Early Career Talks

Poly-gamma-glutamic acid synthesis locus in *Francisella*

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Francisella tularensis causes tularemia. It is a facultative intracellular bacterium for part of its lifecycle and lives part of its life cycle outside of a eukaryotic host. In *Bacillus* species, poly-gamma glutamic acid (γ- PGA) is produced via the genes in the *pgs* locus. γ-PGA is a major virulence and survival factor of anthrax and enables *S.epidermidis* to escape phagocytosis. *B. anthracis* produces an attached γ-PGA capsule. Due to the outer membrane, that mechanism for attaching this type of capsule to the bacteria in gram-negative bacteria is precluded. However, all strains of *Francisella* require this locus for virulence. Our objective is to characterize this *pgs* locus in *Francisella* and whether it can produce γ-PGA. Deletion of these *pgs* genes had a significant inhibitory effect on intracellular growth and results in an attenuated mutant strain in published work. We developed a method to purify γ-PGA from *Francisella*. Using a differential ethanol and water extraction method, we obtained white fluffy material following this extraction. Treatment of the extracted material with DNAse, RNAse and Proteinases removed these contaminants. γ-PGA is a high molecular weight polyamine compound. The purified material was run on an agarose gel and stained alongside PGA standards and is in the range of 50 kDa. The physiological role of *pgs (cap)* locus in *Francisella* is unknown but characterizing the potential formation of γ-PGA in *Francisella* is important in understanding its resistance to stress and its ability to grow in harsh environments such as inside of host cells.

Environmental Regulation of Toxin Production in *Bacillus anthracis*

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Pathogenic *B. anthracis* carries two plasmids: pX01, encoding a tripartite protein exotoxin complex (PA, LF, and EF); and pX02, encoding a poly-D-glutamic acid capsule. AtxA, a multidomain transcription factor, regulates the expression of these virulence genes. AtxA comprises two DNA-binding HTH domains, two PTS regulatory domains (PRD1 and PRD2), and a putative EIIB domain. Glucose and carbon dioxide increase AtxA-dependent toxin gene transcription, as does histidine phosphorylation of PRD1 and PRD2. We propose a histidine-phosphorelay originating from phosphoenolpyruvate (PEP) regulates AtxA via enzymatic activity in the PTS, impacting AtxA activity. Analyses of virulence factors' transcriptional profiling, PA secretion, and a fluorescent reporter strain suggest a synergistic effect of carbon dioxide and sugars on AtxA-dependent toxin production. Mutation analysis identified the cysteine at position 402 in the EIIB domain as essential for AtxA's transcriptional activity. Additionally, deletion of glucose PTS permease ptsG (*ΔptsG*) markedly reduced expression of exotoxin, attenuating infection in a mouse model. However, deletion of pyruvate carboxylase *pyc* (*Δpyc*), a possible enzyme involved in CO_2 sensing and fixation through anaplerosis, minimally affected toxin production. Biochemical and metabolic profiling of mutants highlights glucose as a primary stimulus for toxin production, with CO_2 enhancing AtxA activity through the anaplerotic flux. Our findings propose AtxA as integral to the glucose-PTS system, its transcriptional activity finely tuned by environmental cues. This elucidates the regulatory network governing anthrax virulence, offering potential therapeutic targets.

Harnessing the power of gut microbiome metagenomics analysis to understand prevalence of select potentially pathogenic and zoonotic bacteria in animals (Eunice Ndegwa submitted the abstract)

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Bacterial diseases continue to be important in both human and animal health. Human animal interactions can also result in transmission of zoonotic pathogens that includes bacterial species. In this study we used shotgun metagenomic analysis of gut microbiome to understand the animal prevalence of known select animal and zoonotic pathogens in pre-weaned goats. The bacteria selected include *Campylobacter, Salmonella*, *Mannhemia*, *Clostridial*, *Mycoplasma* and *Mycobacteria*. *Clostridium difficile* was the most common pathogen (50%) detected, followed by Campylobacter *spp* (48%), Salmonella spp (37%), *Clostridium perfrigens* (15%) and *Mannhemia spp* (14%). Both Mycoplasma *spp* and Mycobacteria *spp* were rare and detected in less than 10% of the fecal samples. Campylobacter was detected from one week of age until the day of weaning, while *Salmonella, Clostridial* and *Mannhemia spp* were detected beginning at 2 days of birth until weaning. This information is useful for the One health approach to improving public health by informing risk and implementing control strategies in animals.

Deciphering a Novel Physiological State in Streptomyces minutiscleroticus' Deep Non-Growing Cell

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Recent studies in a bacterial model of longevity reveal intriguing discoveries about the survival mechanisms of free-living organisms facing famine. *Streptomyces flavisclerotic*us, organism of the study, is spore- forming, obligate aerobe, belongs to economically important genus and produces anticancer compound chromomycin. Notwithstanding the long surviving spore form, this study describes findings about the prolonged lifespan of Streptomyces flavisclerotic*us* during its submerged mycelial stage, particularly in high osmolarity medium supplemented with sucrose. Unlike the conventional growth phases in low osmolar growth medium, growth in high osmolar medium apparently lacks the death phase. The quiescent cells that survive extended period of nutrient starvation are vegetative cells as they are killed by heat and sonication, unlike their spore form. They are metabolically active as exponential cells as evidenced by the comparable (i) rate of oxygen consumption, (ii) growth inhibition by PMF dissipating agent CCCP, and accumulation the PMF sensitive dye DiOC6(3), (iii) de novo transcriptional induction of GFP synthesis, (iv) growth inhibition by inhibitors of protein synthesis and cell division suggesting active growth-like stage. Preliminary Transcriptome/ proteome analysis suggests significant up-regulation of ribosomal and transcriptional proteins. Exponentially growing cells transferred from high osmolarity medium unlike that from low osmolarity into nonfermentable 20% sucrose is sufficient for their extended viability, suggesting that the transcriptional changes under these conditions are necessary and sufficient for extended survival in non-growing state. The mechanism of how the quiescent cells is formed and the potential for heterologous synthesis given their active metabolic state requires to be appreciated.

Acute Inflammatory Cytokine Profiles and Dynamic Changes of Serum Anti-MPXV IgG Profiles in Mpox (Monkeypox) Virus (MPXV) and HIV Co-infected Young Adults

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Mpox (monkeypox) virus (MPXV) belongs to *Orthopoxviruses* (OPXV) which include variola major virus (VARV) and vaccinia virus (VACV). Since MPXV spread around the world in 2022, ~40% of mpox cases have been reported in the HIV-infected population. Little is known regarding acute inflammatory cytokine profiles and dynamic changes of anti-MPXV-IgG profiles among the patients affected by the 2022-2023 outbreaks, especially HIV+MPXV co-infected cases. Herein, we determined acute inflammatory cytokine profiles for three HIV-infected young male adults with PCR-confirmed MPXV. IL-2, IL-5, IL-8, IL-10, IL-13 and TNFα were significantly elevated in the three patients' sera within a month post mpox onset (MPO), with 4- to 20-fold changes, consistent with their mild clinical manifestations. Next, we employed a novel, xMAP (Luminex) based, 15-plex anti-OPXV serology assay to characterize dynamic changes in anti-MPXV-IgG profiles. Compared to healthy controls, the infected patients' sera yielded significantly elevated fluorescent intensity against VARV-A36R, VACV-A27L, VACV-A33R, VACV-L1R, MPXV-A29, MPXV-A35R, MPXV-B6R, and MPXV-E8L, with anti- MPXV-A35R, anti-MPXV-B6R and anti-MPXV-E8L yielding >500-fold changes. Anti-MPXV-B6R titers of a case significantly enhanced from 1: 400 at 2 MPO to 1:3200 at 6 MPO, and then remained 1:3200 at 15 MPO. Titers of other antibodies of the three cases showed 2-fold increase from 2-6 MPOs, with no difference between 6 and 15 MPO. Altogether, acute inflammatory cytokine profile had a correlation with the mild clinical manifestations in three youths with MPXV+HIV co-infection. Anti- MPXV-IgG remained stable within 15 MPO. A35R, B6R and E8L represent MPXV immune targets for sensitive serologic identification of MPXV infection.

Interspecies Surfactants Serve as Public Goods Enabling Surface Motility in *Pseudomonas aeruginosa*

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In most natural environments, bacteria live in polymicrobial communities where secreted molecules from neighboring species alter bacterial behaviors including motility, but such interactions are understudied. *Pseudomonas aeruginosa* is a motile opportunistic pathogen that exists in diverse multispecies environments such as the soil and is frequently found in human wound and respiratory tract co-infections with other bacteria including *Staphylococcus aureus*. Here we show that *P. aeruginosa* can co-opt secreted surfactants from other species for flagellar-based surface motility. We found that exoproducts from *S. aureus* and other bacteria enabled *P. aeruginosa* to switch from swarming to an alternative surface spreading motility on semi-solid surfaces and allowed for the emergence of surface motility on hard agar where *P. aeruginosa* was otherwise unable to move. The *P. aeruginosa* surface spreading was also facilitated by the addition of numerous exogenous biological and synthetic surfactants. Mutant analysis indicated that this motility was similar to a previously described mucin-based *P. aeruginosa* motility, 'surfing', albeit with divergent regulation. Thus, our study demonstrates that secreted surfactants from the host as well as neighboring bacterial and interkingdom species serve as public goods enabling a major *P. aeruginosa* flagella-mediated surfing-like surface motility, thereby allowing it to access different environmental niches.

Lightning Talks

A Synthetic Antimicrobial Peptide Inspired by American Alligator Combats Multidrug Resistant Gram-negative Bacteria.

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Antimicrobial peptides (AMPs) are found in living organisms as a part of the innate immune response to pathogens. There is an urgent need to develop novel antimicrobials to tackle the rapid increase in antimicrobial resistance. GATR-3 is a synthetic AMP that we designed from a parent cryptic peptide discovered in American alligator. We evaluated GATR-3 against multidrug resistant (MDR) gram-negative bacteria. *Acinetobacter baumannii* is gram-negative bacteria that causes hospital-acquired infections resulting in pneumonia, sepsis, and severe wound infections and is difficult to treat due to MDR. We tested GATR3 activity against a panel of 8 MDR *A. baumannii* strains as well as time-kill kinetics. Biofilm inhibition and eradication were assessed. GATR3 mechanism of action was determined. Finally, toxicity of GATR3 was evaluated. GATR3 showed fast, potent antimicrobial activity with MIC of 4 µg/ml against MDR *A. baumannii* and a loss of bacterial membrane integrity. The peptide had biofilm-inhibitory activity at 4-16 µg/ml and eradicated established biofilms at 32 µg/ml. Hemolysis and cytotoxicity assays were performed at 1, 10, and 100 µg/ml peptide. We used the waxworm *Galleria mellonella* as an *in vivo* model to assess GATR3 toxicity. In summary, GATR3 exhibits potent antimicrobial activity against MDR *A. baumannii* strains by targeting bacterial membranes. Biofilms are difficult to treat and eradicate; however, GATR3 inhibited biofilm formation and also eradicated preformed biofilms at the indicated concentrations. GATR3 showed little cytotoxicity. Overall, GATR3 presents a promising therapeutic candidate to treat MDR *A. baumannii* infections. (W81XWH2110214 JW200188 to MVH).

Using Synthetic Herpes Simplex Virus to Study Viral Pathogenesis and Develop Cancer Therapeutics

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Background: Herpes Simplex Virus Type 1 (HSV-1) is a linear dsDNA virus typically associated with ulcer development in the mouth and genital mucous membranes. Often overlooked, is the versatile DNA structure that enables HSV-1 to serve as a platform to study viral pathogenesis. HSV-1 also possesses an inherent oncolytic quality, allowing it to function as a selective therapeutic that can be programmed to target and kill malignant tumor cells. **Methods:** Various endonucleases were used to isolate the genome into 11 smaller fragments. To study the effects on viral pathogenicity and oncolytic properties, we synthesized and inserted novel sequences, with a YCpBAC sequence to allow for selection and transformation into yeast spheroplasts for TAR cloning *in vitro*. Fragments containing a cell death gene coding regions were also tested for viability, by running quantification assays and infection tests on cell lines. **Results:** Isolated band size was correlated with theoretical values, indicating accurate fragment construction. After screening on -His and -Ura plates, a 200µL aliquot of 40ng/µL TAR2 sample was determined to yield a satisfactory 85% success rate in yeast assembly. The ideal quantity for viral replication in Vero E6 cells was determined to be around 1.0×10^7 cells/cm^2 providing 2.5ml of 100x viral solution. **Conclusions:** Successful reassembly of HSV-1 demonstrates the feasibility of using HSV-1 as a therapeutic vehicle, useful in treating cancers and understanding neurological diseases. These findings provide a strong foundation for subsequent modifications of HSV-1, which will focus on its effects on various cancer cell lines.

Identification of a Second Branched-Chain Fatty Acid Synthesis Pathway in *Staphylococcus aureus*

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Staphylococcus aureus is an opportunistic human pathogen that is the leading cause of skin and soft tissue infections. The bacterium is also a major cause of devastating invasive infections including endocarditis, osteomyelitis, and bacteremia. Thus, a better understanding of pathways for bacterial membrane biogenesis is critical to manage antibiotic resistance and combat staphylococcal infections. Branched-chain fatty acids (BCFAs) are the predominant fatty acids in staphylococcal membrane phospholipids and are synthesized in part by the branched-chain a-keto acid dehydrogenase (BKDH) complex. Because BCFAs fluidize the membrane, BKDH-deficient strains are BCFA auxotrophs in laboratory culture and do not grow without exogenous fatty acid or branched- chain carboxylic acid (BCCA) supplementation. Exploiting this phenotype, we identified suppressor mutants that regained the ability to grow in the absence of exogenous BCCAs. The mutations result in overexpression of a putative acyl-CoA synthetase named MbcS. Biochemical analyses reveal MbcS is a bona fide acyl-CoA synthetase with high affinity for BCCAs and is required to scavenge the exogenous carboxylic acids for BCFA synthesis. We demonstrate that MbcS is also required by *S. aureus* to utilize the isoleucine derivative 2-methylbutyraldehyde as a precursor for BCFA synthesis. Our findings suggest a second pathway to BCFA synthesis that is strongly downregulated during laboratory growth. The implications for MbcS-dependent BCFA synthesis during colonization and infection will be discussed.

APOE Genotype-Dependent Effects of Exercise on Gut Microbial Composition in Mice.

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Recent research has demonstrated the importance of the gut bacteria in controlling cardiovascular neurological, and metabolic function. Exercise which impacts all of these physiological processes has also been shown to impact the gut microbiome, particularly those that regulate short chain fatty acid (SCFA) production, including *Ruminococcaceae* and *Faecalibacterium*. Apolipoprotein E (APOE) is a cholesterol trafficking protein and it's e4 variant (APOE4) is the most prevalent genetic risk factor for Alzheimer's disease (AD) and increases risk for dyslipidemia, metabolic syndrome, and cardiovascular disease. According to recent research, APOE2/E3 genotype carriers have greater amounts of *Ruminococcaceae* than APOE4 carriers. Additionally, in humans and mice with the APOE4/E4 genotype, the numbers of bacteria that produce SCFA are reduced.

The aim of this study is to determine and compare the impact of APOE genotypes on changes in the gut microbial composition induced by exercise. A total of sixteen 10-month-old mice, homozygous for APOE3 and APOE4 genotypes were used. Mice from each genotype were randomly assigned into either exercise (EX) or sedentary (SED) groups. Mice in the exercise groups were placed in a cage containing a voluntary run-wheel for 8 weeks. Following a series of behavioral tests, mice were sacrificed, and tissue and fecal samples were collected. DNA extractions and bacteria 16S rRNA gene amplification were conducted from fecal-impacted colon tissue.

APOE genotype affected microbiome composition with respect to specific species known to impact SCFA production. Exercise tended to alter microbiome diversity in an APOE genotype-dependent manor. Different levels of voluntary exercise could contribute to the observed microbiome differences caused by exercise. On the other hand, variation in microbiome composition might affect activity levels. Further research is required to clarify the direction of this influence.

SroA Modulates the Levels of Staphyloxanthin Pigment in *Staphylococcus aureus*

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Staphylococcus aureus is a pathogen notorious for causing serious infections worldwide. Its ability to evade host immune defenses and develop antibiotic resistance allows it to persist and spread in hospitals and communities. Without new treatment strategies, antibiotic-resistant S. aureus infections are projected to become a leading cause of death worldwide. Characterizing novel virulence factors or regulators of virulence factors will assist us in developing new treatments. A critical virulence factor for *S. aureus* immune evasion is the carotenoid pigment Staphyloxanthin (STX). STX enhances the ability of *S. aureus* to cause invasive infections with high mortality, including sepsis and bacteremia. STX protects *S. aureus* from reactive oxygen species released by phagocytic immune cells and is essential for pathogenesis. The multi-step biosynthesis of STX is encoded by the crtMNOPQ operon, which is primarily regulated by the alternative sigma factor SigB. However, additional regulatory factors likely exist but remain undiscovered. We recently discovered a novel *S. aureus* protein, SroA, that may act as a major effector of virulence. We created a mutation in sroA and noticed that the cells exhibited increased pigment. This led us to hypothesize that SroA may act to modulate STX synthesis in *S. aureus*. To confirm the SroA effect on STX synthesis, we methanol extracted STX from wild type and sroA mutants and quantitatively measured them. Our results demonstrate a statistically significant (P-value < 0.001) increase in STX amounts in sroA mutants, suggesting that SroA regulates STX synthesis in S. aureus.

Resistance of FPN Q248H to Hepcidin and Its Effects On HIV-1 Infection

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Increased iron stores correlate with rapid AIDS progression in HIV-1-infected patients, in some iron-loaded patients. Ferroportin (FPN) is the only known ferrous iron (Fe^{2+}) transporter present mainly in enterocytes, macrophages, and hepatocytes. FPN exports Fe^{2+} from the cytoplasm, whereupon the iron is oxidized at the basolateral side of the cellular membrane and loaded in transferrin. FPN is negatively regulated by hepcidin, a short peptide secreted by the liver. The FPN Q248H mutation is linked to changes in iron load in Africans. Our study explored the effect of FPN Q248H mutation on HIV-1 infection. HEK 293T cells were transfected with FPN-expressing vector, and sensitivity to physiologic hepcidin concentrations and ferritin concentrations were evaluated using immunoblotting and fluorescence analysis. A knock-in *Slc40a1*^{Q248H} mouse model was developed to study the effect of FPN Q248H mutation on iron load. Mouse-adapted Eco-HIV-1 virus was used to infect mouse spleenocytes ex vivo. FPN Q248H mutant showed decreased sensitivity to hepcidin and lower ferritin concentrations in HEK 293T cells and human primary monocytes. Mice with the FPN Q248H mutation exhibited increased splenic and liver iron levels, and also demonstrated increased serum transferrin saturation. Ex vivo infection of splenocytes showed decreased HIV-1 replication in splenocytes from FPN Q248H mice compared to control WT mice. These findings suggest that FPN Q248H mutation may protect from HIV-1 infection and reduce the negative consequences of HIV-1 infection by moderately increasing iron load. The *Slc40a1*^{Q248H} mouse model may be useful for further exploring the effects of FPN Q248H mutation on HIV-1 infection.

2-Bromopalmitate Depletes Lipid Droplets to Inhibit Viral Replication

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The global impact of emerging viral infections emphasizes the urgent need for effective broad-spectrum antivirals. Lipid droplet (LD) is a potential target for developing broad-spectrum antivirals. We comprehensively utilized 2-BP, alongside other palmitoylation inhibitors such as cerulenin and 2-fluoro palmitic acid (2-FPA), as well as the enhancer palmostatin B and evaluated their impact on LD and the replication of coronaviruses at non-cytotoxic concentrations. While cerulenin and 2-FPA exhibited moderate inhibition of viral replication, 2-BP exhibited a much stronger suppressive effect on MHV-A59 replication, although they share similar inhibitory effects on palmitoylation. As expected, palmostatin B significantly enhanced viral replication, it failed to rescue the inhibitory effects of 2-BP, whereas it effectively counteracted the effects of cerulenin and 2-FPA. This suggests that the mechanism underlying how 2-BP inhibits viral replication is beyond palmitoylation inhibition. Further investigations unveil that 2-BP depletes LDs, a phenomenon not exhibited by 2-FPA and cerulenin. Importantly, the depletion of LDs was closely associated with the inhibition of viral replication because the addition of oleic acid to 2-BP significantly rescued LD depletion and its inhibition of MHV-A59. Our findings indicate that the inhibitory effects of 2-BP on viral replication primarily stem from LD disruption rather than palmitoylation inhibition. Intriguingly, fatty acid (FA) assays demonstrated that 2-BP reduces the FA level in mitochondria while concurrently increasing FA levels in the cytoplasm. These results highlight the crucial role of LDs in viral replication and uncovers a novel biological activity of 2-BP. These insights contribute to developing the broad-spectrum antiviral strategies.

Candida auris Lipid Metabolism Genes Upregulated in Skin-Like Media

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Candida auris is one of four pathogens designated as a critical priority group member in the WHO's 2022 Fungal Priority Pathogens List and is one of five pathogens designated as an urgent threat in the CDC's 2019 Antibiotic Resistance Threats Report. *C. auris'* high mortality rate (~50%), high antifungal resistance profile, and high transmission rates in healthcare facilities underlie its threat to global health. While most *C. auris* clinical cases are bloodstream infections, skin is the primary site of colonization. *C. auris* persists on skin for extended periods of time and is considered the major risk factor for development of life-threatening infections. A significant gap in knowledge regarding *C. auris* skin colonization is how it grows in the nutrient-poor environment of the skin. I conducted an exploratory screen that revealed that *C. auris* upregulates key lipid metabolism genes in artificial sweat media, which contains skin-like nutrients, compared to rich media. Preliminary data has shown that without key lipid metabolism genes *C. auris* mutants grow poorly in the artificial sweat media compared to rich media in vitro. Currently, I have screened a *C. auris* mutant library to find and validate the most important genes required for lipid metabolism and growth. Next, I will perform comparative genomics to study genes, perform additional in vitro studies, and use an in vivo model to study longitudinal colonization of *C. auris* strains. Collectively, this work will advance our understanding of how *C. auris* can utilize skin-like nutrients, grow, and persist on skin.

Time Series Analysis of SARS-CoV-2 N-genes detected in wastewater samples in Baltimore

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Since it was declared a pandemic by the World Health Organization in March 2020, Sars-CoV-2 has been known to exhibit notoriety due to the number of people infected and dead over a period of three years. This study was to determine the presence of Sars-CoV-2 RNA viral fragments in wastewater samples from two wastewater treatment plants in Baltimore over a period of one year. The samples were concentrated by the Polyethylene Glycol 8000 (PEG) method, and RNA fragments were purified using the QiAmp Viral RNA Mini Kit. RT-PCR and qPCR assays were performed, and Cq values below 40 were analyzed and presented as gene copies/L. N1 and N2 genes were detected in both WWTP samples, with N1 having log10 gene copies ranging from 1.38 - 3.34 gc/l and N2 ranging from 1.88-3.20 gc/l. Covid19 hospitalization cases in Baltimore County and City were observed to be positively correlated with the copies of viral RNA detected in the WWTP-A with the N1 and N2 genes having 0.30/0.32 and 0.32/0.40 respectively. On the contrary, the NCov genes (N1 and N2) from both WWTPs exhibited a very weak positive and negative relationship with wastewater physical parameters such as pH, electrical conductivity, total dissolved solutes, salinity, and temperature. The reduction in positive correlation with hospitalization cases could be attributed to an increase in immunity amongst the population in both counties studied. There is a need to ascertain the effect of physical parameters changes from the sampling point to the processing point on the capture and detection of Sars-CoV-2 viral RNA in wastewater.