Rio Grande Branch - American Society for Microbiology Meeting 2021

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1. Tracing and Tracking Genetic Variation in SARS-CoV-2 Genome

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The COVID-19 pandemic is caused by SARS-CoV-2 virus. By April 7, 2021, more than 1 million viral genomes have been sequenced and available in the public domain. The record-high amount of genome data gives unprecedented genome epidemiological insight into the virology of SARS-CoV-2 and epidemiology of the COVID transmission, which helps define the changing landscape of the pandemic. Phylogenetic analysis has identified four major clades, S, L, V, and G, at the beginning of the pandemic in January 2020. Now clade G and its derivatives GH, GV, GR, and GRY have become dominant globally since April 2020. The emergence of new variants, especially mutants in Spike proteins, has caused serious concerns about the impact on the effectiveness of vaccination. In this study, we tracked the temporal and spatial dynamics of these clades and the temporal occurrence of the variable sites in the S gene. Tracing and tracking genome variation provides invaluable information to foresee the evolving trend of the CoV-2 genomes while the clinical and immunological intervention takes place.

2. *Prevotella* Differentially Regulates the Inflammatory Response of Human Monocytes to SARS-CoV-2 Spike Glycoproteins

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Patients suffering severe COVID-19 show an aggressive and excessive immune response against the SARS-CoV-2 coronavirus,

a phenomenon known as a cytokine storm. If left untreated these patients face the risk of tissue damage, multi-organ failure and death. Besides treatments targeting the viral infection, other treatments aim to reduce or regulate the inflammatory process in COVID-19 to avoid the development of related complications. A high relative abundance of *Prevotella copri* has been reported in patients with newly diagnosed rheumatoid arthritis (RA). On the other hand, it has been observed that *Prevotella histicola* can modulate the inflammatory manifestations of autoimmune diseases like multiple sclerosis, and it is now being evaluated as a monoclonal microbial treatment in COVID-19.

We aim to evaluate the inflammatory response in human monocytes to various forms of SARS-CoV-2 Spike glycoproteins, upon pre-inoculation with three different species from the genus *Prevotella*.

Dual THP-1 cells harboring two plasmid-reporter systems for transcription factor NF- κ B, and for interferon regulatory factors (IRFs), were exposed to *P. histicola*, *P. copri*, and *P. oralis* at a multiplicity of infection of 10:1 for 4 h. Cells were then stimulated with various forms of the SARS-CoV-2 Spike glycoprotein for 4 h.

An increase in NF- κ B activation was observed in response to any of the evaluated SARS-CoV-2 S glycoproteins when monocytic cells had been pre-inoculated with *P. histicola* and *P. copri*. The effect was majorly observed when a stabilized trimer of the S glycoprotein was used for stimulation. Inversely, a reduction in IRF activation was observed when monocytic cells had been pre-inoculated with *P. histicola* and *P. copri*. No difference was observed for either transcription factor readout if cells were pre-inoculated with *P. oralis*.

Exposure of human monocytes to certain commensal species of *Prevotella* leads to differential activation of inflammatory pathways in response to SARS-CoV-2 glycoproteins. Contrary to what is observed in tissue, *P. histicola* increases the inflammatory response of blood immune cells, such as monocytes to SARS-CoV-2 S glycoproteins. The simultaneous decrease in IRF activation may translate into a reduction of type I IFNs, which appear important in controlling viral infections.

3. Inactivation of Bacteria in Circulating Air Using Needle Point Bipolar Ionization

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Airborne transmission of SARS-CoV-2 viruses in school environments has been an area of concern during the pandemic. Several methods to reduce transmission have been proposed including face coverings, reduction in the number of students in classrooms, increased ventilation and filtration in HVAC systems, and the use of inactivating agents including ultraviolet light. Although the viral load is presumably very small if all building occupants utilize face coverings, any interventions that can contribute to further reduction in the number of viral particles in circulating air is of paramount importance. Needle point bipolar ionization (NPBI) is a novel system that can be installed in existing HVAC systems to reduce viral numbers. NPBI generates positive and negative ions, which can inactivate microorganisms and promote formation of larger aggregates that can be easily removed by air filtration using HEPA or MERV filters. The objective of this study was to determine if an NPBI system installed in the science wing at one campus of El Paso Community College was effectively removing bacteria from circulating air as compared to a different wing without NPBI. An Andersen single-stage impactor at 9 L/min with blood agar plates was used to collect air samples for 1-h periods in a classroom where in-person instruction has been taken place and in restrooms of the NPBI vs. the non-NPBI wing. The average of three samples collected on different days was calculated. Results indicate that the number of bacteria collected in the non-NPBI classroom and men's restroom was 10 times higher than in the NPBI classroom and men's restroom. The number of colonies was small in the women's restrooms and no significant difference was observed. The results indicate that the NPBI system can effectively inactivate bacteria in circulating air. Future studies will determine if viruses are being inactivated as well.

4. Utilization of Human Vitreous Humor for an *In Vitro* Granuloma System to Study *Mycobacterium tuberculosis* Infection

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Granuloma formation is the hallmark of tuberculosis. Generated in an immune microenvironment that responds to *Mycobacterium tuberculosis* (Mtb) infection, it also provides a niche in which mycobacteria can survive. Thus, more detailed studies of Mtb infectivity are essential to understand the complexity and chronic nature of the disease. *In vitro* models for studying granuloma formation have been insufficient in recreating the microenvironment of an infected human host. An extracellular matrix (ECM), thought to play a major role in macrophage activation, establishes the environment necessary to generate an immune response to infection. Current commercially available ECMs are limited to collagen and synthetic matrices, and although these substances facilitate granuloma formation, they do not reflect the complexity of the human tissue.

This study utilized human vitreous humor (hVH) to assess the interaction between human ECM and macrophages

in granuloma formation. THP-1 human monocytic cells were seeded on hVH obtained from human cadavers and then cultured under three different conditions: hVH only, hVH + vitamin D, and hVH + PMA for 3 days. The system was evaluated for granuloma formation with and without the inoculation of Mtb for 10 days.

We observed that Mtb-exposed THP-1 monocytes can form granulomas in hVH as early as day 7. Treatment with Vit D led to a faster granuloma formation (day 6) of a larger area. Granuloma formation in PMA-transformed cells or in Mtb-unexposed cells under any treatment was unremarkable.

Our data suggest that hVH serves as a viable ECM able to activate macrophages upon exposure to Mtb, providing a more physiologically comparable model to study TB pathogenesis.

5. *Helicobacter pylori* Infection Exacerbates DNA Damage and Decreases Tumor Latency in DNA Polymerase Deficient Mice

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Helicobacter pylori is also an important risk factor that induces chronic inflammation and DNA damage to promote gastric carcinoma. Mutation in DNA polymerase beta (POLB) has been reported approximately in 40% tumors and impact on base excision repair (BER) efficiency. Reduced BER capacity associated with mutation in *POLB* gene and increased DNA damage generated by *H. pylori* infection may give rise to the accumulation of DNA damage and accelerate gastric cancer development. We examined whether the contribution of mutation in *POLB* repair genes to GC risk could be modified by *H. pylori* infection. Our study revealed that *H. pylori* infection in *POLB* mutant mice did not alter the stomach colonization. However, *H. pylori* infection exacerbates oxidative DNA damage and proliferation in *POLB* mutant mice. Further, we show that *POLB*-deficient animals display more severe gastric lesions that are precursors of gastric cancer after chronic infection with *H. pylori*. These data demonstrate that the repair of DNA lesions formed by reactive oxygen and nitrogen species (RONS) during *H. pylori* induced chronic inflammation is important for protection against stomach carcinogenesis.

6. Metformin Reduces Survival Rate, Fecundity and Immunity During Bacterial Challenge Through AMPK Activation

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Metformin is a glucose lowering and insulin-sensitizing agent which helps to control blood sugar level in mammals through activation of 5'-AMP-activated protein kinase (AMPK). AMPK is an intracellular serine/threonine kinase and a key energy sensor that is activated under metabolic stress. AMPK

governs a series of biological process to maintain the energy homeostasis in response to metabolic stresses through ATP depletion. AMPK is activated when cellular energy is low, which shuts down anabolic pathway and concurrently turns on the catabolic pathway to produce ATP. This process continues throughout the metformin feeding which creates a nutrient deficient environment and does not provide enough immunity against microbial community. We investigated the role of AMPK in regulating the ATP homeostasis by feeding *Aedes aegypti* with metformin and observing its effect on survival, fecundity and immunity against bacterial challenge. Our result demonstrated that AMPK activation through metformin increased mortality rate in dose dependent manner where 20 mM had the greater mortality than 15 mM, 10 mM and 5 mM, respectively. Similarly, AMPK also significantly reduced the fecundity rate of these mosquitoes in a dose dependent manner ($10\text{ mM} < 5\text{ mM} < 2\text{ mM} < 0\text{ mM}$). Additionally, in response to larger bacterial load, we also saw dose-dependent mortality rate where 10 mM concentration showed higher mortality than 5 mM and 2 mM in comparison to the control (Mantel-Cox, $P < 0.0001$).

7. Identification, Characterization, and Gene Expression Analysis of Thioester-Containing Proteins (TEPs) in the Squid *Euprymna scolopes*, a Molluscan Model

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Thioester-containing proteins (TEPs) are pattern recognition receptors that have been identified in all major metazoan groups. Although these receptors are one of the most ancient immune molecules, the majority of them are not well characterized. Based on amino acid sequence homology and the presence of protein domains, TEPs have been traditionally categorized into different subfamilies: alpha-2-macroglobulin (A2M), complement component C3 (C3), insect TEPs (iTEPs), and macroglobulin complement related proteins (MCR). The majority of the members of this superfamily share several protein domains in common including a highly reactive thioester region (TER) and multiple macroglobulin domains. This study focuses on the identification, characterization, and gene expression of TEP genes in the squid *Euprymna scolopes*, a mollusc that forms a mutualistic association with the bacteria *Vibrio fischeri*. Using *in silico* methods, we identified eight *E. scolopes* TEPs which grouped into the mentioned subfamilies: two A2M-like molecules (Es-A2M-1, Es-A2M-2), two C3-like transcripts (Es-C3-1, Es-C3-2), three iTEP homologs (Es-TEP1-3), and one MCR transcript (Es-MCR1). NCBI protein BLAST search revealed that four of the *E. scolopes* TEPs have a putative TER (Es-C3-1, Es-TEP-1-2, and Es-A2M-2). Gene expression studies were conducted using real-time quantitative PCR (RT-qPCR) in juvenile squid colonized by *V. fischeri* for 24 h and 48 h. Results showed that three TEP genes (Es-C3-1, Es-C3-2, and Es-MCR-1) were significantly down-regulated in colonized juveniles at 48 h compared to uncolonized (con-

trol) juveniles, suggesting that growth and colonization of the symbiont *V. fischeri* are permitted by modulating the squid's host immune system.

8. Expression of the Immune-Related Thioester-Containing Proteins (TEPs) in the *Biomphalaria glabrata* Embryonic (Bge) Cell Line

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The *Biomphalaria glabrata* embryonic (Bge) cell line is the only molluscan cell line available and was established over 40 years ago from embryos of a susceptible snail strain. Since then, Bge cells have been used in numerous studies aiming at a better understanding of host-parasite interactions, especially in the research of snail-schistosome recognition and defense mechanisms. To define the role of thioester-containing proteins (TEPs) in snail defense we have tested the expression of these proteins in Bge cells. TEPs are a diverse family of proteins in which most of them are characterized by the presence of an active thioester domain used to bind target molecules. Traditionally, TEPs are divided into three groups: alpha-2-macroglobulin, complement-like, and insect-TEPs. In preliminary results we confirmed the presence and expression of *B. glabrata* TEPs in Bge cells, including members of all three major groups. Furthermore, we are studying TEPs transcript expression in Bge cells in response to microbial products exposure, including lipopolysaccharide, peptidoglycan, and beta glucan. This study aims to further our understanding the role TEPs have in snail cells and help to identify potential transcription factors (TF) associated with them. Currently, we are testing Rel Rel/DIF (dorsal-related immunity factor), Relish, STAT (signal transducer and activator of transcription (STAT)), and CREB (cAMP response element-binding protein) as potential transcription factors associated with TEPs signaling responses. These studies aim to better characterize the role of TEPs in *B. glabrata* immune sensing and the associated signaling pathways playing a role in snail-pathogen interactions.

9. Role of Rv2275 and Cyp121 in the Pathogenesis of *Mycobacterium tuberculosis*

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In 2019, tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), was still one of the leading causes of death worldwide. In most Mtb strains, the Rv2275 and cyp121 (Rv2276) genes encode for a cyclodityrosine synthetase and a cytochrome P450 enzyme, respectively, and form a functional operon. Rv2275 uses two molecules of Tyr-tRNA^{Tyr} as substrates to form a cyclodityrosine (cYY) intermediate, which is then converted into mycocyclosin by Cyp121 after the C-C bond formation between the phenolic rings. Cyp121 was previously

shown to be essential for Mtb growth under standard laboratory conditions, making this enzyme an attractive target for the development of novel antitubercular drugs. However, it is still unclear whether the “essentiality” of Cyp121 is due to the need for the mycrocyclosin and/or the accumulation of toxic cYY. In this study, we evaluated the importance of the mycrocyclosin biosynthetic pathway for infectivity and pathogenesis of Mtb using two approaches. First, we tried CRISPR interference to knockdown cyp121 expression, but we have failed to effectively repress cyp121 transcription.

Second, we combined a “gain-of-function” approach with site-directed mutagenesis. We successfully transformed *Mycobacterium smegmatis* with plasmids encoding for the operon with wild-type genes or variants with missense mutations in either Rv2275 or cyp121. Growth studies revealed that the expression of a functional Rv2275 is sufficient to block growth, presumably due the accumulation of toxic cYY. The impact of mycrocyclosin on *M. smegmatis* cells remains unclear, as Cyp121 may not be functional without its native redox partner(s). These experiments are being repeated in Mtb strains.

10. Immunomodulatory Effect of *Lepidium meyenii* in the Inflammatory Response of Human Macrophages to BCG

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Lepidium meyenii (L.m.), also known as maca, is an Andean crop used medicinally for multiple purposes. Studies have shown an immunomodulatory anti-inflammatory effect in murine macrophages. Bacillus Calmette-Guerin (BCG) is the vaccine used to prevent forms of tuberculosis other than pulmonary. We aimed to assess the effect of L.m. on the inflammatory response of human macrophages to mycobacteria. Human monocytic THP-1 cells bearing two plasmid reporter systems for NF- κ B and IRF activation, were differentiated into macrophages and then treated with L.m. at concentrations of 1 μ g/mL, 5 μ g/mL and 10 μ g/mL for 48 h. Cells were then infected with *Mycobacterium tuberculosis* (Mtb), *Mycobacterium smegmatis*, and BCG for 24 h.

L.m.-treated cells showed a dose response increase in the activation of NF- κ B. After infection with BCG we observe a reduction of the NF- κ B activation in cells treated with 1 and 5 μ g/mL of L.m. Cells treated with 10 μ g/mL showed a reverse to values similar to untreated cells. No effect was observed in IRF activation. A decrease in the number of internalized BCG was observed in L.m.-treated cells.

Our results indicate that L.m. exerts an immunomodulatory effect on the NF- κ B activation of human macrophages upon mycobacteria challenge. This is in line with a previous report on the anti-inflammatory effect of L.m. on an acute hepatitis murine model. Our findings could potentially translate into a beneficial effect in the exacerbated inflammatory response associated with BCG or even active TB.

11. Identifying Innate Immune Receptors Causing Production of Inflammatory Cytokines by Infection of *Francisella tularensis*

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Francisella tularensis is a pathogenic species and a causative agent of tularemia. This bacterium is incredibly infectious and often lethal in that *F. tularensis* causes the overproduction of pro-inflammatory cytokines. The objective of this study is to identify the innate immune receptors responsible for the production of inflammatory cytokines in response to *F. tularensis* infection. The study uses an immortalized C57BL/6 bone marrow macrophage (BMMac) cell line as the control and knock-out BMMac that are deficient in various innate immune receptors. The cells are passaged and seeded before infecting them with *F. tularensis* for 2 h. The cells are treated with a high dose of gentamicin for 2 h followed by a low dose of gentamicin for an incubation period of 24 h. The supernatants from these cells are then collected and analysis was processed by ELISA, an enzyme-linked immunosorbent assay, to measure production of inflammatory cytokines. These data were compared with the positive control of wild type BMMac and the negative control of uninfected BMMac cells. Analysis of these mutants reveals the involvement of innate immune receptors in triggering the pro-inflammatory cytokine storm in response to *F. tularensis* infection that leads to death of the host.

12. COVID-19 Associated Pulmonary Aspergillosis: A Pandemic Superinfection

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Viral pneumonias increase the risk of secondary bacterial or fungal superinfections, including invasive pulmonary aspergillosis (IPA). COVID-19 associated pulmonary aspergillosis (CAPA) may represent an additional cause of morbidity and mortality in COVID-19 infected patients.

A 78-year-old woman with a history of chronic obstructive pulmonary disease and hypertension presented with hematemesis. She was diagnosed with COVID-19 two weeks prior and reported a 40-pound weight loss. Due to respiratory distress and to protect her airway, she was intubated and started on treatment for COVID-19 and gastrointestinal bleed with dexamethasone, remdesivir, and pantoprazole, respectively. She was extubated a few days later and subsequently developed hemoptysis with preliminary respiratory cultures growing *Aspergillus* species. CT scan of her thorax illustrated multiple cavitary nodules. A serum galactomannan PCR test was negative, but (1-3)- β -D-glucan assay was significantly elevated with a level greater than 500 pg/mL (positive when greater than 80 pg/mL). She was started on voriconazole, while further identification of *Aspergillus* species was pending. She was discharged with a plan to check a voriconazole level after 10 days, in addition to repeating a CT scan of the thorax after 4

weeks. *Aspergillus fumigatus* was later identified.

This case demonstrates an example of probable superimposed IPA infection associated with COVID-19. Patients can present with variable symptoms with risk factors, including corticosteroid therapy, previous lung damage, lymphopenia, mechanical ventilation, and broad-spectrum antibiotics. Although (1-3)- β -D-glucan is not specific for IPA, a study unrelated to COVID-19 demonstrated that two consecutive serum (1-3)- β -D-glucan tests can generate a specificity of 90% for IPA. Recent literature has defined CAPA into three categories: proven, probable, and possible infection. Proven infection relies on histopathology and/or direct microscopic detection of fungal characteristics consistent with *Aspergillus* species, while probable infection can be diagnosed by microbiology and/or imaging. These factors are crucial to diagnose and treat CAPA, which may increase overall mortality in COVID-19 patients.

13. Who Will Be Dead After We Save the Bats? Implications of UV-C Treatment on Cave Microbial Communities

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White-nose syndrome (WNS), a fungal disease caused by *Pseudogymnoascus destructans*, is devastating bat populations in the United States. Researchers searching for a treatment for WNS have found that UV-C treatment is effective in killing *P. destructans* on bats and on cave surfaces; however, previous research has shown that cave microorganisms can be inhibited or killed by UV-C. Cave microorganisms play crucial roles in cave ecosystems through nutrient cycling. Furthermore, the bat microbiome could provide natural defenses, supplying secondary metabolites that fend off invading pathogens. We investigated whether UV-C treatment would have potential collateral damage to native cave bacterial species. Culture samples were inoculated on-site from caves in Oregon Caves National Monument and sub-cultured in the lab. From 1,237 subcultures, the 16S rRNA gene was sequenced and 100 unique bacterial cultures were identified. Our initial testing of the UV-C effects on these cultures failed to show a strong killing effect of the UV-C. Research revealed that this could be due to the shadowing or shielding effect of the bacterial cells in streak inoculations. Serial dilutions were done to evaluate if the concentration of cells impacts the effectiveness of UV-C, resulting in our changing the inoculation method to serial dilutions instead of streaking. We subjected six slow- and six fast-growers from the original 100 cultures to UV-C radiation at various doses. Our results show that sensitivity to UV-C varies across isolates, revealing that slow growers appear to be more sensitive compared to fast-growers. Notably, our results highlight that even at a higher dose of UV-C, individual cells

are able to shield and protect cells underneath. Addressing the shielding effect is important as it could have implications for the proposed UV-C treatment in caves. Additionally, the results of this study illuminate the potential consequences of UV-C on native microbial cave ecosystems.

14. Warburg Effect in Anopheles Mosquito Anti-Bacterial Immunity

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Otto Warburg discovered cancer cells favor increased glycolysis with pyruvate being converted to lactate rather than acetyl-CoA for the tricarboxylic acid cycle, and this is termed the Warburg effect. Warburg metabolism has been found to occur in mammalian rapidly proliferating cancer and immune cells. *Anopheles* species mosquitoes, the vectors for malaria, rely on their metabolic system to provide energy and intermediates for their innate immune system, so called immunometabolism. We posited Warburg metabolism is involved in *Anopheles* sp. mosquito's immune response. We designed an anti-bacterial immunity model using intrathoracic inoculations of *Escherichia coli* K12, avirulent bacterium, and *Enterobacter* sp. Ag1, virulent bacterium isolated from the *Anopheles* sp. midgut, to test the immunometabolism response. We found a statistically significant doubling in lactic acid upon Ent sp. immune challenge as compared to injection injury controls using a lactate assay (*t*-test, *P* = 0.0016). This provides evidence to support increased Warburg metabolism in immune challenged mosquitoes. We then fed *Anopheles* sp. mosquitoes dimethyl fumarate (DMF), a GAPDH inhibitor, and challenged the mosquitoes with *E. coli*. We found a significant mortality was observed in DMF treated mosquitoes compared to control (Mantel-Cox, *P* < 0.001). By inhibiting the utilization of glycolysis and Warburg metabolism, decreased survival related to immune challenge is observed, showing Warburg metabolism is crucial for mosquito immunity. We believe current cancer therapeutics targeting Warburg metabolism can be an effective vector control strategy to prevent mosquito-borne diseases.

15. Development of Procedures for Detection and Isolation of *E. coli* O157:H7 in Sprouts and Sprout Irrigation Water

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E. coli O157:H7 has been implicated in foodborne disease outbreaks with sprouted seeds. Spent irrigation water has been used in compliance programs for detecting *E. coli* O157:H7 in sprouts. However, detection and isolation of the pathogen by standard cultural methods can be difficult due to the high background microflora associated with these matrices. We optimized procedures for isolation of *E. coli* O157:H7 from artificially contaminated sprouts and from sprout irrigation water.

Sprouts or spent sprout irrigation water were inoculated

with *E. coli* O157:H7 and enriched in: (1) modified buffered peptone water + pyruvate (mBPWp) for 5 h at 37 °C, followed by addition of acriflavine (A), cefsulodin (C), vancomycin (V) and further incubated at 42 °C, (2) mBPWp with CV held at 42 °C with shaking and (3) mBPWp with CV held at 42 °C without shaking. Enriched samples were streaked, diluted and plated onto selective agars or treated with Dynabeads MAX *E. coli* O157 for immunomagnetic separation (IMS) then streaked for cultural recovery. Acid treated samples (broth and IMS) were also culturally detected. Real-time PCR detection of *E. coli* O157:H7 was done by screening for *stx1*, *stx2* and *wzy* gene targets. Samples collected for shotgun metagenomic analysis were sequenced using Illumina technology and a k-mer based method was used for bacterial taxa identification.

Isolation of *E. coli* O157:H7 was difficult from the enrichments due to excessive growth of competing organisms on the selective plates. Acid treatment of the enrichment broths and IMS beads improved recovery of the inoculated pathogen. The relative abundance of *E. coli* O157:H7 was found to be between 15% and 30 % for test conditions (2) and (3) but less than 3% for test condition (1).

Optimized enrichment procedures for effective isolation of *E. coli* O157:H7 were developed for sprouts and sprout irrigation water. The examination of bacterial communities during the enrichment provided an additional tool improving current cultural methods for pathogen detection in difficult matrices.

16. Detection of Antibiotic Resistance and Characterization of Bacterial Communities in the Socorro, NM, Wastewater Treatment Plant and Surrounding Environment

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The β -lactam antibiotics are some of the most prescribed antibiotics in the US. These drugs can be degraded by bacterial enzymes called β -lactamases. Extended-spectrum β -lactamases (ESBLs) are able to degrade many types of β -lactam antibiotics, including carbapenems, known as drugs of last resort. The bacteria which produce ESBLs and the genes that encode ESBLs are thought to get into the environment and circulate back into the clinic through wastewater treatment plants (WWTPs). For this project, the presence of seven ESBL genes (*bla*NDM, *bla*IMP, *bla*VIM, *bla*OXA-48, *bla*KPC, *bla*SPM, and *bla*GES), five of which are actively tracked in the clinic, and β -lactam-resistant bacteria were evaluated at three sampling sites within the Socorro, NM, WWTP (raw sewage, aerobic digester, and treated wastewater) and two environmental sites upstream and downstream from the WWTP. Next, the isolation of bacteria resistant to Meropenem (a carbapenem-class antibiotic) was attempted for each sample. Finally, the taxonomic composition of the bacterial community for each sample was determined using 16S rDNA sequencing. Of the seven genes, five were detected: *bla*IMP, *bla*VIM, *bla*OXA-48, *bla*KPC, and *bla*GES (four of which were the clinically-tracked genes) in various samples, but all of these genes were detected in the treated

wastewater. Meropenem-resistant bacteria were isolated from both raw sewage and treated wastewater; however, none of the target genes in this study were detected in the isolates. The taxonomic study showed significant changes in the bacterial community composition between the raw sewage and treated wastewater, with a shift toward bacteria that are more likely to produce ESBLs.

17. Exploring Surface Water as the Reservoir of CRE Infecting Patients in SE New Mexico

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Antibiotic-resistant infections are a worldwide threat and have risen because of the complex community networks humans have formed throughout the years. This interconnection between humans and their environment, such as prolonged hospital stays, has caused treating antibiotic-resistant infections increasingly difficult, even ones that were formerly treatable. Once antibiotic resistance is established in a pathogen population, human activities can lead to the transfer of antibiotic resistance genes (ARGs) throughout the environment, including reservoirs such as surface waters. Recently, four cases of carbapenem-resistant *Enterobacteriaceae* (CRE), an emerging antibiotic resistant infection, have been found in three adjacent counties of SE New Mexico. The New Mexico Department of Health could find no identifiable health-care source; the only commonality was the Pecos River that traverses these counties. We sampled river water at five different locations in the affected counties, and screened the bacterial population for the presence of CRE-related genes, particularly the VIM gene common to the four patients. We found the presence of four carbapenem-resistance genes within four out of the five different bacterial communities. VIM was one of the genes present and was only detected in sample site four. Others, such as OXA-48, NDM, and GES were also present within the community. Sample site two did not harbor any of the carbapenem-resistance genes. These findings could help unveil the role the environment plays as a reservoir for antibiotic resistance and give insight on the evolution of emerging pathogens in surface water to predict and control potential outbreaks.

18. Identification of Carbapenem Resistant Enterobacteria in Water From the Rio Grande River

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Carbapenem-resistant *Enterobacteria* (CRE) are considered one of the top urgent antibiotic resistant (AR) health threats (CDC, 2019). CRE include bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) that no longer respond to carbapenems, a last antibiotic resource for AR infections. Common carbapenemases

produced by CRE include KPC, NDM and VIM. Because CRE is a bacterial infection mostly diagnosed at hospitals, they should not be part of other environments. However, as urbanization grows along with antibiotic overuse, sewage systems from hospital settings seem to contribute to the spreading of AR genes to the ecosystem. In this study, we aimed to identify the presence of KPC genes of CRE in water samples collected from the Rio Grande River to understand if genes of top AR bacteria have reached our natural water resources.

A total of 15 water samples were randomly collected along a 26 km segment of the Rio Grande River. After filtration and bacterial growth, isolated colonies were analyzed using the MicroScan AutoSCAN 4. NBPC 34 Microscan Panels were used to identify Gram Negative (GN) isolates and for determination of their corresponding antimicrobial susceptibility patterns. Selected GN isolates were screened with CHROMagar™ KPC and then confirmed the presence of KPC genes by PCR.

Out of a total of 28 GN isolates that were analyzed, 11 (39.3%) were identified as positive for CRE/KPC. The types of GN isolates with positive identification of CRE include: *Citrobacter freundii* complex 1 (9.1%), *Klebsiella oxytoca* 2 (18.2%), and *Klebsiella pneumoniae* 8 (72.7%). After DNA isolation, all 11 (100%) positive GN isolates were confirmed positive by PCR.

The identification of CRE in natural water resources represents a major concern for health professionals. Further research and strict surveillance programs that monitor the presence and spreading of these AR bacteria in our ecosystem are needed as water from the Rio Grande River is used as potable water or recreational purposes for the community.

19. Characterization and Host Determination of Four Novel Phages for Potential Use in Phage Therapy

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The overuse and misuse of antibiotics worldwide have resulted in a decrease in efficacy of drugs used to counter bacterial infections. Once easily treated infections now pose a greater risk to human health as the bacteria responsible become increasingly resistant to treatment with antibiotics. To curb the threat of antibiotic resistant pathogens, new drug therapies along with an expansion in public attention are increasingly necessary. As of 2019, the bacterium *Pseudomonas aeruginosa* has been classified by the CDC as a serious threat in the Antibiotic Resistance Threats Report. Multidrug resistant *P. aeruginosa* can often cause nosocomial infections and is easily spread to those with cystic fibrosis, severe burns, or surgery wounds. *P. aeruginosa* produces biofilms that provide the bacteria within extensive protection and defense from environmental challenges via properties of the extracellular matrix, such as stress-resistance and pathogenesis. Bacteriophages, viruses that specifically target bacteria, have been shown to penetrate and disrupt biofilms. The potential that phages have demonstrated against bacterial biofilms suggests that they may be an ideal mechanism for their eradication.

The combination of phages, also called a phage cocktail, may be an effective tool against multidrug resistant pathogens in a strategy known as phage therapy. However, phages must be characterized before therapeutic application so that phages used are appropriate and specific to an individual bacterial infection. Here we characterize and determine the host range of four novel phages for their potential inclusion in phage banks associated with various phage therapies. Four novel lytic phages were isolated from sewage and their host ranges were tested using a CDC panel of antibiotic-resistant *P. aeruginosa* strains. About 58% of the AR panel strains tested were susceptible to infection from two of the novel sewage phages.

20. Presence of *Wolbachia* Within the Order Hymenoptera From the El Paso, TX Region

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The order Hymenoptera is highly diverse and members play a variety of ecological roles in the environments they are present in. Members of this order have been found to be hosts for the bacterium *Wolbachia*. As a widespread endosymbiont, *Wolbachia* has proven to affect its hosts in a variety of ways that may reveal insight into applications such as vector control. In certain hosts, *Wolbachia* has the ability to affect immune systems and reproductive viability. It is here that interest in the order arose. Members in the order Hymenoptera display a reproductive strategy known as haplodiploidy, which in some insects such as those in the order Coleoptera has been associated with *Wolbachia* presence. The nature of the transmission of *Wolbachia* in the order is not clearly understood, nor its prevalence in the American Southwest. As such, we aimed to survey the order by focusing on collecting and analyzing ants by PCR using a Sigma-K kit followed by gel electrophoresis. Ants were collected from eight different sites in El Paso, TX, using pantraps left out for a 24-h period, with five ants per site being examined. Collection sites were chosen due to accessibility, avoidance of nest overlap and prior scouting for hives at said sites. The ants, identified to genus, included *Solenopsis*, *Prenolepis*, *Monomorium*, and *Pheidole*. Thus far, samples from sites 6 and 7, which include East and Northeast El Paso showed the 438 bp rRNA band characteristic of the presence of *Wolbachia*. Moving forward, we aim to survey the region and see if horizontal transmission between ants and related kin, particularly bees, is occurring.

21. A Comparative Study of Techniques for Differential Expression Analysis on Transcriptome of *E. coli* Responses to Below Background Radiation and Natural Background Radiation

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Recent advances in next-generation sequencing technology have brought a wide variety of bioinformatic programs and software tools for analysis of RNA-sequencing data. However, there is no universal pipeline tool that can be used for all cases of RNA-seq. In this comparative study we evaluate the performance of four different software tools: DESeq2 (DNASTAR), EdgeR (DNASTAR), CLC genomic and Partek Flow for identification of differentially expressed genes using RNA-Seq of *E. coli*. The RNA-seq used in this study are from the study of biological effects of below-background radiation on *E. coli* grown at below background radiation underground at Waste Isolation Pilot Plant (WIPP) in Southern NM. For the control experiments, KCl, Pozzolan and Tuff were used as natural/normal background radiation. The results indicated different software tools gave different detection of differential expression when low radiation (minus) was compared to other treatments. All four software analyses suggested *E. coli* highest response was to the KCl treatment. A total number of 89 genes from EdgeR, 94 genes from DESeq2, 69 genes from Partek flow, and 114 genes from CLC were differentially expressed in minus versus KCl treatments. Among them, 55 genes were commonly detected across all four software analyses. When considering the fold-change and coverage among all tested tools, here we propose DESeq2 (DNASTAR) as an appropriate software tool for differential gene expression study of our current RNA-seq data.

22. *Deinococcus radiodurans* RecO and RecR Proteins Stimulate RecA Nucleoprotein Filament Formation on SSB Bound DNA

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Deinococcus radiodurans is an extremely radiation resistant organism, with the ability to survive hundreds of DNA double-strand breaks. The capacity to withstand such a detrimental type of DNA break stems from the efficiency of the DNA repair machinery of this organism. *D. radiodurans* recombination mediator proteins RecO and RecR have been shown to be required for radiation resistance in this organism. In *E. coli*, the RecOR proteins have been reported to stimulate RecA filament nucleation onto SSB coated single-stranded DNA (ssDNA). However, how *D. radiodurans* RecO and RecR work to mediate RecA filament formation is still not well understood. In this study, we further elucidate the biochemical mechanism of RecOR function; we observe that *D. radiodurans* RecO and RecR work together to assist RecA nucleation onto SSB coated ssDNA. We also find that RecO and RecR alter the kinetics of the RecA protein rate of ATP hydrolysis when bound to ssDNA, an effect not previously described for other homologs. Potential mechanisms for this kinetic stimulation will be discussed.