays an important role in innate immunity and that regulation of cell fate may be central to its protective role.

11:20 a.m.-11:40 a.m.

Role of Lipids in Crosstalk between *S. aureus* and Host

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Staphylococcus aureus lipases play a role in nutrient acquisition and protection from toxic fatty acids. Alterations in the host lipidome by staphylococcal lipases could also affect Lipid-mediated signaling. The process of wound healing requires lipid signals and interference with lipid signaling pathways can arrest the healing process. *S. aureus* is a leading cause of wound infections and is associated with delayed wound healing. Wounds that fail to heal within a normal period of time are the leading cause of amputations in the U.S. but pathology of delayed healing remains poorly understood. We hypothesize that lipid-mediated Crosstalk between the host and *S. aureus* plays a central role in delaying wound healing. In this study, we tested our hypothesis that this cross-talk centers around the biofilm phenotype and that *S. aureus* lipases modify host lipids necessary for healing. Using a lipidomic approach, we investigated the effect of a wildtype strain and isogenic lipase mutants on eicosanoids in wounded 3-dimensional tissue culture models of skin. We also investigated the effect of select host lipids on biofilm formation and on expression of the gene encoding the secreted lipase, LipA. Exposure of wounded model skin to a *lipA*-deficient strain lead to a dramatic increase in prostaglandin E 2 (PGE 2) and other pro-inflammatory host lipids but the PGE 2 response induced by the *lipA*-positive wildtype strain was minimal. *S. aureus* responded to PGE 2 through increased biofilm formation and *lipA* transcript levels. In conclusion, LipA may inhibit PGE 2 signaling and PGE 2 induces *lipA* and biofilm formation.

11:40 a.m.-12:00 p.m.

Host and Bacterial Proteases Influence Virulence in a Murine Model of Enterococcal Catheter-Associated Urinary Tract Infection

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Enterococcus faecalis is a leading causative agent of catheter-associated urinary tract infection (CA-UTI), one of the most common nosocomial infections. Although the molecular details of how enterococci survive and persist in the bladder are not well understood, it is known that their ability to grow and form catheter biofilm is dependent upon host fibrinogen (Fg). Here, we examined the contribution of bacterial and host proteases to enterococcal-Fg interaction. Analysis of a mutant panel affecting the two major secreted proteases of *E. faecalis* OG1RF revealed that while the loss of either GelE or SprE had no effect on virulence in a murine CAUTI model or for the ability to form Fg-dependent biofilm in urine, the loss of both resulted in attenuation and defective biofilm formation. Mutants lacking GelE, but not SprE, lost the ability to degrade Fg in media. However, both proteases could degrade Fg upon incubation in urine. This was due to the fact that while activation of SprE in media required cleavage by GelE, SprE could be activated by a host trypsin-like protease in urine. Finally, catheter-implanted mice were treated with a protease inhibitor cocktail to inhibit both host- and bacterial-derived proteases. While this treatment did not reduce bacterial titers, it dramatically reduced catheter-induced inflammation and significantly inhibited dissemination from bladder to kidney. Taken together, these data show that both bacterial and host proteases contribute to CA-UTI, that GelE and SprE are functionally redundant in vivo and that host proteases play a major role in promoting inflammation and dissemination.

ICG+P POSTER SESSION "A" ABSTRACTS

MONDAY, OCTOBER 10, 2016
12:30 P.M.-2:30 P.M.

POSTER NUMBERS AND PRESENTERS

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A02 Ana Castro
A03 Brian Cheng
A04 Jorge Durand-Heredia
A05 Jennifer Endres
A06 Kyle Grim
A07 Bryan Hancock
A08 Timothy Hermanas
A09 Lorne Jordon
A10 Gabriela Kaus
A11 Jeff Kavanaugh
A12 Kyle Kinney
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A15 Robert McKee
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A17 Kimberly Perez
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A26 Andy Weiss
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A28 Anne-Marie Leuck

POSTER SESSION "A" ABSTRACTS

Robert S. Daum.[1]

[1]University of Chicago, Departments of Microbiology and Pediatrics; [2]University of Southern California, Keck School of Medicine

Staphylococcus aureus is the leading cause of skin infection and certain invasive diseases. There is an urgent need to develop an effective vaccine to prevent *S. aureus* infections. However, recent failure in human vaccine trials, despite immunogenicity has indicated that an *S. aureus* vaccine has not yet been identified. We tested a putative stress response protein, SACOL1789, as a vaccine candidate against S. aureus infection in the murine soft tissue and skin infection (SSTI) and bacteremia models. 7 weeks old BALB/c and B-cell deficient (BKO) BALB/c mice were immunized on d0 and d21. Blood serum was collected on d35 for ELISA analysis prior to infection. We found that immunization of SACOL1789 protects BALB/c mice with decreased lesion size and reduced bacterial load compared with controls. However, protection was not observed in the bacteremia model. We further evaluated whether protection in the SSTI model can be replicated in B-cell deficient (BKO) BALB/c mice. Protection observed in BKO BALB/c mice indicated that B cells are not essential. We performed ELIspot on splenocytes extracted from immunized mice and detected increased levels of IL-4, but not IFN-g and IL-17A. This suggests that SACOL1789-driven protection is dependent on IL-4 production and possibly the IL-4 dependent Th2 cell-mediated pathway. It further suggests that immunogenicity alone will not identify candidate protective antigens and that antigens protective against SSTI may not be identical to those protecting against bacteremia.

Poster # A04

CotY and ExsY: providing the foundations for *Bacillus anthracis* exosporium

Jorge M. Durand-Heredia, Molecular Pathogenesis & Therapeutics Graduate Program, Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

George C. Stewart, Department of Veterinary Pathobiology, Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

Bacillus anthracis is a Gram-positive, soil-dwelling, endospore-producing and zoonotic pathogenic bacterium that infects ruminants causing the lethal septicemic disease known as anthrax. Human infections manifest in cutaneous, gastrointestinal, or pulmonary anthrax, depending on the route of spore entry into the patient. *B. anthracis* spores possess structural similarities with *B. subtilis* spores, but have an additional outermost protective layer, the exosporium. The exosporium consists of a proteinaceous basal inner layer surrounded by a hair-like nap outer layer (predominantly composed of the collagen-like glycoprotein BclA). During the initial stages of infection, spores through their exosporia interact with the host innate immune system. Thus the composition of the exosporium, and its function, is essential to an understanding of the *B. anthracis* infectious process. Approximately twenty proteins are known exosporium components. Two of these proteins that reside within the basal layer, CotY and ExsY, share extensive sequence similarity and are thought to be important in exosporium assembly. Despite their extensive sequence similarity, they localize predominantly to different regions of the exosporium and perhaps associate with different protein partners. In this study, we created deletion mutants lacking *cotY* and *exsY* genes, both singly and in combination and examined the effects of loss of CotY and ExsY proteins on exosporium assembly and composition. A model for assembly of this outermost spore layer is proposed based on our findings.

Poster # A05

Use of the Nebraska Transposon Mutant Library to identify *S. aureus* cell wall-associated proteins important for pathogenesis in a murine skin abscess model of infection

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Since the early 1990's, the community-associated USA300 lineage of *Staphylococcus aureus* has been responsible for an epidemic of skin and soft tissue infections in the US. Presumably, a unique combination of virulence factors and metabolic characteristics has made this strain ideally suited to colonize and/or infect a human host. Identifying virulence determinants of the USA300 lineage could lead to significant findings for treatment of infections. To study the surface proteins important in the pathogenesis of abscess formation, we tested mutants affecting LPXTG-motif protein production from the Nebraska Transposon Mutant Library (NTML) in a murine skin abscess model of infection. Interestingly, only two mutants were deficient in abscess formation. The first mutant, SAUSA300_1327 locus, which is a homolog to the Fnb proteins, had bacterial burdens similar to the wild-type strain. However, most mice infected with this mutant did not develop dermonecrosis. The second mutant contained an insertion in the serine-aspartate repeat-containing protein C, *sdrC.* Our studies demonstrated that it had a significantly reduced ability to form abscesses where only two out of eight animals had detectable abscesses by day 7. In agreement with this is that a mutation in sortase, *srtA*, which is a mutant defective in the anchoring of LPXTG motif cell wall proteins only had detectable abscesses in three out of eight animals. Purified SdrC generated IgG titers four logs higher than mock treated animals and the bacterial burdens in abscesses of animals immunized with 100 µg of purified protein showed reduced bacterial burdens within the abscess. Overall, these results provide a comprehensive comparison of the contributions of *S. aureus* cell wall proteins in abscess formation and provide valuable information about the composition of future anti-staphylococcal vaccines.

Poster # A06

Two zinc acquisition systems contribute to the ability of *Staphylococcus aureus* to resist host-imposed metal starvation.

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Staphylococcus aureus is an important human pathogen that causes devastating disease. During infection, vertebrates restrict bacterial access to essential nutrients, a process termed nutritional immunity. The host protein calprotectin contributes to this process by limiting the availability of manganese and zinc. Calprotectin binds manganese and zinc with nanomolar and picomolar affinity, respectively. Despite calprotectin's high affinity for zinc, sequestration of manganese is necessary for maximal antimicrobial activity. Consistent with this observation, concentrations of calprotectin which reduce the intracellular content of manganese do not reduce the intracellular content of zinc. Together, these data suggest that *S. aureus* successfully competes with calprotectin for zinc. However, the zinc uptake systems expressed by *S. aureus* remain unknown. Examination of the regulon of Zur, the zinc uptake regulator, identified two potential zinc importers. The first importer, AdcABC, is a member of a conserved family of zinc transporters. The second importer belongs to a family not previously associated with zinc transport. Both systems are induced in the presence of calprotectin, with the non-canonical transporter being the most highly induced gene set following exposure to calprotectin. Loss of AdcA alone did not significantly increase the sensitivity of *S. aureus* to calprotectin. However, loss of both AdcA and the non-canonical transporter resulted in hypersensitivity to calprotectin. A mutant lacking the non-canonical transporter alone was also more sensitive to

calprotectin-imposed zinc sequestration, suggesting that it serves as the primary transporter under zinc-deplete conditions. A mutant lacking both systems is less virulent, suggesting these transporters are necessary for full virulence.

Poster # A07

Identification of a Group B streptococcal factor that contributes to tight junction disruption in brain endothelium

Bryan Hancock[1], Andres Bermudez[1], John Lopek[2], David Gonzalez[2], Kelly Doran[1]

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Streptococcus agalactiae (Group B *Streptococcus,* GBS) is a Gram-positive pathogen that can cause bacteremia, sepsis, and meningitis. GBS must cross the blood-brain barrier (BBB) in order to cause disease. The BBB is comprised of a single layer of cells joined by protein complexes known as tight junctions that act as a non-permissive barrier between the blood and cerebral spinal fluid. We have characterized a host transcription factor, Snail1, a global regulator of tight junction proteins that is upregulated during GBS infection. Snail1 upregulation in response to GBS infection is necessary and sufficient for disruption of BBB integrity. Our preliminary experiments suggested that the GBS factor responsible for Snail1 induction is proteinacious. Further, we treated GBS with a variety of enzymes prior to infection and subsequently analyzed the Snail1 transcript via qPCR. We have observed that treatment of GBS with proteases reduces Snail1 activation. Purified GBS cell wall extracts upregulate Snail1 nearly 30 fold. Interestingly, cell wall extracts from a GBS mutant that lacks capsule (HY106) and the membrane anchor for lipoteichoic acid, *ΔiagA,* exhibited a reduced ability to activate Snail1. We hypothesized that this double mutant is missing a key factor responsible for Snail1 activation. To investigate we have begun proteomic analysis of cell wall extracts of GBS wild type and the HY106 *ΔiagA* mutant using mass spectrometry. Our results have revealed a number of differentially expressed protein products that we are investigating further using gene ontology analysis to identify the specific protein(s) responsible for Snail1 upregulation.

Poster # A08

A cyclic-di-GMP signaling system in spores of Bacillus anthracis

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The signaling molecule bis-(3'-5')-cyclic-dimeric guanosine monophosphate (c-di-GMP) is a central regulator of diverse cellular functions, including biofilm formation, toxin production and virulence, in bacteria. The ability to generate c-di-GMP can be predicted by the presence of diguanylate cyclases (DGCs) (characterized as GGDEF domain proteins) and degraded by phosphodiesterases (PDEs) (characterized as EAL domain proteins). To date, the majority of phenotypes analyzed have been performed in gramnegative bacteria with recent work revealing that c-di-GMP plays a role in mediating phenotypes in gram-positive bacteria as well. The genome of Bacillis anthracis, causative agent of Anthrax, is predicted to encode at least nine putative GGDEF/EAL-domain containing proteins. We analyzed the expression of the putative GGDEF/EAL domain proteins as mCherry reporter proteins and found that several are expressed during sporulation. In mature spores, it appears that BAS2357, BAS3594, BAS3899, and BAS3954 are present. When expressed in a cotE null background, which lacks an attached exosporium, BAS3899 and BAS3954 were no longer spore associated. This suggests these two proteins are found in the exosporium or interspace layers of the spore. BAS2357 and BAS3594 are retained in the exosporium lacking spores. Also, mature spores appear to have the ability to generate c-di-GMP de novo from GTP. The incubation of mature spores with GTP appears to be sufficient for the generation of c-di-GMP and was only detected with GTP was added. Spores from mutants lacking c-di-GMP-associated genes have been prepared and studies are underway to identify which of these proteins are responsible for the diguanylate cyclase activity.

Poster # A09

RNPP Family Transcription Factors in *Enterococcus faecalis*

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Department of Molecular Biosciences | University of Kansas | Lawrence, Kansas, United States

Infections caused by enterococci are a serious threat to human health, as they represent one of the three most common hospital-acquired pathogens in the United States and around the world. A dichotomy exists between the many benefits of enterococci as a commensal organism and the harmful effects caused to its host in an infective state. Due to its opportunistic nature, enterococci readily transition from being commensals to pathogens. We recently discovered a peptide transporter, PptAB, involved in the export of small peptide pheromones that induce a mating response in plasmid harboring donor cells. This same peptide transporter was also shown to contribute to biofilm formation and recent evidence suggests that it contributes to virulence in a catheter-associated UTI (CAUTI) model. In Gram-positive bacteria, the RNPP family of transcription factors are known to contribute to a variety of cellular processes, including biofilm formation and pathogenesis and their activity is regulated by binding to small peptides. Here we identify five uncharacterized and predicted RNPP homologs in *E. faecalis* V583 by querying the genome for characteristic elements of the RNPP family. We generated gene deletion mutants for each predicted RNPP homolog and examined these mutants for affects on biofilm development and compared their role in pathogenesis using a mouse model of a CAUTI by using a mixed infection of fluorescently labeled parental and mutant strains. We found that one such homolog displayed an increase in biofilm biomass and resulted in a significant increase in catheter and bladder colonization compared to the parental strain.

Poster # A10

***Clostridium difficile* toxin gene expression is bistable**

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Clostridium difficile is a major cause of hospital-acquired infections leading to antibioticassociated diarrhea. The disease is mediated via the action of two exotoxins TcdA and TcdB. TcdA and TcdB are encoded on a pathogenicity locus (PaLoc), with three additional toxin-related genes: *tcdR, tcdC* and *tcdE*. TcdR is an alternative sigma factor that is essential for transcription of *tcdA* and *tcdB*. Previous studies focused on toxin regulation in whole cell populations, thus studying the average response of the population. Here, we used a reporter mCherryOpt to study toxin regulation in individual *C. difficile* cells. We found toxin gene expression is bistable; in stationary phase, the population is divided into a subset of cells that express *tcdA*, "Toxin-ON", and a

group that fails to express *tcdA* "Toxin-OFF." Expression of *tcdA* is bistable in multiple ribotypes and preliminary results suggest that *tcdR* and *tcdB* are also expressed in a bistable manner. Additionally, we demonstrate that bistable *tcdA* expression is dependent upon TcdR and induction of *tcdR* using an exogenous promoter results in uniform levels of *tcdA* expression that correlate with *tcdR* levels. These studies provide new insight into toxin gene expression in *C. difficile*. They raise additional questions including what are the global regulators involved in bistable toxin expression and what is the biological consequence of bistable toxin expression. Future studies will analyze the effect of global regulators on the bistability of toxin expression. Determining the mechanism of bistable toxin regulation may lead to a greater understanding of *C. difficile* infections.

Poster # A11

The role of cysteine protease, ScpA, in the regulation of *Staphylococcus aureus* biofilms

Jeffrey S. Kavanaugh[1], Kristopher P. Heilmann[1], Joe M. Mootz[1,2], and Alexander R. Horswill[1]

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The opportunistic pathogen *Staphylococcus aureus* causes infections ranging from simple skin infections to life-threatening ailments, such as sepsis, endocarditis, and osteomyelitis. Higher colonization rates, immunosuppressive conditions, increases in antibiotic resistance, and the greater use of surgical implants all contribute to increasing infection rates. Approximately 70%of injuries to active duty US service members are orthopedic related and therefore have a high risk of developing complicating, chronic infections due to formation of biofilms in which *S. aureus* attaches to and grows on the medical implant material. These biofilm infections are intractable due to their inherent resistance to antimicrobial therapies and host immune defenses, but *in vitro* studies have shown *S. aureus* biofilms can disperse through the action of proteases that are produced by *S. aureus* under regulation by the *agr* quorum-sensing system. This suggests novel therapies aimed at the *agr* quorum-sensing system and *S. aureus* proteases hold the promise of eradicating chronic biofilm infections. Developing these therapies requires a greater understanding of *agr* expression within the host, and identification of the proteins that are the targets of the *agr*-regulated proteases. We have found that *S. aureus* biofilms are particularly sensitive to the cysteine protease Staphopain A (ScpA), and proteomic studies have identified a number of surface proteins, including SdrDE, Atl, ClfAB, Eap, FnBPAB, IsaA, as potential ScpA targets. Ongoing studies aimed at validating our proteomic findings indicate a significant role for fibronectin binding proteins (FnBPAB), alone and in combination with clumping factor A (ClfA), in stabilizing *S. aureus* biofilms.

Poster # A12

***Staphylococcus aureus* Superantigen Activation of Non-hematopoietic Cells Contributes to Disease**

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Staphylococcus aureus is the leading cause of infective endocarditis (IE). IE is a life-threatening condition of the heart characterized by the formation of vegetative lesions on the inner leaflet of the valves. Recent studies have shown the importance of superantigens (SAgs) in the establishment of IE. Currently, the working model is that the immune dysregulation caused by SAgs makes it difficult to clear the vegetation leading to persistent bacteremia and more complications. Further research has found that SAgs have an additional binding site region, called the dodecapeptide, that is highly conserved among SAgs and can interact with epithelial cells. Interactions with the epithelium may explain the body's decreased ability to heal the initial site of damage and further explain the mechanism by which SAgs contribute to IE. To test whether the dodecapeptide could play a role in wound healing and the development of IE, alanine scanning was performed on the dodecapeptide region of TSST-1 SAg. Human vaginal epithelial cells were then treated with the respective mutants and relative cytokine expression levels measured by qRT-PCR. Wound healing was also assessed using a scratch wound assay. Results showed that certain amino acids within the dodecapeptide are important for TSST-1 to induce cytokine expression in epithelial cells. TSST-1 also causes a lag-effect during cellular migration. In vivo studies using SAg deficient strains are consistent with in vitro observations. These findings elucidate potential mechanisms of SAgs during the development of IE and should lead to improved treatment.

Poster # A13

The bacterial invasin Internalin B enhances vertical transmission of *Listeria monocytogenes*

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Listeria monocytogenes (*Lm*) is a facultative gram-positive intracellular bacterium that causes severe infections in pregnant women that can lead to abortion, still-birth, and disseminated fetal infection. Previous studies have shown that subpopulations of *Lm* exhibit different tissue tropisms, resulting in the acquisition of novel target organ replication niches. As an example, a clinical *Lm* isolate, 07PF0776, was found to have an enhanced ability to target cardiac tissue based on amino acid variations present within Internalin B (InlB), a bacterial surface protein associated with host cell invasion. Given that the mammalian receptor bound by InlB, Met, is abundantly expressed by placental tissue, we examined *Lm* 07PF0776 for its ability to be transmitted from mother to fetus following bacterial infection of pregnant mice. Pregnant Swiss-Webster mice infected with cardiotropic *Lm* 07PF0776 exhibited significantly enhanced transmission of *Lm* to placentas and fetuses compared to non-cardiotropic *Lm*. Increased expression of InlB in *Lm* 07PF0776 enhanced fetal infection efficiency by more than two-fold such that nearly 90% of placentas were infected, and also enhanced fetal transmission of noncardiotropic strains. This data suggests that InlB plays a critical role in vertical transmission of *Lm* via direct invasion of placental and fetal cells.

Poster # A14

Functional Analysis of Urease in *Staphylococcus aureus*

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Staphylococcus aureus is one of the most important pathogens that cause opportunistic infections in both the community and health care facilities. Urease, which catalyzes the conversion of urea into ammonia and CO2, is crucial to niche adaptation of many bacteria such as *Helicobacter pylori*. However, the function of urease in the pathogenesis or host colonization of *S. aureus* is relatively unknown. We hypothesize that urease functions to rescue acid-induced cell death via ammonia generation and subsequent pH homeostasis. To investigate this hypothesis,

POSTER SESSION "A" ABSTRACTS

growth experiments were performed with *S. aureus* USA 300 LAC JE2 wildtype and urease deficient strains (JE2 *ureB*::ΦNΣ) measuring pH, colony-forming unit (cfu), and multiple metabolite levels following growth in Tryptic Soy Broth (TSB) containing 45mM glucose with or without the addition of 10mM urea. In addition, respiration and reactive oxygen species production was evaluated by flow cytometry with 5-cyano-2,3-ditolyltetrazolium chloride (CTC) and hydroxyphenyl fluorescein (HPF) staining. JE2 *ureB*::ΦNΣshows a significant defect compared to wild type in viability, and ability to restore pH in acidic conditions created by 45mM glucose due to the lack of ammonia generation in the presence of urea. Comparing to wildtype, the urease deficient strain has reduced respiration and increased reactive oxygen species level. In conclusion, our data demonstrate that urease facilitates pH homeostasis, rescues respiration and prevents ROS generation thus contributing to survival under acid stress in the presence of urea.

Poster # A15

c-di-GMP regulation of putative colonization factors in *Clostridium difficile*

Robert W. McKee, Naira Aleksanyan, Rita Tamayo

Clostridium difficile is the most common cause of antibiotic associated diarrhea in the U.S. Two exotoxins, TcdA and TcdB, are responsible for disease symptoms. However, the factors that allow *C. difficile* to colonize the intestine remain largely unknown. The second messenger cyclic diguanylate (c-di-GMP) regulates the production of host colonization factors in several bacterial pathogens. We thus tested whether c-di-GMP affects the ability of *C. difficile* to attach to human intestinal epithelial cells. We induced expression of *dccA*, which encodes a c-di-GMP synthase, to artificially increase intracellular c-di-GMP levels in *C. difficile,* then evaluated adherence to Caco-2 and HT-29 cells *in vitro*. *C. difficile* with elevated intracellular c-di-GMP levels showed significantly increased attachment to both cell types compared to the controls. We previously reported that c-di-GMP promotes biofilm formation by positively regulating the production of Type IV pili (T4P), so we hypothesized that c-di-GMP induced TFP facilitate attachment. However, mutants lacking TFP attached as well as wild type bacteria to HT-29 cells. To identify additional factors that might contribute to c-di-GMP induced adherence, the transcriptomes of *C. difficile* with wild type and artificially elevated intracellular c-di-GMP were compared using RNAseq. Among the many genes regulated at the transcriptional level, we identified 6 genes encoding putative adhesins. Ongoing experiments will determine the roles of these factors in attachment of *C. difficile* to host cells. Knowledge of factors involved in host cell attachment and intestinal colonization could lead to the identification of new therapeutic targets for *C. difficile* infection.

Poster # A16

Characterization of a biofilm regulatory protein A (BrpA) homologue in group B *Streptococcus* innate immune resistance phenotypes

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Streptococcus agalactiae (Group B *Streptococcus*, GBS) colonizes the rectovaginal tract in 20-30% of healthy women; however, during pregnancy GBS can be transmitted to the newborn, potentially causing severe disease including pneumonia and sepsis. Routine screening and intrapartum antibiotic prophylaxis have failed to completely prevent GBS transmission, and GBS remains an important cause of neonatal morbidity and mortality. We are seeking novel targets for therapeutic interventions that either attenuate GBS virulence or enhance host innate immune function. Here we investigate a GBS surface protein that is a member of LytR-Cps2A-Psr enzyme family with roles in cell wall polymer biosynthesis. This GBS protein shares homology with BrpA from *S. mutans,* which contributes to that pathogen's acid and oxidative stress responses, antimicrobial resistance, biofilm formation and blood survival *in vivo*, but possesses a unique C-terminal domain. Using insertional mutagenesis, we have generated a GBS *brpA* knockout mutant. Compared to the wild-type parent strain, GBS Δ*brpA* shows a 100 -fold increased susceptibility to killing by primary human neutrophils and stimulates increased neutrophil reactive oxygen species. In contrast, GBS Δ*brpA* was not more susceptible to endogenous antimicrobials LL-37 or lysozyme, nor hydrogen peroxide, in minimum inhibitory concentration assays. Ongoing work seeks to assess the virulence capacity of GBS Δ*brpA* in an *in vivo* sepsis model, and to define the morphology, antimicrobial susceptibility, and immune cell interactions controlled by this regulatory factor. We suspect that *brpA* is likely an important virulence determinant of GBS and may be a useful target in design of therapeutic or antibacterial strategies.

Poster # A17

Frequency of *Staphylococcus epidermidis* Small Colony Variant Formation is Reduced by Lysosomal Alkalinization

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Small colony variants (SCVs) are naturally occurring, slow-growing subpopulations of bacteria that emerge in response to diverse environmental pressures. SCVs have decreased sensitivity to antimicrobial activity and are associated with recurrent infection. We previously found low pH and the intracellular environ to promote formation of *S. epidermidis* SCVs. Herein, we treated infected host cells with lysosomotropic alkalinizing agents to examine whether neutralizing lysosomal pH would reduce the formation of *S. epidermidis* SCVs. *S. epidermidis* strain RP62A and two *S. epidermidis* prosthetic joint infection isolates were studied. Human lung fibroblast MRC5 cells were infected at a multiplicity of infection of 1. Daily, cells were washed with PBS and lysostaphin or daptomycin (to kill extracellular bacteria) and fresh medium supplemented with lysosomotropic alkalinizing agents (20 μM chloroquine or 5 mM ammonium chloride) was added. At 0, 3, 5 and 7 days post infection, host cells were lysed and quantitative cultures performed. SCVs were identified on the basis of their size and reduced pigmentation. Using an average percentage of SCVs from three independent experiments, infected host cells treated with chloroquine or ammonium chloride had an average 3- and 2-fold decrease in the frequency of SCVs, respectively, compared to untreated infected cells 7 days after infection. No differences were observed in total colony counts between cells treated with or without the agents. Lysosomal pH neutralization of cells infected with intracellular *S. epidermidis* via alkalinizing agents chloroquine or ammonium chloride reduces the percentage of SCVs formed during infection.

Poster # A18

Sex bias in severity of *Staphylococcus aureus* skin infection

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Numerous studies have reported sex bias in infectious disease susceptibility, with bias direction dependent upon both the

pathogen and the site of infection. *Staphylococcus aureus* is the most common cause of skin and soft tissue infections (SSTI), yet sex bias in susceptibility to *S. aureus* skin infection has not been described. A search of the Cerner Health Facts® database revealed an odds ratio of 2.4 for male incidence of *S. aureus* SSTI versus female (71//100,000 vs 30/100,000; population >33 million). While this difference likely results from both behavioral and biological factors, we sought to investigate the physiological basis of this bias using male and female mice in a *S. aureus* dermonecrosis model. Consistent with the epidemiological data, female mice subcutaneously infected with *S. aureus* showed significantly reduced dermonecrosis and increased bacterial clearance compared to age-matched males. Importantly, protection in females was disrupted by ovariectomy and restored by exogenous administration of 17β-estradiol (E2). Furthermore, bone marrow neutrophils from female mice showed significantly increased phagocytosis and superior *S. aureus* killing *ex vivo* compared to those from male or ovariectomized female mice. While many questions clearly remain, this work suggests that innate differences in neutrophil bactericidal capacity may be one driver of sex bias in *S. aureus* skin infection. Understanding the physiologic factors underlying this bias holds promise for identifying novel therapeutic targets to promote host innate defense against *S. aureus* skin infection. Such host-targeted strategies may prove essential in combatting the growing antibiotic resistance crisis.

Poster # A19

Growth of *Staphylococcus aureus* USA300 JE2 in Complete Defined Medium

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Growth of *Staphylococcus aureus* in Tryptic Soy Broth (TSB) has been well defined. However, certain metabolic experiments require a defined medium where the components are known. Variations of a Complete Defined Medium (CDM) have been recently used by multiple laboratories to address certain hypotheses; however, metabolic profiles of these media have not been comprehensively performed. Growth of *S. aureus* JE2 was assessed in CDM, which contained 18 amino acids excluding glutamine and asparagine, and was compared to CDM containing 14 mM glucose and TSB. Growth in CDM resulted in rapid catabolism of 8 amino acids (Ala-Ser-Gly-Thr-Arg-Pro-Glu-Asp) whereas catabolism of histidine was detected by 12 hours of growth. Acetate was detected via catabolism of the pyruvate generating amino acids and was subsequently utilized as a secondary carbon source. In contrast, growth in CDM containing 14mM glucose (CDMG) resulted in rapid uptake of glucose but no utilization of proline or arginine or histidine even after 12 hours of growth. In addition, acetate consumption appeared to be impaired in CDMG resulting in growth arrest and was not dependent upon CcpA. Based on amino acid analysis, addition of 10X Ser, Gly and Ala resulted in an increased growth yield of *S. aureus* JE2 in CDMG (OD_{600} 4.4 to 8.7) following 10 hours of growth. In addition, the buffering components of CDM [KH_2PO_4 (3g/L) and Na_2HPO_4 (10g/L)] were replaced with K_2HPO_4 (2.5g/L) and NaCl (5g/L) resulting in similar buffering capacity as observed in TSB.

Poster # A20

Development of HAMLET as an adjuvant for the treatment of drug-resistant *Staphylococcus aureus* infections

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major multi-drug resistant pathogens responsible for community- and hospital-acquired skin, lung and bloodstream infections. Hospitalizations have increased in the US over the last number of years from around 300,000 to 500,000 cases annually and the proportion of those infections caused by MRSA now account for over 60%, resulting in over 19,000 deaths. The decline of new antibiotics annually approved for marketing in the US, combined with the emergence of multi-drug resistant pathogens, results in a critical need for the development of new therapeutic agents and novel strategies to combat antimicrobial resistance. HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a protein-lipid complex from human milk, with both tumoricidal and bactericidal activities. It can have direct bactericidal activity on pathogens such as *Streptococcus pneumoniae.* However, it can also potentiate the effect of broad spectrum of antibiotics on multi-drug resistant pathogens, such as *S. aureus,* where it has no direct bactericidal activity. Further, HAMLET inhibits the development of antibiotic resistance and reduces the burden of CFUs in the lungs of mice when administered as a single dose intranasally. To validate the potentiation effect of HAMLET on a clinically relevant population of MRSA, we have confirmed its activity against a collection of community- and hospital-acquired clinical isolates exposed to various classes of antibiotics *in vitro*, using Minimal Inhibitory Concentration (MIC) assays. We are further testing its activity with bactericidal and biofilm assays. These preliminary data highlight the potential of HAMLET as a novel antimicrobial adjuvant against multi-drug resistant *Staphylococci*.

Poster # A21

Molecular typing of *Staphylococcus aureus* isolated from patients with autosomal dominant hyper IgE syndrome

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Autosomal dominant hyper IgE syndrome (AD-HIES) is a primary immunodeficiency caused by a loss-of-function mutation in the Signal Transducer and Activator of Transcription 3 (STAT3). This immune disorder is clinically characterized by increased susceptibility to cutaneous and sinopulmonary infections, in particular with *Candida* and *Staphylococcus aureus.* It has recently been recognized that the skin microbiome of patients with AD-HIES is altered with an overrepresentation of certain Gram-negative bacteria and Gram-positive staphylococci. However, these alterations have not been characterized at the species- and strain-level. Since *S. aureus* infections are influenced by strain-specific expression of virulence factors, information on colonizing strain characteristics may provide insights into host-pathogen interactions and help guide management strategies for treatment and prophylaxis. The aim of this study was to determine whether the immunodeficiency of AD-HIES selects for unique strains of colonizing *S. aureus*. Using multi-locus sequence typing (MLST), protein A (spa) typing, and PCR-based detection of toxin genes, we performed a detailed analysis of the *S. aureus* isolates (n=13) found on the skin of twenty-one patients with AD-HIES. We found a low diversity of sequence types, and an abundance of strains that expressed methicillin resistance, Panton-Valentine leukocidin (PVL), and staphylococcal enterotoxins K and Q (SEK, SEQ). Our results indicate that patients with AD-HIES often carry antibiotic-resistant strains that harbor key virulence factors.

POSTER SESSION "A" ABSTRACTS

Poster # A22

Generation of a stable plasmid for in vitro and in vivo studies of S*taphylococcus*

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A major short-coming to plasmid-based genetic tools is the necessity of using antibiotics to ensure plasmid maintenance. While selectable markers are very powerful, this practice is not always practical, such as during in vivo models of bacterial infection. During previous studies, it was noted that the uncharacterized LAC-p01 plasmid in *Staphylococcus aureus* USA300 isolates was stable in the absence of a known selection, and therefore could serve as a platform for new genetic tools for Staphylococcus species. LAC-p01 was genetically manipulated into an E. coli-S. aureus shuttle vector that remained stable for at least 100 generations without antibiotic selection. The double and single-stranded (dso and sso) origins were identified and found to be essential for plasmid replication and maintenance, respectively. By contrast, deletion analyses revealed that none of the four LAC-p01 predicted open reading frames were necessary for stability. Subsequent to this, the shuttle vector was used as a platform to generate two plasmids. The first plasmid (pKK22) contains all genes native to the plasmid for use in S. aureus USA300 strains, while the second (pKK30) lacks the four predicted open reading frames for use in non-USA300 isolates. pKK30 was also determined to be stable in S. epidermidis. Moreover, pKK22 was maintained for 7 days post-inoculation during a murine model of S. aureus systemic infection, and successfully complemented an hla mutant in a dermonecrosis model. These plasmids that eliminate the need for antibiotics during both in vitro and in vivo experiments are powerful new tools for studies of *Staphylococcus.*

Poster # A23

***Staphylococcus aureus* von-Willebrand factor-binding protein interacts with fibrinogen via unique binding sites**

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Staphylococcus aureus is a world-wide threat to human health, causing mild to life-threatening diseases, such as infective endocarditis and pneumonia. The bacterium expresses a multitude of virulence factors, including cell-wall anchored and secreted proteins, that contribute to the pathogenicity of the organism. Two of the secreted proteins of S. aureus, von Willebrand factor-binding protein (vWbp) and Coagulase (Coa) can cause blood coagulation by their ability to interact with prothrombin and fibrinogen and are proved virulence factors. Although the interaction between Coa and fibrinogen has been well studied, it is not clearly understood as to how vWbp interacts with fibrinogen. In this study, we showed that vWbp interacts with fibrinogen via unique binding sites. Both the N-terminus and C-terminus of vWbp harbor fibrinogen-binding activities; however, they recognize fibrinogen differently. The N-terminus of vWbp interacts with fibrinogen when either coated on a microtiter plate or in the fluid phase; whereas, the C-terminal protein seems to specifically recognize fibrinogen residing in the fluid phase. In addition, their binding sites on fibrinogen are also distinct. N-terminal vWbp binds to the fibrinogen β-chain. However, the C-terminus of vWbp does not bind specifically to the fibrinogen-β chain. To conclude, both the N-terminus and C-terminus of vWbp bind to fibrinogen, but they do not share the same binding site. This information advances our knowledge on how vWbp interacts with fibrinogen.

Poster # A24

Growth environment-dependent plasticity of coagulase-negative *staphylococci* fatty acid composition and susceptibility to antimicrobial fatty acids

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The balance of straight-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs), and occurrence of straight-chain unsaturated fatty acids (SCUFAs) in *Staphylococcus aureus* is determined by the nutritional growth environment. *S. aureus* is not considered to be part of the natural skin microbiota in contrast to various coagulase-negative staphylococcal species, which dominate the skin community. Since skin is protected by various antibacterial fatty acids, we sought to determine growth environment influence on coagulase-negative staphylococcal fatty acid composition, and the response of these organisms to free fatty acids. Growth in Mueller-Hinton broth resulted in increased proportions of BCFAs (63-84%) and lowered proportions of SCFAs (16-37%) more than compared to growth in Trypic Soy Broth (53-75% BCFAs, 23-50% SCFAs), a phenomenon first described in *S. aureus.* Growth in human serum stimulated resulted in incorporation of preformed SCUFAs into fatty acid profiles (*S. aureus,* 45%; *S. epidermidis,* 65%; *S. hominis* and S. *saprophyticus,* 55%; *S. haemolyticus*, 35%; *S. auricularis* and *S. capitis*, 25%); BCFAs, which are biosynthesized, were decreased to as little as 8% of the total. High proportions of oleic (C18:1Δ9) and linoleic (C18:2Δ9,12) acids were incorporated by the coagulase-negative staphylococci, whereas S. aureus did not incorporate linoleic acid. All species were susceptible to 32 – 64 μM sapienic acid (C16:1Δ6), and 256-512 μM palmitic acid (C16:0). Coagulase-negative staphylococcal fatty acid profiles were highly influenced by the growth nutritional environment, particularly by amino acids and fatty acids, and are expected to impact membrane biophysics and possibly antimicrobial susceptibility and virulence.

Poster # A25

The cell surface adhesin BspC contributes to Group B Streptococcal meningitis

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Streptococcus agalactiae (Group B *Streptococcus,* GBS) is a Gram-positive bacterium typically found within the human gastrointestinal and urogenital tract. GBS is currently the leading cause of neonatal meningitis due to its multiple virulence factors. In order to gain access to the central nervous system, GBS must penetrate the blood-brain barrier (BBB), which is composed of specialized cells known as human brain microvascular endothelial cells (hBMEC). The mechanisms of the BBB crossing are not well understood. We hypothesized that a member of the Antigen I/II family of cell surface anchored proteins, BspC, may promote GBS interaction with brain endothelium. Antigen I/II family proteins are known virulence factors that promote colonization of the oral cavity by other streptococci. To assess whether BspC contributes to GBS-BBB interaction, we performed allelic replacement of the bspC gene. Scanning electron microscopy revealed that the

ΔbspC mutant exhibited altered surface appearance and decreased ability to interact with neighboring cells. To examine the role of BspC in the interaction of GBS with host cells we infected hBMEC with wild-type (WT) and the ΔbspC mutant and found that the mutant exhibited reduced ability to interact with endothelium. Further, using a murine model of hematogenous meningitis, we observed that mice challenged with the ΔbspC mutant exhibited a significant decrease in mortality and less bacterial brain loads compared to WT infected mice. These results suggest that BspC promotes virulence and BBB passage. Ongoing studies are underway to determine the exact mechanism of how BspC contributes to the pathogenesis of GBS meningitis.

Poster # A26

Stepwise identification of virulence-related sRNAs in *Staphylococcus aureus*

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Staphylococcus aureus is an important human pathogen that has the capacity to cause devastating infections in almost every site within its host. Throughout the infectious process, the precise temporal utilization, and thus regulation, of numerous virulence determinants is vital for pathogenic success. Although great strides have been made in our understanding of proteinaceous factors that control disease causation, other regulatory molecules, such as small regulatory RNAs (sRNAs), have received less attention. To address this, we catalogued and annotated all known *S. aureus* sRNAs across a number of different strains. This was necessitated by the observation that almost all transcripts of this kind had previously been identified and independently reported an average of 3.5 times. From this work, we were able to identify 39 new and undescribed sRNAs, leading to the annotation of 303 such transcripts in S. *aureus* USA300. We next performed RNAseq experiments with different staphylococcal species to understand which sRNAs contribute to *S. aureus* specific behavior. In so doing we identified numerous widely conserved sRNAs within the *staphylococci,* but, more importantly, 137 that are only found in *S. aureus*. These findings were then compared to Tn-seq data generated from a murine model of wound infection, resulting in the identification of 28 *S. aureus*-specific sRNAs that are required for full virulence. For selected candidates we employed transcriptomic and proteomic approaches to characterize potential targets and cellular functions of these overlooked regulatory molecules.

Poster # A27

Inactivation of the Pta-AckA pathway impairs fitness and sporulation of *Bacillus anthracis.*

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Appropriate response to rapid environmental changes is an essential feature of all living organisms. Numerous studies of bacterial pathogenesis directly linked basic metabolic processes during adaptation to sporogenesis and virulence. In this work we highlight the impact of the Pta-AckA pathway on *Bacillus anthracis* fitness, metabolism, and sporulation. We demonstrate that disruption of the Pta-AckA pathway in *B. anthracis* causes a drastic inhibitory effect on growth during overflow metabolism, alters metabolic and energy states of bacteria, and impairs sporulation. Particularly, inactivation of the Pta-AckA pathway increases glucose consumption, affects intracellular ATP and NAD/NADH levels, leads to a metabolic block at the pyruvate node, and redirects carbon into the TCA cycle and PHB biosynthesis pathway. Analyses of the formation of spores and accumulation of PHB granules during growth in sporulation and glucose-enriched media revealed glucose-mediated and PHB-independent inhibition of the sporulation process in the pta and ackA mutants. Furthermore, transmission electron microscopy (TEM) experiments demonstrate the critical role of the Pta-AckA pathway during sporogenesis as inactivation of this pathway impairs development and composition of the mature endospore. Taken together, the results of this study provide an important insight into basic physiological processes and metabolic requirements during aerobic growth and sporogenesis of *B. anthracis.*

POSTER SESSION "A" ABSTRACTS

Poster # B01

Determining mechanisms of β-lactam resistance in *Clostridium difficile*

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Clostridium difficile causes severe antibiotic-associated diarrhea and colitis. *C. difficile* is an anaerobic, Gram-positive spore former that is highly resistant to a wide variety of antibiotics. Antibiotic resistance in *C. difficile* plays an important role in its life cycle by allowing the pathogen to cause disease in antibiotic-treated patients. *C. difficile* exhibits resistance to many β-lactam antibiotics, particularly the cephalosporins. However, the mechanisms of β-lactam resistance in *C. difficile* are unknown. In other bacterial species, β-lactam resistance is often mediated by the production of β-lactamase enzymes, which cleave β-lactam rings. Our data demonstrate that *C. difficile* can hydrolyze the cephalosporin nitrocefin, indicating production of a β-lactamase. To identify the gene encoding this β-lactamase, we examined expression of a variety of putative β-lactamases in the presence of β-lactams. A putative operon encoding a potential β-lactamase was identified that was highly upregulated in response to these drugs. Using the TargeTron method, we generated an insertional mutant to abolish expression of this operon in the commonly used historical isolate 630Δerm. We determined the effects of these genes on β-lactam resistance by examining growth of the parent and mutant in a variety of β-lactams. The mutant strain was found to exhibit decreased resistance to some, but not all, β-lactams. We also examined β-lactamase activity in the mutant strain directly by assessing its ability to hydrolyze nitrocefin. By identifying genes and mechanisms responsible for β-lactam resistance in *C. difficile,* it may be possible to identify novel targets for the prevention of β-lactam-associated *C. difficile* infections.

Poster # B02

Biofilms: A Developmental niche for Vancomycin-Intermediate Resistant *Staphylococcus aureus.*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has become resistant to a multitude of different antibiotics. Vancomycin is the drug of choice of treatment for many MRSA infections. However, vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains have emerged. One mechanism for the increased antibiotic (vancomycin-intermediate) resistance is due to acquisition of various mutations within different genes that alters the cell physiology making vancomycin ineffective. Biofilm formation is a bacterial survival mechanism that can lead to mutations within the bacterial genome and allow for advantageous traits such as increased antibiotic resistance. Biofilms are also a site for increased horizontal gene transfer. The biofilm environment is harsh, having niches that are often nutrient and oxygen deficient which damages DNA. This DNA damage induces the SOS response to fix double-stranded breaks in DNA, and enables bacterial survival. This repair often results in mutations. We hypothesize that vancomycin intermediate resistance is an unintended consequence within the *S. aureus* biofilm environment. To assess this, both wildtype and RecA/LexA biofilms will be grown. Efficiency of plating techniques will be performed to quantify the subpopulation of biofilm-derived *S. aureus* cells that have developed vancomycin intermediate resistance. The expression of the RecA and LexA transcripts in biofilm-derived cells will be evaluated through qRT-PCR and used to quantify their activity in these growth conditions. The results will be compared to the results from wildtype and RecA/LexA planktonic cultures and used to evaluate the role of the *S. aureus* biofilm environment in the development of vancomycin intermediate resistance.

Poster # B03

Long-acting Nanoparticles Inhibit against Virulent *Mycobacteria tuberculosis* and Promoting Phagosome in Macrophages

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Mycobacteria tuberculosis (M.tb) is the causative agent of tuberculosis (TB). Multi-drug resistant M.tb is one of serious threats in preventing the spread of TB, urging new treatments and novel drugs. Nanoparticles are good carriers to deliver drug to target sites while protecting drugs from degradation. In this work, six different types of nanoparticles were prepared to deliver gallium (Ga (III)) and rifampin to M.tb infected-macrophages and reduce M.tb growth. Although all nanoparticles varied in size, morphology and ζ-potentials, our results indicate that they can be used as potential drug carriers. Folate- or mannose-conjugated block copolymers encapsulating Ga (III) showed increased uptake by macrophages and sustained Ga release in macrophages for 15 days, resulting in significant inhibition of virulent M.tb growth in human monocyte-derived macrophages. Nanoparticles with dendrimers encapsulating Ga or rifampin also showed promising anti-tuberculosis activities. In addition, co-localization of phagocytized nanoparticles with M.tb in phagosomes and detection of mature cathepsin D (34 kDa, lysosomal hydrogenase) implicate that the nanoparticles appeared to promote maturation of phagosome, which would be expected to increase macrophage-mediated killing of the organism. Targeted delivery of drugs like Ga (III) and rifampin in the form of nanoparticles to macrophages offers a promising approach against virulent M.tb and drug resistant M.tb.

Poster # B04

Pyruvate Oxidase as a link between capsule production and metabolism in *Streptococcus pneumoniae*

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Amongst bacterial pathogens, *Streptococcus pneumoniae* is one of the most prodigious producers of hydrogen peroxide, which has been implicated in competition between other bacterial colonizers of the nasopharynx and in damage to the host epithelium. However, the role during invasive disease has been less clear. In this study, we demonstrate a role for the main hydrogen peroxide producing enzyme, pyruvate oxidase, in the production of the polysaccharide capsule. Deletion of pyruvate oxidase resulted in a loss of capsule production, which completely abrogated the invasive capacity of the *pneumococcus*. Loss of pyruvate oxidase significantly lowered cellular levels of acetyl-CoA, a co-factor

ICG+P POSTER SESSION "B" ABSTRACTS

TUESDAY, OCTOBER 11, 2016
7:30 P.M.-9:30 P.M.

POSTER NUMBERS AND PRESENTERS

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B02 Jenelle Chapman
B03 Seoung Choi
B04 Haley Echlin
B05 Brintha Girinathan
B06 Adam Grossman
B07 Luke Handke
B08 Tramy Hoang
B09 Jason Joyner
B10 Karan Gautam Kaval
B11 Yuzo Kevorkian
B12 Jessica Kubicek-Sutherland
B13 McKenzie Lehman
B14 Nicole Massa
B15 Bindu Nanduri
B16 Srivatsan Parthasarathy
B17 Alyssa Medler
B18 Nicole Putnam
B19 Liwen Deng
B20 Belkys Sanchez
B21 Kristie Schmidt
B22 Gwenn Skar
B23 Brian Wilkinson
B24 Nitija Tiwari
B25 Matthew Turner
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POSTER SESSION "B" ABSTRACTS

required in capsule synthesis. These data suggest that depletion of this critical co-factor leads to loss of capsule. To test this hypothesis, two complementary approaches were undertaken. First, pyruvate oxidase was deleted in other serotypes. Deletion of pyruvate oxidase correlated with capsule defects only in pneumococcal strains with capsules harboring acetylated sugars, consistent with the hypothesis that the defect in capsule biosynthesis results from acetyl-CoA limitation. Second, an additional metabolic pathway for the biosynthesis of acetyl-CoA was independently deleted. This mutant mimicked the acapsular phenotype of the pyruvate oxidase mutant while retaining hydrogen peroxide production. These data indicate a cellular hierarchy for the competition for key metabolites such as acetyl-CoA that are required for multiple essential bacterial components. Together, these data establish a critical link between central pneumococcal metabolism and capsule production.

Poster # B05
Effect of mutation in *C. difficile* sin locus

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Clostridium difficile is the major cause of nosocomial diarrhea and pseudomembranous colitis. It produces dormant spores, which serve as infectious vehicle responsible for transmission of the disease and persistence of the organism in the environment. In *Bacillus subtilis*, the sin locus (sinRI) is responsible for sporulation inhibition. A homolog of sinR encoding gene is present in *C. difficile* genome and was predicted to have a similar role. To understand the role of sin in *C. difficile*, we constructed and characterized sin mutants in R20291 and JIR8094 *C. difficile* strains. We found that *C. difficile* sin mutant is asporogenic in nature, indicating that products of sin locus have positive influence on sporulation in *C. difficile.* Other than being asporogenic, sin mutant in R20291 strain also displayed decreased toxin production and motility. Transcriptome analysis further revealed reduced expression of sporulation and motility-associated genes in the sin mutants when compared to the parental strains.

Poster # B06
Antimicrobial activity of lignin against *Staphylococcus aureus* biofilms

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As modern medicine edges closer to the post-antibiotic era, innovative and novel approaches to controlling antibiotic resistant bacteria must be developed. In particular, biofilms formed by pathogenic bacteria can significantly exacerbate tolerance to antibiotics, antiseptics, and the host immune system. The phenolic plant cell wall polymer lignin is known to be a strong antioxidant and to have antimicrobial activity. Using lignin extracted from the waste stream of a lignocellulosic biorefinery, lignin suspensions were added to preformed biofilms of wildtype *S. aureus* clinical strain UAMS-1, isogenic agr UAMS-155, and agr ica+ *S. aureus* 15981. The viability of all biofilms was significantly inhibited by lignin in vitro, as determined by enumeration of colony forming units after 24 hours incubation. Lignin-rteated biofilms were dispersed more easily than nontreated biofilms, suggesting that lignin may interfere with cellular functions that may directly or indirectly affect biofilm adhesion and/or dispersal. Phenolic compounds are known to disrupt a number of cell membrane functions including electron transport, proton motive force, and membrane permeability leading to dysregulation of membrane potential and intracellular pH. Therefore, ongoing and future studies will be aimed at testing the effect of lignin on these phenotypes. It is hypothesized that one or more of these combined effects on the *S. aureus* cell membrane makes industrial lignin an effective inhibitor of S . aureus biofilm growth and viability.

Poster # B07
MntC-dependent manganese transport is essential for S. aureus oxidative stress resistance and virulence.

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Staphylococcus aureus is a successful human pathogen that has developed several approaches to evade the immune system, including strategies to resist oxidative killing by immune cells. This resistance is mediated by production of manganese-requiring superoxide dismutase (SOD) enzymes. *S. aureus* encodes three manganese transporters, MntABC, MntH and MntX, yet their relative contribution to manganese transport has not been well-defined in clinically relevant isolates. For this purpose, insertional inactivation mutations were introduced into mntC, mntH, and mntX individually and in combination. In microbroth dilution studies with a free radical generating compound, mntC was necessary for resistance to oxidative stress. In contrast, strains with intact mnt displayed a minimal compound sensitivity phenotype that was only revealed in mntC strains, and no phenotype was observed with mntX strains. Similar results were obtained with SOD activity assays, where MntC alone was associated with robust SOD activity. In addition, mntC strains were attenuated in a mouse sepsis model of infection. To further link these observations to manganese transport, a MntC protein lacking manganese binding activity was designed, expressed, and purified. While circular dichroism experiments demonstrated that the secondary and tertiary structure of this protein were unaltered, a defect in manganese binding was confirmed by isothermal titration calorimetry. In contrast to complementation with wild-type mntC, introduction of the manganese-binding defective allele into the chromosome of a mntC strain did not restore resistance to oxidative stress. These data underscore the importance of MntC-dependent manganese transport in *S. aureus* oxidative stress resistance and virulence.

Poster # B08
Regulation of *Staphylococcus epidermidis* icaADBC by IcaR and TcaR

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Staphylococcus epidermidis is a nosocomial pathogen commonly associated with biofilm related infections. Accordingly, the ability to form biofilms is the most significant virulence factor for this pathogen. Staphylococcal biofilms are heterogeneous and are composed of proteinaceous, nucleic acid, and polysaccharide components. Polysaccharide intercellular adhesin (PIA) is one such component shown to be significantly correlated with biofilm formation. PIA is produced by the products of the icaADBC operon and regulation involves a complex system of genetic regulators, including the direct repressors IcaR and TcaR. Studies of icaADBC regulation in a constitutive strain (*S. epidermidis* 1457) and an inducible strain *(S. epidermidis* CSF 41498) suggest that PIA synthesis, and thus biofilm formation, is regulated differently within these groups. In 1457, we observe that icaADBC is primarily repressed by TcaR. In contrast, deletion of icaR in CSF 41498 increases PIA synthesis while inactivation of tcaR does not greatly affect icaADBC transcription. Data from DNase footprinting experiments suggests that IcaR and TcaR bind to the same sequence at the icaADBC promoter region, indicating that IcaR and TcaR are competing for regulation of icaADBC. Overall, our data suggest that icaADBC regulation, and PIA synthesis, is carefully regulated in *S. epidermidis.* And this occurs possibly through binding competition between the direct repressors IcaR

and TcaR.

Poster # B09

Virus-Like-Particle (VLP) Vaccine Induces Immune Protection from *Staphylococcus aureus* agr-Mediated Virulence

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Staphylococcus aureus is the leading cause of skin and soft tissue infections (SSTIs) in the United States. Mounting antibiotic resistance requires innovative treatments such as ones that inhibit *S. aureus* pathogenicity and support innate immune clearance. *S.* aureus coordinates virulence factor expression through the density-dependent accessory gene regulator (agr) operon via secretion of cyclic autoinducing peptides (AIPs). S. aureus lacking agr fails to cause dermonecrosis in mouse models of SSTI and is more readily cleared compared to agr positive isolates. Therefore, we hypothesized that vaccination against *S. aureus* AIP could generate protective immunity against subsequent SSTI challenge. Because *S. aureus* AIPs are too small to stimulate a natural immune response (7-9 amino acids), we engineered a virus-like-particle (PP7-VLPs) for surface presentation of a modified autoinducing peptide sequence (AIP1S). VLP-based vaccines allow multivalent presentation of target antigens and are highly immunogenic due to their repetitive, virus-like structure. As expected, vaccination with PP7-AIP1S induced AIP1-specific antibodies, and transcriptional analysis of skin from vaccinated and challenged mice showed that PP7-AIP1S vaccination limits agr-activation in vivo. Most importantly, in a challenge model of *S. aureus* SSTI, PP7-AIP1S vaccinated mice showed significantly reduced dermonecrosis and increased bacterial clearance compared to control vaccinated mice, demonstrating the efficacy of this vaccination approach. To the best of our knowledge, this is the first report of an efficacious, VLP-based vaccine which induces immune control of *S. aureus* AIP1-regulated virulence. These data suggest that PP7-AIP1S vaccination could be an effective tool to limit *S. aureus* pathogenesis during SSTI.

Poster # B10

Structural and Regulatory Features of Ethanolamine Utilization in *Enterococcus faecalis*

Karan Gautam Kaval[1], Wade Winkler[2] & Danielle Garsin[1]

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Enterococcus faecalis, a Gram-positive, commensal inhabitant of the human gastrointestinal tract, is also known for causing life-threatening nosocomial infections. It successfully colonizes the gut where there is much ethanolamine, a breakdown product of the cell membrane phospholipid, phosphatidylethanolamine. Ethanolamine can serve as a valuable source of carbon and nitrogen to bacteria like E. *faecalis* that possess an ethanolamine utilization (eut) locus. The eut genes encode metabolic enzymes as well as structural components that enclose the enzymes within a proteinaceous shell, forming capsid-like structures called bacterial microcompartments (BMCs). Eut BMCs function to catabolize ethanolamine in a manner that separates the toxic intermediates from the rest of the cytoplasm. Gene expression of both the enzymatic as well as the shell proteins is under the control of a 2-component regulatory system consisting of the EutV and EutW proteins. TEM analysis done on the wild type strain, grown under inducing conditions, showed the clear presence of BMCs within its cytoplasm as compared to the ΔeutVW strain. Ongoing studies are focused on ascertaining the spatio-temporal regulation of these BMCs and defining their structural features. To this end, we are generating antibodies against and GFP tagging the Eut proteins. We are also perfecting a protocol for the biochemical purification of whole BMCs from *E. faecalis* cells. Progress on these fronts will be presented.

Poster # B11

A strain--specific requirement for the Csp family of germination regulators in *Clostridium Difficile*

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Clostridium difficile Infection (CDI) is a major healthcare--associated disease that is transmitted by *C. difficile's* metabolically dormant spore form. Upon entering the gut, *C. difficile* spores germinate and outgrow to produce vegetative cells that release disease--causing toxins. In *C. difficile,* germination depends on the Csp family of subtilisin--like serine (pseudo)proteases and the cortex hydrolase SleC. The pseudoprotease CspC acts as the primary germinant receptor that initiates germination upon binding specific bile salts. CspC activates the protease CspB, which in turn removes an inhibitory pro--peptide from SleC. Active SleC degrades the protective cortex layer, allowing spores to outgrow and resume metabolism. We previously showed that the pseudoprotease CspA domain, which is initially produced as a fusion to CspB, controls the incorporation of CspC germinant receptor in mature spores. However, study of the individual Csps was complicated by polar effects on cspC expression caused by the TargeTron mutagenesis method and the requirement for multi--copy plasmid complementation, which can cause experimental artifacts. To overcome these limitations, we have used allele--coupled exchange to create individual deletions of the regions encoding CspB, CspA, and CspC. Our results indicate that the CspB domain is necessary for stabilizing CspA in sporulating cells. However, unlike previously characterized disruptions in JIR8094, these mutants do not completely lose their ability to germinate, suggesting a strain specific requirement for Csp family regulation in the 630Δerm strain background. Our findings point toward a currently unknown mechanism of germination in some strains of *C. difficile.*

Poster # B12

Rapid Detection of Gram-Positive Bacterial Pathogens in Patient Serum

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The incorrect diagnosis of bacterial pathogens results in the misuse of antibiotics, a major contributor to the evolution of antibiotic resistance. The ability to rapidly identify a bacterial pathogen would facilitate more effective treatment strategies reducing the unnecessary use of antibiotics. Current diagnostics of bacterial pathogens often require cell growth and are therefore less capable of informing timely treatment options. Here, we describe the development of a rapid assay (<45 min) to identify Gram-positive bacteria directly in human serum without any growth or isolation required using a waveguide-based optical

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biosensor developed at the Los Alamos National Laboratory (LANL). We have developed fluorescence-based immunoassays specifically targeting the Gram-positive biomarker lipoteichoic acid (LTA), which is not found in Gram-negative bacteria. LTA is an amphiphilic molecule with both hydrophobic and hydrophilic portions. Our approach, termed membrane insertion, exploits the biochemistry of the molecule by allowing it to passively insert itself in a lipid bilayer thereby trapping it for probing with our fluorescently labeled α-LTA antibody. Using this assay, we have successfully detected in human serum 100 µg/ml of LTA derived from *Streptococcus pyogenes* as well as 100 µg/ml of LTA derived from *Staphylococcus aureus*, and we expect a limit of detection less than 10 µg/ml in the optimized assay based on sensitivities observed herein. We intend to combine this rapid assay with others currently in development into a diagnostic tool for point-of-care distinction between bacterial and viral infections, as well as for Gram status determination of bacterial pathogens.

Poster # B13

Amino acid metabolism is important during a *Staphylococcus aureus* skin abscess infection

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It is well characterized that *Staphylococcus aureus* has the ability to colonize a wide variety of tissues including heart, bone, and soft tissue. Each of these niches pose unique challenges in which the bacterium must adapt not only its multitude of virulence factors, but also its metabolism to proliferate during an infection. Unfortunately, there is little known about the nutrients available at the various host microniches. We hypothesize within the skin abscess, lactate, peptides and free amino acids are the primary carbon sources available. Utilizing a skin abscess model as our basis for understanding amino acid metabolism within a host, we sought to determine the metabolic pathways required for *S. aureus* proliferation with amino acids as the sole carbon source. We demonstrate that in media lacking glucose, *S. aureus* utilizes proline or arginine to synthesize glutamate, an amino acid at the junction of nitrogen and carbon metabolism. Glutamate can be converted to 2-oxoglutarate via glutamate dehydrogenase (GudB), which can then be cycled through gluconeogenesis if other energy sources are not present. Additionally, we show that host proteins associated with an abscess, such as collagen, act as a reservoir of essential amino acids such as proline and arginine. Moreover, the gudB mutant had decreased fitness in an in vivo skin abscess model compared to wildtype, suggesting that amino acid metabolism is important for *S. aureus* proliferation in a skin abscess. Overall, these studies begin to elucidate the nutrients available within an abscess and identify novel targets, including key metabolic pathways, for future therapeutics of *S. aureus* abscess infections.

Poster # B14

Development of a high throughput assay to identify inhibitors of the oligopeptide permease in *Enterococcus faecalis*

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Enterococcus faecalis uses a peptide based quorum signals for cell-to-cell communication and these signals are involved in conjugation and biofilm development. The generation of peptide signals require several processes related to peptide processing, secretion, and importation. Through a genetic screen our laboratory identified two complementary peptide importation pathways and disruption of both pathways blocks peptide signaling. In order to identify chemical compounds that inhibit peptide-based signaling we developed a whole cell luciferase-based assay and have begun to screen small chemical compound libraries for inhibitors of oligopeptide permease.

Poster # B15

Regulation of *S. pneumoniae* virulence by polyamine transport

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The global burden of pneumococcal pneumonia in adults, caused by *Streptococcus pneumoniae* (pneumococcus), remains a major health risk. Current polysaccharide based vaccines are not effective against all pneumococcal serotypes and the emergence of multi-drug resistant strains mandate the development of novel therapeutics. Polyamines are ubiquitous small cationic molecules that are important for virulence of human pathogens, including pneumococcus. We previously reported that deletion of polyamine transport operon in *S. pneumoniae* resulted in an attenuated phenotype in murine models of pneumococcal pneumonia. Polyamine transport genes are conserved among *pneumococci*, and constitute an attractive anti-virulence target for drug discovery. However, a comprehensive description of either the host innate immune mechanisms that are specific to polyamine transport deficient *pneumococci* or polyamine-dependent pneumococcal gene/protein expression for rational drug design is not available, and is the focus of this study. We conducted mass spectrometry based expression proteomics to compare murine lung response to infection with wild type (WT) or polyamine transport deficient *S. pneumoniae* TIGR4 (ΔpotABCD), and identify differences in pneumococcal protein expression in vitro. Our results show that polyamine synthesis compensates for transport in vitro. Impaired polyamine transport led to altered expression of virulence factors including capsule, PcpA, PsaB, PiaA. Capsule dot blot assays with ΔpotABCD show reduced capsule compared to WT. Intranasal infection with WT showed inhibition of a neutrophil elastase, which was lost when animals were infected with ΔpotABCD. Taken together, these results could explain the observed enhanced opsonophagocytic killing and reduced virulence of polyamine transport deficient *pneumococci.*

Poster # B16

Development of a Drip-Flow Biofilm Competition Assay for Screening *Enterococcus faecalis* mutants

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Enterococcus faecalis is a common Gram-positive commensal of the intestinal tract, but is also well known as a leading cause of nosocomial infections. *E. faecalis* utilizes biofilm formation as one of the successful infection strategies, and this strategy has been implicated in several infectious diseases including endocarditis and urinary tract infections (UTI). Recent evidence suggests that static biofilms do not closely mimic relevant in vivo conditions seen in endocarditis and UTI. The drip-flow bioreactor, which grows biofilm in a continuous supply of growth media, provides a physiologically more relevant experimental model as there is continuous flow of nutrients over the biofilm surface, and this also provides a moderate level of sheer stress. This also provides an environment to grow multiple strains in the same run for directed

competition assays. We have engineered a system to express fluorescent markers with non-overlapping spectra in order to conduct directed competition assays in the drip-flow reactor. We have focused our efforts on quorum sensing systems, including Fsr and a series of mutants predicted to encode members of an emerging family of transcription factors, namely the RNPP family. We provide evidence that this system allows a sensitive measure of differences in how biofilms form under semi-flow conditions.

Poster # B17

Development of an Oto-protective Commensal Bacteria to Counteract the Ototoxicity of Aminoglycoside Treatments

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Aminoglycoside induced ototoxicity is a major cause of deafness and hearing loss in patients treated for serious bacterial infections. It is hypothesized the structure of mitochondrial ribosomes in inner ear hair cells are inadvertently targeted by aminoglycoside treatment, often resulting in hair cell apoptosis and permanent hearing loss. Aminoglycoside antibiotics are predominantly used in serious gram negative infections, such as *Pseudomonas aeruginosa,* but can also be used to treat multidrug resistant *Mycobacterium tuberculosis.* As with all antibiotic resistance, mechanisms exist which allow for some species of bacteria to resist treatment. One mechanism used by prokaryotes are aminoglycoside modifying enzymes (AME). These enzymes can alter the structure of the aminoglycoside in a variety of ways, depending on the location and the reaction they catalyze, rendering the antibiotic ineffective. We hypothesize that engineering a secreted AME into commensal nasopharyngeal flora and subsequent colonization of the cochlea with this modified strain will provide a way to alleviate ototoxicity by reducing hair cell apoptosis. We will utilize an in vivo mouse model to measure the protectiveness of cochlear colonization during aminoglycoside treatment. These experiments will direct us to develop a bio-engineered strain of commensal bacteria or viral gene delivery system that provides a protective function against ototoxicity often seen in patients who require long-term aminoglycoside treatment, such as CF patients and patients with MDR-TB infections.

Poster # B18

The role of innate immune recognition during *S. aureus* osteomyelitis

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Staphylococcus aureus is the leading cause of invasive bone infection (osteomyelitis), leading to inflammation and dysregulated interactions between boneforming osteoblasts and bone-resorbing osteoclasts. We have previously determined that alpha-type phenol soluble modulins (PSMs), which are abundantly produced by USA300 lineage strains, mediate cell death of skeletal cells. However, alterations in bone remodeling may also be due to host factors that impact osteoclast physiology during osteomyelitis. We hypothesize that *S. aureus* or its secreted products can modulate osteoclast cell biology and bone homeostasis through ligation of osteoclast pattern recognition receptors (PRRs) and the concomitant induction of inflammation. We discovered that stimulation with toxin-deficient bacterial supernatants can modulate differentiation of osteoclasts (osteoclastogenesis) from myeloid cells with and without the canonical osteoclast differentiation factor RANK-Ligand (RANKL). Importantly, skeletal cells express PRRs, but the contribution of osteoclast lineage PRRs towards pathogen clearance, inflammation, and bone remodeling during osteomyelitis has not yet been explored. To test the role of PRR signaling pathways in osteomyelitis pathogenesis, we utilized mice lacking the critical PRR and IL-1R signaling adaptor MyD88. *S. aureus* supernatant could no longer alter osteoclastogenesis in myeloid progenitor cells lacking MyD88. To address the contribution of MyD88-dependent signaling in vivo, we used a post-traumatic *S. aureus* murine osteomyelitis model. In this model, MyD88 was critical for control of *S. aureus* replication and dissemination during bone infection. Collectively, our data demonstrate that *S. aureus* modulates osteoclastogenesis in a MyD88-dependent manner, and innate sensing through MyD88 is important for limiting pathogenesis during osteomyelitis.

Poster # B19

Characterization of vaginal colonization by *Staphylococcus aureus*

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Staphylococcus aureus is an important organism responsible for nosocomial and community acquired infections in humans, and cases of community associated methicillin-resistant *S. aureus* (CA -MRSA) infection have continued to increase despite widespread preventative measures. Reports have suggested an increase in CA-MRSA infections in pregnant and postpartum women as well as outbreaks in newborn nurseries. *S. aureus* has been reported to colonize the vagina in up to 22% of pregnant women, however little is known about the specific bacterial factors that promote vaginal colonization and subsequent infection. We hypothesize that S. aureus must express factors that mediate interaction with vaginal epithelium and compete or cooperate with other common colonizers in order to promote niche establishment. We have adapted a mouse model of Group B Streptococcal vaginal colonization and demonstrate that both invasive and colonizing MRSA isolates, CA-MRSA USA300 and MRSA-252, persist in the murine vaginal tract. Further we have also demonstrated that USA300 attaches to human vaginal epithelial cells (HVEC) in vitro. We have previously demonstrated that Fibrinogen (Fg) is an important vaginal matrix component for bacterial colonization, thus we sought to investigate the importance of MRSA-Fg interactions for vaginal cell interaction. We have constructed single and combination mutants of Fg adhesins including ClfA, ClfB, FnbpA, FnbpB, and SdrCDE. We observed that inactivation of multiple adhesins reduced Fg binding and the ability to adhere to HVEC compared to the parental strain. Future studies aim to determine the importance of Fg binding to MRSA vaginal colonization and polymicrobial interactions within the vaginal tract.

Poster # B20

Electron transport chain is linked to disulfide bond formation in the Gram-positive biofilm-forming bacterium *Actinomyces oris*

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The Gram-positive bacterium *Actinomyces oris* is a key colonizer in the development of oral biofilms, due to its ability to form biofilm and interact with other microbes via its adhesive pili. Folding of nascent pilus proteins, termed oxidative protein folding, is mediated by two thiol-disulfide oxidoreductases MdbA and VKOR. MdbA catalyzes disulfide bond formation of protein precursors emerging from the Sec translocon, whereas VKOR reactivates MdbA. How VKOR is reactivated remains unknown. Here, we report a large-scale transposon screen of *A. oris* Tn5 mutants that identified clones defective in coaggregation with the co-colonizer *Streptococcus oralis.* We obtained 37 independent mutants, 13 of which completely failed to aggregate with *S. oralis*, and the remainder exhibited a range of phenotypes from severely to weakly defective coaggregation. While the former were found to have Tn5 insertion into genes encoding the pilus assembly machine, the latter mapped to several uncharacterized protein-encoding genes and various nuo genes predicted to encode subunits of NADH dehydrogenase (complex I) of the electron transport chain (ETC). Electron microscopy of in-frame deletion mutants of nuo genes and the adjacent ubiE gene, coding for an essential component of menaquinone biosynthesis, revealed severe defects in pilus assembly, as well as defects in bacterial coaggregation. Significantly, the ubiE mutant was defective in reoxidation of VKOR. We propose here a model of how the ETC is involved in pilus assembly and polymicrobial interactions.

Poster # B21

The response of *Streptococcus pyogenes* to tryptophan starvation

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Streptococcus pyogenes is auxotrophic for most amino acids including tryptophan (W). As a result, the pathogen's response to amino acid starvation is thought to be critical to maintaining infection and causing disease. Most bacteria respond to amino acid limitation via the stringent response, which is a global regulatory response that represses stable RNA synthesis and the initiation of cell division. The response is mediated by the secondary messenger (p)ppGpp, which is synthesized by RelA. Prior studies have characterized the stringent response in *S. pyogenes* by starving cultures for branched-chain amino acids. We are interested in the response to W starvation because it is the least abundant amino acid and it is specifically degraded in the human host during the inflammatory response to infection. Using defined media, we examined the regulatory response to W starvation with qRT-PCR in both wild-type *S. pyogenes* and a *relA* mutant. In the *relA* mutant, but not the parental strain, transcripts containing W anti-codons were significantly more abundant than those that lacked W anti-codons. Moreover, this unusual de-coupling of transcription and translation was associated with greater viability, or persistence, of the *relA* mutant compared to the parental strain when starved for W. While paradoxical, the response is similar to obligate intracellular bacteria that lack a *relA* gene. In these pathogens, the unusual response has also been associated with persistence. Our results suggest the codon content of at least some genes may be important in mediating the regulatory response to amino acid starvation.

Poster # B22

Abstract Title: CCR2 does not significantly influence early responses to CNS catheter infection but is essential for long term resolution

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Infants are at high risk of *S. epidermidis* ventriculoperitoneal shunt infection. Our mouse model of infant CNS catheter infection has shown an increase in parenchymal spread of infection in infant mice. We also observe a decrease in peripheral immune cells such as monocytes and macrophages in infant mice compared with adult mice. We hypothesized that the decrease in peripheral immune cell responses allowed for the parenchymal spread of infection in infant mice. To evaluate this hypothesis, we infected adult CCR2 KO mice with *S. epidermidis* to attempt to replicate the infant phenotype of increased parenchymal spread of infection in the absence of a significant peripheral immune cell infiltrate. CCR2 is a key receptor for chemokines responsible for monocyte recruitment, such as CCL2, and its absence in the CCR2 KO mice has resulted in the lack of recruitment of monocytes and macrophages to the CNS in other animal models. The lack of peripheral immune cell infiltrate did not result in an increase in clinical illness or mortality in the CCR2 KO mice compared with wild type adult mice. At early time points post infection, there was not a significant difference in the bacterial growth kinetics between wild type and CCR2 KO adult mice, suggesting that the increased parenchymal spread of infection seen in infant mice is not due to the lack of peripheral immune cells as the absence of these cells in the adult mice does not replicate the infantile bacterial growth phenotype. Similarly, the CCR2 KO mice did not replicate the infant phenotype of attenuated inflammation, with no significant difference in levels of pro-inflammatory chemokines and cytokines in the CCR2 KO versus WT adult mice, as opposed to the decrease seen in infant mice. Age-dependent differences in resident glia, such as microglia and astrocytes, may play a more important early role in shaping the inflammatory response and controlling the spread of infection than peripheral immune cells. Interestingly, in the CCR2 KO mice we see an increase in bacterial burdens and persistence of infection at 8 weeks post-infection that we have not previously observed in adult or infant wild type mice, suggesting that CCR2-dependent recruitment of inflammatory cells is important in the long term resolution of infection. This may also explain the behavioral abnormalities, in terms of abnormal nestlet behavior, observed in the CCR2 KO mice at 8 weeks post infection. Collectively, these findings suggest that CCR2-dependent signaling plays a key role in the long term responses of CNS catheter infections with S. epidermidis and that resident glia are likely the drivers of early post-infection responses. Future studies will include characterization of the astrocytes and microglia in adult versus infant mice, as well as evaluation of bacterial burdens at 8 weeks post infection in CCR2 KO mice infected as infants. Better understanding of the immune mechanisms that place infants at higher risk of these infections can be used to guide future screening and adjunctive therapies.

Poster # B23

Growth environment-dependent plasticity of coagulase-negative staphylococci fatty acid composition and susceptibility to antimicrobial fatty acids

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The balance of straight-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs), and occurrence of straight-chain unsaturated fatty acids (SCUFAs) in *Staphylococcus aureus* is determined by the nutritional growth environment. *S. aureus* is not considered to be part of the natural skin microbiota in contrast to various coagulase-negative staphylococcal species, which dominate the skin community. Since skin is protected by various antibacterial fatty acids, we sought to determine growth environment influence on coagulase-negative staphylococcal fatty acid composition, and the response of these organisms to free fatty acids. Growth in Mueller-Hinton broth resulted in increased

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proportions of BCFAs (63-84%) and lowered proportions of SCFAs (16-37%) more than compared to growth in Trypic Soy Broth (53-75% BCFAs, 23-50% SCFAs), a phenomenon first described in *S. aureus*. Growth in human serum stimulated resulted in incorporation of preformed SCUFAs into fatty acid profiles (*S. aureus*, 45%; *S. epidermidis*, 65%; *S. hominis* and *S. saprophyticus*, 55%; *S. haemolyticus*, 35%; *S. auricularis* and *S. capitis*, 25%); BCFAs, which are biosynthesized, were decreased to as little as 8% of the total. High proportions of oleic (C18:1Δ9) and linoleic (C18:2Δ9,12) acids were incorporated by the coagulase-negative staphylococci, whereas *S. aureus* did not incorporate linoleic acid. All species were susceptible to 32 – 64 μM sapienic acid (C16:1Δ6), and 256-512 μM palmitic acid (C16:0). Coagulase-negative staphylococcal fatty acid profiles were highly influenced by the growth nutritional environment, particularly by amino acids and fatty acids, and are expected to impact membrane biophysics and possibly antimicrobial susceptibility and virulence.

Poster # B24

Study of the regulatory mechanisms of the *S.aureus* SrrAB two-component system

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Antibiotic resistant *Staphylococcal aureus,* commonly known as Methicillin-Resistant *Staphylococcus aureus* (MRSA) are a major cause of hospital infections. Thus, there is an urgent need for identifying novel targets/mechanisms for antibiotic development. Bacterial two-component systems (TCS) contribute to infections by regulating toxin production, antibiotic resistance and survival. TCSs are composed of a membrane bound histidine kinase (HK) and a cytoplasmic response regulator (RR). The HK senses extracellular stimuli through its extracellular sensor domain and undergoes autophosphorylation at a conserved histidine residue in the cytoplasmic kinase domain. The phosphorylated HK then transfers the phosphoryl group to a conserved aspartate residue in the RR, which in turn binds DNA to control gene expression. SrrAB TCS is a regulator of *S. aureus* virulence factors including toxic shock syndrome toxin-1 (TSST-1), which causes toxic shock syndrome. SrrB is a dual function kinase/phosphatase capable of regulating the level of phosphorylation of SrrA. Here, we present data showing that the PAS domain effects SrrB kinase and phosphatase function. Preliminary data indicates that the presence of the PAS domain increases the rate of autophosphorylation and has an inhibitory effect on SrrB phosphatase activity. We also identified heme as a ligand for the PAS domain, which could be a potential mechanism of regulating SrrAB TCS. Crystal structure of the apo form of the PAS domain shows a putative binding pocket which provides insights into potential residues involved in heme binding. Together, our data suggest that heme binding to the SrrB PAS domain may be involved in redox regulation of the SrrAB TCS.

Poster # B25

Characterization of the cid operon in *Streptococcus mutans* with a focus on competence

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Genetic exchange by *Streptococcus mutans* is an important contributor to virulence and hardiness within the oral microbiome. The SMU_1701c/1700c (*cidAB)* pair is an operon in which the "A" component is classified as a membrane protein with similarity to the holin-class family of bacteriophage proteins. Previous work has shown that a *S. mutans* UA159 strain with a *cidB* deletion (Δ*cidB*) displays increased sensitivity to aerobic growth and oxidative stress. These findings indicate the importance of *cidAB* to *S. mutans* physiology and stress response. In this present study, a Δ*cidB* mutant was found to have decreased competence, a phenotype which could be rescued by addition of synthetic competence stimulating peptide (sCSP). Preliminary data using a CSP-responsive PcipB-lacZ reporter assay also suggested that the *cidB* mutant may contain slightly decreased extracellular levels of endogenous CSP. This result correlated with recently-published RNAseq data demonstrating decreased expression of *comA*, which is involved in CSP transport. The genetic neighbors of *cidB*, designated SMU_1703c/1702c and SMU_1699c/1697c, encode putative proteins which are co-transcribed with *cidAB*. The upstream pair SMU_1703c/1702c are currently annotated as integral membrane proteins, with SMU_1703c recently reported to play a role in biofilm formation and stress tolerance. The downstream pair SMU_1699c/1697c are annotated as a radical SAM family enzyme and rRNA methylase, respectively. On-going and future efforts will characterize the contribution of these ORFs to *S. mutans* physiology and virulence, as well as determine if they are functionally related to CidA/B.

Poster # B26

Development of the Only Human Cathelicidin into Two Different Anti-Staphylococcal Biofilm Strategies

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Bacterial biofilms constitute a challenging medical problem as they are difficult to treat. To develop anti-biofilm methods, we have been making use of the only human cathelicidin as a template. LL-37, one of the cleaved forms in human skin and neutrophils, is known for both antimicrobial and immune modulation properties. Our identification of the major antimicrobial region of human LL-37 has laid a foundation for our development (Li, X. *et al*. 2006 *J. Am. Chem. Soc. 128*: 5776-85). A synthetic peptide FK-16 corresponding to residues 17-32 of LL-37 is proved to be most potent against methicillin-resistant *Staphylococcus aureus* (MRSA) USA300. Previously, we demonstrated the anti-biofilm capability of an engineered LL-37 peptide both *in vitro* and *in vivo* (Mishra, B. *et al*. 2016 *ACS Med. Chem. Lett*. 7: 117-121; Wang, G., *et al*. 2014 *ACS Chem. Biol.* 9: 1997-2002). In this poster, we present an alternative antibiofilm approach via site-specific or orientation-specific covalent immobilization of FK-16 on the titanium surface. This peptide-coated titanium surface showed a broad-spectrum activity against the ESKAPE pathogens, including *Enterococcus faecium*, MRSA, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In addition, the coated surface reduced the biofilm formation of *S. aureus* USA300. Therefore, it is possible to develop two antibiofilm strategies based on one molecule.

Poster # B27

Group B streptococcal CovR regulation impacts cellular in Group B streptococcal CovR regulation impacts cellular interaction and induction of host autophagy in brain endothelium.

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Group B *Streptococcus* (GBS) is the leading cause of meningitis in human newborns. In order to cause meningitis blood-borne bacteria must penetrate the blood-brain barrier (BBB), which consists of a specialized layer of human brain microvascular endothelial cells (hBMEC). These cells form the first line of defense between invading pathogens and the host nervous tissue. To study the contribution of different bacterial components to GBS BBB infection, we used a mutant lacking CovR, a response regulator of the CovR/S two component regulatory system that regulates key virulence elements including □-hemolysin production. We also engineered double and triple mutant strains lacking CovR, capsule (CspE) and □-hemolysin (ClyOp) production. We observed that deletion of the capsule facilitated GBS entry into hBMEC, however, all CovR deficient mutants where either not able to invade brain endothelium or did not survive intracellularly. We hypothesize that autophagy may be an important host defense to protect brain endothelium from intracellular bacteria helping to prevent pathogen traversal into the brain. Upon autophagy activation the microtubule-associated protein 1 light chain 3 (LC3) is lipidated to its activated form, LC3-II. We have previously demonstrated that GBS infection induces autophagy in hBMEC. Here we observed that infection with the CovR mutant or the CovR/CspE double mutant resulted in increased LC3-II compared to infection with the WT or other mutant strains. This was independent of caspase 3 activation and cell death. Further studies will investigate the mechanisms of CovR regulation for autophagy induction as well as the role of autophagy in host defense.

Poster # B28

Staphylococcus caprae produces an 8-residue autoinducing peptide that is a potent inhibitor of _Staphylococcus aureus_ quorum sensing and virulence

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The coagulase negative staphylococcal species *S. caprae* is primarily an animal-associated organism that colonizes goats and sheep, but can also be found on human farmers who work with these animals. We found that *S. caprae* secretes a quorum quencher that is active against *S. aureus*. Biochemical and genetic characterization revealed that the quorum quencher was the native autoinducing peptide (AIP) of *S. caprae*, and mass spectrometry demonstrated that the AIP is an 8-residue thiolactone ring. A synthetic version of the peptide was a potent quorum quencher of *S. aureus* agr types I, II, and III, and a weak quorum quencher of agr type IV. In a murine model of dermonecrosis infection, the synthetic AIP inhibited lesion formation, *S. aureus* growth, and *S. aureus* agr induction *in vivo*. This work identifies a novel quorum quencher, is the first characterization of a *S. caprae* AIP, and suggests the importance of interactions between commensal staphylococcal species and pathogenic *S. aureus*.

POSTER SESSION "B" ABSTRACTS

2016 ICG+P TRAVEL AWARD RECIPIENTS

The ICG+P provides funding for travel awards to all students and postdoctoral trainees who present either an oral or poster presentation during the conference. The awards help to defray meeting registration and travel-related expenses for eligible participants. If you are a awardee, you will be contacted by the conference organizer during the meeting to complete the necessary paperwork and awards are given after the conference.

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Kyle Grim
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Karan Gautam Kaval
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Kyle Kinney
Christina Krute
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Jakub Kwiecinski
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Ping Xu
Wei Xu

OUT AND ABOUT IN OMAHA

ICG+P is held in the heart of Omaha, Nebraska. Omaha is considered one of the top places to live, work and raise a family in the country, earning high rankings nationwide in several sectors.

Old Market District

Step outside the front doors of the Embassy Suites Omaha Downtown/Old Market, and you will be immersed in the Old Market neighborhood with its myriad of restaurants, pubs, shopping, art galleries and museums including the Bemis Center for Contemporary Arts and the Durham Museum. Most restaurants in the Old Market are causal dining and do not require reservations.

Downtown

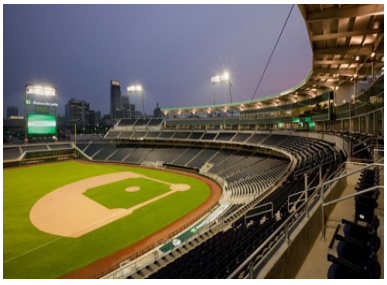
West of the Old Market, is the heart of downtown Omaha with its primary business towers: Woodman of the World Tower, First National Bank Tower, Union Pacific Center, and several other commercial and residential towers. The Orpheum Theater is a historic entertainment venue of Omaha that continues to bring in top productions from across the globe. The Omaha Children's Museum and the Rose Theater provide wonderful adventures for our young and young-at-heart visitors.

Just north of the hotel, Omaha opens up to several walking opportunities such as a quick stroll to the Gene Leahy Pedestrian Mall flanked by the Holland Center for Performing Arts. One can wander through sculptures of a wagon train or wild bison or geese at First National's Spirit of Nebraska's Wilderness and Pioneer Courage Park. Further west, but still is easy walking distance from downtown, is the Joslyn Art Museum and the Omaha Civic Auditorium.

A slightly longer venture leads east from the hotel to the Missouri riverfront which encompasses the Heartland of America Park and Fountain adjacent to the ConAgra campus; Lewis & Clark Landing; and the Bob Kerrey Pedestrian Bridge that connects Omaha with Council Bluffs, which is home to three major casinos.

North Downtown District

Omaha's North Downtown district hosts several key sporting venues, including TD Ameritrade Park, home of baseball's NCAA College World Series; Creighton University soccer at Morrison Stadium; and hockey at CenturyLink Center Omaha, the latter puts on major concerts and conventions for the city. This neighborhood also supports entertainment and shopping including Film Streams and Hot Shops Art Center.



South Omaha District and Midtown Crossing

A little further afield are several not-to-be-missed tourist destinations around Omaha. South of downtown Omaha is the world renowned Omaha's Henry Doorly Zoo, Lauritzen Gardens and Kenefick Park, El Museo Latino, and Sokol Auditorium. Westward, between downtown Omaha and the campus of the University of Nebraska Medical Center is Midtown Crossing, a newly regenerated neighborhood with outstanding dining, shopping, and entertainment opportunities.