

Use of qualitative and quantitative microbial data to determine if turkey re-hang and post-chill sampling are predictive of *Salmonella* enterica contamination in ground turkey.

by

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Abstract

Salmonella is a major foodborne pathogen posing significant economic and public health risks worldwide. Poultry products serve as its primary reservoir, making the consumption of contaminated poultry a leading cause of salmonellosis. To mitigate this risk, the U.S. Department of Agriculture Food Safety Inspection Services (USDA-FSIS) has progressively strengthened the *Salmonella* Regulatory Framework for Raw Poultry Products since 1996. Currently, industry stakeholders are required to monitor microbial indicators at re-hang and post-chill as a means of assessing processing control. However, numerous studies have demonstrated that *Salmonella* prevalence in final products, such as ground meat, cannot be reliably predicted from these sampling locations, therefore, the efficacy of re-hang and post-chill sampling assessing *Salmonella* in ground turkey remains uncertain. This study aimed to evaluate the effectiveness of microbial sampling to measure *Salmonella* in ground turkey by quantifying microbial indicators and detecting *Salmonella* at key processing steps: re-hang, post-chill, and grinding. A total of 455 samples were collected from 40 commercial turkey barns at a poultry processing facility (re-hang = 200, post-chill = 200, and grinding = 55 samples). Indicator microorganisms, including Aerobic Plate Counts (APC), Enterobacteriaceae (EB), *E. coli*, and *Salmonella* were detected and quantified using different methods. Significant differences ($P < 0.0001$) were observed among sampling locations, depicting high levels for all indicator microorganisms at re-hang, a systematic reduction after post-chill, and re-emergence at grinding. *Salmonella* detection was significantly higher in ground samples (30.97%) compared to re-hang and post-chill samples (6.19% and 3.1% respectively). Furthermore, no relationship ($P > 0.05$) was observed between *Salmonella* presence and microbial indicators at any sampling location. While strong positive association were identified between re-hang and post-chill indicator counts ($P < 0.0001$), poor

linear regressions were observed between re-hang and grinding, as well as post-chill and grinding ($P>0.05$). These findings suggest that relying solely on microbial indicator quantification is insufficient for risk assessment of final product safety. Process control measures based on indicator microorganisms does not accurately reflect *Salmonella* contamination in ground turkey, highlighting the need for more robust pathogen-specific detection strategies.

Keywords: *Salmonella enterica*, indicator microorganisms, process control, re-hang, post-chill, ground turkey.

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Dedication

This achievement is a tribute to my parents—the foundation of my life. They are my rock, my lighthouse, my unweaving anchor. I come from a family filled with love and warmth, and as I reflect on my journey, I now see the immense sacrifices they made. Their tireless, selfless efforts ensured that our table was always full of food and that we received a solid education, even when the weight of those sacrifices went unseen. Every step I’ve taken, every place I’ve visited, and every decision I’ve made is a reflection of their dedication. My gratitude towards them is immense. Their relentless drive to see me and my siblings succeed has shaped the very core of who I am. No words can truly capture the depth of my appreciation. Not a single day goes by without me missing them and feeling profoundly thankful to life and God for granting me the privilege of experiencing such love and sacrifice.

Chapter 1 - Literature Review

1.1 *Salmonella enterica*

1.1.1 *Salmonella* Characteristics, Taxonomy, and Biology

Salmonella is a genus of rod-shaped, gram-negative, facultative aerobic, motile, and non-spore-forming bacteria of the family *Enterobacteriaceae* (Olajide & LaPointe, 2022). This genus comprises two genomic species, *Salmonella bongori* and *Salmonella enterica*. *Salmonella bongori* is primarily associated with cold-blooded animals (e.g., reptiles and amphibians) and has a more limited genetic diversity compared to *Salmonella enterica*, hosting only 20 different known serotypes, which suggests reduced adaptability (Brenner et al., 2000). On the other hand, *Salmonella enterica* is subdivided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. Among these, *Salmonella enterica* subsp. *enterica* (henceforth referred to as *Salmonella*) predominantly colonizes the intestinal tract of humans and warm-blooded animals (Grimont et al., 2000). So far, *Salmonella* comprises over 2,500 serotypes, defined by distinct surface structures (antigens) that differentiate groups within the species, thus, this vast diversity is classified using the traditional serotyping of *Salmonella* which follows the White-Kauffman-Le Minor scheme (Andrews-Polymenis et al., 2010; CDC, 2022). Serotyping is based on the determination of somatic O antigens (which identifies the group), flagellin H antigens (which defines the specific serotype), and in some cases, capsular antigens (Vi), by reaction with specific antisera (Banerji et al., 2020; Le Minor et al., 1990; Ryan et al., 2017). More specifically, the O antigens are distinguished by their different chemical make-up, whereas the H antigens are distinguished by the protein content of the flagella. Therefore, scientists determine the serotype based on the distinct combination of O and H antigens, which create a unique

identification code which is called “antigenic formula” (CDC, 2022). The specific antigenic formula consists of three positions: the first position represents the O antigens, the second and third positions the two different flagellin H antigens all separated by colons (Banerji et al., 2020). For example, the antigenic formula of 6,7,14:r:1,5 represents the serotype Infantis, while the formula 6,8,20:e,h:1,2 represents serotype Newport (Franklin et al., 2011; Herrera-León et al., 2004). Indeed, for over 70 years serotyping has been the cornerstone of public health monitoring and epidemiological investigations of *Salmonella* infections in humans and animals, enabling scientists to detect outbreaks and trace them to their sources (Achtman et al., 2012).

Infections with *Salmonella* serotypes are classified into two categories depending upon the type of disease they cause: typhoidal salmonellosis (e.g., serotypes Typhi and Paratyphi), and nontyphoidal salmonellosis (e.g., serotypes Typhimurium and Enteritidis). Typhoidal serotypes are human-restricted and cause typhoid fever, while nontyphoidal *Salmonella* causes gastroenteritis in a wide range of hosts. This disease can occur after the consumption of animal-source foods such as poultry, eggs, seafood, beef, and pork, plant-based foods such as fruits and vegetables, or even direct and indirect contact with *Salmonella* carriers (Basler et al., 2016; Brands et al., 2005; CDC, 2023b; Fazza et al., 2021; Hilbert et al., 2012; Rortana et al., 2021; Wang et al., 2023). While most infections result in gastroenteritis ranging from mild to severe, vulnerable populations—including children under 5, pregnant women, the elderly, and immunocompromised individuals—are at a higher risk of getting salmonellosis and are more likely to have severe symptoms (FDA, 2023).

The classification and transmission of *Salmonella* highlight its significance as a public health and economic concern. With nontyphoidal strains causing widespread foodborne illness, understanding its impact on healthcare, economics, and prevention efforts is essential.

1.1.2 Economic Impact of *Salmonella*

The global economic burden of foodborne diseases (FBD) is substantial, varying based on a country's level of economic development. According to the World Bank Group (2018), FBD in low and middle-income countries leads to approximately USD 95.2 billion in annual productivity losses, alongside an additional USD 15 billion spent on healthcare costs associated with treating these illnesses. High-income nations, such as the United States, also face significant financial burdens from foodborne illnesses. In the U.S., illnesses linked to meat and poultry consumption alone account for economic losses of up to USD 20.3 billion annually, with *Campylobacter*, *Salmonella*, and *Toxoplasma gondii* being the primary contributors. Among these, *Salmonella* stands out as particularly costly (Scharff, 2020). According to the Economic Research Service of the U.S. Department of Agriculture (USDA-ERS), foodborne *Salmonella* infections impose an estimated annual burden of USD 3.7 billion, encompassing medical expenses, lost wages, and societal costs associated with preventing fatalities (USDA-ERS, 2015). On an individual level, each case of salmonellosis costs an estimated USD 4,312 per affected American (90% CI, 1,558–10,042) (Scharff, 2012). Beyond the direct costs and personal suffering, unsafe food undermines socioeconomic development by straining healthcare systems, disrupting trade and tourism, and slowing economic growth in affected nations (FAO, 2018). In the United States, the financial burden of foodborne *Salmonella* infections alone highlights the need for more robust prevention and control measures. However, the true impact of *Salmonella* extends far beyond economic losses since it is a major public health concern due to its widespread occurrence. Understanding the full scope of *Salmonella* on society comprehends not only the economic part but also its impact on public health, its transmission, and its prevalence on food products.

1.1.3 Public Health Impact of *Salmonella*

Foodborne diseases remain a pressing global public health concern, affecting populations across all continents, and as stated by the World Health Organization (WHO), an estimated 600 million individuals—approximately one in ten people worldwide—fall ill each year due to the consumption of contaminated food, resulting in 420,000 deaths annually (WHO, 2024). In addition to these numbers, *Salmonella* is a leading contributor, responsible for an estimated 90 million cases of gastroenteritis and approximately 155,000 deaths globally each year (Galán-Relaño et al., 2023).

Focusing on the Americas (North, Central, and South), 77 million people fall ill, and more than 9,000 die due to the consumption of contaminated food yearly, and also, FBDs are responsible for 95% of diarrheal cases in the Region (WHO, 2019), underscoring their significant burden on public health systems. Focusing more specifically on the United States, data suggest that 31 known foodborne pathogens, including bacteria, viruses, and parasites, collectively account for approximately 9.4 million cases (90% CI 6.6-12.7 million), 55,961 hospitalizations (90% CI 39,534-75,741), and 1,351 deaths annually (90% CI 39,534-75,741) (Painter et al., 2013; Scallan et al., 2011). Within this context, *Salmonella* emerges one more time as a major pathogen of concern, with the Centers for Disease Control and Prevention (CDC) estimating it causes 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths annually in the U.S. alone (CDC, 2023a).

Of the 9.4 million foodborne illnesses cases in the U.S., the following numbers highlight the relative contributions of various pathogens. Norovirus accounts for the highest proportion of illnesses (58%), followed by non-typhoidal *Salmonella* spp. (11%). However, in terms of severe outcomes, the order gets inverted, with *Salmonella* leading the majority of hospitalizations

(35%), followed by norovirus (26%). Notably, *Salmonella* also leads the foodborne disease-associated mortality, accounting for 28% of deaths, followed by *T. gondii* (24%), and *Listeria monocytogenes* (19%) (Scallan et al., 2011).

These findings align with other studies highlighting the impactful threat that *Salmonella* poses among foodborne pathogens, particularly in terms of hospitalizations and mortality. The data underscore the critical need for targeted interventions to mitigate *Salmonella* contamination and reduce the public health burden of foodborne diseases on a global and national scale.

1.2 *Salmonella* in the Poultry Industry

1.2.1 *Salmonella* Habitat and Infection

Multiple studies state that *Salmonella* naturally resides in the gastrointestinal system of several animal species, due to the warm constant temperature (i.e., between 35-37 °C is the optimal which is body temperature), as well as the high concentration of free amino acids and sugars (Jajere, 2019; Winfield & Groisman, 2003). However, the pathogen can also persist in various environments outside the host (e.g., soil, water, food, and surfaces), but this is mainly attributed to fecal contamination and/or cross-contamination (Grimont et al., 2000). Despite *Salmonella*'s ubiquity, the pathogen tends to prefer warm-blooded animals, and some serotypes showcase host specificity. For example, the serotype Dublin is commonly found in cattle, Choleraesuis in pigs, and Gallinarum in poultry, however, most of the serotypes are present in a wide variety of hosts such as serotypes Typhimurium and Enteritidis which infect both humans and animals (WHO, 2018).

Salmonella's pathogenicity relies on various virulence factors to colonize its hosts by invading, attaching, and overcoming intestinal defenses like gastric acid and gastrointestinal proteases. Key factors include flagella, capsules, plasmid adhesion systems, and type 3 secretion

systems encoded on the *Salmonella* pathogenicity island (SPI)-1 and SPA-2, and other SPIs (Jajere, 2019), letting the pathogen thrive in its natural habitat. In terms of the food industry, poultry serves as the main reservoir for various *Salmonella* serotypes among food-producing animals. Once it colonizes live birds and depending on the serotype, poultry can experience either lethal systemic infections or long-term asymptomatic infections, often referred to as a “carrier state”. This is because the bacteria have evolved to survive the host’s immune response (Velge et al., 2022) and this can be due to their particular lymphatic system (LS). The LS in poultry compresses lymphoid tissue, lymph vessels, and lymphoid organs, but it lacks encapsulated lymph nodes, therefore, it is less developed than the LS of mammals, meaning they have a weakened system which plays a key role in *Salmonella* colonization (Ceccopieri & Madej, 2024; Khamas & Rutllant, 2024).

1.2.2 *Salmonella* Transmission and Harborage in Poultry

Salmonella can be transmitted through two primary routes in poultry: vertical or transovarial transmission (i.e., from hens to eggs) and horizontal or fecal-oral transmission (e.g., bird to bird by non-hereditary means) (S. Shaji et al., 2023).

Vertical transmission involves the transfer of the bacteria from parent birds (e.g., hens) to their next generation (e.g., eggs) by infecting the ovary and oviduct tissue, allowing the bacteria to enter the yolk, shell membrane, and albumen before the eggshell forms and spawning (Liu et al., 2023). More concretely, after the pathogen invades the host’s intestinal, and epithelial cells, it gets spread through the body inside macrophages, which later migrate to other tissues, such as the liver, spleen, and lymph nodes, facilitating a systemic spread of the infection (Gantois et al., 2009; Winter et al., 2010). Once *Salmonella* colonies pass through the gastrointestinal tract (GIT) and reach the cloaca, their ability to exploit the shared connection between the

reproductive and excretory systems in poultry, enables them to colonize the reproductive tract and ascend to the ovary (Liu et al., 2023). Indeed, in commercial flocks, the control of the dissemination of *Salmonella* is complicated due to numerous contamination sources but mostly due to the vertically integrated system; this consists in a business model where a single company owns multiple stages of production such as breeder flocks, hatchery, grow-out flocks, transportation, processing plant, etc. encouraging the easy dissemination of the bacteria (Clauer, 2023).

Moreover, horizontal transmission consists on the mechanism of *Salmonella* colonization from an infected bird to a healthy bird or due to contact with contaminated environmental sources and vectors (S. Shaji et al., 2023). Referring to bird-to-bird contamination by non-hereditary means, studies state that *Salmonella* can colonize the GIT of healthy birds through several routes after contact with contaminated birds and they include: the mouth, cloaca, eyes, nasal passages, and navel of newborn birds (Byrd et al., 1998). In terms of contact with contaminated sources, fecal matter from infected birds spreads the pathogen, infecting the flock, water, litter, feed, and vectors (e.g., cockroaches, rodents, insects) (Snyder & Weimer, 2020). Once the birds consume or come into contact with these contaminated sources, *Salmonella* can colonize their GIT and initiate infection, perpetuating the cycle of contamination and spreading the pathogen further throughout the flock. Horizontal transmission demonstrates that pathogens can spread effectively through direct or indirect contact, enhancing their potential for widespread dissemination (Angulo et al., 2006).

At plant processing level, cross-contamination, poor hygiene, and biofilms are major contributing factors for *Salmonella* transmission, especially if food products are not submitted to a lethal treatment at the end of processing (Carrasco et al., 2012). Cross-contamination occurs

when contaminated surfaces or water come into contact with food products, with ground meats (e.g., ground turkey) being particularly at risk due to their increased surface area and repeated bacterial mixing during grinding (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024). Indeed, 25% of foodborne outbreaks are closely associated with cross-contamination events involving deficient hygiene practices, contaminated equipment, processing or inadequate storage (Munther et al., 2016). Food handlers also contribute to contamination, as personnel plays a significant role in transmission. Studies have shown that up to 40% of *Salmonella* isolates have been recovered from workers' hands, highlighting a critical contamination route (Cui et al., 2015). Lastly, *Salmonella* can form biofilms on surfaces, and once they are formed, normal cleaning and disinfecting procedures such as washing with chlorine or iodine sanitizers may not remove the entire biofilm. Indeed, It has been reported that *Salmonella* can survive as long as 28 days on stainless steel under tempered and dry conditions and other strains for up to 6 months in dry environments (Gong & Jiang, 2017).

The individual routes of *Salmonella* transmission at different production stages underscore the need for specific and targeted interventions (e.g., biosecurity in farms and standardized effective disinfection of equipment in plants). Moreover, the assessment of *Salmonella* prevalence on ground turkey at critical steps in poultry processing facilities can help to develop and implement targeted strategies to reduce the risk of salmonellosis.

1.2.3 *Salmonella* Prevalence and Outbreaks in Poultry Products

Poultry, in particular chicken and turkey, are frequently asymptotically colonized by *Salmonella*, facilitating its transmission to humans through table eggs and poultry meat (Antunes et al., 2016). This makes *Salmonella* prevalence a persistent concern in the poultry industry, with poultry products identified as a significant and major source of contamination and transmission.

Between 1998 and 2021, The Interagency Food Safety Analytics Collaborations (IFSAC) reported a total of 1,322 outbreaks in the U.S. territory, 987 of which were caused by *Salmonella* (IFSAC, 2024). Over 23% of these foodborne *Salmonella* illnesses were linked to the consumption and or contact with poultry and/or poultry products (USDA-FSIS, 2022b), with chicken accounting for 18.6% and turkey for 5.5% (Cason et al., 2024).

In the United States, live poultry-associated salmonellosis (LPAS) outbreaks have been documented since the 1990s, involving 2,630 illnesses, 387 hospitalizations and 5 deaths from 1990 to 2014 (Basler et al., 2016). These outbreaks highlight that contamination can occur at early stages of the poultry production chain and not necessary only from raw, undercooked, or contaminated final products. On the other hand, a notable recent outbreak in 2024 linked to infected eggs resulted in 93 illnesses, and 34 hospitalizations but no deaths (Centers for Disease Control and Prevention CDC, 2024). However, actual case numbers are likely underreported as many individuals recover without seeking medical care. Shifting to major salmonellosis outbreaks in the U.S. related to chicken and turkey, it is included the 2013 multistate *Salmonella* Heidelberg outbreak, with 634 cases, 212 hospitalizations and no fatalities linked to Foster Farms chicken products (rotisserie chicken and boneless skinless chicken tenderloins) sold at different retail stores (CDPH-FDB, 2014). Multiple strains of *Salmonella* Heidelberg identified in the outbreak were found to be resistant to several commonly prescribed antibiotics, emphasizing the public health risk (CDC, 2014; USDA-FSIS, 2013). At the Foster Farms facilities, *Salmonella* was only isolated from the exterior portion of the chicken, and all samples of meat collected from internal portions of the chicken tested negative for *Salmonella*, suggesting that chicken products were likely to be contaminated by cross-contamination (CDPH-FDB, 2014). Moreover, in 2017-2019, a multistate *S. Reading* outbreak caused 356 cases across 42

different states, linked to widespread turkey contamination, leading to recalls of approximately 300,000 pounds of ground turkey due to improper handling and preparation of the contaminated product in accordance with FSIS guidelines (Hassan et al., 2019). Even though the particular serotype it is uncommonly associated with human illness (Webb et al., 2022), patients reported exposure to various turkey products (turkey products, raw turkey pet food, and live turkeys) that were contaminated with the serotype. This event marked the first collaboration between the CDC and FSIS with the National Turkey Federation (NTF), highlighting the need for supply chain-wide safety interventions (Hassan et al., 2019). In addition, in 2011, a *Salmonella* Hadar outbreak tied to contaminated turkey burgers resulted in 19 illnesses over 13 states, 4 hospitalizations, and the first USDA-FSIS recall of raw poultry due to multidrug-resistant (MDR) *Salmonella* (Green et al., 2014). *S. Hadar* accounted for nearly 14.7% (72/491) of *Salmonella* isolates from retail meats in 2008, a notable increase from the average of 6.6% seen from 2002 to 2006., becoming the most common serotype in ground turkey (Green et al., 2014; Karp et al., 2017).

Turkey-associated *Salmonella* outbreaks are primarily linked to serotypes Hadar, Saint Paul, Enteritidis, Heidelberg, Typhimurium and Newport (Jajere, 2019; Tobolowsky et al., 2022). Additionally, other studies state that Corvallis, Kentucky, Bredeney and Virchow serotypes tend to be present in ground turkey and whole turkey parts as well (Erol et al., 2013). Alongside these serotypes, *Salmonella* Hadar, Typhimurium, and Muenchen are under the supervision of the Food Safety Inspection Services (FSIS), since they have been highly associated with poultry products. Consequently, the recurring outbreaks associated with *Salmonella* in poultry products, specifically in turkey, underscore the critical need for enhanced process controls and comprehensive food safety interventions across the supply chain.

Implementing stricter biosecurity measures, improving slaughter and processing safety, and educating consumers on proper food handling is essential to reducing the burden of salmonellosis. Addressing these issues not only mitigates public health risks but also ensures a safer and more sustainable poultry industry.

1.3 Poultry Regulatory Framework

The FSIS of the U.S. Department of Agriculture (USDA) has a mission to ensure that meat, poultry, and egg products are safe, wholesome, and properly labeled (USDA-FSIS, 2022a). In support of this mission, the FSIS established a *Salmonella* verification testing program in 1996 as part of the “Pathogen Reduction; Hazard Analysis Critical Control Point Systems” (PR/HACCP) final rule (61 FR 38806) (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024a). This program had the aim to reduce pathogenic microorganisms in meat and poultry products, reduce foodborne illnesses associated with the consumption of these products, and provide a new framework to modernize the current system of meat and poultry inspection (FSIS, 1996). This regulation required that all establishments must develop and implement written Sanitation Standard Procedures (SSOPs), conduct regular microbial testing at slaughter establishments to demonstrate process controls, establish a pathogen reduction performance standard for *Salmonella*, and design and implement a HACCP system to improve the safety of their products (FSIS, 1996). In fact, in the decade of the 90s, the performance standard for *Salmonella* in ground turkey was ruled by 49.9% of positives allowed, meaning that 29 of 53 samples were permitted to test positive after microbial sampling, numbers that nowadays, will exceed more than three times the current acceptable as regulations have become stricter.

Twenty years later, in 2016, the FSIS published an updated “Performance Standards *Salmonella* Verification Program for Raw Poultry Products”, outlining that the new performance standards for comminuted chicken and turkey products are still represented as a fraction of the maximum allowable *Salmonella*-positive results over a targeted number of samples collected and analyzed in a 52-week moving window (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024a). As of 2016, the maximum permissible percentage in turkey carcasses was 7.1% of positives, meaning 4 of 56 carcasses were allowed to test positive for *Salmonella*, and for ground turkey, the maximum acceptable was 13.5%, being represented by 7 of 52 samples (USDA-FSIS, 2021). However, while the results of FSIS’ *Salmonella* verification sampling show that the current prevalence-based performance standards approach has been effective in reducing the proportion of poultry products contaminated with *Salmonella*, these measures have yet to have an observable impact on human illness rates, which have remained consistent over two decades (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024b). Therefore, in 2024, the FSIS proposed a new draft regulatory framework announcing a stricter regulation considering three components: 1) establishments must characterize *Salmonella* as a hazard likely to occur at receiving, 2) enhance establishments’ process control monitoring and FSIS verification, and 3) implement an enforceable final product standard that would define whether certain raw poultry products contaminated with certain levels and/or serotypes of *Salmonella* are adulterated (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024b).

Currently, in raw turkey carcasses and ground turkey, the FSIS has tentatively determined that these products will be categorized as adulterated if contain any type of *Salmonella* at or above 10 colony forming units/per milliliter or gram (10 CFU/mL(g)) in analytical portion (i.e.,

mL of rinsate or gram of product) and contain any detectable levels of the serotypes Hadar, Typhimurium, and Muenchen, since they have been highly associated with poultry products (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024b). If they exceed the permitted limit, all products in the lot represented by the sample would be prohibited from entering commerce.

Nevertheless, despite all the implemented efforts to reduce the rates of salmonellosis and its impact on public health, enhanced process controls and microbial monitoring programs (MMPs) are still required to effectively understand and predict how *Salmonella* contamination occurs.

1.4 Microbiological Sampling of Raw Poultry

1.4.1 Process Control

Process control is defined as the procedure or set of procedures designed by an establishment to provide control of those operating conditions that are necessary for the production of safe and wholesome food (USDA-FSIS, 2011). Likewise, statistical process controls provide a powerful mechanism for establishments to monitor and interpret the data collected for ongoing HACCP verification as well as it provides an early warning if their process is not functioning as designed allowing them to take corrective actions to bring the process back in control (FSIS, 2021). Under the final rule to modernize poultry slaughter inspection, all poultry processing establishments are required to develop, implement, and maintain written procedures to prevent contamination of carcasses and parts by enteric pathogens and fecal contamination throughout the entire slaughter and dressing operation. At minimum, these procedures must include sampling and analysis for microbial organisms in accordance to the sampling location and frequency requirements to monitor their ability to maintain process control

(CFR, 2014). Currently, the poultry industry is making use of several physical and antimicrobial interventions to improve the food safety and shelf life of poultry meat. They include scalding, on-line spray washing, chilling, dip treatment, and antimicrobial ice (Kataria & Morey, 2020). These interventions aim to optimize microbial load reduction, with continuous monitoring providing critical data on their effectiveness every time they are assessed.

The FSIS categorizes establishments based on the *Salmonella* verification sampling results into three categories. Category 1 is defined as consistent process control and contains those establishments that have achieved 50% or less *Salmonella* maximum allowable percent positive during the most recently completed 52-week moving window (7.1% for turkey carcasses and 13.5 for comminuted turkey). Establishments falling under category 2 are defined as variable process control and are those that have results greater than 50% of the maximum allowable percent positive during any completed 52-week moving window over the previous three months, even though they have met *Salmonella* maximum allowable percent positive for all completed 52-week moving window. Establishments that have exceeded the maximum permitted percent positive for *Salmonella* during any 52-week window during the previous three months are included in category 3, which denotes a highly variable process control (Chavez-Velado et al., 2024; U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024b).

Indeed, *Salmonella* verification testing period from December 2023 through December 2024, all young turkey carcass processors fell under category number one, meanwhile, when it comes to comminuted turkey, processors fell under the three categories disproportionately (28.89% under category one, 24.44% under category two, 17.78 under category three and 28.89 under N/A) (FSIS, 2025). This data demonstrates that after the chilling step, further processes,

such as de-bone, mixing, and grinding, contribute to cross-contamination affecting directly final products.

1.4.2 Sampling Locations and Frequency

Simply testing finished product will not typically provide sufficient information to detect and correct vulnerabilities at specific steps in the HACCP system (FSIS, 2021). Therefore, establishments operating under traditional inspection must collect and analyze samples for microbial organisms at the hot re-hang (henceforth referred to as re-hang) and post-chill points in the process. Re-hang is the location right after carcass hocks are cut, and the carcasses are ready to enter the viscera removal process, whereas post-chill is the point after the carcass exits the chiller and after all slaughter interventions are completed. In fact, the FSIS considers the microbial characteristics of poultry carcasses at re-hang to be a valuable source of data about how well an establishment is minimizing contamination with enteric pathogens and fecal material on live birds coming to slaughter and on carcasses throughout the evisceration and dressing process (FSIS, 2015). Similarly, the microbial characteristics of poultry carcasses at post-chill demonstrate how well an establishment is minimizing contamination during chilling and the overall effectiveness of any antimicrobial interventions the establishment has chosen to apply throughout its process (FSIS, 2015). If the samples collected at both locations exceed the allowed limits (specified later in the section of indicator microorganisms), it will indicate that the establishment may not be maintaining process control and is less likely to meet the performance standards. Conversely, if the values are lower than the established limits, it demonstrates that the facility is maintaining process control. However, the FSIS states that there are no identified index organisms that directly reflect the presence or absence of enteric pathogens in poultry (e.g., *Salmonella*), therefore, the Agency recommends the testing of enteric pathogens at least

intermittently and compare its results against the presence or absence of other non-pathogenic organisms (i.e., Aerobic Plate Counts, Enterobacteriaceae, *E. coli* or Coliforms) to assess whether it is maintaining process control (FSIS, 2015).

According to the Code of Federal Regulations (CFR) 9 CFR 381.65, establishments must, at minimum, collect and analyze samples at a frequency proportional to the establishments' volume of production. For instance, when poultry facilities are processing turkey carcasses, the sampling frequency is once per 3,000 carcasses, but at minimum once each week or operation, whereas for chicken, the frequency decreases down to once per 22,000 carcasses at least once per operating week (CFR, 2014). In addition, carcasses must be randomly collected and different selection methods can be used, but always implementing the randomization to ensure that the testing data are not biased (FSIS, 2015).

Microbial testing at re-hang and post-chill stages is integral to assessing process control in poultry processing, as it helps monitor contamination levels and the effectiveness of antimicrobial interventions. However, the reliance on indicator microorganisms that do not directly indicate the presence of enteric pathogens such as *Salmonella* raises concerns about the adequacy of such testing in not only representing the microbial safety of the final product (e.g., ground turkey), but also, the decision-making process of the poultry industry.

1.4.3 Aerobic Plate Counts (APC)

Aerobic plate counts (APCs), or also Standard Plate Counts (SPCs), are very widely used in the industry to assess the microbial load of fresh meat and poultry products for determining overall product quality and for providing possible information on product safety and shelf life (Jay, 2002). Typically, two 50 cm² areas of turkey carcasses are swabbed before and after chilling, and the samples are tested for various indicator microorganisms. For Aerobic Plate

Counts, the expected median value before chilling is 1,800 CFU/mL, which decreases significantly—by a factor of 100—to 18 CFU/mL following the chilling process (FSIS, 2015).

In a recent study, Kang *et. al*, reported counts of 4.70, 3.68, and 3.34 Log CFU/g of APC after chilling turkey carcasses in three different subzero saline solutions respectively, and numbers which exceed significantly the allowable limit (Kang et al., 2021).

1.4.4 Enterobacteriaceae (EB)

Enterobacteriaceae is a wide family composed of 10 genera and 20 clinically significant species, including the genera *Salmonella* (Farmer et al., 2010; Schwan et al., 2022). Most of the genera and species that are within the family are small gram-negative rods, non-spore producers, motile, and are sugar fermenters often with gas production (Farmer et al., 2010). These microorganisms are also considered indicators for food quality and sanitation, and even though its presence in food does not indicate an apparent hazard to public health, it may indicate poor sanitation, hygiene, and handling practices (Marriott et al., 2018). In contrast, *E. coli* counts are highly correlated to Enterobacteriaceae counts, which are commonly used in slaughterhouses as indicators of fecal as well as environmental contamination (Barco et al., 2014). For EB counts, the maximum limit allowed of cells before chilling is 50 CFU/mL, while after chilling, the counts should be less than 1.2 CFU/mL (FSIS, 2015).

Whyte *et. al* reported EB counts in 2001 and 2004 as high as 3.48 and 3.49 Log CFU/g after the chilling step, highlighting the potential deficiencies in process control or antimicrobial interventions (Barco et al., 2014).

1.4.5. *E. coli* and Coliforms (ECC)

Coliforms are part of the *Enterobacteriaceae* family and include genera such as *Citrobacter* and *Enterobacter* and species such as *Escherichia coli* (Schwan et al., 2022). Also,

they are a group of gram-negative, non-spore forming, rod-shaped aerobes and facultative anaerobes that inhabit the intestinal tract of all vertebrates (Hammad et al., 2022). Therefore, the presence of coliforms in food matrices and water indicates the possible presence of pathogenic bacteria. Also, it is often used as an indicator of hygiene because of its association with fecal contamination (Anand & Griffiths, 2011; Leclerc et al., 2001). Coliforms have a maximum allowable limit of 40 CFU/mL before chilling, with levels expected to drop to below 1.2 CFU/mL after chilling. Similarly, generic *E. coli* counts are limited to 22 CFU/mL prior to chilling, and like coliforms, should also fall to less than 1.2 CFU/mL post-chill (FSIS, 2015).

Counts of 1.65 and 2.09 Log CFU/g were reported for *E. coli* and Coliforms after chilling respectively (Kang et al., 2021), suggesting that the numbers exceed the FSIS' post-chill expectations of less than 1.2 CFU/mL. This discrepancy indicates that the current interventions may not be sufficiently microbial loads to meet the established standards.

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**Chapter 2 - Use of qualitative and quantitative microbial data to
determine if turkey re-hang and post-chill sampling are predictive
of *Salmonella enterica* contamination in ground turkey**

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2.1 Introduction

Foodborne diseases (FDBs) remain a significant global concern, impacting both public health and the economy. The World Bank (2018) estimates that contaminated food costs the global economy over USD 110 billion annually due to productivity losses, product recalls, traceability investigations, fines, and healthcare expenditures (Huyck & Syders, 2021). In the United States, the economic burden of foodborne illnesses—primarily caused by *Salmonella enterica* (henceforth referred to as *Salmonella*), *Campylobacter*, *Toxoplasma gondii*, and *Listeria monocytogenes*—is approximately USD 75 billion (Hoffmann et al., 2024). Among these, *Salmonella* has emerged as the costliest pathogen, with estimated productivity losses escalating from USD 3.7 billion in 2013 to USD 17.1 billion in 2024 (Hoffmann et al., 2015; Hoffmann et al., 2024; USDA-ERS, 2015). The sharp increase is primarily driven by improved estimates of the frequency and cost of chronic sequelae, which now account for 54% of the total cost, compared to just 15% in earlier assessments.

Beyond economic implications, *Salmonella* is the leading cause of bacterial foodborne illness and death worldwide. The World Health Organization (WHO) reports that over 600 million people suffer from FDBs annually, resulting in 420,000 deaths, with *Salmonella* being the primary contributor accounting for 90 million cases and 155,000 deaths globally (15% and 36.9% respectively) (Galán-Relaño et al., 2023; WHO, 2024). In the United States, 9.4 million cases (90% CI [6.6–12.7 million]) of FBD are documented by the Centers for Disease Control and Prevention (CDC) every year. Of those cases, *Salmonella* is responsible for 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths per year (CDC, 2023a; Hoffmann et al., 2024; Scallan et al., 2011). Given its widespread impact, the infection caused by this pathogen, known as salmonellosis, presents with gastroenteritis characterized by abdominal pain fever, and

diarrhea, with severity influenced by host susceptibility and ingested dose (Langridge et al., 2012). The YOPI group (Young, Elderly, Pregnant, and Immunocompromised individuals) is particularly vulnerable (FDA, 2025).

The extensive burden of salmonellosis is intrinsically linked to the pathogen's natural reservoirs and its ability to persist in various environments, facilitating its transmission through the food chain. *Salmonella* primarily resides in the gastrointestinal tract of various animal species, where the optimal temperature (35-37 °C), abundance of free amino acids and sugars, and pathogenicity support its survival (Jajere, 2019; Winfield & Groisman, 2003). However, the pathogen can also persist in external environments—such as soil, water, food, and surfaces—mainly due to fecal and/or cross-contamination (Grimont et al., 2000). Colonization and contamination of *Salmonella* can occur via vertical and horizontal transmission, biofilms, vectors (e.g., cockroaches, rodents, insects), and cross-contamination (Carrasco et al., 2012; Gong & Jiang, 2017; Syamili Shaji et al., 2023; Snyder & Weimer, 2020). Numerous studies have shown that poultry serves as the main reservoir for various *Salmonella* serotypes (Antunes et al., 2016; Jajere, 2019; Syamili Shaji et al., 2023; Winfield & Groisman, 2003). Given that, the gastrointestinal tract of poultry serves as *Salmonella*'s primary habitat, its presence in poultry-derived products (e.g., such as eggs and meat), is biologically expected yet undesirable for consumers; its presence remains a significant food safety concern due to the risk of contamination and potential transmission to humans. Notably, poultry products account for a substantial proportion of foodborne outbreaks: between 1998 and 2022, 19.7% of cases were linked to chicken and 5.25% to ground turkey (IFSAC, 2024). In addition, the CDC has documented 75 outbreaks associated with turkey, including 11 specifically from ground turkey,

with serotypes Hadar, Anatum, and Enteritidis, currently posing the greatest risk (Centers for Disease Control and Prevention CDC, 2024).

Recognizing the substantial role of poultry products in foodborne outbreaks, regulatory agencies have implemented stricter control measures to mitigate *Salmonella* contamination and reduce public health risks. Since the Modernization of Poultry Slaughter Inspection, regulatory standards imposed by the Food Safety Inspection Services (FSIS) have progressively reduced allowable *Salmonella* levels in poultry. In 1996, the acceptable limit for *Salmonella*-positive ground turkey samples was 49.9%, decreasing to 13.5% by 2016 (FSIS, 1996; USDA-FSIS, 2021). In 2024, a new rule classified raw chicken and turkey products as adulterated if they contained ≥ 10 CFU/ml(g) in analytical portion (i.e., mL of rinsate or gram of product) and contain any detectable levels of Hadar, Typhimurium, or Muenchen, effectively restricting their market entry (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024b). Despite these stricter regulations, human *Salmonella* infection rates have remained unchanged for two decades, indicating gaps in contamination control and surveillance strategies (U.S. Department of Agriculture Food Safety Inspection Services USDA-FSIS, 2024b).

Currently, FSIS requires all poultry meat processors to sample 1 out of every 3,000 turkeys in the facility to demonstrate process control (9 CFR 381.65(g)) (CFR, 2014). This sampling is based on component two of the “Proposed Regulatory Framework to Reduce *Salmonella* Illnesses Attributable to Poultry”, which recommends to establishments to test for the presence of indicator microorganisms (Aerobic Mesophiles, Enterobacteria, and *E. coli*) at hot re-hang and post-chill locations in order to predict the presence of *Salmonella* in the final product (ground turkey) (USDA-FSIS, 2022a). Commonly, Aerobic Plate Counts are used to assess general microbial contamination, Enterobacteriaceae as a potential indicator of *Salmonella*

levels, and *E. coli* as fecal contamination indicator (Barco et al., 2014; Jay, 2002; Marriott et al., 2018; Schwan et al., 2022). However, although these microorganisms and locations are effective against each other when sampling carcass surfaces, recent scientific publications reinforce the argument that *Salmonella* can also be internalized within the carcass rather than solely present on the surface (Cui et al., 2015; Vargas et al., 2023; Wu et al., 2014). Among other reasons, and thus, the indicator microorganisms are not able to predict the presence of pathogens such as *Salmonella* in the final product from these locations due to the abysmal differences between the microbial load of whole bird surfaces versus ground turkey, which may lead to unreliable results (Bueno López et al., 2022; Cason et al., 2024; De Villena et al., 2022; NACMCF, 2024; Richards et al., 2024; Vargas et al., 2023). However, the effectiveness of re-hang and post-chill sampling in predicting *Salmonella* prevalence in ground turkey remains uncertain. Apart from that, it is important to know that research on turkeys is very limited compared to chickens and broilers.

Understanding the relationship between microbial indicator levels at these processing stages and *Salmonella* contamination in ground turkey may enhance process control strategies and risk assessment frameworks. Therefore, this study aims to evaluate the efficacy of re-hang and post-chill microbial sampling as a process control measure for ground turkey by detecting and quantifying *Salmonella* and microbial indicators at re-hang, post-chill and grinding processing steps.

2.2 Materials and Methods

2.2.1 Samples collection

From May 2023 to June 2024, samples were collected from 40 commercial turkey barns from a poultry processing facility. For each barn, samples were collected from three different processing steps: hot re-hang—henceforth referred to as re-hang—(where carcass hocks are cut, and the carcasses are ready to enter the viscera removal process), post-chill (where carcasses have exited the last chiller) and grinding. At the end of the study, 455 samples had been collected (re-hang = 200, post-chill = 200, and grinding = 55).

For re-hang and post-chill sampling respectively, ten turkey carcasses of the same barn were randomly chosen and two 10×10 cm areas were swabbed aseptically (Figure 1) using a sterile template and premoistened sponges with neutralizing buffer[®] (Neogen, Lansing, MI). Per location, five samples were analyzed using Petrifilms[®] (Neogen, Lansing, MI) for indicator microorganisms, and the other five using the BAX[®] Real-Time PCR system and SalQuant[®] assay (Hygiena, Camarillo, CA) for *Salmonella* detection and quantification. For grinding sampling, two samples of ground turkey, representing the final product, were aseptically collected from two combo bins per barn. Turkey parts used for grind samples were scheduled for the first grind of the barn, to prevent cross-contamination.

Information about the commercial barns was shared prior to each sampling trip to ensure samples were taken from the targeted lots across the processing lane. Upon collection, samples were stored in a cooler with ice packs and shipped overnight to the Meat Microbiology Laboratory located at Kansas State University, Department of Animal Science and Industry to be further processed and analyzed within 24 hours.

2.2.2 Samples enrichment

Samples analyzed for indicator microorganisms did not require additional enrichment broth if they were collected using a premoistened sponge (re-hang and post-chill) since sponges already contained 10 mL of neutralizing buffer. For ground turkey, 225 mL of Peptone Water™ (PW) (Becton, Dickinson and Company, Franklin Lakes, NJ) were added to 25 g of products (1:10). Tenfold dilutions were created for all samples followed by plating onto Petrifilms®. For the samples screened for *Salmonella*, 50 mL of Buffered Peptone Water (BPW) (Romer Labs, Union, MO) pre-warmed at 42 °C was added to re-hang and post-chill samples followed and were hand-stomached for 1 minute. For ground turkey, 25 g of product were enriched with 225 mL of BPW pre-warmed at 42 °C and homogenized. *Salmonella*-screening samples followed two consecutive incubation periods prior the creation of lysates. The first period corresponded to quantification lysates after an incubation period of 6 h at 37 °C, and the second to detection lysates after incubation period of 24 h at 42 °C.

2.2.3 Quantification of indicator microorganisms

For each sample, tenfold dilutions were created up to 10^{-6} for re-hang and ground turkey and 10^{-2} for post-chill samples. Aliquots of 1 mL from appropriate dilutions were plated in duplicate onto Aerobic Plate Counts (APC), Enterobacteriaceae (EB), and *E. coli* (EC) Petrifilms® for quantification of indicators microorganisms (Neogen, Lansing, MI). Per Official methods AOAC 990.12, APC petrifilms were incubated at 35 ± 1 °C for 48 ± 3 h and count plates with rounded red colonies were recorded as Aerobic Counts. Per AOAC Official Methods 2003.01, EB petrifilms were incubated at 37 ± 1 °C for 24 ± 2 h and red colonies associated with entrapped gas, and red colonies with both yellow zones and entrapped gas, were recorded as

Enterobacteriaceae. Per AOAC Official Methods 998.08, EC petrifilms were incubated at 35°C ± 1 for 24 ± 1 h and blue colonies with entrapped gas were recorded as *E. coli*. Results within the quantifiable range were collected, averaged, and converted to Log₁₀ CFU/cm².

2.2.4 Lysets for *Salmonella* detection and quantification

The BAX[®] real-time PCR system (Hygiena, Camarillo, CA) was used for detection, and the BAX system SalQuant[®] assay (Hygiena, Camarillo, CA) was used to quantify *Salmonella*, following manufacturer instructions. Lysates were created after the first and second incubation periods for quantification and detection. They only differed in the enrichment incubation time (6 and 24 hours). For all samples, an aliquot of 5 µL of the enrichment was transferred to 200 µL of lysis reagent in cluster tubes. This reagent consisted of 150 µL of protease for every 12 mL of lysis buffer (Hygiena, Camarillo, CA). Next, cluster tubes containing the samples were taken to thermocyclers to complete 20 min at 37 °C, 10 min at 95 °C, and finally cooled down to 4 °C for five min. Prepared lysates were stored for up to 48 hours at 4 °C.

2.2.5 PCR analysis for *Salmonella* detection and quantification

All samples were screened utilizing the detection lysates (24 h). Samples that were positive for detection were then quantified using the quantification lysets (6 h). Briefly, PCR tablets were hydrated with 30 µL of the detection lysates and allowed to sit in the cooling block between 10≥30 min before loading into the BAX[®] PCR equipment. Binomial results for *Salmonella* detection were recorded as negative or positive for every sample. A second PCR was run for positive samples using the SalQuant[®] assay, this time using the quantification lysates following the procedure described for the first PCR. Results for the quantification assay consisted of a cycle threshold (CT) value that was then used to estimate the level of *Salmonella*

through standard curves (provided with SalQuant[®] assay). These estimations were reported in CFU/cm² for re-hang and post-chill, and CFU/g for ground turkey. All values were then converted to a logarithm (i.e., base 10) scale. Samples with a negative result for quantification assay were considered *Salmonella* detectable but not quantifiable (*Salmonella* below 1 CFU/unit).

2.2.6 Experimental design and statistical analysis

This research was a longitudinal study under a Completely Randomized Design (CRD) with sampling location (i.e., re-hang, post-chill, and grind) considered as the fixed effects and barns and sampling trips as the random effects. Barns served as the experimental units whereas microbial loads and *Salmonella* served as the observational units. Sub-sampling at each location was conducted to provide precision to the study. To manage the data of the carcasses' sub-sampling, individual sub-sample quantitative measurements were log₁₀-transformed and averaged for analysis.

Microbial indicators and *Salmonella* detection data analyses were conducted using a generalized linear mixed model (GLIMMIX), and a binomial distribution was fitted to *Salmonella* data. Additionally, a linear regression model was applied, with re-hang and post-chill as independent variables (i.e., when re-hang was compared to post-chill or grind, and when post-chill was compared to grind), and post-chill and grind as dependent variables (i.e., when compared to re-hang). To support and complement the linear regression associations, PROC CORR was conducted to provide Pearson correlation coefficients for microbial sampling at each location. All data were analyzed using the Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC, USA).

2.3 Results

This study quantified microbial indicators and *Salmonella* (APC, EB, and EC) at three key processing stages for ground turkey production (hot re-hang, post-chill, and ground turkey), in order to identify the effectiveness of microbial indicator testing for predicting *Salmonella* contamination. Moreover, linear regression analysis was utilized to evaluate if microbial indicator testing at rehang and post-chill were indicative of ground turkey microbial performance.

Figure 2 presents the estimated Log CFU/cm²(g) for indicator microorganisms present in each sample type. Significant differences were observed in the level of indicator microorganisms at each of the three sampling locations ($P < 0.0001$). For all indicators, the highest counts were observed at re-hang (3.76 and 2.98 Log₁₀ CFU/cm²), with a systematical reduction at post-chill (0.50 Log₁₀ and 0.31 CFU/cm²) and re-emergence at grinding (2.70 Log₁₀ and 1.55 CFU/g) for APC and EB respectively. *E. coli* was present at all processing steps in a naturally lower concentration compared to the other indicator microorganisms, with 2.27, 1.40, and 0.05 Log₁₀ CFU/g(cm²) respectively. Results for estimated *Salmonella* detection rates (%) at each processing step are presented in Figure 3. Overall, *Salmonella* detection was highest in samples at the grinding stage (30.97%) as compared to re-hang (6.19%) and post-chill (3.1%) samples. In contrast, no observable difference was identified in *Salmonella* detection between re-hang and post-chill samples.

The relationship between *Salmonella* and microbial indicator data at re-hang and post-chill locations was intended to be determined, however, due to the scarcity of data for *Salmonella* detection and quantification, linear regression analysis was not possible. Thus, descriptive statistics and a coarse comparison (*i.e.*, analyzing the data with detection as the

treatment effect) were used to assess the influence of microbial indicator levels on *Salmonella* contamination. Overall, *Salmonella* was found in 43/455 (9.45%; Table 1), and from all the positives, 74.42% (32/43) of samples were below the detectable limit of quantification (Limit of Detection (LOD) 1 CFU/mL(g)), meaning that almost 3/4 of the samples were detectable but non-quantifiable. Among the samples enumerated for *Salmonella*, the mean concentration was 1.45 Log CFU/unit., with the lowest count being 1 Log CFU/unit and the highest 4.25 Log CFU/unit. Figure 4 shows the estimated level (Log CFU/unit) for each microbial indicator based upon detection (positive vs. negative) for all sample types. Regardless of microbial indicator type, no observable differences in the microbial level were found between positive and negative samples ($P > 0.05$). The levels of all indicator microorganisms were slightly higher in *Salmonella*-positive samples compared to *Salmonella*-negative samples. This data is solely descriptive and does not formally evaluate relationships.

Finally, the efficacy of re-hang and post-chill microbial sampling as process control for ground turkey test results was evaluated. Linear regression between re-hang and post-chill sample types revealed a significant ($P < 0.0001$) and strong positive relationship for APC and EB counts (intercept = 0.663; slope = -2.047; Adj R^2 = 0.742 and intercept = 0.480; slope = -1.185; Adj R^2 = 0.691 respectively), meaning that these two indicators have predictive values when it comes to re-hang and post-chill sampling (Figures 5 and 8). However, this was not observed in *E. coli* (P = 0.350; intercept = 0.002; slope = -0.003; Adj R^2 = -0.003) (Figure 11).

In terms of re-hang and grind processing steps, APC and EB did not reveal an observable relationship between the two locations (P = 0.379 and P = 0.108 respectively) (Figures 6 and 9). Also, the predictive values of both microorganisms are depicted as a low and negative association (intercept = -0.107; slope = 3.016; Adj R^2 = -0.006 and intercept = -0.128; slope =

1.941; Adj $R^2 = 0.043$ respectively), showcasing a poor predictive power from re-hang to grind. In addition, *E. coli* presents itself as a significant value ($P = 0.038$), however, its predictive power is also poor and negative (Adj $R^2 = 0.087$) (Figure 12).

Lastly, linear regression between post-chill to grind is quite interesting. Figures 7, 10, and 13 show that the counts of all indicator microorganisms (i.e., in the order: APC, EB, and *E. coli*) did not reveal an observable difference between the two locations ($P = 0.541$, $P = 0.240$, and $P = 0.560$), and also, showed weak and negative predictive association (intercept = -0.097; slope = 2.663; Adj $R^2 = -0.016$, intercept = -0.165; slope = 1.605; Adj $R^2 = 0.011$, and intercept = -3.711; slope = 1.371; Adj $R^2 = -0.018$ respectively). Indicator data appears to be a reliable measure for process control between re-hang and post-chill, however, these measurements do not seem to extend effectively through the grinding process.

Pearson correlation coefficients were calculated to further support the linear regression results (Table 2). These correlations mirrored the trend observed in the regression analysis, showing strong positive correlation between re-hang and post-chill Aerobic Counts and *Enterobacteriaceae* (0.863 and 0.833 respectively). Conversely, when comparing re-hang or post-chill to grinding, correlation coefficients for both microorganisms did not demonstrate a strong relationship (APC = -0.145 and -0.101; EB = -0.261 and -0.193).

2.4 Discussion

This study showed that there is a systematic reduction of all indicator microorganisms from re-hang to post-chill but a re-emergence in the final product. Re-hang exhibited a predictable trend, with the highest APC, EB, and *E. coli* detected at this location (3.76 Log₁₀, 2.98, and 2.27 CFU/cm² respectively). This observation is consistent with the initial contamination from slaughter and de-feathering processes, where exposure to environmental and fecal contaminants is at its peak (Marmion et al., 2021). At the slaughter step, it is also notable that the carcass remains highly contaminated by feces, dirt, feathers, and other external contaminants (Boubendir et al., 2021; Marmion et al., 2021). On the other hand, scalding and de-feathering may produce a significant increase in the risk of cross-contamination (Hayama et al., 2011). Carcass scalding occurs at temperatures of around 52.5 °C, reducing the microbial carriage on the surface of poultry carcasses (Harris et al., 2018). However, the temperature may be insufficient to prevent the spread of microbes to carcasses since APCs comprise a wide type of microorganisms that thrive at different temperatures. Alongside that, the stiff rubber fingers of de-feathering equipment can act to harbor physical contaminants such as feces and dirt, disseminating any associated microbes among products (Cason et al., 2004; Pacholewicz et al., 2015; Rouger et al., 2017). Although this study did not formally evaluate the sources of contamination, it was hypothesized that the fecal matter of deceased birds after hocks cutting (i.e., they lose neuromuscular control, resulting in the gradual expulsion of residual cecal contents) was one of the factors that also contributed to higher overall numbers of microbial indicators since carcasses contacted one another.

A similar trend was depicted by post-chill processing resulting in a significant reduction of all indicators to 0.50, 0.31, and 0.05 Log₁₀ CFU/cm² for APC, EB, and *E. coli* respectively.

This behavior is expected since the chiller is the main microbial intervention in poultry production and its purpose is to reduce and limit the growth of both pathogenic and spoilage microorganisms (James et al., 2006). However, the reductions achieved by the chillers can vary depending upon the exposure time in the chiller, antimicrobial solutions used and their concentration, and sampling method. Studies have achieved as high as 6.1 Log₁₀ CFU/carcass reduction in broilers after immersion in the chiller with 45 ppm of PAA (peroxyacetic acid) (Thames et al., 2022) and as low as 0.63 Log₁₀ CFU/cm² after water immersion with 50 ppm of chlorine, at 3 °C during 45 min in chickens (Mast & MacNeil, 1978). Differences in microbial concentrations might be caused in both studies by the time of immersion and antimicrobial solution implemented as well as the different sampling methods used (i.e., Thames et. al (2022), collected samples using the swab test of 5×5 cm area; Mast & MacNeil (1978), collected samples through the whole carcass rinse technique). For reference, the samples collected in this study were submitted to a chiller with four interconnected immersion tanks for 2.5 hours in water at 4 °C with PAA concentrations of 100, 30, 30, and 60 ppm respectively.

The significant re-emergence of all microorganisms at grinding was observed for all indicators (2.70, 1.55, and 1.40 Log₁₀ CFU/g respectively for APC, EB, and *E. coli*). It has been widely documented that the grinding process step (i.e., when a de-boned whole muscle is transformed into smaller pieces) has an influence on overall microbial load (Barros et al., 2007; Kebede & Getu, 2023; Rouger et al., 2017; Siriken, 2004; Thomas, 2021). Grinding increases the surface area exposed to potential contaminants, therefore providing more niches for microbial attachment and growth (McKinley & Avens, 1981). In addition, the grinding equipment can serve as a reservoir for bacteria if not adequately sanitized, suggesting cross-contamination during mechanical grinding and handling (Westhoff & Feldstein, 1976). Also, ground turkey is

often produced by combining meat from multiple carcasses, re-introducing, or amplifying microbial contamination since bacteria are naturally present in the environment and animals (NTF).

Location differences were also observed not only for indicator microorganisms but for *Salmonella* as well. Data shows that *Salmonella* prevalence was significantly higher at grinding (30.97%), compared to re-hang and post-chill (6.19% and 36.1% respectively). The prevalence differences between carcasses (re-hang and post-chill) and ground turkey demonstrate the challenges faced by integrators and the unreliability of carcass data in predicting the final product *Salmonella* (Cason et al., 2024). Higher levels of *Salmonella* at grinding may not be solely due to cross-contamination after post-chill but also due to internalization of *Salmonella* within skin, tissue, and bones. The role of systemic infection and its impact on the prevalence and numbers of *Salmonella* in ground poultry components are poorly understood (Rimet et al., 2019), however, recent data supports that pathogens can be also present inside carcasses rather than only on their surfaces. Richards et al. (2024) suggested that the overall *Salmonella* load on carcasses poses a higher risk at post-chill compared to re-hang, as surviving serotypes are the potential primary source of contamination in ground meat. In addition, our study did not evaluate the presence of *Salmonella* in whole muscles nor bones, nevertheless, Cui et al. (2015) detected *Salmonella* internally in drumstick bones and spleens at low levels in turkeys (9.3% and 6.7% respectively), and also, reported that when *Salmonella* presence reached higher levels (neck skin (42%)) it may indicate a flock with greater potential for *Salmonella* contamination of ground turkey. Moreover, Wu et al. (2014) reported contaminated neck skin and bones containing internalized *Salmonella*, categorizing them as potential sources of this pathogen in ground

chicken. These may support the fact that internal parts of poultry, besides their gastrointestinal tract, may also contribute to *Salmonella* prevalence.

The food processing industry relies on microbial indicator organisms to evaluate microbial control effectiveness, particularly when pathogen data are limited or challenging to obtain in commercial settings (Mead, 2007). The objective is to track indicator organisms levels across different processing stages, assuming that pathogen loads will exhibit similar trends to closely related indicator organisms (Saini et al., 2011; Schaffner & Smith-Simpson, 2014). Specifically in this study, *Salmonella* quantification was too scarce to establish a linear regression between indicator microorganisms and *Salmonella* at each processing stage evaluated. However, no significant differences were observed ($P>0.05$) between the indicator's levels from samples that tested negative for *Salmonella* compared to the ones that tested positive (Figure 4). This may indicate that indicator levels are not predictive of *Salmonella* contamination. This has been shown in previous studies conducted in broilers (Bueno López et al., 2022; De Villena et al., 2022; NACMCF, 2024).

Linear regression analyses revealed that there is a strong positive relationship between microbial indicators of re-hang and post-chill ($P<0.05$) but minimal to negligible predictive associations between re-hang to grind ($P>0.05$), and post-chill to grind ($P>0.05$) were found for all indicators. However, it is important to note that this relationship is based on whole carcass samples, where surface sampling was performed at both locations, whereas ground turkey consisted of a more alternated sample, which introduces the internalization of *Salmonella* factor, influencing the microbial dynamics. Indicator organisms such as EBs or APCs have been used by the industry as gauges of process control and to measure microbial reduction on carcasses from slaughter to post-chill (Williams et al., 2015). Statistically, a consistent and predictable

trend is observed between re-hang and post-chill, as high microbial counts at re-hang naturally decline following antimicrobial interventions at post-chill, therefore, leading to a strong association. This trend is not solely seen in poultry products or microbial indicators. Bueno López et al. (2022) reported similar behaviors in pork carcasses, showing significant microbial reductions of APCs and EBs between gambrelling (i.e., when pork carcasses are suspended by the hind legs using a gambrel after de-hairing, which is the equivalent of re-hang in our study) and post-chilling. Moreover, Richards et al. (2024) detected significant reductions in *Salmonella* prevalence between re-hang and post-chill chicken carcasses (48.4% and 7.9%). These studies reinforce the notion that microbial counts consistently decline following antimicrobial intervention stages; however, this trend remains confined to the re-hang and post-chill locations, with no significant predictive relationships extending beyond these points in the processing chain. In terms of the relationship between re-hang and post-chill to grind (both $P > 0.05$), similar results were reported by Vargas et al. (2023), who revealed low correlations when comparing APC and EB counts with *Salmonella* levels on broilers, with significant variability between sampling locations. It is important to note that currently, at re-hang and post-chill the carcasses are either sampled with surface swabs, or whole carcass rinses (Mast & MacNeil, 1978; Thames et al., 2022), conversely, at grinding, a portion of the already ground turkey is usually collected. Besides the difference between sampling methods, there is an abysmal difference between the microbial load of the surface of whole birds and ground meat. Ground turkey has also been subjected to more interventions, which adds more uncertainty to the product and making it difficult to compare the microbial populations from one to the other. In addition, after properly tested and chilled, the carcasses are typically cut and deboned to accommodate a variety of different products (i.e., drumsticks, thighs, leg quarters, etc.), steps that can actively

contribute to higher levels of microbial loads since they come with overall low microbial counts from the chiller, consequently, disrupting the potential relationship between re-hang/post-chill to grinding. Overall, Pearson correlation coefficients reinforce the finding in the linear regressions, highlighting that while re-hang and post-chill surface sampling accurately predict microbial contamination for indicator microorganisms, this predictive power is not carried over to the ground turkey samples.

The low linear regressions and Pearson correlation coefficients obtained in this study suggest that risk assessment on a final product should not rely solely on indicator organisms' quantification from early processing stages. Indicator's counts are not enough for accurate decision-making on pathogen levels.

2.5 Conclusions

Indicator microorganisms depicted a reduction in microbial contamination from re-hang to post-chill with further re-contamination in ground turkey after processing. Data from this study supports the use of indicator organisms as a reliable tool for demonstrating process control between re-hang and post-chill processing. However, between re-hang and post-chill, indicator testing does not appear to effectively reflect *Salmonella* contamination. Additionally, data suggests that microbial indicator testing at both re-hang and post-chill processing stages is not a reliable indicator of overall microbial contamination or specifically *Salmonella* contamination in ground turkey. In addition, both indicator organism and *Salmonella* data suggest post-chill re-contamination, likely due to cross-contamination or internalization within carcasses. Lastly, the low linear regressions obtained in this study suggest that contamination of final product should not rely solely on indicator organisms' quantification.

In summary, while progress is being made in controlling *Salmonella* contamination during certain stages, the grinding process remains a high-risk area requiring additional focus and intervention. The use of indicator organisms and process monitoring shows promise but needs to be refined to ensure effectiveness across all stages of poultry processing.

2.6 Figures and tables

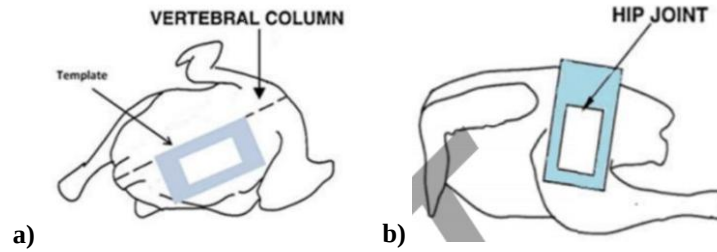


Figure 1. Sampling methodology by area. **a)** Once the tail was located, a 10×10cm area was sampled starting just above the tail and it extended forward along either side of the vertebral column. **b)** The sampling area started from the hip joint, covering the thigh area. Per carcass, both zones were swabbed with a single sponge 10 times vertically and 10 horizontally, using a different side of the swab per zone.

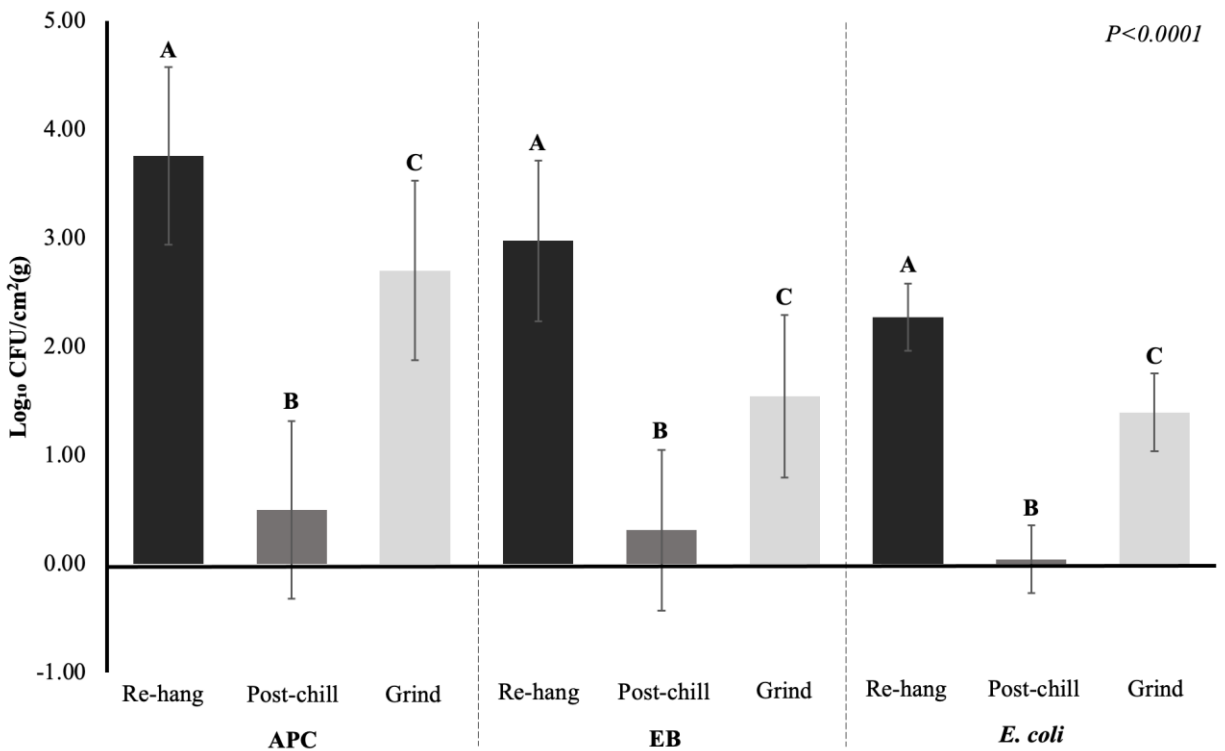


Figure 2. Estimated Log₁₀ (CFU/cm²(g)) for indicator microorganisms present in each sample type. Whiskers depict 95% confidence intervals. Different letters indicate significant differences (P<0.05).

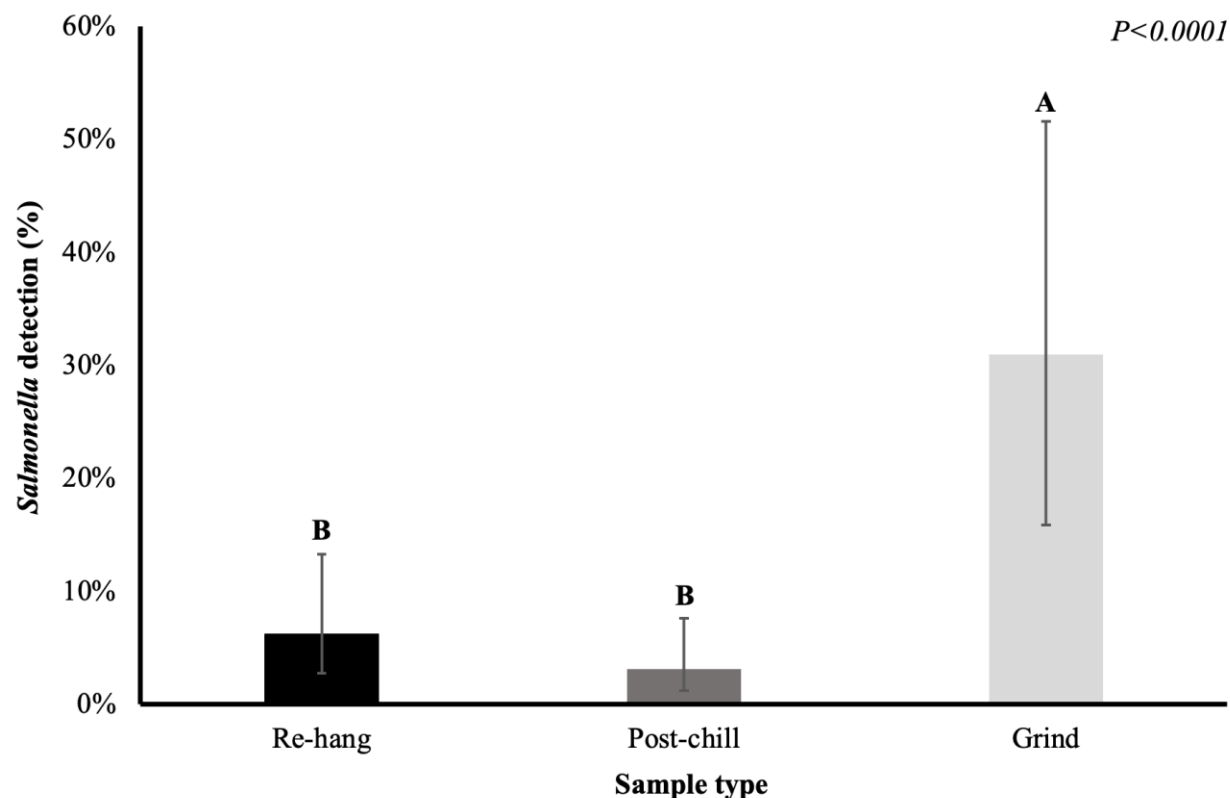


Figure 3. Estimated *Salmonella* detection rates (%) for each sample type. Whiskers depict 95% confidence intervals. Different letters represent significant differences ($P < 0.05$).

Sample type	N° of positive samples	Percentage of positive samples (%)	N° of quantifiable samples	Percentage of quantifiable samples (%)
Re-hang	17/200	8.50	7	41.17
Post-chill	9/200	4.50	2	22.20
Grind	17/55	30.91	2	11.76
Total	43/455	9.45	11	25.58

Table 1. Descriptive analysis of positive and quantifiable *Salmonella* data from sample types.

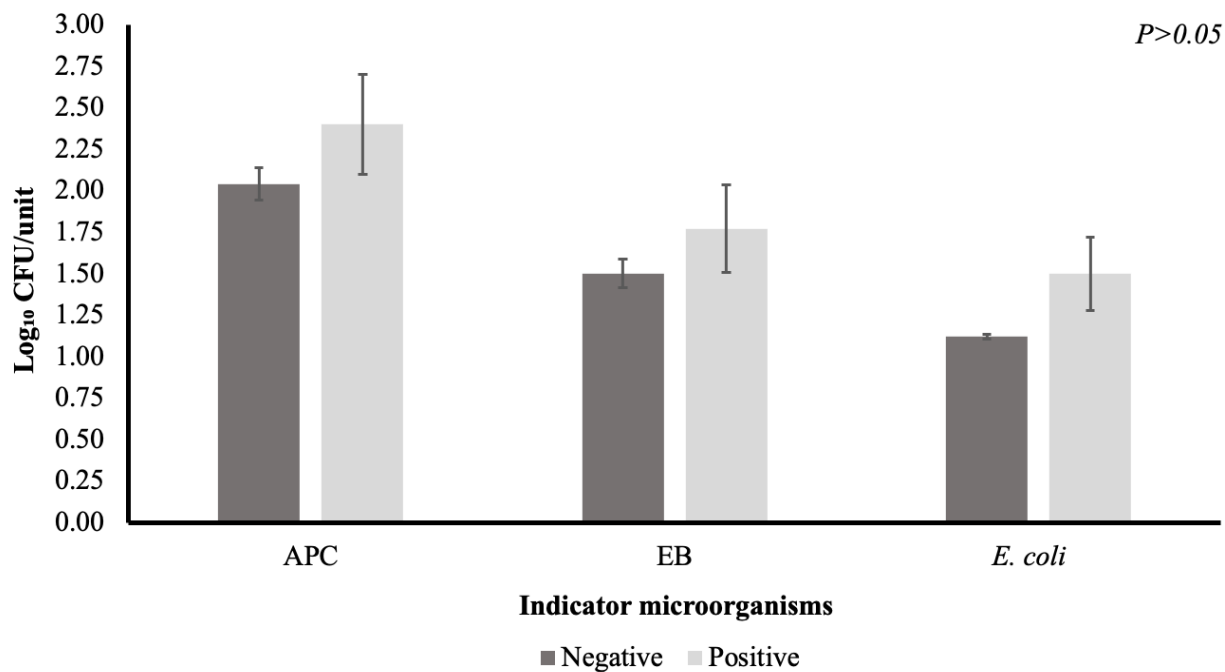


Figure 4. Estimated *Salmonella* detection rates (%) for all samples based upon microbial indicator level. Whiskers depict the Standard Error of the Mean (SEM). No significant differences ($P > 0.05$).

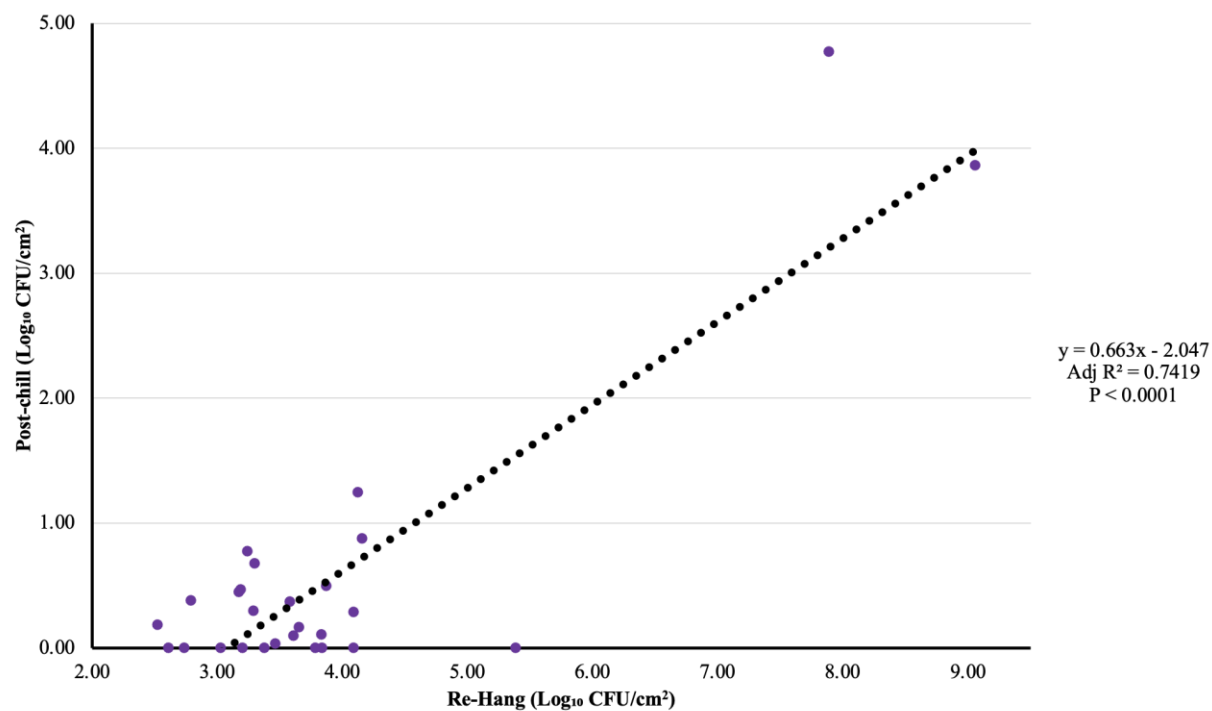


Figure 5. Linear regression of APC results between re-hang and post-chill samples.

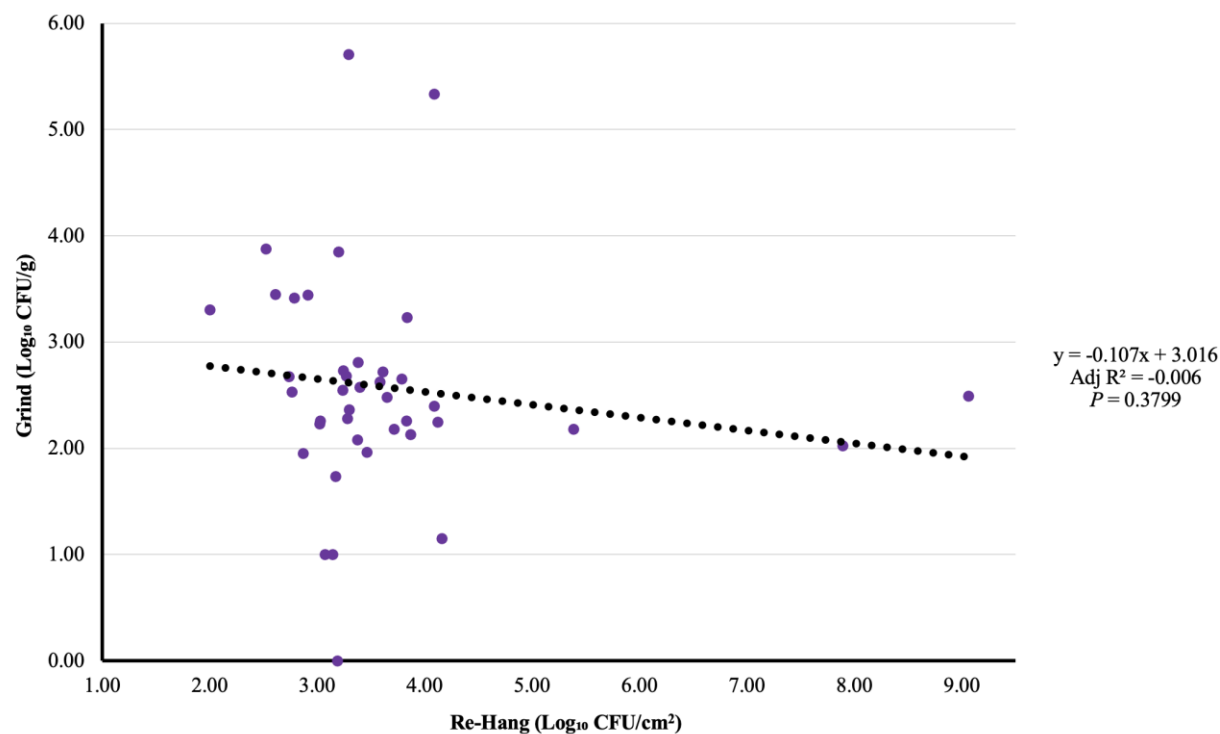


Figure 6. Linear regression of APC results between re-hang and grind samples.

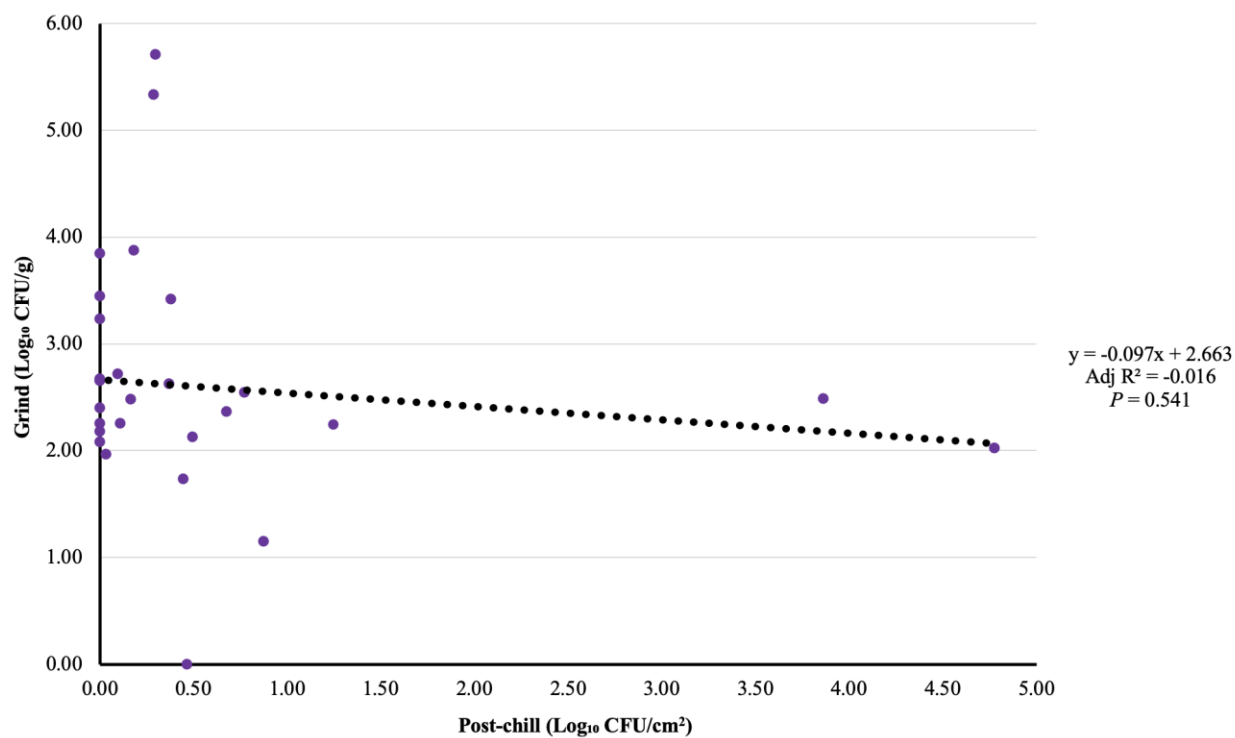


Figure 7. Linear regression of APC results between post-chill and grind samples.

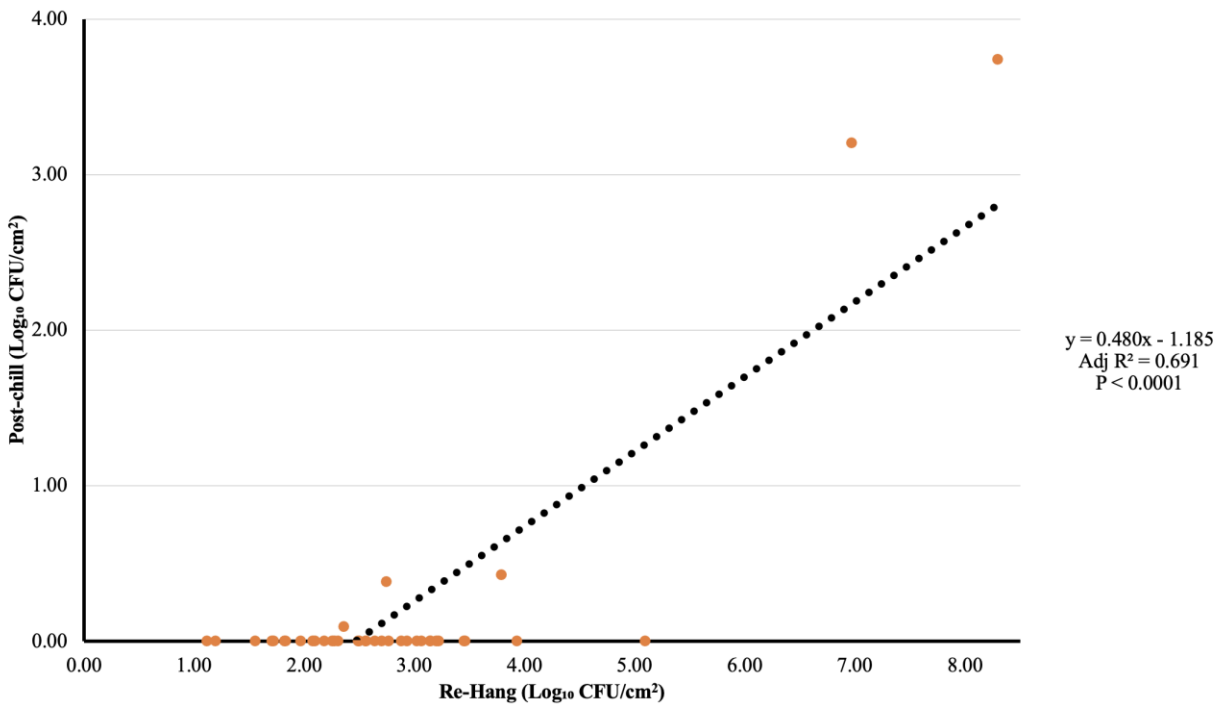


Figure 8. Linear regression of EB results between re-hang and post-chill samples.

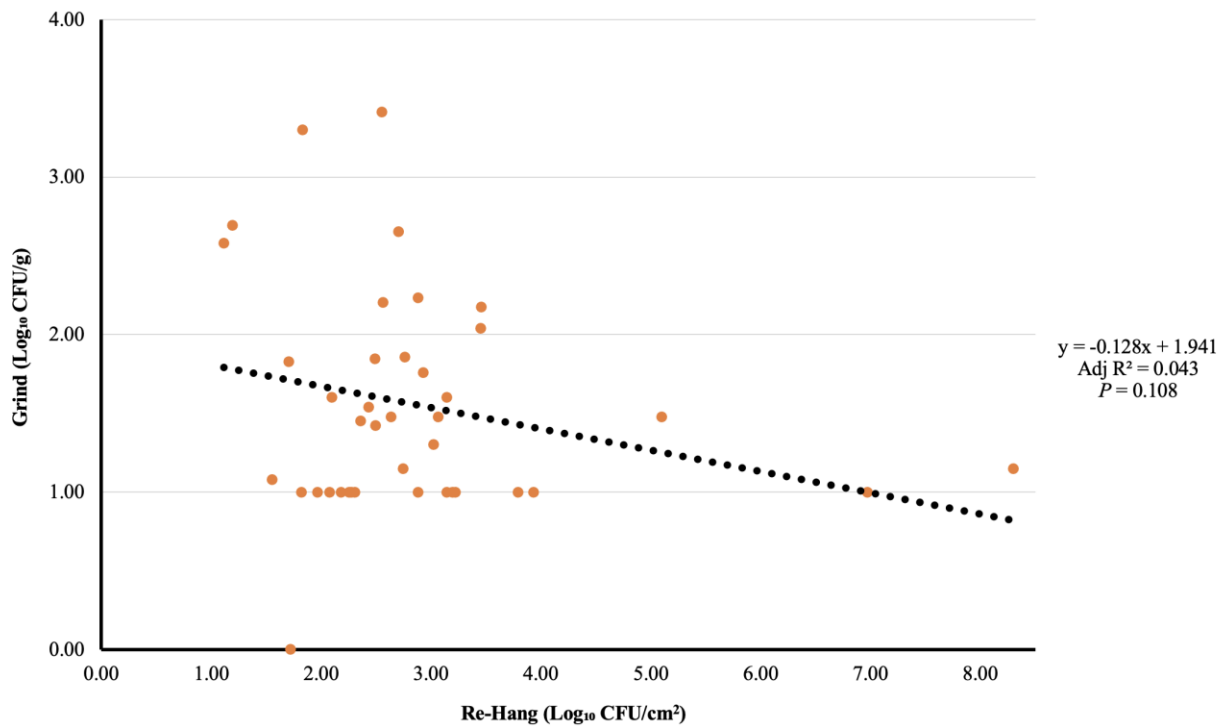


Figure 9. Linear regression of EB results between re-hang and grind samples.

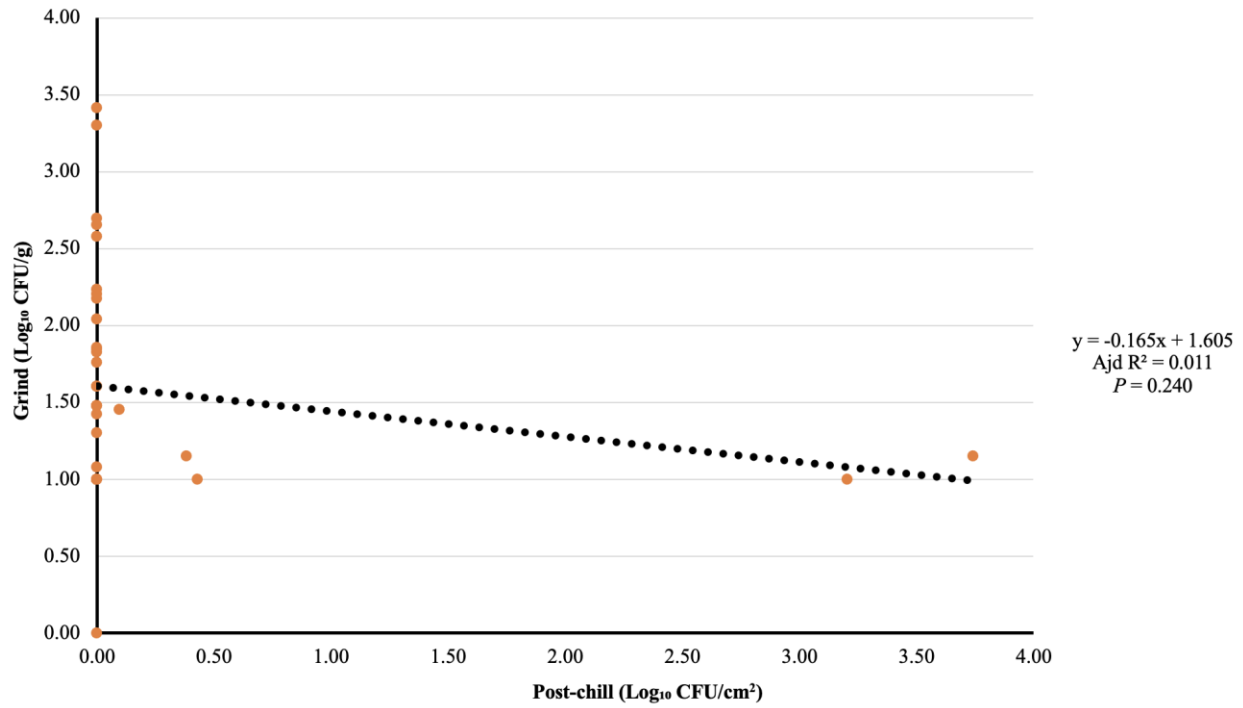


Figure 10. Linear regression of EB results between post-chill and grind samples.

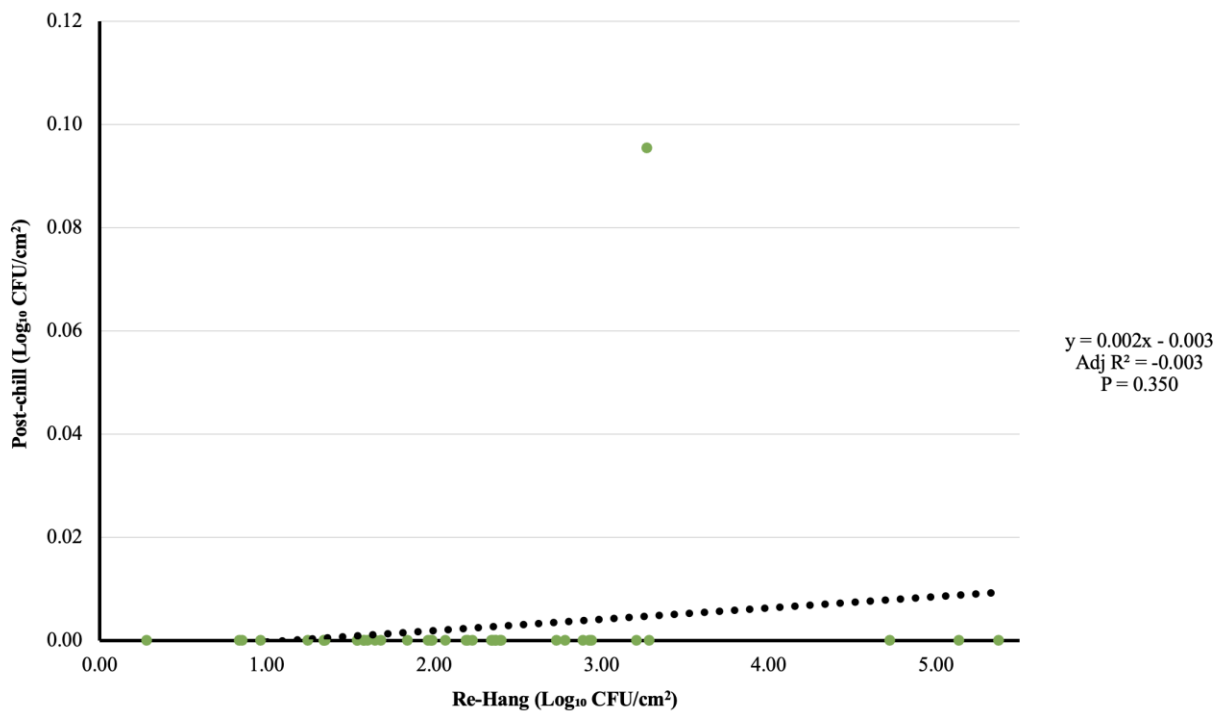


Figure 11. Linear regression of *E. coli* results between re-hang and post-chill samples.

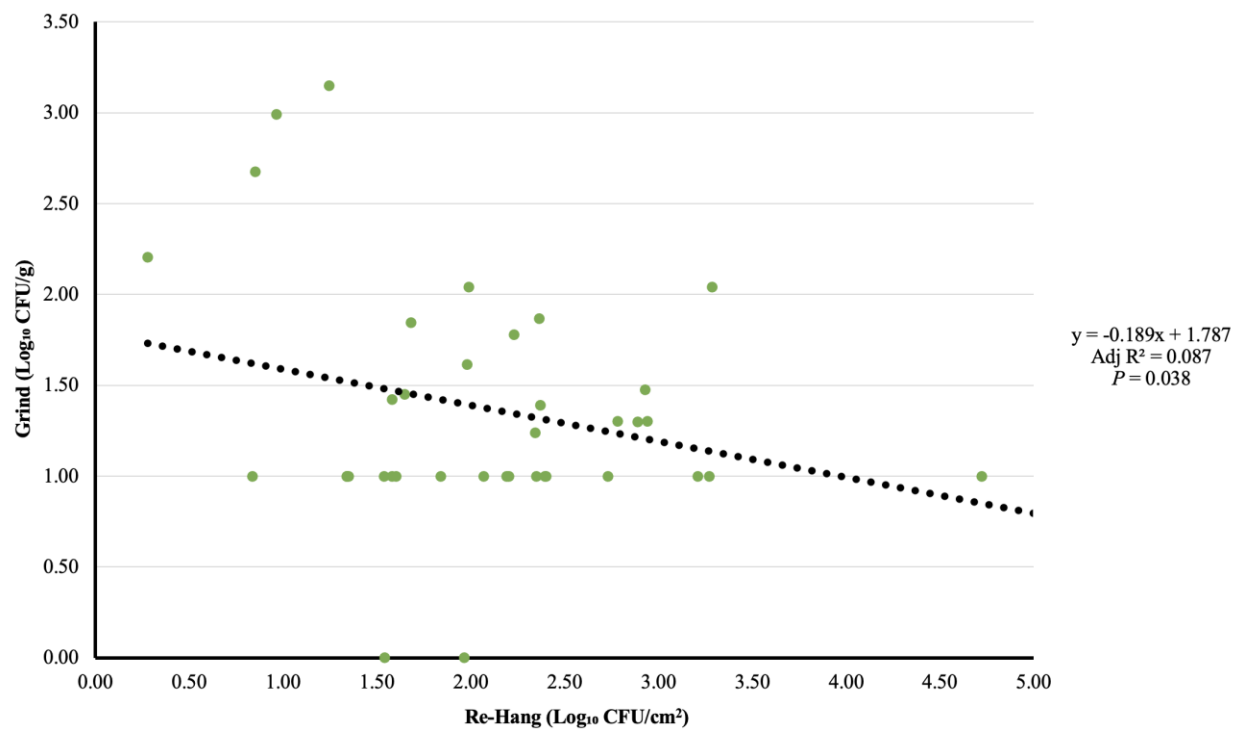


Figure 12. Linear regression of *E. coli* results between re-hang and grind samples.

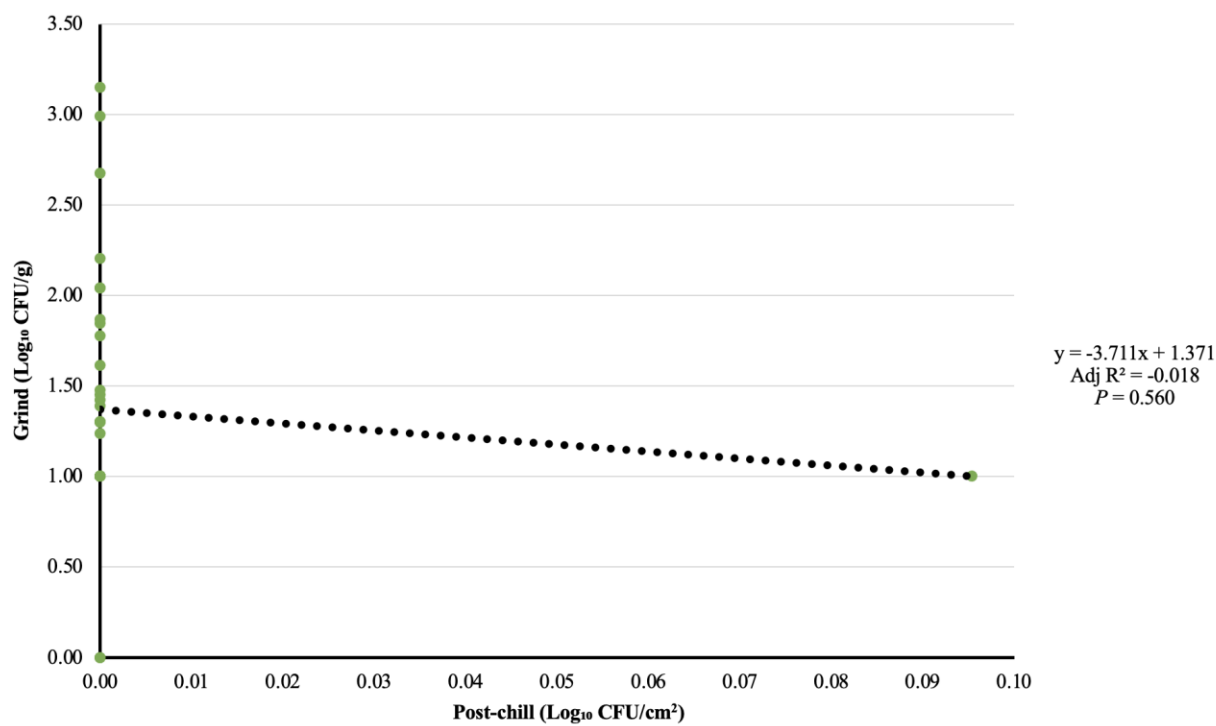


Figure 13. Linear regression of *E. coli* results between post-chill and grind samples.

	Re-hang APC	Re-hang EB	Re-hang <i>E. coli</i>	Post-chill APC	Post-chill EB	Post-chill <i>E. coli</i>	Grinding APC	Grinding EB
Re-hang APC								
Re-hang EB	0.96486 ***							
Re-hang <i>E. coli</i>	0.85556 ***	0.92642 ***						
Post-chill APC	0.86287 ***	0.80068 ***	0.66823 ***					
Post-chill EB	0.90157 ***	0.83324 ***	0.64146 ***	0.94352 ***				
Post-chill <i>E. coli</i>	0.06435 -	0.11174 -	0.15567 -	0.14703 -	0.04991 -			
Grinding APC	-0.14458 -	-0.19739 -	-0.34586 -	-0.10081 -	-0.06716 -	-0.06739 -		
Grinding EB	-0.22661 -	-0.26116 -	-0.25548 -	-0.23543 -	-0.19276 -	-0.14144 -	0.33757 *	
Grinding <i>E. coli</i>	-0.29652 -	-0.30749 -	-0.33380 *	-0.13812 -	-0.15192 -	-0.09628 -	0.32383 *	0.50729 **

Table 2. Pearson correlation coefficients. This table reports pairwise correlation coefficients between the microbial contamination at each of the different processing steps.

(-) Depicts no observable significant correlation between variables.

(*) Depicts a significant probability of <0.05.

(**) Depicts a significant probability of <0.001.

(***) Depicts a significant probability of <0.0001.

2.7 References

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