CodY upregulates SaeR/S two-component system activity by increasing branched-chain fatty acid Synthesis

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Staphylococcus aureus is an opportunistic pathogen that can impact both human and animal health. It is recognized for its role in skin and soft tissue infections, endocarditis, and bacteremia, emphasizing the interconnectedness of health across species. The success of S. aureus as a pathogen is attributed to its repertoire of virulence factors that enhance host colonization and facilitate evasion from the host immune response. The production of these factors is controlled, in part, by a complex network of regulatory proteins such as CodY, which monitors nutrient availability. When *S. aureus* is deprived of certain amino acids, CodY becomes inactive and activates the SaeR/S Two-Component System (TCS). This in turn upregulates toxins and other virulence factors. Herein, we show that reducing CodY activity in *S. aureus* cells does not alter SaeR or SaeS expression levels. Rather, SaeS kinase activity is enhanced in CodY-deficient cell membranes. We discovered that CodY- deficient cell membranes have increased levels of branched-chain fatty acids (BCFAs) compared to wild-type cell membranes. Blocking BCFAs synthesis substantially reduced SaeR/S activity, which was complemented chemically and genetically. Single-gene knockouts and epistasis experiments revealed that CodY constrains Sae activity by repressing genes for *de novo* branched-chain a-keto acid synthesis and by repressing genes that encode permeases for branched-chain amino acid import. These results reveal a novel method of post-transcriptional virulence regulation via BCFA synthesis, linking CodY activity to virulence regulation in *S. aureus*.

A one-year genomic investigation of Pseudomonas aeruginosa epidemiology and nosocomial spread at a large U.S. hospital.

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Pseudomonas aeruginosa is a versatile, opportunistic human pathogen causing a wide range of infections in the clinical setting in addition to proliferating in the hospital environment. A thorough understanding of circulating lineages both from patients and environmental reservoirs is essential to rapidly detect outbreaks and promote mitigation efforts. Here, we investigated the population structure, prevalence of resistance, and transmission of *P. aeruginosa* at a single U.S. hospital. A complete set of 438 clinical isolates, collected between January to December 2022, and 65 environmental *P. aeruginosa* (cultured from 56 swabs across 5 floors, 7 wards, and 44 rooms) were analyzed. WGS and *in silico* core-genome MLST identified a heterogenous population with **182** STs represented. High-risk pandemic lineages ST-235 (n=16), ST-253 (n=14), ST-244 (n=10) were observed, however a single lineage, ST-621 (n=21) was dominant due to an ongoing outbreak. Environmental sampling confirmed ST-621 was endemic in the hospital, with isolates recovered from multiple wards and floors (from drains/sinks/supply-carts) sharing a high level of genetic relatedness to patient isolates (<10 SNPs). Notably, within the clinical isolates, 7 transmission clusters associated with 7 lineages were detected (<15 SNPs), grouping isolates from 40 patients. Overall, 22% of the whole population were non-susceptible to fluoroquinolones, 16% to carbapenems (despite <1% carbapenemase carriage), and two isolates carried an extended-spectrum β-lactamase gene ($bla_{\text{CTX-M-15}}$ and $bla_{\text{GES-1}}$, respectively). Notably, the ST-621 transmission clusters were enriched with MDR isolates. This study highlights infection control challenges associated with *P. aeruginosa* widespread environmental reservoirs likely sustaining the spread of protracted MDR outbreak clones.

Identification of bacterial copper systems used in marine copper surface colonization

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Copper can be potentially toxic for bacterial cells when it is present in excess amounts due to its redox potential. Copper-based antimicrobial paints are frequently used to inhibit the biofouling of marine vessels. However, some bacterial species can overcome this copper challenge and colonize the surfaces. The early adherent bacterial population plays an important role because of its ability to produce extracellular polymeric substances (EPSs), forming a thin layer of organic matter that traps nutrients from the water and protects other colonizers by blocking the toxic antifouling (AF) coatings. Thus, it is of interest to study the factors that drive the initial colonization of copper surfaces. Bacteria harbor diverse regulatory mechanisms that respond to intracellular and extracellular copper and maintain copper homeostasis in cells. Our comparative genomics study of the copper-regulatory signal transduction system on marine bacteria examined the influence of the environment on the presence, abundance, and diversity of copper-associated signal transduction systems across species isolated from marine sources like biofilm and seawater. Our study demonstrated that the distribution of the copper regulatory components in marine species is mainly influenced by phylogeny; however, the abundance of certain copper regulatory components is also influenced by the environment in which these bacteria were initially isolated. For instance, bacteria isolated from the biofilm and sediments displayed more homologs of the copper-associated system than bacteria found in the seawater. Among the diverse copper regulatory systems found across marine bacteria, we aim to identify copper regulatory pathways found in the copper surface colonizing bacterial communities by metagenomic study. The functional features of copper regulation shared by these colonizers can act as an indicator of marine biofouling of copper surfaces.

Understanding the Effect of Cold Atmospheric Plasma on Interactions Between Human Pathogens and Fresh Produce

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Cold atmospheric plasma (CAP) is a surface modification technology that produces oxidative species that inactivate microorganisms. CAP has been shown to be an effective sanitization technology for use on a variety of food products, leafy greens are a uniquely promising application. CAP products, such as reactive oxygen and nitrogen species (RONS), UV light, and ozone, are environmental stressors with antimicrobial effects that plants already encounter in their natural environment, and still respirating fresh produce will produce a stress response when treated by CAP. This response may be beneficial to both the nutritional quality and safety in spinach. Baby spinach that had undergone a 60 second CAP treatment did not need to be directly exposed to the electrode to experience the 3 log CFU/g reduction in *E. coli* as a directly exposed leaf. Baby spinach leaves also significantly ($p<0.05$) increased their flavonoid content by 1.20 ± 0.98 mg/g after a 60 second, 120-watt CAP treatment. The flavonoid content of kale treated by the same device significantly decreased by 1.35 ± 0.13 mg/g after a 60 second, 120-watt CAP treatment. Kale not directly exposed to CAP source also experienced a loss of flavonoids (1.10 ± 0.36 mg/g) suggesting that CAP treatment parameters should change for different leafy greens species and varietals. Experiments are underway to evaluate the effect of CAP treatment on the stress response and microflora of sweet basil partially exposed to CAP to understand the systemic plant response and its implications for the safety of leafy greens and fresh herbs.

Using Sand and Zero-valent Iron Filters to Control Foodborne Parasites in Irrigation Water

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Numerous multistate foodborne outbreaks of cyclosporiasis linked to fresh produce underscore the need to reduce parasite contamination of fruits and vegetables. Sand filtration, a common method to improve irrigation water quality, may effectively reduce parasite introduction to produce. This study evaluated the efficacy of sand and zero-valent iron (ZVI) filters to reduce levels of *Eimeria tenella*, a surrogate for *Cyclospora cayetanensis*, in irrigation water intended for fruits and vegetables. *E. tenella* oocysts (~500,000) in 100 mL of sterile deionized water were filtered through 100% sand and 50% ZVI/sand filters at a rate of 1.0 L/min. The inoculum was followed by a 1 L flush, and then backflushed with 1 L of sterile deionized water. *E. tenella* oocysts were enumerated from filtered effluent fractions (100 mL pre-elution; 5 x 200 mL effluent samples) and from backflush (1 L). Samples were concentrated by gravity settling and oocysts were counted on McMaster counting chambers. Filtration trials with both filter types (100% sand and 50% ZVI/sand) were performed in triplicate (n = 3). ZVI/sand filters reduced *E. tenella* in effluents by 99.9%, whereas sand filters reduced *E. tenella* by 55%. Most of the oocysts that transited the filters were recovered in the first 400 mL of filtration effluent. After backflushing, 4% and 9% of the oocysts in the inoculum were recovered for the 100% sand and 50% ZVI/sand filters, respectively. These findings demonstrate that ZVI/sand filtration of irrigation water may be an effective pre-harvest intervention to help mitigate the risks of foodborne parasites.

Evaluation Of Ultraviolet B Treatment For Improved Quality And Safety Of Coconut Water

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UV-C light is potent antimicrobial; however, it triggers photo-oxidation of compounds like fructose, that generates reactive oxygen species (ROS), thereby, deteriorates the quality of fructose-rich products especially fruit juices. In this context, whether UV-B can produce similar antimicrobial effect or provoke similar ROS generation in the presence of fructose is not studied. Therefore, in the present study we compared UV-B (312 nm) and UV-C (254) light for their antimicrobial effect, photo-reactivation potential and ability to generate reactive oxygen species (ROS) and consequent ascorbic acid degradation in fructose enriched coconut water. UV-B caused 4.5±0.19 log inactivation in *E. coli* K12 at 0.45 J/cm^2 and inactivation kinetics followed Weibull model, compared to log-linear 4±0.05 log reduction at 0.2 J/cm^2 dose of UV-C. A lower extent of UV-A (360 nm) induced photo-reactivation was observed in UV-B treated samples compared to UV-C. UV-B mediated microbial inactivation was largely caused by DNA damage and impaired metabolic activity of *E. coli*. UV-B treatment did not cause membrane damage, or thiol oxidation. Compared to UV-C, UV-B produced lower concentration of ROS from fructose. At a dose required for 4 log reduction of *E. coli* K12 by UV-B, only 8% ascorbic acid degraded. These results show that UV-B is an attractive alternative to UV-C treatment.

Discovery of Medically Important Lytic Bacteriophages from the Environment

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Antibiotic resistance is increasing and we may soon have no antibiotics to treat bacterial infections. Annually, three million antibiotic-resistant infections occur in the United States and 40,000 people die of these infections. Lytic bacteriophages are viruses that kill bacteria. Bacteriophages are host specific. Finding bacteriophages that can kill antibiotic-resistant bacteria is very important because these phages may be an important alternative when faced with dwindling antibiotic options. Environmental samples (hospital wastewater, ocean water, pond water, and ocean sand) were analyzed for lytic bacteriophages against several human pathogens. Numerous lytic *Pseudomonas aeruginosa* bacteriophages were discovered in hospital wastewater and several were isolated from ocean water. *Pseudomonas aeruginosa* is a serious threat to humans and animals due to its virulence and antibiotic resistance. Additionally, I characterized the spectrophotometric activity of two known bacteriophages, T2 and T4 against *E. coli* to design a more rapid bacteriophage screening program. These phages displayed a time and concentration-dependent decrease in optical density (OD) absorbance indicative of a signal of bacteriophage activity against susceptible bacterial host. In such, I hope to next test this methodology with unknown environmental samples for possible evidence of bacteriophages. The ultimate goal of my research remains to shorten the time from the discovery of bacteriophages in the environment to the treatment of patients with antibiotic-resistant infections.

The Effect of Mucus-like Viscoelastic Stress on Giardia Growth

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Microorganisms' ability to survive in mucus, a viscoelastic, non-Newtonian fluid, often holds the key to infection. *Giardia lamblia* is a flagellated mucus-interacting intestinal protozoan parasite, yet most *in vitro Giardia* cultures and experiments are conducted in low viscosity, Newtonian culture media or buffered salt solutions. Thus, there is insufficient understanding of how mucus's mechanical and chemical components act to affect *Giardia* behaviors. Here, we investigate how *Giardia* behavior changes in viscoelastic environments created by adding biocompatible polymers to culture media. The polymers offer a physiological-relevant viscoelasticity, tunable depending on concentration. We performed experiments in a novel micro-culture chamber that permits systematic imaging of culture surfaces. We found no significant change in cell doubling time between the Newtonian and the viscoelastic media. Furthermore, a significant fraction of cells was attached to the ceiling of the culture chambers in viscoelastic media instead of the predominant floor attachments found in Newtonian media. Finally, *Giardia* grew in aggregates in viscoelastic media compared to dispersed growth in Newtonian media. In addition, preliminary transcriptome analyses revealed a small number of genes with significantly altered expression levels in viscoelastic cultures, with surface proteins as the most prominent gene category. The absence of a growth rate effect, coupled with the aggregate formation, suggests a nuanced effort to conserve energy. Our studies are the first effort in the field to investigate how viscoelastic materials alter *Giardia* growth patterns and transcriptome. Our results provide insights into cell responses to mucus-associated mechanical stresses.

Genetic and Enzymatic Characterization of Amy13E from Cellvibrio japonicus Reclassifies it as a Cyclodextrinase also Capable of α-diglucoside Degradation

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Cyclodextrinases are Carbohydrate Active enZymes (CAZymes) involved in the linearization of circular amylose oligosaccharides, and primarily thought to function as part of starch metabolism. Previous reports describe bacterial cyclodextrinases that also possess additional enzymatic activities on linear malto- oligosaccharides. Additionally, environmentally rare α-diglucosides, kojibiose (α-1,2), nigerose (α-1,3), and isomaltose (α-1,6), like cyclodextrins, are becoming prevalent in the foods, supplements, and medicines humans consume. These carbohydrates subsequently feed the human gut microbiome, making it increasingly important to understand the bacterial metabolism of these sugars. Previous genome sequencing of three *Cellvibrio japonicus* strains adapted to utilize these α-diglucosides identified multiple, but uncharacterized, mutations in each strain. One of the mutations identified was in the *amy13E* gene, which was annotated to encode a neopullulanase. In this report, we functionally characterized this gene and determined it in fact encodes a cyclodextrinase with additional activities on α-diglucosides. Deletion analysis of *amy13E* found that this gene was essential for kojibiose and isomaltose metabolism in *C. japonicus*. Interestingly, an Δ*amy13E* mutant was not deficient for cyclodextrin or pullulan utilization in *C. japonicus*, however, heterologous expression of the gene in *E. coli* was sufficient for cyclodextrin-dependent growth. Biochemical analyses found that *Cj*Amy13E cleaved multiple substrates, with a preference for cyclodextrins and maltose, but did not cleave pullulan. Our characterization of the *Cj*Amy13E cyclodextrinase is useful for refining functional enzyme predictions in related bacteria, and for engineering enzymes for biotechnology or biomedical applications.

Comparison of Cross Protection Against Salmonella Granted by Commercial Whole Cell Bacterin and SRP Vaccines in Chickens

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Control of *Salmonella* infections is one of the top priorities for poultry producers due to the potential of the bacteria to infect humans and cause severe disease. *Salmonella* is typically controlled in poultry through biosecurity and vaccination. *Salmonella*e exist as multiple serotypes, which have been identified based on somatic O-antigens, and generally these antigens do not confer cross protective immunity when used in inactivated whole cell bacterin vaccines. Siderophore receptors and Porins (SRPs) are highly conserved pore proteins on the surface of gram-negative bacteria, including *Salmonella*, that transport essential nutrients and iron, which are necessary for bacterial growth. Antibodies produced against *Salmonella* SRP proteins have been shown to be cross-protective and not serotype-specific. As such, the comparison of commercially licensed vaccines utilizing whole cell bacteria and vaccines using SRP proteins from *Salmonella Enteritidis* as antigens were compared for protection against homologous and heterologous *Salmonella* challenge in chickens. The presence and abundance of *Salmonella* in internal organs and feces were evaluated following simultaneous intraperitoneal and oral challenges. Results indicate that SRP vaccines do offer heterologous protection against different serotypes of *Salmonella* and could provide broad protection of commercial poultry against *Salmonella* challenge.

Global Surveillance of Hypervirulent and Resistant-Virulent Klebsiella pneumoniae Lineages

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Klebsiella pneumoniae (*K. pneumoniae*) is a leading cause of hospital acquired multidrug resistant infections. In contrast, the community acquired hypervirulent *K. pneumoniae* (hvKp) pathotype are generally susceptible to most antibiotics. Genomic studies have identified determinants of hypervirulence, often located on large virulence plasmids, that correlate with the hvKp phenotypes. Recently, the emergence of resistant and virulence-carrying *K. pneumoniae* lineages were detected, generally due to resistant strains acquiring virulence plasmids. Alarmingly, large hybrid plasmids that co-harbor resistance and virulence genes have now been reported in isolates from multiple countries. Here, we conduct a retrospective surveillance study of hvKp and resistant-virulent lineages circulating globally. Illumina sequencing was performed on a global collection of 1,472 *K. pneumoniae* collected between 2011-2021 in collaboration with the US DoD's GEIS branch. During the study period, 222 *K. pneumoniae* isolates (15%) were identified as carrying at least 1 acquired virulence biomarker (*iuc, iro*, *peg-344*, *rmpA*, and/or *rmpA2*). From this subset, 200 were resistant-virulent, co-carrying a ESBL and/or carbapenemase gene in addition to ≥1 virulence biomarker. Using long read sequencing, we generated closed reference sequences for 51 isolates that were selected based on genetic diversity including acquired virulence genes, AMR genes, and sequence type. Closed plasmid sequences identified the genetic context of virulence and resistance genes in addition to novel hybrid plasmids, allowing for the characterization of >10 distinct types of hvKp and/or hybrid plasmids. This project identifies emerging hypervirulent and resistant-virulent lineages of *K. pneumoniae* and highlights the diversity of plasmids harbored by these lineages.

Understanding the Microbial and Environmental Drivers of Benthic Marine Invertebrate Settlement

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The long-term survival of coral reef ecosystems depends on the successful settlement and recruitment of benthic marine invertebrates, a process mediated by interactions with microbial biofilms. In turn, the composition and structure of these biofilms is determined by environmental parameters and ecological processes like succession. We are using amplicon and shotgun metagenomic sequencing and fluorescence *in situ* hybridization paired with microscopy to characterize the composition, functional potential, and spatial structure of benthic microbial communities, respectively, from monthly timepoints throughout the coral spawning season in Roatán, Honduras across spatial scales ranging <1m to 1km. These characterizations will be correlated with environmental conditions, including temperature and nutrient concentrations, and patterns of invertebrate settlement to identify important parameters driving these environment-microbe-invertebrate interactions. Initial microbial community analyses reveal strong patterns of succession, with communities becoming more complex over time, and community structuring based on microhabitat conditions associated with the top and bottom surfaces of settlement tiles. The tops of tiles experienced high light conditions and were more exposed, and they contained a higher percent cover of fleshy macroalgae, while the bottoms of tiles had higher cover of microbial biofilms and all observed benthic invertebrate settlers. Correlational analysis is ongoing and will be used to holistically describe the suite of biotic and abiotic interactions that drive settlement of benthic marine invertebrates. These results will help to explain where and why larvae choose to settle and how the process of settlement will change as environmental conditions continue to shift in the face of ongoing environmental change.

Fine-scale spatiotemporal variations in bacterial community diversity in agricultural pond water

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Freshwater microbial communities associate with ecological processes underpinning overall water quality. However, dynamic relationships between specific taxa and the spectrum of water properties remain poorly understood, especially in understudied systems such as ponds. Here, we aimed to determine the impacts of daily sampling time (9:00, 12:00, 15:00) and sampling depth (0, 1, and 2m) on the microbial diversity and co-occurring physicochemical water quality measurements in a model irrigation pond. 16S *rRNA* gene sequencing analysis demonstrated that microbial diversity increased throughout the day with depth-dependency. Although relative abundances of dominant taxa (e.g., *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*) appeared to be relatively stable throughout the water column, fluctuations in less abundant members and, at the finer taxonomic scale, many genera underscored the observed microbial diversity transitions across space and time. Most water quality properties, notably pH, DO, and temperature, significantly yet weakly associated with water microbiome profiles. Additionally, chlorophyll, phycocyanin, conductivity, and colored dissolved organic matter exhibited relationships with α-diversity and a variety of key bacterial taxa. Further investigation of sediment microbiomes uncovered important associations with sampling location, i.e., distinctions at the bank vs. interior sites of the pond, which has implications for variations in the water column based on internal mixing and resuspension. Overall, the results of this work emphasize the importance of accounting for time of day and water sampling depth when surveying the microbiomes of irrigation ponds and potentially other small freshwater sources.

Role of Aggregative Adherence Fimbriae from Enteroaggregative Escherichia coli Isolates in Biofilm and Virulence

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Enteroaggregative *E. coli* (EAEC) is associated with acute and chronic diarrhea worldwide. Furthermore, among deployed military personnel, EAEC is the second most common bacterial cause of travelers' diarrhea. EAEC creates thick biofilms on the intestinal mucosa. Although biofilm formation is believed to be important for EAEC to cause illness, the EAEC biofilm has yet to be well characterized. Typical EAEC strains encode and produce one of five different aggregative adherence fimbriae (AAF). The AAF mediates aggregative adherence to tissue culture cells, but the degree of importance of each of the five AAF types in biofilm formation and pathogenesis is unknown. In this study, we characterized the biofilm from ten recently isolated EAEC strains, including two strains of each AAF type. We observed that AAF type did not correlate with the level of biofilm produced. However, deletion of the AAF fimbrial subunit gene led to a large reduction in biofilm formation for all EAEC tested. In contrast, only some AAF-mutant strains were attenuated for colonization in an antibiotic-treated mouse colonization model. A preliminary test of an AAF4 mutant strain in the *Galleria mellonella* (wax moth) larvae pathogenesis model indicated that the mutant strain was attenuated for virulence. We predict that the other AAF mutant strains will also be attenuated in *Galleria*. Overall our results suggest that AAF fimbriae from EAEC play an important role in biofilm formation and virulence.

Interspecies secreted surfactants induce emergent motility in Pseudomonas aeruginosa

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Bacteria often live in complex multispecies communities where secreted molecules from neighboring species can affect bacterial behaviors. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently co-isolated from the respiratory tracts of people with cystic fibrosis and are associated with increased disease severity. Here, we hypothesize that *P. aeruginosa* may alter its motility in the presence of *S. aureus* secreted products. To test this, we observed *P. aeruginosa* motility on agar plates containing a percentage of *S. aureus* supernatant under conditions where *P. aeruginosa* would normally swarm. Instead of swarming, *P. aeruginosa* slid down and spread over the plate in a way distinct from other established motility phenotypes. By testing motility appendage deficient *P. aeruginosa* mutants, we found that this motility is flagellar-dependent but pili-independent. Next, we tested supernatants from multiple species and found that only strains that secrete surfactants (*S. aureus, P. aeruginosa,* and *B. subtilis* 3814) could induce the motility in *P. aeruginosa*. Further mutants of these species which lacked the production of the respective surfactants were unable to exhibit the emergent motility, indicating that these secreted surfactants induce this motility in *P. aeruginosa*. We also observed that addition of biological and synthetic surfactants also led to this motility. Finally, transcriptomics revealed that cells undergoing this motility downregulated metabolism and respirations pathways and upregulated genes for bacteriophage proteins, as well as citrate and acetoin metabolism. Overall, we describe an emergent motility displayed by *P. aeruginosa* in the presence of interspecies secreted surfactants which is distinct from established motility phenotypes.

High-frequency monitoring of fecal indicator bacteria and associated particle size distributions in the sandy creek bottom sediments

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Variability of concentrations of the fecal indicator bacteria (FIB) *Escherichia coli* and enterococci in sediments was studied at large temporal scales such as seasons, and large spatial scales encompassing different land use. The knowledge about smaller-scale variability remains sparse. This work aimed to research the small-scale variability of *E. coli* and enterococci in a mountain creek with sandy bottom sediments. Sediment samples were taken weekly for a year in triplicate from bottom plots at sampling sites in forested headwaters, an agricultural area, and a mixed urban-agricultural area. The average weekly change in concentrations was from two times at the forested site to five times at the urban-agricultural site. Mean relative deviations from averages across sampling locations showed the significant trend of increase from -25% at the forested site to 45% at the urban-agricultural site. This trend was also well pronounced when data were grouped into cold and warm seasons. The sediment particle size distributions were significantly different among the three sites and between cold and warm seasons. Rankings of sediment fine mass fractions and FIB concentrations were positively correlated at two of three sampling sites in more than 70% of observation dates. The uncertainty of sediment FIB concentrations needs to be evaluated before designing sediment FIB monitoring and using the results to estimate the effect of sediment FIB on microbial water quality.

Organic fertilizers support survival of pathogenic and non-pathogenic Escherichia coli in soils and sporadic transfer to Romaine lettuce

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E. coli O157:H7 is a bacterial foodborne pathogen previously associated with leafy green outbreaks in the U.S. Organic fertilizers (like heat treated poultry pellets (HTPP), or seabird guano (SBG) are commonly used when growing organic lettuce. The role of these fertilizers in the survival of E. coli in soils and transfer to lettuce has not been evaluated. Romaine lettuce was grown in a Biosafety level 2 growth chamber with controlled light, temperature, and relative humidity. Soil was side-dressed with either HTPP, HTPP with corn steep liquor (CSL), SBG, SBG with CSL, or left unamended (UA, no fertilizer). Soils were co-inoculated with non-pathogenic *E. coli* TVS 353 and two *E. coli* O157:H7 REP strains and their longitudinal survival was evaluated over 28 days. On day 28, Romaine lettuce was harvested, and *E. coli* levels were quantified. Initial levels of *E. coli* on day 0 were ca. 5 log CFU/g soil. By day 28, all soil samples contain less than ca. 2 log CFU/g except one treatment. No leaf sample contains a quantifiable amount of *E. coli*. On Day 28, 13.3% (6/45) and 11.1% (5/45) of Romaine lettuce leaf samples contained *E. coli* TVS 353 and E. coli O157:H7, respectively. Our study demonstrated that *E. coli* can survive in soils containing organic fertilizers for at least 4 weeks, but their level of transfer to romaine lettuce is low. These results showed that transfer of *E. coli* O157:H7 from soils containing organic fertilizers to Romaine lettuce is sporadic.

Host Factors Associated with Failure of Fecal Microbiota Transplant for Recurrent Clostridioides difficile Infection: 9-year Real World Experience in a Dedicated C. difficile Clinic

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Abstract: *Clostridioides difficile* infection (CDI) poses a significant burden on the healthcare system due to high rates of recurrence. Fecal microbiota transplantation (FMT) is an effective treatment of recurrent CDI (rCDI). We have been providing care for rCDI patients at the Complicated C. *difficile* Clinic at the University of Virginia since 2013. A thorough review of patients' medical records who underwent FMT between 2013 and 2022 was conducted to assess performance of FMT, in addition to identifying factors associated with either success or failure. Primary outcome was failure of FMT, defined as either rCDI or death within one year. Data on demographics, comorbid medical conditions, CDI history, and laboratory values prior to FMT were collected to identify factors that determine FMT outcome. 240 patients underwent FMT: 70.4% were female, median age was 69, and median episodes of CDI was 4. 24.6% experienced failure within the year (18.3% had rCDI and 7.1% died). Older age (≥ 70), male sex, history of ≥4 episodes of CDI, hypertension, diabetes mellitus, malignancy, elevated TSH levels, anemia, and low zinc levels were linked to failure. Our study showed that FMT continues to hold up as effective treatment for rCDI, as well as identified new factors associated with failure of FMT. These results underscore the importance of recognizing factors that can predict failure of FMT and the need for additional research to clearly define causality. The association between elevated TSH and failure is a new finding and should be explored further.