DISSERTATION

FROM COMPUTATION TO COMMUNICATION: UNVEILING *SALMONELLA* METABOLIC PLASTICITY AND PUBLIC PERCEPTIONS OF THE MICROBIAL WORLD USING MULTI-OMICS AND THEMATIC ANALYSIS

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ABSTRACT

FROM COMPUTATION TO COMMUNICATION: UNVEILING *SALMONELLA* METABOLIC PLASATICITY AND PUBLIC PERCEPTIONS OF THE MICROBIAL WORLD USING MULTI-OMICS AND THEMATIC ANALYSIS

Research and communication on microorganisms and microbiomes has become increasingly important in recent decades due to evolving threats posed by infectious diseases and microbial contributions to ecological systems. Antibiotic resistance presents a significant challenge to global health equity, with nontyphoidal *Salmonella* infections being a prominent concern. Despite its prevalence and impact, *Salmonella* infections lack effective vaccines, posing a serious threat to vulnerable populations. Concurrently, misconceptions and misinformation about microorganisms and microbiomes can arise given the dynamic nature of scientific research which can hinder effective science communication and health outcomes. Despite this, little is known about public perceptions of microorganisms and microbiomes, impeding our ability to create effective, tailored science messaging. Both basic pathogen research and science communication research are essential to identify targeted prevention strategies and to understand public perceptions of microorganism and microbiomes.

This dissertation spans microbiome and science communication research, employing both qualitative and quantitative methods. The overarching research goals of this dissertation are to 1) lay the groundwork for therapeutics by studying *Salmonella* metabolism and metabolic plasticity, 2) develop a multi-omics repository to expand the

usability of our omics datasets, and 3) understand public perceptions of microorganisms and microbiomes to improve future microbial science communication efforts. Chapter 1 as the introductory chapter reviews the current state of *Salmonella* and science communication research, providing a context for the new research presented in this dissertation. Through a multi-omics approach, Chapter 2 explores the metabolic strategies of *Salmonella* under different diet backgrounds and over time, offering insights into potential therapeutic targets. Chapter 3 introduces the CBA_DREAMM database, facilitating centralized storage and sharing of multi-omics datasets to enhance communication of our research and collaboration in microbiome research. Chapter 4 investigates public perceptions of microbes and microbiomes in the United States, revealing a need for tailored science communication efforts. Additionally, the study emphasizes the importance of clear communication, trust, and emotions, like apathy, in science communication. Chapter 5 is the conclusion, summarizing findings from Chapter 2, 3, and 4 and describing future directions. By bridging natural and social sciences, this dissertation aims to inform strategies for tackling global issues by advancing microbiome and science communication research.

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"I'm glad to be with you, Samwise Gamgee… here at the end of all things." (1)

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"Jesus Christ, my Lord and God, I give thanks for your loving kindness and all the blessing You have richly bestowed upon me." -A Prayer of Thanks

Reference:

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Chapter 1: Overview of Current State of *Salmonella* and Science Communication research

1.1 Pathogen Research in Changing World

The need for basic pathogen research has become more evident in recent decades as evolving pathogen threats change our world. Many factors contribute to the increase in infectious disease threats including rapid urbanization, climate change, land-use change, and improved access to travel (1). These changes disproportionally affect low- and middle-income countries resulting in increased morbidity, mortality, and further spread of infectious disease (1–4). One of the major evolving pathogen threats is antibiotic resistance. To improve health outcomes and equity worldwide, we must work to address antibiotic resistance globally.

The Center for Disease Control (CDC) reports about 3 million people contracting an antibiotic-resistant infection per year in the United States alone (5). This includes many gastrointestinal pathogens, like nontyphoidal *Salmonella*. According to the CDC's 2019 report, drug-resistant nontyphoidal *Salmonella* is a "serious threat" resulting in 212,500 infections and 70 deaths annually (5). Nontyphoidal *Salmonella* remains one of the leading causes of diarrhea globally causing approximately 150 million illness and 60,000 deaths per year (3, 5). With no available vaccines (3, 4), the young, elderly, and immunocompromised individuals rely on broad-spectrum antibiotic treatments. Antibiotics are also the only line of defense against the emergence of invasive non-typhoidal salmonellosis which is prevalent in sub-Saharan Africa and associated with a 20-25% case fatality rate without treatment (3, 4). Together, this data exemplifies a need

for basic pathogen research to identify targeted therapeutics and proactive prevention strategies.

Even with solutions in hand, basic research must not work in a silo. Having effective science communication initiatives, community buy-in, and stakeholder involvement in solutions are critical for the success of any research-driven public health campaign. As we have seen from the COVID-19 pandemic, misinformation can result in reduced compliance with public health measures and avoidance of medical treatment, in the worst cases leading to loss of life (6–8). Science communication research works to understand the public perceptions of these phenomenon and test interventions to improve future science communication outcomes, yet much of this work still needs to be done (9–14). Furthermore, determining how to best communicate science is critical for public health outcomes and ensuring future science literacy (9, 14, 15). As a graduate student during one of the most significant pandemics in recent history, it is clear to me that there is a need for basic pathogen research to lay the foundation for developing novel therapeutics and science communication research to understand how best to communicate these findings.

1.2 *Salmonella*: The Swiss Army Knife of Pathogens

There are approximately 2,500 different serovars of *Salmonella enterica* identified with gastroenteritis predominately caused by *Salmonella enterica* serovar Typhimurium (*Salmonella*) (16). Typified by a self-limiting inflammatory gastrointestinal infection in humans, *Salmonella* enters the gastrointestinal tract typically through ingestion of contaminated food and must use its metabolic capacity to outcompete native microbiota and the host immune system (17–19).

A review by Hume et. al. (19) terms *Salmonella* as the “Swiss Army Pathogen” for its diverse array of virulence genes and metabolic functions that allow it to be a successful pathogen. In a healthy gut, commensal microbes produce short chain fatty acids (SCFAs) that maintain gut homeostasis and modulate epithelial metabolism, through complex interaction based on diet, microbiome membership, and the host, as depicted in **Figure 1.1** (20–22). Upon infection, *Salmonella* uses type 3 secretion system (T3SS) genes and effector proteins to invade host gut epithelial cells and survive in macrophages (19, 23, 24). Consequently, the innate immune system initiates an inflammatory response characterized by oxygenation of the gut lumen, along with neutrophils and inflammatory monocytes generating reactive oxygen and nitrogen species (17, 19, 24). This inflammatory response generates respiratory electron acceptors and donors *Salmonella* can use to proliferate and depletes strict, native anaerobes in the gut (17, 25–30). This unique metabolism makes development of targeted therapeutics challenging, and *Salmonella* interesting to study from a microbial ecology perspective.

While *Salmonella* infection typically results in depletion of the commensal microbiota, factors such as diet can impact whether a productive infection occurs (31–36). In gnotobiotic mouse models or mice with reduced microbiomes, the addition of specific microbes enhances colonization resistance against *Salmonella* (31, 37–39). Contrastingly, diet alone is sufficient to break colonization resistance by reducing commensal microbes (31, 34, 40). Given the vast adaptive potential of *Salmonella*, evaluating *Salmonella* metabolism under different diet backgrounds furthers our understanding of *Salmonella* metabolic plasticity. The mechanisms of how *Salmonella*

responds to different diets or while competing with the microbiome throughout infection remains to be fully understood. Knowledge about which microbes and what conditions facilitate or impede *Salmonella* infection can be used for the development of therapeutics, but the study of microbial communities during *Salmonella* infection has been challenging.

Historically, *Nramp1* -/- mice were used due to their susceptibility to systemic *Salmonella* infection. Yet, disease progression of *Salmonella* Typhimurium does not result in systemic infection in humans. Additionally, regardless of *Nramp1* status, antibiotics are typically given prior to inoculation to allow *Salmonella* to colonize which impedes studying *Salmonella* in a microbiome context. Having a higher initial inflammatory response, *Nramp1* +/+ mice have become more common for evaluating the impacts of *Salmonella* during inflammation (41, 42). Notably, these mice display disease progression more analogous to the disease manifestation in humans where *Salmonella* does not become systemic, but rather remains localized to the gut. Of the *Nramp1*+/+ mouse models, CBA mice tolerate a high *Salmonella* load and are susceptible to *Salmonella* infection without antibiotic pretreatment, allowing a longer temporal infection study period (43–45). Using this model, our research group developed a CBA genome database, foundational to studying *Salmonella* and the gut microbiome during infection (45).

1.3 The Need for Omics Data Stewardship

Excitingly, we are now poised to answer questions and reaffirm long-held assumptions about the gut microbiome and pathogen interactions due to the invention of omics technologies. Omics technologies refer to the technologies that allow us to

examine all the genes (metagenomics), mRNA (metatranscriptomics), proteins (metaproteomics), and metabolites (metabolomics) in a sample at a particular time (46, 47). The generation of omics technologies has revolutionized the field of microbial ecology, where we can begin to understand the metabolic capacity, expression, and chemical exchange of the microbiome. Omics research has expanded our knowledge of microbial abundance, expression, and interaction across biomes from human health to climate change (48–53). The power of these tools to identify biological signals increases when they are integrated, providing a foundation to unravel intricacies of complex systems (47, 54).

With the cost of sequencing plummeting in the last decade and increased accessibility to more powerful computational infrastructures, it is now more affordable than ever to deeply sequence samples, enabling the identification of rare microbial members, constructing genomes of microbes that have yet to be cultured in a laboratory, and understanding the complex network of microbial relationships within an environment (55). Additionally, the creation of portable sequencers, like the MinION from Nanopore, allow this research to occur in the field in real-time on a laptop or even a cell phone (56). With the ability to generate vast amounts of data, findable, accessible, interoperable, and reusable (FAIR) data management principles are necessary to maximize added value and equity of this research (57–59). Furthermore, work within the field of microbiome science needs to standardize best practices, promote data sharing, improve annotation resources, and provide training for the next generation of scientists.

The Microbial Ecosystem Lab not only does microbiome research in a variety of biomes, but also has created tools for the broader scientific community (43, 45, 53, 60–

62). This includes a microbial metabolism annotation tool, which allows scientists to go from a series of base pairs from a sequencer to genes, and ultimately assigned functions for the microbiome of interest. Our laboratory's tool DRAM (Distilled and Refined Annotation of Metabolism), can be applied across thousands of genomes in customizable formats (60). DRAM can provide raw annotation information for metagenome assembled genomes (MAGs) and specific distilled outputs for metabolisms of interest, which was critical to our *Salmonella* research. As part of the project, we have also generated genomes databases as community resources for a variety of ecosystems, including the CBA mouse gut (45, 53). Prior to my PhD, microbiota in CBA mice were previously not represented in murine microbiome databases, either by amplicon or genome-based information. To address this, we generated a CBA mouse gut genome database containing 160 dereplicated MAGs and 609 dereplicated viral MAGs, supporting enteric pathogen research (45). As multi-omics research continued on my project, large amounts of data across diets, infection, and time were generated. My stewardship of this data resulted in the creation of a data repository, CBA_DREAMM (CBA Data Repository of Expression, Amplicon, Metagenomic, Metabolites). Not only does this work align with our dedication to improve barriers in the microbiome research, but it also integrates multi-omics data in a sharable format with the scientific community.

1.4 Science Communication and Society

"*Nothing in science has any value to society if it is not communicated.*" - Anne Roe (63)

The federal government funds about half of scientific research and is the highest funder of basic research (64). Government spending cuts on research primarily impact

basic research, and funding has declined in recent years (65, 66). Despite this, findings from basic scientific research influence policies and therapeutic practices affecting the daily lives of many. Clear communication about research is lacking and can result in misinformation (6, 8). This is most notably exemplified during the COVID-19 pandemic which emphasized many failures in our public health system including science communication messaging (7, 67–69). With the rise of misinformation and decrease science funding, science communication has become more essential than ever.

Science communication is a vast field of social science research dedicated to the study of communicating scientific knowledge, bridging science and society. There are many benefits of science communication including but not limited to 1) sharing findings and excitement, 2) educating others, 3) increasing appreciation for science, 4) influencing people's opinions, behaviors, and policy, 5) including stakeholders in the generation of solutions, and 6) promoting diversity, equity, and inclusion (70, 71). Additionally, scientists themselves also benefit from science communication including increased scientific impact, networking opportunities, learning from stakeholders, and addressing misinformation in their field (71–73).

Recent publications have studied public perceptions and tested communication interventions for specific infectious diseases, finding misinformation related to causes and prevention strategies (10, 11, 74–76). Misinformation has been shown to delay health behaviors and avoidance of disease interventions (8, 67, 69). Others have shown that scientists' deficient perception of the public, believing the public to be ignorant about science, impedes their intentions to correct misinformation (77). Together, these results highlight the need for increased science literacy and improved science

communication metrics. While there is extensive literature related to science communication and public understanding about specific pathogens (10, 11, 74–76), little is known about public perceptions of microbes and microbiomes in general. Microbes and microbiomes are essential to our ecosystems and used in a variety of industries unrelated to disease (78, 79). Complicating matters, people have different psychological, cultural, and political reasons for their perceptions on any given subject (13, 71, 80). Understanding public perceptions allows science communicators to target messaging based on previous knowledge and address the concerns of their audience (71, 80, 81).

Science communication efforts can tailor communication offering more effective connection between federal agencies, researchers, and the public. For example, decades of research on public perceptions of Americans on climate change led to the identification of six groups of Americans: alarmed, concerned, cautious, disengaged, doubtful and dismissive (13, 82–84). This research was foundational in crafting specific communication to meet the needs of each of these groups, with the goal of facilitating changes for a low-carbon future. Research, like this, is limited for microbes and microbiomes even though microbes and microbiomes drive many ecological functions and potential solutions for complex problems. Science communication research of public perceptions of microbes and microbiomes will identify what people know, how they know it, and will inform future science communication efforts.

1.5 Goals of this Dissertation

Together, this work spans across the disciplines of microbiology and science communication using both quantitative and qualitative research methods. It aims to 1)

understand the factors influencing *Salmonella* metabolism during infection, 2) optimize data sharing via construction of a repository to promote FAIR principles, and 3) advance our knowledge of public perceptions of microbes and microbiomes to guide future science communication efforts.

We approach our first goal using a multi-omics approach in a murine mouse model. Chapter 2 reveals the diverse metabolic strategies and dynamic nature of *Salmonella* metabolism in two diet backgrounds and over time. This research expanded upon existing theories of metabolic capacity and identified new metabolisms of interest that may be critical to stages of infection. Upon further research, this work could be used to develop targeted therapeutics.

Given the abundance of data generated in this work and across our research team, the generation of a repository with associated metadata was created. Chapter 3 highlights the construction of this resource for the microbiome science community. The repository, CBA_DREAMM (CBA Data Repository of Expression, Amplicon, Metagenomic, Metabolites), contains 16S rRNA amplicon sequencing, a genome database containing metagenome assembled genomes and viral metagenome assembled genomes, metatranscriptomic sequencing, and both targeted and untargeted metabolomics data. FAIR data principles were used to guide the development of this tool (57). All metadata, methods, and annotations are included to increase the accessibility of the data. Additionally, computationally expensive processes including database generation and gene mapping files are provided. Having a data repository such as this provides opportunities for labs with limited computational resources to contribute to this work and fosters opportunities for collaboration.

We use a qualitative approach, semi-structure interviews, to gain insight on public perceptions of microbes and microbiomes. Chapter 4 describes the findings of an inductive thematic analysis of interview data to inform and improve future science communication efforts. These provide a foundation for science communication intervention research and act as a call-to-action for scientists to join public discourse about microbes and microbiomes.

We conclude this dissertation with Chapter 5, a summary of the impact of the work presented here and future directions for the fields of microbiology and science communication research. This section describes how this thesis spans natural science and social science to address knowledge gaps in mechanisms of *Salmonella* metabolism and public perceptions of the microbial world. This thesis also depicts the importance of data resource sharing to foster equity in future microbiome research.

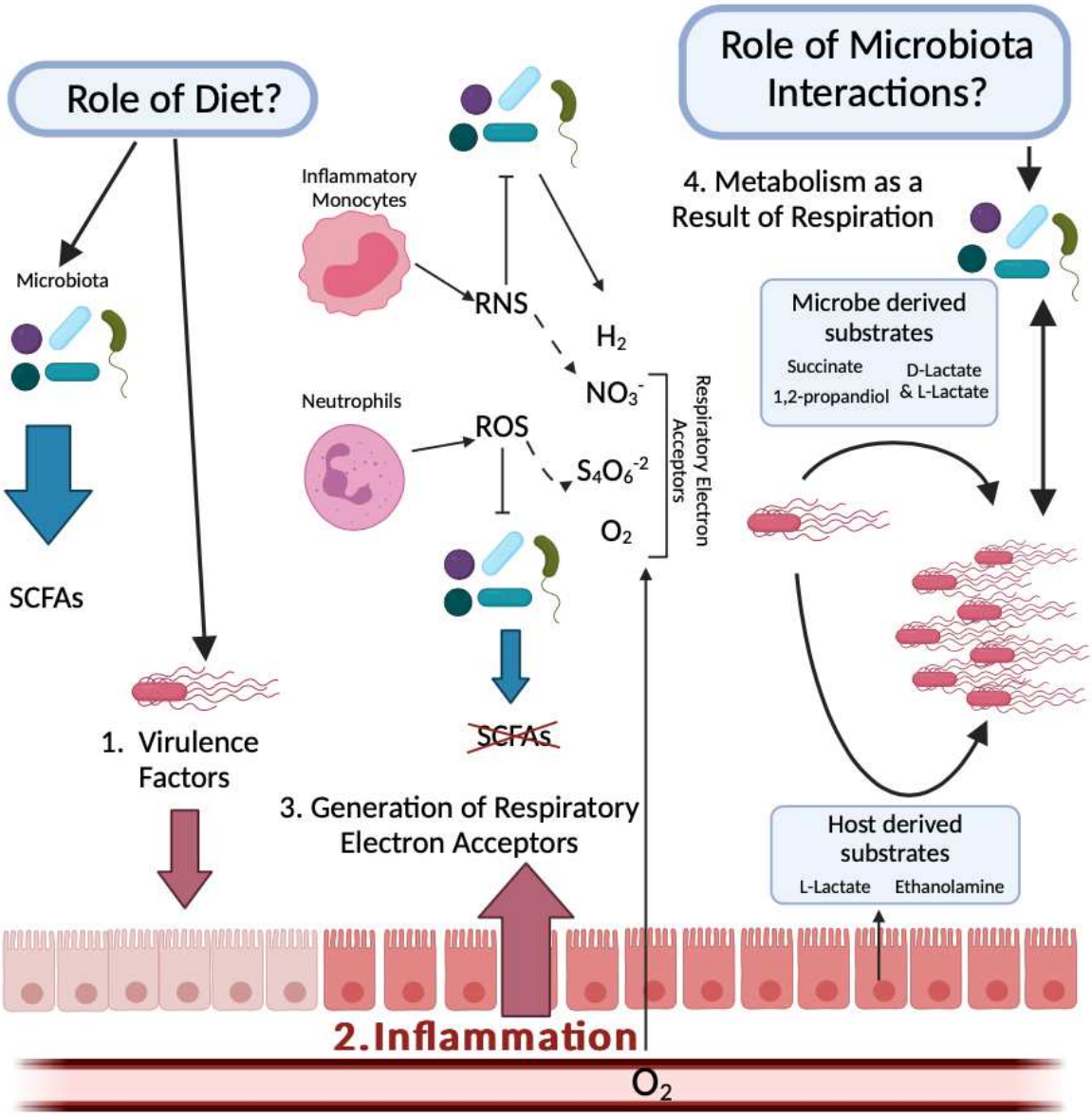

Figure 1.1 Conceptual model of *Salmonella* infection and ongoing research questions. This model describes the interplay between the microbiome, host, and pathogen in a specific diet background. Components of *Salmonella* pathogenesis and resulting outcomes are shown specifically: 1) virulence factors 2) host inflammation 3) generation of respiratory electron acceptors, including reactive nitrogen species (RNS) and reactive oxygen species (ROS) and 4) metabolism as a result of respiration.

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Chapter 2: Time resolved multi-omics reveals diverse metabolic strategies of *Salmonella* during diet-induced inflammation

2.1 Summary

With a rise in antibiotic resistance and chronic infection, the metabolic response of *Salmonella enterica* serovar Typhimurium to various dietary conditions over time remains an understudied avenue for novel, targeted therapeutics. Elucidating how enteric pathogens respond to dietary variation not only helps us decipher the metabolic strategies leveraged for expansion but also assists in proposing targets for therapeutic interventions. Here, we use a multi-omics approach to identify the metabolic response of *Salmonella enterica* serovar Typhimurium in mice on both a fibrous diet and high-fat diet over time. When comparing *Salmonella* gene expression between diets, we found a preferential use of respiratory electron acceptors consistent with increased inflammation of the high-fat diet mice. Looking at the high-fat diet over the course of infection, we noticed heterogeneity of samples based on *Salmonella* ribosomal activity, which separated into three infection phases: early, peak, and late. We identified key respiratory, carbon, and pathogenesis gene expression descriptive of each phase. Surprisingly, we identified genes associated with host-cell entry expressed throughout infection, suggesting sub-populations of *Salmonella* or stress-induced dysregulation. Collectively, these results highlight not only the sensitivity of *Salmonella* to its environment but also identify phase-specific genes that may be used as therapeutic targets to reduce infection.

2.2 Introduction

Salmonella enterica serovar Typhimurium (*Salmonella*) is a leading cause of gastrointestinal disease worldwide, posing serious public health risk due to increasing

antibiotic resistance (1, 2). One challenge of controlling this pathogen is its broad metabolic capacity and adaptability to its environment. Recent studies have demonstrated that *Salmonella* infection can be modified with a robust microbiome and through diet manipulation (3–7). However, mechanisms explaining these diet-based phenomena remain understudied. Here we address this knowledge gap by leveraging deeply sequenced, time-series transcriptomics, to reveal the metabolism of *Salmonella* throughout infection.

As a facultative anaerobe, *Salmonella* outcompetes the native microbiota through stimulation of the host's inflammatory response and numerous virulence factors (8–11). In addition to oxygen diffusion into the gut lumen, the subsequent inflammatory response results in the generation of reactive oxygen and nitrogen species which produce respiratory electron acceptors such as nitrate, nitrite, dimethyl sulfoxide (DMSO), trimethylamine N-oxide (TMAO), fumarate, tetrathionate and thiosulfate (12–18). Furthermore, various carbon sources become available to *Salmonella* with inflammation. *Salmonella* can utilize host and microbial metabolic end products, such as lactate and ethanolamine, as well as microbial derived succinate, and more energetically favorable carbon sources (18–24). Most studies evaluating *Salmonella* substrate and electron acceptor use focus on a single compound. Moreover, these studies often do not track metabolism under different dietary conditions or over time, primarily focusing on late-stage infection processes.

Diet is a critical driver of gut microbiomes, influencing the gut metabolic landscape and microbial membership, which can alter colonization resistance against *Salmonella* (25–28). For example, high-fat diets (HFDs) result in increased inflammation

and host susceptibility to infection (29–31). Furthermore, prior research demonstrated that pre-treatment with a high-fat, low-fiber, Western diet was sufficient to break pathogen colonization resistance, resulting in increased susceptibility to *Salmonella* (3). Given the expansion of the Western diet globally (32), studying *Salmonella* pathogenesis and physiology in more realistic diet backgrounds is needed.

Changes in the microbial membership, chemical landscape of the gut, and host response are dynamic factors exploited by pathogens like *Salmonella* (9, 11, 13). Yet, time-series studies are limited in this field. Here we investigate *Salmonella* metabolic processes by analyzing gene expression from CBA mice fed fibrous or high-fat diets and over time. Pairing 16S rRNA sequencing, metatranscriptomic sequencing, lipocalin-2 analysis, and both targeted and untargeted metabolomics, we revealed known and previously unrecognized metabolic strategies that distinguish early, peak, and late infection phases. These data emphasize the importance of environmental context to *Salmonella* metabolism and demonstrate preferential expression of metabolic and pathogenic pathways by diet and infection phase. These key pathways could be targeted to abate enteric infection.

2.3 Results and Discussion

2.31 High-fat diet increases inflammation and *Salmonella* respiratory electron acceptor utilization

Using fecal samples, we assessed the role of diet on *Salmonella* infection by comparing the effects of a fibrous chow diet (Chow) or a high-fat diet (HFD) on *Salmonella* relative abundance, *Salmonella* gene expression, and mouse inflammation (**Figure 2.1A**). First, we used 16S rRNA amplicon sequencing (16S) to screen the

relative abundance of *Salmonella* per mouse and compared microbial community metrics between the two diets. We assessed nine *Salmonella* infected mice on days 8 (HFD) and 11 (Chow), along with paired pre-infection samples from the same mice (n=18). Days 8 (HFD) and 11 (Chow) were chosen as late infection samples based on disease severity (33), *Salmonella* relative abundance, and sample availability (see methods).

All selected mice, regardless of diet, were classified as high responders with *Salmonella* relative abundance >25% (**Figure 2.1B, top**) (9, 33). The HFD (97%) had a slightly higher average *Salmonella* relative abundance compared to the Chow (82%). Despite these slight differences, there was no significant difference in microbial diversity between the two treatments at late infection (ANOVA, p=0.386 and p=0.133) (**Figure S2.1**). Regardless of dietary treatment, the paired pre-infection samples exhibited decreased microbial richness and Shannon's diversity compared to their respective post-infection samples. Consistent with reports from others (3, 34), Chow pre-infection samples had significantly higher microbial diversity than HFD pre-infection samples (ANOVA, p<0.001 and p=0.008, respectively). This finding suggests that diet disrupts the microbiome, potentially impacting *Salmonella* physiology.

Along with *Salmonella* relative abundance, we measured lipocalin (Lcn-2) concentrations, which is a host-derived protein indicating inflammatory status (35). Lcn-2 concentrations (ng/g of feces) illustrated a significant increase in inflammation in HFD mice compared to Chow mice (**Figure 2.1B**, bottom) (ANOVA, p<0.001). Together, our 16S and lipocalin analyses illustrate that while diet alone can reduce microbial diversity,

the presence of *Salmonella* results in more pronounced inflammation in HFD mice during late infection.

To ensure higher fidelity of our experimental results and address potential strain heterogeneity that may have developed during laboratory maintenance of this strain (36–39), we constructed a draft genome for this *Salmonella* isolate. This pangenome was derived from a combination of short and long read sequencing (see methods). This strain-resolved genome shared 4597 called genes with 99.99% average nucleotide identity to the previously published *Salmonella* ATCC genome (SAMN08777876). Our sampling averaged 27.19 Gbp of sequencing per sample, generating 1,363,165,050 reads. The internally derived genome was used to map metatranscriptomic sequences from nine fecal samples (Chow=4, HFD=5) and resulted in consistent read mapping regardless of diet.

Prior reports have suggested that inflammation increases electron acceptor availability (8, 11, 12, 20), which favors *Salmonella* growth during infection. As such, we hypothesized that we would see increased respiratory electron acceptor expression concurrent with increased inflammation in the HFD. *Salmonella* gene expression revealed that oxygen (*cyoA* and *cydA*), nitrate and nitrite (*narZ*/*narG*, and *napA*), DMSO (*dmsA*), TMAO (*torC*), and fumarate (*frdA*) utilization genes were differentially expressed in the HFD compared to the Chow diet **(Figure 2.1C, Data set S2.2)**. Tetrathionate reduction (*ttrA*), while detected, did not show significant expression differences across diet treatments.

Additionally, when *Salmonella* encodes multiple genes for utilizing an electron acceptor, like oxygen and nitrogen, we observed increased expression of genes that

function optimally at higher substrate concentrations and are more energetically favorable. Specifically, in the HFD, *Salmonella* preferentially expressed the low-affinity oxygen (*cyoA*) and nitrate (*narZ/narG*) utilization genes, compared to the less inflamed Chow treatment where *Salmonella* activated the high-affinity oxygen (*cydA*) and nitrate (*napA*) utilization genes (40–44). Collectively, our results indicate that *Salmonella* exploits HFD-induced inflammation and suggests that *Salmonella* can finely tune its energetic strategy to local chemical conditions.

2.32 *Salmonella* respiration is structured by infection phase

Given the elevated inflammation and metabolic response in HFD mice, we were interested in observing the progression of *Salmonella* metabolism throughout infection. We collected fecal samples from five HFD mice 1, 2, 3 and 6 days before *Salmonella* inoculation, and continued daily sampling after inoculation until sacrifice (day 8). Fecal samples were processed for 16S rRNA amplicon sequencing (16S), metatranscriptomics (metaT), lipocalin-2 ELISA assays (Lcn-2), targeted short-chain fatty acids (SCFAs) metabolomics, and untargeted metabolomics (LC-MS) (**Figure 2.2A**). Amplicon sequencing was used to profile *Salmonella* relative abundance across all 60 samples, guiding metatranscriptomic sample selection (**Data set S2.1**). We collected metatranscriptomes during infection days 1, 3, 5, and 8, as well as day -1, analyzing 5,339,114,584 reads from 25 samples, with an average depth of 32.25 Gbp per sample (**Data set S2.2**).

Timeseries amplicon data showed that all HFD mice became high responders by day 5, but we note that there was heterogeneity among mice in the timing of peak *Salmonella* relative abundance (**Figure S2.2**). Consequently, we used expression of the

single-copy S3 ribosomal protein (*rpsC*) from *Salmonella* to group samples by infection phase relative to each mouse over time (**Figure 2.2B**). As shown in Figure 2B, peak expression of *Salmonella* varied over time and between mice. Using the relative increase of S3 gene expression per mouse, we clustered the samples into 3 infection phases: early (9 samples), peak, (5 samples), and late (6 samples) (**Figure 2.2C**) (see methods).

Using our metatranscriptomics data and the sample grouping described above, we compared expression of oxygen (*cyoABCDE* and *cydAB*), nitrate (*narGVZ*), TMAO (*torA*), tetrathionate (*ttrS*), thiosulfate (*phsABC*) and hydrogen (*hybC*) utilization genes (**Figure 2.3**). These genes were differentially expressed between the infection phases according to DESeq2 or clustering by GeTMM normalization (**Data set S2.2**). Our findings revealed selective utilization of respiratory electron acceptors during infection phases (**Figure 2.3**). Early and late infection phases exhibited increased expression of anaerobic respiration genes (*narGVZ*, *phsABC*), while peak phase showed increased expression of aerobic respiration genes (*cyoABCDE* and *cydAB)*. Of these respiratory complexes, only the catalytic subunits for tetrathionate showed no differential temporal signal; however, the sensor for tetrathionate (*ttrS*) was distinctive in the early samples. Consistent with prior research, this respiratory capacity provides *Salmonella* a competitive advantage against obligate fermentative microorganisms prevalent in the pre-infection gut (8, 33, 45–47).

Our findings indicate differential electron acceptor use along the infection gradient. It was not surprising to see oxygen use, the most energetically favorable electron acceptor, at peak infection when *Salmonella* ribosomal protein expression was

also highest, as the lumen becomes more oxygenated in response to *Salmonella* (13). Interestingly, genes encoding anaerobic respiration were more highly expressed in the early and late infection phases. These data also demonstrate that multiple electron acceptor genes are activated simultaneously in the same infection phase, possibly reflecting sub-population responses across the gut habitats (38, 48, 49), or co-metabolic regulatory control under common redox transcriptional regulators (50, 51), findings warranting further investigation. This dynamic gene expression highlights how an energetically versatile bacterium like *Salmonella* rapidly optimizes its energetic strategies to changing local chemical conditions during infection and as a consequence of host-pathogen-commensal microbiota interactions (23, 52–54).

2.33 Targeted and untargeted substrate profiles revealed during infection

Prior studies have reported the multitude of electron donors *Salmonella* can competitively utilize during respiration. In some cases, it is thought that the pathogen utilizes lower-energy carbon substrates not viable for commensal obligate fermenters. Some of these include ethanolamine and 1,2-propanediol, which have been suggested to be important for *Salmonella* expansion over commensal microbes (18, 20, 22, 55). Additionally, higher energy carbon sources, such as mannitol, arabinose, and galactitol, have been studied in relation to intracellular survival, *Salmonella* expansion, or competition (4, 6, 23, 56).

While tracking the expression of genes that utilize these carbon sources throughout different infection phases, we noticed significant expression changes for galactitol (*gatD*) during early infection. Notably, mannitol (*mtlA*, *mtlD*) and arabinose (*araA*, *araB*, *araD*) were expressed across all infection phases and did not uniformly

show enrichment over any infection phase. Additionally, ethanolamine (*eutC*) and 1,2-propanediol (*pduC*) were not discriminate of a particular infection phase but instead predominately expressed during both the peak and late phases. In summary, our study design allowed us to track gene expression of substrate use over time, adding new insights into *Salmonella* occupancy throughout infection.

Furthermore, our untargeted approach provided the potential to discover new putative substrates that may support *Salmonella* expansion, especially in this less-explored high-fat diet model. To do this, we examined the global clustering of substrate related gene expression, comparing it with our metabolite data from HFD infected, HFD uninfected, and Chow uninfected mice (**Data set S2.3, Data Set S2.4**). The genes for utilizing carbon substrates clustered by infection phase (MRPP, p=0.002), but not by mouse or time point (MRPP, p>0.05) (**Figure 2.4A**). Examining the differential expression of these carbon utilization genes across infection phases revealed distinct metabolic patterns. Early samples expressed a broader range of substrates, marked by differential expression of D-xylose isomerase (*xylA*). Supporting this, our metabolite data denoted consumption of xylose in the infected HFD samples **(Figure S2.3)**.

We also saw expression of carnitine utilization genes (*caiABCT*) (**Figure 2.4B**), which were differentially expressed in late samples compared to peak samples. Carnitine has been shown to stimulate anaerobic growth of *Salmonella,* which our respiration data suggest occurs at the early and late phases (**Figure 2.3**) (57, 58). Along with gene expression in late samples, we detected carnitine at day 7, the late phase of infection in HFD-infected mice **(Figure S2.3)**. Further research is needed to understand

the impact of these metabolisms on *Salmonella* growth and physiology, as well as the interactions with the surrounding community.

Consistent with aerobic respiration being a hallmark of peak infection, we observed simultaneous differential expression of genes for utilizing isocitrate, succinate, and malate *(icdA*, *mdh*, *sdhABCD*, *sucABCD*). It is possible that these genes were co-expressed with respiration due to roles in transforming intermediates of the tricarboxylic acid cycle, a critical component of aerobic respiration. However, it has also been shown that *Salmonella* can utilize microbially derived succinate as a substrate during aerobic respiration (21). Here the metabolite data showed less coordination with gene expression data, as succinate increased in the HFD-infected samples **(Figure S2.3)**. It is possible that succinate was not used by *Salmonella* more than its microbial production, or that the metabolite was additionally host-derived, as indicated in other HFD mouse studies (59). Unraveling the complex interactions of the host-microbiome-pathogen food web are warranted for this important gut metabolite (60).

Our targeted and untargeted transcriptomic approaches revealed the significance of lactate to overall *Salmonella* energy metabolism. Of the three lactate dehydrogenase genes *Salmonella* contains (*ldhA*, *dld,* and *lldD*), the *ldhA* and *dld* genes encode an enzyme specific for the D-isomer of lactate, while the *lldD* gene encodes a protein with specificity for the L-isomer (19). It is thought that the host only produces the L-lactate isomer, while the microbial members can produce both isomers. Studies with gnotobiotic or microbiota-reduced mice have demonstrated the importance of L-lactate dehydrogenase (lldD) for *Salmonella* in utilizing host-derived lactate (19). Our targeted data show that the *lldD* gene was a core member of the transcriptome, detected across

all time points but not distinguished by infection phase (**Figure S2.4A**). However, our untargeted approach revealed that genes for utilizing D-lactate (*ldhA* and *dld*), likely derived from microbial production, were differentially expressed during peak infection when *Salmonella* was likely most rapidly growing based on respiration genes and ribosomal protein expression (**Figure 2.4B**). Additionally, our metabolite data confirmed elevated levels of this compound at day 7 of infection relative to non-*Salmonella* inoculated mice on either diet **(Figure S2.3)**, indicating production exceeding consumption during the late phase of infection. This finding suggests new cross-feeding between the microbiome and *Salmonella*.

2.34 Non-nutritional genes expression patterns have implications on pathogenesis and horizontal gene transfer

Beyond nutritional requirements, we mined our data for other genes that were differentially expressed between phases and found categories of *Salmonella* pathogenesis genes which could be potential targets for therapeutic interventions. Volcano plots revealed gene expression patterns associated differentially expressed genes between phases (early to peak/peak to late) with the following categories: (i) not significant (5549/6473), (ii) conjugation genes (22/NA), (iii) motility genes (9/28), (iv) outer membrane genes (33/13), (v) phage-like genes (22/4), (vi) other significant genes (940/141), and (vii) hypothetical genes (127/17) (**Figure 2.5**, **Data set S2.2**).

In early samples, we observed an upregulation of conjugation, motility, and fimbriae related genes (**Figure 2.5A and Figure S2.5**). Conjugation facilitates the spread of virulence genes within *Salmonella* populations, influencing pathogen evolution (61). Additionally, motility and fimbriae genes support *Salmonella* movement and adhesion, which assist interactions with colonocytes and trigger the immune response

(62–65). Noteably, motility genes were also differentially expressed in the late phase compared to the peak phase. We consider it possible that *Salmonella* has enhanced chemotaxis to find nutrient sources during the late phase or for environmental entry (66, 67).

The expression of many pathogenesis genes could not be discriminated by infection phase. For instance, type III secretion protein genes (*invAG*, *sptP*, *sspH12*, *srfJ*) **(Figure S2.4**, **Data set S2.2)** were detected and highly expressed in all infection phases. Given their presumed role in initiation of infection and the inflammatory response, it was somewhat surprising that these genes were not discriminant of early expression but instead, seemed active over the course of infection. We consider it possible that sub-populations of *Salmonella* may be infecting host cells continually or inflammation-induced envelope stress might alters the expression of these genes, explaining their chronic expression (48, 68–70).

Comparatively, peak samples differentially expressed various outer membrane associated genes (**Figure 2.5B, Figure S2.6**). Many of these genes were responsible for colanic acid synthesis (*wcaACDEFIJLM*). These genes protect bacteria from osmotic stress and are linked to biofilm formation (71–73). Additionally, cellulose synthase (*bcsA*), a sigma factor regulating genes controlling biofilm formation (*rpoS*) and a biofilm-dependent modulation protein were differentially expressed in the peak phase. Other biofilm-related genes (*adrA*, *csgACEFG*, *bcsBCE*, *mrlA*, *ompR*) are highly expressed in the peak and late phases. This aligns with previous findings identifying *Salmonella* luminal biofilms, where nitrate mediates two *Salmonella* populations resulting in virulent, planktonic cells and survival-adapted biofilm cells (74). Moreover,

we detected differential expression of phage-like genes in both early and late infection. These prophage regions of the *Salmonella* genome carry virulence factors and are important for infection (47, 75, 76). Inflammation has been shown to boost prophage transfer between *Salmonella* species (77), but the role of phage in controlling *Salmonella* pathogenesis requires further investigation.

2.35 Conceptual model of *Salmonella* metabolism

In conclusion, this research contributes to the development of a conceptual model illustrating how the high-fat diet background impacts *Salmonella* gene expression **(Figure 2.6, Data set S2.2).** We show that *Salmonella* responds to a highly inflamed gut environment and tactically uses respiratory electron acceptors and carbon sources over time. For example, our findings indicate differential isomer utilization of lactate, a critical gut SCFA (19). These findings highlight the potential microbial cross-feeding as well as affirm lactate consumption across infection phases, supporting the importance of this metabolite to *Salmonella*. When possible, we supported the gene expression data with metabolite data to provide additional insights into gut metabolite transformations (**Figure 2.6**).

We also provide evidence for expression of genes related to pathogenesis, motility, and biofilms over the course of infection (**Figure 2.6**). Surprisingly, virulence factor genes were not confined to the early stages when they are thought to function, but expressed across infection, indicating a heterogeneity in infection processes that were active even in a well-controlled, clonal experimental mouse model. Our discoveries benefitted from a well-established intellectual framework from years of detailed, curated pathogen physiological inquiry (11, 13, 78). This infrastructure

provided a solid foundation that we could both validate and build upon. Simultaneously, our work opens new avenues for research, offering fresh perspectives and opportunities for further exploration. Moreover, this study offers a distinctive outlook on the early phases of *Salmonella* infection, providing gene expression data on days 1, 3, and 5 post-inoculation. These insights may enable research into curbing *Salmonella* proliferation during this critical period.

2.4 Conclusion

Despite being one of the most studied microbes, knowledge of *Salmonella* metabolism and pathogenesis in relevant diet contexts and across infection phases remains limited. Here, we addressed this knowledge gap using a multi-omics approach, allowing us to examine existing theories (targeted approach) and develop new potential hypotheses (untargeted approach). In the targeted approach, we applied existing scientific knowledge to investigate specific genes previously implicated in *Salmonella* metabolism (4, 12, 13, 19–22), while the untargeted approach mines the data generated here for newly expressed functionalities discriminant of different infection phases. This work demonstrates the importance of time-dependent analysis in comprehending the finely-tuned gene expression of *Salmonella* in response to the dynamic pathobiome environment.

This research lays the foundation for understanding how *Salmonella* pathogenesis and metabolism change under realistic dietary conditions within a dynamic gut ecosystem. Unraveling the intricacies of *Salmonella* metabolism can reveal key interaction junctures with the host and surrounding microbiota. The practical implications of this study may extend to the development of targeted therapeutics

designed to disrupt specific pathogenic pathways or molecules, with the aim of minimizing adverse effects on host and microbiome functionalities.

2.5 Materials and Methods

2.51 Strains and media

Salmonella enterica serovar Typhimurium strain 14028 was cultured at 37 °C in Luria-Bertani (LB) broth overnight. This culture was washed and resuspended in water for inoculation.

2.52 Mouse experimentation and sample collection

Female CBA/J mice from The Jackson Laboratory (Bar Harbor, ME) were housed by treatment, with five mice per cage. Mice were fed either a fibrous chow diet (with 5.8% fat and 18.3% fiber, formula 7012, Teklad Diets) or a high-fat, no-fiber diet (with 36% fat and 0% fiber, formula F3282, Bio Serv) for six days before infection. Five HFD mice and 12 Chow mice were not inoculated with *Salmonella*, while the remaining mice (HFD=5, Chow=43) were inoculated with 10^9 CFUs of *S. enterica* Typhimurium strain 14028 via oral gavage on day 0 without treatment throughout the course of infection. Mice for multi-omics analysis were selected based on fecal sample availability. High responders among chow-fed, infected mice were chosen based on *Salmonella* reaching ≥25% relative abundance at any timepoint. Noteably, unlike HFD mice, most Chow mice (n=30) are not high responders. Animal experimentation was approved by the Ohio State University Institutional Animal Care and Use Committee (IACUC; OSU 2009A0035). HFD mice were sacrificed on day 8 due to severe disease, following IACUC protocols, while Chow mice were sacrificed on day 16. Fecal samples were collected daily starting at diet transition until sacrifice (except on day -5 and -4) on

autoclaved aluminum foil, transferred into labeled microcentrifuge tubes, and flash-frozen in liquid nitrogen. Samples were stored at -80°C until further processing.

2.53 DNA and RNA extraction and sequencing

Total nucleic acid was extracted using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research) and stored at −20 °C until amplicon sequencing could be performed. Amplicon sequencing was submitted to Argonne National Lab at the Next Generation sequencing facility, using the Nextera XT DNA Library Preparation kit (Illumina) (**Data set S1)** and the Illumina MiSeq with 2 x 251 bp paired-end reads following HMP protocols (81). PCR amplification (30 cycles) of the V4 hypervariable region of the 16S rRNA gene was conducted with universal primers 515F and 806R, with the 515F primer containing a unique barcode.

RNA was extracted using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research) and stored at −80 °C until metatranscriptomic sequencing could be performed. RNA clean-up and library prep were performed using either the Ribo-Zero(TM) rRNA Removal Kit (Epicentre) with the Illumina Truseq Stranded RNA LT kit (Illumina) or Zymo-Seq RiboFree Total RNA Library Kit (Zymo Research)(**Data set S2.1)**. Chow samples were sequenced on the Illumina HiSeq2500 platform using 151bp paired-end reads at the Genomics Shared Resource facility at the Ohio State University. High-fat diet samples were sequenced on the NovaSEQ6000 platform on a S4 flow cell using 151bp paired-end reads at the University of Colorado-Anschutz Medical Campus at the Genomics Shared Resource Center.

2.54 16S rRNA Amplicon Sequencing Analysis

Data was processed using Qiime2 2019.10 (82) with specific steps described here. In short, raw data fastq files were demultiplexed in Qiime2. Then DADA2 was used for quality filtering, dereplication, denoising, removing chimeras and merging sequences. Amplicon sequence variant (ASV) taxonomy was determined via SILVA release 132 SSU Ref NR 99 (83). Counts were filtered to ASVs with at least 10 reads in at least 5 samples. ASV feature table and taxonomic assignment are included (**Data set S1**).

2.55 Long read sequencing and *Salmonella* pangenome generation

Genomic DNA for long read sequencing was extracted from our *Salmonella enterica* serovar Typhimurium strain 14028 isolate using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research). Library preparation was performed using the Genomic DNA by Ligation (SQK-LSK 109) kit by Oxford Nanopore following the manufacturer's instructions and sequenced on the Flongle Flow Cell (R9.4.1) (Oxford Nanopore Technologies, Oxford, UK). Bases were called using Guppy (v 5.0.11), assembled using Flye (v2.8.3), and polished with long reads (84, 85). Our pangenome was created by concatenating called genes [DRAM (v1.4.0) (86)] from our highest quality *Salmonella* short read metagenome assembled genome (33) and our best long read *Salmonella* assembled genome. After filtering duplicate genes at 99% minimum sequence identity using Mmseqs2 (Release 7-4e23d) (87), our *Salmonella* pangenome is 99.99% identical to the Joint Genome Institute *Salmonella* isolate. Genes were annotated using DRAM (v1.4.0) (86).

2.56 Metranscriptomics Data Analysis

Reads were quality trimmed and had adapters removed using bbduk.sh (v38.89) (88) and mapped to our *Salmonella* pangenome using bowtie2 (v2.4.5) (89) using flags -D 10 -R 2 -N 0 -L 22 -i S,0,2.50. Mapping files were filtered for high sequence identity (≥97%) using reformat.sh (88) and sorted by sequencing name using Samtools (v.1.9) (90). Counts were generated using htseq using flags -a 0 -t CDS -i ID --stranded=reverse (v21.0.1) (91) and normalized using DESeq2 (92) or GeTMM (93) in R. For DESeq2 normalization, groups were based on infection phases (early, peak, and late). Infection phases were determined by GeTMM normalized *Salmonella* S3 ribosomal protein (*rpsC*) expression. Samples were grouped into infection phases (early, peak, and late) based on the greatest increase in *Salmonella* S3 ribosomal protein per mouse, which is the peak sample for that mouse. Any samples before the peak S3 ribosomal protein expression were the early phase, whereas any samples after were the late phase. The five samples prior to *Salmonella* infection on day -1 were used as a control for nonspecific mapping.

2.57 Lipocalin-2 ELISA Assay

Fecal samples were homogenized in PBS containing 0.1% Tween 20 (100 mg/ml) for 20 minutes then the resulting suspension was centrifuged at 12,000 rpm for 10 minutes at 4 °C. The inflammation marker, Lipocalin-2, was measured from the resulting supernatant using the Duoset murine Lcn-2 ELISA kit (R&D Systems, Minneapolis, MN). Measuring lipocalin-2 (Lcn-2) is a tractable, sensitive marker of host inflammation (35).

2.58 Metabolomics Sequencing and Analysis

For untargeted metabolomics, we used a 1mL solution of three solvents (water/methanol/dichloromethane,1/2/3, v/v/v) to extract metabolites from fecal samples, disrupted with a sonicator(Bioruptor®, Diagenode, Belgium). The resulting aqueous layer suspension was analyzed using Ultimate 300 liquid chromatography coupled to Thermo Q-Exactive plus mass spectrometer (Thermo Fisher Scientific, CA, USA) coupled to a mass spectrometer with two different separation columns (reverse phase liquid chromatography and hydrophilic interaction liquid chromatography (HILIC)) for metabolome analysis. For reverse phase separation, water with 0.1% (v/v) formic acid and acetonitrile with 0.1% (v/v) formic acid were used as mobile phases. The flow rate was set at 0.3 mL/min with the gradient as follows: 2% B for 0-2 min; 2%-30% B for 4 min; 30%-50% B for 8 min, 98% B for 1.5 min, and held at 98% B for 1min, then returning into initial gradient for equilibrium for 1.5 min. For HILIC separation, ACQUITY UPLC® BEH HILIC 1.7 µm (2.1 X 150 mm) was used. Water/acetonitrile with 0.1% formic acid and 10 mM ammonium formate were prepared as solvent A (95/5, v/v) and solvent B (5/95, v/v). For gradient elution, 99% B was held for 2 min, gradually reduced to 75% B for 7 min and reduced again to 45% B for 5 min. The gradient was held at 45% B for 2 min, returned to the initial gradient and re-equilibrated for 5min. The flow rate was set at 0.3 mL/min. The quality control (QC) sample was prepared for each sample and analyzed after every 6 samples. For data processing, peak-picking and metabolome annotation were processed with MS-Dial (v.4.90) (94).

For targeted metabolomics, the short chain fatty acids were extracted as described above, and prepared using a previously published method (95). Briefly, 200 mM 3-NPH (3-nitrophenylhydrazine), 200 mM EDC (*N*-(3-dimethylaminopropyl)-*N*′-

ethylcarbodiimide) and pyridine were added to extracted fecal SCFAs. Isotope-labeled SCFAs ($^{13}C_2$- acetic acid, $^{13}C_3$- propionic acid, and $^{13}C_4$ – butyric acid) were added as an internal standard before derivatization. LC-MS/MS analysis of SCFAs was conducted using the ultimate 300 liquid chromatography and Thermo Quantiva Triple Quadrupole mass spectrometer (Thermo Fisher Scientific, CA, USA). Total run time for LC was 10 min with water with 0.1% of formic acid as mobile phase A and acetonitrile with 0.1% of formic acid as mobile phase B. The gradient started with 2% B, held for 0.5 min, linearly increased up to 98% B for 8 min, and re-equilibrated in 2% B for 1.5 min. Multiple concentration of standard SCFAs (acetic acid, propionic acid, and butyric acid) were prepared alongside fecal SCFAs for quantitative analysis. The collected MS data were analyzed with Skyline (96).

2.59 Statistical analysis and Data Availability

Alpha diversity metrics, richness and Shannon's diversity, and significance values were calculated in R using the vegan package (v2.5-7) (97). To compare carbon utilization expression patterns among samples, Bray-Curtis dissimilarity was calculated using 406 *Salmonella* carbon utilization genes annotated by DRAM (**Dataset S2.3**). Annotation calls for CAZymes, central carbon, hydrocarbon, and pyruvate metabolism were selected as well as carbon associated genes from our *Salmonella* DRAM module. Non-parametric multidimensional scaling (NMDS) plots were created using R (ggplot2 package v3.3.5, and the vegan package (v2.5-7) for visualization and non-parametric-fit quality was determined by stress value (97–99). Significance of infection phase carbon utilization expression differences was determined by analysis of similarity (ANOSIM) and multiple response permutation procedure (MRPP) (97). Heatmaps were generated

with GeTMM normalize expression scaled by gene using the R package pheatmap (v1.0.12) (100).

All data files and R scripts to generate figures are available in Github at https://github.com/Kokkinias/HFDtimeseries. All *Salmonella* MAGs and raw data is deposited at the National Center for Biotechnology Information (NCBI) under accession number PRJNA348350. The gene delineated *Salmonella* pangenome is available in Zenodo at DOI: 10.5281/zenodo.10479610. All supplemental files and figures are described in **Appendix A**.

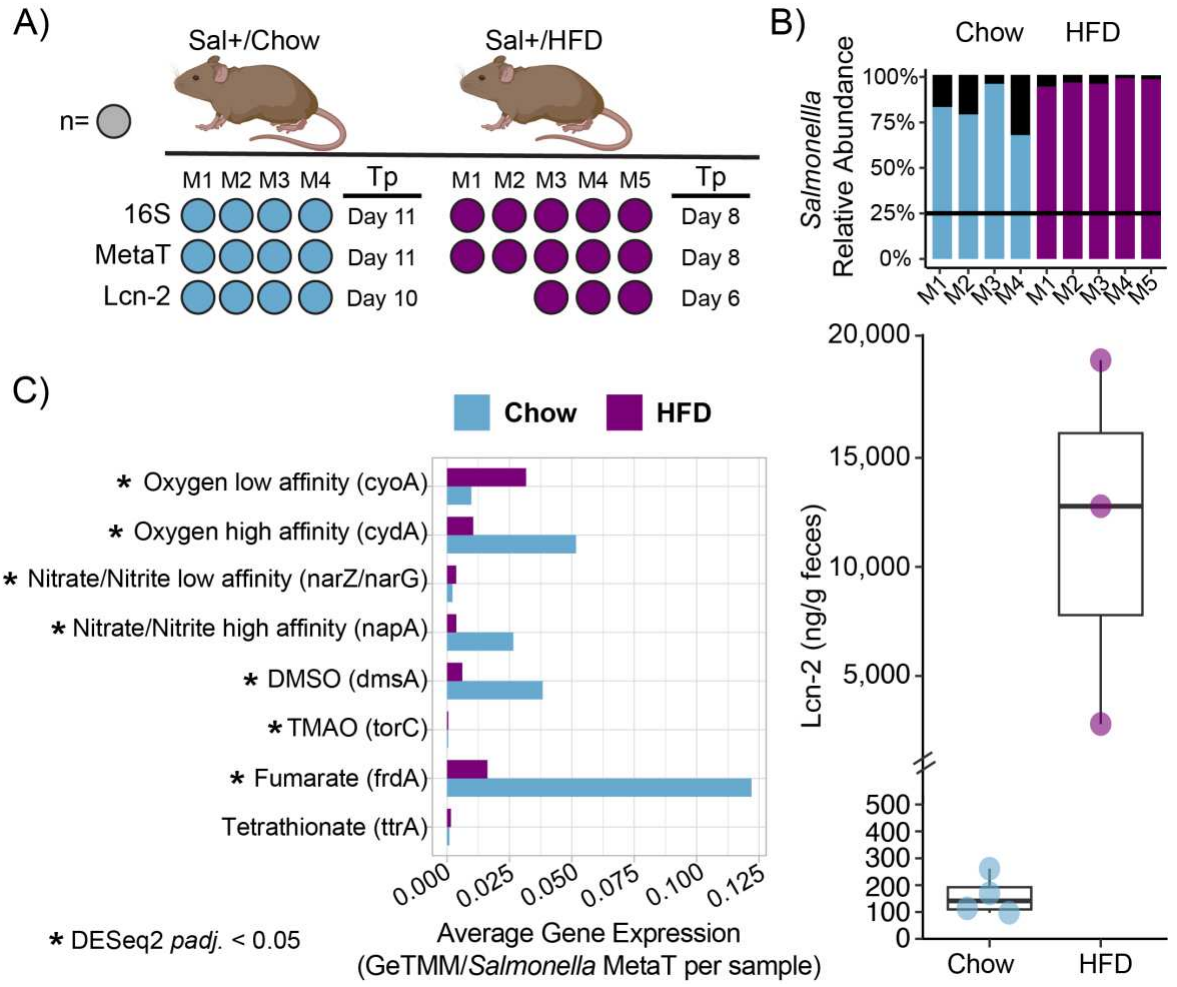

Figure 2.1: Increased inflammation and use of respiratory electron acceptors when comparing HFD and Chow mice

Experimental design figure describes the number of mice, the dietary treatment, and types of analysis that were conducted. 16S rRNA amplicon sequencing (16S), metatranscriptomics (MetaT), and lipocalin-2 ELISA assays (Lcn-2) were conducted on fecal samples from mice fed either chow diet (Chow, blue) or high-fat diet (HFD, purple) with timepoint (Tp) indicated. B) Box plot shows the median and Q1/Q3 -/+ 1.5 interquartile range of concentrations of Lipocalin-2 (ng/g), as a measure of inflammation, from fecal samples for Chow and HFD mice. Stacked bar chart shows *Salmonella* abundance (colored blue or purple based on diet) for each mouse relative to the rest of the microbial community (denoted by black bars) as determined from 16S rRNA amplicon sequencing. Black line denotes samples that are high responders (25% *Salmonella* relative abundance). C) Bar chart shows average *Salmonella* gene expression as gene length corrected trimmed mean of M-values (GeTMM) for key respiratory electron acceptors between Chow and HFD mice. Asterisk (*) indicates that the gene was significantly expressed between dietary regimes by DESeq2 (*adjusted p* value <0.05).

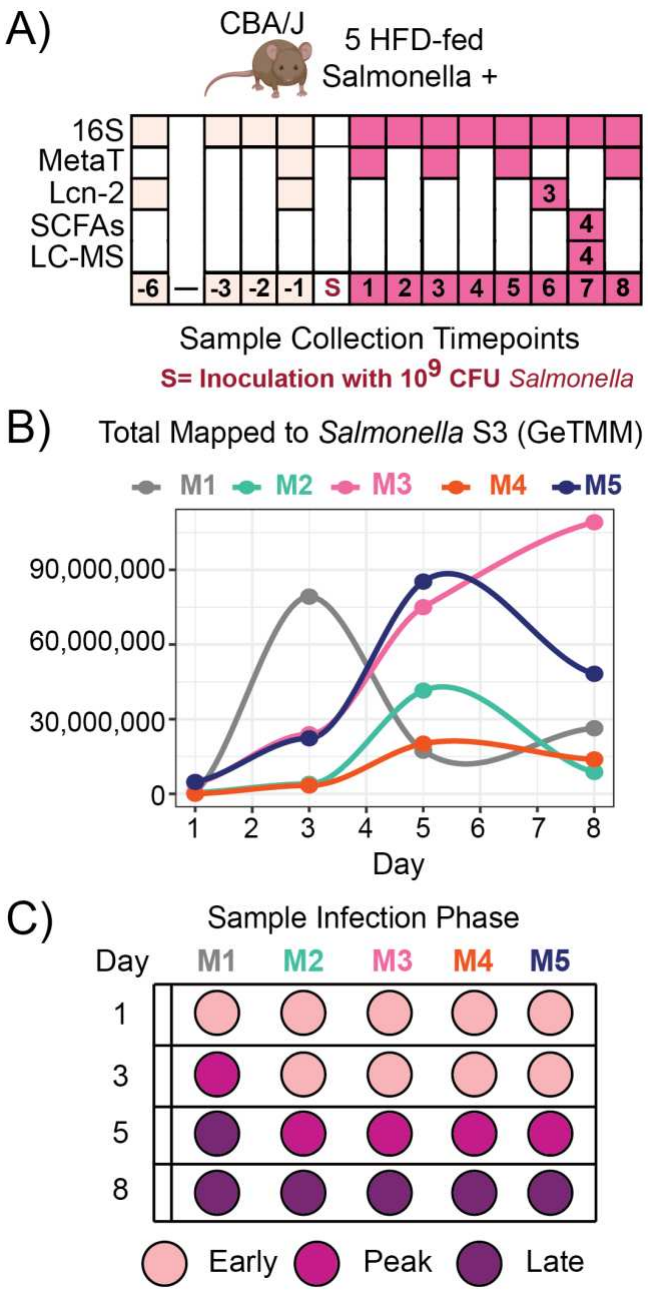

Figure 2.2: *Salmonella* infection heterogeneity over time
A) High-fat diet (HFD) experimental design of 5 mice describes the types of analysis that were conducted on fecal samples over time. 16S rRNA amplicon sequencing (16S), metatranscriptomics (MetaT), lipocalin-2 ELISA assay (Lcn-2), targeted short-chain fatty acids (SCFAs) metabolomics, and untargeted metabolomics (LC-MS) were conducted prior to infection (light pink) and after inoculation with 10^9 CFU *Salmonella* (dark pink). Analyses were performed from fecal samples from all 5 mice, unless noted otherwise by numbers within colored boxes. B) The line plot shows normalized single-copy marker gene expression (GeTMM) of the *Salmonella* S3 ribosomal protein per mouse over time. C) Given the heterogeneity of *Salmonella* gene expression over time, samples were grouped into infection phase (early: light pink, peak: dark pink, and late: purple).

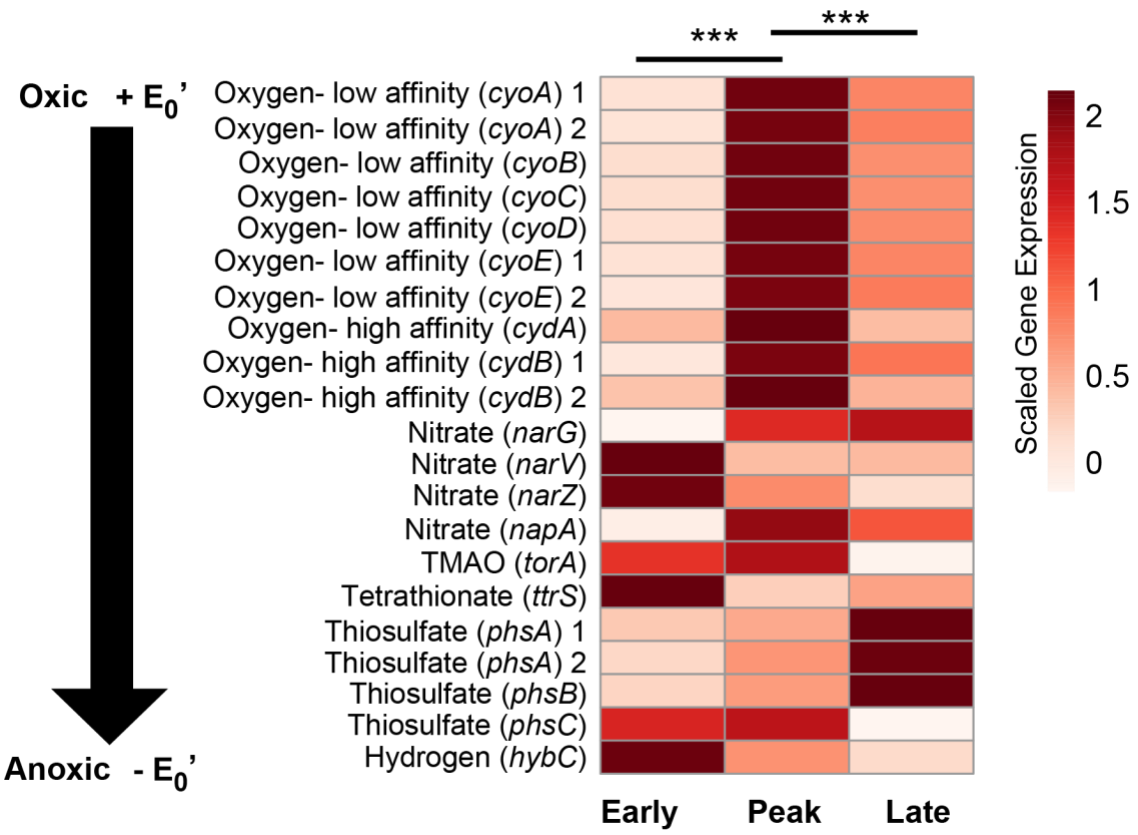

Figure 2.3: Respiratory electron acceptors utilization by infection phase
Heatmap of the mean, normalized gene expression from respiratory electron acceptor utilization genes mapped to the *Salmonella* pangenome shows patterns between infection phases. Genes are listed from oxic to anoxic (black arrow), and 15 samples are grouped by infection phase (early, peak, and late). Asterisks indicate statistical significance between phases where *** is a *p* value of <0.001.

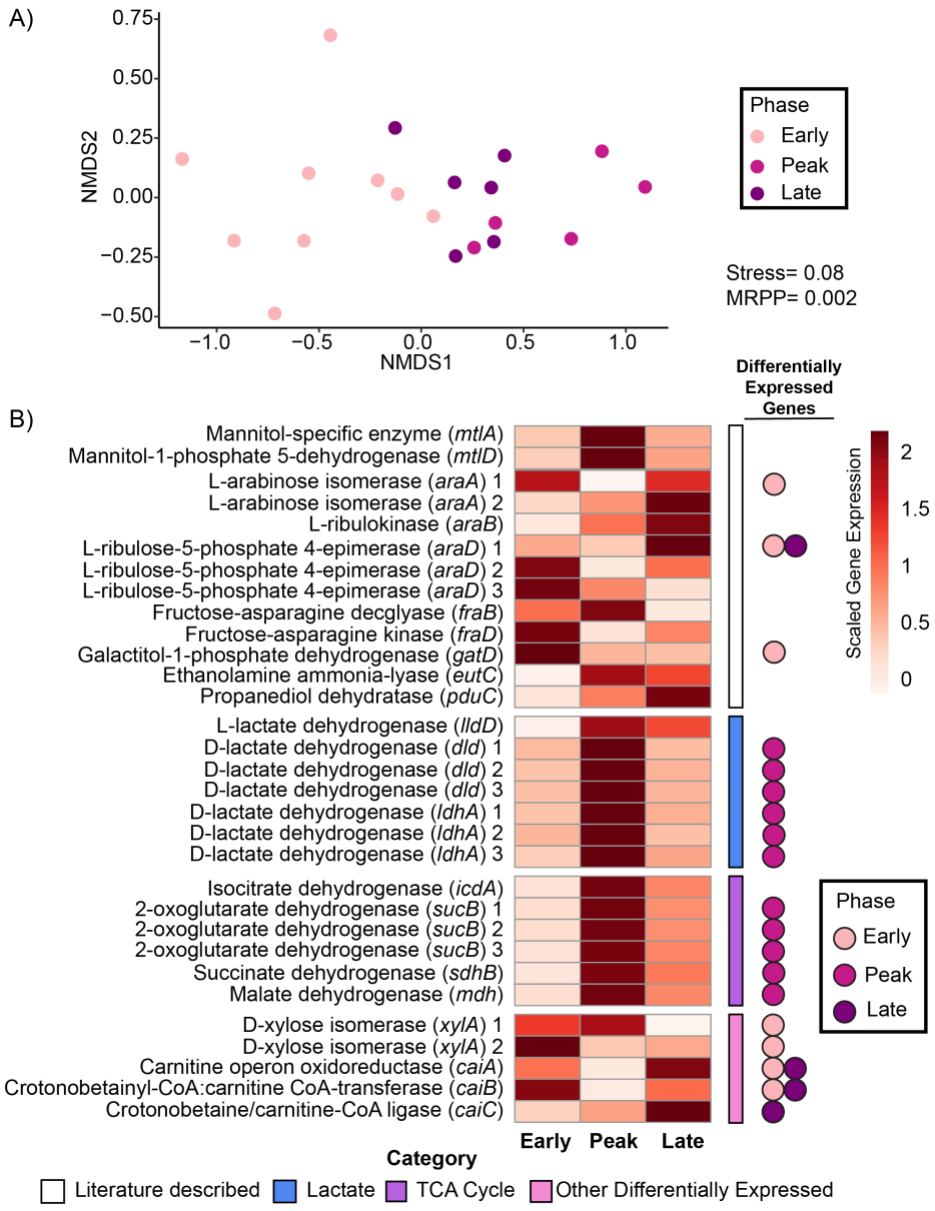

Figure 2.4: Differential expression of *Salmonella* carbon utilization genes by infection phase

A) Non-parametric multidimensional scaling (NMDS) depicts annotated *Salmonella* carbon utilization gene expression of samples colored by infection phase (early: light pink, peak: dark pink, and late: purple). Bray-Curtis dissimilarity matrix from early, peak and late samples (stress=0.08) show significant grouping of *Salmonella* carbon utilization genes by infection phase (MRPP, *p*=0.002) as found in **Data set S2.3**. B) Heatmap of the mean, normalized carbon utilization gene expression from our *Salmonella* pangenome shows patterns of carbon utilization by infection phase. Genes are grouped by carbon categories (Literature described carbon: white, SCFA: blue, TCA cycle: purple, Other differentially expressed carbon: bright pink). Genes that were characteristic of a specific phase (DESeq2 *padj.* >0.05) are denoted on side by colored circles (early: light pink, peak: dark pink, and late: purple) (**Data set S2.2**).

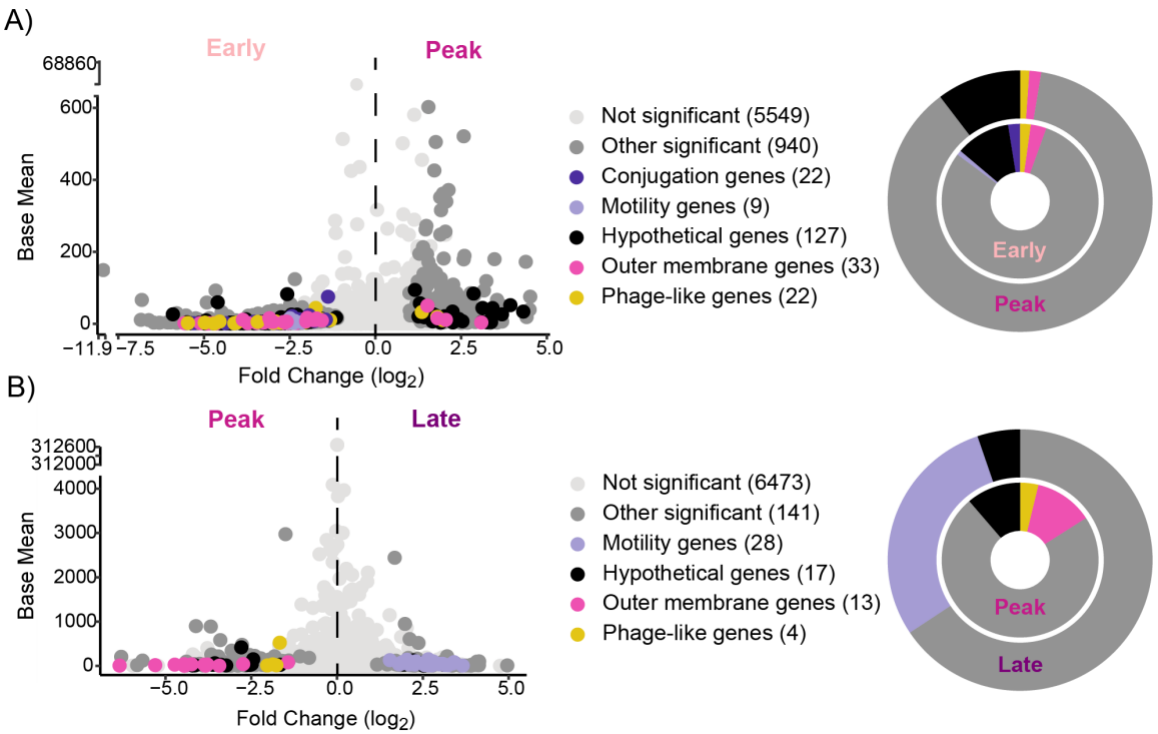

Figure 2.5: *Salmonella* pathogenesis gene expression between infection phases
Volcano plots (left) display differentially expressed genes between (A) early and peak or B) peak and late samples. Each point represents a single gene, with point color indicating significance (DESeq2, *padj.* >0.05) and annotation category. Numbers in parentheses next to each category indicate the number of genes in the *Salmonella* pangenome represented. Donut plots (right) highlight the proportion of significant genes within each category.

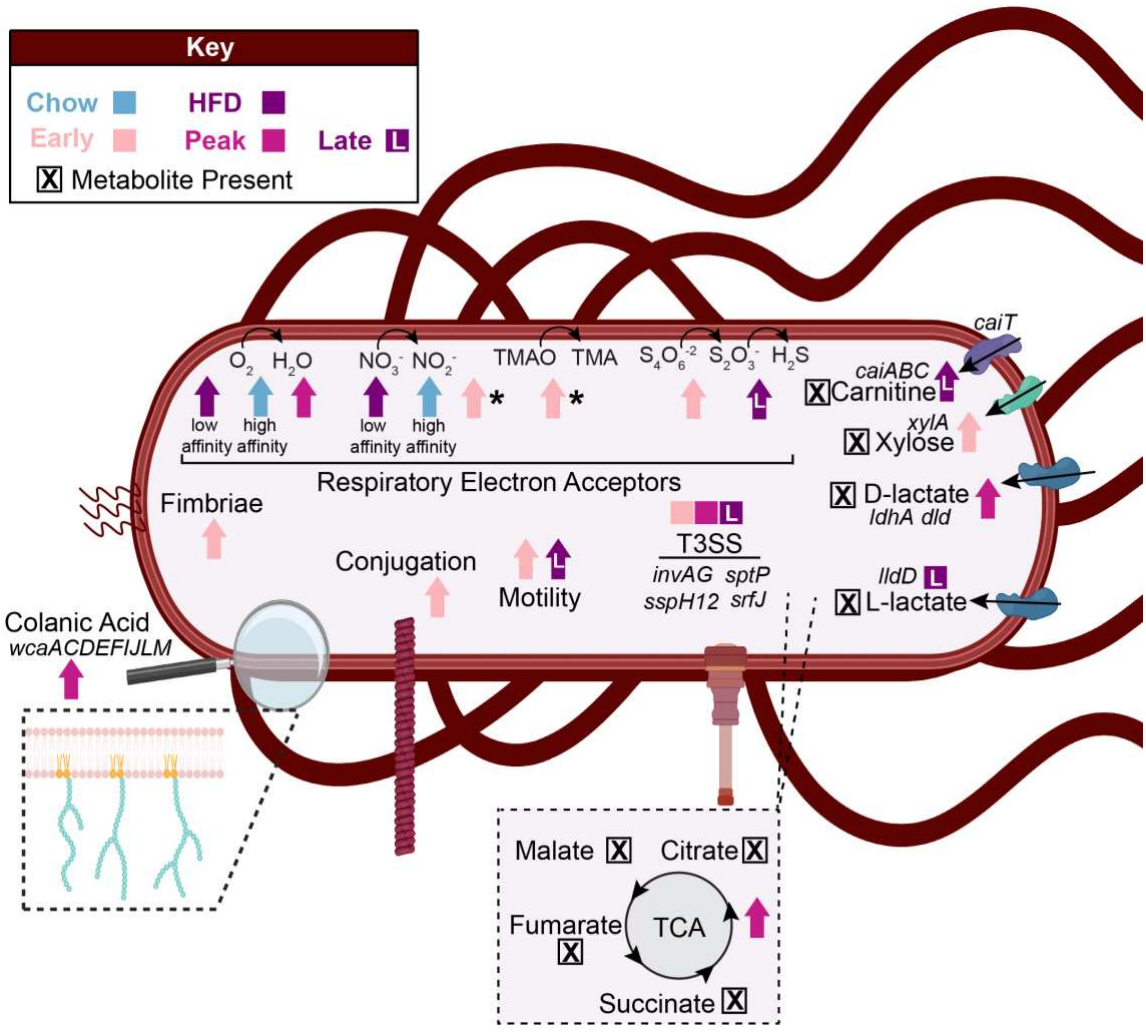

Figure 2.6: Conceptual model of findings across diet treatments and infection phases

Conceptual model summarizes our findings with regards to respiration, carbon utilization, other pathogenesis pathways, and non-differentially expressed active genes. Arrows indicate the treatment (chow: blue, high-fat diet: purple) or infection phase (early: light pink, peak: dark pink, late: purple with the letter "L") with differential expression. Asterisk (*) next to an arrow indicates differences between DESeq2 and GeTMM, where DESeq2 was prioritized for figure creation. Boxes with an "X" show metabolite presence (**Data set S2.2, Data set S2.4**).

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Chapter 3: CBA_DREAMM: a diet and time-resolved multi-omics CBA gut microbiome resource

3.1 Summary

The CBA mouse is an important model for studying gut microbial interactions; however, a microbiome infrastructure for this model did not exist. We announce a fecal CBA-specific microbiome resource that spans diet, time, and pathogen gradients. This resource includes amplicon, metagenomic, and metatranscriptomic sequencing, and metabolomics, to unveil microbial functionalities.

3.2 Introduction

The CBA mouse model is commonly used for immunology and enteric pathogen research, particularly for evaluating the impacts of *Salmonella enterica* serovar Typhimurium (*Salmonella*) during inflammation (1, 2). Notably, these mice display disease progression analogous to the disease manifestation in humans with localized gut infection rather than systemic spread. Specifically, CBA mice tolerate a high *Salmonella* load without antibiotic pretreatment, allowing a longer temporal infection study period and study of commensal microbes (3–5).

Additionally, diet plays a crucial role in chemical dynamics, microbial composition, and pathogenesis in the gut (6, 7). Studies illustrate that high-fat diets can break colonization resistance to enteric pathogens (8–11). Shifts towards high-fat diets and a rise in antibiotic resistance emphasizes the necessity for targeted therapeutics (12, 13). Understanding the metabolic relationships of pathogens, like *Salmonella*, over time and in different diet backgrounds is challenging but essential for designing interventions that minimize harm to the host and microbiome.

By pairing multi-omics technologies, we can better characterize these microbial interactions, yet robust genome-resolved resources in mouse models with representative diets across time are limited. To address this, we presented CBA_DREAMM (CBA Data Repository of Expression, Amplicon, Metagenomic, Metabolites) which incorporates 16S rRNA amplicon sequencing, metatranscriptomic sequencing, bacterial metagenome assembled genomes (MAGs), viral MAGs, as well as both targeted and untargeted metabolites across various time points, diets, and pathogen perturbations. This resource serves as a comprehensive tool for research in pathogenesis and in the CBA mouse model.

3.3 Results and Discussion

Together, CBA_DREAMM contains 1754 16S rRNA amplicon samples, 17 deeply sequenced metagenomic samples (972 Gbp), 61 time-series metatranscriptomics samples (1521 Gbp), 2742 untargeted metabolomics samples, and 13 targeted short chain fatty acid samples, adding diet and time dimensionality (3, 5, 14–16). Fecal samples were collected from mice infected with *Salmonella* and pretreated with a high-fat diet, uninfected mice fed a high-fat diet, mice infected with *Salmonella* and fed a fibrous chow diet, uninfected mice fed a fibrous chow diet, and mice fed a fibrous chow diet and inoculated with dextran sodium sulfate as an inflammatory control. Specific methods for mouse experimentation with approval by the Ohio State University Institutional Animal Care and Use Committee (IACUC; OSU 2009A0035), DNA and RNA extraction, bacterial and viral genome generation, metatranscriptomic analysis, and targeted short chain fatty acid (SCFA) and untargeted metabolomics are detailed here (3, 5, 14–16).

Amplicon sequencing raw data for all samples was processed using Qiime2 2019.10 with specific steps described here (17). In short, raw data fastq files were demultiplexed in Qiime2. Then DADA2 was used for quality filtering, dereplication, denoising, removing chimeras and merging sequences. Amplicon sequence variant (ASV) taxonomy was determined via SILVA release 132 SSU Ref NR 99 (18) (see data accessibility). Our genome-resolved metagenome database was assembled using previously published methods (5, 15). Critical to this mission was the collection of deep metagenomic sequencing, with over 11 times more sequencing depth per sample (mean ~57 Gbp/sample) than the 5 Gbp commonly used in most gut metagenome studies (19–21).

Together, we provide i) 23,668 amplicon sequencing variants, ii) 970 MQHQ bacterial MAGs, iii) 2351 viral MAGs, of which 160 bacterial genomes and 609 viral genomes are unique when dereplicated (5), iv) ~25 Gbp/sample of metatranscriptomic data across time and in two distinct diet backgrounds, v) 1220 annotated metabolites through untargeted metabolomics, and vi) targeted metabolite concentrations of three important SCFAs. Novelty highlighted in this work includes identifying MAGs at early infection time points (e.g. day 1 after inoculation), phage genomic content (viral MAGs) which are known drivers of microbiomes, high-fat diet relevant MAGs, and the ability to detect metabolic nuance through our multi-omics approach. For example, in a fibrous chow diet background, *Salmonella*-induced inflammation reduced the abundance of dominant members like *Clostridia*, favoring the survival of *Lactobacillus* and *Enterococcus* (3, 5). Lastly, the amplicon and SCFA analyses highlighted the role in pathogen dosing and inflammation in microbiome restructuring (3). Ultimately, these tools become more

powerful when combined, describing complex interaction across time, diet, and the pathobiome. These insights may lead to the development of rationally designed therapeutics in the context of an ever-changing microbial landscape. Multi-omics data tools, such as CBA_DREAMM, are crucial for advancing knowledge of these complex gut ecosystems and enabling researchers with limited computational resources access to valuable information.

3.4 Data availability statement

The sequence data supporting the results of this article are available in the National Center of Biotechnology Information (NCBI) under Bioproject number PRJNA348350. All sequencing outputs, taxonomy, and annotations are included on Zenodo (https://doi.org/10.5281/zenodo.10929037)

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Chapter 4: Examining Public Perceptions of Microbes and Microbiomes in the United States

4.1 Summary

Within a changing research and media landscape, misconceptions and misinformation about microorganisms and microbiomes can arise, necessitating improvements in science communication practices through insights in public perceptions of the microbial world. Yet, little is known about public perceptions of microorganisms and microbiomes, making it difficult to develop tailored messaging. Here we perform an inductive thematic analysis with interviews and surveys from thirty adults across the United States to identify key factors to enhance science communication efforts about microorganisms and microbiomes. Together, our results underscore the importance of 1) recognizing the existing and desired future knowledge of an audience, 2) aligning with broader socio-scientific issues that resonate with people in relevant channels using social networks, 3) fostering collaboration between microbiologists, social scientists, and communicators to improve messaging, and 4) appealing to people's values and emotions to establish meaningful connections.

4.2 Introduction

Microorganisms (microbes) and microbiomes are vital to life on earth and drive processes related to carbon and nutrient cycling, produce secondary compounds like antibiotics, and facilitate gas exchange which have ramifications on human, animal, plant, and soil health (1–3). Despite their broad impacts, “microbes” is a vague term that could refer to bacteria, viruses, fungi, or protozoa (4). The term “microbiome” describes microbes from an ecological perspective and refers to a community of microorganisms

and their genomes in an environment at a particular point in time, including both biotic and abiotic factors (5). The importance of science literacy of microbial terms and functions becomes more apparent with the increase of emerging infectious diseases and global environmental challenges related to climate change (6–9). During the COVID-19 pandemic, the complex, ever-changing landscape of microbial research made science communication efforts challenging, which gave rise to misinformation and an “infodemic”, which was defined as the existence of an excessive amount information on a subject some of which may be false or misleading (10–13). With the abundance and availability of information, discerning what is factual and false can be difficult.

At the same time, advances in science pave the possibility of new therapeutics through mechanistic discoveries about microbes and microbiomes (14, 15). To highlight these discoveries, there has been a global increase in media coverage and the growth of a multi-billion dollar pre- and probiotic industry based on microbiome research which often overstate the efficacy or importance of research findings (16–18). With increased opportunity for awareness of microbial functions and their impacts, understanding public perceptions of microbes and microbiomes to guide science communication efforts is essential to public health and scientific advocacy.

Microbes are often associated with negative outcomes such as disease, even though much less than 1% of microbes are considered pathogenic (19). This association with disease can lead to uncertainty and negative emotions, like fear or anxiety (6, 20–22). Importantly, negative emotions can influence thoughts, learning, and decision-making (23–26). These perceptions can influence health behaviors which can lead to the rejection of therapeutic interventions, increase health disparities, or in some

cases, result in loss of life (10, 27, 28). Work in the fields of science education have investigated the perception of school-aged children related to microbes and found that students associate microbes with something harmful (22). Additionally, studies have examined public perceptions of specific infectious diseases such as COVID-19, Mpox, West Nile virus, or malaria, finding misconceptions about causes and prevention strategies as well as emotions such as fear and apathy (29–32). Together, misconceptions about microbes can delay lifesaving preventative care and can result in noncompliance with public health recommendations (6, 10, 33–37). Similarly, perceived risk research on fecal microbiome transplants found feelings of disgust stimulated a greater perceived risk of microbiome research and therapies (20). While this body of research examines perceptions of disease-causing agents or therapeutics, little is known about public perceptions of microbes and microbiomes in general. Furthermore, few studies have asked for stakeholder input into future microbial science communication.

Knowledge about the audience is essential for any communication campaign but is especially important with complex subjects that are characterized by uncertainty or elicit emotions like fear (10, 25, 26, 38–41). Different audiences have different perceptions of such issues, developed through personal experience and beliefs which can influence health behaviors and actions (30, 35–37, 42). To create tailored messaging to meet science communication goals, there needs to be an understanding of public perceptions. Research in public perceptions of climate change has identified six worldviews towards climate change amongst Americans: alarmed, concerned, cautious, disengaged, doubtful and dismissive (43, 44). Climate change communication

research has focused on targeting messaging to these groups to evoke change (44–47). Such audience analysis regarding microbial perceptions is lacking.

Multiple theories examine how people respond to infectious disease and elements to consider in communication research. Briefly, the Theory of Motivated Information Management provides a framework for analyzing how individuals manage emotions and information in the context of uncertainty, with a focus on how people assess the outcomes of their information-seeking and the efficacy of the communication process they undertake to gain that information (41). Correspondingly, Lasswell's model of communication (48, 49) frames communication into five factors: 1) Who is the communicator? 2) What is the message? 3) What channel or medium is it being shared through? 4) Who is the audience? 5) What is the effect? Using these theoretical frameworks, our goal was to identify previous knowledge, sources of information, desired messages, and suggestions for future science communication efforts about microbes and microbiomes. Specifically, we utilize our interview data and the existing theoretical frameworks to address the following research questions:

RQ1: What do people know about microbes and microbiomes?
RQ2: Where and why have they learned this information?
RQ3: What do people want to know about microbes and microbiomes?
RQ4: How do people want that information shared and who do they think should share that information?
RQ5: How do emotions impact public perceptions about microbes and microbiomes?

Through these interviews, participants share their experiences and preferences for future microbial science communication campaigns. We accessed where people have learned about microbes and microbiomes, what microbial information people want, how to share it, who should share it, and how other interests and emotions might affect

the reception of that information. Together, these results can be used to create more effective, relevant science communication about microbes and microbiomes.

4.3 Methods and Materials

4.31 Participant Recruitment and Interviews

In the summer of 2023, adults across the United States were recruited via flyers and social media to participate in semi-structured interviews about microbes and microbiomes. Thirty participants were selected for be interviewed on a first-come first-served basis. The interview script was developed to stimulate conversation around microbes and microbiomes. Questions pertained to definitions of microbes and microbiomes, their functions, personal and peer perceptions of these topics and science communication. As interviews proceeded, the interview script was revised to include follow-up questions commonly mentioned by the participants. A final version of the interview script is available in Appendix B (**Supplemental Files S4.1**).

These interviews were conducted either in-person or via Zoom based on the preference and physical location of the participant. All interviews were recorded with the participants approval. Interviews were on average ~27 minutes and a survey with demographics questions as well as a question regarding science curiosity was sent out via email after the interview. The science curiosity survey question was developed based on a previously published science curiosity scale (50, 51). A document containing the questions listed in the survey is provided in Appendix B (**Supplemental Files S4.2**). The survey had a 60% (18/30) response rate. Our participants were predominately white (43%), between 25 and 34 years of age (30%), and all had received at least a high school diploma or equivalent (**Figure 4.1**). Demographics information provided during the interview were also included for participants who did not

complete the survey. Participants were compensated for their time with a $25 gift card. Participants' transcripts were deidentified and transcribed before analysis. This study was approved by the Colorado State University Institutional Review Board (#4304).

4.32 Interview Analysis

After transcription of the recorded interviews, we performed inductive thematic analysis. Two researchers (KK and TP) reviewed five interviews to identify recurring themes and trends in the data. After the two researchers met to discuss their notes, they collapsed similar themes to create an initial codebook. The constant comparison method was used to compare quotes to themes, modify descriptions and examples, and determine if additional themes were necessary (52). Together, these researchers revised the codebook until they were confident it captured most common themes in the data and no new themes emerged. A final copy of the codebook can be found in **Table 4.1**. To determine intercoder reliability, 10% of interviews (that is, three interviews) were independently coded using the codebook and codes were compared within each line as defined by MAXQDA software (v 22.8.0) (53). Cohen's κ intercoder score was acceptable (κ=0.783) as was percent agreement (95%) (54). These tests were used to identify nuance in how the two researchers were coding the data and were discussed among the researchers. The remaining interviews were divided among the two researchers (KK and TP) for coding using the MAXQDA software (v 22.8.0) (53). A frequency table of the sub-theme examples was generated by KK and TP (**Table 4.2)**. Sub-theme examples are listed if at least two participants mentioned that example.

4.33 Statistics

Figures and statistics were generated using Excel or R (ggplot2 package v3.3.5, dplyr package v1.1.0, readxl package v1.4.2, and viridis package v0.6.2) (55–58) and modified using Adobe Illustrator (v27.6.1). All available scripts for generating the figures can be found here (https://github.com/Kokkinias/Public-Perceptions-of-Microbes-and-Microbiomes.git).

4.4 Results

Our interviews revealed four main themes: (i) previous knowledge, (ii) topic of interest that led to microbial knowledge, (iii) information seeking processes, and (iv) emotions affecting microbial worldview as indicated in **Table 4.1**. Additionally, 11 sub-themes, descriptions of each sub-theme, and example quotes are provided in **Table 4.1**. Sub-theme examples and percent of participants who mentioned each sub-theme example are listed in **Table 4.2**.

4.41 Previous Knowledge about Microbes and Microbiomes

To answer question RQ1, we analyzed what participants knew about microbes and microbiomes, particularly their features or functions. The data revealed that many participants were aware of the connection of microbes and disease, but many participants were confused about the definition and differences between different types of microbes (i.e. viruses, bacteria, and fungi).

> "… I don't think I know the difference between like bacteria and viruses and microbes and how they all like interact." (P11)

> "I don't know if they [mushrooms] are microbes…" (P10)

Despite this lack of clarity around definitions, nearly all participants were able to identify a positive function of microbes (93%) or microbiomes (93%), citing familiarity with the existence of "good" and "bad" microbes.

> "… I'm aware that they're not all bad, and I think I've heard somewhere that it's like 80% of microbes are maybe even higher than that are beneficial or completely neutral to the human body." (P12)

Furthermore, some individuals are unfamiliar with term "microbiome" but can describe what a microbiome is often using word etymology or describing a microbiome without using the term.

> "I'm not familiar with [what a microbiome is], but I might assume that... they're like a group or a general environment description for microbes, their atmosphere or their context." (P5)

Other participants had heard of the term and could provide functions of the microbiome usually associated with the human gut microbiome.

> "…it [the human gut microbiome] impacts other systems of your body. So, if you have the right bacteria swirling around in there and your like immune system might be better… gut health is actually connected to not having Alzheimer's … It can potentially impact a lot of your other systems and your ability to stay healthy." (P4)

Despite some participants not being able to define the terms "microbes" or "microbiomes", all participants were able to identify at least one positive, negative, or neutral feature and function of both. Most participants (83%) described microbes as "small" or "something you can't see" and 70% mentioned disease as a negative function. More participants described neutral (100%) or positive (93%) features and functions of microbiomes than negative (53%) features and functions.

4.42 Past Information Seeking Processes and Topics of Interest

In response to interview questions related to RQ2, about where and why they learn about microbes and microbiomes, participants describe learning this information through formal education, word of mouth, books, research articles, news media, TV/movies, podcasts, social media, the internet, and advertisements for products. Overall, the concept of microbes was learned through formal education. Alternatively,

the concept of microbiomes, specifically the gut microbiome, was learned through social media or advertisements. Most participants stated they learned microbial information from formal education (80%) but either currently receive information from social media or suggested sharing microbial information via social media (56.7%). During an interview, a participant mentioned prebiotics and probiotics; upon further questioning from the interviewer about where they had seen information about this, the participant said:

> “Pretty much everywhere now… The health food store that I go to in [a city in the east coast].” (P25)

There were also individuals who displayed source amnesia, an inability to recall where, when or how information has been acquired, which can result in the spread of misinformation (59).

> “Definitely personal conversations. It's hard to pinpoint. It's honestly hard to distinguish whether I heard the word “microbe” or if it was lumped into conversations about health in general.” (P11)

Participants described topics of interest that led to microbial knowledge. These topics seems to help introduce people to concept of microbes and microbiomes indirectly through other interests in fields such as nature, food systems, or health and wellness. Some specific interests included climate change, gardening, composting, farming or agriculture, exercise and fitness, nutrition and gut health, and an interest in natural remedies or holistic medicine.

> “I'm also super interested in being healthy… I'm very active. I go to the gym, so naturally that led to, “OK, what should I be eating?”, which led to, “OK, what's healthy?”, “What's not?”, which led to deep dives into “OK, like, this is bad for you?” (P21)

> “Because I've been so interested in this topic, I would say of gardening and of learning about like homesteading and stuff that naturally leads itself to learning more about the natural gut biome...”. (P22)

In the post-interview survey science curiosity question, participants described the science seeking behavior or their science interests they participated in within the last year. Over half of survey respondents described having an interest in science (61%), an interest in nature (72%), and consumed science on social media (56%) (**Figure 4.2**). People with an interest in other fields, such as health and wellness, described more positive functions than negative functions of microbes and microbiomes compared to those who didn't mention a special interest. Additionally, people with these interests were more likely to define the term "microbiome" or mention non-human related functions of microbiomes like soil health.

4.43 Desired Information about Microbes

During interviews, participants shared information they would like to know personally and information they thought others would benefit from (RQ3). Usually, the information a participant thought would be useful for them was described as also being useful for others and related to the perception of others' knowledge of microbes and microbiomes. The most commonly desired information was general knowledge about microbes and microbiomes such as definitions about these terms. This was often mentioned when describing what others should know about microbes. Many participants (53.3%) also wanted information on disease avoidance and human health benefits. Some participants (16.7%) wanted more microbial information related to climate change solutions or other special interests.

> "I would love to learn more... as it relates to climate change and some of the toxic chemicals and products that we have created. And how we could potentially treat those problems with microbes. Also, would love to learn at a high level what we've learned as it relates to viruses and immunity and how a better understanding of microbes can help us avoid pandemics in the future." (P3)

> “What are they? What's their effect on us humans? On animals?... If they are dangerous or not? How to use them in your favor? How to prevent them harming us? Where are they? What is it?... Where do we find them? What's their lifespan too?” (P5)

More broadly, some participants expressed a desire for themselves or others to understand the ecological roles of microbes (46.7%) and other human-centric benefits (53.3%).

> “We should also know their essential to our existence, because I believe that human existence is dependent on so many factors, and I think microorganisms and microbes are one of the factors. So, I think it will be important for us to also know the role they play in the ecosystem.” (P7)

Those who knew about functions of microbiomes also wanted others to know about the positive role microbes and microbiomes can play.

> “I would mainly just like them to know that… it's a much bigger part of our lives than is readily apparent… I think I would like people to know that they're not all pathogens. Microbes aren't just things that cause disease. It's so, so much bigger than that.” (P13)

Many participants wanted to learn and share information about how microbes and microbiomes impacts on human health. Some with an interest in nature emphasized the importance of understanding their role in natural ecological processes or climate change.

4.44 Future Information Seeking Processes

When describing how they wanted microbe-related messages shared in future science communication efforts (RQ4), participants discussed information sharing channels, communicators, and communication techniques that would be beneficial or should be avoided. Formal education, word of mouth, books, research articles, news media, TV/movies, podcasts, social media, the internet, and advertisements for products were identified by participants as channels to share microbe-related information in the future. Yet regardless of age, there was a greater emphasis on using

various social media platforms (Instagram, TikTok, Reddit, etc.) due to perceived reach to a variety of audiences.

> “Unfortunately, it [social media] is probably the best place. I hate social media, but I will admit that I am on it. It's just the quickest access to information when you need it, and social media does a great job of taking information and condensing it into really short videos. That is not great for our attention span; however, if you need a quick answer, it's good for that.” (P21)

> “Reddit. That's explained like I'm five in words that I can understand…” (P16)

Participants had a wide array of suggestions as to who they would like to share microbe-related information which included specific individuals they knew, professionals (doctors, scientists, science communicators, or social media influencers), educational institutions (museums, universities, and TED conferences), and governmental institutions (e.g., the Centers for Disease Control and Prevention or the National Institutes of Health). Most commonly, participants wanted the “experts” sharing information about microbes and microbiomes or collaborations between researchers and communicators. The “experts” participants referred to were commonly scientists or others who are “doing the research”, yet some doubted the science communication skills of scientists, suggesting collaborations with individuals with those skills.

> “If someone was a microbiologist, if someone was probably a registered dietitian, I'd probably also listen to that… like doctor, like a nurse practitioner or a regular physician.” (P21)

> “The information should come from scientists that have the research and things to back it up. I think they're maybe not the best people to making those graphics just because they maybe don't have… the design background. So, I guess it would be someone either from the company or the department that works more in your marketing department just because I think they have a better idea of how to visually appeal to an audience. I think they should work together.” (P24)

Regardless of the communicator, trust was an important factor described by many participants (46.7%). Some participants were hesitant in recommending government

institutions related to how they share information or the public's trust in these institutions.

> "That's a good question. I feel like if it came from some official source... By official I mean, the CDC or something governmental. Maybe I might be less inclined to click on it, because I might just assume that it was gonna be kind of boring. But on the other hand, there needs to be some credibility, because I don't want to just live in a world where we believe whatever people say on the Internet… I guess there are people who have made careers out of science communication, who have degrees in various science fields, so I guess that's kind of the group I would look towards." (P19)

> "So, if it's a government source telling people to do things, I think a lot of people are going to be wary of that just off the bat." (P22)

Participants also described an array of science communication techniques, most of which have supporting evidence of efficacy (60–65). Many participants described the effectiveness of "short" video content using "simple" language as an effective means of sharing information.

> "I personally just enjoy the video format and I think a lot of other people do as well. I don't think I'm just projecting that, maybe I am, but I'm imagining maybe more or the short video. So, think kind of like 5 minutes or less. Think of like Tik-Toks or Instagram reels and sort of very brief discussions that can have powerful visual aids attached to them…. Then you can keep their interest, and they can spend 5 minutes on it, 10 minutes on it." (P13)

Additionally, some recommended connecting to people's interests, using humor (6.7%), and having scientists or communicators share their identities outside of their career (16.7%).

> "… in a very non-scary, non-sterile way… when I think of people talking about microbiology, I think of people talking and jargon talking very dry. Maybe there's a whiteboard behind them and they're going to show me something with their dry erase marker that I'm probably not going to remember or pay attention to. So… maybe someone who's dressed in just everyday clothes and they're like, "Hey. I'd love to tell you something" that would work for me… but if it was told in a way that was humorous, most people are receptive to humor. So, if someone could find a way to say it in a way that's entertaining like with jokes, that would be great." (P21)

> "I think if you can like lay the groundwork of like, you know, "I'm a scientist and I love, you know, working in this lab and it's so great. And like, I love this, and I also love my cat. And I love gardening. And I love like my grandma and her cookies that she makes

> me", I just feel like to me that just, like, makes them seem like a real human and like if they're more relatable to me..." (P22)

Overall, participants described wanting "experts" that they trust to be sharing information about microbes and microbiomes. They describe using literature supported science communication techniques and prefer a video format possibly via social media to share this information.

4.45 Emotions affecting Microbial Worldview

Emotions affect how and when we perceive, seek, and obtain information (24, 25, 41). Given the role that emotions can play in information processing, questions related to RQ5 help us understand how the publics' emotions affect their microbial worldview and perceive others. These emotions related to microbial information fell into two main categories: emotions towards self and perceptions of others. The most common emotions towards self were a lack of self-efficacy (96.7%), apathy (56.7%), curiosity (43.3%), self-efficacy (43.3%), cognitive overload (26.7%), and fear (23.3%) about microbes and microbiomes. Predominately, individuals experienced a lack of self-efficacy during interviews, with some doubting their own abilities and knowledge of the subject and expressing that they felt ignorant in front of the interviewer. Others had this lack of self-efficacy transform into curiosity about microbes, or they portrayed experiencing cognitive overload.

> "I feel like I have like an okay grasp on how digestion is affected by them [microbes]. I'd be curious if it's like is affecting other systems in the body. I am curious about… what they're doing in the air if they're doing anything... I guess I'd be curious what advances are being made right now… like fuel or climate related things... I don't know if they're working on like cancer treatments or stuff like that." (P18)

> "One of the hurdles that you have is that you don't know who's putting up material. The individuals, or if they represent an organization and many times it's very contradictory. That one person's telling you to do this and the other person's telling you, "No, do that". It's a juxtaposition between the two of them. They're clashing and you have no idea as to who I am going to listen to at this point. Do I listen to one? Do I listen to the other? Do I

> listen to neither? They [the public] can't listen to both, because the information is contradictory." (P27)

Participants' perceptions of others fell into six categories: disease-focused, ignorant, intimidation, apathy, knowledgeable, and expertise. Most participants (90%) assumed that others knew about as much as or less than they knew about microbes and microbiomes.

> "I definitely think they if they're not like you studying this [the interviewer], they know just as much as me, which is nothing or less than that. Maybe I just think that to make myself feel better, but I do. I feel like people don't really know what a microbe is, especially since it's like something we use to advertise to people…[the term "microbe"] a buzzword sometimes. I don't think people know very much about them." (P9)

Similarly, some participants (56.7%%) described feelings of apathy in themselves and would often ascribe it to others as well (50%).

> "This probably isn't a good answer, but I'm kind of apathetic. There's not like anything, I just need to know." (P30)
>
> "My guess [is] the average Joe could care less about it." (P26)

Participants (70%) perceived those with a background in science, technology, engineering, or math (STEM) fields or a medical degree as having microbial "expertise". At the same time, some participants (66.7%) noted that people without a STEM background are knowledgeable about microbes, primarily through media or experience with disease.

> "I think a lot of people don't know what they are. They're not scientists… it [the term "microbes"] sounds intimidating and complicated, so I feel like, unless you're a scientist… maybe if you have a special interest in that kind of research, you probably just know that they are small." (P24)
>
> "I feel like at this point with COVID, we've all had a lot of knowledge or education about cover(ing) your cough, wear(ing) a mask, stay(ing) home when you're sick. Although a lot of people still aren't doing those things. So, I guess we could probably use a reminder now and then." (P19)

Generally, participants thought that others had equivalent or less knowledge than they did about microbes and microbiomes. These perceptions did not apply to those who

study microbes and microbiomes. Some describe feeling apathetic, fearful, or overwhelmed with information about microbes and microbiomes while others felt curious or confident about their knowledge especially when it related to other interests that had. Understanding emotions that underlie the publics' perceptions of microbes, microbiomes, and others illustrates potential barriers and opportunities for science communication.

4.5 Discussion

With microbial research constantly evolving and an unprecedented wealth of information available, it is overwhelming for both scientists and non-scientists to navigate information seeking and sharing about microbes and microbiomes. Uncertainty in science is an ever-present reality that challenges science communication efforts (25, 38–41). Similarly, there is much investigation about how to communicate concepts about microbes and microbiomes (20, 22, 29–32), but many of these studies have focused on a particular infectious disease and have not addressed microbial worldview holistically. This study investigates public perceptions of microbes and microbiomes through semi-structured interviews, identifying current microbial knowledge, channels of microbial information, and future directions for science communication. Using the Theory of Motivated Information Management which exemplifies how people deal with uncertainty, we evaluated how individuals' emotions affect their microbial worldview (41). We show that stakeholders are considering what, when, how, and who should be sharing microbial information from their personal experience.

4.51 Targeting Audience Pre-existing and Desired Future Knowledge

To generate effective science communication strategies about microbes and microbiomes, it is important to identify audience's existing knowledge and knowledge gaps (RQ1). Similarly, it is also beneficial to identify what people want to know or think their peers should know about the microbial world (RQ3). Our work notes that while individuals are familiar with the relationship between microbes and disease at a broad level, some participants are also aware of some positive functions of microbes and microbiomes. Creating awareness of the many industries that microbes already are a part of, such as food, cosmetics, or pharmaceutical industries, may garner greater appreciation and less fear of microbes (9, 14, 35, 42, 66).

Most participants wanted to know general information about microbes and microbiomes including definitions. The data indicate that participants may not know distinct differences between microbes or are unfamiliar with microbe-related terms. Understanding general knowledge about microbes and microbiomes was cited by almost all participants (76.7%) as important for microbial messaging. Lacking this knowledge may lead to confusion about therapeutic interventions or science communication efforts using these terms. For example, other studies have shown low perceived risk and knowledge about antibiotic resistance despite it being among the top ten threats for global health (67–69). Clarifying this confusion with targeted interventions may reduce misconceptions and improve health behaviors around issue like antibiotic resistance.

Interestingly, many participants mentioned other interests they had that led them to microbial knowledge. Interests related to human health, specifically nutrition and gut health, and holistic and natural remedies were most common among participants.

These interests acted as a catalyst for learning microbial information through leisure activities. These participants were also more aware of functions of microbes and microbiomes typically related to their interests. This corresponded with what participants wanted to know about microbes which generally had a human-centric focus, either as pathogens or how they benefit us. Specifically, people wanted information they could use that could directly impact their lives, which includes disease prevention messaging. Describing how microbes and microbiomes play a role in systems that individuals already have an interest in may be an effective way to share microbial information, and such a strategy could be applied for introducing other scientific topics that have indirect or direct relationships to areas of interest for specific audiences. Deliberate science communication efforts targeting information the public wants to know will aid in crafting meaningful messages which can result in increased scientific engagement.

4.52 Aligning Microbial Messaging to Socio-scientific Issues Using Relevant Channels and Social Networks

When asking individuals where they learned information about microbes and microbiomes (RQ2) and where information should be shared in the future (RQ4), many mentioned school settings. Science education research has identified multiple intervention strategies to improve science literacy in the classroom (7, 8, 42, 64, 66, 70, 70–73). While it is important for school-aged children to learn microbial information, research and circumstances arise where scientific consensus about microbes changes, and there is still a need to share information with those no longer in school.

When describing instances where participants received knowledge as adults, information was received via social media, internet searches, and word of mouth which can include more misconceptions or misinformation (10, 11, 27, 28, 34, 74, 75). Even

though other research and our participants both perceive that social media is not a trustworthy way to receive information (76), our participants report consuming and suggest sharing science information via social media. The reach of social media to more individuals was mentioned as a benefit of social media as a channel. Recommendations include working with content creators that individuals already engage with to broaden the reach of science messaging. These channels are spaces where individuals already received information and can be targeted by science communicators in collaboration with existing content creators to correct misinformation.

Additionally, empowering individuals to correct misinformation within their social groups or social media has been proposed as a strategy and highlights the importance of science literacy and identity (11, 60, 77–81). Particularly, individuals with interests that led them to microbial information often wanted others to know about the positive functional roles of microbes and microbiomes. They often described sharing what they learned to others through personal interactions. Other participants explained learning about microbes and microbiomes through personal interactions with family or friends with special interests or science education. These relationships may be utilized to share messaging to those within social networks. Future work should examine the role of these interactions in acquiring microbial knowledge compared to other non-social learning methods. Knowledge about where individuals receive information about microbes and microbiomes and their interests can be leveraged for future science communication campaigns by tapping into existing channels of sharing information, topics that individuals will seek out, or social networks.

4.53 Fostering Collaboration between Microbiologist, Social Scientists, and Communicators

When examining who participants want communicating microbial messages (RQ4), our results highlight the importance of scientists and doctors as a part of the science communication efforts (82–84). Participants' perceptions of others were often worse or similar to how participants viewed themselves, which did not apply to how participants viewed those with a STEM background. Many individuals desired information to come from researchers with scientific training. Any reservations about scientists and doctors sharing this information related to the delivery of the microbial information. While participants wanted scientists and doctors to share microbial information, there was a lack of confidence in how effectively those messages would be shared. Scientists were often stereotyped as passionate but boring communicators (85, 86). To address this, science communication initiatives like "TED talks" have shown success in sharing science stories with non-scientists (63, 87, 88), yet further work needs to be done to communicate research in realistic and entertaining ways. Furthermore, the expertise of those in communication fields are needed to refine science messaging and incorporate best practices. Participants want concise messaging from trusted sources in plain language with exciting visuals. Communicating in this way requires skill, knowledge of your audience, and expertise in evidence-based science communication. This may be done through integration of science communication into formal scientific training which has been shown to increase science identity and inclusion in science (89–94) as well as collaboration between communicators and scientists. Some scientists' perceptions of the public as unable to comprehend or uninterested in science provides another barrier to science

communication (79, 95–97). Stepping away from the deficit model of science communication and valuing the expertise of the public would encourage scientists to engage in conversations with the public but also may increase their willingness to correct misinformation (79, 92). Our data also suggest that scientists embrace their identity outside of science to increase their relatability when participating in science communication (92, 98–100). Sharing their identity may have the added benefit of encouraging others with a shared identity to pursue a career in science (90, 91, 101, 102).

When advising scientists and doctors as communicators, participants typically mentioned the concept of “trust” and “trusted sources”. People tend to trust others they have a personal relationship with but trust different professionals based on their profession (11, 34, 76, 77). For example, despite a slight decline in confidence in recent years, public trust in scientists and medical scientists remains high while trust in journalists and political leaders is comparatively low (76). In accordance with this research, some participants advised against sharing science messaging from governmental institutions stating that others may view those sources as more biased (76, 103). Participants instead recommended sharing information using channels that individuals already engage with, specifically citing social media. Some participants also mentioned specific social media influencers as trusted sources, typically associated with other interests. Benefits of scientists engaging on social media platforms include dispelling misinformation, increasing the visibility of research findings, and professional networking (104–106). Despite this, many scientists remain reluctant to share science on social media platforms, not knowing where to begin and struggling to incorporate it

into the workday (104, 105, 107). Through collaboration with institution communicators and existing content creators as well as promoting media training for scientists and graduate trainees, scientists can increase their participation in science conversations on social media and with through relationships with people in their daily lives.

4.54 Appealing to People's Values and Emotions

Perceptions of self and others impact information processing and must be understood when crafting communications (RQ5) (23, 79, 94, 101). Feeling apathetic, fearful, or overwhelmed with information about microbes and microbiomes can inhibit information processing (24–26, 108, 109). Additionally, increases levels of intolerance to uncertainty are associated with reduced self-efficacy levels (110, 111). Addressing negative emotions through targeted messaging can improve understanding and appreciation for science (25, 66, 109, 112, 113). Creating relevant, unified messaging that addresses uncertainty will increase public understanding and self-efficacy (25, 35, 38, 39). While participants viewed those with STEM trainings as "experts", they often lacked self-efficacy, which impacts perceptions and health behaviors (23, 35, 42, 101). Participants often had a lack of self-efficacy especially when they did not express an interest that led them to microbial knowledge. Adult learning research indicates, as our participants recommended, that "fun" increases motivation and attention, improving learning outcomes (65, 114). Connecting microbial information to fields that interest people already can make the "intimidating" task of learning about microbes and microbiomes fun. Future work should examine the benefits of interventions that connect microbial information to participants other interests and subsequent effects on emotions

as well as benefits of interventions connecting microbial researchers to people with shared identities via boundary spanning.

4.55 Limitations

According to our data, our participants are highly educated with 80% of our participants having a bachelor's degree or higher compared to the United States as a whole (37.7%) (115). These values may be elevated since participant recruitment occurred in the vicinity of a college town in a highly educated state. Confidence in scientists has been shown to increase correlated to education level and income, which may have affected participant responses (116). Capturing perspectives of older individuals, individuals from more regions of the United States, individuals without college education, non-white individuals, and more men may identify additional themes not depicted here. Future work should examine how generable these themes are to others across the United States through large scale surveys. Additionally, participants responded to a flyer about microbes and microbiomes which might attract individuals who have some knowledge about them. While this study provides a basis for the publics' perceptions of microbes and microbiomes, future study design can test proposed interventions to provide relevant microbial science communication on specific stakeholders and communities.

4.6 Conclusion and Establishing the Groundwork for Future Research

Together, this work identifies the importance of 1) recognizing the pre-existing and desired future knowledge of audiences (RQ1/RQ3), 2) connecting with larger socio-scientific issues people already care about on social media and through social networks (RQ2/RQ4), 3) collaboration between microbiologists, social scientists, and

communicators (RQ4), and 4) connecting with people's values and emotions (RQ5). Specifically, interests like nutrition and gut health may lead people to microbial knowledge and may be leveraged to share microbial messaging. People may benefit from knowledge about differences between microbes and the ecological functions they have in systems that people value. Additionally, our results indicate that individuals want this information communicated on social media and from scientists with whom they share social identities. Information should be unified, clear, and fun to mitigate the distress of scientific uncertainty and other negative emotions while increasing self-efficacy and science literacy. For the most effective communication, scientists should partner with science communication researchers and communicators, implementing tested communication techniques. These findings set the foundation for research to guide future microbial science communication interventions. With this qualitative study, we have identified several themes and topics of interest to audiences, which sets the foundation for quantification, via large landscape surveys. Ultimately, implementation of this work will lead to improved appreciation for and working knowledge of microbes and microbiomes.

Chapter 4: Tables

Table 4.1: Codebook
Codebook including themes, sub-themes, descriptions of sub-themes, and example quotes from participants generated through inductive thematic analysis.

Themes	Sub-themes	Description	Example Quotes
Previous Knowledge	Microbes Features and Functions	A physiological feature or function of microbes	"It's something microscopic, so it's something that you can't see with the naked eye. It's a small life form. Affects our daily lives and you... they are necessary. That can sometimes be bad." (P11)
	Microbiome Features and Functions	A physiological feature or function of microbiomes	"Because there are lots of bacteria that grow in the soil and break down different nutrients and breakdown some of the fibrous materials and leftover parts that are in soil. So, like when we till our gardens turns over the bacteria as well. That's in there and helps break it up." (P8)
Topics of interest that led to microbial knowledge	Nature	An interest in nature or the environment	"One thing that that comes to mind is… I'm pretty passionate about climate change, and I have found some of the research at a high level very interesting… [I am interested] in a microbe that could eat away at plastic..." (P3)
	Food systems	An interest in food systems such as gardening, farming, agriculture, etc.	"I'm very conscious of the environment. I'm very conscious of my family, with what they eat. I like to garden, I'm a beekeeper." (P25)
	Health and Wellness	An interest in health and wellness including exercise, nutrition, gut health, holistic or natural remedies, etc.	'I'm just a person that is interested in fitness and nutrition. You know, there's a lot of "gut biome" stuff that comes up." (P15)
Information seeking processes (past and desired future)	Communicator	A person, professional, educational institution, or governmental institution who has or should communicate about microbes/microbiomes	"Yeah, my brother went on a real kick about it [the gut microbiome]. So, he lectured me about it for a while. This was probably five or six years ago. So, I got some information from him." (P4)
	Channel or Medium	A channel or medium where participants have received microbial information or think should be used to	"Where I'm at in life now.... Probably just see it on Instagram or a cool YouTube video. I think that's where I do most of my learning. It's either a friend recommends me a book, or I just am on YouTube at like 11:30 at night

		share information in the future	and I'm just clicking whatever sounds interesting." (P12)
	Communication techniques	A communication technique that should be used to communicate information about microbes/microbiomes	"I'm more of like a visual learner, so I would like some sort of video that kind of shows what's going on, how they interact with everything. It'd be kind of hard, since they're so small, but you know, diagrams help." (P17)
	Desired information	A topic about microbes and microbiomes that participants would like to know or think others should know	"I think we should demonstrate the significance of them and show why they, why they're necessary for us. And how they help us. How they help us humans and our just our daily lives." (P10)
Emotions affecting microbial worldview	Emotions towards self	A participants' emotions about themselves related to microbes/microbiomes	I want to find out more about it [microbes and microbiomes]. I'm interested now. I feel like going to the library tomorrow and just like read up more about it… and seek more information… So maybe ask my doctor for information about that. (P29)
	Perceptions of others	Characteristics ascribed towards others by the participant related to microbes/microbiomes	"I feel like the general perception from people who don't have a STEM background is that like microbes are bad. Like, you have to disinfect everything because you don't want the microbes. People that have the STEM backgrounds… are very excited about microbes and all of the potential implications that they have for understanding certain processes in the world." (P1)

Table 2: Frequency of Sub-theme Examples
Table lists the percent of participants (n=30) who mentioned the listed example of each sub-theme. Sub-theme examples are listed from greatest to least frequency of mention within each sub-theme.

Themes	Sub-themes	Sub-themes Examples	% of participants mentioning this example (n=30)
Previous knowledge	Microbes Features and Functions	a) Neutral Feature or Function b) Positive Feature or Function c) Negative Feature or Function	96.7% (29) 93.3% (28) 70% (21)
	Microbiomes Features and Functions	a) Neutral Feature or Function b) Positive Feature or Function c) Negative Feature or Function	100% (30) 93.3% (28) 53.3% (16)
Topics of interest that led to microbial knowledge	Nature	a) Climate Change	16.7% (5)
	Food systems	a) Gardening and Composting b) Farming/ Agriculture	16.7% (5) 13.3% (4)
	Health and Wellness	a) Nutrition and gut health b) Holistic & natural medicine c) Exercise and fitness	36.7% (11) 30% (9) 6.7% (2)
Information seeking processes (past and desired future)	Communicator	a) Profession (i.e. doctors, scientists, etc.) b) Individual c) Educational Institutions (i.e. museums, universities) d) Governmental agencies (i.e. NIH, CDC)	56.7% (17) 43.3% (13) 40% (12) 23.3% (7)
	Channel/ Medium	a) Class b) Social media c) Internet d) Word of mouth e) News f) Source Amnesia g) Advertisements h) Books i) Podcast j) TV/Movies k) Research Articles	80% (24) 56.7% (17) 46.7% (14) 43.3% (13) 43.3% (13) 36.7% (11) 30% (9) 26.7% (8) 26.7% (8) 20% (6) 13.3% (4)
	Communication techniques	a) Visual format (i.e. video, diagrams) b) Trusted sources c) Plain language d) Short e) Connect to interests f) Partner with communicators g) Shared identity h) Humor	63.3% (19) 46.7% (14) 43.3% (13) 36.7% (11) 33.3% (10) 30% (9) 16.7% (5) 6.7% (2)

	Desired information	a) General microbial knowledge (i.e. definitions)	76.7% (23)
		b) Disease avoidance and Health Promotion	53.3% (16)
		c) Microbial Benefits to humans	53.3% (16)
		d) Ecological roles of microbes	46.7% (14)
		e) Microbial Benefits to the environment	30% (9)
		f) Microbial Benefits to industry	23.3% (7)
Emotions affecting microbial worldview	Emotions towards self	a) Lack of Self-efficacy	96.7% (29)
		b) Apathy	56.7% (17)
		c) Self-efficacy	43.3% (13)
		d) Curiosity	43.3% (13)
		e) Cognitive overload	26.7% (8)
		f) Fear	23.3% (7)
	Perceptions of Others	a) Ignorant	90% (27)
		b) STEM Expertise	70% (21)
		c) Knowledgeable	66.7% (20)
		d) Disease-focused	63.3% (19)
		e) Apathy	50% (15)
		f) Intimidation	36.7% (11)

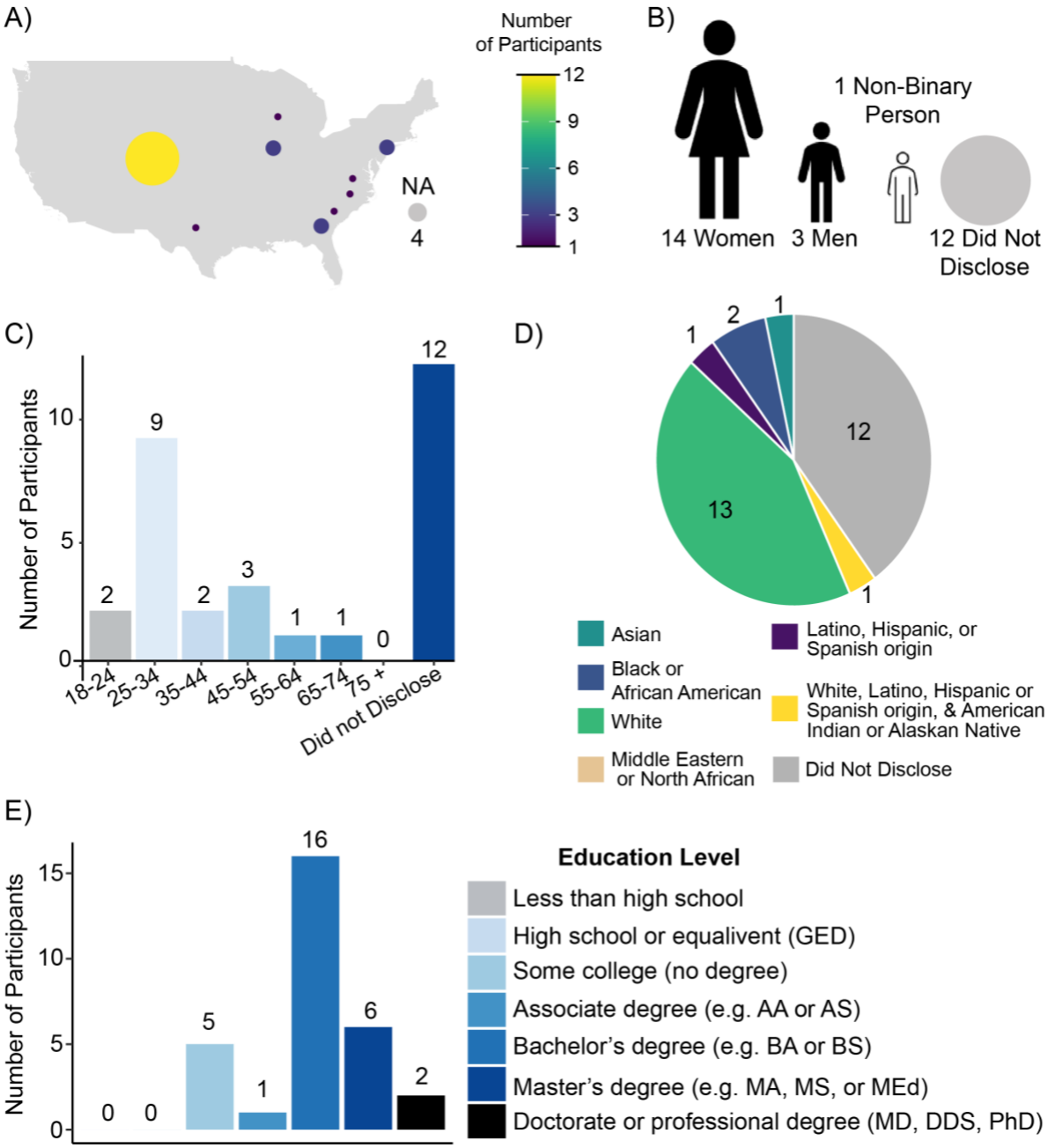

Figure 4.1: Participant Demographics
Demographics of participants are depicted including A) where participants live in the United States, B) participants' gender (women, men, or non-binary), C) age range of participants, D) participants' ethnicity, and E) participants' education level.

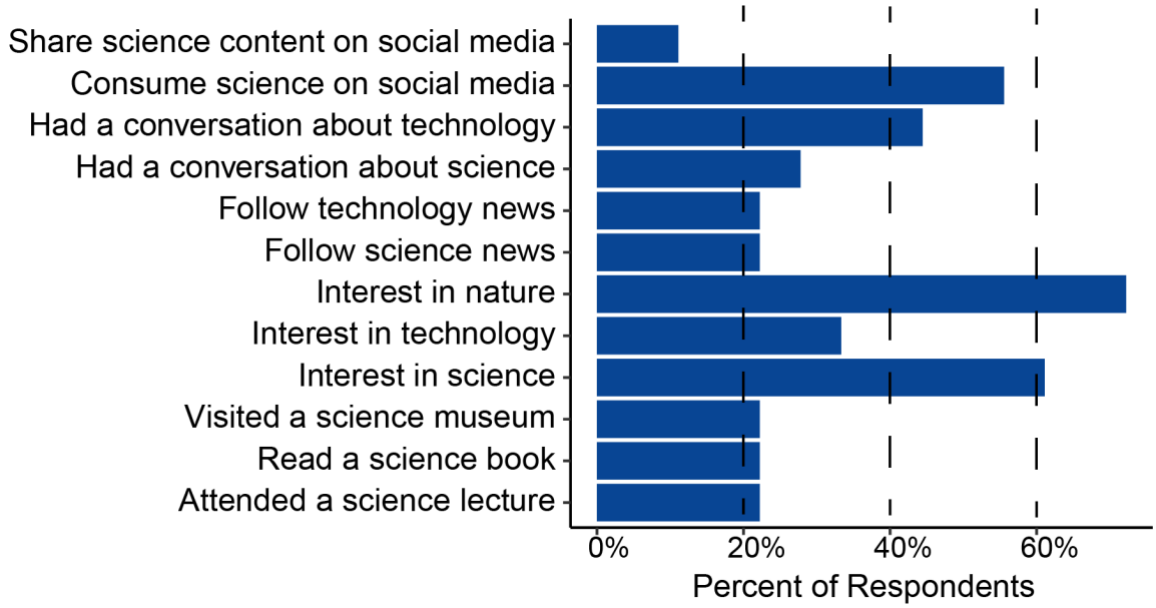

Figure 4.2: Science Curiosity
Bar chart of survey respondents' science curiosity within the last year.

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Chapter 5: Summary and Future Considerations

Knowledge of microbes and microbiomes will provide insights to their contributions and may lead to the development of strategies to tackle global issues such as antibiotic resistance and climate change. Communicating about these complex scientific topics both within science communities and with the general public remains a challenge requiring research into best practices. Working towards these goals, my dissertation spans both microbiome and science communication research.

In chapter 2, I used multiple omics technologies to explore *Salmonella* plasticity in different diet backgrounds and over time to identify potential therapeutic targets and understand the gut microbiome interactions. Notably, I identified a redox gradient of *Salmonella* gene expression based on diet, where *Salmonella* used more energetically favorable genes for oxygen and nitrate respiration in a high-fat diet compared to a fibrous chow diet, and based on infection phase, where the peak phase was characterized by oxygen utilization genes. Through this targeted and untargeted approach, substrate profiles were also shown to change by infection phase, detecting more diverse use of substrates by *Salmonella* in the early phase compared to the peak or late phases of infection. We also provided nuance to the lactate economy of the gut, showing expression of *Salmonella* D-lactate utilization genes which may suggest potential microbial cross feeding. Lastly, exploring non-nutritional *Salmonella* gene expression patterns revealed phase specific expression of *Salmonella* pathogenesis and horizontal gene transfer genes. These included differential expression differences in motility, conjugation, and biofilm-associated genes. Some research has been done to

examine the relationship between the gut microbiome and *Salmonella* during infection (1–8).

Given the potential of gut microbes to cross feed or compete with *Salmonella* for substrates (1, 3, 5–10), future work should examine interactions between *Salmonella* and the gut microbiome over time, conducting competition experiments with persistent commensal microbes or developing targeted therapeutics disrupting a combination of pathways listed here. Similar to previous research that discovered inflammation increases phage transfer between *Salmonella* species (11), our work noticed differential expression of phage-related genes. It would be interesting to examine the predator-prey relationship between the gut virome with a dominant organism, like *Salmonella*, or to examine conditions where *Salmonella* phage may become lytic. This may affect *Salmonella* gene content and pathogenesis within the host.

Similarly, my research highlighted that the role of biofilms in *Salmonella* infection within the gut remains to be fully understood. Non-murine hosts have been shown the potential to develop biofilms in the gut lumen (12, 13). Our work adds to this body of knowledge showing that *Salmonella* biofilm-associated genes are expressed in the highly inflamed high-fat diet mouse model. Further work visualizing biofilms in this dietary regime would confirm the potential of *Salmonella* to generate biofilms in the gut lumen and expand our knowledge of biofilm triggers within the gut. This may be a factor in chronic *Salmonella* carriage (14, 15) and would have implications on treatment.

Finally, it was surprising that pathogenesis genes associated with type 3 secretion systems, typified by initiation of infection, were expressed throughout infection. Sub-populations of other pathogens during chronic infection with distinct

phenotypes or dysregulation of master controls have been observed (16, 17). Determining the extent each of this may contribute to this observation would help explain why some *Salmonella* pathogenesis genes are continuously expressed.

With the vast amount data produced by omics technologies, data management and data sharing are crucial to the advancement of the field of microbiome science. Chapter 3 describes the CBA_DREAMM database which provides a centralized location for the data generated from our team. This multi-omics database houses 16S amplicon sequencing, metagenomic sequencing, metatranscriptomic sequencing, and both targeted and untargeted metabolomics across the *Salmonella* infection gradient, in different dietary regimes, and over time. While this repository models FAIR principles, much work needs to be done as a community to implement and maintain data sharing principles. Due to the vast generation of microbiome data and data repositories to store this data, microbiome data often lacks necessary metadata or computationally expensive data processing files (18–20), both of which are needed to contextualize findings. Moving towards more equitable data sharing practices allows for linked metadata studies which can examine patterns across large, diverse data sets. This mission's importance prompted the NIH to provide funding for data management and for training for early career scientists (21). There has also been discussion among journals to standardize principles of data management for publication. Individual labs and institutions can provide support and incentives for implementing data management and data sharing initiatives.

Communicating science has a wide array of benefits to the scientific community and to society, yet to do it well, one must understand the perceptions of their audience.

Surprisingly, few studies examine public perceptions of microbes and microbiomes in general, usually focusing on specific pathogens or other features related to disease (22–35). Chapter 4 discusses the findings of our thematic analysis and survey on public perceptions of microbes and microbiomes in the United States. Interestingly, participants described special interests, such as an interest in climate change, as a method of obtaining microbial information. Future work should examine the benefits of interventions that connect microbial information to participants other interests or to scientists who share personal identities and the subsequent effects on their emotions towards microbes. Participants also shared that they want collaboration between scientists and communicators to share microbial messaging, preferably using channels like social media or through word of mouth. Further research should examine the role of these interactions in acquiring microbial knowledge compared to other non-social learning methods. People wanted to know definitions of microbes and microbiomes, disease avoidance, health benefits and benefits to people in general. This messaging should be in a visual format, plain language, and entertaining according to our data. Other than people in STEM fields, participants viewed themselves and others as ignorant or apathetic about microbes and microbiomes. Yet, some people, usually those with special interests, perceived themselves and others as knowledgeable. Together this work can guide both future large landscape survey studies as well as the creation of interventions to improve microbial messaging. Furthermore, future study design can test proposed interventions to provide relevant microbial science communication on specific stakeholders and communities.

Through a combination of microbiome and science communication research, this thesis identified factors influencing *Salmonella* metabolism during infection and public perceptions of microbes and microbiomes. It highlights the importance of understanding gut microbiome interactions over time during infection. Moreover, it introduces the CBA_DREAMM database for centralized data storage and emphasizes the necessity of equitable data sharing practices. This thesis also investigates public perceptions of microbes and microbiomes in the United States, revealing a desire for collaborative science communication efforts and interventions tailored to people's interests. This research aims to inform the development of strategies to address global challenges and improve microbial science communication. I feel fortunate to have contributed to this work and am excited to see how these discoveries develop and how others expand on this progress.

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Appendices

Appendix A: Chapter 2 Supplemental Files and Figures

Data set S2.1.xlsx: Data set S2.1 contains metadata about the multi-omics performed, ASV feature count table, SILVA taxonomy (132-99-515-806), diversity metrics, and lipocalin-2 concentrations (ng/g of feces).

Data set S2.2.xlsx: Data set S2.2 contains raw, unfiltered mapping to the Salmonella pangenome, gene length and strandedness information, GeTMM normalized gene counts, DESeq2 differentially expressed genes by each phase (early, peak, post), DRAM (v1.4.0) annotations, and a list of genes depicted in figure 6.

Data set S2.3.xlsx: Data set S2.3 contains a GeTMM normalized Salmonella carbon gene expression matrix, annotations of the Salmonella carbon gene from CAZyme and DRAM module step form, and the DRAM module step form (https://github.com/WrightonLabCSU/DRAM).

Data set S2.4.xlsx: Data set S2.4 contains abundance and annotations of untargeted metabolites and abundance of targeted short-chain fatty acids (acetate, propionate, and butyrate).

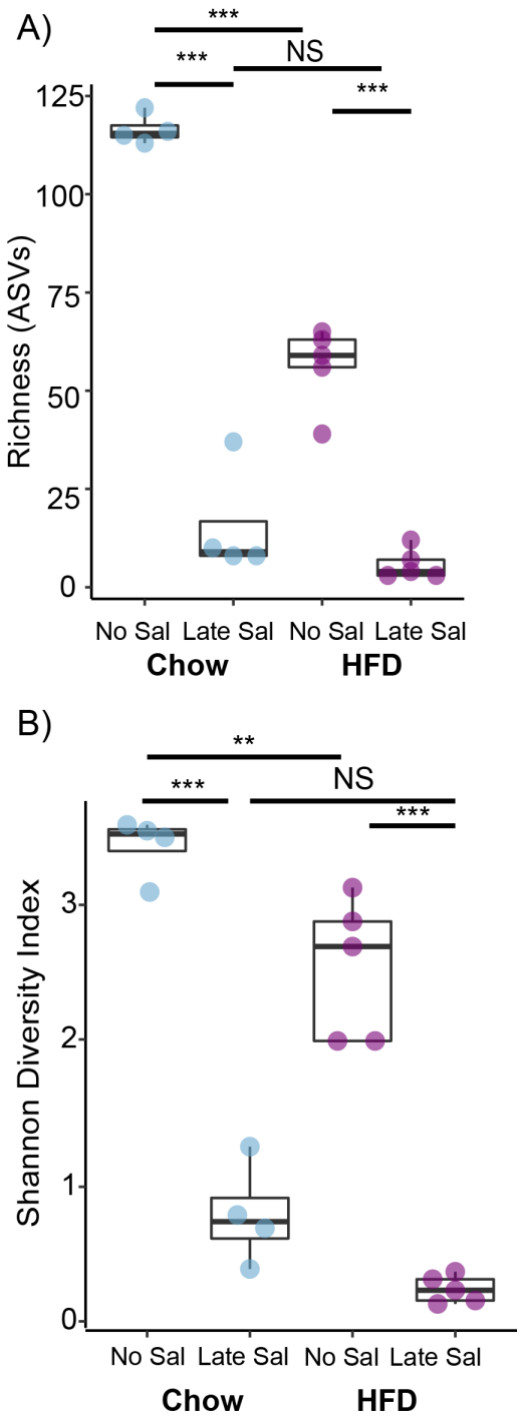

Figure S2.1: Microbial Diversity between Chow and HFD mice
A) Richness and B) Shannon's diversity metrics between chow (Chow) and high-fat diet (HFD) mice fecal samples prior to infection (day -1) and during late infection (day 11 or day 8, respectively). Asterisks indicate statistical significance where ** is a *p* value of <0.01 and *** is a *p* value of <0.001. NS indicates that there is no statistically significant difference.

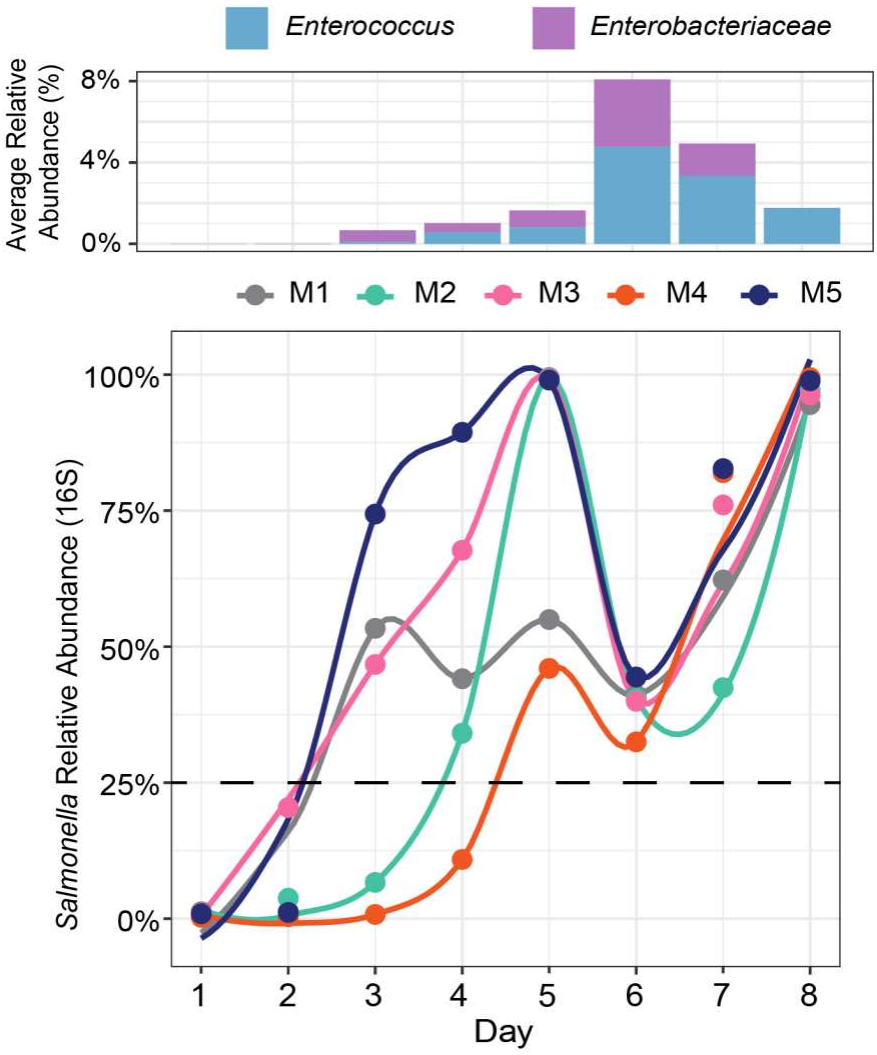

Figure S2.2: 16S *Salmonella* relative abundance per mouse over time
The line plot (bottom) shows 16S rRNA gene relative abundance of *Salmonella* per mouse over time, and the bar chart (top) shows the average relative abundance of one *Enterococcus* amplicon sequencing variant (ASV) and one Enterobacteriaceae ASV over time.

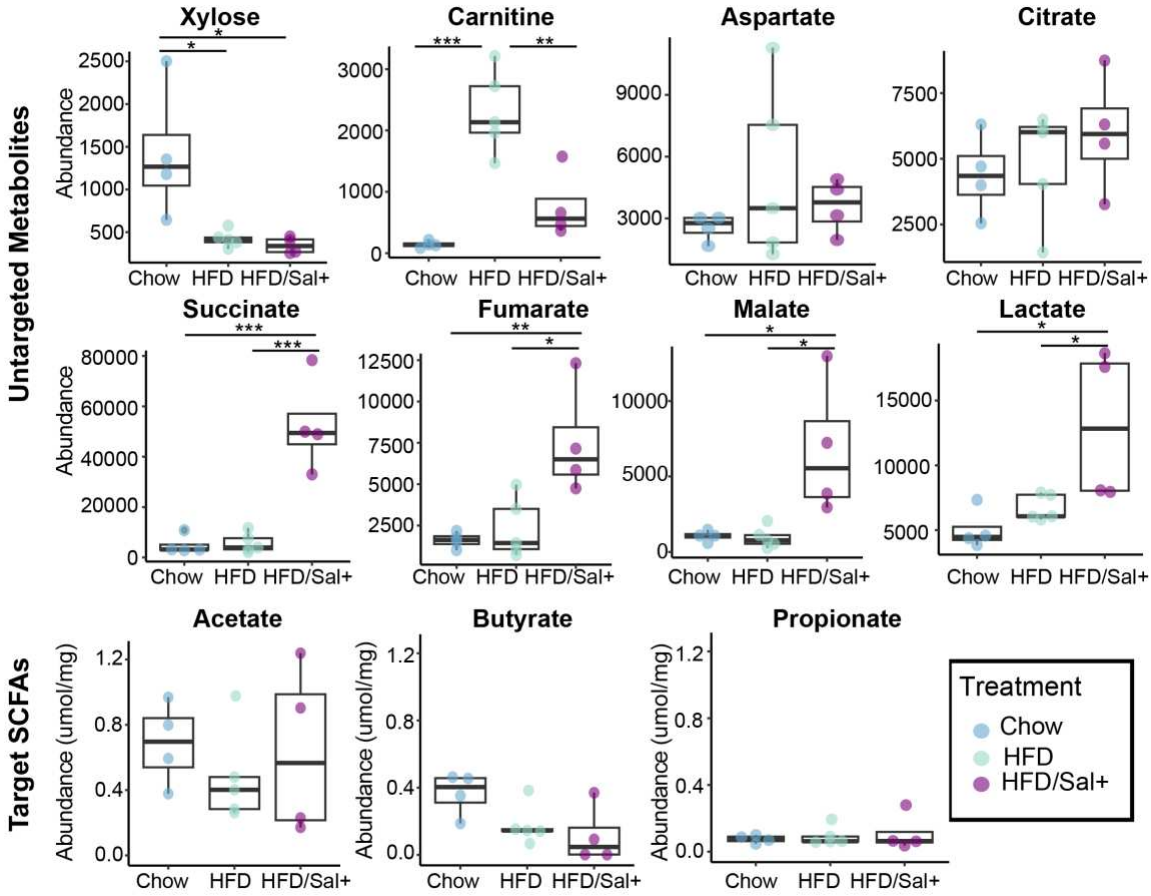

Figure S2.3: Metabolite abundance during late infection phase across treatments Boxplots depict untargeted (xylose= C00181, carnitine= C00487, aspartate=C00049, citrate= C02226, succinate= C00042, fumarate= C00122, malate= C00497, lactate= C00256) and targeted (acetate, butyrate, propionate) metabolite abundance in fecal samples of uninfected chow (Chow, blue), uninfected high-fat diet (HFD, light green), and infected high-fat diet (HFD/Sal+, purple) mice on day 7. Asterisks indicate statistical significance where * is a *p* value of <0.05, ** is a *p* value of <0.01, and *** is a *p* value of <0.001.

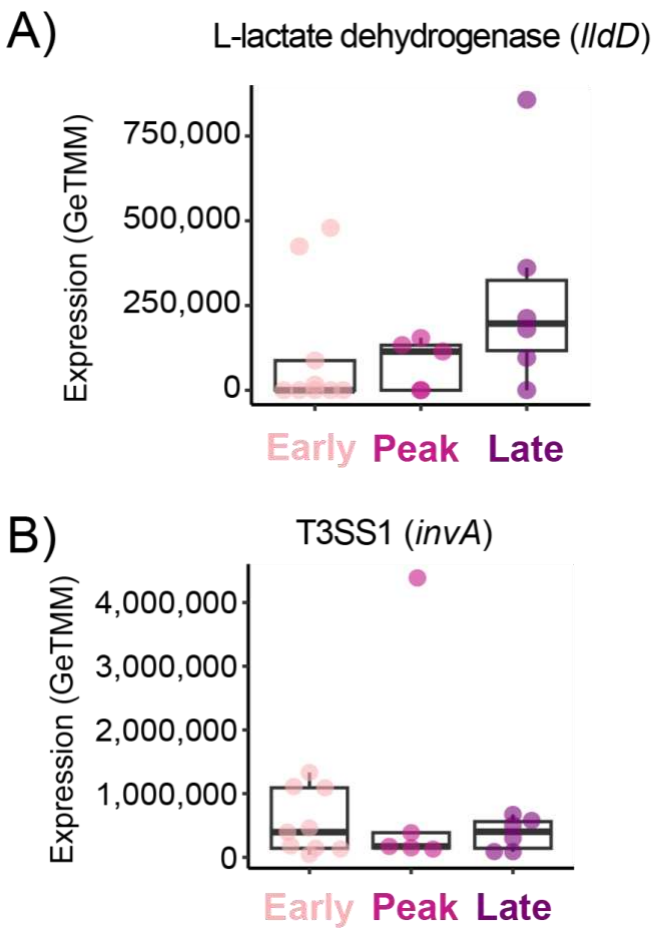

Figure S2.4: Other active, non-differentially expressed genes
Boxplots show normalized gene expression of A) L-lactate dehydrogenase (*lldD*) and B) a type III secretion protein (*invA*) across infection phases.

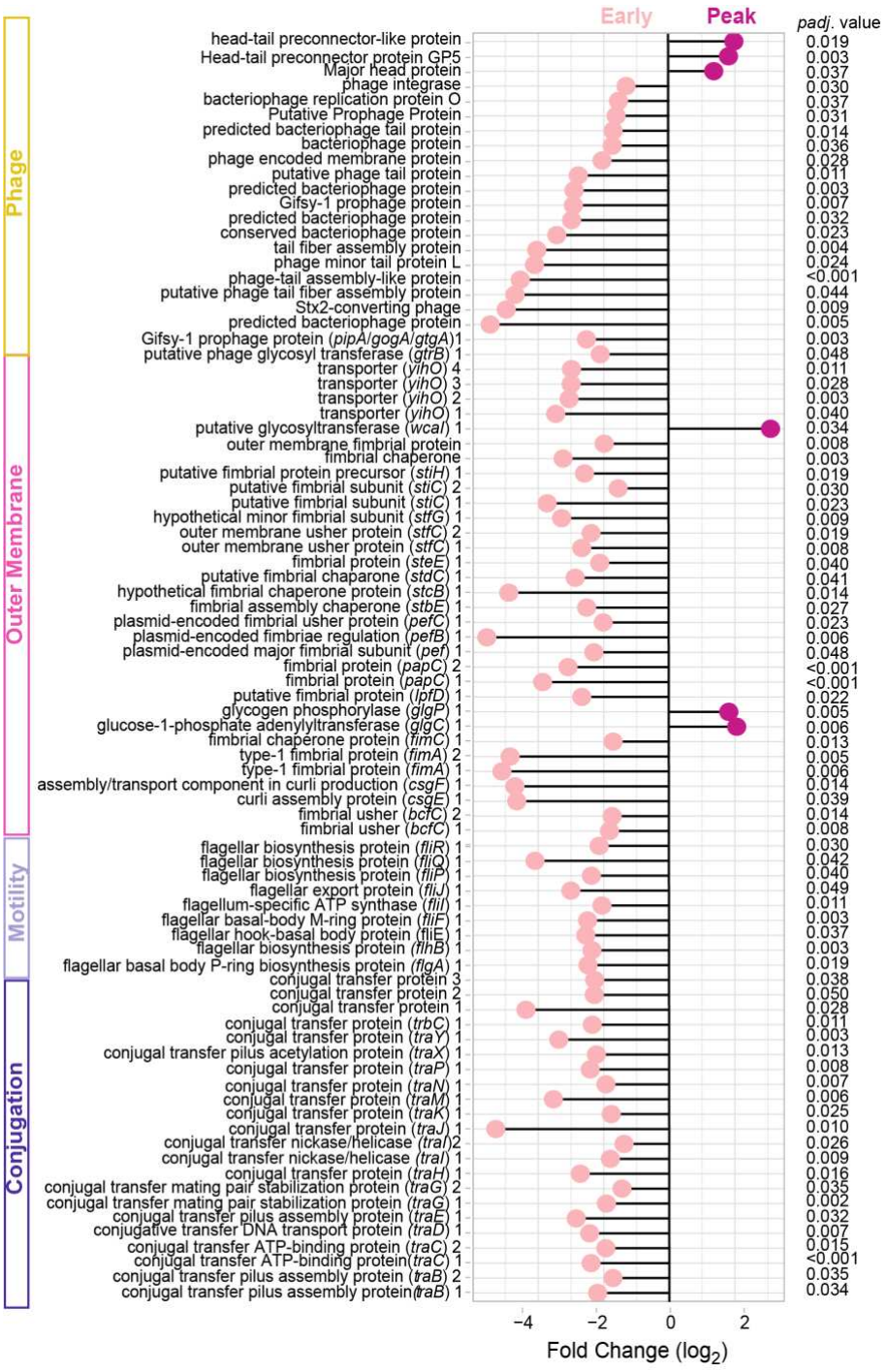

Figure S2.5: Differential expression of pathogenesis genes between early and peak phases

Lollipop plot of fold change (log_2) of differentially expressed (DESeq2, *padj.* >0.05) pathogenesis pathway genes between early (light pink) and peak (dark pink) infection phases. Genes are ordered by gene categories: conjugation (purple), motility (light purple), outer membrane (pink), and phage-like genes (yellow). Adjusted *p* values are listed in the *padj*. value column.

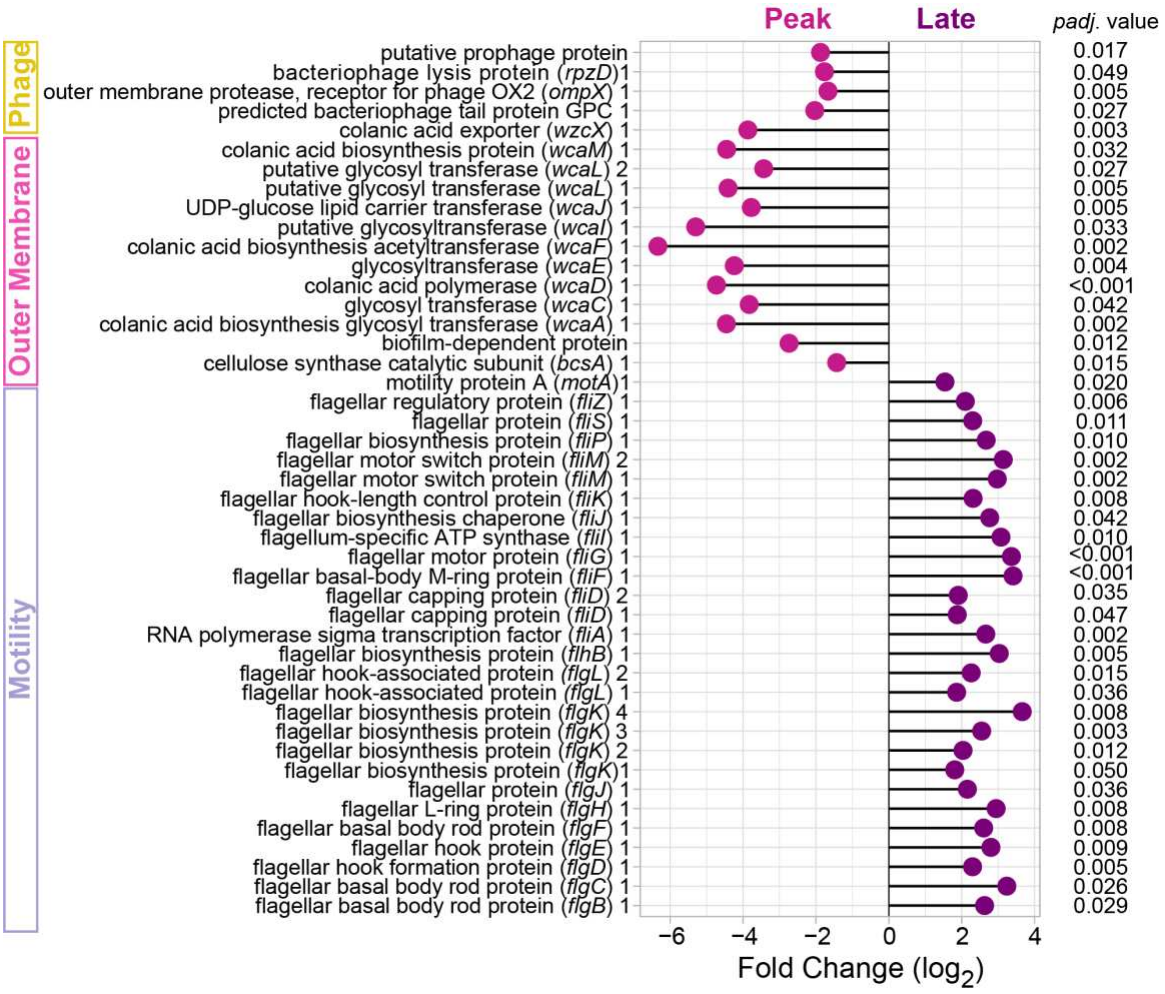

Figure S2.6: Differential expression of pathogenesis genes between peak and late phases

Lollipop plot of fold change (log_2) of differentially expressed (DESeq2, *padj.* >0.05) pathogenesis pathway genes between peak (dark pink) and late (purple) infection phases. Genes are ordered by gene categories: motility (light purple), outer membrane (pink), and phage-like genes (yellow). Adjusted *p* values are listed in the *padj*. value column.

Appendix B: Chapter 4 Supplemental Files

Supplemental files S4.1.docx: This file contains the facilitation guide for interviews which contains the purpose of each question set and interview questions. Highlighted questions were added in the interview process.

Supplemental files S4.2.docx: This file contains a list of the survey questions sent to each participant after completing the interview.

Appendix C: Additional Accomplishments and Awards

This section is written to highlight the additional accomplishments and awards I received during my PhD. In 2021, I was awarded the NIH Ruth Kirstein Institutional National Research Service Award Training Grant “Infectious Disease Research and Response Training Program”. This appointment included cohort training in science communication, admittance to in-house research retreats, and travel funds that allowed me to attend two international conferences to present my work. In 2022, I was nominated for the Philanthropic Educational Organization (P.E.O.) Scholar Award, a merit-based award for women pursuing a doctoral-level degree who have gone above and beyond to conduct research and to engage in philanthropy. While I did not receive this award, I was grateful to get to the final stage after being nominated by a local chapter of the organization. In 2023, I received the Microbiology, Immunology, and Pathology Graduate Student Excellence in Leadership award for exemplary leadership while at Colorado State University. This award was in recognition of my leadership as the President of the Microbiology, Immunology, and Pathology department’s graduate student organization, member of the department’s diversity, equity, and inclusion committee, member of the departments’ seminar committee, and participation in science education across campus. Collectively, these roles were pivotal to my PhD experience and strengthened my leadership and science communication skills. I am grateful for all those who worked to improve our department’s seminar and promote equity within our department. More information on this award can be found here (https://www.linkedin.com/pulse/presenting-2023-mip-graduate-student/).