LISTERIA MONOCYTOGENES AND DRAIN MICROBIOMES IN RETAIL DELIS DURING COVID-19 AND AN ASSESSMENT OF MICROBIAL CONTAMINATION IN SENEGALESE PEANUTS

by

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To my family and friends,

Without whom this would not have been possible.

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ABSTRACT

Foodborne illnesses have global impacts and research institutions, government agencies, and the private sector have made significant efforts to understand the causative agents of foodborne illness and to discover new ways to combat them. There are a number of foodborne pathogens of interest (e.g. *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes*) and other microorganisms that impact food safety and security on a global scale. Additionally, COVID-19 was declared a global pandemic in March 2020; the pandemic greatly impacted research efforts for more than a year. This dissertation discusses three different studies that highlight my contribution to the efforts to mitigate foodborne illness both domestically and abroad. The chapters of this dissertation describe (i) the impact of COVID-19 on *Listeria monocytogenes* in retail deli departments, (ii) the microbial consortia inhabiting retail deli drains and drain biofilms, and (iii) microbial contamination of peanuts produced and sold in the Senegalese peanut basin.

Chapter 1: Given the diversity of the studies contained in this dissertation, I began with a review of literature for the various chapters discussed here. This chapter begins with an introduction to the COVID-19 pandemic due to its significant effects on the research described here. The review then briefly summarizes the current knowledge of *Listeria monocytogenes* and its importance in the retail deli environments, as well as the microbial ecology of drains and biofilms in food processing. Additionally, this chapter ends with a summary of the current literature in regard to peanut production, consumption, and concerns associated with foodborne illness derived from peanut consumption.

Chapter 2: "*Listeria monocytogenes* prevalence in retail delicatessen departments decreased during the first year of the COVID-19 pandemic" describes a study investigating *L. monocytogenes* contamination in retail deli departments during COVID-19 and validating a predictive risk model associated with the enhanced cleaning and sanitation procedures utilized in response to the pandemic. This study was conducted in 44 retail deli departments across seven states in the US. The results showed that *L. monocytogenes* prevalence decreased from 5.8% positive prior to March 2020 to 4.3% during the pandemic. No *L. monocytogenes* was found on the scales or trashcans, which were factors previously correlated with high *L. monocytogenes*

prevalence (>10%). The predictive model accurately predicted high *L. monocytogenes* in 10/17 stores with high prevalence ($\alpha < 0.0001$, $\beta=0.1186$) during the COVID-19 pandemic. Cleaning and sanitation protocols were the factors most highly correlated with high *L. monocytogenes* prevalence in the conducted survey. These results indicated that the heightened awareness of personal hygiene and cleaning and sanitation due to COVID-19 likely reduced the prevalence of *L. monocytogenes* in retail delis.

Chapter 3: "Characterization of retail delicatessen drains and biofilms using 16S rRNA metataxonomic and shotgun metagenomic sequencing" was a study designed to understand the microbial ecology of retail deli drains and biofilms harvested from deli drains. In this study, 14 biofilms were harvested from drain trenches and environmental sponge samples were collected from the surface of the same drain covers. 16S rRNA gene sequencing was used to characterize the microbiome of the biofilms and sponge samples and shotgun metagenomics analysis was conducted on nine biofilms with ≥10 ng/µL DNA. While *Pseudomonas* spp. dominated the microbiomes of the biofilms and drain surfaces, the microbial consortia inhabiting each location of the drains was vastly different. Additionally, shotgun metagenomics revealed that pathogenic bacterial species were in low abundance in the biofilms, and rare taxa reside in the same biofilms. Common sanitizer resistance genes (*qacEΔ1*, *qacE*, and *qacL*) were observed in the biofilms as well, indicating possible increased tolerance to quaternary ammonium-based sanitizers.

Chapter 4: "Microbial contamination patterns in peanuts produced and sold in the Senegalese peanut basin" was a study conducted as part of the USAID Feed the Future Food Safety Innovation Lab (FSIL) in collaboration with the Senegalese Institute of Agricultural Research (ISRA). A survey was conducted of 198 households that produce peanuts in Senegal and 198 peanut samples were collected for microbial analysis. These peanut samples were evaluated for Enterobacteriaceae, coliforms, and total yeast and mold concentrations, then observed populations (log CFU/g) were correlated with survey questions related to producer knowledge of microbial contamination and storage methods utilized by producers. The results indicated that peanuts were heavily contaminated with the fecal indicators Enterobacteriaceae and coliforms (13.0% and 13.6% above detection limit [5.0 log CFU/g], respectively). Only 22.7% and 18.7% of producers reported they had heard of pathogenic bacteria or aflatoxins, respectively, before this study. Additionally, the combination of storage container type and whether the peanuts were store off the ground were predictive of bacterial contamination. This study provides preliminary data to inform

future studies which should assess prevalence of pathogenic microorganisms (e.g. *Salmonella* spp. and *E. coli*) and evaluate preventive measures to be utilized during harvest and storage to minimize the risk of microbial contamination of peanuts.

CHAPTER 1. LITERATURE REVIEW

1.1 Impacts of the COVID-19 pandemic on food safety

COVID-19 is a highly contagious respiratory disease that is caused by severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2 (WHO, 2020b). The first known case of COVID-19 was identified in Wuhan, Hubei Province, China in November 2019 (Huang et al., 2020). As of October 20, 2021, 243.6M cases have been reported globally, with 46.2M in the United States alone (worldometer.com, 2021). Although precise host origin remains unclear, (Huang et al., 2020), current consensus is that the virus emerged from a non-domestic animal in a local seafood market, commonly known as a "wet market." The World Health Organization (WHO) declared COVID-19 a global pandemic on March 11, 2020 (WHO, 2020c); since this announcement, countries have implemented preventive measures to slow the spread of COVID-19, such as social distancing, quarantining, isolation, and new vaccinations and treatments to combat the virus. However, these policies also disrupted many business and research ventures both domestically and abroad, and many livelihoods were compromised by business closures and decreased consumer spending (WHO, 2020a).

In response to COVID-19, many local governments and retail companies have implemented measures to minimize the risk of SARS-CoV-2 transmission between employees and customers (Shumsky et al., 2021). These contact prevention measures typically included one-way isles, occupancy limits, increased disinfection frequency of high-touch surfaces, reduced operating hours to allow for third-party cleaning, and safe shopping hours for vulnerable groups (Shumsky et al., 2021). While SARS-CoV-2 is not considered a foodborne pathogen, many retail grocery chains still took precautions to prevent spread of disease through food handling by removing self-service deli bars and reducing the number of products sliced and sold in the store, thus increasing pre-packaged food sales. Additionally, the heightened awareness of personal hygiene, human interaction, and cleaning and sanitation that resulted from the pandemic has likely had a positive impact on environmental contamination of foodborne pathogens, such as *L. monocytogenes*. In 2020, 363 food recalls were issued, of which 141 occurred in the first quarter (January to March), however, only 79 and 52 were reported in the second and third quarters, respectively; a 44% decrease from 2019 (Shumsky et al., 2021). *L. monocytogenes* was responsible for 47 recalls in

2020, a decrease from the 60 recalls due to *L. monocytogenes* contamination in 2019 (Food Safety Magazine, 2020).

1.2 Introduction to *Listeria monocytogenes* and the retail deli environment.

The Centers for Disease Control and Prevention (CDC) estimate that 31 pathogens cause approximately 37 million illness each year in the United States, of which 9.4 million are foodborne; the CDC estimates that 5.5 million (58.5%) of these are caused by bacteria (Scallan et al., 2011). Additionally, foodborne pathogens are responsible for 55,961 hospitalizations and 1,351 deaths annually in the US (Scallan et al., 2011). Of the bacterial foodborne pathogens, *Listeria monocytogenes* is the second leading cause of death, accounting for 1,455 hospitalizations and 255 deaths (15.9% mortality rate) in the US. Approximately 99% of these hospitalizations are attributed to consumption of contaminated foods (Scallan et al., 2011), particularly ready-to-eat (RTE) deli meats (USDA & FDA, 2003).

1.2.1 *Listeria spp.* and *L. monocytogenes*.

Listeriae are rod-shaped, gram-positive, facultative anaerobes that are widely distributed in the environment (Schmid et al., 2005). The bacterial genus *Listeria* has 22 recognized species, 11 of which have been described since 2009, including *L. monocytogenes, L. seeligeri, L. ivanovii, L. welshimeri, L. marthii, L. innocua, L. grayi, L. fleischmannii, L. floridensis, L. aquatica, L. newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia, L. booriae, L. cossartiae, L. farberi, L. immobilis, L. portnoyi,* and *L. rustica* (Carlin et al., 2021; Orsi & Wiedmann, 2016). While most *Listeria* spp. are considered apathogenic, species within the genus, *L. monocytogenes* and *L. ivanovii,* are known to cause disease in humans and animals (Schmid et al., 2005).

L. monocytogenes is the *Listeria* species most commonly associated with human disease and thus has warranted significant attention by food safety experts. *L. monocytogenes* is ubiquitous and has been isolated from diverse environments, including soil (Vivant et al., 2013), food manufacturing (Muhterem-Uyar et al., 2015; Tompkin, 2002), and retail grocery environments (Burnett et al., 2020; Pouillot et al., 2012; Simmons et al., 2014). The pathogen can not only survive, but grow, in adverse conditions typically used for pathogen control in food processing, such as refrigeration temperatures, low pH, and high salt concentrations (Gandhi & Chikindas,

2007). Between 2002 and 2006, the USDA issued 333 food recalls of which 32.4% (108) were due to *L. monocytogenes* (Drevets & Bronze, 2008). A 2003 FDA-USDA-CDC quantitative risk assessment determined that the majority of listeriosis cases are due to consumption of contaminated RTE food products, particularly RTE deli meats (USDA & FDA, 2003). While the prevalence of *L. monocytogenes* prevalence in deli meat samples has declined at the manufacturing level, the frequency of human listeriosis cases has not reduced as expected. Pradhan et al. (2010) estimated that 83% of human listeriosis cases associated with consumption of RTE deli products were likely attributed to contamination at the retail level.

1.2.2 Listeriosis and mechanisms of disease.

L. monocytogenes is the causative agent of listeriosis (Gandhi & Chikindas, 2007). Once consumed, *L. monocytogenes* is capable of surviving the harsh conditions associated with mammalian gastrointestinal tracts. The internalin A (*intA*) and internalin B (*intB*) genes encodes proteins necessary for invasion of epithelial cells of the intestine (Gaillard et al., 1991), where the pathogen is consumed by intracellular phagosomes. Listeriolysin O (LLO) and two phospholipases are involved in lysis of the phagosomal membrane and escape of the cell into the cytoplasm, while ActA aids in cell-to-cell spread (Pizarro-Cerda et al., 2012). *L. monocytogenes* cells can ultimately reach the liver, allowing spread to other tissues, including the central nervous system (Marquis et al., 2015).

Listeriosis is most common in newborns, the elderly, pregnant women, and immunocompromised individuals (YOPIs). While it was previously known that *L. monocytogenes* is transmitted through oral ingestion, it was verified as a foodborne pathogen in the 1980's (Schlech et al., 1983). There are two forms of listeriosis caused by *L. monocytogenes*: (i) noninvasive gastrointestinal listeriosis and (ii) invasive listeriosis (Allerberger & Wagner, 2010). In healthy individuals, listeriosis usually presents with febrile gastrointestinal symptoms, including fever, diarrhea, nausea, headache, and joint pain (Allerberger & Wagner, 2010; Lecuit, 2007). In otherwise healthy individuals, symptoms are usually mild and self-limiting. However, immunocompromised individuals typically present with the more severe, invasive form of listeriosis, which often manifests as septicemia, meningitis, and encephalitis (Allerberger & Wagner, 2010; Drevets et al., 1995). Additionally, invasive listeriosis can be orally contracted by pregnant women and transmitted from the mother to the fetus through the placenta, leading to

abortion, stillbirth, sepsis, or meningitis of the newborn (Allerberger & Wagner, 2010; Lecuit, 2007).

1.2.3 *Listeria* in Food and the Retail Food System.

L. monocytogenes is a foodborne pathogen associated with a variety of food products, including meats, cheeses, and produce (Jordan & McAuliffe, 2018). *L. monocytogenes* is an opportunistic pathogen that grows and survives in diverse environments (Olaimat et al., 2018). The pathogen has been isolated from a multitude of sources, including processed meats and cheeses, produce, dairy products, soil, silage, and livestock (Beresford et al., 2001). The common occurrence of *L. monocytogenes* in agriculture systems contributes to the introduction of the pathogen to foods and food system environments by humans or raw ingredients (Hammons & Oliver, 2014). *L. monocytogenes* is sensitive to high acidity, pressure, and high temperatures; thus, the pathogen can be killed via thermal processing methods (Hammons & Oliver, 2014). Therefore, it is unlikely that raw ingredients cause illness in RTE products and *L. monocytogenes* contamination in RTE foods is more likely attributed to cross-contamination during processing and handling post-lethality treatment (Forauer et al., 2021; Hammons & Oliver, 2014; Pradhan et al., 2010).

The open structure and continuous movement of consumers provide numerous entry points for the introduction of *Listeria* spp. to retail environments (Forauer et al., 2021). Surfaces that are rarely cleaned thoroughly accumulate soils and food residues, creating niches for biofilm formation and *Listeria* survival (Forauer et al., 2021; Hammons & Oliver, 2014). Pooled water in poorly designed facilities and drains where soils and foods can accumulate are harborage points for *Listeria* spp. (Hammons et al., 2017). Additionally, persistent *L. monocytogenes* and other *Listeria* spp. are often found in corners and niches of difficult-to-clean equipment, including deli case coils, scales, slicers, and sinks (Etter et al., 2017; Hammons et al., 2017; Tompkin, 2002; Wu et al., 2020a). *Listeria* spp. can also be spread from non-food contact surfaces (NFCS) to food contact surfaces (FCS) through human contact, improper sanitation and handling, and aerosols from high pressure hoses, which can ultimately create new niches for *L. monocytogenes* harborage and cross-contaminating RTE deli products (Forauer et al., 2021; Hammons et al., 2017; Saini et al., 2012).

1.2.4 Control Strategies for *L. monocytogenes* at retail.

The ubiquitous nature of *L. monocytogenes* makes it difficult to control and items from all food categories have been the source of listeriosis outbreaks (Lianou & Sofos, 2007). Food products that are more likely to be associated with listeriosis outbreaks include products that are susceptible to post-lethality contamination and have an extended shelf-life under refrigerated conditions (Hammons & Oliver, 2014; Lianou & Sofos, 2007). Additionally, at the retail level, there is a risk of cross-contamination from the flow of people and products (Forauer et al., 2021; Maitland et al., 2013); it is important to ensure that only deli employees have access to the RTE deli department to minimize the risk of *L. monocytogenes* contamination from raw product departments (Hammons & Oliver, 2014). The US Interagency Retail *Listeria monocytogenes* Risk Assessment Workgroup (Akingbade et al., 2013) reported on five key findings to reduce the risk of *L. monocytogenes* in retail deli departments: (i) control growth with growth inhibitors and temperature control, (ii) control cross contamination, (iii) control contamination at its source (e.g. incoming products and raw ingredients), (iv) continue sanitation and improve these practices by making deli areas easier to clean and maintain, and (v) identify key routes of contamination (e.g. the deli slicer). Additionally, the workgroup recommends verifying that cleaning was performed correctly and providing leadership and support for food safety measures thereby creating a culture that values food safety (Hammons & Oliver, 2014; Powell et al., 2011; Wu et al., 2020b).

Our group has worked extensively to identify challenges in facility design, niches that could harbor *L. monocytogenes,* and practices that may increase the likelihood for high *L. monocytogenes* prevalence. Studies have shown that NFCS often are significant harborage points for *L. monocytogenes* in retail deli departments; the most common harborage points are drains, floor-wall junctions, standing water, and squeegees (Burnett et al., 2020; Etter et al., 2017; Hammons et al., 2017; Simmons et al., 2014; Wu et al., 2020c). A longitudinal study by our group isolated *L. monocytogenes* from 25/30 stores and identified persistent *L. monocytogenes* subtypes (isolated at least two times from the same deli) in 14 of these stores (Simmons et al., 2014). In a follow-up study, Etter et al. (2017) implemented enhanced sanitation standard operating procedures (SSOPs) utilizing recommendations from relevant stakeholder groups in 30 retail delis. The enhanced SSOPs had limited impact on environmental *L. monocytogenes* in retail deli departments and did not impact persistent subtypes. Another study (Hammons et al., 2017) evaluated the efficacy of an aggressive, third-party, deep cleaning SSOP (DC-SSOP) in nine retail

delis. The DC-SSOP did reduce *L. monocytogenes* by up to 25.6% during the three-month follow-up period and had varying effects on persistent isolates; however, the DC-SSOP did not eliminate persistent *L. monocytogenes* in this study and reductions were not maintained over time (Hammons et al., 2017).

There was a strong correlation between *L. monocytogenes* prevalence and certain deli employee behaviors (e.g. washing hands, changing gloves, etc) in a comprehensive survey distributed to managers of the 30 retail stores participating in the enhanced SSOP and DC-SSOP studies (Wu et al., 2020a). Therefore, Wu et al. (2020c) implemented an employee-executed SSOP (EE-SSOP), complimented with employee and management training and facility improvements, in seven stores identified to have high *L. monocytogenes* prevalence. The EE-SSOP immediately reduced *L. monocytogenes* from 15.2% to 5.8% after deep-cleaning and maintained a reduction of 10.8% during the six-month follow-up period (Wu et al., 2020c). Taken together, these data indicate that a commitment to food safety by supervisors and managers and a strong food safety culture within the organization are critical in mitigating *L. monocytogenes* is (Neal et al., 2012; Wu et al., 2020b).

1.2.5 Understanding the microbial ecology of retail deli environments.

Built environments, including food processing and retail food establishments, are characterized by the microbial communities that establish themselves to the surfaces of equipment and other areas in the environment (De Filippis et al., 2021). In food processing environments, these communities often contain an array of microbes, including spoilage microorganisms and pathogens that establish persistence in niche locations (hard-to-clean surfaces), often through the formation of biofilms (Bridier et al., 2015). Spoilage microorganisms harbored within the microbiome of food processing and retail environments can negatively affect the shelf-life and quality of RTE foods sold at retail. While there is limited research evaluating the microbial ecology of retail grocery environments, different groups have studied the microbiome of drains in food processing facilities and various RTE food products. For example, Dzieciol et al. (2016) studied water and biofilms from drains in a cheese processing facility and identified the predominate phyla found in drains consisted of product-, fermentation-, and food spoilage-associated phylotypes. This study also found that the microbial ecology of drain water was significantly different than the community forming biofilms in drains and suggested that these biofilms may be a source of cross

contamination of spoilage bacteria (Dzieciol et al., 2016). Recent research of the microbiome of various RTE retail products (romaine lettuce, cabbage, deli meats, and chicken legs) has offered characterization of the bacterial ecology of these foods. Higgins et al. (2018) observed differences in the predominate phyla within the microbiome of produce and meat products; they reported that these communities may differ based on store size (small vs. large). Another study (Hoisington et al., 2016) investigating the indoor airborne microbial diversity in 13 U.S. retail grocery stores found that the most prevalent genera found in HVAC filter dust samples are closely associated with human oral and skin microbiomes; thus the microbial communities found in indoor air are likely influenced by the occupants within these retail environments (Hoisington et al., 2016). The open environments of retail delis coupled with high traffic from store visitors, likely introduce pathogens and spoilage bacteria, thus increasing the chances of cross-contamination.

Additionally, there is a high risk of cross contamination of pathogens from NFCS to FCS during daily operations (Hoelzer et al., 2011), making persistent *L. monocytogenes* an environmental health concern. Persistent *L. monocytogenes* subtypes are commonly found in niche locations, such as drains and floor wall junctures (Simmons et al., 2014). This persistence is likely due to the biofilm-forming ability of *L. monocytogenes* and tolerance to sanitation (Folsom & Frank, 2006); it has been established that complex, multigenus biofilms containing *L. monocytogenes* have even higher sanitizer tolerance than *L. monocytogenes* alone (Fagerlund et al., 2017). The efficacy of cleaning and sanitizing chemicals utilized in food processing environments is typically determined based on tests against planktonic bacteria, not biofilms, which may explain the inability of current intervention protocols to reduce biofilm-forming pathogens prevalence at retail (Coughlan et al., 2016; Fagerlund et al., 2017). A recent study (Fagerlund et al., 2017) identified that the predominate subspecies of background microbiota on conveyor belts in meat processing facilities shifted when the biofilms were subjected to cleaning and sanitation cycles (Fagerlund et al., 2017). Another study (Fox et al., 2014) evaluating *L. monocytogenes* in food processing environment floor drains identified differences in the microbial communities between drains with *L. monocytogenes* present and those without. Additionally, some studies have shown that the microbial communities residing in these niches can promote the survival of *L. monocytogenes* (Carpentier & Chassaing, 2004), while others have shown certain bacterial species can inhibit *L. monocytogenes* growth (Fox et al., 2014; Langsrud et al., 2016; Rodríguez-López et al., 2018). Since retail deli environments provide a known risk for cross-

contamination of *L. monocytogenes*, it is important to consider the microbiome of the built environment found in retail deli departments and understand how the ecology of these environments can influence the persistence of the pathogen.

1.3 Introduction to food safety and peanut production in Senegal, Africa

The peanut (*Archis hypogaea* L.), an annual legume, is also known as the groundnut, earthnut, monkeynut, and goobers (Guchi, 2015; Surendranatha Reddy et al., 2011). Peanuts rank as the world's 13th most important food crop and 4th most important oilseed crop (Surendranatha Reddy et al., 2011). Peanuts are important to global consumption because they are highly nutritious food stuffs and a source of many micronutrients, including vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron, and potassium (Guchi, 2015); thus, peanuts are a significant nutritional source in food insecure countries across the globe.

1.3.1 Foodborne illness and food safety research on the African continent.

According to the World Health Organization (WHO), the burden of foodborne diseases in greatest in African countries (WHO, 2015). WHO estimated that foodborne hazards are responsible for 135 million acute illnesses and approximately 180,000 deaths each year in Africa; children under the age of five are the most at risk, especially for diarrheal pathogens such as *Escherichia coli*, non-typhoidal *Salmonella enterica* serovars, and *Campylobacter* spp. (McDonnell, 2019). In Africa, microbial pathogens (e.g. toxigenic *E. coli*, *Salmonella* spp., *Campylobacter* spp., and norovirus) account for approximately 80% of the foodborne disease burden (Jaffee et al., 2019). The Global Food Safety Partnership (GFSP; Ababa, 2019) recently reported that more than half of the donor-funded food safety projects in Sub-Saharan Africa are focused on food safety of commodities for export, with minimal efforts focused on food safety for domestically consumed foods. In addition to illness, foodborne disease is responsible for an estimated $16.7 billion a year in human capital losses across the African continent (Ababa, 2019). Additionally, estimated productivity losses associated with unsafe foods in Africa approach $20 billion a year, with an additional $3.5 billion attributed to the cost of treating these illnesses (Jaffee et al., 2019).

Traditionally, African policymakers and international research donors (e.g. USAID, The World Bank Group, and European Commission) have focused resources and research on food

production and food security; however, food safety is slowly gaining importance in various development agendas (Arias-Granada et al., 2020). While data of foodborne disease for individual countries are limited, international development agencies recognize the need for food safety research in middle- and low-income countries and have started focusing resources to identify at-risk commodities and ways to mitigate associated microbial contamination risks. GAFP estimates that international donors spent $383 million to support food safety projects in Senegal between 2010 and 2017 (Arias-Granada et al., 2020). This research has focused on high risk foods, such as meat and poultry (Pouillot et al., 2012; Stevens et al., 2006; Vounba et al., 2019), seafood products (Coly et al., 2013; Demoncheaux et al., 2012), raw milk (Breurec et al., 2010), and drinking water (Rondi et al., 2014; Sorlini et al., 2013). However, data offering characterizations of bacterial contamination of staple crops and commodities, including peanuts, maize, and leafy vegetables, are limited. Food safety research of these commodities in Senegal has predominately focused on managing aflatoxin contamination, which is produced by the fungi *Aspergillus flavus* (Senghor et al., 2020). Exposure to aflatoxins is a significant risk factor for hepatocellular carcinoma (Liu & Wu, 2010). As more than 55 million people worldwide are exposed each year to high (above acceptable daily intake levels) levels of aflatoxin, significant efforts have focused on minimizing aflatoxin contamination of commonly eaten foods. Many of the pre- and post-harvest management practices aimed at reducing aflatoxin contamination of peanuts (Torres et al., 2014), may also be useful in managing bacterial contamination in peanuts. Humans, livestock, and other animals are known reservoirs of these bacterial pathogens. In Senegal, livestock and other animals are often near households and peanut storage areas, increasing the likelihood of cross-contamination. Thus, proper storage of harvested peanuts (in sealed storage containers away from animals) and heat treatment before consumption may be important mitigation strategies to reduce the burden of foodborne disease associated with bacterial contamination of peanuts.

1.3.2 Peanut production and consumption in Africa.

Senegal, like many countries in Sub-Saharan African countries with developing economies, relies heavily on agricultural production for food and income (Thuo et al., 2014). In 2020-2021, Senegalese peanut production was estimated at 1,797,000 tons (USDA-FAS, 2021). More than 60% of this production occurs across five regions of Senegal: Fatick, Kaolack, Kaffrine, Louga, and Thies regions (Dieme et al., 2018). Peanuts cover approximately 40% of the

cultivatable land in these regions, thus making the area known as the Senegalese Peanut Basin (Thuo et al., 2014). In Senegal, Peanuts are generally cultivated by smallholder and resource-poor famers (Faye et al., 2018; Tarawali & Quee, 2014) in a country that is highly susceptible to climate change. Over the past 30 years, peanut production has decreased due to high temperature and drought conditions (Faye et al., 2018). Thus, researchers at the Senegalese Institute of Agricultural Research (ISRA) and at various international development agencies have focused on developing drought-resistant types of peanuts and identifying harvest and storage methods to improve crop yields under these conditions (Faye et al., 2018; Roudier et al., 2014; Thuo et al., 2014).

Senegal is one of five countries (Argentina, US, Sudan, Senegal, and Brazil) accounting for 71% of total world peanut exports (Torres et al., 2014). Peanuts are the fourth leading revenue generating export in Senegal and one of the main sources of income for Senegalese smallholder farmers, making it an important cash crop in Senegal (Dieme et al., 2018; Faye et al., 2018; Georges et al., 2016). However, the nutritional value of peanuts highlights the importance of domestic consumption as well. Domestic consumption of peanuts was approximately 79,000 metric tons in 2019, nearly 5.6% of the total national production (Aria-Granada et al., 2020). Peanuts are consumed raw, roasted, boiled, as oil extracted from the kernel, or in oilcake or peanut butter forms (Guchi, 2015). These multiple uses make peanuts a useful cash crop for domestic and international consumption. However, microbial contamination during the production, harvest, storage, and consumption stages can affect the quality, safety, and value of Senegalese peanuts in both local and global markets.

1.3.3 Food safety concerns with peanuts.

Peanuts are often considered microbiologically "safe" foods due to low water activity (< 0.75) and additional thermal processing steps commonly utilized before consumption. While peanuts can be consumed raw, this rarely occurs and most are subjected to roasting and further processing (e.g. peanut butter), thus subjecting peanuts to a heat lethality step (Indiarto & Rezaharsamto, 2020). As such, peanuts have traditionally been viewed as insignificant risks for foodborne illness and, to the best of our knowledge, the burden of disease associated with bacterial contamination of peanuts is unknown. However, peanuts have recently been associated with foodborne pathogen outbreaks, including a 1996 outbreak of *S. enterica* ser. Mbandaka in Australia, a 2001 outbreak of *S. enterica* ser. Stanley in Australia and Canada, and a 2009 outbreak

of *S. enterica* ser. Typhimurium in the U.S. (Harris et al., 2019). It is likely that poor handling before and during processing, and storage post-processing is responsible for any outbreaks associated with peanuts (Chang et al., 2013). Thus, it is important to focus research efforts on proper handling, storage, and cooking methods for peanuts produced in middle- and low-income countries, especially those utilized for domestic consumption.

1.3.4 Effects of COVID-19 on food security.

The COVID-19 pandemic has impacted a growing number of developing economies by reducing incomes and disrupting supply chains, reversing years of development gains (The World Bank, 2021). These impacts have led to increases in global food insecurity, causing a rise in chronic and acute hunger in countries susceptible to farming and economic shocks (Middendorf et al., 2021; The World Bank, 2021). It is expected that the poorest and most vulnerable countries will most greatly feel the effects of the COVID-19 pandemic (Mardones et al., 2020). In Senegal specifically, a survey was conducted to understand smallholder farmer perceptions of anticipated impacts of COVID-19. The survey was distributed to farmers in three agricultural sectors (cropping, livestock, and horticulture; Middendorf et al., 2021) , and respondents from all three sectors expressed concern over access to critical inputs, ability to plant, reduction of crop yields and labor resources, and ability to feed and sell livestock. Additionally, respondents reported concerns related to access to food for their households, that markets would by disrupted or close altogether, and that the price of food would greatly increase (Middendorf et al., 2021). Similar concerns are likely rampant across all food insecure economies. Studies are further warranted to determine actual effects of the pandemic on farming and human well-being; however, due to the continued emergence of SARS-CoV-2 variants, it is likely the pandemic and its impacts on food security will continue into the foreseeable future.

CHAPTER 2. *LISTERIA MONOCYTOGENES* PREVALENCE IN RETAIL DELICATESSEN DEPARTMENTS DECREASED DURING THE FIRST YEAR OF THE COVID-19 PANDEMIC

2.1 Abstract

The purpose of this study was to measure the prevalence of *L. monocytogenes* at retail and validate a predictive statistical model for assessing *L. monocytogenes* contamination risk in similar businesses. Notably, the study took place March 2020 to March 2021 which coincided with the COVID-19 pandemic. Environmental samples were collected twice from 44 retail deli departments across seven states during the 12-month period. Each sample was tested for *L. monocytogenes* and other *Listeria* spp.; confirmation was conducted via whole genome sequencing and PCR amplification of the *sigB* gene. A Firth's bias-corrected logistic regression model was developed to predict the probability of a deli having high *L. monocytogenes* prevalence (>10%); samples collected between March 2020 and March 2021 were used to validate the model. A 117-question survey was developed based on previous work in retail deli departments and distributed to store or deli managers once during sampling. Survey responses were correlated with *L. monocytogenes* using linear regression and ANOVA statistical models. *L. monocytogenes* prevalence decreased from 5.8% prior to March 2020 to 4.3% during the pandemic. Squeegees and standing water had the highest *L. monocytogenes* prevalence of all sampling sites in the study; *L. monocytogenes* was not found on scales or trash cans. The model accurately predicted high *L. monocytogenes* prevalence in 10/17 sampling events with high prevalence and low risk for all 42 sampling events with low *L. monocytogenes* prevalence ($\alpha < 0.0001$, $\beta=0.1186$). Additionally, our study found that cleaning and sanitation protocols correlated with lower *L. monocytogenes* prevalence. As the COVID-19 pandemic likely heightened awareness of disease transmission and the importance of sanitation to prevent spread of SARS-CoV-2, the results suggest that there may have been additional benefits (i.e., reduced *L. monocytogenes* prevalence and therefore reduced risk of transmission to foods) beyond decreasing SARS-CoV-2 transmission.

2.2 Introduction

In November 2019, the first case of COVID-19 was identified in Wuhan Province, China; the World Health Organization (WHO) declared COVID-19 a global pandemic on March 11, 2020

(WHO, 2020c). In response, countries have implemented preventative measures to slow the spread of COVID-19. Many retail stores and local governments have implemented measures to reduce the transmission of SARS-CoV-2 between customers and employees, including one-way aisles, reduced operating hours, occupancy limits, and "safe shopping" times for vulnerable groups (Shumsky et al., 2021).

In 2020, 363 food recalls were issued, of which 141 occurred in the first quarter (January to March). However, only 79 and 52 recalls were reported in the second and third quarters, respectively, a 44% decrease from 2019 (Shumsky et al., 2021). *L. monocytogenes* was responsible for 47 recalls in 2020; this is a decrease from the 60 recalls due to *L. monocytogenes* contamination in 2019 (Food Safety Magazine, 2020). While SARS-Cov-2 is not a foodborne pathogen, the pandemic has heightened awareness of personal hygiene and shown the importance of cleaning and sanitation protocols to reduce disease transmission, likely reducing environmental contamination of foodborne pathogens.

L. monocytogenes is among the deadliest bacterial foodborne pathogens in the United States, resulting in an estimated 1,600 cases of foodborne illness and 260 deaths each year (Scallan et al., 2011). A 2003 FDA-USDA-CDC quantitative risk assessment determined that the majority of listeriosis cases are due to consumption of contaminated ready-to-eat (RTE) food products, particularly RTE deli meats (USDA & FDA, 2003). Despite the substantial decline in *L. monocytogenes* prevalence in deli meat at the manufacturing level, the frequency of human listeriosis cases have not reduced as expected. Pradhan et al. (2010) estimated that 83% of human listeriosis cases associated with consumption of RTE deli products were likely attributed to contamination at the retail level.

It is well-established that *L. monocytogenes* is readily found throughout retail deli departments. Niches within these departments provide environments conducive to persistent strains, increasing the likelihood of cross-contamination from deli non-food contact surfaces (NFCS) to food contact surfaces (FCS) (Pradhan et al., 2010). Recent studies have found *L. monocytogenes* at least once in more than 60% of stores sampled and have determined that *L. monocytogenes* prevalence can range from 0% to greater than 30% per store (Etter et al., 2017; Forauer et al., 2021; Simmons et al., 2014). Many of these studies also focused on measuring the efficacy of different sanitation standard operating procedures (SSOP) to reduce persistent *L. monocytogenes* prevalence. Etter et al. (2017) reported that enhanced daily SSOPs did not

significantly reduce *L. monocytogenes* in all 30 delis enrolled in the study. A follow-up study (Hammons et al., 2017) determined that novel, aggressive, deep-cleaning SSOPs (DC-SSOP), performed by a third-party cleaning service immediately reduced *L. monocytogenes* in half (2/4) of the delis with high prevalence (>10%; Hammons et al., 2017). These reductions, however, were not sustained over time. The reestablishment of *L. monocytogenes* in these delis could be attributed to the fact that third-party deep cleans do not address employee behavior and routines associated with appropriate food safety practices (Wu et al., 2020c). In response, Wu et al. (2020c) implemented employee executed deep-cleaning SSOPs (EE-SSOP) with appropriate training and targeted maintenance programs. The EE-SSOPs were effective in immediately reducing *L. monocytogenes* prevalence from 15.2% to 5.8% among the seven stores enrolled and sustaining a 10.8% reduction on non-food contact surfaces (NFCS) throughout the follow-up period (Wu et al., 2020c). These data indicate that training of employees on food safety behaviors, individualized cleaning and sanitation practices appropriate to each facility, and identification of high-risk deli departments are important measures to reduce *L. monocytogenes* prevalence in retail deli departments.

The goals of this study were to (i) identify common changes in protocols and operations at the retail level in response to COVID-19, (ii) determine the prevalence of *L. monocytogenes* in retail deli departments during the pandemic, (ii) externally validate a previously developed predictive risk model to identify retail deli departments at risk for high prevalence of *L. monocytogenes* under extreme conditions, and (iii) elucidate relationships between facility design, management strategies, cleaning and sanitation practices, and *L. monocytogenes* prevalence during this unprecedented time.

2.3 Materials and Methods

2.3.1 Overall study design.

Environmental samples were collected from forty-four retail delicatessen establishments across seven states (A-G) from Fall 2019 to Spring 2021. Corporate retail food safety experts representing each retail establishment were asked to include delis with and without perceived food safety challenges, locations with differing community demographics, and facilities of varying size and age. Ten sampling sites were selected based on the sites that were most highly correlated with

the predictive risk models described in Section 2.7 (Table 2.1). These sites included: deli floor drain, trash can, scale touch points, cold room drain, cold room storage rack, floor-wall juncture under the three-basin sink, wheeled cart handle, one-basin sink interior, squeegee head, and standing water in the deli area; each site was sampled twice in one year during daily operations in each store. A total of 19 stores were sampled once prior to the initiation of safer-at-home guidance beginning in March 2020, while the second sampling event occurred during the pandemic. Additionally, 15 stores were sampled two times between March 2020 and March 2021, and nine stores were only sampled once in January 2021. A survey of the facility design, management practices, and cleaning and sanitation frequencies was conducted concurrently with *L. monocytogenes* sample collection. Four corporate retail food safety experts and sanitarians, previously trained in environmental sample collection by Purdue University, conducted aseptic environmental sampling. Samples were shipped on ice to Purdue University within 24 h of sampling. All *L. monocytogenes* isolates were confirmed by whole genome sequencing and other *Listeria* spp. were identified by *sigB* gene sequencing.

2.3.2 Collection of environmental samples

Sample collection was conducted as previously described by Burnett et al. (2020), with minor modifications. All sampling sites were sampled using EZ Reach Dual Sponge samplers (World Bio Products, LLC, Bothell, WA). Two sponge samplers pre-moistened with Hi-cap Neutralizing Buffer (20 mL) were used for each site: one for isolation of *L. monocytogenes* and other *Listeria* spp. Sponges were shipped to food safety personnel trained to complete the sampling within one day prior to the sampling event. Each surface is described in Table 2.1. After sampling, sponges were separated and aseptically placed into individual pre-labeled stomacher bags, stored on ice, and shipped overnight to Purdue University for further processing.

2.3.3 *Listeria* spp. and *L. monocytogenes* detection and isolation

L. monocytogenes and *Listeria* spp. were detected as previously described by Simmons et al. (2014) using the U.S. Food and Drug Administration Bacterial Analytical Manual with modifications (REF). Colonies displaying morphology for *L. monocytogenes* or *Listeria* spp. on chromogenic plating medium (LMPM; R&F Products, Downers Grove, IL) were randomly

Table 2.1. Sampling site descriptions and *L. monocytogenes* and *Listeria* spp. prevalence among the ten sampled sites before and during the Covid-19 pandemic.

		No. of positive samples/total samples tested (%)			
		L. monocytogenes		***Listeria* spp.**[1]	
Site Name	**Description**	**Before**[2]	**During**[3]	**Before**	**During**
Cold storage room drain	Floor drain cover located inside or directly in front of the cold storage room	0/19 (0.0)	4/58 (6.9)	3/19 (15.8)	6/58 (10.3)
Trash can	Large trash can used only in the deli area	0/19 (0.0)	0/59 (0.0)	3/19 (15.8)	0/59 (0.0)
Contact surfaces of scale	The entire contact surface of a single front of house scale and all 90° angles	0/19 (0.0)	0/59 (0.0)	1/19 (5.3)	1/59 (1.7)
Cold room storage racks	Rack in cold room used to store deli meats	0/19 (0.0)	2/59 (3.4)	0/19 (0.0)	3/59 (5.1)
Floor/wall juncture under three-basin sink	The floor-wall junction beneath the three-basin sink	1/19 (5.3)	2/59 (3.4)	2/19 (10.5)	4/59 (6.8)
Deli area floor drain	Floor drain cover located in the high traffic area of the deli department	1/19 (5.3)	5/59 (8.5)	4/19 (21.1)	9/59 (15.3)
Wheeled cart handle	The handle of mobile carts used in the deli area to transport food.	0/19 (0.0)	1/58 (1.7)	1/18 (5.6)	0/58 (0.0)
Interior of the single-basin hand-washing sink	Interior of single-basin handwashing sink including corners and drain cover	1/17 (5.9)	1/59 (1.7)	0/17 (0.0)	0/59 (0.0)
Squeegee or other floor cleaning equipment	Surface of squeegee, mop, broom, or other floor cleaning equipment	4/12 (33.3)	5/52 (9.6)	4/12 (33.3)	4/52 (7.7)
Standing water (pools)	Standing (pooled) water in the deli area or cold storage room	3/13 (23.1)	5/54 (9.3)	1/13 (7.7)	6/54 (11.1)

[1] *Listeria spp. includes all Listeria spp. other than L. monocytogenes.*
[2] *Before: Stores sampled Oct. 2019 through Jan. 2020*
[3] *During: Stores sampled July 2020 through March 2021*

selected, re-streaked to LMPM, and incubated at 35°C for 48 h. In cases where no presumptive *L. monocytogenes* or *Listeria* spp colonies were produced on LMPM, two *Listeria*-like colonies were randomly selected from Modified Oxford Agar (MOX; Difco) and sub-streaked to LMPM. For each presumptive positive sample, four colonies were sub-cultured in 10 mL Brain Heart Infusion broth (BHI; Difco), incubated with shaking at 36°C for 24 h, and frozen at -80°C in 20% glycerol. For presumptive *L. monocytogenes* positive samples, a single isolate was sequenced by whole genome sequencing. For each presumptive *Listeria* spp. sample, one isolate was confirmed by *sigB* PCR-amplicon sequencing.

2.3.4 Whole genome sequencing of *L. monocytogenes*

One isolate from each *L. monocytogenes* positive site was sequenced. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the CDC PulseNet protocol (https://www.cdc.gov/pulsenet/pathogens/protocols.html) for gram-positive bacterial cells with minor modifications. Briefly, *L. monocytogenes* isolates were grown on LMPM agar at 35°C for 48 h and a single colony was selected and grown overnight (36°C, 24 h, shaking) in 10 mL BHI broth. Cells were harvested (1 mL overnight culture) in a 1.5 mL microcentrifuge tube by centrifuging for 10 min at 7500 rpm. The cell pellet was resuspended in 180 µL enzymatic lysis buffer and incubated at 56°C for 30 mins with vortexing (5 s) every 10 min. RNase A (4 µL) was added and the solution was incubated at room temperature (~21.5°C) for 5 min. After incubation, 25 µL Proteinase K and 200 µL AL buffer was added, followed by incubation at 56°C for 30 min. Molecular-grade ethanol was added (200 µL; 200 proof) and the solution was vortexed for 6 s. DNA clean-up and elution were performed following the CDC Pulsenet protocol. Determination of DNA quality was performed using the NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA). Extracted DNA was stored at -20°C for one week, then shipped on dry ice to the University of Georgia Center for Food Safety for library preparation and sequencing. Libraries for the individual *L. monocytogenes* isolates were generated using the Nextera XT DNA Library kit (Illumina, San Diego, USA) following the manufacturer's protocols and sequenced on an Illumina MiSeq System to obtain 250bp paired-end reads.

Raw reads were assembled into contigs using the Shovill pipeline (https://github.com/tseemann/shovill). This pipeline uses the SPAdes assembler (v3.14.1; Bankevich et al., 2012) to assemble Illumina paired-end reads into a draft genome. Isolates were

then subtyped using a 7 gene multi-locus sequence type (MLST) scheme (Ragon et al., 2008) as developed by Torsten Seeman (https://github.com/tseemann/mlst). For each clonal complex, single nucleotide polymorphisms (SNPs) were called using the Snippy pipeline (https://github.com/tseemann/snippy), a variant calling and core genome alignment pipeline. SNP-based phylogenies for each clonal complex were clustered using FastTree (Price et al., 2009). *L. monocytogenes* sequences have been deposited in the NCBI Sequence Read Archive (BioProject accession number PRJNA758968).

2.3.5 PCR-based identification of *Listeria* spp. using sigB gene sequencing

PCR amplification and sequencing of the *sigB* gene was performed on a single isolate for each positive site to confirm and speciate *Listeria spp*. PCR amplification was performed using GoTaq™ G2 Colorless Master Mix (Promega Corp., Madison, WI). Primers used for PCR amplification and nucleotide sequencing, as well as PCR amplification conditions, were as previously described by Nightingale et al. (2007). PCR products were purified using the ExoSAP-IT PCR product cleanup reagent (Applied Biosystems, Foster City, CA). All purified PCR products were shipped to Eurofins Testing Laboratory (Eurofins USA, Luxemborg) to Sanger sequence *sigB* amplicons. Sequences were visually inspected and edited using SnapGene viewer (SnapGene, Chicago, IL) and queried against the National Center for Biotechnology Information (NCBI; Bethseda, MD; Altschul et al., 1990) RefSeq Genome Database using the NCBI Nucleotide BLAST Local Alignment Search Tool.

2.3.6 COVID-19 debrief with corporate food safety experts

Debriefing meetings were held with corporate retail food safety experts from each of the four companies with stores enrolled in the study to share study results and discuss policy and practice changes made in response to the pandemic. Each retailer was asked a series of ten questions to gain insight into changes in company policy, practices, or observed changes in personnel hygiene and interactions. These questions included major changes in cleaning and sanitation protocols, employee hygiene, glove use, employee interactions, product availability, operating hours, and total time dedicated to cleaning and sanitation. Retailers were also asked to report any observed changes that were not implemented as company policy, as well as any additional information regarding operational changes to their stores in 2020 and 2021.

2.3.7 Validation of predictive model

Predictive risk models (A & B) were developed as previously described by Wu et al. (2020c). Briefly, models were developed from previously collected data (Simmons et al., 2014; n = 30 delis, 28 sites, 6 months) with forward stepwise selection using Firth's bias-corrected logistic regression models and Proc Logistic in SAS v9.4 (SAS Institute, Cary, NC). Models were validated using the 70/30 exclusion rule where 70% of the data were used to train the model and 30% of the data were used to internally validate the model. Model cutoff values were selected to control type II error ($B < 0.05$), minimizing the risk that the model would fail to identify a deli with high *L. monocytogenes* prevalence. Additionally, the models were further validated by Wu et al. (2020c) using data collected during a longitudinal study (n = 30 stores, 28 sites, 6 months). Here, model A (drains present) was externally validated using 10 sampling sites from 10 stores sampled in one or two events during the COVID-19 pandemic. Ten stores were only sampled one time due to travel restrictions and changes in job requirements resulting from the pandemic. All stores enrolled in the study had floor drains, thus model B (no floor drains) was not evaluated in this study.

2.3.8 Facility design and management practices survey development and data collection

A survey was developed based on our previous work in retail deli and produce departments (Wang, 2014; Wu et al., 2020a). The final survey consisted of 117 total questions, with 83 questions related to facility design and management practices and 34 for questions associated with cleaning and sanitation frequencies. Prior to the study, the survey and study protocol were approved by the Purdue Institutional Review Board (protocol IRB-2020-1133). Managers from each of the thirty-five stores enrolled in the study were recruited to complete the survey. Surveys were distributed in-person by trained personnel conducting sample collection. Survey responses were then matched with *L. monocytogenes* prevalence for each store.

2.3.9 Statistical Analysis of infrastructure and management survey

All statistical analyses for the survey were performed in SAS v9.4 (SAS Institute) and performed as previously described (Wu et al., 2020a). Responses to the 117 survey questions were categorized as categorical or continuous variables. Each response was treated as a predictor

variable (x-variable) and correlated with *L. monocytogenes* prevalence (y-variable). Pearson correlation was used to correlate *L. monocytogenes* prevalence with continuous variables and ANOVA was used for categorical variables ($\alpha < 0.05$). Tukey pairwise comparisons were made on all significant variables and crosstabulations were conducted to visualize the correlations between the significant predictor variables and *L. monocytogenes* prevalence. Finally, a generalized linear regression model using Proc GLM was preformed to detect significant predictor variables in an interdependent context and multicollinearity (continuous variables = Pearson correlation, categorical variables = Fisher's exact test, and continuous*categorical = ANOVA with Tukey's pairwise comparisons; $\alpha < 0.05$) was conducted to evaluate the strength of the study.

2.4 Results and Discussion

In this study we evaluated the predictive power of a previously developed model for detecting retail delicatessen environments at risk of high *L. monocytogenes* prevalence (>10%) during the COVID-19 pandemic. We also conducted a risk assessment survey to determine risk factors for increased *L. monocytogenes* prevalence under these conditions. Our data indicated that (i) *L. monocytogenes* is highly prevalent in some stores even during increased cleaning and sanitation frequency, (ii) the regression model did not accurately predict stores at high risk of *L. monocytogenes* during the pandemic, and (iii) survey questions related to cleaning and sanitation of floors and drains were the most strongly correlated with *L. monocytogenes* prevalence. To our knowledge, this is the first study assessing *L. monocytogenes* prevalence in retail delicatessen departments during a global pandemic and provides insights into the challenges of high *L. monocytogenes* prevalence despite increased awareness and/or execution of cleaning, sanitation, and personnel hygiene.

2.4.1 Changes made in retail deli departments in response to the COVID-19 pandemic

Corporate retail food safety experts were asked a series of questions regarding changes made within their company in response to COVID-19 during a debrief meeting post-sample collection (Table 2.2). We found that none of the retailers enrolled in our study made changes to cleaning and sanitation of NFCS or FCS, however, all retailers reported increasing frequency of cleaning of high touch surfaces (HTS). Most commonly (3/4), companies assigned this task to at least one employee per shift, and this individual cleaned HTS every hour. Two retailers reported

decreased access to cleaning and disinfectant products due to lack of supply. Thus, one retailer changed chemical companies while the other reported increasing the strength of sanitizers to reach disinfectant-grade for use as a disinfectant. All the companies shortened their operating hours. Only three retailers used the extra time for third-party cleaning at the end of daily operations; the fourth retailer reported that they returned to normal operating hours after a month and did not use a third-party cleaning company during the pandemic. Overall, employees at all four companies utilized more personal protective equipment (e.g., gloves, masks, etc.; PPE); only one company reported limited access to PPE (e.g., gloves), prompting employees to use other utensils in place of gloves.

An increase in overall sales was observed by all companies; however, each retailer stated that there was a decrease in deli products sliced, packaged, and sold at the deli. Additionally, all companies reported a significant decrease in daily customer traffic while their ecommerce rates increased dramatically. All companies closed their hot cases and self-service salad bars in 2020. Finally, none of the retailers implemented changes in employee scheduling as the number of employees scheduled was dynamic and based on sales. Despite increased sales, additional work hours were generally not scheduled because employees were often out sick or had to quarantine due to potential exposure to COVID-19.

To limit the spread of COVID-19, the CDC recommended that individuals limit face-to-face contact, wear a mask, and social distance (maintain ≥ 6 feet distance; CDC, 2021). In response, many retail grocery chains implemented occupational limits and one-way aisles to minimize the transmission of SARS-CoV-2 among customers while shopping. Implementation of these practices, however, varies widely between companies and geographic locations (Shumsky et al., 2021). Retail grocery stores have been linked to outbreaks of COVID-19 between customers and employees (Hayden, 2020), most commonly in stores within low-income communities. To minimize risk to employees, the CDC recommended that all retail workers practice proper hand hygiene, clean all HTS frequently, limit close contact with others, and wear a face covering at all times (CDC, 2020). In this study, retailers reported following each of these guidelines, especially in the early stages of the pandemic. As the pandemic continued through summer 2020, retailers reported difficulties acquiring commonly used cleaning products and disinfectants. In response,

Table 2.2. Effects of Covid-19 on retail grocery operations reported by corporate food safety experts during the debrief meetings.

	Retail Corporation*			
Responses to Covid-19	**A**	**B**	**C**	**D**
Cleaning and Sanitation				
Cleaning protocols for NFCS, FCS, and TP	.	X	X	.
Frequency of cleaning of NFCS, FCS, and TP	X	X	X	X
Changes in cleaning and sanitation products (due to access)	.	X	X	.
Increased strength of sanitizers	.	.	X	X
Third-party cleaning after daily operations	X	.	X	X
Store Operations				
Changes in flow of product in deli departments	X	X	X	X
Removal of self-service in delis	X	X	X	X
Shortened hours of operation	X	X	X	X
Specialized hours for immunocompromised and senior citizens	X	.	X	X
Employees				
Scheduling fewer employees	.	.	X	.
Protocols to reduce interactions between employees (e.g. staggered break times, sections of deli department, etc.)	.	X	.	X
Increased volume of PPE (e.g. gloves, masks, etc)	X	X	X	X
Decreased access to PPE (e.g. gloves, masks, etc)	.	X	.	.

***** *The "X" indicates changes that were made by each retil corporation; "." indicates changes that were not made during COVID-19.*

EPA expedited its review program for new emerging viral pathogen claim submissions and List N: disinfectants for use against SARS-CoV-2 additions (EPA, 2020). Additionally, the Food and Drug Administration (FDA), released their "Best Practices for Retail Food Stores, Restaurants, and Food Pick-Up/Delivery Services During the COVID-19 Pandemic" in April 2020. This guidance recommended that all foodservice establishments frequently sanitize FCS and utensils, clean and disinfect HTS, floors, and counters, discontinue all self-service stations, and encourage customers to wear masks and maintain social distancing while in line (FDA, 2020). Increased awareness of personal hygiene and more frequent cleaning and disinfection likely had the added benefit of reducing foodborne pathogen contamination, including *L. monocytogenes*, during the pandemic (Beach, 2021). Additionally, the CDC speculated that the 26% decrease in incidence of infection caused by foodborne disease during COVID-19 is likely attributed to pandemic-related travel restrictions, restaurant closures, and decreased and delayed health care-seeking (Ray et al., 2021).

2.4.2 A total of 4.3% (24/566) of environmental samples tested positive for *L. monocytogenes* during pandemic-influenced conditions

In this study *L. monocytogenes* was found at least once in 31.6% (6/19) of stores sampled before the onset of the COVID-19 pandemic (Figure 2.1); 5.8% (10/174) of samples taken before COVID-19 tested positive for *L. monocytogenes*. In contrast, 44 stores were sampled at least once between March 2020 and March 2021 with *L. monocyt*ogenes isolated at least once in 36.4% (16/44) of these stores; however, only 4.3% (24/566) of all samples collected during the pandemic were positive (Figure 2.2). Additionally, 26.3% of the stores (5/19) sampled before increased guidance to stay home was established in the U.S. had high *L. monocytogenes* prevalence (>10%), while only 18.1% (8/44) of stores had >10% prevalence following stay-at-home mandates or guidelines. Similar to prior studies, there was significant variability in *L. monocytogenes* prevalence among the stores in this study (Etter et al., 2017; Hammons et al., 2017; Sauders et al., 2009; Simmons et al., 2014). However, the trends in the data presented here differ from past studies evaluating *L. monocytogenes* prevalence in retail deli departments. For example, in a four-month longitudinal study of 30 retail deli departments, Etter et al. (2017) reported that 9.7% of all environmental surfaces sampled were positive for *L. monocytogenes* and contamination ranged from 0% to 60% in the delis studied. Of the 30 stores, eight stores had high (>10%) *L.*

monocytogenes prevalence while 13 stores had low (<1%) prevalence. Additionally, Simmons et al. (2014) reported a 9.5% average *L. monocytogenes* prevalence rate among all sampled surfaces and prevalence in the 30 studied delis was 0% to 35%; eight stores had high prevalence and nine stores had low *L. monocytogenes* prevalence. The prevalence reported in those studies was much higher than in the current study, where only 5.8% and 4.3% of all environmental samples taken before and during the pandemic, respectively, were positive for *L. monocytogenes* and prevalence rates in stores ranged from 0% to 30% prior to, and 0% to 22% during COVID-19.

Squeegees and other floor cleaning equipment had the highest *L. monocytogenes* prevalence (14.1%; 9/64) among the sites tested in this study (Table 2.1). Prevalence on squeegees decreased from 33.3% (4/12) prior to the pandemic to 9.6% (5/52) during COVID-19. A similar trend was observed with standing water samples where a total of 11.9% (8/67) samples were positive for *L. monocytogenes* including 3/13 (23.1%) prior to and 5/54 (9.3%) during COVID-19. Prior studies evaluating environmental contamination of *L. monocytogenes* (Burnett et al., 2020; Hammons et al., 2017; Simmons et al., 2014) reported the pathogen was most commonly isolated from cold storage room drains (30-40% of samples) and floor wall junctures under single basin or three-basin sinks (20-60%). Hammons et al. (2017) also reported that 40.6% of standing water samples were positive for *L. monocytogenes* during operations. However, Simmons et al. (2014) and Hammons et al. (2017) reported *L. monocytogenes* prevalence of 3.9% and 7.4%, respectively, on scales during operation and 5.0% and 5.6%, respectively, on trash cans. This differs from the current study where *L. monocytogenes* was not found on scales or trash cans.

Retail deli departments have been a primary focus of *L. monocytogenes* research due to their potential for cross-contamination to RTE deli products (Simmons et al., 2014). Prior studies have shown that listeriosis cases associated with RTE deli meats are likely due to meats sliced at retail rather than prepackaged products (Endrikat et al., 2010; Gombas et al., 2003; Pradhan et al., 2010). Additionally, many studies have shown that *L. monocytogenes* is highly prevalent and able to persist in retail grocery environments (Burnett et al., 2020; Etter et al., 2017; Forauer et al., 2021; Hammons et al., 2017; Sauders et al., 2009; Simmons et al., 2014). However, recent studies on deep-cleaning intervention strategies (Etter et al., 2017; Hammons et al., 2017; Wu et al., 2020c) have shown deep-cleaning can effectively reduce *L. monocytogenes* in retail deli environments.

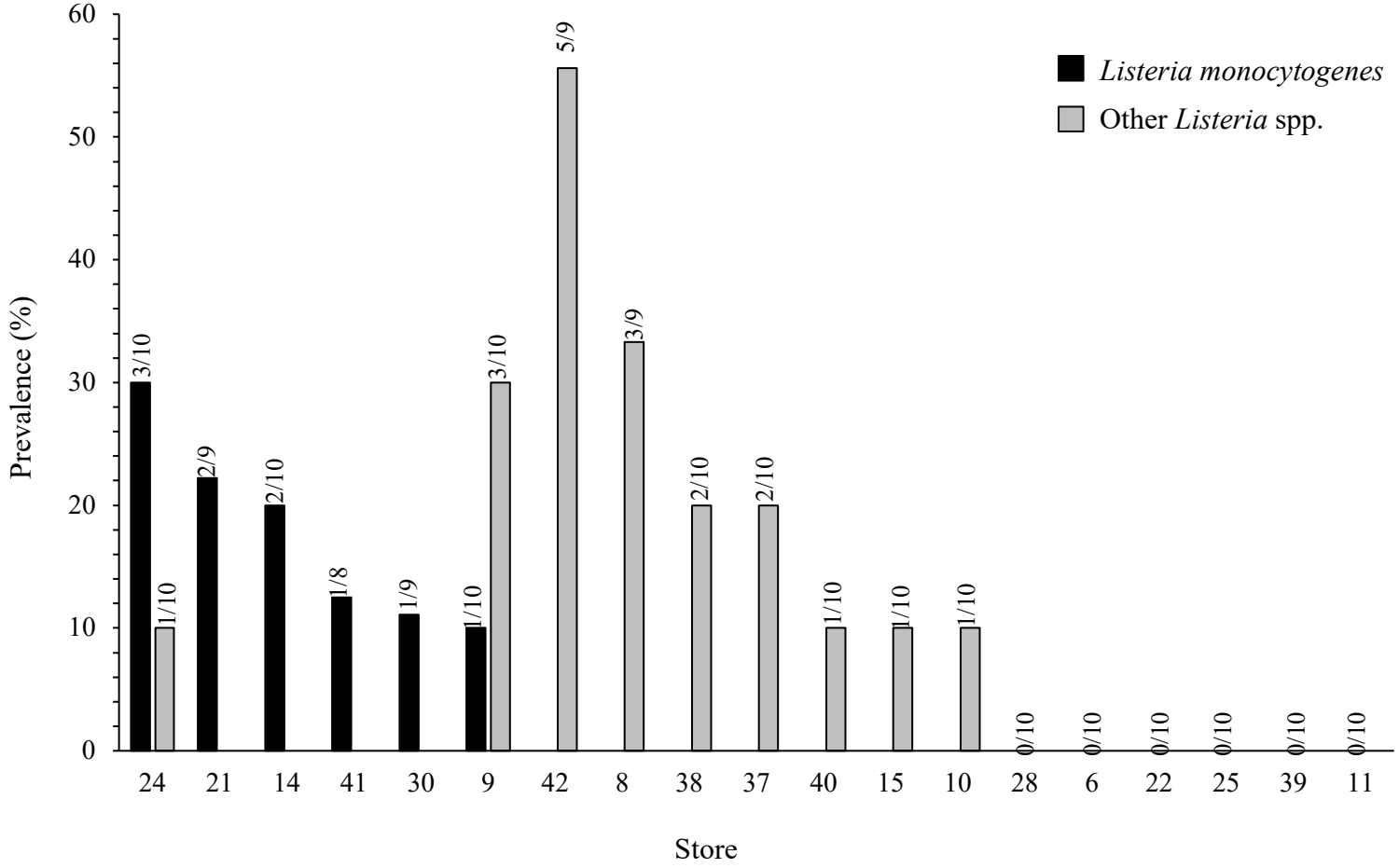

Figure 2.1. *Listeria monocytogenes* and other *Listeria* spp. prevalence for each store that was sampled before Covid-19 (Oct 2019 to February 2020). Numbers above the bars indicate the number of positives/total number of samples tested for each store.

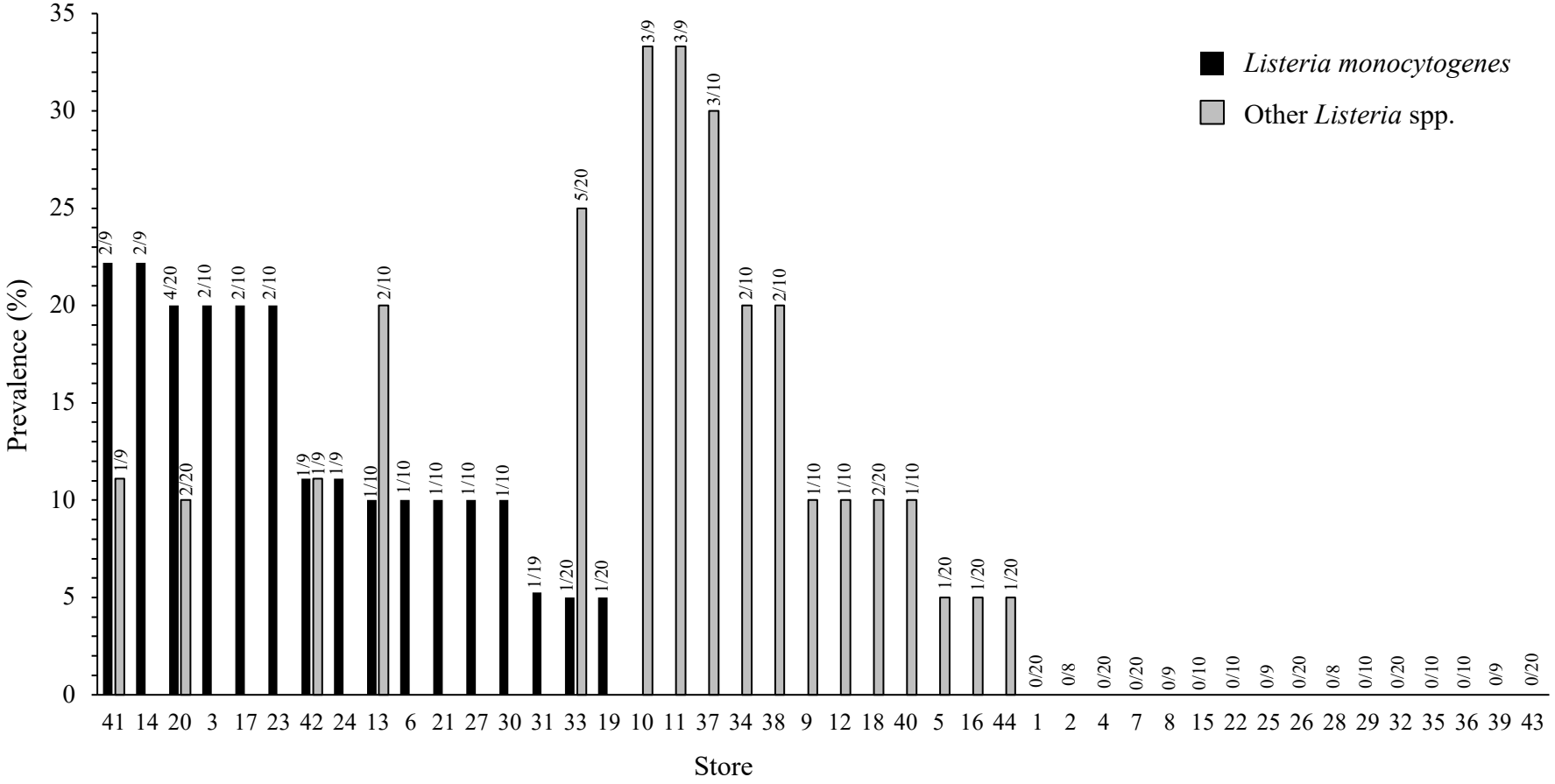

Figure 2.2. *Listeria monocytogenes* and other *Listeria* spp. prevalence for each store that was sampled during Covid-19 (March 2020 to March 2021). Numbers above the bars indicate the number of positives/total number of samples tested for each store.

2.4.3 The predictive risk model underpredicted stores at risk for high *L. monocytogenes* environmental prevalence during COVID-19.

Validation of the drain-associated predictive risk model (model A) was conducted to evaluate the accuracy of the model under modified hygienic conditions resulting from the COVID-19 pandemic included cold storage room drains, deli area drains, scales, trash cans, and cold storage room racks. The model accurately predicted *L. monocytogenes* risk in 52/59 sampling events ($\alpha < 0.0001$, $\beta = 0.1186$), including 42/59 delis with low *L. monocytogenes* prevalence (≤10%) and 10/59 with high prevalence (>10%). Deli area drains were the most influential factor in the model where detecting *L. monocytogenes* in deli drains increased the probability of high *L. monocytogenes* prevalence by 160-fold (CI_{95} = 26.2, 982.8) compared to delis that did not have positive drains (Table 2.3a). Additionally, the probability of high *L. monocytogenes* prevalence increased 75-fold if *L. monocytogenes* was isolated from trash cans (CI_{95} = 1.9, >999.9) or scales (CI_{95} = 1.9, >999.9). However, *L. monocytogenes* was not detected on scales or trash cans during this study, thus reducing the probability of predicting potentially high-risk delis. This likely contributed to the increased number of falsely predicted low risk stores (11.9%; 7/59 sampling events) observed here.

Risk-based predictive models to assess foodborne pathogen contamination are highly sought-after tools for *L. monocytogenes* control in response to the Food Safety Modernization Act (FSMA) and updated Codex Alimentarius guidelines (Codex Alimentarius, 2007; FSMA, 2020). Predictive modeling is now commonly used to evaluate pathogen growth and inhibition in various food matrices and processing environments (Buchanan & Whiting, 1996; Jarvis, 2016; Wu, Hammons, Wang, et al., 2020). A recent study (Gallagher et al., 2016) indicated that control of cross-contamination and sanitation were important factors to mitigate *L. monocytogenes*, however, the study concluded that reduced *L. monocytogenes* contamination cannot be attributed to a "simple solution." Wu et al. (2020c) developed a predictive risk model for *L. monocytogenes* in retail deli environments using previously collected data from 30 deli departments. This study indicated that the five most highly correlated sites with high *L. monocytogenes* prevalence were cold storage room floors, deli area drains, scales, trash cans, and cold storage room racks (Table 2.3b). The authors (Wu et al., 2020c) validated the model by screening 50 stores from six states and found that it conservatively predicted stores at risk for high *L. monocytogenes*. The model identified 13 stores with potentially high prevalence and seven of these were confirmed to have

high *L. monocytogenes* prevalence. The same trend was not observed under the pandemic response conditions observed in this study; the model underpredicted stores at high risk for *L. monocytogenes*.

Table 2.3. Odds ratio estimates for sites in the drain associated predictive risk model. A) Odds ratio estimates for validation of the model during Covid-19 and B) Odds ratio estimates of the originally developed model.

A.

	Odds Ratios of the Model During COVID-19		
Sampling Site	**Point Effect**	**95% Wald's Confidence Interval**	
Cold Storage Room Drain	28.9	8.5	97.8
Trash Can	75.2	1.9	>999.9
Scale	75.5	1.9	>999.9
Cold Storage Room Racks	21.1	3.2	139.1
Deli Area Drain	160.1	26.2	982.8

B.

	Odds Ratios of the Original Model		
Sampling Site	**Point Effect**	**95% Wald's Confidence Interval**	
Cold Storage Room Drain	46.6	10.0	217.3
Trash Can	169.7	3.7	>999.9
Scale	2.2	0.02	218.0
Cold Storage Room Racks	20.1	1.6	260.9
Deli Area Drain	240.7	31.8	>999.9

Human factors, such as attitudes, perceptions, and behaviors, are vital to improving food safety (Wu, Hammons, Silver, et al., 2020). Studies have shown that food safety climate and culture are both organizational and behavioral challenges in food processing and retail environments (De Boeck et al., 2015; Griffith et al., 2010; A. Neal et al., 2000; Wu, Hammons, Silver, et al., 2020). Wu et al. (Wu, Hammons, Silver, et al., 2020) reported that a greater commitment to food safety by both management and employees resulted in lower *L. monocytogenes* prevalence. This indicates that employee commitment to food safety can reduce *L. monocytogenes* in food processing and retail deli departments. Communication and training associated with preventing the spread of SARS-CoV-2 significantly increased employee

commitment to improved personal hygiene (e.g., handwashing) and greater frequency of cleaning and disinfecting of HTS in retail deli departments. Outbreaks and food recalls associated with common foodborne pathogens (e.g., *Salmonella enterica* and *L. monocytogenes*) have decreased since March 2020 (Storey, 2021). It is expected that CDC recommendations to prevent SARS-CoV-2 transmission have had positive effects on foodborne illness control by reducing environmental contamination of pathogens on HTS and FCS (e.g., scales and trash cans). Ultimately this valuable observation also has a deleterious effect on the accuracy of the predictive model evaluation.

2.4.4 Seventeen factors significantly correlated with *L. monocytogenes* prevalence.

During this study, 43 of the 44 surveys were fully completed and returned. A correlation analysis found that 17 variables significantly ($\alpha < 0.05$) correlated with *L. monocytogenes* prevalence (Table 2.4). These data suggest the primary theme of *L. monocytogenes* control is associated with cleaning and sanitation protocols. These 17 predictor variables were utilized in a generalized linear regression model, and prevalence data with $\log_{10}$ transformation resulted in a moderate linear fit ($R^2 = 0.6403$, MSE = 0.1709); overall, the model was significant ($P = 0.0141$). Our study indicated that limitations in cleaning and sanitation protocols were indicative of potential *L. monocytogenes* prevalence during the COVID-19 pandemic. Changing disposable gloves after having contact with NFCS ($P = 0.0248$) and keeping cleaning and sanitizing records as part of the SSOP ($P = 0.0404$) were correlated with lower *L. monocytogenes* prevalence. Management at 9/43 (20.9%) of the stores reported that they were unaware of glove changing frequency and five (55.6%) of these stores had high *L. monocytogenes* prevalence (>10%). In stores where records were part of the cleaning and sanitizing SSOP, 78.8% (26/33) had ≤ 10% *L. monocytogenes* prevalence; of the 10 stores where records were not kept, 50.0% (5/10) had high prevalence (>10%). The FDA Food Code requires that foodservice employees change their gloves between customers and wash their hands before donning new gloves (Lubran et al., 2010). Additionally, studies have shown that proper handwashing and glove use is associated with lower prevalence of pathogens and other bacteria in deli environments (Lubran et al., 2010; Lynch et al., 2005; Wu et al., 2020b). However, Lubran et al. (Lubran et al., 2010) reported that only 1/21 individuals in an observational study of food safety practices in retail deli departments changed their gloves between customers.

2.4.4.1 Involvement of management is important in reducing L. monocytogenes *prevalence in retail deli departments.*

We also found that delis with managers who were unaware of the cleaning and sanitizing equipment and procedures used in their deli departments were strongly correlated with high *L. monocytogenes* prevalence ($P < 0.05$). Managers in 15 delis were unaware of the sanitizing equipment that are used to clean deli floors ($P = 0.0285$) and seven of these delis (46.7%) had high *L. monocytogenes* prevalence. Managers in 12 stores were unaware of the procedures used to clean the floors in the deli retail area ($P = 0.0025$) and seven of these delis (58.3%) had high *L. monocytogenes* prevalence. Additionally, 13 managers were unaware of which sanitizing equipment are used on cold room floors ($P = 0.0247$) and six (46.2%) of these delise had high *L. monocytogenes* prevalence. In a multicollinearity analysis (Fisher's exact test), these three questions strongly correlated with each other ($P < 0.05$), suggesting that if managers were unaware of cleaning and sanitizing procedures, they were also unaware of the equipment used to sanitize the floors in the delis and cold rooms.

It is established that the management system is important to creating a strong food safety culture (Griffith et al., 2010; Neal et al., 2012; Powell et al., 2011; Wu et al., 2020b). Wu et al. (2020b) previously reported that there were perceived differences in devotion to food safety between managers and employees with both parties believing that they had a greater commitment to food safety than the other. Knowledge gaps have been reported in food systems within the level of employment in addition to between employees and managers (Nayak & Waterson, 2017). It is likely that these gaps can cause miscommunication within an organization which may ultimately affect food safety culture and climate. For example, adequate training on cleaning and sanitation procedures can improve employees' perception of management's commitment to food safety (Wu, Hammons, Silver, et al., 2020), improve attitudes toward training and food safety (Soon et al., 2012), and ultimately reduce *L. monocytogenes* prevalence in food processing and retail environments (Wu, Hammons, Wang, et al., 2020). In a recent food safety culture study, Wu et al. (2020b) found that a greater sense of commitment to food safety practices strongly correlated with lower *L. monocytogenes* prevalence in retail deli environments.

Table 2.4. Significant predictor variables for *L. monocytogenes* prevalence ($\alpha < 0.05$) amongst heightened awareness of cleaning, sanitation, and personnel hygiene during the Covid-19 pandemic.

Identifier	Question	Outcome ($P_{adj} < 0.05$)	*P*-value
Q15	How many hours a day are dedicated to cleaning tasks in the deli area during operation?	Cleaning for 2 or 3 hours a day was correlated with lower *L. monocytogenes* prevalence compared to cleaning a) 1 hour b) 4 hours c) 5 hours d) >5 hours	0.0107
Q2_23	Which department(s) do the deli area employees work in?	Having deli employees who also work in the produce department was correlated with higher *L. monocytogenes* prevalence.	0.0425
Q18	Are disposable gloves changed after touching non-food contact surfaces (e.g., cart handles, hand wash sink basin, drain cover, etc)?	Employees changing disposable gloves after touching NFCS was correlated with lower *L. monocytogenes* prevalence.	0.0174
Q21	When are floor surfaces in the deli retail area cleaned relative to other areas in the deli retail environment?	Nature of correlation not clear	0.0317
Q24	Are records of past cleaning and sanitizing kept as part of SSOPs?	Keeping records of cleaning and sanitizing as part of an SSOP was correlated with lower *L. monocytogenes* prevalence.	0.0404
Q38_2	What type of drain is present in the deli area?	Having a trench drain with automatic flushing in the deli area was correlated with higher *L. monocytogenes* than stores with a) catch basin floor drains or b) trench drains without automatic flushing.	0.0174
Q61_2	The deli retail area is cleaned at the end of daily operation by who?	Delis with a designated cleaning crew clean the retail deli area after daily operations was correlated with high *L. monocytogenes* prevalence.	0.0291

Q71	Is the handwashing sink shared between the deli area and other departments?	Sharing a handwashing sink with other departments was correlated with higher prevalence compared to a) not sharing a sink or b) not having a sink present in the deli.	0.0336
Q77_1	What department(s) is sharing the wheeled carts?	Sharing a cart with the bakery correlated with higher *L. monocytogenes* prevalence.	0.0174
Q86_6	What sanitizing equipment is used on floors?	Stores with management that was unaware of which sanitizing equipment was used on floors correlated with higher prevalence.	0.0285
Q90_5	What procedures are used to clean and sanitize the floor of deli *retail* area?	Using spray-on foam sanitizer to clean and sanitize the floor of the deli retail area correlated with lower prevalence.	0.0038
Q90_7	What procedures are used to clean and sanitize the floor of deli *retail* area?	Having managers who were unaware of the procedures used to clean and sanitize the floor of the deli retail area correlated with higher *L. monocytogenes* prevalence.	0.0025
Q91_5	What procedures are used to clean and sanitize the floor of deli *prepare* area?	Using spray-on foam sanitizer to clean and sanitize the floor of the deli prepare area correlated with lower prevalence.	0.0179
Q94_6	What sanitizing equipment is used on the deli cold room floor?	Stores with managers who were unaware of the equipment used to clean the deli cold room floor correlated with higher prevalence.	0.0247
Q94_7	Is a foam sanitizer used to clean the deli cold room floor?	Using a foam sanitizer to clean the deli cold room floor was correlated with lower *L. monocytogenes* prevalence.	0.0400
Q99_7	Is a foam sanitizer used to clean the drains on the floor?	Using a foam sanitizer to clean the drains on the floor was correlated with lower *L. monocytogenes* prevalence.	0.0400
Q99_8	Is a foaming drain cleaner used to clean the drains on the floor?	Using a foaming drain cleaner to clean the drains was correlated with higher prevalence.	0.0174

The COVID-19 pandemic has greatly influenced personal hygiene and cleaning and sanitation practices across the globe. This heightened awareness of highly transmissible diseases has resulted in greater frequency of cleaning, disinfection, and hand washing, as well as decreased social interaction. The retailers participating in the current study reported that the pandemic has increased employee commitment to personal hygiene and company SSOPs during daily operations. Taken together, employee commitment and greater frequency of cleaning and sanitation has likely resulted in a reduction of *L. monocytogenes* prevalence in retail delicatessen departments.

2.4.4.2 Use of foaming sanitizers resulted in lower **L. monocytogenes** *prevalence.*

Our data indicated that "low" prevalence (≤10%) stores were more likely to report using foaming sanitizers on floors and drains ($P < 0.05$). Overall, no stores that reported using spray-on foam sanitizers to clean and sanitize deli retail floors (17/43) had >10% prevalence, while 46.2% (12/26) of stores that did not report using spray-on foam sanitizers had high prevalence. Additionally, nine stores reported using foam sanitizer to clean and sanitize the deli cold room floors, all of which (100%) had ≤10% *L. monocytogenes* prevalence. Managers' awareness of which procedures are in place for cleaning and sanitizing floors in the deli retail area was highly correlated with the use of a foam sanitizer on the deli floors and drains ($P < 0.05$) in a multicollinearity analysis.

Cleaners and sanitizers are incorporated into good manufacturing practices to prevent microbial contamination and biofilm formation (Cruz & Fletcher, 2012). Specific niches in food processing and retail environments have been found to harbor persistent *L. monocytogenes*. Such niches include drains, floor-wall junctures, and hard-to-clean pieces of equipment (Fagerlund et al., 2020; Hammons et al., 2017; Simmons et al., 2014). Sanitation is used as a preventive and corrective action for *L. monocytogenes* control; however, studies have shown that even with deep-cleaning events, traditionally used cleaners and sanitizers are not sufficient to eradicate *L. monocytogenes* from retail environments if niches remain (Etter et al., 2017; Fagerlund et al., 2017, 2020; Hammons et al., 2017; Wu et al., 2020c). Alkaline foam or gel treatments are commonly used to clean equipment, floors, and walls in food processing facilities. Acidic foam cleaners and enzymatic cleaners are also occasionally used in food processing environments (Fagerlund et al., 2020). Several groups have evaluated biocontrol strategies for *L. monocytogenes* in food

processing and retail facilities to competitively inhibit *L. monocytogenes* growth and disrupt biofilm formation in these environments (Gray et al., 2018; Rodríguez-López et al., 2018; Zhang et al., 2021). Further studies are necessary to determine if the biocontrol mechanism of these cleaners and sanitizers is effective or if the protocols used with the foaming cleaner are responsible for reducing *L. monocytogenes* in retail deli departments.

2.4.5 *L. monocytogenes* isolates clustered by store and state

Five clusters of genetically similar *L. monocytogenes* isolates were obtained in this study, of which three were found in multiple stores and states. These clusters were defined as including *L. monocytogenes* sequence types that match a central genotype of at least six of seven housekeeping genes (Chen et al., 2016). The naming scheme used in this study incorporated the sequence type and associated cluster. For example, ST5C5 was cluster 5 within the seven gene MLST sequence type 5. ST5C5 was the most isolated clone (26/37), followed by ST6C6 (5/37) and ST85C7 (3/37). ST5C5 was isolated 26 times from 11 stores across four states. Additionally, ST6C6 was isolated five times from two stores in a single state, and ST85C7 was isolated three times from three stores across two states. Within ST5C5, the isolates grouped into 10 closely related clades that commonly clustered by store and state from which the isolates were recovered (Figure 2.3). Isolates from the same store were usually < 2 SNPs different and isolates found in different stores but the same state were < 3.5 SNPs different. These results are in agreement with prior studies that found that *L. monocytogenes* sequence types and pulsed-field gel electrophoresis (PFGE) types can be found in multiple stores and across multiple states (Burnett et al., 2020; Simmons et al., 2014; Stasiewicz et al., 2015). Stasiewicz et al. (Stasiewicz et al., 2015) reported that there is a possibility for common clones and highly conserved genetics in various stores across the U.S. Wang et al. (Y. Wang et al., 2018) reported that if the genetic distance between *L. monocytogenes* isolates is 20 SNPs or less, it is highly likely they were isolated from the same facility. The data presented here and by Burnett et al. (2020), however, suggest that highly related clones (<20 SNPs different) can be found in several facilities in multiple states across the US.

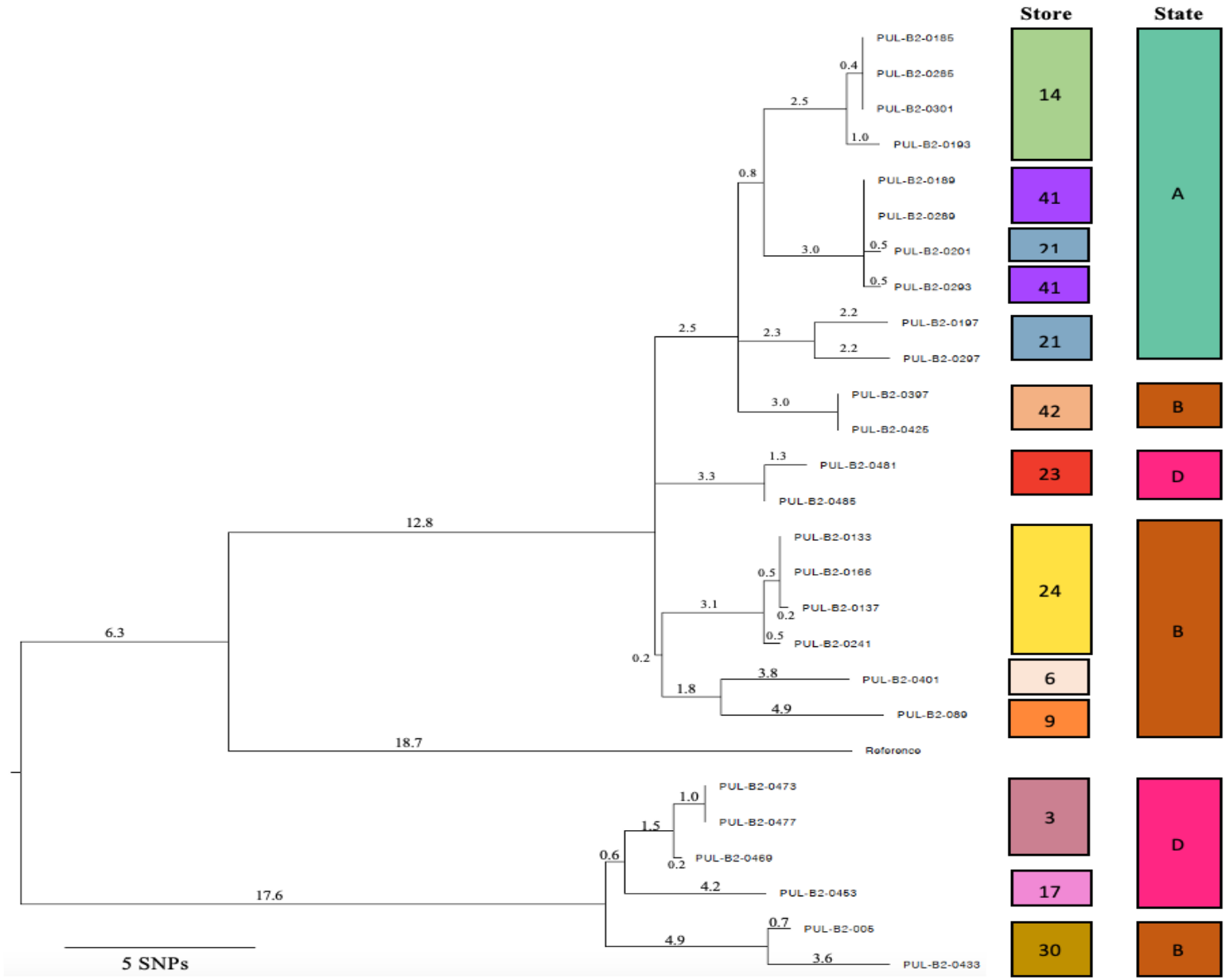

Figure 2.3. SNP-based phylogeny of ST5 clonal complex 5 isolates and geographic location by store and state (A-G)

2.4.6 Study limitations.

The COVID-19 pandemic began after the initiation of this study. Due to lockdown situations and heightened control of traffic in retail grocery stores, 29 of the 44 stores were only sampled one time during the pandemic; only 15 of 44 stores were sampled two times during the study, decreasing the power of the study. The food safety experts at each of the companies enrolled in our study were concerned about introducing new people to the environment and disrupting the work of their employees. Another limitation related to the pandemic was that the survey was not always completed by store managers or deli managers due to decreased capacity of store employees. In some cases, the food safety experts that attended each sampling event would fill out the survey questions that captured objective company-wide policies and the store manager completed only the knowledge and opinion questions.

2.5 Conclusions

To our knowledge, this is the first study to investigate *L. monocytogenes* contamination in retail deli departments during a global pandemic and the resulting behavior and sanitation modifications. Our data suggest that greater awareness of sanitation practices and reduced physical interaction between employees and customers resulted in lower *L. monocytogenes* prevalence in retail deli departments. This study also determined that *L. monocytogenes* was lower on HTS in comparison to prior studies in retail deli departments (Hammons et al., 2017; Simmons et al., 2014; Wu, Hammons, Wang, et al., 2020). Thus, the model evaluated here was not accurately predictive of stores at risk for high *L. monocytogenes* prevalence during COVID-19. This study highlights the impact of improved cleaning and sanitation practices, greater frequency of disinfecting high-touch surfaces, and increased awareness of personal hygiene in reducing risk of *L. monocytogenes* in retail deli departments and we recommend these practices continue in perpetuity.

2.6 Acknowledgements

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CHAPTER 3. CHARACTERIZATION OF RETAIL DELICATESSEN DRAINS AND BIOFILMS USING 16SRNA AMPLICON AND SHOTGUN METAGENOMIC SEQUENCING

3.1 Abstract

Drains located in retail deli departments are known harborage points for pathogenic and spoilage microorganisms. Previous studies have used sequencing approaches to study the microbial consortia inhabiting drains and biofilms in various food processing environments. None of these studies, however, have focused on retail. The primary objective of this study was to characterize the microbiome of retail deli drains and drain biofilms through shotgun metagenomic and 16s rRNA amplicon sequencing approaches. Sponge samples were collected from the surfaces of retail deli drain covers and biofilms were harvested from inside deli drain trenches from fourteen retail delis in three major US cities. All samples were subjected to 16s rRNA gene sequencing and shotgun metagenomic sequencing was performed on nine biofilm samples (≥10 ng/µL DNA). Across all samples, *Pseudomonas* spp. were most abundant; the remaining genera differed by sample, city, and sequencing method. Shotgun metagenomics provided greater resolution of biofilms; more than 27 times the number of unique genera were observed than with 16s gene sequencing. Common sanitizer resistance genes (*qacEΔ1* and *qacE*) were observed in all biofilms. Metagenomic analysis revealed a low number of reads assigned to *L. monocytogenes* and other pathogens. While mean alpha levels were similar between biofilm and sponge samples evaluated via 16sRNA amplicon sequencing, a greater range of diversity was observed between biofilms than sponge samples. Additionally, beta diversity differed (P=0.01) between biofilms and sponges. While studies traditionally have focused on detection of specific microorganisms of interest, this study indicates that drain biofilms may harbor microorganisms that are not observed through traditional methods of environmental sampling. Collection of drain biofilms and use of culture-independent approaches would provide a more complete picture of the deli drain microbiome.

3.2. Introduction

Microorganisms often develop survival mechanisms to adapt to stress conditions, including the formation of biofilms (Kumar et al., 2017). Biofilms are microbial communities that adhere to solid surfaces and produce extracellular polymeric matrices that protect the embedded bacteria

(Alvarez-Ordóñez et al., 2019). The resulting communities can consist of a single species (monospecies) or multiple genera (multigenus). Complex biofilms often harbor multiple microbial genera or species, including pathogenic and spoilage microorganisms, and pose a serious problem in the food industry (Fagerlund et al., 2021). Many pathogens are able to form biofilms with resident microbiota, including *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes. L. monocytogenes* is of particular concern in retail deli departments due to its association with ready-to-eat (RTE) foods, specifically RTE deli meats (USDA & FDA, 2003). It is also known that biofilms formed by spoilage microorganisms and pathogens are a likely source of cross-contamination in food processing facilities (Alvarez-Ordóñez et al., 2019; Chmielewski & Frank, 2003; Coughlan et al., 2016). In turn, pathogens may be introduced to vulnerable groups via biofilm-mediated cross-contamination in retail environments.

The microbiota of food processing facilities can influence the growth of pathogens through competitive inhibition or cooperative survival methods. For example, *Pseudomonas* spp. are commonly found in biofilms containing *L. monocytogenes* indicating a synergism between the two organisms, while inhibition of *L. monocytogenes* is associated with the production of antagonistic compounds in dual species biofilms by *Bacillus cereus* and lactic acid bacteria (Fagerlund et al., 2021). Additionally, biofilm matrices protect the microorganisms by providing a physical barrier against environmental stressors (i.e., temperature, cleaning and sanitizing agents, and antimicrobials), forming an ecological niche and allowing complex interactions between microbes (Chmielewski & Frank, 2003; Fox et al., 2014; Kumar et al., 2017). Bacteria are highly social organisms that utilize quorum sensing, a chemical communication system and gene regulation mechanism (Preda & Sandulescu, 2019; Williams & Cámara, 2009). Quorum sensing is involved in regulation of hundreds of bacterial genes associated with surface adhesion, detachment, and toxin production (Preda & Sandulescu, 2019). Increased survival mechanisms have been reported in multigenus biofilms compared to their single species counterparts (Fagerlund et al., 2017); for example, several studies have reported increased sanitizer resistance and persistence of pathogenic microorganisms in biofilms compared to planktonic cultures (Coughlan et al., 2016; Fagerlund et al., 2017; Folsom & Frank, 2006; Frank & Koffi, 1990).

While biofilm formation and control strategies have been investigated in various food processing environments, to our knowledge, there are no studies evaluating the microbial composition of biofilms in retail deli environments. Additionally, while numerous groups have

studied artificially developed biofilms in food processing environments, the microbial composition of natural biofilms as they exist in these environments has not yet been evaluated. This knowledge gap prompted us to study the composition of biofilms in retail deli department drains. Therefore, the goals of this study were to (i) characterize the microflora of drains in retail deli departments and (ii) identify bacterial species that may influence the colonization potential of *Listeria monocytogenes* in retail deli department drains.

3.3. Materials and Methods

3.3.1 Overall study design

A total of 14 retail deli departments from three major US cities (A, B, and C) were enrolled in this study. Retail chains that voluntarily participated in the study were asked to select deli establishments serving communities with differing demographics, facilities of different sizes and ages, and establishments with and without perceived food safety challenges. Collection of deli area drain biofilm samples was performed by a corporate food safety and sanitation expert previously trained to conduct aseptic environmental sampling by Purdue University. All samples were shipped on ice within 24 h of collection to Purdue University.

3.3.2 Biofilm sample collection and DNA extraction

To collect samples, the drain cover was removed to allow access to the drain trench and a sterile FisherBrand High Precision #22 style disposable scalpel (Fisher Scientific, Waltham, MA) was used to aseptically remove a section of biomass from the drain trench. The section of biomass was immediately placed in a sterile 50 mL conical centrifuge tube (Fisher Scientific). The samples were stored on ice and shipped overnight (within 24 h) to Purdue University. Upon arrival, the conical tubes with the section of biomass were stored at -20°C until DNA extraction.

DNA was extracted from each biofilm sample using the Qiagen DNeasy Powerbiofilm kit (Qiagen, Hilden, Germany). Approximately 0.10 g of biomass was aseptically removed from each sample and placed in a 2 mL collection tube, centrifuged (13,000 *x g,* 1 min), and excess liquid removed. The biofilm material was resuspended in 350 mL Solution MBL (provided), transferred to a PowerBiofilm Bead Tube. Solution FB (100 µL) was added before incubating the mixture at 65°C for 5 min. After incubation, the samples were bead beat using a Vortex Adaptor (Qiagen) for

15 min. The samples were then centrifuged (13,000 *x g,* 1 min) and the supernatant transferred to a new 2 mL collection tube. Solution IRS (100 µL) was added to the collection tube before incubation (4°C, 5 min). After incubation, samples were centrifuged (13,000 *x g,* 1 min) and the supernatant was transferred to a new 2 mL collection tube. Solution MR was added (900 µL) before 650 µL of supernatant was loaded into an MB spin column and centrifuged (13,000 *x g,* 1 min). The spin column was then placed in a clean 2 mL collection tube. Solution PW (650 µL) was added and the mixture was centrifuged (13,000 *x g,* 1 min). The flow-through was discarded and 650 µL ethanol was added to the spin column followed by centrifugation (13,000 *x g,* 1 min). The flow-through was discarded a second time and samples were centrifuged once more (13,000 *x g,* 2 min). Finally, the spin column was placed in a final 2 mL collection tube, 100 µL ethanol was added to the center of the filter membrane, samples were centrifuged (13,000 *x g,* 1 min), and the spin column discarded. Extracted DNA was stored at -20°C for one week, then shipped on dry ice to the Center for Food Safety at the University of Georgia for library preparation and sequencing.

The libraries for each sample were prepared using the Illumina 515F with barcode/806R primer set to amplify the V3 and V4 regions of the 16s rRNA gene. The 16s rRNA amplicons were sequenced on an Illumina MiSeq system at the University of Georgia, Center for Food Safety (300x2). Additionally, samples with > 10 ng/µL DNA (a total of nine samples) were shipped on dry ice to Microbial Genome Sequencing Center (MiGS; www.migscenter.com) for library preparation and shotgun metagenomic sequencing as per the MiGS protocol. The nine samples used for shotgun metagenomics were sequenced on an Illumina NextSeq 2000 system at MiGS center (150x2).

3.3.3 Drain sponge sample collection and DNA extraction

Sponge samples were collected using EZ Reach Dual Sponge samplers (World BioProducts, LLC, Bothel, WA) premoistened with High-Cap neutralizing buffer (20 mL). Two sponge samples were taken from each sample site: one sponge was used for 16s rRNA gene sequencing and the other for detection and isolation of *Listeria* spp. The *Listeria* spp. results are reported in another study (Britton et al., *accepted*). After sampling, sponges were placed into individual pre-labeled stomacher bags, stored on ice, and shipped overnight to Purdue University. Once received, sponge samples for 16s rRNA gene sequencing were held at -20°C for 6-8 months

until transportation (on ice, overnight) to the University of Georgia, Center for Food Safety for DNA extraction. To maximize cell recovery from the sponge samples, 50 mL phosphate buffered saline (PBS; 1X; Difco, BD) was added to each sample bag and samples were stomached (7 min, 230 rpm; Stomacher 400 Circulator, Seward Ltd., Worthington, West Sussex, UK). The resulting homogenate was transferred into a 50 mL conical tube (Fisher Scientific) and centrifuged (4°C) at 1,871 x g for 25 min. After centrifugation, the supernatant was removed and the microbial DNA was extracted from the resulting cell pellet using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen). Library preparation and sequencing occurred as described in section 2.2.

3.3.4 16s rRNA gene bioinformatics and statistical analysis

Bioinformatic analysis was performed for both biofilm and drain cover sponge samples. Demultiplexed amplicon sequences were uploaded into the R statistical software (v4.0.3) and amplicon sequence variants (ASV) were assigned with the DADA2 (v1.16) package (Callahan et al., 2016). During ASV assignment, primers, lower quality ends, and chimeras were removed. Taxonomy was conducted using a pretrained Naive Bayes classifier trained with the Ribosomal Database Project (RDP) Classifier (Cole, 2004). Alpha and Beta diversities were calculated and visualized using the PhyloSeq package (McMurdie & Holmes, 2013) in R. Alpha diversity was assessed via Shannon's Diversity Index (measure of richness and evenness) and Simpson's Diversity Index (measure of number of species and relative abundance of each species present). Beta diversity was measured using Bray-Curtis Dissimilarity (a quantitative measure of community dissimilarity). Beta diversity effects were evaluated using the adonis function from Vegan (v. 2.5-7). Bioinformatic analyses code can be found on GitHub ((https://github.com/Brittob/RetailBiofilmStudy).

3.3.5 Shotgun bioinformatics and statistical analysis

The metagenomic sequences were first trimmed and filtered using Trimmomatic (Bolger et al., 2014). Supplied adaptors in the sequence file were removed using the ILLUMACLIP command. Next, the leading three and trailing three nucleotides were removed from each read and sequence reads with < 30 bp were removed, along with their mate-pair reads. Finally, reads were checked for their Phred quality score (Qscore) and nucleotides were removed until the average Qscore was > 33. After trimming and filtering, paired-end reads were merged using the

Fast Length Adjustment of Short Reads (FLASH) software to improve genome assemblies (Magoc & Salzberg, 2011). Sequences were assembled with the metaSPAdes assembler (Nurk et al., 2017); metaSPAdes uses de Brujin graphing methods on short-read sequences to ultimately construct an assembly graph that corresponds to long fragments in the metagenome. The remaining sequences were aligned to the default reference genome and sorted using Burrows-Wheeler aligner (BWA; Li & Durbin, 2010). Metagenome binning was performed using MetaBAT2 (Kang et al., 2019). Read classification was performed using Sepia (https://github.com/hcdenbakker/sepia). Sepia uses a novel taxonomic database developed from the Genome Taxonomy Database (GDTB) release 202 (https://gtdb.ecogenomic.org/), with kmer length of 31 and a minimizer size of 21.

Classified sequences were subsequently uploaded into R studio. Similar to the 16s amplicon sequences, alpha and beta diversities were calculated and visualized using the PhyloSeq package in R studio. Alpha diversity was assessed via Shannon's Diversity Index (measure of richness and evenness) and Simpson's Diversity Index (measure of number of species and relative abundance of each species present). Beta diversity was measured using Bray-Curtis Dissimilarity (a quantitative measure of community dissimilarity). Additionally, the top 10 genera were visualized using ggplot2 (Villanueva & Chen, 2019). 16s targeted gene bioinformatic analyses code can be found on GitHub (https://github.com/Brittob/RetailBiofilmStudy). Sanitizer resistance genes associated with common pathogens of interest (table 3.1) were visualized from the de novo assembly graphs using Bandage (Wick et al., 2015). The genes evaluated here were *qacEΔ1*, *qacE*, and *bcrABC.* The biofilm shotgun metagenomic sequences have been deposited in the NCBI Sequence Read Archive (BioProject accession number PRJNA762323).

3.4. Results and Discussion

3.4.1 General sequencing results for 16s amplicon and shotgun sequencing.

Sequencing of the 16s rRNA V3-V4 gene region of 14 DNA samples isolated from biofilms collected from drains in retail deli departments (and 3 blank samples) generated 4.3M reads with an average of 308,475 reads per sample (range = 131,515 to 576,441; table 3.2). Quality filtering resulted in removal of approximately 18.2% of the reads across all samples;

Table 1. Reference sequences used to identify presence of sanitizer resistant genes (*qacEΔ1*, *qacE*, and *bcrABC)* for pathogens of interest in retail deli environments.

Gene	Microorganism	Reference Sequence	Reference
qacEΔ1	*Pseudomonas aeruginosa*	MKGWLFLVIAIVGEVIATSALKSSEGFTKLAPSAVVIIGYGI AFYFLSLVLKSIPVGVAYAVWSGLGVVIITAIAWLLHGQK LDAWGFVGMGLIIAAFLLARSPSWKSLRRPTPW	https://www.uniprot.org/uniprot/Q7BQY4
qacE	*Escherichia coli*	MKGWLFLVIAIVGEVIATSALKSSEGFTKLAPSAVVIIGYGI AFYFLSLVLKSIPVGVAYAVWSGLGVVIITAIAWLLHGQK LDAWGFVGMGLIVSGVVVLNLLSKASAH	https://www.uniprot.org/uniprot/P0AGC9
bcrA	*Listeria monocytogenes*	MSAKKQDKRAHLLNAAIELLGSNDFDTLTLEAVAKQANV SKGGLLYHFPSKEALYAGITELIFQDFVYRFNELAENDPIE KGKWTRALIHAYTDDLNNSQVLNIASHSFSKLNPTVTENI LVHFEYIQSKIDEDGIDSVLATTIRLTLDGLYYSEFFKLGQV NFDLREKIIEKLIESTT	https://www.uniprot.org/uniprot/I7B1D2
bcrB	*Listeria monocytogenes*	MNPYVLLIGAILFEVFGSSMMKASNGFKKLVPTVGLVIGM GSAFYLLSKALEHIPLGTAYAIWSGGGTALTAIVGILVWKE KFNLKILLGLLIIIAGVVVLKLSH	https://www.uniprot.org/uniprot/I7A797
bcrC	*Listeria monocytogenes*	MKGYVALGIAIIGEIFGTSMLKLSEGFTNIYPTIGVAIGFFIA FYTLSLSLKTLPLSLAYAIWSGVGTALTALIGVLVWNEPFN ILTFIGLVMIVGGVIILNQRSADTKTSTSH	https://www.uniprot.org/uniprot/I7A794

5.7% of the remaining filtered reads were classified as chimeric and removed. All 14 samples were included in downstream analysis. Of the three blank controls included in this analysis, all three sequenced with an average read count of 3,532 reads after filtering. A total of nine biofilm samples were successfully sequenced using shotgun metagenomics analysis; five of the samples did not yield sufficient DNA for sequencing. The nine samples generated 6.9M reads with an average of 771,866 reads per sample (range = 475,916 to 1,089,844 reads/sample). An average of 4.8% of reads were removed by Trimmomatic.

Sequencing of the 16s gene of environmental sponge samples collected from the surface of the deli area drain covers where the 14 biofilms were collected generated 1.9M reads with an average of 154,558 reads per sample; a range of 42,241 to 324,929 reads/sample (table 3.2). Quality filtering resulted in removal of 36% of the reads across all samples and 2.5% of the filtered reads were classified as chimeric. All 12 samples were included in downstream analysis.

3.4.2 Comparison of 16s rRNA targeted gene sequencing and shotgun metagenomic sequencing.

Biofilm samples were collected directly from the drain trench in 14 retail deli departments as described in section 2.2. All 14 biofilms were subjected to 16s rRNA amplicon sequencing and nine biofilms were subjected to shotgun metagenomic sequencing. The sequencing depth chosen in this study (3,269,303 to 7,626,131 reads/sample for metagenomic analysis) provided valuable insight into the diversity of the bacterial communities surviving in biofilms. Shotgun metagenomics provided a higher resolution (species level) than 16s rRNA gene sequencing.

In this study, both approaches provided relatively similar, although not identical, compositions of the drain biofilm microbiome in retail deli departments. The different taxa are hierarchically organized, and the lowest taxonomic levels (genus and species) were the most challenging to identify, so we performed our comparisons at these levels. When we compared the sensitivity of each method, 16s rRNA based analysis of environmental samples provided fewer reads/sample than shotgun metagenomic analysis; thus, the 16s amplicon profiles were only evaluated to the genus level, while the deeper sequencing of the shotgun data allowed for evaluation of the microbiome at the species level.

Both 16s rRNA gene sequencings and metagenomic shotgun sequencing are widely used to investigate the bacterial profile of a variety of environmental systems and are routinely used in

Table 3.2. Total number of sequences remaining after each quality filtering step in DADA2 for 16s rRNA gene sequencing analysis of drain biofilm and surface sponge samples.

Steps of DADA2	Sequences Obtained after Quality Filtering			
	Biofilm Samples		Surface Sponge Samples	
	Total Sequences	Percent	Total Sequences	Percent
Input[1]	4,318,660	100%	1,854,693	100%
Filtered[2]	3,531,868	81.8%	1,178,672	63.6%
Denoised[3]	3,507,495	NA	1,176,248	NA
Merged[4]	3,401,445	78.8%	1,160,126	62.6%
Non-chimeric[5]	3,206,660	74.3%	1,149,465	62.0%

[1] *Input: Total number of forward and reverse reads sequenced.*
[2] *Filtered: Total number of reads remaining after quality score filtering.*
[3] *Denoised: Total number of reads remaining after removal of low quality regions of sequences.*
[4] *Merged: Total number of reads remaining after merging forward and reverse reads.*
[5] *Non-Chimeric: Total number of reads remaining after removal of chimeras; final number of sequences included in downstream analysis.*

metagenomic studies (Durazzi et al., 2021; Shah et al., 2010). 16sRNA amplicon sequencing utilizes PCR amplification of the 16s rRNA gene hypervariable regions (Durazzi et al., 2021); the PCR primers used in 16s rRNA sequencing target the highly conserved regions of the gene (Ranjan et al., 2016), however, these primers can lead to bias in the representation of taxonomic units (Durazzi et al., 2021). Additionally, this method is limited as the taxa are defined by operational taxonomic units (OTUs), which are most commonly analyzed at the phylum or genus level and are limited at the species level (Ranjan et al., 2016). The analysis is often complicated by sequencing error and chimeric sequences caused by PCR amplification (Shah et al., 2010). An alternative to 16s rRNA gene sequencings is whole genomic shotgun sequencing. Shotgun metagenomics is a whole genome sequencing method that targets DNA fragments, which are individually sequenced, assembled, and aligned for taxonomic identification (Brumfield et al., 2020). While shotgun metagenomics does require higher sequencing coverage than 16s rRNA gene sequencing, it is more precise in classifying microbial communities at the species level than 16s targeted sequencing (Ranjan et al., 2016) and can provide genetic contribution of each member of the community by profiling functional genes of each organism (Durazzi et al., 2021).

3.4.3 *Pseudomonas* spp. was the dominant genera in deli drain biofilms, but bacterial diversity differed across cities and individual samples.

The top five phyla and top ten genera for the biofilm samples from 16s amplicon and shotgun sequencing are presented in figures 3.1 and 3.2, respectively. Proteobacteria was the most dominant phylum identified in the biofilm samples by both shotgun and biofilm methods. The next most abundant phylum in the biofilms evaluated with 16s rRNA sequencing was Firmicutes (figure 3.1a), however, shotgun metagenomic analysis revealed that the second most abundant phylum was Actinobacteria (figure 3.1b). Proteobacteria are an abundant phylum that contains gram-negative bacteria including many common pathogenic microorganisms such as *Salmonella* spp., *Campylobacter* spp., *Escherichia* coli, and *Vibrio* spp. (Rizzatti et al., 2017). Proteobacteria are commonly found in RTE food processing environments and are associated with both food spoilage and foodborne disease. To the best of our knowledge, this is the first study to characterize retail deli drain biofilms. However, Dzieciol et al. (2016) characterized drain biofilms and drain water samples in a cheese processing facility and reported that Proteobacteria and Bacteroidetes and Proteobacteria and Firmicutes were the most dominant phyla in biofilms and drain water, respectively. *Pseudomonadaceae* was the dominate family observed in the biofilms collected from retail drains, which is to be expected due to the aerobic, refrigerated conditions of the retail deli departments (Dzieciol et al., 2016; Weinroth et al., 2019).

The predominant genus found in all biofilms by both metataxonomic and metagenomic analysis was *Pseudomonas* spp. (figure 3.2), however, the proportions of the next most dominant genera varied by sample, location, and sequencing method. Samples were collected from three major cities in the US. While *Pseudomonas* spp. was the dominant genus in all three cities, the second and third most dominant genera were different. In the 16s amplicon analysis, city C was characterized by the greatest diversity of bacterial genera with high abundances of *Stenotrophomonas* spp., *Staphylococcus* spp., *Serriatia* spp., *Psychrobacter* spp., *Alcaligenes* spp., and *Achromobacter* spp., while city A and B had less diversity with *Serratia* spp. and *Chryseobacterium* spp., respectively, being the next most abundant genera after *Pseudomonas* spp. A similar trend was observed with the shotgun analysis: city C had the greatest bacterial diversity

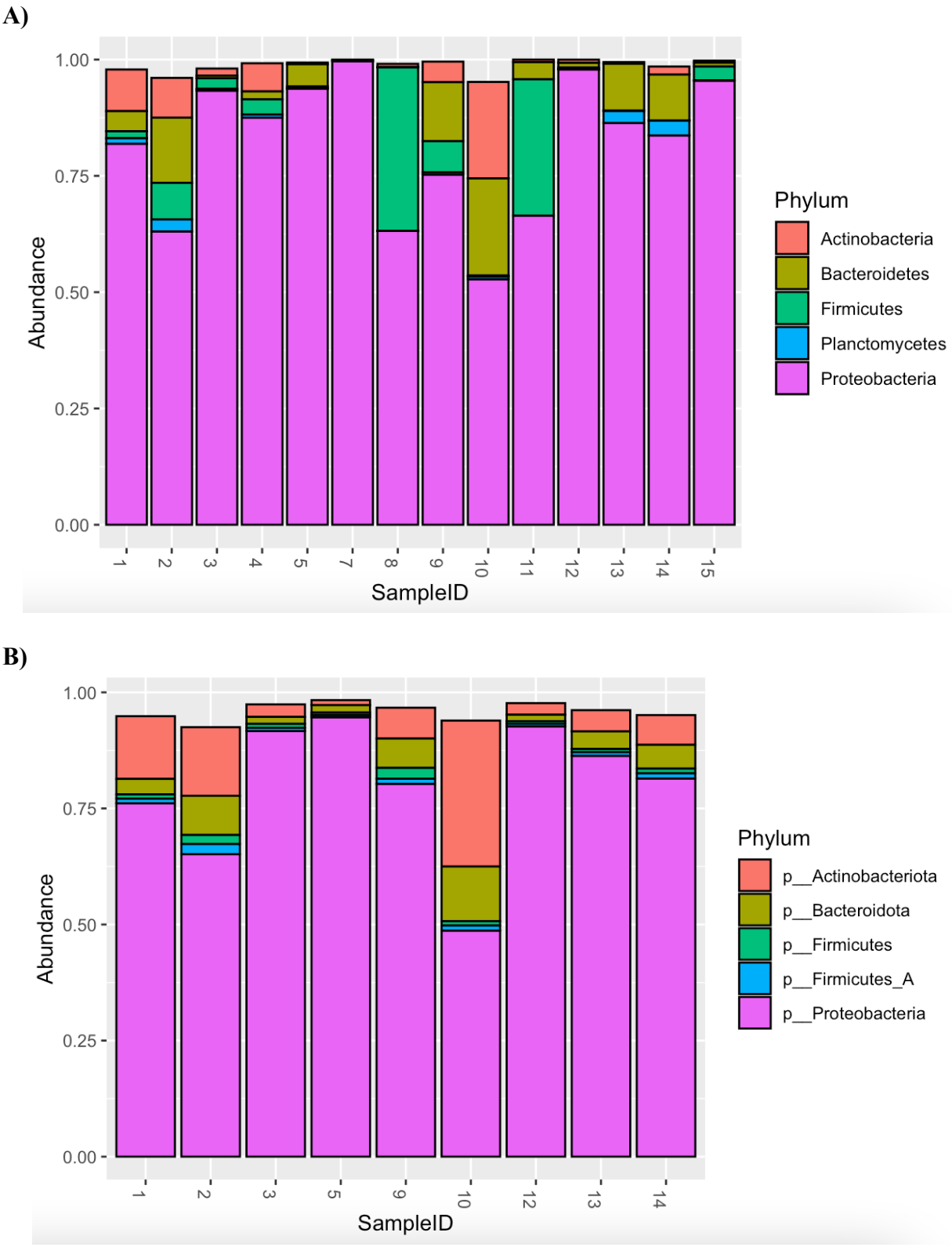


Figure 3.1. Top five phyla observed in biofilms via **A)** 16s rRNA gene sequencing and **B)** shotgun metagenomic sequencing methods.

with *Pseudomonas* spp. being the most abundant genus, however, there was a greater abundancy of *Achromobacter* spp. than found with the 16s analysis. Additionally, in city A, *Serratia* spp. was the second most abundant genus and *Psychrobacter* spp. was second most abundant in city B.

The high abundances of *Pseudomonas* spp. found in the biofilm samples can likely be attributed to the high biofilm forming abilities of the genus, particularly in aerobic environments (Dzieciol et al., 2016; Meliani & Bensoltane, 2015). *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* are common spoilage microorganisms that are often found on floors and drains in food processing environments (Meliani & Bensoltane, 2015). Additionally, *Pseudomonas aeruginosa* is often a focus in hospital drain studies due to its association with nosocomial infections (Fusch et al., 2015; Lalancette et al., 2017). The other highly abundant genera were common spoilage bacteria that are often associated with water or marine environments and food processing environments. *Serratia* spp. and *Psychrobacter* spp. have been observed in meat processing environments (Wagner et al., 2020), cheese processing facilities (Schirmer et al., 2013), and water samples (Møretrø & Langsrud, 2017). The *Serratia* genus also has known biofilm forming capabilities and has been observed as a dominant genus within the environmental microbiome of milk processing facilities (Møretrø & Langsrud, 2017). Furthermore, Cleto et al. (2012) reported isolating culturable bacteria, including *Pseudomonas* spp., *Serratia* spp., *Staphylococcus* spp., and *Stenotrophomonas* spp., after sanitation procedures of milk processing lines. The authors also found that the microorganisms in these mixed species environments are capable of producing various enzymes and had the capacity to form biofilms.

3.4.4 Bacterial abundances differed between biofilms and surface samples using 16s rRNA gene sequencing.

Similar to the biofilm samples, the deli drain surface samples were dominated by genera in the Proteobacteria phylum, such as *Pseudomonas* spp. and *Psychrobacter* spp. However, the drain surfaces showed greater abundances of bacterial genera classified within the Firmicutes phylum, which the biofilms had greater abundances of Bacteroidetes (figure 3.3a). Drain surface samples

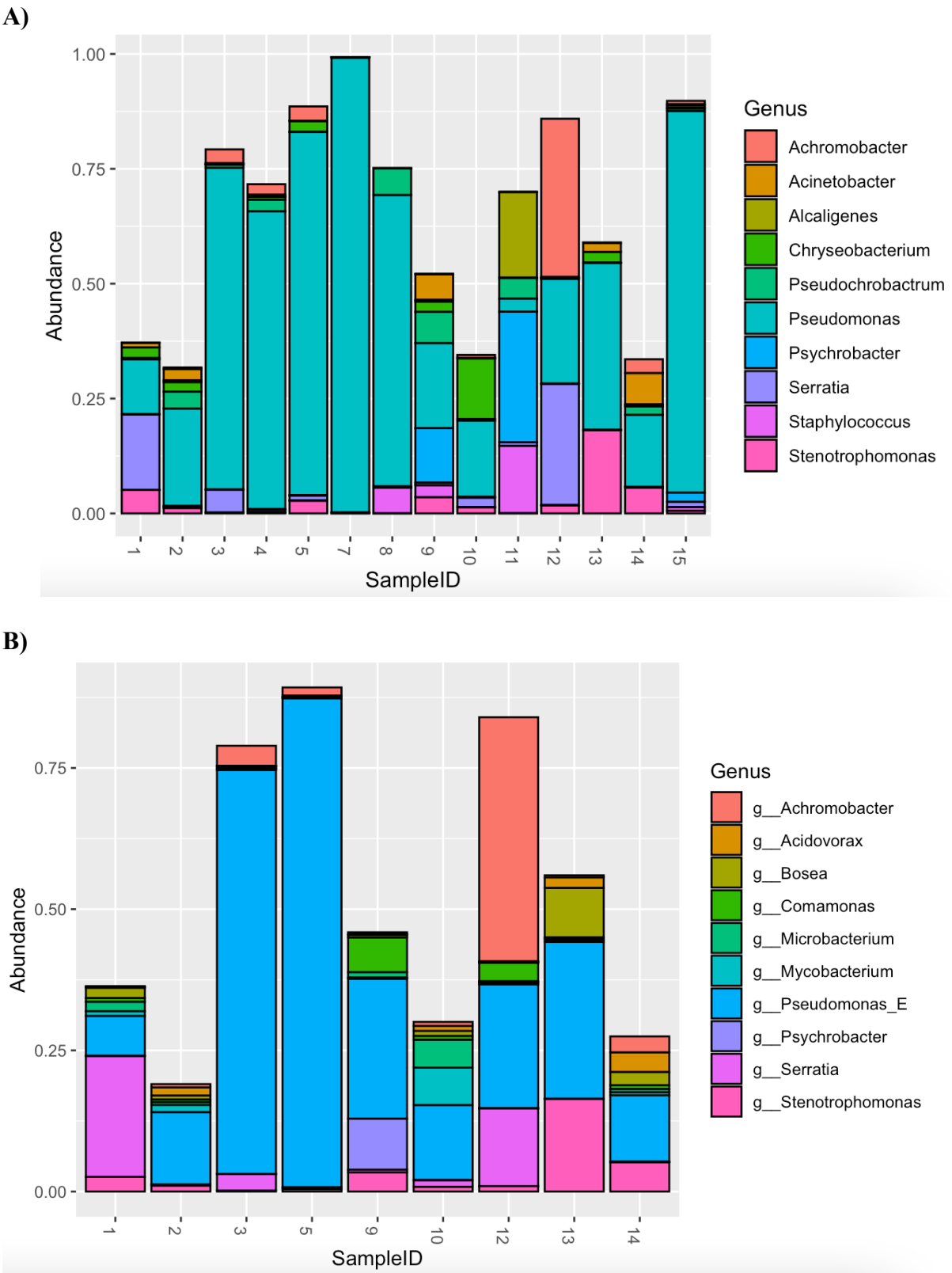
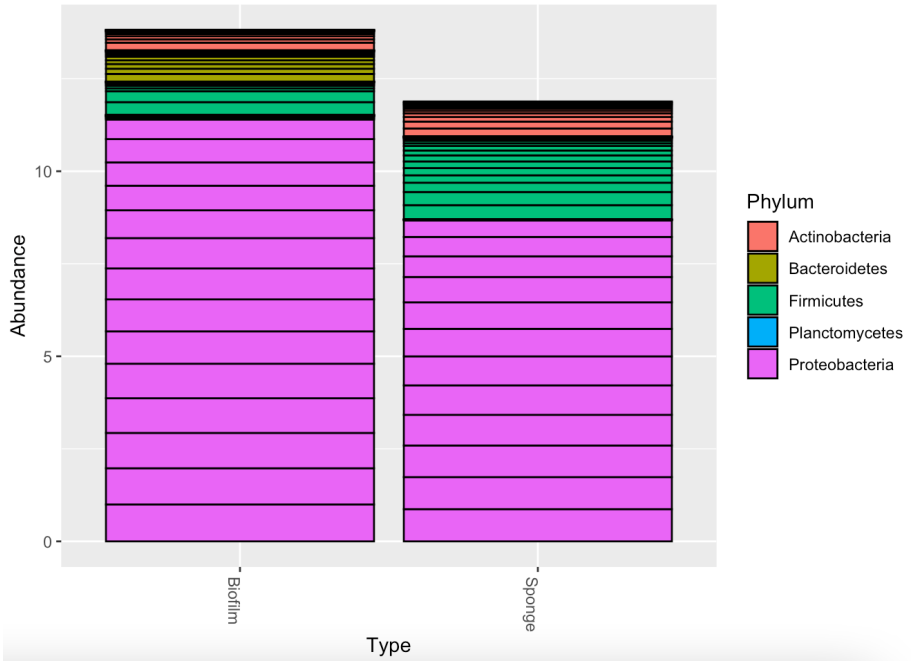


Figure 3.2. Top 10 genera observed in biofilms via **A)** 16s rRNA gene sequencing and **B)** shotgun metagenomic sequencing methods.

had high abundances of *Erwinia* spp., *Methylorubrum* spp., and *Enterococcus* spp., which were not observed in the top 10 genera of the biofilm samples (figure 3.3b). Overall, drain surface samples had less diversity than the biofilm samples with only 349 unique genera identified; 474 genera were observed in the biofilm samples. Drain surfaces had lower alpha diversity calculations than the biofilms collected from inside the drain trench as measured by Shannon's Diversity Index and Simpson's Diversity Index than the biofilm samples (Figure 3.4). Furthermore, when beta diversity was compared, the two sampling methods differed significantly ($P = 0.01$; figure 3.5).

Floor drains in food processing environments have long been considered harborage points for various pathogenic and spoilage microorganisms. To the best of our knowledge, there are no studies comparing the differences of the microbiota that reside on drain surfaces and in drain biofilms, as well as limited data on the microbiome of drains in food processing environments. However, studies have been conducted to understand the microbiome of drains, drain water, and biofilms in various environments, including food processing (Dzieciol et al., 2016; Fagerlund et al., 2021; McHugh et al., 2021) and household drains (McBain et al., 2003). The aforementioned study by Dzieciol et al. (2016) compared the microbiota of drain water samples and drain biofilms in a cheese processing facility and reported there were significant differences in the communities observed in both types of samples, thus suggesting that a sampling plan based on drain water alone may not be sufficient. Similar results were observed in the current study where the microbiota observed on drain surfaces was largely different than the communities collected from drain biofilms. Additionally, studies have shown that in food processing facilities, the resident microbiota may have a competitive or cooperative impact on the persistence of pathogens, such as *L. monocytogenes* (Fagerlund et al., 2021; Fox et al., 2014; Heir et al., 2018). Thus, it is possible that these pathogens may be harbored in drain biofilms even if they are not isolated from drain surfaces via traditional sponge sampling methods. However, monitoring drains is a challenge (Dzieciol et al., 2016); it is likely that a more complete picture of the environment would be obtained if biofilms were also a part of environmental sampling plans.

A)



B)

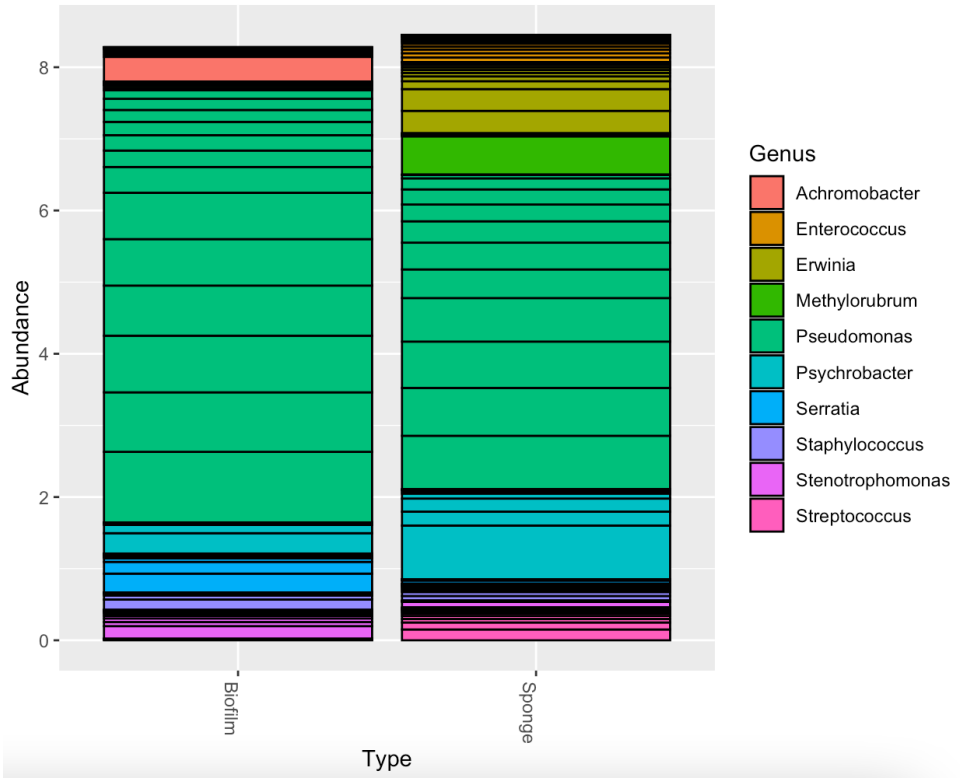


Figure 3.3. Microbial diversity observed in the biofilm and sponge samples evaluated with 16s rRNA gene sequencing. A) Top five phyla observed in biofilms and sponge samples and B) top 10 genera observed in both sample types.

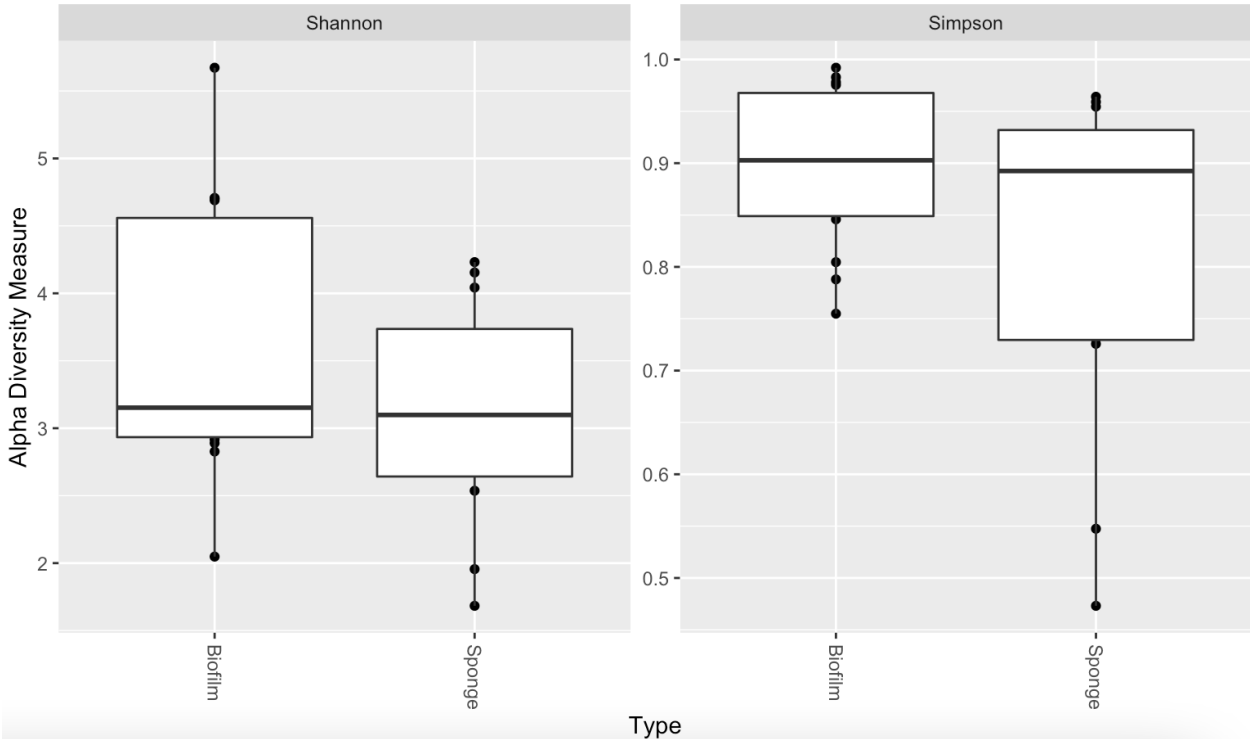


Figure 3.4. Alpha diversity (Shannon and Simpson Diversity Indices) of drain biofilm and sponge samples evaluated via 16s rRNA gene sequencing.

3.4.5 Sanitizer tolerance genes associated with *Pseudomonas* spp. and Enterobacteriaceae were found in abundance in the biofilm samples.

Sanitizer resistance is a major concern often associated with biofilms in the food industry. While there are numerous *qac* genes (*qacA-L*) that can indicate increased tolerance to quaternary ammonium (QAC) in food processing environments, this study focused on tolerance genes most commonly associated with the pathogenic microorganisms observed in abundance in this study. Here, the focus was on sanitizer tolerance genes often found within the genomes of pathogens of interest in retail deli departments (e.g. *P. aeruginosa, Enterobacteriaceae* family, and *L. monocytogenes*), including *qacEΔ1*, *qacE*, and *bcrABC* (table 3.3). The BLAST-query conducted here revealed a high number of hits for *qacEΔ1* in seven of the nine biofilms in this

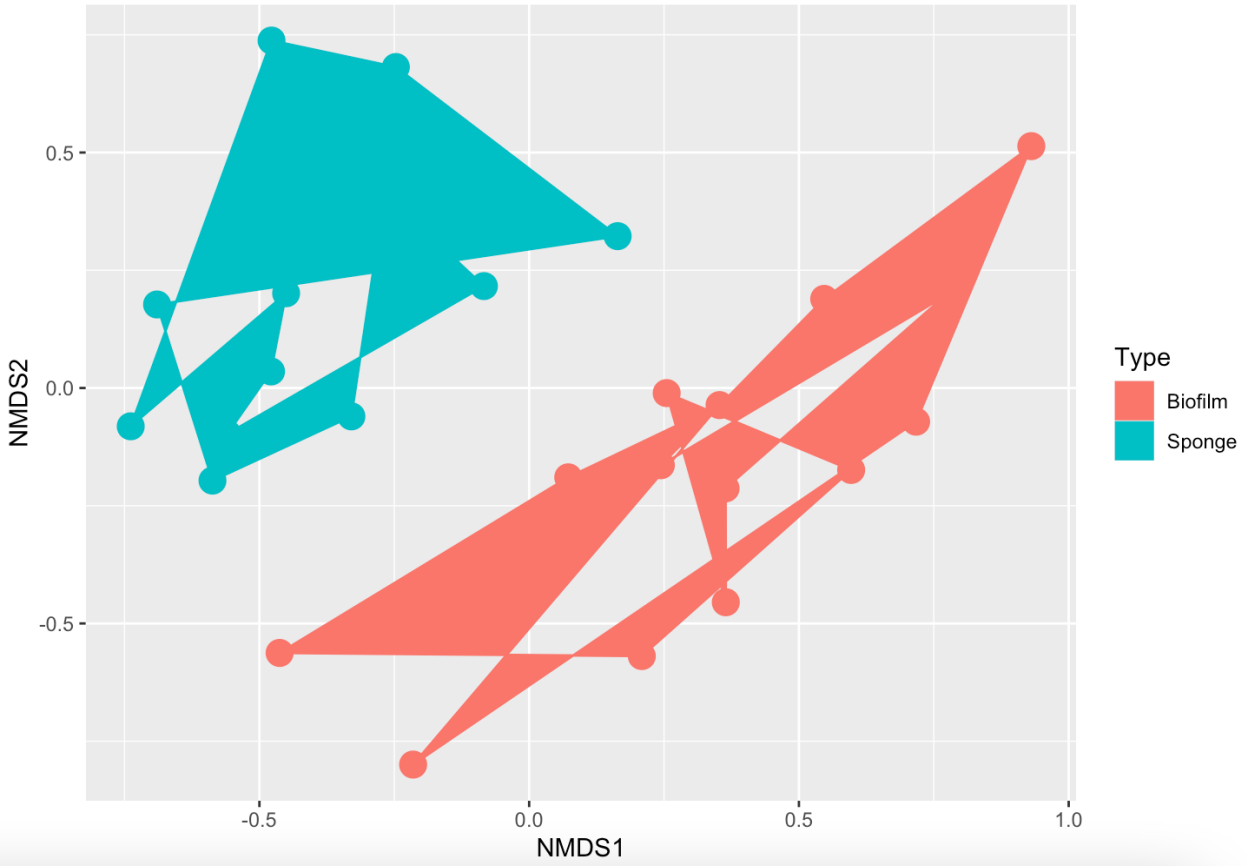


Figure 3.5. Beta Diversity (Bray-Curtis Dissimilarity) of biofilm and sponge samples evaluated via 16s rRNA gene sequencing.

study; these seven biofilms had > 95% sequence similarity with E-values indicating high quality, homologous matches. Additionally, *qacE* genes displayed the greatest percentage of sequence (75-100%) similarity in all nine biofilms. qacE and *qacEΔ1* are known quaternary ammonium tolerance genes in gram-negative bacteria, such as *Pseudomonas* spp. (specifically *P. aeruginosa*) and Enterobacteriaceae (such as *Serratia* spp. and *E. coli*; Sidhu et al., 2004). A significant association between *qacEΔ1* and antimicrobial resistance in *P. aeruginosa* has been observed (Zou et al., 2014). The *qacEΔ1* gene is a deletion mutation of the *qacE* gene (Zou et al., 2014), and has been associated with increased minimal inhibitory concentration of QAC's (Kucken et al., 2000). The high number of high-quality matches to these two antimicrobial- and QAC-tolerance genes indicate the microbial communities in these biofilms may have increased tolerance to common sanitizers used in the retail food industry.

In contrast, the *bcrABC* cassette was not found in any of the biofilms in this study, which is consistent with the classification results indicating minimal reads assigned to *Listeria* spp. The

bcrABC cassette is known as a benzalkonium chloride resistance gene in *Listeria* spp. While there were hits matched to each of the individual *bcrA, bcrB,* and *bcrC* genes within each of the biofilms, the sequence similarities were low (< 60%) and the E-values were much higher than the other genes evaluated here. These genes are typically found as a group in the *bcrABC* cassette, which can be transferred via horizontal gene transfer to the other bacteria within the biofilms (Holmes et al., 2003), and are predicted to help *Listeria* spp. persist in retail and food processing environments (Cherifi et al., 2018; Jiang et al., 2017).

3.4.6 Study limitations

The COVID-19 pandemic began after the initiation of this study. Due to concerns about introducing new people to the store environments and disrupting the work of their employees, retail food safety experts limited access to their stores to collect samples for this study. Therefore, each store was only sampled one time. Additionally, due to the pandemic, heightened awareness of personal hygiene and changes in cleaning and sanitation procedures may have had a significant impact on the microbial composition of retail deli drains.

3.5 Conclusions

The aim of this study was to provide insights into the composition and characterization of retail deli department drains, including the surface microbiome and composition of biofilms in the drain trench. The data presented here show that drain surfaces and drain trench biofilms harbor distinct bacterial communities. Additionally, while *L. monocytogenes* was not isolated through enrichment methods from surface sponge samples, shotgun sequencings indicated that *Listeria* spp., including *L. monocytogenes*, were present in drain biofilms. Collecting biofilms along with drain surface samples could be beneficial and provide a more complete picture of drain microbiomes, thus, increasing the chances of detection and isolation of *L. monocytogenes* during environmental monitoring of retail delis.

Table 3.3. Quaternary ammonium tolerance genes with the greatest similarities (%) and highest query identity identified in each biofilm.

	Quaternary Ammonium Resistance Genes					
	qacEΔ1		***qacE***		***brcABC***	
Biofilm[1]	***Similarity[2]***	***E-value[3]***	***Similarity***	***E-value***	***Similarity***	***E-value***
1	97.92%	8.16e-54	100.00%	1.94e-64	< 60%	≥ 4.18e-23
2	98.26%	2.92e-67	95.83%	2.29e-53	< 55%	≥ 3.23e-20
3	96.88%	4.66e-57	99.09%	4.42e-68	< 53%	≥ 1.32e-20
5	73.96%	1.01e-41	75.46%	5.01e-50	< 60%	≥ 1.73e-20
9	97.92%	3.74e-52	100.00%	6.50e-62	< 58%	≥ 4.70e-23
10	61.98%	7.72e-42	76.36%	2.65e-49	< 53%	≥ 9.67e-20
12	95.83%	1.66e-56	98.18%	1.40e-67	< 53%	≥ 7.27e-21
13	98.49%	4.73e-38	98.49%	1.44e-38	< 50%	≥ 5.85e-22
14	95.83%	3.39e-57	98.18%	2.41e-68	< 53%	≥1.73e-24

[1] The nine biofilm samples with 10ng/uL of DNA for shotgun metagenomic sequencing.
[2] Similarity (%): Sequence similarity over the length of the hit. 100% means the query and node are identical over the length of the hit.
[3]E-value: The BLAST-calculated expect value.

3.6 Acknowledgements

The authors would like to thank the retailers who volunteered and supported the study. We would also like to thank the food safety experts at Diversey, INC. for their support in sample collection. The authors extend gratitude to Rowen Waller and Amy Mann for their assistance with DNA extraction and sequencing of the sponge and biofilm samples.

CHAPTER 4. MICROBIAL CONTAMINATION PATTERNS IN PEANUTS PRODUCED AND SOLD IN THE SENEGALESE PEANUT BASIN

4.1 Abstract

Peanuts and peanut products are significant revenue sources for smallholder farmers in the Senegalese peanut basin. However, microbial contamination during production and storage can greatly affect market access for producers. Peanut products have emerged as possible sources of foodborne illness, encouraging discussions on international standards for peanuts. In this study, we interviewed 198 households throughout the Senegalese peanut basin to assess current production practices, storage methods, and producers' prior knowledge of microbial contamination using a 162-question survey. A member of each household orally completed the survey with a trained enumerator and the results were compared to microbiological results obtained from peanut samples collected at the time of the interview using linear regression and an analysis of variance model. Samples were collected from stored peanuts at each household; peanuts were shelled and total Enterobacteriaceae, coliform, and yeast and mold populations were enumerated. Of the 198 samples analyzed, 13.0% and 13.6% contained concentrations greater than the upper detection limits for Enterobacteriaceae and coliforms, respectively. Likewise, concentrations of yeast and mold were above detection limits in 21.2% of samples. Only 22.7% and 18.7% of producers had heard of pathogenic bacteria or aflatoxins, respectively. There were no significant differences in observed microbial populations between households that took preventative measures against microbial contamination and those that did not. Additionally, four households reported washing their kitchen utensils before using them to eat and 60.1% reported always washing their hands before eating. Enumerators were asked to report peanut storage container type and if the containers were stored off the ground at the time of collection. While the interaction between storage container type and whether the container was stored off the ground was significant for Enterobacteriaceae and coliforms, it was not significant for yeast and mold. Additionally, when storage container type and whether peanuts were stored off the ground were included in the regression model, the model was predictive of contamination levels for Enterobacteriaceae and coliforms. To our knowledge, this is the first study to analyze the relationships among *Enterobacteriaceae*, coliforms, and yeast, and mold contamination and producer knowledge of

Senegalese peanuts. These results provide preliminary data to inform future studies to determine pathogen prevalence and impactful preventative measures to minimize microbial contamination of peanuts produced in Senegal.

4.2 Introduction

The peanut (*Archis hypogaea* L.), also known as the groundnut, is among the world's most important oilseed crops. Five countries (Argentina, the United States, Sudan, Senegal, and Brazil) account for 71% of total world exports of peanuts. In Senegal, peanuts are cultivated in most regions of the country and are one of the main sources of income for many rural smallholder farmers; smallholder farming is defined as a small farm operating under a small-scale agriculture model. As the fourth leading revenue generating export, peanuts are an important Senegalese cash crop. However, microbial contamination during production, harvest, and storage can affect the safety, quality, and value of Senegalese-grown peanuts in the global market.

The World Health Organization (WHO) reported that African and South-East Asian Regions have the highest burden of foodborne disease in the world. Additionally, WHO reported that foodborne hazards are responsible for 91 million illnesses and 137,000 deaths in Africa each year. The continent suffers from the highest rate of foodborne disease globally which results in an estimated $16.7 billion per year in human capital losses. While data relevant to individual countries are limited, international development agencies recognize the need to identify risk associated with various sources associated with microbial contamination. In Senegal, research has focused on meat and poultry products, seafood products (Coly et al., 2013; Demoncheaux et al., 2012), and raw milk (Breurec et al., 2010). Limited data are available for staple crops and commodities, including peanuts (Arias-Granada et al., 2020), of which domestic consumption in Senegal was approximately 79,000 metric tons in 2019 (*Senegal Peanut Meal Domestic Consumption by Year*, 2021), representing 5.6% of the total national production. Peanuts are often considered microbiologically "safe" foods due to low water activity (< 0.75) and additional thermal processing steps commonly utilized before consumption (Chang et al., 2013; Eglezos et al., 2008). To the best of our knowledge, the burden of disease associated with bacterial contamination of peanuts is unknown. However, peanuts have recently been associated with foodborne pathogen outbreaks, including a 1996 outbreak of *S. enterica* ser. Mbandaka in Australia, a 2001 outbreak of *S. enterica*

ser. Stanley in Australia and Canada, and a 2009 outbreak of *S. enterica* ser. Typhimurium in the U.S. (Harris et al., 2019).

Considerable research has focused on managing aflatoxin contamination produced by the fungi *Aspergillus flavus* (Senghor et al., 2020), specifically in developing economies. More than 55 billion people worldwide are exposed to aflatoxins, a significant risk factor for hepatocellular carcinoma (Liu and Wu, 2010). However, few studies have assessed bacterial contamination, including pathogen and fecal indicators such as Enterobacteriaceae and coliforms (Eglezos et al., 2008). Humans, livestock, and other animals are known reservoirs of these organisms (Doyle & Erickson, 2006). In Senegal, livestock and other animals are often near households and peanut storage areas, increasing the likelihood of cross-contamination. While peanuts are commonly further processed before consumption, they are also often consumed raw from roadside vendors. Additionally, it is possible that roasting processes may not fully destroy pathogens on peanuts before consumption (Asiegbu et al., 2020).

To our knowledge, there are no studies evaluating bacterial contamination of peanuts produced in the Senegal peanut basin. This knowledge gap prompted us to explore the relationship among indictors for bacterial contamination of peanuts and current storage methods and producer knowledge of potential contamination. We hypothesized that (i) storage methods would directly impact microbial contamination levels and (ii) limited foundational knowledge of bacteria and cross-contamination pathways would result in higher contamination rates. We were also interested in microbial contamination differences in peanuts intended for household consumption compared to those retained as seed.

4.3 Materials and Methods

4.3.1 Survey development, participation, and data collection

A survey was developed based on previous work with Senegalese groundnut producers. The survey consisted of 162-questions to assess demographic parameters, production activities (i.e., planting, harvest, drying), intended use of peanuts, household storage methods, producer knowledge of microorganisms and their effect on human health, economics, and observation questions completed by enumerators conducting the survey. For this analysis, intended use of peanuts, storage methods, and producer knowledge of microbial contamination of groundnuts were

the primary themes: “intended use”, “storage methods”, and “producer knowledge”, respectively (table 4.1); production methods and marketing strategies data from this survey will be reported in another study (Granada et al, *in prep).* The survey was conducted by enumerators from the Institut Sénégalais de Recherches Agricoles (ISRA) and the Agence Nationale de Conseil Agricole et Rural (ANCAR). Enumerators were trained on survey delivery prior to study initiation. The protocol and survey were approved by Purdue University Institutional Review Board (protocol IRB-2019-783). After training, enumerators were deployed throughout Senegal’s southern peanut basin to visit groundnut producers to verbally administer the survey questionnaire. For this study, 198 households were surveyed, and a single peanut sample (50 g) was collected for microbial analysis from each. Producers were compensated market price for the peanuts that were collected.

4.3.2 Peanut sample collection training, sample collection, and shipment of peanuts

Prior to initiation of the study, enumerators were trained in aseptic technique for peanut collection via teleconference (Cisco Webex, Milpitas, CA). The training included modules for basic microbial contamination concerns for peanuts, proper use of latex gloves to avoid cross-contamination between samples, and appropriate labeling and collection of groundnut samples from smallholder farmers. A new pair of latex gloves was utilized to collect each sample and each sample was placed in a sterile sealable plastic bag. Labels included town and commune nearest the household, survey identification number associated with each sample, and designated use of the sample (i.e., household consumption, for sale to venders, or to be utilized as seeds). Enumerators conducting the survey were also asked to record their observations of storage practices associated with the collected peanut samples. These observations included potential contamination sources (e.g., animals, trash, wastewater) near stored peanuts, visible evidence of fecal matter or fecal odor near the peanuts, and how the samples were stored (i.e., the type of container and location of the stored peanuts). At the end of each collection day, all samples were stored under refrigerated conditions (approximately 4ºC) at ISRA until shipped to Purdue University. Enumerators collected 198 total groundnut samples. Samples were packed into corrugated boxes by ISRA personnel prior to shipping. All samples were transported at ambient temperature from Dakar, Senegal to the Department of Food Science at Purdue University (APHIS permit #PCIP-20-00056); shipping took approximately five days. Upon arrival, samples were immediately placed at 4ºC until microbiological analysis.

Table 4.1. Summary of survey sections and associated question themes

Question Themes[1]	Survey Sections Associated with Theme	Survey Questions Associated with Theme	Total Questions in Theme
Household demographics	1, 2, 10, 12	1-10, 138-142, 153-166	32
Production methods	3, 4, 5	11-70	60
Intended use of peanuts	6	71-96	26
Household storage methods	7	97-120	24
Producer knowledge of bacteria and aflatoxins	8, 11	121-126, 143-152	16
Economics-based questions about assets, income, risk preferences, and interest in Aflasafe™[2]	9, 13, 14, 15	127-137, 167-197	42
Observed information about collected peanut samples	17	198-209	12

[1] Survey questions were grouped into overarching themes for ease of assessment. In this study, the themes of "intended use," "household storage methods," and "producer knowledge of bacteria and aflatoxins" were the primary foci;
[2] Aflasafe™ is a biocontrol product developed to minimize the risk of contamination of peanuts, maize, and other commodities from aflatoxin-producing Aspergillus flavus.

4.3.3 Microbiological analysis

Peanuts collected in-shell were unshelled by hand with gloves sanitized with 70% ethanol. New gloves were used for each sample. Whole peanut kernels were ground with a sanitized stainless-steel Waring 0.75 horsepower bar blender (BB155S; Waring Commercial, Conair Corp., Stamford, CT) on high for 5 s. Ground peanut samples (25 g) were placed into a Whirl-Pak filter bag (24-oz; Nasco, Madison, WI) containing 75 mL of 0.1% phosphate buffered saline (PBS; BD Difco, Franklin Lakes, NJ). Ground samples were mechanically pummeled for 1 min at 235 rpm (Stomacher 400 Circulator, Seward Company, Islandia, NY) and then serially diluted in 0.1% PBS (BD Difco). Dilutions were plated, in duplicate, onto Petrifilm Enterobacteriaceae Count plates (EB; 3M, St. Paul, MN), Coliform Count Plates (3M), *Escherichia coli* Count Plates (3M), and Yeast and Mold Count Plates (3M). Enterobacteriaceae, coliform, and *E. coli* were enumerated after incubation at 35°C, 44°C, and 35°C, respectively, for 24 ± 2 h. Total yeast and mold colonies were counted after incubation at 25°C for 48-60 h. The lower and upper detection limits of the bacterial analyses were 1.3 to 5.0 log CFU/g, respectively, for Enterobacteriaceae, coliform, and *E. coli* count plates; lower and upper detection limits for yeast and mold count plates were 1.3 to 4.6 log CFU/g, respectively.

4.3.4 Statistical Analyses

All Enterobacteriaceae*,* coliform, and yeast and mold populations were expressed as average means for log CFU/g of ground peanuts under the assumption of a lognormal distribution for plate counts; *E. coli* was not detected in this study. All analyses were conducted using SAS 9.4 (Cary, NC, SAS Institute, Inc.). Linear regression (PROC REG) was used to assess the ability of reported storage methods and producer knowledge of microbial contamination to predict Enterobacteriaceae, coliform, and yeast and mold contamination levels. Analysis of variance (ANOVA) and Tukey's pairwise comparison test ($\alpha = 0.05$) was developed using PROC GLM and was used to investigate differences among the average *Enterobacteriaceae,* coliforms, and total yeast and mold population means for all storage methods and differences in producers' knowledge of bacteria and aflatoxins.

4.4 Results

4.4.1 Peanuts collected from Senegalese peanut basin were heavily contaminated with Enterobacteriaceae and other fecal indicators.

Of the 198 peanut samples analyzed for Enterobacteriaceae, 162 samples were within the detection range (1.3 to 5.0 log CFU/g). Approximately13% (26/198) of total samples resulted in greater than 5.0 log CFU/g of Enterobacteriaceae, while only 5% (10/198) of total samples fell below the detection limit (Table 4.2). Similarly, 165 samples were within the detection limit (1.3 to 5.0 log CFU/g) for total coliform populations; 13.6% of total samples (27/198) were greater than the detection limit, however, only 3% (6/198) were below 1.3 log CFU/g. All peanut samples were evaluated for total yeast and mold populations of which 21.2% (42/198) were above the detection limit (4.60 log CFU/g), while a single sample was below the detection limit (1.3 log CFU/g). Average log CFU/g for Enterobacteriaceae, coliforms, and yeast and mold populations are presented in Table 2; samples with populations above and below the detection limits were excluded from these calculations.

There were no significant differences ($P \geq 0.05$) in the concentrations of Enterobacteriaceae, coliforms, or total yeast and mold between samples stored with and without shells (Table 4.3). Producers also reported end uses for the peanuts to enumerators at sample collection. Most of the peanuts (61.1%; 121/198) were intended for household consumption or held as seeds for the next planting season (32.3%; 64/198). Only a single collected sample was intended for sale at local markets (12 producers did not offer an intended use for their remaining crop). Linear regression showed that intended use of the peanuts is a significant predictor of Enterobacteriaceae ($F = 11.71$, $P < 0.001$; $R^2 = 0.052$), coliform ($F = 6.52$, $P = 0.011$; $R^2 = 0.032$), and yeast and mold ($F = 6.64$, $P = 0.011$; $R^2 = 0.033$) contamination. Contamination levels for Enterobacteriaceae, coliforms, and yeast and molds were lower (-0.516, -0.364, and –0.238, respectively) in the samples kept for seed than those intended for household consumption. Additionally, there was a significant difference ($P < 0.05$) in Enterobacteriaceae, coliform, and total yeast and mold concentrations in peanuts intended for household consumption versus those kept for seed (Table 4.4).

Table 4.2. Summary of Enterobacteriaceae, coliform, and yeast and mold populations (log CFU/g ± SD) recovered from Senegalese peanut samples, including detection limits and total samples above and below these limits

	Enterobacteriaceae	**Coliforms**	**Yeast and Mold**
Average log CFU/g ± SD	3.42 ± 1.4	3.42 ± 1.3	3.72 ± 0.8
Detection Limit (log CFU/g)	1.30 to 5.0	1.30 to 5.0	1.30 to 4.6
Total samples below DL (< 1.3 log CFU/g)	10	6	1
Total samples above DL (> 5.0 log CFU/g)	26	27	42

[a-b] *Means without a common superscript letter differ (P < 0.05); =*
CFU: colony forming unit;
SD: standard deviation;
DL: detection limit.

Table 4.3. Adjusted means for Enterobacteriaceae, coliform, and yeast and mold populations (log CFU/g ± SD) recovered from shelled and in-shell peanut samples collected from the Senegal peanut basin

	Microbial Populations ± SD[1]		
	Enterobacteriaceae	**Coliforms**	**Yeast & Mold**
Stored in-shell	3.33 ± 1.2	3.28 ± 1.0	3.54 ± 0.8
Stored without shell	3.52 ± 1.3	3.51 ± 1.2	3.78 ± 0.7

[1] There were no significant pairwise comparisons (P ≥ 0.05);
CFU: colony forming unit;
SD: standard deviation.

Table 4.4. Adjusted means for Enterobacteriaceae, coliform, and yeast and mold populations (log CFU/g ± SD) recovered from Senegalese peanut samples intended for household consumption, seed, or sale.

	Microbial Populations ± SD		
	Enterobacteriaceae	**Coliforms**	**Yeast & Mold**
Household consumption	3.72 ± 1.2^{b}	3.68 ± 1.1^{b}	3.87 ± 0.8^{b}
Held for seed	2.98 ± 1.2^{a}	3.10 ± 1.2^{a}	3.45 ± 0.7^{a}
For sale to vendors[1]	3.89 ± 0.0^{ab}	3.88 ± 0.0^{ab}	4.60 ± 0.0^{ab}
No response	3.69 ± 1.2^{ab}	3.37 ± 1.3^{ab}	3.67 ± 0.7^{ab}

[a-b] Means without a common superscript letter differ (P < 0.05);
[1] Only one sample was reported as "for sale to vendors";
CFU: colony forming unit;
SD: standard deviation.

4.4.2 Previous knowledge of microbial contamination did not predict contamination levels of sampled peanuts.

Only 22.7% (45/198) of surveyed peanut producers reported they had "heard of bacteria (e.g. *E. coli, S. enterica,* coliforms)" prior to the survey. Follow-up questions were asked of those who responded "yes", to determine whether these respondents had knowledge of microbial contamination in foods and whether they took measures if measures were taken to prevent microbial contamination during production and storage. When asked if participants believed

bacteria were harmful to human health, four responded that they did not think bacteria were harmful. Of the 45 producers who reported that they had heard of bacteria, only 26 took measures to prevent bacterial contamination during production; most commonly (18/26 responses), producers indicated they prevented insects from infesting the crop. Several follow-up questions associated with preventive measures for bacterial contamination and possible contamination routes were included in the linear regression model, including whether sand or dust were on the consumed peanuts, whether peanuts were contaminated with fecal matter, and whether participants rinsed their peanuts before consumption. Taken together, knowledge of bacterial contamination and possible measures to prevent contamination were not indicative of Enterobacteriaceae ($F = 2.34$, $P = 0.074$, $R^2 = 0.216$) and coliform contamination ($F = 2.48$, $P = 0.063$, $R^2 = 0.226$) of peanuts. However, peanuts produced by those who took measures to prevent bacterial contamination had, on average, 0.32 log CFU/g more Enterobacteriaceae than those who did not take measures to prevent contamination. Coliform populations were 0.14 log CFU/g higher on peanuts produced by those who reported taking measures to prevent bacterial contamination ($P = 0.695$; Figure 4.1). Average Enterobacteriaceae and coliform populations recovered from peanuts produced by respondents who were aware of bacteria were not statistically significantly different ($P \geq 0.05$) from those who were not (Figure 4.1).

Prior knowledge of aflatoxins, possible preventive measures, and possible contamination sources were included in a linear regression model that was not predictive of yeast and mold contamination ($F = 2.05$, $P = 0.075$, $R^2 = 0.065$). Fecal contamination peanuts intended for household consumption was the only significant variable in the model ($P = 0.029$), however, for every unit increase in present fecal matter on consumed peanuts there was an expected 0.45 log CFU/g decrease in yeast and mold populations. Additionally, there were no significant differences in total yeast and mold counts across the peanut types, regardless of respondents' prior knowledge of aflatoxins ($P = 0.363$) or whether respondents took measures to prevent aflatoxin contamination ($P = 0.10$). Similarly, 18.7% (37/198) of respondents reported that they had heard of prior to the survey; 63.6% (126/198) of producers believed that aflatoxins were harmful to human health, while 24.2% reported that they did not know whether aflatoxins were harmful. However, 74 of 198 producers responded that they took measures to prevent aflatoxin (mold) contamination during the 2019 planting and harvest seasons. While not statistically significant, yeast and mold counts were 0.20 log CFU/g greater on peanuts grown by

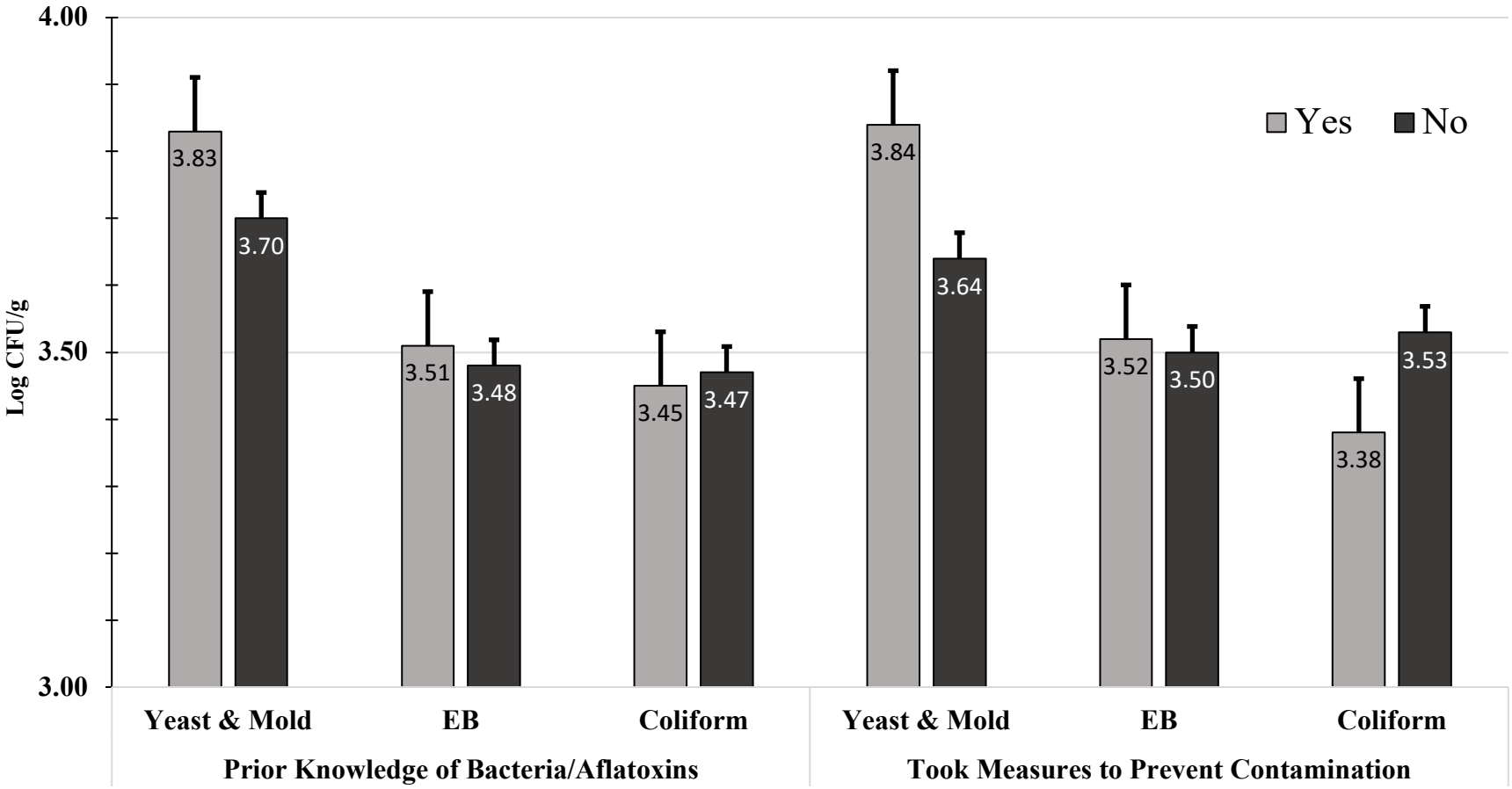


Figure 4.1. Microbial loads (log CFU/g) for yeast and mold, Enterobacteriaceae (EB), and coliforms recovered from peanuts collected from producers with and without prior knowledge of bacteria and aflatoxins, including those who reported taking measures to prevent bacterial and aflatoxin contamination.

producers who reported taking measures to prevent aflatoxin contamination than those who did not (Figure 1).

Beyond production practices, all producers were asked basic hygiene questions regarding use of utensils and hand washing (e.g. use of the utensils, washing utensils before or after use, and storage of the utensils). Of the 198 producers surveyed, 193 reported that they use their hands to consume foods and 131 reported they use a metal spoon to eat. However, only four households wash their kitchen utensils after eating. Additionally, when asked how often households wash their hands before eating, 60.1% (119/198) producers reported always washing their hands, while 26 producers (13.1%) stated that they never wash their hands before eating.

4.4.3 Current variations in storage practices do not impact microbial contamination of peanuts produced in the Senegalese peanut basin.

During sample collection, 40.4% (80/198) of enumerators reported seeing fecal matter near the storage site of the collected peanuts and 20.7% (41/198) of enumerators reported smelling feces in the surrounding area. Enumerators were not asked to differentiate feces sources. Regardless, neither presence of fecal matter nor fecal odor was predictive of Enterobacteriaceae ($F = 0.03$, $P = 0.854$, $R^2 = 0.00$; $F = 2.55$, $P = 0.112$, $R^2 = 0.01$; respectively) in the linear regression model. However, while presence of fecal matter was not a significant predictor of coliform contamination ($F = 0.01$, $P = 0.915$, $R^2 = 0.00$), fecal odor was found to be an adequate predictor of high coliform contamination ($F = 4.30$, $P = 0.039$, $R^2 = 0.022$). Additionally, the interaction between presence of fecal matter and reported fecal odor was not significant for Enterobacteriaceae nor coliform contamination ($P > 0.05$).

In Senegal, livestock and other animals are commonly allowed inside homes and around food storage areas. Livestock are also known reservoirs for pathogens such as *Escherichia coli* and *Salmonella* spp. (Doyle & Erickson, 2006). Enterobacteriaceae and coliforms are indicators for these pathogens, thus yeast and molds were not evaluated in respect to livestock presence or fecal contamination. More than half (56.1%; 111/198) of producers reported that animals were allowed inside their home and 66.7% (132/198) of enumerators observed animals near the storage sites where the peanut samples were collected. However, the reported presence of animals near the peanut storage sites were not predictive of Enterobacteriaceae ($F = 0.56$, $P = 0.455$, $R^2 = 0.003$) or coliform contamination ($F = 0.39$, $P = 0.534$, $R^2 = 0.002$). There were also no mean differences (P

≥ 0.05) in Enterobacteriaceae and coliform populations recovered from the peanut samples stored in the presence of livestock and other animals.

The storage conditions of the peanuts were also recorded by enumerators, including the type of storage container from which the peanut samples were collected and if the container was elevated or stored directly on dirt floors. When all observed storage conditions (six types of storage containers and three ways the peanuts were elevated) were considered in the model, the observed conditions were only slightly predictive of Enterobacteriaceae ($F = 1.96$, $P = 0.047$, $R^2 = 0.088$). The interaction between storage container and whether those containers were stored directly on dirt floors was significant for Enterobacteriaceae ($P = 0.0266$) and coliforms ($P = 0.0441$, but not for total yeast and mold ($P = 0.0595$). Means for Enterobacteriaceae and coliform interactions are presented in Table 4.5. Main effects of storage container type and container elevation for total yeast and mold are presented in Table 4.6 and 4.7 because the interaction was not significant. Samples were predominately collected from woven bags, consistent with data self-reported by peanut producers during the survey; 80.3% (159/198) of samples were from containers stored directly on dirt floors. These results indicate that type of storage container likely does not impact the microbial contamination level on peanuts produced in Senegal and that other productions practices are influencing contamination patterns.

4.5 Discussion

The current study provides initial analyses of Enterobacteriaceae and coliform contamination in peanuts produced by farmers in the Senegalese peanut basin. Enterobacteriaceae and coliforms are indicators of fecal material contamination and potentially pathogenic microorganisms that can lead to foodborne illness (Uçkun and Var, 2018). Total yeast and mold populations were also evaluated. Historically, bacteriological contamination in peanuts has been of minimal concern due to low water activity and minimal moisture content found in peanuts; Codex Alimentarius specifies that peanuts must be ≤ 9-10% moisture content prior to storing or milling (International Nut & Fruit Council, 2019). The International Commission on Microbiological Specifications for Foods states that the level of bacteriological contamination directly reflects the environment where the peanuts are grown and harvested (Nascimento et al., 2018). Thus, high levels of bacterial contamination are not surprising considering the reported storage methods utilized by Senegalese farmers. Additionally, to the best of our knowledge,

international microbiological standards are not available for raw peanuts. However, a technical report from the International Nut and Dried Fruit Council (INC) indicates that roasted peanut products are expected to have <10 CFU/g Enterobacteriaceae, <10/g MPN coliforms, and <100 CFU/g yeast and mold (*Peanut Technical Information*, 2019). The same report indicates that concentrations of E. coli in roasted peanuts should be < 3.6/g MPN *E. coli,* but free of all *Salmonella* spp. Heat lethality treatment of peanuts, including roasting and boiling, are intended to reduce or eliminate pathogens and other microorganisms before consumption. Roasted peanuts were not analyzed in the current study. Thus, we recommend that future studies evaluate the microbiological differences between raw and roasted peanuts produced in Senegal.

Enterobacteriaceae*,* coliform, and yeast and mold concentrations found in shelled peanuts in the current study ranged from 1.3 log CFU/g to 5.0 log CFU/g (Enterobacteriaceae and coliform) and 1.3 log CFU/g to 4.6 log CFU/g (yeast and mold), indicating a wide range of potential contamination. High microbial populations are indicators of poor hygiene practices and unsanitary conditions during production, harvest and/or storage of raw peanuts. However, such contamination characteristis not unique to peanuts from the Senegal peanut basin. In another study (Uçkun & Var, 2018), contamination levels in stored raw peanut collected from Turkish producers were at similar levels to those reported here (coliform concentrations ranged from < 3.0 MPN/g to > 1100.0 MPN/g for coliforms; yeast and mold concentrations ranged from not detectable to 5.3 log CFU/g and 3.7 log CFU/g, respectively) (Uçkun & Var, 2018). Additionally, Asiegbu et al. (2020) (Asiegbu et al., 2020) assessed contamination levels in boiled peanuts sold by street food vendors in Johannesburg, South Africa. Enterobacteriaceae were recovered from all five samples (average concentration of 3.71 log CFU/g). Total aerobic plate counts from boiled and roasted peanut samples were 4.16 log CFU/g and 2.57 log CFU/g, respectively (Asiegbu et al., 2020). These data suggest that microorganisms may not be fully destroyed through heat lethality treatments, product can be re-contaminated, and producers should follow appropriate Good Agricultural Practices to minimize the risk of contamination during production and storage.

In recent years, peanuts and peanut products have been associated with foodborne disease outbreaks, including a multistate outbreak of *S. enterica* ser. Typhimurium associated with peanut butter in the US in 2009 (Centers for Disease Control and Prevention (CDC), 2009). This outbreak highlighted the importance of Good Manufacturing Practices (GMPs) during peanut production, storage, and processing (Chang et al., 2013). While GMPs are commonly recognized as an

Table 4.5. Adjusted means for the interaction between type of storage container and the platform the containers were stored on for *Enterobacteriaceae* (EB) and coliform (CC) populations (log CFU/g) recovered from Senegalese peanut samples.

	Average Least Squares Means for Microbial Populations ± SD							
	Wooden Pallet		**Improved Floor**		**Directly on Dirt Floor**		**Other**	
Storage Container	**EB**	**CC**	**EB**	**CC**	**EB**	**CC**	**EB**	**CC**
Metal Drum	-	-	-	-	1.93 ± 0.2^{ab}	1.65 ± 0.5^{ay}	5.00 ± 0.0^{a}	5.00 ± 0.0^{az}
Plastic Drum	-	-	-	-	1.3 ± 0.0^{a}	1.60 ± 0.0^{ab}	-	-
Jerrycan	-	-	1.93 ± 0.6^{a}	2.44 ± 0.5^{a}	2.88 ± 1.5^{b}	2.90 ± 1.4^{b}	-	-
Woven Bag	3.47 ± 1.6^{a}	3.62 ± 1.3^{a}	3.33 ± 1.2^{b}	3.31 ± 1.1^{a}	3.64 ± 1.2^{c}	3.63 ± 1.1^{c}	4.06 ± 0.4^{a}	4.08 ± 0.6^{a}
Hermetic Bag	-	-	-	-	3.40 ± 1.2^{bc}	3.30 ± 1.1^{bc}	-	-
Plastic Bag	-	-	-	-	4.70 ± 0.0^{bc}	4.39 ± 0.0^{bc}	-	-
In Bulk	-	-	-	-	3.62 ± 1.3^{bc}	3.47 ± 1.2^{bc}	-	-

[a-c] Means in each column without a common superscript letter differ ($P < 0.05$).
[y-z] Least-squares means in each row without a common superscript letter differ ($P < 0.05$).
There were no data available for the treatment combinations with a hyphen (-).
EB: Enterobacteriaceae;
CC: coliforms;
CFU: colony forming units;
SD: standard deviation.

Table 4.6. Adjusted means for Enterobacteriaceae, coliform, and yeast and mold populations (log CFU/g ± SD) for the main effect of type of storage container pooled across how the containers were stored on or off the floor for Senegalese peanut samples.

Treatment	Microbial Populations ± SD[1]
	Yeast and Mold Counts
Metal Drum	3.48 ± 1.2
Plastic Drum	2.38 ± 0.0
Jerrycan	3.39 ± 0.8
Woven Bag	3.77 ± 0.8
Hermetic Bag	3.60 ± 0.6
Plastic Bag	3.62 ± 0.0
In Bulk	4.08 ± 0.6

[1] *There were no significant pairwise comparisons (P ≥ 0.05);*
CFU: colony forming units;
SD: standard deviation.

Table 4.7. Adjusted Means for total yeast and mold counts (log CFU/g ± SD) for the main effect of elevation of sampling containers pooled across storage container type for Senegalese peanut samples.

Type of Elevation on or off Floor	Microbial Populations ± SD[1]
	Yeast and Mold Counts
Wooden Pallet	3.78 ± 0.6
Improved Floor	3.78 ± 0.8
Directly on Dirt Floor	3.69 ± 0.8
Other	4.29 ± 0.6

[1] *There were no significant pairwise comparisons (P ≥ 0.05);*
CFU: colony forming units;
SD: standard deviation.

important intervention protocol in developed economies, strategies to minimize risk of pathogen contamination are not commonly practiced in developing economies. The authors of a Brazilian study (Nascimento et al., 2018) detected *Escherichia coli* and *S. enterica* in seven (1.7%) and nine (2.2%) samples, respectively, collected post-harvest during secondary processing. Additionally, *E. coli* and *S. enterica* were recovered from retail samples (Nascimento et al., 2018). Another study reported 20% (1/5) *S. enterica* prevalence, 40% (2/5) *Staphylococcus aureus* prevalence, and 100% (5/5) prevalence for *Listeria monocytogenes* on boiled peanuts collected from street food

vendors (Asiegbu et al., 2020). *E. coli* was not recovered from any samples in the current study and *Salmonella* spp. prevalence was not determined. Thus, further studies are warranted to determine presence of pathogenic microorganisms in raw and ready-to-eat peanut products in Senegal.

Additionally, yeast and mold populations can indicate potential aflatoxin contamination in raw peanuts. Aflatoxins are produced by the fungi *Aspergillus* section *Flavi* and pose serious risks to human health (Senghor et al., 2020). Significant efforts have been made to determine appropriate GAPs and storage methods that could minimize risk of mold growth and aflatoxin production in developing economies, including use of hermetic storage bags (Martey et al., 2020; Torres et al., 2014; Tubbs et al., 2016). In this study, yeast and mold populations recovered from the peanut samples were on average 0.20 log CFU/g greater on samples from producers who reported taking measures to prevent yeast and mold contamination. Due to the implementation of control measures for yeast and mold during storage, it was expected that contamination would be lower in such peanuts than those of peanuts collected producers reported not taking aflatoxin preventative measures. While 0.20 log CFU/g was not considered biologically significant, this difference does indicate that measures taken by respondents to minimize the risk of yeast and mold contamination were ineffective. Additionally, the high levels of total yeast and mold contamination observed here may also result in substantially reduced shelf-life of the peanuts (Martín et al., 2018). However, high levels of bacteria and yeast and mold may be expected due to timing of collection of samples (March 2020) in relation to the fall harvest of peanuts (October – December 2019).

The development of global microbiological standards to reduce the human health burden associated with foodborne disease highlights the need for building local knowledge of common production practices to improve small producer's access to international markets (Perez-Aleman, 2012). In the current study, less than a quarter of producers surveyed who had heard of bacteria or aflatoxins, fully understood how their crop could become contaminated or appropriate preventive measures they could take to prevent contamination. This was further emphasized by the storage conditions of the collected peanut samples as the majority of samples tested were collected from woven bags stored directly on dirt floors. These results are consistent with previous studies (Abdullahi et al., 2016; Asiegbu et al., 2020; Azeze & Haji, 2017; Mkhungo et al., 2018; Perez-Aleman, 2012) on knowledge and understanding of foodborne illness transmission and

proper food handling in developing economies and reiterates the importance of improving producer food safety knowledge.

4.6 Conclusions

In this study, a survey was developed to identify current production and storage methods utilized by Senegalese peanut producers and to better understand producers' current knowledge of microbiological contamination. Producers were randomly selected to participate in the survey and subsequent peanut sample collection; the survey data was used to predict potential Enterobacteriaceae, coliform, and yeast and mold contamination of Senegalese peanuts. The results of this study indicate that peanuts produced in the Senegalese peanut basin are heavily contaminated by all three categories of microorganisms tested. Storage methods used producers did not impact microbial contamination rates of peanuts produced in Senegal. The survey also indicated that producers utilized similar production methods regardless of their understanding of microbial contamination, suggesting there would be no difference in contamination based on prior knowledge of bacteria or aflatoxins. Further studies are needed to determine the most useful combination of storage methods to minimize microbial contamination. Additionally, producers' self-reported prior knowledge and understanding of bacterial and mold contamination had little to no effect on observed contamination of peanuts. Greater producer understanding of microbiological contamination routes could contribute to better GAPs and ultimately improved human health.

Prevention of contamination with molds, aflatoxins, and bacteria in peanuts is necessary to continuing improving human health in Senegal. While the present study provides limited insight into the microbiological status of peanuts after long-term storage under current production practices, future studies are necessary to further evaluate prevalence of pathogenic microorganisms in peanuts and consequent effects on human health in Senegal. Additionally, studies should be conducted to determine which GAPs and GMPs can be integrated seamlessly into current Senegalese peanut production systems to minimize risk of bacteriological contamination.

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APPENDIX A. LIST OF *LISTERIA MONOCYTOGENES* ISOLATES

Isolate name	Sequence Type*	Clonal Complex	Lineage	Store Number	Sampling Site
PUL-B2-005	ST5	C5	I	30	Squeegee Head
PUL-B2-089	ST5	C5	I	9	Squeegee Head
PUL-B2-133	ST5	C5	I	24	Squeegee Head
PUL-B2-137	ST5	C5	I	24	Standing water
PUL-B2-166	ST5	C5	I	24	Deli Area Drain
PUL-B2-185	ST5	C5	I	14	Standing water
PUL-B2-189	ST5	C5	I	41	Squeegee Head
PUL-B2-193	ST5	C5	I	14	Floor/Wall Juncture
PUL-B2-197	ST5	C5	I	21	Standing water
PUL-B2-201	ST5	C5	I	21	1-Basin Sink Interior
PUL-B2-241	ST5	C5	I	24	Squeegee Head
PUL-B2-285	ST5	C5	I	14	Cold Room Drain
PUL-B2-289	ST5	C5	I	41	Squeegee Head
PUL-B2-293	ST5	C5	I	41	Deli Area Drain
PUL-B2-297	ST5	C5	I	21	Squeegee Head
PUL-B2-301	ST5	C5	I	14	Standing water
PUL-B2-329	ST6	C6	I	20	Deli Area Drain
PUL-B2-333	ST6	C6	I	20	Floor/Wall Juncture
PUL-B2-337	ST6	C6	I	20	Squeegee Head
PUL-B2-341	ST6	C6	I	33	Standing water
PUL-B2-377	ST736	C736	I	19	Cold Room Drain
PUL-B2-397	ST5	C5	I	42	1-Basin Sink Interior
PUL-B2-401	ST5	C5	I	6	Deli Area Drain
PUL-B2-425	ST5	C5	I	42	1-Basin Sink Interior
PUL-B2-433	ST5	C5	I	30	Squeegee Head
PUL-B2-449	.	.	III	17	Cold Room Drain
PUL-B2-453	ST5	C5	I	17	Standing water
PUL-B2-469	ST5	C5	I	3	Deli Area Drain
PUL-B2-473	ST5	C5	I	3	Standing water
PUL-B2-477	ST5	C5	I	3	Floor/Wall Juncture
PUL-B2-481	ST5	C5	I	23	Deli Area Drain
PUL-B2-485	ST5	C5	I	23	Standing water
PUL-B2-493	ST85	C7	II	13	Wheeled Cart
PUL-B2-501	ST85	C7	II	27	Cold Room Racks
PUL-B2-521	ST6	C6	I	20	Standing water
PUL-B2-537	ST85	C7	II	31	Cold Room Racks
PUL-B2-561	.	.	III	29	Cold Room Drain

APPENDIX B. SURVEY INFORMATION, SELF-REPORTED AND OBSERVED BY ENUMERATORS, COLLECTED FROM PRODUCERS AND UTILIZED IN THIS STUDY.

	Survey Information Collected from Producers[1]							
House[2] Number	Intended Use	In-shell or no shell[3]	Heard of Aflatoxins	Take Measure to prevent aflatoxins	Heard of Bacteria	Took measures to prevent bacteria	Storage Container	Platform to Elevate4
1	HC	No Shell	Yes	Yes	No	.	Woven Bag	Other
2	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
3	Seed	In-shell	No	Yes	No	.	Jerry Can	Dirt floor
4	HC	No Shell	No	No	No	.	Woven Bag	Improved Floor
5	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
6	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
7	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
8	Seed	In-shell	No	.	No	.	Woven Bag	Dirt floor
9	Seed	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
10	HC	In-shell	No	Yes	No	.	Woven Bag	Improved Floor
11	HC	In-shell	No	Yes	No	.	Plastic Drum	Dirt floor
12	HC	In-shell	No	No	Yes	No	Woven Bag	Dirt floor
13	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
14	Seed	In-shell	No	No	No	.	Jerry Can	Dirt floor
15	HC	In-shell	No	Yes	No	.	Jerry Can	Improved Floor
16	HC	No Shell	Yes	Yes	No	.	Hermetic Bag	Dirt floor
17	Seed	In-shell	No	No	No	.	.	.
18	Seed	In-shell	No	No	Yes	No	Metal Drum	Dirt floor
19	.	No Shell	No	Yes	Yes	Yes	Hermetic Bag	Dirt floor
20	Seed	In-shell	No	No	Yes	No	Woven Bag	Dirt floor
21	.	No Shell	No	.	No	.	Woven Bag	Dirt floor
22	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
23	HC	No Shell	No	.	No	.	Jerry Can	Dirt floor
24	HC	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
25	HC	In-shell	No	.	No	.	Woven Bag	Improved Floor

26	.	In-shell	No	No	No	.	Woven Bag	Dirt floor
27	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
28	HC	In-shell	No	No	Yes	No	Woven Bag	Dirt floor
29	HC	In-shell	No	Yes	Yes	No	Woven Bag	Dirt floor
30	HC	In-shell	No	No	No	.	Jerry Can	Improved Floor
31	.	In-shell	No	.	No	.	Woven Bag	Dirt floor
32	HC	In-shell	No	.	No	.	Woven Bag	Dirt floor
33	HC	In-shell	Yes	No	No	.	Jerry Can	Dirt floor
34	Seed	No Shell	No	Yes	Yes	Yes	Woven Bag	Dirt floor
35	Seed	In-shell	Yes	No	Yes	No	Woven Bag	Dirt floor
36	HC	In-shell	No	No	Yes	No	.	.
37	HC	In-shell	No	.	No	.	Woven Bag	Dirt floor
38	Seed	In-shell	No	Yes	No	.	Woven Bag	Improved Floor
39	HC	In-shell	No	Yes	No	.	Woven Bag	Wood Pallet
40	.	In-shell	No	No	Yes	Yes	Woven Bag	Dirt floor
41	HC	In-shell	No	No	Yes	Yes	Woven Bag	Dirt floor
42	Seed	In-shell	Yes	Yes	Yes	Yes	Hermetic Bag	Dirt floor
43	HC	In-shell	No	Yes	Yes	No	Jerry Can	Dirt floor
44	Seed	In-shell	No	.	No	.	Woven Bag	Dirt floor
45	Seed	In-shell	Yes	Yes	Yes	Yes	Hermetic Bag	Dirt floor
46	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
47	Seed	No Shell	Yes	No	Yes	No	Woven Bag	Dirt floor
48	HC	No Shell	Yes	Yes	Yes	No	Hermetic Bag	Dirt floor
49	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
50	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
51	HC	In-shell	No	Yes	No	.	Woven Bag	Improved Floor
52	HC	In-shell	No	No	No	.	Woven Bag	Improved Floor
53	HC	In-shell	No	Yes	Yes	Yes	In bulk	Dirt floor
54	HC	In-shell	No	Yes	Yes	Yes	In bulk	Dirt floor
55	HC	In-shell	Yes	Yes	Yes	Yes	Woven Bag	Dirt floor
56	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
57	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
58	Seed	In-shell	No	.	Yes	No	Woven Bag	Dirt floor

59	HC	No Shell	No	Yes	No	.	Woven Bag	Dirt floor
60	Seed	No Shell	Yes	Yes	No	.	Woven Bag	Dirt floor
61	HC	In-shell	Yes	Yes	No	.	Woven Bag	Dirt floor
62	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
63	HC	No Shell	No	No	No	.	Jerry Can	Dirt floor
64	HC	In-shell	No	.	No	.	Woven Bag	Improved Floor
65	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
66	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
67	HC	In-shell	No	.	No	.	Woven Bag	Dirt floor
68	HC	In-shell	Yes	Yes	No	.	Woven Bag	Dirt floor
69	Seed	In-shell	No	Yes	Yes	Yes	.	.
70	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
71	Seed	In-shell	No	Yes	Yes	Yes	Metal Drum	Dirt floor
72	HC	In-shell	No	No	No	.	Woven Bag	Other
73	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
74	Seed	In-shell	No	.	No	.	Woven Bag	Dirt floor
75	.	In-shell	No	No	No	.	Woven Bag	Dirt floor
76	Seed	In-shell	No	Yes	Yes	Yes	.	.
77	Seed	In-shell	No	No	No	.	Woven Bag	Wood Pallet
78	HC	In-shell	Yes	Yes	No	.	In bulk	Dirt floor
79	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
80	Seed	In-shell	No	No	No	.	Woven Bag	Dirt floor
81	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
82	HC	In-shell	No	Yes	Yes	Yes	Woven Bag	Wood Pallet
83	Seed	No Shell	No	.	No	.	Plastic Bag	Dirt floor
84	HC	In-shell	No	.	No	.	Woven Bag	Dirt floor
85	Seed	In-shell	No	No	No	.	Jerry Can	Dirt floor
86	Seed	No Shell	No	.	No	.	Woven Bag	Dirt floor
87	HC	No Shell	Yes	No	Yes	Yes	Hermetic Bag	Dirt floor
88	HC	In-shell	No	No	No	.	Jerry Can	Dirt floor
89	Seed	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
90	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
91	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor

92	HC	In-shell	No	.	No	.	Woven Bag	Dirt floor
93	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
94	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
95	Seed	No Shell	No	.	No	.	Woven Bag	Improved Floor
96	Seed	In-shell	Yes	No	No	.	Hermetic Bag	Dirt floor
97	HC	In-shell	No	Yes	Yes	No	Woven Bag	Dirt floor
98	HC	In-shell	No	No	Yes	Yes	Woven Bag	Wood Pallet
99	HC	In-shell	No	No	No	.	Jerry Can	Dirt floor
100	Seed	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
101	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
102	HC	In-shell	No	.	No	.	In bulk	Dirt floor
103	HC	In-shell	Yes	No	Yes	Yes	Woven Bag	Improved Floor
104	HC	No Shell	No	Yes	No	.	Woven Bag	Dirt floor
105	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
106	Seed	In-shell	Yes	Yes	.	.	Woven Bag	Dirt floor
107	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
108	HC	In-shell	No	.	No	.	Woven Bag	Improved Floor
109	Seed	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
110	HC	In-shell	No	.	No	.	In bulk	Dirt floor
111	Seed	In-shell	No	.	Yes	No	In bulk	Dirt floor
112	HC	No Shell	No	.	No	.	Woven Bag	Dirt floor
113	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
114	HC	In-shell	No	No	No	.	Jerry Can	Improved Floor
115	HC	In-shell	No	No	Yes	No	Woven Bag	Dirt floor
116	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
117	Seed	No Shell	No	Yes	Yes	Yes	Hermetic Bag	Dirt floor
118	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
119	Seed	In-shell	No	Yes	No	.	Jerry Can	Dirt floor
120	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
121	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
122	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
123	HC	No Shell	No	Yes	No	.	Woven Bag	Dirt floor
124	Seed	In-shell	Yes	No	Yes	Yes	Woven Bag	Dirt floor

125	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
126	Seed	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
127	HC	No Shell	No	Yes	No	.	Woven Bag	Dirt floor
128	Seed	In-shell	No	No	No	.	Hermetic Bag	Dirt floor
129	.	In-shell	Yes	Yes	Yes	Yes	Hermetic Bag	Dirt floor
130	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
131	HC	No Shell	Yes	Yes	No	.	Woven Bag	Dirt floor
132	Seed	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
133	HC	In-shell	No	No	Yes	Yes	Woven Bag	Dirt floor
134	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
135	HC	No Shell	No	No	No	.	Woven Bag	Wood Pallet
136	.	In-shell	No	No	No	.	.	.
137	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
138	Seed	In-shell	No	No	No	.	Woven Bag	Dirt floor
139	HC	No Shell	Yes	Yes	Yes	Yes	Hermetic Bag	Dirt floor
140	HC	No Shell	No	.	No	.	Woven Bag	Improved Floor
141	Seed	In-shell	No	No	No	.	Woven Bag	Improved Floor
142	HC	In-shell	No	.	No	.	Woven Bag	Improved Floor
143	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
144	Seed	In-shell	No	Yes	Yes	Yes	Hermetic Bag	Dirt floor
145	Seed	No Shell	No	No	No	.	Woven Bag	Dirt floor
146	HC	In-shell	No	No	No	.	Woven Bag	Improved Floor
147	HC	In-shell	Yes	No	No	.	Jerry Can	Dirt floor
148	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
149	Seed	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
150	Seed	No Shell	Yes	No	No	.	Woven Bag	Dirt floor
151	.	In-shell	No	No	No	.	Jerry Can	Dirt floor
152	.	In-shell	No	No	No	.	Jerry Can	Dirt floor
153	HC	In-shell	No	No	Yes	Yes	Woven Bag	Dirt floor
154	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
155	.	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
156	HC	In-shell	Yes	Yes	No	.	Hermetic Bag	Dirt floor
157	HC	In-shell	No	Yes	No	.	Woven Bag	Improved Floor

158	Seed	In-shell	Yes	Yes	Yes	No	Metal Drum	Other
159	Seed	No Shell	No	No	No	.	Woven Bag	Dirt floor
160	Seed	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
161	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
162	Seed	In-shell	No	.	Yes	No	Woven Bag	Improved Floor
163	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
164	Seed	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
165	Seed	In-shell	No	.	No	.	Woven Bag	Dirt floor
166	Seed	In-shell	No	.	No	.	Woven Bag	Dirt floor
167	Seed	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
168	Seed	In-shell	No	.	No	.	Woven Bag	Improved Floor
169	HC	In-shell	No	Yes	No	.	Woven Bag	Dirt floor
170	Seed	In-shell	No	Yes	No	.	Jerry Can	Dirt floor
171	HC	In-shell	No	No	No	.	Jerry Can	Dirt floor
172	Seed	No Shell	Yes	No	No	.	Woven Bag	Dirt floor
173	Sale	In-shell	No	No	No	.	Woven Bag	Dirt floor
174	HC	In-shell	No	No	Yes	Yes	Woven Bag	Dirt floor
175	Seed	In-shell	No	No	No	.	Jerry Can	Dirt floor
176	HC	In-shell	No	No	No	.	Woven Bag	Improved Floor
177	Seed	In-shell	No	No	Yes	No	Woven Bag	Dirt floor
178	HC	In-shell	No	No	Yes	No	Woven Bag	Dirt floor
179	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
180	HC	In-shell	Yes	No	No	.	Hermetic Bag	Dirt floor
181	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
182	HC	In-shell	No	No	No	.	Woven Bag	Dirt floor
183	HC	In-shell	Yes	No	No	.	Woven Bag	Dirt floor
184	Seed	In-shell	No	Yes	No	.	Hermetic Bag	Dirt floor
185	Seed	In-shell	No	Yes	Yes	No	Woven Bag	Dirt floor
186	Seed	In-shell	No	No	No	.	Woven Bag	Wood Pallet
187	HC	In-shell	No	.	No	.	Woven Bag	Dirt floor
188	.	In-shell	Yes	Yes	No	.	Woven Bag	Dirt floor
189	HC	No Shell	Yes	Yes	No	.	Woven Bag	Dirt floor
190	HC	In-shell	No	No	Yes	No	.	.

191	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
192	HC	No Shell	Yes	Yes	Yes	Yes	Hermetic Bag	Dirt floor
193	Seed	In-shell	No	.	No	.	Woven Bag	Improved Floor
194	Seed	In-shell	No	.	No	.	Woven Bag	Dirt floor
195	HC	No Shell	No	No	No	.	Woven Bag	Dirt floor
196	HC	In-shell	No	Yes	No	.	Woven Bag	Other
197	Seed	In-shell	No	Yes	No	.	Woven Bag	Improved Floor
198	Seed	In-shell	No		No	.	Woven Bag	Dirt floor

[1] Observational and self-reported data collected from the survey of Senegalese peanut producers;
[2] Randomized house number for surveyed peanut producers;
[3] Peanuts were stored in-shell or without a shell;
[4] Type of container the peanuts were stored in;
[5] Type of platform the container holding the peanuts were stored on;
HC: intended for household consumption;
Seed: intended to be utilized as seed the next year;
".": a missing data point.

CURRICULUM VITAE

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EDUCATION:

Purdue University December 2021
Doctor of Philosophy, Food Science – Food Safety West Lafayette, IN
Major Advisor: Haley F. Oliver, Ph.D.
Current GPA: 3.97

Colorado State University August 2018
Master of Science, Animal Science – Meat Science Fort Collins, Colorado
Thesis Title: *Effects of Antimicrobial Interventions on Food Safety and An Assessment of the Colorado Pork Supply*
Major Advisors: Keith Belk, Ph.D. and Dale Woerner, Ph.D.
GPA: 3.85
Bachelor of Science, Animal Science December 2015
Current GPA: 3.79*, Cum Laude*

Redlands Community College May 2013
Associates of Science, Animal Science El Reno, OK
GPA: 3.90, *President's Academic Achievement Award*

PROFESSIONAL EXPERIENCE:

Purdue University West Lafayette, IN
Graduate Research Assistant *August 2018-Present*

- Lead research projects testing environmental samples and microbial contamination of various food products
    - Managed and conducted research to develop a predictive risk model for *Listeria monocytogenes* in retail delicatessen departments.
    - Collaborated with Ag. Economics dept. to conduct an analysis of microbial contamination on peanuts and developed a training for capacity building projects in Senegal, Africa.
    - Assist with research related to sanitation protocols, characterizing bacterial populations persistent in food production environments, and in conducting surveys of food processors.
- Mentored undergraduate and helped train and develop their technical research skills
- Lead educational workshops and lectures related to meat safety and food microbiology

Colorado State University Fort Collins, CO
Graduate Research Assistant *August 2016-August 2018*

- Conduct research in the area of meat quality and meat microbiology
    - Managed multiple projects evaluating antimicrobial interventions against pathogens such as *Escherichia coli, Salmonella* spp., and *Listeria monocytogenes.*

- Led a large shelf-life study of ground beef treated with various antimicrobial interventions, with and without antioxidant addition, in modified atmosphere packaging.
- Assisted with other studies including in-plant validations, trained and consumer sensory panels, food microbiology, shelf-life, and nutritive analysis projects.
- Certified in HAACP and to work in a Biosafety Level II Laboratory

Boot Barn, INC. Fort Collins, CO
Key Holder *August 2015-July 2016*

- Managed a team of sales associates and provided assistance with technical duties on a daily basis
- Applied critical thinking and problem-solving skills to provide a positive experience for all
- Utilized clear communication and motivational tactics to encourage our team to achieve goals

Magnum Feedyard, LLC Wiggins, CO
Feedyard Operations Summer Intern *May-August 2015*

- Received guidance on and assisted with all daily production activities
- Further developed analytical thinking skills to quickly make decisions and solve problems
- Gained a working knowledge of cattle feeding and its importance to the meat industry

Colorado Pork Producers Council Denver, CO
Executive Summer Intern *May-August 2014*

- Assisted the CPPC executive director with preparation of all summer meetings and presentations
- Developed an understanding of international pork markets while at USMEF
- Gained valuable communication skills with language barriers present during assignment to a commercial swine facility

RESEARCH EXPERIENCE

Refining a *Listeria monocytogenes* Predictive Risk Tool for Retail Deli Departments
August 2019 – Present

- Validate the performance of a recently developed predictive tool based on mathematic modeling for assessing *Listeria monocytogenes* contamination risk at retail.

Evaluating Microbiological Contamination of Senegalese Groundnuts
October 2019 – June 2020

- Evaluate the presence of *Enterobacteriaceae*, coliforms, and yeast and molds on groundnuts from producers and those sold at market in Senegal, Africa.

Nisin inhibition of *Listeria monocytogenes* growth in a smoked whitefish salad blend
January 2019

- Evaluated the efficacy of nisin to inhibit the growth of *Listeria monocytogenes* inoculated in a smoked whitefish salad blend over a 42-day shelf-life period.

Assessment of the Pork Supply in Colorado
July 2018

- Identified the processors in Colorado who harvest pigs and characterized the size and scope of these operations, as well as established a ranked set of desired traits for producers selling live animals to processors.
- Determined opportunities for the Colorado pork industry to improve quality and relationships between producers, processors, and consumers of pork products.

Antimicrobial effects of chemical solutions, alone or in combination with the surfactant, Disponil DB, against inoculated bacterial populations on beef carcass surface tissue.

May 2018

- Evaluated the antimicrobial efficacy of combining an alkyl polyglycoside surfactant with peroxyacetic acid (PAA), a sulfuric acid and sodium sulfate blend (SSS), and PAA acidified with SSS, against inoculated nonpathogenic *Escherichia coli* biotype I on prerigor beef carcass surface tissue samples.
- Spray treated the beef tissue samples to simulate a carcass wash intervention system to determine the efficacy against *E. coli.*

Antimicrobial effects of peroxyacetic acid acidified with acetic acid or a sulfuric acid and sodium sulfate blend when applied to inoculated prerigor beef surface tissue.
October 2017

- Evaluated the efficacy of acidifying peroxyacetic acid with acetic acid or a sulfuric acid and sodium sulfate blend against nonpathogenic *Escherichia coli* biotype I on inoculated prerigor beef carcass surface tissue samples compared to peroxyacetic acid alone for use as an antimicrobial intervention system.

Evaluation of the addition of different organic acids to acidify peroxyacetic acid
August 2017

- Evaluated the efficacy of peroxyacetic acid acidified with lactic acid, acetic acid, citric acid, and a sulfuric acid and sodium sulfate blend against nonpathogenic *Escherichia coli* biotype I on inoculated prerigor beef carcass surface tissue samples immediately after treatment.

Antimicrobial efficacy of the addition of surfactant to a sulfuric acid and sodium sulfate blend against *Listeria monocytogenes* inoculated on whole cantaloupes
June 2017

- Evaluated the efficacy of a sulfuric acid and sodium sulfate blend, with and without the addition of surfactant, against *L. monocytogenes* inoculated on whole cantaloupes immediately after treatment and after 24 h of aerobic storage at 4ºC.

Effect of surfactant addition on the antimicrobial effects of a sulfuric acid and sodium sulfate blend on beef tissue and chicken parts
May 2017

- Evaluated the efficacy of adding a surfactant to a sulfuric acid and sodium sulfate blend (SSS), peroxyacetic acid (PAA), and PAA acidified with SSS for use against *E. coli* inoculated on beef trimmings and *Salmonella* inoculated on chicken wings immediately after treatment.

Determination of shelf life properties of ground beef treated with various antimicrobials, with and without antioxidant addition, and packaged in modified atmosphere packaging in dark storage and retail display settings
October 2016 to December 2016

- Ground 80% lean beef trimmings, applied four different antimicrobial treatments, with and without antioxidant addition, and packaged in plastic overwrap, then placed into modified atmospheric packaging.
- Evaluated shelf life properties utilizing trained color panelist evaluation, objective color spectrophotometer color evaluation, and microbiological analysis to determine color and microbiological changes during dark storage and retail display.

Antimicrobial efficacy of peroxyacetic acid, a sulfuric acid and sodium sulfate blend, and peroxyacetic acid acidified with a sulfuric acid and sodium sulfate blend against nonpathogenic *Escherichia coli* biotype I inoculated on beef head meat and trimmings
October 2016

- Evaluated the efficacy of peroxyacetic acid (PAA), sulfuric acid and sodium sulfate blend (SSS), and PAA acidified with SSS against nonpathogenic *Escherichia coli* biotype I inoculated on beef head meat immediately after treatment.
- Evaluated the efficacy of PAA, SSS, and PAA acidified with SSS against nonpathogenic *E. coli* biotype I inoculated on 50% lean beef trimmings immediately after treatment, 24 h post-treatment, and after grinding (with and without the addition of surfactant).

Assisted with multiple Colorado State University Center for Meat Safety & Quality research projects with other master and PhD students
August 2016 to July 2018

- Including: In-plant food safety validations, trained sensory panelist – flavor, odor, and color evaluation, NCBA National Beef Quality Audit – In-plant audits, In-plant product collections, Warner-Bratzler shear for operator, and more.

EXTENSION EXPERIENCE

Microbiological techniques workshop for food industry food safety and quality personnel
January 2020 to March 2020

- Hands-on microbiological techniques workshop for a food company, including common foodborne pathogens and methods for identification of pathogens in food products.
- Training goals were to ensure food safety and quality personnel were proficient in microbiological techniques (i.e. aseptic technique, gram staining, media prep, etc) and that personnel understand common microbial contamination of food products.

- Included 12 training participants and 3 food safety and quality supervisors.

Sample collection and aflatoxin testing training for Senegalese enumerators and researchers at the Institut Senegalais de Recherches Agricoles (ISRA; Senegalese Institute for Agricultural Research)
August 2019 to May 2020

- Develop and implement training protocols for in-country sample collection and aflatoxin testing. Training goals were to prepare enumerators and laboratory technicians to complete sample collection and aflatoxin testing aseptically to prevent further contamination of samples and minimize sampling bias.
- Due to Covid-19, training was conducted via the Webex platform with an interpreter and informative handouts were translated and utilized during collection in the field.
- Included approximately 10-15 survey enumerators collecting samples, 4 laboratory technicians conducting aflatoxin testing, and 2 supervisors at ISRA to oversee

Improving pork quality and processing for small and very small meat processors in Colorado
August 2017 to July 2018

- Understand meat quality and production practices associated with small and very small processing facilities in Colorado. Establish a ranked set of priorities for pigs produced in the state.
- Identify opportunities for improvement of pork quality and production, discuss these opportunities with processors, and work closely with Colorado Pork Producers Council to improve relationships with producers, processors, and consumers.
- Included 41 survey respondents (information used to understand pork processing in Colorado), 10 processors were visited, and the Colorado Pork Producers Council Executive Director.

EXTRAMURALLY FUNDED RESEARCH GRANTS

1. **Agency:** Foundation for Meat and Poultry Research and Education
Title: Effect of antimicrobial treatment of beef cheek meat and tongues on pathogen survival/death and product flavor volatiles during refrigerated and frozen storage.
Duration of Funding: September 2017 – September 2018
Role: Co-Investigator
Responsible for: $74,972

2. **Agency:** USDA Agriculture and Food Research Initiative – PreDoctoral Fellowship
Title: Characterization of Microbial Communities Among *Listeria monocytogenes* in Retail Environments
Duration of Funding: July 2020 – July 2022
Role: Project Director
Responsible for: $120,000

PUBLICATIONS

Peer-Reviewed Publications

B.C. Britton, I. Geornaras, J.O. Reagan, S. Mixon, D.R. Woerner, and K.E. Belk. 2020. Antimicrobial efficacy of acidified peroxyacetic acid treatments against surrogates for enteric pathogens on prerigor beef. Meat and Muscle Biology. doi:10.22175/mmb.10992.

Weinroth, M.D., **B.C. Britton**, and K.E. Belk. 2018. Genetics and microbiology of meat. Meat Science. 144:15-21. doi:10.1016/j.meatsci.2018.04.017.

Weinroth, M.D., **B.C. Britton**, K.R. McCullough, J.N. Martin, I. Geornaras, R. Knight, K.E. Belk, and J.L. Metcalf. 2019. Ground beef microbiome changes with antimicrobial decontamination interventions and product storage. PloS one. E0217947. https://doi.org/10.1371/journal.pone.0217947

A. Sundarram[+], **B.C. Britton[+],** J. Liu, K. Desiree, R. Ogas, P. Lemaster, B. Navarrete, H. Nowakowski, M.K. Harrod, D. Marks, P.D. Ebner, and H.F. Oliver. 2021. Lytic capacity of commercial *Listeria* phage against *Listeria* spp. with varied genotypic and phenotypic characteristics. Foodborne Pathogens and Disease. https://doi.org/10.1089/fpd.2020.2897

[+]*Authors contributed equally*

B.C. Britton, I. Sarr, and H.F. Oliver. 2021. Enterobacteriaceae, coliform, yeast, and mold contamination patterns in peanuts compared to production, storage, use practices, and knowledge of food safety among growers in Senegal. *Inter. J. Food Microbiol.* https://doi.org/10.1016/j.ijfoodmicro.2021.109437

B.C. Britton, K.T. Cook, S.T. Wu, J. Burnett, R.C. Waller, H.C den Bakker, and H.F. Oliver. *Listeria monocytogenes* prevalence in retail delicatessen environments decreased during the first year of the COVID-19 pandemic. *Submitted. Food Control. October 2021.*

Publications in Preparation for Submission

B.C. Britton, K.T. Cook, S.T. Wu, J. Burnett, H.F. Oliver, and H.C den Bakker. Characterization of retail delicatessen drains and biofilms using 16s rRNA metataxonomic and shotgun metagenomic sequencing. *In preparation for submission to Appl. Environ. Microbiol. December 2021.*

Thesis

Britton, B.C. 2018. M.S. Thesis publication. Effects of Antimicrobial Interventions on Food Safety and An Assessment of the Colorado Pork Supply. Colorado State University, Fort Collins.

TEACHING EXPERIENCE

Teaching Assistant Experience

Graduate Student Teaching Assistant, Purdue University Food Science
2019

- Assist professor with laboratory duties (i.e. media preparation, cleaning, etc.).

- Teach undergraduate students basic food microbiology laboratory techniques.
- Aid students with office hours and additional course explanation.
- Proctor exams.
- Assist in teaching the following courses:
  - FS 363 – Food Microbiology Laboratory
  - FS 361 – Food Plant Sanitation

Graduate Student Teaching Assistant, Colorado State University Animal Sciences
2016 to 2018

- Assist professor with course duties.
- Grade examinations and coursework.
- Assist undergraduates with understanding livestock evaluation.
- Aid students with office hours and additional course explanation.
- Proctor exams.
- Assist in teaching the following courses:
  - ANEQ 250 – Live Animal and Carcass Evaluation

Undergraduate Student Teaching Assistant, Colorado State University Animal Sciences
Fall 2015

- Assist professor with laboratory duties.
- Grade laboratory write-ups and examinations.
- Assist undergraduates in exam reviews and additional course explanation.
- Assist in teaching the following course:
  - ANEQ 320 – Principles of Animal Nutrition

Guest Lectures

Pathogenic *Escherichia coli*, Food Microbiology (FS 553), Fall 2020
Meat Microbiology Lecture, Advanced Meat Science (ANSC 552), Fall 2019
Principles of Sanitation, Aseptic Processing Technologies (FS 535), Spring 2019 & 2021
Universal Lessons in Food Safety, Intro to Environmental Health (HSCI 575), Spring 2019 & 2020

ABSTRACTS/POSTERS/PRESENTATIONS

Abstracts

Britton, B.C., I. Sarr, and H.F. Oliver. 2021. Enterobacteriaceae and coliform contamination patterns in peanuts produced and sold in the Senegalese Peanut Basin. International Association for Food Protection. Annual Meeting (Abstract).

Britton, B.C. and H.F. Oliver. 2020. Refining a *Listeria monocytogenes* Predictive Risk Tool for Retail Deli Departments. International Association for Food Protection Annual Meeting. (Abstract).

Britton, B.C. and H.F. Oliver. 2019. Nisin Inhibition of *Listeria monocytogenes* In A Whitefish Salad Blend. International Association for Food Protection Annual Meeting. (Abstract).

Britton, B.C., I. Geornaras, J.O. Reagan, and K.E. Belk. 2018. Antimicrobial effects of chemicals, alone or in combination with an alkyl polyglycoside surfactant, against inoculated bacterial

populations on beef carcass surface tissue. Global Food Science Student Competition, Wuxi, China. (Abstract).

Britton, B.C., I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, J.O. Reagan, and K.E. Belk. 2018. Antimicrobial effects of peroxyacetic acid acidified with various acids when applied to inoculated prerigor beef carcass surface tissue. American Meat Science Association Reciprocal Meat Conference. (Abstract).

Britton, B.C., I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, J.O. Reagan, and K.E. Belk. 2018. Evaluation of antimicrobial solutions, with and without a surfactant, for reducing inoculated bacterial populations on beef trimmings, chicken wings, and cantaloupes. International Association for Food Protection Annual Meeting. (Abstract).

Britton, B.C., K.M. Thomas, I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, J.O. Reagan, and K.E. Belk. 2017. Effect of surfactant addition on the antimicrobial activity of a sulfuric acid-sodium sulfate blend when applied to inoculated beef tissue and chicken parts. Annual JBS-CSU Research Symposium. (Abstract).

Britton, B.C., K.R. McCullough, I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, J.O. Reagan, and K.E. Belk. 2017. Effect of acidified peroxyacetic acid on the microbiological and color characteristics of beef tissue. International Association for Food Protection Annual Meeting. (Abstract).

Final Reports

Britton, B.C., and H.F. Oliver. 2019. Nisin inhibition of *Listeria monocytogenes* growth in a smoked whitefish salad blend. Final Report prepared for Acme Smoked Fish Corp.

Britton, B.C., K.E. Belk, J.N. Martin, R.J. Delmore, and D.R. Woerner. 2018. An assessment of the Colorado Pork Industry. Final Report prepared for Colorado Pork Producers Council.

Britton, B.C., I. Geornaras, D.R. Woerner, J. L. Metcalf, and K.E. Belk. 2018. Antimicrobial effects of chemical solutions, alone or in combination with the surfactant, Disponil DB, against inoculated bacterial populations on beef carcass surface tissue. Final Report prepared for Zoetis.

Britton, B.C., I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, and K.E. Belk. 2017. Antimicrobial effects of peroxyacetic acid acidified with acetic acid or a sulfuric acid and sodium sulfate blend when applied to inoculated prerigor beef surface tissue. Final Report prepared for Zoetis.

Britton, B.C., I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, and K.E. Belk. 2017. Evaluation of the addition of different organic acids to acidify peroxyacetic acid. Final report prepared for Zoetis.

Britton, B.C., I. Geornaras, D.R. Woerner, R.J. Delmore, J.N. Martin, and K.E. Belk. 2017. Effect of surfactant addition on the antimicrobial effects of Centron/Amplon on beef tissue, chicken parts, and cantaloupe. Final Report prepared for Zoetis.

Britton, B.C., K.R. McCullough, D.R. Woerner, R.J. Delmore, I. Geornaras, and K.E. Belk. 2016. Centron/PAA shelf-life study. Final report prepared for Zoetis.

SOCIETY MEMBERSHIP AND INVOLVMENT:

Institute of Food Technologists, Member June 2019-Present

IFT Student Association Purdue Chapter Secretary	2019-2020
International Association for Food Protection, Member	May 2017-Present
American Meat Science Association, Member	August 2016-Present
Food Science Graduate Student Association Purdue Chapter, Member	August 2018-Present
FSGSA Purdue Chapter Vice President	*2019-2020*
Purdue Meat Science Quiz Bowl, lecturer	May 2019
American Meat Science Association Iron Chef Competition, Participant	June 2017
Animal Science Graduate Student Association CSU Chapter, Member	August 2016-July 2018
CSU Meat Animal Evaluation Team Member	January 2015-June 2015
CSU Intercollegiate Livestock Evaluation Team, Member	August 2013-Dec. 2014
CSU Block and Bridle Association, Member	August 2013-Dec. 2015
CSU Chapter Secretary	*2014-2015*

AWARDS AND HONORS:

Meat Industry Suppliers Alliance Scholarship	2019 & 2020
IFT Graduate Student Scholarship	2020
IAFP Student Travel Scholarship	2020
3rd Place, Food Science Dept. 3MT competition, Purdue University	2019
Doctoral Fellowship Recipient, Purdue University	2018-2020
1st Place, Iron Chef Competition, 70th Reciprocal Meat Conference	2017
Colorado Graduate Grant Recipient	2017-2018
Dr. Kurt S. Bucholz Endowment Scholarship	2014
Abby Scott Memorial Scholarship	2014
Colonel Arthur C. Allen Endowment Scholarship	2014
Dr. Jerry Bohlender Memorial Scholarship	2013 & 2014
Phi Theta Kappa Academic Scholarship	2013

CERTIFICATIONS

FSPCA Preventative Controls (PCQI) for Animal Foods	2021
FSPCA Preventative Controls (PCQI) for Human Foods	2021
British Retail Consortium Global Standards (BRCGS) Certification	2021
Safe Quality Food (SQF)/Internal Auditing Certification	2021
Institutional Review Board (IRB), Purdue University Human Research Protection Program	2019-Present
Biosafety Levels I & II, Purdue University Radiological & Environmental Management Services	2018-Present
Hazard Analysis Critical Control Points (HACCP) Certification	2018
Pork Quality Assurance Certification, PQA Online Training	2014