ASM WASHINGTON, D.C. BRANCH
SPRING 2019 MEETING
Clinical and Public Health Microbiology
March 23, 2019
Abstracts & Agenda
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<th>Start</th>
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| 10:00      | 10:45     | **Welcome**  
Denise M. Akob, Ph.D.  
President, DC Branch of ASM                                   |
| 10:45      | 11:00     | **Invited Talk:**  
The Public Health Laboratory Response to Ebola  
Anthony Tran, DrPH, MPH, D(ABMM)  
Director, DC Public Health Laboratory                                    |
| 11:00      | 11:30     | **Identification of Candida auris and Other Pathogenic Yeasts by MALDI-TOF Mass Spectrometry of Membrane Lipids  
Lisa Leung, Ph.D.  
Postdoctoral Fellow, Maryland Department of Health                          |
| 11:30      | 11:45     | **Corynebacterium pseudodiphtheriticum** exploits **Staphylococcus aureus** virulence components to compete for the human nasal colonization niche  
Britney L. Hardy  
Graduate Student, Uniformed Services University                                    |
| 12:00      | 1:00      | **Lunch (provided)**                                                        |
| 1:30       | 2:00      | **Invited Talk:**  
Catch me if You Can: The Spread of Antibiotic Resistant Neisseria gonorrhoeae as it Relates to Biological Fitness  
Ann Jerse, Ph.D.  
Professor, Uniformed Services University                                      |
| 2:00       | 2:15      | **Culture aeration alters the role of the Pseudomonas aeruginosa PrrF sRNAs in antimicrobial activity against Staphylococcus aureus**  
Luke K. Brewer  
Graduate Student, University of Maryland Baltimore                          |
| 2:15       | 2:30      | **Novel Small Molecule Inhibitors of Borrelia burgdorferi**  
S. Stephanie Garcia-Buntley, Ph.D.  
Postdoctoral Fellow, Uniformed Services University                           |
| 2:30       | 4:00      | **Poster session and Coffee Break**                                          |
| 4:00       | 5:00      | **ASMDL Keynote Address:**  
Lessons Learned from an Accidental Pathogen  
Barbara I. Kazmierczak, M.D., Ph.D.  
Professor, Yale University School of Medicine                                |
| 5:00       | 5:15      | **Concluding Remarks**  
Kileen Shier, Ph.D., D(ABMM), MLS(ASCP)CM  
President-Elect, DC Branch of ASM                                              |

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Level: Intermediate  
Contact Hours: 3.0  
Fee: $5
Multi-omic insights into effects of drought on soil microbiome

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Soil microbiome responses to changing environmental conditions are manifested as shifts in community structure and/or modification of activity. However, molecular-level details underlying functional responses of soil microbiomes to perturbation are largely unknown. Here, we demonstrate a multi-omics approach to determine the impact of environmental perturbations on the soil microbiome across taxonomic and functional levels. Kansas native prairie soil samples from three field locations were either treated with glycine as a model root exudate or perturbed by changing moisture conditions. The microbiome response was assessed using a suite of omics measurements: 16S rRNA amplicon sequencing, metagenomics, metatranscriptomics, and metabolomics. The soil microbiome responded to glycine at the functional level, but not at the community structure level. In contrast, soil drying shifted both the microbiome composition and function. A major challenge in soil microbial ecology is the extraordinary phylogenetic and functional diversity of the soil microbiome in association with the physico-chemical complexity of the soil habitat. Here by using a multi-omics approach, we elucidated the phenotypic response of the soil microbiome across different levels of expression; thus, providing a proof-of-concept for use of this approach to assess key physiological traits expressed by the soil microbiome.
Clinically Isolated Thiamine Auxotrophs of *Neisseria gonorrhoeae* Demonstrate Increased Susceptibility to Host Innate Defenses

Nelson C. E. Dozier\(^1\), Nazia Rahman\(^1\), Adriana Le Van\(^1\), William M. Shafer\(^2\), Eric C. Gargas\(^1\), and Ann E. Jerse

\(^1\)Uniformed Services University, Bethesda, Md, USA; \(^2\)Emory University, Decatur, Ga, USA

Thiamine pyrophosphate (TPP) affects many metabolic pathways within the cell. We recently demonstrated that a genetically defined *Neisseria gonorrhoeae* (Gc) mutant that cannot biosynthesize thiamine pyrophosphate (TPP) is more susceptible to killing by neutrophils, cationic antimicrobial peptides (CAMPs), and reactive oxygen species (ROS) in vitro, and attenuated for experimental infection of mice that have an inflammatory response. Here we investigated the susceptibility of five clinical TPP auxotrophs to ROS and CAMPs as a first step towards understanding the consequence of thi auxotrophy during human infection. Gc isolates in the USUHS Gc Resistance and Reference Repository isolated between 2014 and 2017 were screened for the capacity to grow on medium without TPP. Five thi auxotrophs were identified among the 89 isolates tested (5.6%). Auxotrophs were tested for susceptibility to paraquat and colistin (polymyxin E) using standard methods. Four of the auxotrophs exhibited increased susceptibility to paraquat and colistin. Auxotroph 4097, in contrast, showed a ~2-fold greater resistance to 0.0195 mM of paraquat and colistin compared to a wild-type Gc strain, suggesting this isolate may carry a compensatory mutation(s). We conclude that clinically isolated thi auxotrophs are more susceptible to ROS and CAMPS. We hypothesize that these isolates may have a lesser ability to withstand oxygen-dependent and –independent effectors of the host inflammatory response and that selection for compensatory mutations during infection may be one mechanism by which thi auxotrophs remain in circulation. Analysis of WGS data is underway to identify the genetic basis of thi auxotrophy and possible compensatory mutations.
Surveillance of Antibiotic-Resistant Neisseria gonorrhoeae through the U.S. DoD Global Emerging Infectious Disease Surveillance (GEIS) Program: Differences in antibiotic resistance in US military sites


The Global Emerging Infectious Surveillance Program of the U.S. Department of Defense, Armed Forces Health Surveillance Branch supports a repository for Neisseria gonorrhoeae (GC) clinical isolates recovered from seven routine care at U.S. Military Treatment Facilities in the continental U.S. (CONUS) and at several overseas (OCONUS) labs through collaborative surveillance projects. Here we report the use of phenotypic data in conjunction with molecular typing and whole genome sequencing (WGS) of GC to describe the antimicrobial resistance trends from isolates collected from CONUS sites from 2014 to 2018.

We found variability among resistance isolates from the different study sites that was not dramatically different from that which is reported in the civilian population. The number of tetracycline resistant isolates appears to be increasing over time, with about 8 percent of isolates resistant, and 29% of isolates were ciprofloxacin resistant, which is higher than the national statistics, although this percentage varied by study site. Isolates from naval sites tended to have a higher resistance to ciprofloxacin than from army sites, which may suggest networking differences or therapeutic selective pressure. While no β-lactamase positive isolates were identified, reduced susceptibility to penicillin was increased (present in 82% of all isolates). Results from NG-MAST typing and WGS analysis suggest genetic diversity among US-based isolates. Interestingly, 5 unreported NG-MAST types were identified and none of the 16 most common European NG MAST types was found among CONUS isolates. Continued surveillance is on-going to further define the antibiotic resistance trends in military populations.
Sulforaphane as an Adjunctive Epigenetic Therapy for *Neisseria gonorrhoeae* infections

Rok Tkavc\(^1\,^2\) and Ann E. Jerse\(^1\)

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*Neisseria gonorrhoeae* (Ng) is the agent of gonorrhea, one of the most prevalent sexually transmitted infections. Due to its constitutive competence and high frequency of spontaneous mutations, Ng develops antibiotic resistances relatively quickly. There is currently no single class of antibiotic for first-line treatment of gonorrhea, and dual therapy with ceftriaxone and azithromycin is now the recommended therapy. However, isolates that are resistant to both of these antibiotics have emerged. With no vaccines available, Ng represents a serious public health problem.

Cruciferous plants such as broccoli have developed an efficient defense mechanism against pests. Cell wall damage activates an enzyme myrosinase, which transforms glucoraphanin into glucose and sulforaphane (SFN), which repels arthropods. Exposure of human cells to SFN leads to induction of phase 2 and inhibition of phase 1 detoxification enzymes. SFN also inhibits human histone deacetylases, which leads to expression of cationic antimicrobial peptides and other innate defense genes.

One approach towards combating antibiotic resistant pathogens is to stimulate the host’s production of antimicrobial factors. We recently demonstrated that supernatants from SFN-treated endocervical tissue culture cells decrease the viability of Ng (Yedery et al., in preparation) and in preliminary studies, that supernatants from SFN-treated cells increase the potency of antibiotics against Ng. Here we report that SFN promotes a dose-dependent inhibition of bacterial adhesion to cervical cells and internalization. We also show that SFN induces genes that encode host factors with antibacterial activity. These results strongly support SFN as a promising candidate for adjunctive epigenetic therapy.
Preliminary Characterization of Biofilm Formation by *Helicobacter pylori* Strain G27

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*Helicobacter pylori* is a Gram-negative pathogen that colonizes the gastric mucosa of humans and non-human primates. Infection with *H. pylori* is often asymptomatic, though infected individuals possess an increased risk for development of gastric-related illnesses. Current research indicates that *H. pylori* is capable of forming biofilms, a mode of life that could contribute to treatment difficulty. Comparison between biofilm studies conducted in different labs is difficult given how the assays are performed; it is unknown how different conditions might affect *H. pylori* biofilm formation. We characterized the biofilm formation ability of the *H. pylori* lab strain G27 under different conditions. We also sought to characterize the extracellular matrix of the biofilm using chemicals and enzymes was used to break down components of the ECM and fluorescent confocal microscopy to visualize the extracellular matrix. In addition, we tested the anti-biofilm peptides IDR-1018 and DJK-5 against *H. pylori* biofilms. Our results indicate that while different rich media did not greatly alter final biofilm mass, surface selection had a significant effect on biofilm formation. The addition of DNase I or Proteinase K each lead to dispersal of the biofilm, in a time and dose dependent manner, indicating the importance of extracellular DNA and proteins to the structure of the extracellular matrix at different stages of biofilm development. We found that both anti-biofilm peptides were effective against biofilms, though the effects differed based on when the peptides were added. Together these results revealed numerous interesting avenues for future investigations of *H. pylori* biofilm formation.
Neisseria gonorrhoeae infection of the uterus produces a distinct immune response compared to lower genital tract infection in a female mouse model

Allison Costenoble-Caherty and Ann Jerse

Neisseria gonorrhoeae is one of the leading causes of pelvic inflammatory disease (PID) in women. Gonococcal PID is a condition resulting from cervical infection (the lower reproductive tract or LRT) ascending to the uterus and/or fallopian tubes (collectively called the upper reproductive tract or URT). Left untreated, PID can cause ectopic pregnancy, infertility, and chronic pain. Many factors contributing to PID remain undefined, including host and bacterial factors that allow colonization of the upper reproductive tract. In addition, very little is known about the immune response to URT infection and how it differs from LRT infection. This includes whether a protective immune response to infection in the URT would involve the same components as that to LRT infection. These questions have relevance for vaccine design, as an effective gonorrhea vaccine would have to protect against both LRT and URT infection.

We have recently developed a female mouse model of URT infection that utilizes human transferrin supplementation to support URT infection. Using this model, we have shown that both URT and LRT infection result in a similar bacterial burden. However, surprisingly, LRT infection results in higher and longer lasting levels of local inflammation as evidenced by local cytokines and chemokines. We further explored whether induction of a Th1/Th2 response via blocking of TGF-beta would elicit a protective immune response resulting in clearance of infection as previously shown in a mouse model of LRT infection. We have shown that blocking of TGF-beta does not appear to be protective in URT infection.
Characterization of Attenuated *Francisella tularensis* LVS Phagosomal Transporter Mutants

B. E. Hobbs and E. M. Barry, Ph.D.
Center for Vaccine Development and Global Health, University of Maryland, School of Medicine, Baltimore, MD USA

*Francisella tularensis* (Ft) is a Gram-negative, facultative intracellular bacterium that is a Tier 1 Select Agent of concern for biodefense. A family of 9 *Francisella* phagosomal transporter (fpt) genes encoding members of the Major Facilitator Superfamily of transporters has been identified as critical to the pathogenesis of Ft and potential targets for attenuation and vaccine development. The ability of Ft to survive and replicate to high numbers within macrophages is key to pathogenesis. Deletion mutations have been generated in 8 fpt genes in LVS and infection assays within mouse peritoneal macrophages demonstrated altered intracellular replication kinetics by 5/5 fpt mutant strains tested in comparison to parental LVS. Mutant phenotypes varied but included defective intracellular replication, delayed egress, inability to re-infect neighboring cells, and altered host cell death kinetics. Mutations in fptA, fptB, fptE, and fptF resulted in reduced intracellular replication and reduced cytotoxicity, as well as attenuation in the C57BL/6J mouse model of respiratory tularemia. LVSΔfptG exhibited the most significant defect in vitro. It was unable to escape the host cell and re-infect neighboring cells, and induced alterations in host cell cytokine responses. We hypothesize that fptG is essential for the timely escape of Ft from the cell. These results support a fundamental necessity for fpt gene products in the pathogenesis of Ft, and ongoing analysis will further our understanding of these roles.
Rationally Designed Short Antimicrobial Peptide Variant Derived from Snake Venom-associated Cathelicidin Demonstrates Anti-Infective and Anti-Cancer Therapeutic Potential

Brian Nguyen, Saswata Sahoo, Justin Davis, Barney Bishop

Due to the increasing concern regarding the spread of multi-drug resistant bacteria, cationic antimicrobial peptides (CAMPs) have emerged as potential leads for the development of new therapeutics and treatment strategies. These peptides exhibit a positive charge and typically target bacterial membranes. Cathelicidins are a prominent class CAMPs produced by vertebrates.

The 34-amino acid residue cathelicidin, NA-CATH, was identified from the venom glands of the Chinese cobra (Naja atra). This helical conformation consists of an N-terminal amphipathic helix followed by an unstructured hydrophobic C-terminal tail. The helical region of NACATH includes a pair of semi-conserved 11-residue segments referred to as ATRA motifs, which contain a combination of positively charged and hydrophobic residues. The 8 non-polar C-terminal residues which lack any defined structure in the presence of anionic surfactants is referred to as the hydrophobic tail domain (HYD).

A synthetic 11-residue peptide, ATRA-1, was generated that reproduces the sequence of the first ATRA motif of NA-CATH. This peptide served as the template for the design of two additional peptides ATRA1-R3W2, which incorporated sequence changes that were intended to improve antimicrobial effectiveness. Because the unstructured C-terminal HYD segment may contribute to the high antimicrobial potency of the NA-CATH parent peptide, an additional ATRA-1 peptide variant, ATRA1-R3W2-HYD, was produced. This peptide incorporated the HYD tail of NA-CATH. Both of these new peptides exhibited promising results when evaluated against a panel of Gram-negative and Gram-positive bacteria, and they showed low host cytotoxicity. In conclusion, the culminated variant, ATRA1-R3W2-HYD shows potential for further therapeutic development.
Deep sequencing to track temporal patterns of the *Eonycteris spelaea* virome

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As the number of bats that roost in urban areas continues to increase, spillover from bats to humans in Southeast Asia has gained recognition as a potential source of pandemic infections. Bats are predicted to host more than 200 known viruses, including several RNA viruses that have caused recent outbreaks in humans (and in some cases also livestock) with devastating consequences. Determining the aspects of bat physiology and/or lifestyle that make them excellent virus repositories requires thorough documentation of the viruses that exist in bats. In this study, deep sequencing of viral RNA extracted from swabs of four body sites per bat per timepoint is used to characterize the virome of a captive *Eonycteris spelaea* colony in Singapore. To our knowledge this is the first study that uses probe-based viral enrichment combined with high-throughput sequencing to create a viral profile from multiple swab sites on each individual bat and its cohort. This work has led to the characterization of the *E. spelaea* virome, including several novel viruses. Zoonotic-related viruses of the Coronaviridae, Paramyxoviridae, Reoviridae and Astroviridae families were evaluated longitudinally for discernable temporal patterns in detection and genetic variability. Given the known risk for bat-human zoonoses, a more complete understanding of the viral dynamics in Southeast Asian bats would have significant implications for disease control. The finding of this study will be of significant interest to U.S. Department of Defense personnel stationed in the Asia-Pacific region and regional public health laboratories engaged in emerging infectious disease surveillance efforts.
Effects of 400 Hz vibrations on biofilms of *Pseudomonas fluorescens*

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Background: Biomass formation is dependent on quorum sensing, a method of bacterial communication that regulates gene expression. The use of audible acoustic vibration therapy to influence quorum sensing within biofilms has not been extensively researched.

Materials and Methods: Samples were developed using set suspensions of *P. fluorescens* transferred to a microplate. The plates were exposed to 400 Hz vibrations for 10 minutes and incubated overnight. Serial plating was performed with a subset of samples to determine CFU compared to an untreated control. Crystal violet staining of a second subset of treated/untreated samples was used to determine the remaining biomass.

Results: Treated samples from both trials demonstrated statistically insignificant decreased biomass compared to the control (p = 0.098 and p=0.198, respectively). Growth at dilutions of 1:10, 1:100, and 1:200 were all too numerous to count, as was growth for the 1:1000 control plate. Treated 1:1000 plates had an average of 1.69 x 10^5 colonies (27.8 colonies/1mm^2 area) and 1.52 x 10^5 colonies (25 colonies/1mm^2 area), for trials 1 and 2, respectively.

Discussion: Biofilms are a persistent threat to health; endocarditis caused by biofilms of *Staphylococcus* is a fatal example. It is possible that exposure to vibrations may contribute to decreased biomass for *P. fluorescens* biofilms, but this needs to be confirmed with additional trials.

Acknowledgements: Research funded by NSF grants 1643498 and 1503192.
Bacteriophage-derived endolysins have great potential as alternative antimicrobial agents for Gram-positive bacterial infectious diseases as they are peptidoglycan hydrolases that can destroy susceptible bacteria when applied exogenously. Due to the modular structure of endolysins, engineering methods can be used to improve their properties or change their host range via manipulation of the functional domains. The multimeric endolysin, PlyC, has potent activity on groups A, C, and E streptococci, as well as *Streptococcus uberis*, but is devoid of activity on other streptococci such as *S. agalactiae* (i.e. group B strep), *S. mutans*, or *S. pneumoniae*. PlyCA, the enzymatically active domain of PlyC, consists of two catalytic domains, GyH, a glycosyl hydrolase, and CHAP, a cysteine, histidine-dependent amidohydrolase/peptidase. Notably, GyH and CHAP have been shown to work synergistically to achieve lytic rates ~100 fold higher than comparable single catalytic domain endolysins. In this work, we provide a new design of chimeric endolysins to take advantage of the synergistic effects of PlyCA. ClyX-1 was created via fusing the pneumococcal Cpl-1 cell binding domain (CBD) in between the GyH and CHAP catalytic domains of PlyCA. This chimera displayed ~100 fold increase in activity in vitro against *S. pneumoniae* compared to the parental Cpl-1 enzyme. ClyX-2 was then created using a similar strategy by fusing the broad host range PlySs2 CBD between GyH and CHAP catalytic domains. ClyX-2 not only demonstrated wild-type PlyC activities on groups A, C and E streptococci, but now included high levels of activity (i.e. 20-50 fold higher than PlySs2) against *S. mutans* and *S. agalactiae*. Moreover, this design format (i.e. CBD in the middle of two catalytic domains) can also be applied to other enzymes in order to achieve similar synergistic results. CHAP or GH25 catalytic domains were added to the C-terminus of full-length Cpl-1 and PlySs2, respectively, and displayed synergistic effects. To date, with the exception of PlyC, two catalytic domains in one endolysin have not shown synergism, even in enzymes that naturally contained two catalytic domains. Our work suggests a novel design for adopting the synergy of two catalytic domains for increased lytic activity.
Copper Resistance is Important for Virulence of *Acinetobacter baumannii*

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*Acinetobacter baumannii* is a highly drug resistant pathogen that can cause severe infections, which can be life threatening due to limited treatment options. Copper is inherently antimicrobial and has potential for therapeutic use against multidrug-resistant bacteria. Clinical isolate AB5075 is a model strain to study modern antibiotic resistant strains, and is also relatively copper resistant. In AB5075, we identified putative copper resistance components and obtained mutant strains containing independent transposon insertions into 21 of these putative copper resistance genes. Eight of the strains displayed a copper sensitive phenotype (copB, copA/cueO, copR/cusR, copS/cusS, copC, copD, pcoA, pcoB). Putative functions include copper transport, oxidation, sequestration, and regulation. We used ICP-mass spectrometry to investigate whether the mutant strains were able to properly mobilize excess copper; many of the strains accumulated more intracellular copper than the wildtype strain, which could explain the inability to grow/survive in high concentrations of copper. We found that in a biofilm structure all of the mutant strains were still subject to killing by copper. We are currently investigating the role of these copper resistance genes in *A. baumannii* virulence using two established models: *Galleria mellonella* and murine pneumonia. Crucially, our preliminary data shows that many of the mutant strains are attenuated in these models of infection. Overall, we have shown that copper possesses antimicrobial activity against drug-resistant *A. baumannii* and copper sensitivity is further increased when copper homeostasis mechanisms are interrupted. Importantly, these proteins are important for full virulence of *A. baumannii* and may represent novel drug targets.
Host suppression of quorum sensing during catheter associated urinary tract infections

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Chronic bacterial infections on medical devices, including catheter-associated urinary tract infections (CAUTI), are associated with bacterial biofilm communities that are refractory to antibiotic therapy and resistant to host immunity. Previously, we have shown that Pseudomonas aeruginosa can cause CAUTI by forming a device-associated biofilm that is independent of known biofilm exopolysaccharides. Here, we show by RNA-seq that host urine alters the transcriptome of P. aeruginosa by suppressing quorum sensing regulated genes. P. aeruginosa produces acyl homoserine lactones (AHLs) in the presence of urea, but cannot perceive AHLs. Urea inhibits perception by preventing the uptake of AHL, suggesting that P. aeruginosa has a pathway to import these quorum sensing molecules into the bacterial cytoplasm. Quorum sensing-regulated processes in clinical CAUTI isolates are also inhibited by urea. These data show that urea in urine is a natural anti-quorum sensing mechanism in mammals.
Molecular Characterization of the Conjugative qacA-positive plasmid pC02

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*Staphylococcus aureus* is a common culprit of hospital and community acquired infections, and some *S. aureus* plasmids have been shown to carry genes that affect resistance to biocides. Among these genes, qacA encodes the QacA Multidrug Efflux Pump that imparts decreased susceptibility to chlorhexidine, which is a common biocide that is used ubiquitously in healthcare facilities. We previously identified a novel conjugative qacA-positive plasmid, pC02, from the clinical *S. aureus* isolate C02. We were able to demonstrate conjugative transfer of pC02 to *S. aureus* and *Staphylococcus epidermidis* strains. In silico sequence analysis of pC02 suggested that the plasmid is part of the pWBG749-family of conjugative plasmids and that it contains two origins of transfer (oriT). Indeed, we found that both oriTs were functional and could mediate plasmid transfer. Furthermore, depending on which oriT was utilized, partial transfer of pC02 was consistently observed at defined frequencies. To define the ability of the pC02 plasmid to utilize different oriTs, we screened the mobilization ability of nonconjugative plasmid variants engineered to contain unique oriT family inserts from pC02 as well as various sequenced Staphylococcal plasmids. The oriT-OTUNa family was transferred at the highest frequency; additional oriT-families were also transferred but at lower frequencies. pC02 was maintained at a low copy number and was stably maintained; stability of partially transferred plasmids was dramatically decreased. Given the increasing use of chlorhexidine, we speculate that the conjugative plasticity of pC02 could contribute to the spread of qacA across Staphylococcal strains and species.
Pathogen-shed, urinary proteins in tuberculosis patients include drug targets and markers of antibiotic resistance

Ruben Magni, Lance Liotta, and Alessandra Luchini

We used a nanotechnology based pre-analytical processing method, mass spectrometry analysis, and a novel bioinformatics pipeline in order to investigate the proteome shed by *Mycobacterium tuberculosis* in the urine of infected patients. Previously unattained analytical sensitivity was ensured by our sample concentration method, affinity hydrogel particles, which selectively sequester and preserve target protein analytes from solution and yield hundreds-fold sensitivity enhancement depending on the initial volume of sample. Mass spectrometry analysis results were filtered with a bioinformatics pipeline that ensured 100% amino acid sequence identity with *Mycobacterium tuberculosis (Mtb)* proteins, evolutionary taxonomic verification for related pathogens, and lack of overlap with human or *Mtb* organisms. We analyzed 150 urine samples that were collected from patients with microbiology confirmed pulmonary tuberculosis, adult and pediatric, in presence or absence of HIV co-infection, and with extrapulmonary tuberculosis, including tuberculosis meningitis and pleural effusion, ganglionar tuberculosis, and ocular tuberculosis. We identified more than one hundred proteins, including known or highly promising drug targets (LppX_LprAFG lipoprotein, Alanine racemase, thymidylate synthase, PadR-like family transcriptional regulator, leucyl aminopeptidase, integral membrane indolylacetylinositol arabinosyltransferase EmbB), and proteins known to be involved in mechanisms of drug resistance (ABS transporter ATP-binding protein, Taurine ABC transporter permease protein, TetR family transcriptional regulator). Pathogen-shed urinary proteins in patient affected by *Mtb* infection at different sites hold great potential as markers for diagnosis and drug resistance.
Sequence Determination of Hybridoma Antibody Transcripts Targeting Virulent or Immunogenic Factors of *Mycobacterium tuberculosis*

Hui-Chen (Jane) Chang Foreman, Andrew Frank, and Timothy Stedman  
BEI Resources, ATCC. 10801 University Boulevard, Manassas, VA 20110-2209

*Mycobacterium tuberculosis* (*Mtb*), a causative agent for Tuberculosis (TB), remains one of the most challenging pathogens to control. Lacking protective vaccines and a rapid, effective diagnostic tool hamper TB control. Only in recent years has TB antibody development offered new potential to control TB infections. Knowledge of the paratope sequences of Mtb antibodies enables engineering diagnostic and therapeutic tools. Currently, few validated TB antibody sequences are available. Here, we identified the sequence of functional variable immunoglobulins (IgVs) expressed in 14 hybridomas encoding antibodies to Mtb targets with potential therapeutic/diagnostic value: 1) Mpt64, a mycobacterial diagnostic peptide; 2) Ag85 complex, the most immunogenic Mtb protein to date; 3) the *Mtb* bacterial surface components: glycolipid LAM, lipoprotein LprG and HBHA, an epithelial cell adhesion factor; 4) the *Mtb* enzymes: Superoxide Dismutase SodA, and Catalase KatG; 5) the *Mtb* regulatory factors PhoS1/PstS1, factors within the ABC transporter system; and 6) the *Mtb* Heat Shock Proteins HspX, DnaK, and GroES. We isolated the paratope-determining CDR 1-3 regions of the heavy and light chains of these IgVs using a 5'RACE-PCR amplification from the cDNA of each hybridoma via an isotype-specific primer for each of the light chains of Ig/Ig_1, 2, and 3 and heavy chains of IgG1, IgG2a, IgG3 and IgM. Using an Illumina NGS_MiSeq, 2X150bp_pair-wise sequencing platform, we generated 28 IgV libraries. Most libraries contained sufficient reads and coverage for de novo assembly of Ig chains via a bioinformatics algorithm workflow for analysis of Ig sequences. Thirty-three (33) putative TB IgV sequences were identified.
A dedicated diribonucleotidase resolves a key bottleneck as the 1 terminal step of RNA degradation

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Degradation of RNA polymers, an ubiquitous process in all cells, is catalyzed by specific subsets of RNases. After endoribonuclease cleavage, the RNA fragments are thought to be degraded stochastically by a group of 3'-5' exoribonucleases that together recycle RNA fragments into nucleotide monophosphate and in γ-proteobacteria comprise up to eight distinct enzymes. Among them, Oligoribonuclease (Orn) is unique as its activity is required for clearing short RNA fragments, which is important for transcriptional control, signaling, and overall cellular fitness. However, the structural and molecular basis of Orn's unique cellular function remained unclear. Here we show that Orn exhibits exquisite substrate preference for diribonucleotides. Co-crystal structures of Orn with substrates reveal an active site optimized for diribonucleotides that does not accommodate longer substrates. While other cellular RNases process oligoribonucleotides down to diribonucleotide entities, our functional studies demonstrate that Orn is the one and only diribonucleotidase that completes the terminal step of the RNA degradation pathway. Together, our studies indicate RNA degradation as a step-wise process that ends with a dedicated enzyme for the clearance of a specific intermediate pool, diribonucleotides, that affect cellular physiology and viability.
Mechanisms of toxin regulation in *Bacillus anthracis*

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*B. anthracis*, a spore forming, Gram positive aerobic rod bacterium, is virtually capable of causing anthrax in almost all mammals. Full virulence in *B. anthracis* is attained only if it has two plasmids: pX01, which encodes a tripartite protein exotoxin complex (protective antigen-PA, lethal factor-LF and edema factor-EF); and pX02, which encodes poly-D-glutamic acid capsule. Expression of these virulence genes is controlled by AtxA, a multidomain transcription factor. AtxA has two DNA binding HTH domain, two PTS (Phosphoenolpyruvate Sugar Phosphotransferase System) - regulatory domain (PRD1 and PRD2) and an EIIB domain. Phosphorylation of conserved H199 in PRD1 and H379 in PRD2 has opposing effect on the activity of AtxA, where later is inhibitory to toxin gene transcription. Among cellular and extracellular factors, presence of glucose and CO$_2$ in its environment is essential for AtxA dependent gene transcription. We aim to identify the sensor for CO$_2$ and/or the sugar transporter component(s) of AtxA EII-complex to better understand the mechanism of toxin regulation in *B. anthracis*.

Transcriptional profiling of pX01 encoded virulence factors, secretion of PA and fluorescent reporter strain suggested the synergistic effect of CO$_2$ and mono or di or trisaccharides or sugar alcohols on AtxA dependent gene transcription. This is important because EII-complexes are involved in transport of specific sugar, but AtxA seems omnivalent. Inhibition of active transport system using nigericin and CCCP confirmed the dependency of AtxA on sugar transport. Crosslinking of cellular protein followed by AtxA pull-down, co-precipitated some putative interacting partners which will be identified by mass-spectrometry. Towards identifying the underlying mechanism, I performed pathway analysis using two separate datasets from public domain. A significant induction of pst- operon, regulating intracellular inorganic phosphate (Pi) concentration, was observed under toxin producing conditions. The phosphorylation of PRD in EII-complex depends on the availability of high energy phosphate carrying metabolites like phosphoenolpyruvate (PEP) whose turnover is maintained by the intracellular Pi concentration (EMP Pathway). This observation was confirmed through quantification of total Pi using 31P-NMR and Malachite green assay, where the atxA null mutant was having 20 percent lesser Pi concentration as compared to wild type (Wt). This is suggestive of AtxA being an intermediate in sugar scavenging mechanism by PTS during starvation and/or anaerobic condition like presence of CO$_2$. The findings will help to control the toxin production in *B. anthracis* by targeting one master regulator.