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Microbiota Characterization of Poultry Processing Systems and Associated Microbiological Sampling Materials Collected at Commercial Processing Facilities

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Food Science

Jennifer A. Wages
University of Central Arkansas
Bachelor of Science in Biology, 2001
University of Arkansas
Master of Science in Operations Management, 2010

May 2020
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

____
Steven Ricke, PhD
Dissertation Director

____
B.J. Bench, PhD
Committee Member

____
Steven Foley, PhD
Committee Member

____
Michael Johnson, PhD
Committee Member

____
Young Min Kwon, PhD
Committee Member

Abstract

The poultry industry and associated regulatory bodies use whole bird carcass (WBC) rinsates to evaluate different stages of broiler processing systems for the prevalence of food-borne pathogens, including *Salmonella* spp. and *Campylobacter* spp. Within industry and research groups, the same sample collections are enumerated to determine *E. coli*, *Enterobacteriaceae* (EB), and Aerobic Plate Count (APC) microorganisms. Analysis of these indicator microorganisms provides numerical data that can be used to demonstrate the effects of specific process control steps where low occurrences of target pathogens hinder the exclusive use of prevalence data. With the utilization of next generation sequencing (NGS), including analysis of 16s rDNA sequences, a more complete characterization of the microbial communities present (the microbiota) can be identified. Microbiota analysis applied to samples collected within the pre-evisceration stages of a commercial broiler processing facility highlighted shifts in enteric microorganisms that were not fully recognized by traditional microbiological culture methods and provided a better understanding of cross-contamination events within those stages of the process. Additionally, microbiota data provided a more complete evaluation of spoilage and sanitary dress indicator microorganisms which were not identified using the traditional culturing methodologies. In addition to evaluating the processing system, microbiota analysis has also helped to identify how a change in the applied rinse solution could affect downstream microbiological analyses. Cultured microbiological methods indicated significant differences ($P \leq 0.05$) in APC levels of the two rinsates, but no significant differences in EB, *Salmonella* spp. or *Campylobacter* spp. prevalence. In contrast, beta diversity analysis of the microbiota compositions of the two rinsate types and associated matrices used to assess those levels of

microorganisms revealed a marked difference ($P < 0.05$) at the operational taxonomic unit (OTU) level.

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Dedication

I want to dedicate this work to my parents, Phillip and Janice Ford, who have always instilled in me a desire to reach farther and learn more. I also want to give a special “shout out” to my nieces, Sarah and Samantha Thiele, who are just beginning their journeys into higher educations---I wish you all the best and can’t wait to see how far you will go.

And to my husband, Travis Wages, for always supporting my efforts and for pushing me to do my best and persevere even when I didn’t believe there was any end in sight, and most of all, for TCB when my work or academic endeavors did not allow me to do my part.

Published Papers

Section II: Microbiome Comparisons of Poultry Carcass Samples at Pre-Evisceration Sites Within Commercial Processing Facilities.

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1 Introduction

Raw poultry carcasses and parts are commonly implicated as contributing sources of food borne-disease caused by *Salmonella* spp. and *Campylobacter* spp. Commensal within the gastrointestinal tract (GIT) of broilers, these human pathogens are often liberated from the feathers, feet, crop, and GIT of processed carcasses. To aid in control of these microorganisms and limit the amount of contamination and cross-contamination events, multi-hurdle interventions including the use of carcass and equipment washes, product chilling, and the application of antimicrobials are commonly employed. To measure antimicrobial efficacy of applied control steps, whole bird carcass (WBC) rinses are collected at sites that represent pre-intervention(s) and post-intervention(s) locations within the processing system and evaluated for target pathogenic microorganisms as well as indicator and spoilage microorganisms. The microbiological evaluations of these rinses provide both processors and regulatory bodies quantifiable information that can be used to assess the performance of specific processing steps and interventions within a poultry harvest system.

While the determination of indicator microorganisms and food borne pathogen prevalence levels using industry-specific culture based methodologies are informative for both processors and researchers, co-currently determining the microbiota present in associated samples enhances the microbiological findings. Furthermore, identifying how these shifts may be altered with changes in collection and sampling methods, helps to identify and explain conflicting results from different sample sets. A greater understanding of the microbial populations present can be used to directly inform food safety quality assurance decisions in poultry processing systems.

2 Literature Review

The commercial processing of poultry to produce food is a complex process that requires an extensive network of equipment, automation and process oversight to remain efficient, while also maintaining quality and food safety standards (Chao et al., 2014; Handley et al., 2015; Zweifel et al., 2015). Every stage of processing live poultry can be directly influenced by several factors along the farm to fork continuum that may or may not be controllable but can have an influential impact on the health of the birds being reared and the quality of product being produced (Brown, 2000; Buncic and Sofos, 2012; Rajan et al., 2017; Roto et al., 2015). As such, the industry employs a wide range of policies and procedures to maintain certain standards (fecal and ingesta contamination, temperature control) which are governed by federal government regulatory bodies so that risk associated with consumption of the food products is reduced or altogether mitigated (Erdtsieck 1995; Sams and McKee, 2010; USDA, 1996; USDA, 2016). Other standards or practices including scalding temperatures and chemical processing aids are facility dependent and based on the commercial company or the target consumer's preferences (Belluco et al., 2016; Bowker et al., 2014; Buhr et al., 2014; Erdtsieck, 1995; Sams and McKee, 2010; Smith, 2014).

Policies and procedures often act as intervention strategies and target a specific aspect of the processing system to reduce both the microbial and organic loads on the birds and carcasses within each stage of the process. Intervention efficacies are often determined by enumerating indicator microorganisms which provide a general microbial evaluation of the processing stage and the resulting product (Belluco et al., 2016; Milios et al., 2014). Aerobic plate count organisms, and *Enterobacteriaceae* organisms including *Escherichia coli* are relatively simple to identify and quantify, while also occurring at high enough levels to be used as an adequate

measure of slaughter process control (Altekruse et al., 2009; Belluco et al., 2016 González-Miret et al., 2001).

While the determination of indicator microorganisms and target pathogen prevalence levels within any stage of the poultry process can be informative for the processor, a more complete understanding of the microbial shifts using next generation sequencing (NGS) could enhance these evaluations by providing a more robust, exhaustive identification and measurement of the microbiome. Furthermore, identifying the microorganisms along with the genetic diversity, or microbiome, in a poultry processing system (from farm to fork) can be informative for food safety quality assurance planning as it will facilitate a greater level of understanding of the microbial community present during any stage of the process.

2.1 Live Bird: Vertical Integration

Most commercial poultry operations in the United States are vertically integrated so that the company owns and thereby controls all aspects of the growth, development and processing (Cowen et al., 2003; Dorea et al., 2010; Northcutt and Buhr, 2010). This level of integration typically means that the company owns not only the processing facilities where the food product is produced, but also the breeding stock, hatcheries, and feed mills associated with the live production aspect of the operations (Cowen et al., 2003; Durrant, 1995; Liljebjelke et al., 2005; Northcutt and Buhr, 2010). Also within the span of the processor's controls, is the live production environment, which is typically handled on a contract farm. Under contract, the farmer will be expected to adhere to specific standards of feed, feed supplementation, treated water, and environmental controls so that fully grown poultry flocks will be uniform in size, shape and yield (Northcutt and Buhr, 2010). Additional controls involving litter treatments, vaccinations, biosecurity measures, routine cleaning of transport equipment, antibiotics, and

other medications are utilized to not only ensure the flock meets the physical attributes required, but also ensure that the flock is healthy, free of disease, and undesirable or pathogenic microorganisms are eliminated or at least minimized (Dorea et al., 2010; Newell and Davison, 2003; Sams and McKee, 2010). However, even with controls in place, unwanted or undesirable microorganisms may be introduced and have the potential to proliferate (Gabriel et al., 2006; Handley et al., 2015; Liljebjelke et al., 2005; Stanley et al., 2014). Due to proximity and density of the population within a commercial grower setting, any infections or disease can be easily spread from bird to bird and rapidly infect an entire flock (Cox and Pavic, 2010; Jay, 2012; Korver, 2012; Newell and Davison, 2003).

The microbial populations within the live production and rearing farm environments directly influence the gastrointestinal tract (GIT) of individual chicks starting at hatch (Deusch et al., 2015; Grau, 1986; Oakley et al., 2014; Rinttilä and Apajalahti, 2013). As chicks mature, the GIT will undergo microbial shifts within each area or organ, resulting in a relatively stable but distinct and diverse microbiome (Deusch et al., 2015; Pan and Yu, 2014; Rinttilä and Apajalahti, 2013; Roto et al., 2015). Some GIT microbial inhabitants will aid in proper digestion, nutrition, immune responses, and protection from environmental stresses (Deusch et al., 2015; Gabriel et al., 2006, Oakley et al., 2014; Roto et al., 2015). Other microorganisms present may have little or no effect on the host's health (Bailey, 1988; Bolder, 1998; Deusch et al., 2015). Some microorganisms, if not eradicated somewhere along the farm to fork continuum, can cause shelf-life and quality issues in the final product (Deusch et al., 2015; Gabriel et al., 2006; Nychas et al., 2008) as well as infections in humans (Bailey, 1986; Barbut, 2002; Bolder, 1998; Deusch et al., 2015; Oakley et al., 2013). In order to reduce the levels of unwanted or harmful microorganisms from the final product, the poultry industry typically uses multi-hurdle

intervention systems to manage aspects of the process, from grow-out to final product, and reduce population levels of all microorganisms (Cox and Pavic, 2010; Gragg and Brashears, 2014; Horrocks et al., 2009; Nagel et al., 2013; Stopforth et al., 2007).

2.2 Live Bird: Intervention Systems

The earliest stages of the production of poultry food products, including egg hatching, grow-out, and harvesting of the live birds, can elicit considerable impact on the microbial quality of the final product (Mulder, 1999). Intervention practices involving diet phase programs and restrictions (Ravindran, 2014), diet and water supplementation (Alali et al., 2013; Anderson et al., 2005; Byrd et al., 2001; Chaveerach et al., 2004; Hermans et al., 2012), vaccination programs (Barrow et al., 2003; Davies, 2005; Dorea et al., 2010; Korver, 2012), as well as cleaning, sanitation (Davies and Wray, 1994;) and biosecurity measures to lessen the risk of any adverse effects are applied to varying degrees within the industry (Cox and Pavic, 2010; Horrocks et al., 2009; Mulder, 1999). The degree to which steps within the live production process have the most impact on the microbial quality of the final product are not fully understood and are subject to debate (Lahellec and Colin, 1985; Mulder, 1999), and in some applications, the impact may be negated by marginal implementation and management practices (Davies, 2005; Dorea et al., 2010; Newell et al., 2011).

As the GIT of commercial poultry is relatively short with limited surface area for absorbing nutrients, consumed feed can be transported relatively rapidly (2 to 5 hours) through the upper regions of the GIT and subsequently deposited in the ceca (Pan and Yu, 2014; Ravindran, 2014; Sergeant et al., 2014; Svihus et al., 2002). Due to this short duration and the high demand of nutrients needed to support the rapid growth within the muscular tissue of commercial reared birds, flocks are typically fed *ad lib*, although the diet components will

change based on the age of the birds and the nutrient needs during each stage of growth (Aviagen, 2014; NRC, 1994; Ravindran, 2014) . Typical feeding programs will include phases of feed identified as “prestarters”, “starters”, “growers”, and “finishers” (Aviagen, 2014; Ravindran, 2014; Roush et al., 2004). Each stage or phase of the diet will address specific energy needs as well as ensure that the economic impact associated with feeding is optimized (Miranda et al., 2015; Ravindran, 2014, Trevisan et al, 2014). It is well documented that changes in diet cause microbial shifts within the GIT of poultry (Oviedo-Rondón, 2009; Pan and Yu, 2014), although the shifts or the origins of the changes may not be well understood (Dunkley et al., 2009; Ramirez et al., 1997; Roto et al., 2015).

Results from practices involving the supplementation of feed and water further highlight the challenges involved with understanding the microbiota shifts produced in the GIT of poultry (Chambers and Gong, 2011; Pan and Yu, 2014). Water treatment trials performed by Hermans et al., (2012) have shown a reduced colonization of *Campylobacter* within a flock when water was supplemented with emulsions of medium chain fatty acids (MCFA). Supplementations of MFCA were shown to reduce the *Campylobacter* populations in the upper and central part of the GIT, thereby possibly reducing the probability of microorganisms colonizing the lower (cecum) parts of the GIT (Hermans et al., 2012). Additionally, acidification treatments have been shown to limit the proliferation of *Campylobacter* in drinking water, thereby eliminating or mitigating the risk associated with this route of transmission (Chaveerach et al., 2004; Hermans et al., 2012).

Evaluations of other water treatments have also shown reduced incidences of *Salmonella* colonization within the GIT of poultry, although the level of reductions were not the same within all regions of the GIT (Alali et al., 2013). In trials performed by Alali et al., (2013), the use of

the non-pharmaceutical compounds in including organic acid mixtures, lactic acid, levulinic acid and essential oils, all reduced the incidence of *Salmonella* colonization in the crops for both inoculated birds and close proximity birds. Even with the reduced colonization in the crops, however, there was no reduced incidence of *Salmonella* within the ceca (Alali et al., 2013).

The environment and equipment used in the early stages of poultry production (egg laying, hatching, and transport to farm) have also been identified as sources of cross-contamination when sanitation and handling practices are marginal or when equipment and ventilation designs do not allow for proper cleaning and sanitation cycles between flocks (Davies, 2005; Davies and Wray, 1994). Sanitization of hatching eggs is typically performed at both the farm and the hatchery (Davies and Wray, 1994). In comparing intervention strategies for egg disinfection, Davies and Wray (1994) found that the most effective disinfection practice involved a dip treatment at the farm, a fog treatment during transport, followed by formaldehyde fumigation after receipt at the hatcher. This practice led to no detectable incidences of *Salmonella* in the egg handling and sorting areas of the hatchery (Davies and Wray, 1994). Conversely, eggs that were only sanitized by being dipped into a sanitizing solution at the farm followed by a disinfectant fogging at the hatchery, resulted in a 28.6% *Salmonella* incidence level within the egg handling sorting areas, which is similar to the incidence levels experienced during the hatching process when disinfectant or fumigation were not conducted at the hatchery (Davis and Wray, 1994).

Air handling and ventilation in hatcheries has also proven to be an important factor in the control of *Salmonella* (Davies, 2005; Davies and Wray, 1994). Airflow from air handling units that became contaminated with *Salmonella* can cause the bacteria to become established in other areas of the hatchery (Davies, 2005; Davies and Wray, 1994). Furthermore, if building design and air handling is not adequate, air sourced from "dirty" areas may be circulated to "clean"

areas, directly compromising any cleaning and sanitation practices that may be occurring in these areas (Davies and Wray, 1994).

As the live production process involved with rearing a commercial poultry flock is vast and complicated, clear and concise standards are subject to interpretation as well as economic constraints to maintain cost effectiveness (Barbut, 2002; Barrow et al., 2003; Bolder, 1997; Corry et al., 2007b; Davies, 2005). Some practices have been identified with the successful production of specific pathogen free flocks, but instituting these practices in a commercial setting are typically cost prohibitive and are not sustainable for long term duration (Barbut, 2002; Bolder, 1997; Callicott et al., 2008). Even with specific pathogen free flocks, microorganisms that cause spoilage and other quality issues will still need to be addressed (Bolder, 1997). While it is understood that live production practices to reduce the microbial load of a poultry flock should be utilized whenever possible, the limitations associated with some of these applications further highlight the importance of intervention strategies within the processing facility (Barrow et al., 2003; Corry et al., 2007b; Kaneene and Potter, 2003; Lahellec and Colin, 1985).

2.3 Poultry Processing

Poultry processing is a multi-step system that begins with the harvesting of live birds on farms and ends with the corresponding human food products emerging from the facility. After live birds are harvested from the grow-out farm, the birds are transported to the abattoir for processing to undergo several process steps including dressing operations, evisceration and chilling (Barbut, 2002; Bolder, 1998; Maurer, 2003; Schilling et al., 2014). Due to the complexity of the process and the risk of cross-contamination within the system, processors apply intervention steps involving chemical, physical and biological components to help reduce the risk of cross-contamination between individual birds and among flocks (Escudero-Gilete et

al., 2013; Linaou et al., 2012; Zweifel and Stephan, 2012). These treatments work individually or in tandem to produce a multi-hurdle system, so that if the system is operating efficiently, each step within the process generates carcasses with reduced microbial populations (Linaou et al., 2012; Smith et al., 2015; Zweifel and Stephan, 2012). (See Figure 1: Multi Hurdle Intervention Poultry Processing). Each step of the process can have profound effects on the final product and must be managed so that the processor is not only compliant with regulatory standards related to control of fecal contamination and contamination of pathogenic microorganisms, but can also produce a safe and quality product while remaining profitable (Lyon et al., 1991; Schilling et al., 2014; Yegani and Korver, 2008; Zweifel and Stephan, 2012).

2.3.1 Live Haul and Transport

Prior to harvest and transport, birds will often be subject to a period of feed withdrawal, which empties the contents of the GIT and may reduce the likelihood of fecal contamination during processing (Durrant, 1995; Maurer, 2003; Mead, 2012; Northcutt and Buhr, 2010; Wabeck, 1972; Wesley, 2009) and produce a carcass with a lower microbial load compared to carcasses produced from birds that have not undergone a feed withdrawal period (Izat et al., 1989). There is evidence to suggest that the timing of this withdrawal can adversely affect the rate of contamination due to the possibility of increased fecal shedding during transport (Mead, 2012; Wesley, 2009), and/or the weakening of the intestinal linings leading to rupturing of viscera during processing. Suggested feed withdrawal times of 8 to 16 hours can result in the intestines having the least amount of fecal material (Durrant et al., 1995; Mainali et al., 2009). In cases where transport and staging time of the birds exceeds this optimum time, an increase in the incidence of *Salmonella* contamination of processed carcasses has been observed (Buncic and Sofos, 2012; Mainali et al., 2009). This could be attributed to the birds consuming litter and

fecal material in the absence of feed which could serve to increase the incidence of pathogenic organisms in the crop (Byrd et al., 2001; Corrier et al., 1999; Ramirez, 1997).

Equipment, vehicles, and personnel involved in the harvesting and transport of the flock also provide opportunities for cross-contamination (Bunic and Sofos, 2012; Lahellec and Colin, 1985; Slader et al., 2002). Slader et al. (2002) reported that washing and disinfection steps used to clean bird transport crates significantly reduced the levels of *Campylobacter* present, but did not completely eradicate the microorganism from all crates. Visual inspection crates after washing and disinfection demonstrated that in many cases, organic material was still present which could provide microorganisms a protective niche from the washing process (Slader et al., 2002). This was further highlighted when birds from previously *Campylobacter*-free flocks became contaminated during collection and subsequent transport to the processing facility (Slader et al., 2002). While the overall levels of *Campylobacter* contamination were at relatively low levels and were reduced after processing to 20 ± 9 most probable number (MPN) per carcass, the presence of *Campylobacter* on the crates could be exemplary of the possibility of cross-contamination between flocks due to the reuse of contaminated equipment (Slader et al., 2002).

2.3.2 Slaughter

After unloading the birds are placed on shackles within a rail system, stunned until rendered unconscious, and incorporated into the slaughter system (Maurer, 2003; Sams and McKee, 2010; Parry, 1995). The unconscious birds are moved through the cutting machine which uses a blade to cut the jugular veins and carotid arteries of the neck allowing the blood to drain (Maurer, 2003; Sams and McKee, 2010; Parry, 1995). Proper exsanguination is necessary to help ensure a timely, humane death, as well ensure sufficient blood loss is achieved to minimize residual blood

within the bird (Barbut 2002; Gregory 1995; Sams and McKee, 2010). Insufficient blood loss could cause quality issues in the final product due to discolorations, as well as potentially influencing shelf-life stability (Gregory 1995; Sams and McKee, 2010). After sufficient blood loss, the carcasses subsequently progress through the processing system through a series of stages, involving feather removal, evisceration, and product chilling (Buncic and Sofos, 2012; Handley et al., 2015; Sams and McKee, 2010).

2.3.3 Scalding and Feather Removal

After slaughter, carcasses are subjected to a scalding process to help open the feather follicles and aid in the subsequent removal of the feathers via a feather removal system (Allen et al., 2003a; Barbut, 2002; Bolder, 1998; Maurer, 2003). Feather removal in a commercial poultry processing facility is typically performed by treating exsanguinated carcasses with heated water which acts to loosen the muscles surrounding the follicles so that the feathers can be removed by mechanical action. Mechanical action to remove the feathers typically involves revolving rubber extrusion or pickers which pull or otherwise remove the feathers from the epidermis of the carcass (Barbut, 2002; Bolder, 1998; Buncic and Sofos, 2012; Cason et al., 2000; Kim and Doores, 1993; Nde et al., 2007; Sams and McKee, 2010). Actual scalding temperature and dwell time can vary due to the desired end product and/or facility/company preferences (Bolder, 1998; Maurer, 2003; Okrend et al., 1986; Sams and McKee, 2010) but generally, the process involves submerging carcasses in 48 to 60°C water for 2 to 4 minutes (Barbut, 2002; Bolder, 1998; Kim et al., 1993b; Maurer, 2003; McKee et al., 2008; Sams and McKee, 2010).

Changing the temperature and/or dwell time will change the appearance of the skin due to loss of the layer of waxy cuticle or stratum corneum (Maurer, 2003; Sams and McKee, 2010) and will result in a carcass that has been either subjected to a hard or soft scald (Barbut, 2002;

Bowker et al., 2014; Maurer, 2003; Sams and McKee, 2010). Typically, a hard scald will be at higher (55 to 62°C) temperatures for shorter periods of time (45 to 90 s) and will result in the loss of most of the cuticle generating a lighter colored carcass with a smooth surface (Barbut, 2002; Bowker et al., 2014; Buhr et al., 2014; Luján-Rhenals et al., 2017; Maurer, 2003; Sams and McKee 2010; Schilling et al., 2014; Shackelford, 1987; Smith, 2014). Alternatively, a soft scald will be conducted at lower (48 to 54°C) temperatures for longer periods of time (120 to 210 s) and most of the cuticle will remain intact, and in some cases, the result will be a yellow tinged carcass (Barbut, 2002; Bowker et al., 2014; Buhr et al., 2014; Luján-Rhenals et al., 2017; Maurer, 2003; Sams and McKee, 2010; Schilling et al., 2014; Smith, 204). A medium scald may also be performed by utilizing temperatures ranging from 54 to 58°C, with dwell times up to 120 s (Barbut, 2002; Buhr et al., 2014). This scald type would typically be reserved for mature birds where the processed meat will undergo further processing steps including breading and frying (Barbut, 2002). Regardless of the temperature and dwell time of this part of the process, it is generally assumed that this step can help to reduce the organic load as well as the microbial load that may be present on the feathers and skin surface of individual carcasses, but may also lead to opportunities of cross-contamination of other carcasses (Allen et al., 2003a,b; Barbut, 2002; Bolder, 1998; Clouser et al., 1995a; Dickens and Whittemore 1997; Nde et al., 2007; Shackelford, 1987) and could increase the overall prevalence of microorganisms in the processing system (Allen et al., 2003a,b; Buncic and Sofos, 2012).

The scald time and temperature will have an effect on the upper levels of the epidermis, but can also effect other attributes of the bird and the final product produced (Buhr et al., 2014). If temperatures are not sufficiently high enough, the picking efficiency of the feather removal process could be reduced (Buhr et al., 2014; Sams and McKee, 2010) and lead to extra

processing steps that would be cost prohibitive in a commercial setting. Conversely, temperatures that are too high can begin to denature proteins on the skin's surface and dermal tissue of the bird, especially in those regions that have a limited number of feathers or protection from exposure to these temperatures (Buhr et al., 2014). The denaturation of the proteins can cause bands of discoloration and influence the texture and appearance of the final product (Buhr et al., 2014; Sams and McKee, 2010; Schilling et al; 2014). Exposure to high temperatures can also have an adverse effect on the final yield associated with the processed bird, as liquefied fat could be lost from the bird during processing (Buhr et al., 2014; Sams and McKee, 2010). Overall, the scald process step can have a significant impact on the visual and physical attributes of the muscle meat, but can also have profound effects on the surface properties of the skin surface (Clouser et al., 1995a,b; Sams and McKee, 2010; Thomas and McMeekin, 1980; 1982).

A scalding treatment and subsequent feather removal will remove layers of the epidermis and leave behind a completely different surface than previously present (Thomas and McMeekin, 1982). This new surface may be generally smoother and less hydrophobic due to the removal of the cuticle, and will possess grooves, pockets and feather follicles that can serve as harborage areas or reservoirs for organic material, debris and microorganisms (Thomas and McMeekin, 1980; Thomas et al., 1987). In turn, the niche areas and surface properties of the skin may provide any microorganisms that are present potential protection from exposure to washes and antimicrobial interventions utilized during this and subsequent stages within the poultry production process (Kim and Doores, 1993; Thomas and McMeekin, 1982).

Equipment design in an immersion scalding process has been identified as an important consideration to combat cross-contamination that may occur in the scalding and defeathering processes (Barbut, 2002; Bolder, 1998; Buncic and Sofos, 2012; Sams and McKee, 2010;

Waldroup et al., 1993). Scald systems can be improved by the use of pre-scald brushes and by increasing the influx and movement of clean water in the tanks (Bolder, 1998; Sams and McKee, 2010). Besides the mechanical removal provided by these features, designing a scald system in which the influx of fresh water is incorporated at the end stages of the scald process allows the carcasses to move through cleaner water while progressing through the system, which in turn, may aid in the reduction of microorganisms and organic material the carcasses are exposed to during this stage of the process (Barbut, 2002).

Waldroup et al. (1993) found that microbial populations of the drip from whole bird carcasses (WBC) treated within a counter-current, multiple stage scalder when compared to a conventional, non-counter current flow scalder were significantly lower. Reductions of 0.5 log cfu/mL APC, 0.7 log cfu/mL coliform counts and 0.6 log cfu/mL *E. coli* counts were observed when the counter-current samples were compared to the conventional scalder. Additionally, *Salmonella* incidence for counter-current samples was 10% less than the conventional scalder samples. Whole bird carcass sampling at post evisceration and post chilling did not show that reductions observed with the counter-current scalder were evident in the other stages of the process as the *Salmonella* incidence at post chilling was higher for the carcasses treated with the counter-current scalder.

Cason and Hinton Jr (2006) described a scalding equipment design involving the use of a counter flow system with three tanks in succession, resulting in coliform and *E. coli* $\log_{10}$ cfu/mL levels in the final scald tank being at least 3.0 $\log_{10}$ cfu/mL lower than those levels seen in the first tank. Additionally, *Salmonella* was only identified in the first and second scalder tanks while there was no detectable incidence of *Salmonella* in the final scalder tank (Cason and Hinton Jr, 2006). When the corresponding carcasses were tested by performing WBC rinses, 18

out of 36 carcass rinses tested had *Salmonella* present and, coliform and *E. coli* levels were at least 1.5 log_{10} cfu/mL higher than the scald waters (Cason and Hinton Jr, 2006).

Along with equipment design, antimicrobial and chemical interventions applied to the scald tanks have been shown to have some efficacy in reducing the microbial load of scald waters so that cross-contamination between carcasses can be mitigated (Humphrey et al., 1981; Maurer, 2003; Okrend et al., 1986). The application of an acetic acid treatment in scalder systems has been shown to reduce the thermal death rate of certain *Salmonella* serovars in treated scald waters (Okrend, et al., 1986), suggesting that acid applications in a scalder system could help to reduce the incidence of *Salmonella* in this part of the process. Even when significant reductions in aerobic plate counts (APC, 2.25 log cfu/mL) and *Enterobacteriaceae* (greater than 3.88 log_{10} cfu/mL) counts in acetic acid treated scald water occurred, the corresponding WBC rinses did not prove to have significantly different APC and *Enterobacteriaceae* loads compared to WBC rinses from an untreated scalder system (Lillard et al., 1987). Treatments utilizing an alkaline scalder additive have been shown to be successful at reducing *Salmonella* prevalence in the scalder water as well as resulting in greater than 1.50 log10 cfu/sample *Salmonella* reductions in rinses from the associated treated WBC (McKee et al., 2008).

The design and function of the picking cabinets can also impact the microbial loads of the carcasses during this step of the processing system (Dickens and Whittemore, 1997). In the picking cabinets, fingers, or protrusions, will work to massage and remove feathers from the carcass (Barbut, 2002; Maurer, 2003; Sams and McKee, 2010). While removing feathers, the resulting drumming action can release fecal and ingesta material from the carcass, and the rotation of the equipment and movement of the carcasses may help to spread this material and

any microorganisms that are potentially present (Berrang et al., 2001; Dickens and Whittemore, 1997). The fingers used to remove the feathers will directly contact the skin, feathers, and organic material present on all carcasses, increasing the likelihood of cross-contamination (Barbut, 2002; Berrang et al., 2001; Dickens and Whittemore, 1997).

Cross-contamination can also occur from the production of aerosols, resulting in the transfer of microorganisms through water droplets which likely become airborne due to the actions and movements within the picking cabinets (Allen et al., 2003a,b; Barbut, 2002; Berrang et al., 2004). The effect of aerosol cross-contamination was observed when a carcass previously inoculated with a marked microorganism was processed through a system containing one of two different defeathering systems (Allen et al., 2003a,b). The marked organism was recovered from carcasses preceding and following the inoculated carcass, indicating that the production of aerosols as well as the direct contact of the protrusions involved in feather removal support proliferation and dissemination of microorganisms at this stage of the process (Allen et al., 2003a,b).

The scalding and picking stages of the poultry process can be a point of cross-contamination between carcasses due to the comingling of carcasses in the scalder water and within the picking process, as well as due to the production of aerosols which transfer the microorganisms in water droplets (Allen et al., 2003b; Berrang et al., 2004; Clouser et al., 1995a,b; Grau, 1986; Lillard et al., 1987; Lues et al, 2007; Okrend et al., 1986; Shackelford, 1988). Any cross-contamination during these steps may have limited impact on the overall microbial load of carcasses, especially when compared to the later stages of processing, including evisceration, spray washing, and chilling steps (Cason et al., 1997,1999; Munther et al., 2016; Rivera-Perez et al., 2014). Theoretically, reductions in microbial counts at this early

stage of the processing system could help ensure that the later stages of processing are both effective and efficient (Okrend et al., 1986), although the complexity of the processing system does not necessarily allow for the comparisons of microbial loads in scalder waters to be wholly indicative of microbial loads of corresponding carcasses (Cason et al., 2000).

During scalding, the microorganisms present on the skin and feathers of the carcass will be subjected to the heat treatment, but due to the short dwell time (45 s to 210 s), many microorganisms will survive on the skin of the carcasses, as well as within the treatment water itself (Carrasco et al., 2012; Yang et al., 2001). This would especially be true of microorganisms that have heat resistance characteristics which can result in those microorganisms not only surviving the heat treatment process, but also increasing the microbial load of the scalder water, thereby increasing the probability of cross-contamination among carcasses (Humphrey, 1981). Survival of some microorganisms is also enhanced by the attachment properties of the cells which allow some microorganisms to become "embedded" in crevices, liquid films or otherwise attached to skin (Corry et al., 2007b; Kim et al., 1993a; Tamblyn and Conner, 1997; Thomas and McMeekin, 1982). Another concern can be the amount of organic material present in the scalding and picking processes, as some studies have indicated that the thermal death rate of microorganisms associated with poultry can be directly influenced by the organic materials present (Humphrey, 1981). One explanation of this could be explained by the debris providing niches of protection which allow survival, but also by the chemical changes occurring within the scald water (Humphrey, 1981). A major component of poultry feces, ammonium urate, disassociates in water to produce uric acid and ammonium hydroxide, and has been shown to influence the pH level of the water to produce a more acidic scald water which could induce a

stress response of some microorganisms, allowing for limited protection against heat stress that may be encountered during the scalding process (Humphrey, 1981).

2.3.4 Evisceration

After scalding and feather removal, the heads are removed from the necks and the carcasses progresses to the evisceration stage, where both inedible and edible viscera are separated from the carcass (Maurer, 2003; Sams and McKee, 2010). In order to accomplish evisceration, the carcasses progress through a series of automated steps that have three main objectives: opening the body cavity, removal of the viscera including the intestinal tract and associated organs, heart and lungs, and removal of the giblets (Bolder, 1998; Maurer, 2003; Sams and McKee, 2010). To aid in evisceration steps and maintain consistencies so that the automated equipment can function efficiently, the carcasses are inverted and suspended on shackles that are transported on a rail system. Typically, the use of rotating equipment along with guide bars and framework serve to position the carcass so that the automated equipment will be able to perform efficiently and effectively. Vent cutters begin the evisceration process by excising and removing the lower part of the intestines; the opener will subsequently enter the cavity and act to widen the cavity and the opening previously exposed by the vent cutter (Maurer, 2003; Sams and McKee, 2010). The larger opening allows for easier removal of the viscera. The viscera must accompany the carcass after it has been removed so that it and the associated carcass can be inspected for disease and contamination by regulatory personnel working within the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) regulatory personnel (USDA, 2014). Some systems allow for the complete removal and placement of the viscera pack in a tray or on a shackle line in direct proximity to the carcass, while other systems allow the viscera to remain connected to the cavity, but hanging on

the outside of the carcass (Barbut, 2002; Maurer, 2003; Sams and McKee, 2010; Schilling et al., 2014; Smith, 2014). In systems where the viscera is still attached to the carcasses, there is an additional step in which the viscera are completely removed from the bird. Once the viscera are removed, the crop of the carcass is subsequently removed by a probe pushing it through the cavity of the carcass to the opening at the neck (Parry, 1995; Schilling et al., 2014; Smith, 2014). After the cropper, the lungs and remnant tissues are removed by either automated or manual vacuum probes and the carcass passes through the neck breaker and subsequent neck puller which serves to break the backbone and remove the neck from the carcass (Maurer, 2003; Parry, 1995; Sams and McKee, 2010; Schilling et al., 2014; Smith, 2014).

2.3.5 On-line and off-line reprocessing

Effective evisceration is an important step in the production of poultry as the viscera have been identified as a potential source of contamination due to the diverse population of organisms present in the GIT of commercial birds (Cunningham, 2012; Gabriel et al., 2006). During evisceration, there is an increased likelihood of contamination as failure to fully remove and/or rupture the viscera could increase the prevalence of unwanted microorganisms on carcasses and equipment (Buncic and Sofos, 2012; Russell and Walker, 1997; Sams and McKee, 2010). Also of concern in the evisceration process is that the constant reuse of equipment in this step of the process which could lead to cross-contamination between carcasses (Bailey et al., 1986; Buncic and Sofos, 2012; Mead, 1995; Sams and McKee, 2010). To determine the efficacy of the evisceration systems and to satisfy regulatory requirements related to the production of wholesome product, routine visual inspections of the external and internal surfaces of the carcasses determine if additional trimming or washing (reprocessing) is required (USDA, 2014).

Extensive off-line reprocessing is not favorable for the processor as it requires additional labor to handle and manage the reprocessing steps, as well removing carcasses from the main processing line, resulting in processing delays (Fletcher et al., 1997). Although it is generally recognized that fecal and ingesta contamination cannot be avoided in the slaughter process (Buncic and Sofos, 2012), regulatory policies have required controls to minimize the presence of these contaminants, and have enacted a zero-tolerance policy on fecal and ingesta material entering the chiller (USDA, 2014). This policy requires that all carcasses contaminated with fecal and ingesta, regardless of the level of the contaminant, to be reprocessed to remove the contamination (USDA, 2014). However, reprocessing carcasses that have minimal contaminants has not been necessarily shown to significantly change the overall microbial loads of the carcasses (Bilgili et al., 2002; Giombelli et al., 2015; Stefani et al., 2014). Furthermore, research has concluded that fecal contamination does not necessarily indicate that the affected carcass or carcasses are more likely to have higher microbial loads when compared to non-contaminated or inspection passed carcasses (Bilgili et al., 2002; Jimenez et al., 2002). Findings published by Jimenez et al. (2002), revealed that microbial loads on non-contaminated carcasses collected after evisceration exhibited a higher incidence of *Salmonella* (20%) when compared to contaminated carcasses (12.5%), and this trend persisted after chilling when non-contaminated carcasses also demonstrated a greater level of *Salmonella* incidence.

In the past, carcasses with visual fecal contamination on the outside of the carcass would have been removed from the processing system and reprocessed to trim and/or wash the affected area(s) while carcasses with contaminated cavities would have been removed from the system and condemned (Blankenship et al., 1975). Blankenship et al. (1975) provided evidence that when comparing carcasses categorized as one of the following: passed inspection, condemned

and washed, or condemned and removed from the process, the average bacterial counts for total plate counts (TPC), *Enterobacteriaceae* and *Clostridia* were generally very similar and although there were some statistically significant differences, the differences were relatively small (less than 0.5 log_{10}). Additionally, the authors suggested that since the wash system used in this process was a 5-sec wash utilizing only 200 mL of water per carcass, improvements in the wash system could lead to even smaller differences of bacterial counts between condemned and inspection-passed carcasses (Blankenship et al., 1975).

Wash systems including inside-outside bird washes (IOBW) along with on-line reprocessing (OLR) (also known as continuous online processing or COP) have become more commonly utilized by processors to reduce the microbial loads of carcasses on the processing line while also minimizing the number of carcasses requiring off-line reprocessing (Giombelli et al., 2015; Kemp et al., 2001; Northcutt et al., 2003; Smith, 2014). The IOBW systems help to remove any visible organic material from both the inside and outside of the carcass and these "cleaner" carcasses progress into an OLR system typically equipped with an antimicrobial wash or rinse (Bashor et al., 2004; Buncic and Sofos, 2012; Kemp et al., 2001; Schilling et al., 2014). These systems may not eliminate the need for all off-line reprocessing, but the number of carcasses requiring off-line reprocessing should be greatly reduced (Buncic and Sofos 2012; Fletcher et al., 1997). Additionally, some wash systems have been shown to reduce microbial populations of all treated carcasses (Kemp et al., 2001; Northcutt et al., 2003) although the efficacy of the systems for these purposes are subject to debate and dependent on the spray volume and pressure, type and concentration of the antimicrobial component as well as application temperatures (Bashor et al., 2004; Corry et al., 2007b; Northcutt et al., 2005).

In findings published by Kemp et al., (2001), an IOBW system followed by an OLR system equipped with acidified sodium chlorite (ASC) reduced *E. coli* counts by 1.78 logs cfu/mL, *Campylobacter* counts by 1.75 logs cfu/mL and *Salmonella* incidence by 21.6%, on fecal contaminated carcasses compared to the standard off-line reprocessing washing practices. These results are similar to findings published by Bashor et al., (2004) in which a wash system equipped with both chlorinated water and a processing aid, trisodium phosphate (TSP) or ASC, resulted in reduced *Campylobacter* counts of 1.03 log cfu/mL and 1.26 log cfu/mL, respectively.

Spray and wash systems equipped with pH neutral quaternary ammonium compounds, such as cetylpyridinium chloride (CPC), have also been shown to be affective at treating carcasses prior to the chilling step (Arritt et al., 2002; Oyarzabal, 2005). These compounds have not only been observed to be germicidally effective against a wide range of microorganisms but have also been reported to inhibit the attachment of certain microorganisms to poultry skin (Arritt et al., 2002; Breen et al., 1995; Oyarzabal, 2005). When CPC, TSP and ASC compounds were applied to inoculated poultry skin, 0.5% CPC was the most affective compound at reducing *Campylobacter jejuni* counts for all contact times studied (Arritt et al., 2002). *Campylobacter jejuni* reductions achieved with 0.5% CPC averaged 2.89 log cfu/skin, while reductions achieved with 10% TSP and 0.1% ASC were 1.63 and 1.52 log cfu/skin, respectively. However, efficacy of the CPC was markedly reduced when the concentration was reduced to 0.1%, and at this level, CPC was generally less effective than the 10% TSP and 0.1% ASC compounds (Arritt et al., 2002).

Pre-chiller intervention systems involving chlorinated washes and rinses with or without an additional antimicrobial component have been utilized by poultry processors to reduce the number of carcasses requiring off-line reprocessing not only due to fecal contamination but to

also reduce the risks of cross-contamination (Oyarzabal, 2005). Efficacy of these washes are dependent on several factors including carcass dwell time, spray volume, and antimicrobial intervention concentrations (Arritt et al, 2002; Bashor et al., 2004: Keener et al., 2004). Furthermore, efficacy can be measured by not only the germicidal activity of the intervention but also the physical action and removal of microorganisms as well as the ability of the corresponding intervention to inhibit attachment of microorganisms to the carcasses (Arritt et al., 2002). If interventions are managed and operating as intended at this stage of the poultry process, then the efficacy of the entire processing system should be enhanced due to the decreased organic and microbial loads that are entering the chilling systems and subsequent processing stages (Yang et al., 1998).

2.3.6 Chilling

After evisceration, the chilling component of poultry processing serves to reduce the microbial population on the carcasses as well as aids in the transformation of muscle proteins to meat (Barbut, 2002; Bowker et al., 2012; James et al., 2006; Petrak et al., 1999; Sams and McKee, 2010; Sofos et al., 2013; Veerkamp, 1995; Zhang et al., 2011). Chilling is usually accomplished with the use of chilled air or water although other forms of coolants such as liquid nitrogen and solid carbon dioxide have been employed (Veerkamp, 1995; Zhang et al., 2011). In the US, the most common form of chilling is the immersion chilling system which utilizes tanks of chilled water with augers or paddles to move the carcasses through the system (Dickens and Whittemore, 1995; Northcutt et al., 2008; Veerkamp, 1995). Efficient chilling is accomplished by managing both the temperature of the water and the contact between the water and the carcasses (James et al., 2006; Veerkamp, 1995). To manage water temperatures, fresh water that is chilled to 0 to 4°C is added at a rate comparable to the outflux of water so that water levels are

maintained and adequate to meet the chilling standards required of carcasses exiting the chiller (USDA, 2014). The amount of fresh, chilled water will help determine the efficiency of the chilling process but this efficiency must be balanced with the costs associated with operating heat exchangers as well as the efforts to conserve water for sustainability purposes (Northcutt et al., 2008). Mechanical or air agitation and the countercurrent flow of incoming water within the chiller tanks also help to manage the efficiency of the chilling processes, by working to "wash" the carcasses while also increasing the contact between individual carcasses and the chilled water, thereby increasing the heat exchange efficiency of the chilling process (Bowker et al., 2014; Northcutt et al., 2006; Petrak et al., 1999; Sofos et al., 2013; Veerkamp, 1995).

Similar to immersion scalders, the co-mingling of carcasses within the chiller can represent an opportunity for cross contamination to occur (Carrasco et al., 2012; Lillard, 1990; Mohamed et al., 2014). Along with maintaining chilled temperatures of the chiller water and the fresh water being added to the system, the addition of an antimicrobial component has been shown to aid in reducing microbial loads on carcasses as well as help mitigate the cross-contamination risks associated with co-mingling of carcasses (Bauermeister et al., 2008; Veerkamp, 1995). Chlorine compounds possess enhanced antimicrobial efficacies when used at lower temperatures, and therefore have been routinely used in chiller applications, although some of chlorine's efficacy is diminished when there are high organic loads or when the pH increases above 7.0 (Bauermeister et al., 2008; Lillard, 1980; Russell and Axtell, 2005; Wabeck et al., 1968). While the application of chlorine compounds has been shown to provide antimicrobial reductions in immersion chillers, the variable organic loads inherent in commercial processing systems can often overwhelm the chlorine levels and limit the establishment of residual levels of freely available chlorine (Russell and Axtell, 2005; Zweifel and Stephan, 2012).

Chiller treatments with ozonated water have been found to be effective due to the strong oxidative potential, broad range of antimicrobial activity, and minimal impact on organoleptic properties of ozone (Bolder, 1997; Fabrizio et al., 2002; Sheldon and Brown, 1986; Zweifel and Stephan, 2012). The efficacy of ozone is dependent on the residual levels available after application; a process or solution that has a high ozone demand will require a higher level of ozone so that a residual level of ozone is established (Kim et al., 2003; Zorlugenc and Zorlugenc, 2012). Filter treatments, the addition of complimentary compounds or applying a separate intervention to act in tandem to the ozone treatments may serve to increase the efficiency of ozone applied to a system with a high ozone demand (Kim et al., 2003). The widespread use of ozone in commercial poultry processing settings may be somewhat limited due to health hazards to personnel within the establishments, as sensitivity to ozone differs from person to person and higher concentrations of ozone may not be feasible for application in all processing areas (Bolder, 1997; Zorlugenc and Zorlugenc, 2012).

The use of generally recognized as safe (GRAS) organic acids as antimicrobial components in poultry chilling operations has been identified as a feasible option due to a low economic impact coupled with a high level of effectiveness (Mani-Lopez et al., 2012; Ricke, 2003; Tamblyn and Conner, 1997; Zweifel and Stephan, 2012). Unfortunately, the use of acids can greatly lower the pH levels of the chill water which can adversely affect the visual appearance of carcasses by causing darkening or yellowing of the carcass tissue (Bilgili et al., 1998; Dickens et al., 1994; Dickens and Whittemore, 1994; Zweifel and Stephan, 2012). Treatment of poultry meat with acids has also been observed to result in meat with undesirable flavor and odor profiles, although these side effects are specific to the acid, concentration, temperature and contact time utilized (Bauermeister et al., 2008a,b; Bolder, 1997; Keener et al.,

2004; Mani-Lopez et al., 2012). Adverse sensory properties that result from acidic treatments can sometimes be mitigated by adding a separate compound which will minimize the negative aspects of the low pH levels, while still maintaining the antimicrobial efficacy of the acid compound (Bauermeister et al., 2008a,b). Peroxyacetic acid compounds have become commonly used in poultry processing as the antimicrobial qualities of the acetic acid in combination with hydrogen peroxide have proven to be effective with limited adverse effects on visual and sensory attributes of the final product (Bauermeister et al., 2008a,b). Regardless, of whether used alone or in combination, there is concern about using organic acids at sub-lethal levels as this environment has been shown to induce acid resistance and virulence properties of the targeted microorganisms (Mani-Lopez et al.; 2012; Ricke, 2003).

2.3.7 Post-Chilling

Ineffective chilling can have both food safety and quality implications for a processor (James et al., 2006; Sams, 1999). The quality of meat produced from a slow chilling can result in muscle meat that is pale, soft and exudative (PSE), which will be both aestically and texturally unappealing (James et al., 2006; Sams, 1999). Conversely, fast chilling can result in muscle fibers constricting, or "cold shortening" leading to the production of tough meat (James et al., 2006). Quality of the final product can also be impacted if the meat temperature has not been reduced enough to slow or prevent the outgrowth of spoilage microorganisms (James et al., 2006). Along with controlling spoilage microorganisms, the chilling process has been shown to be effective at reducing the prevalence of pathogenic microorganisms that pose food safety risks (James et al., 2006).

As more stringent standards have been placed on poultry processors to reduce the prevalence of pathogens on raw poultry carcasses and parts, post-chill intervention treatments

have been become more commonly utilized in poultry processing systems (Nagel et al., 2013). Post chill intervention applications usually involve a chemical intervention applied via a spray or dip tank to carcasses directly after exiting the chiller. While the chemical intervention at the post-chill application may be the same or similar as the chemical component in pre-chill interventions and chiller systems, the contact time, chemical concentration, and organic load on the carcasses will be different (Arritt et al., 2002; Nagel et al., 2013). As previously discussed, effectiveness of chemical interventions is directly influenced by the organic and microbial loads that may be present on the carcasses during treatment and overall, post-chill applications may prove to be effective, but cannot be used to overcome inadequate interventions applied in previous stages of the poultry processing system.

2.3.8 Multi-Hurdle Intervention Poultry Processing Schematic

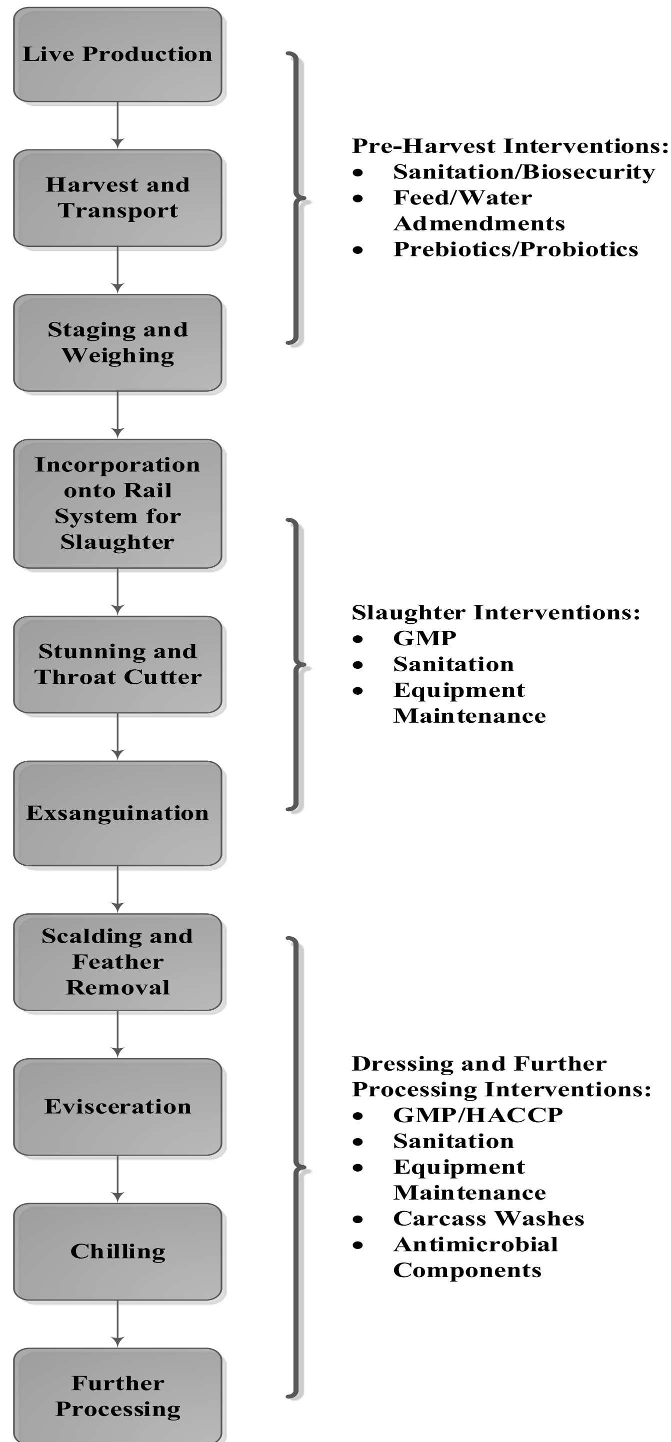

Figure 1. Multi-Hurdle Intervention Poultry Processing (Adapted from Barbut, 2002; Chen and Wang, 2003)

2.4 Poultry Processing: History of Food Safety

The publication, "The Surgeon General's Report on Health and Promotion of Disease and Prevention" in 1979, led to different sectors of the US government becoming unified in providing measurable and meaningful improvements in the health of the US population (PHS, 1979). In support of these objectives, a program entitled "Promoting Health/Preventing Disease: Objectives for the Nation", which outlined 226 objectives known as the "1990 health objectives" were proposed to improve the health of the nation's population by 1990 (PHS, 1980). When a midcourse review was performed in 1985, noted improvements in human health had already been achieved, but it was also apparent that several of the goals of the original program would not likely be achieved and further, new challenges had increased the number of objectives that needed to be addressed (U.S. Department of Health and Human Services, 1986). To address these changes, the program and the associated objectives expanded, and with the publication of "Healthy People 2000", specific goals focused on food safety and reducing illnesses associated with food borne diseases were introduced (U.S. Department of Health and Human Services, 1991). Review of the achievements obtained from the Healthy People 2000 and 2010 initiatives, showed improvements in food safety and the reduction of some food borne illnesses, but also identified areas requiring additional improvements (U.S. Department of Health and Human Services, 2000; 2010). To help achieve Health People 2020 goals, USDA-FSIS was charged with reducing the amount of food borne illnesses caused by poultry, and instituted policies to help achieve a 25% reduction in human salmonellosis cases and a 33% reduction in human campylobacteriosis cases attributed to poultry by the year 2020 (USDA, 2015).

In the US, it is estimated that foodborne pathogens are responsible for approximately 9 million illnesses, more than 50,000 hospitalizations and 1000 deaths, annually (Scallan, et al.,

2011) and three of the four highest ranking causative agents for these illnesses (*Salmonella*, *Campylobacter* and *Clostridium*), are pathogenic organisms in which poultry serve as natural reservoirs/sources (Hayama et al., 2011). In a report released by the Interagency Food Safety Analytics Collaboration (IFSAC) Project, four pathogens including *Salmonella* and *Campylobacter* were identified as high priority due to the severity of the illnesses that can occur, as well as the impact intervention strategies may have on the persistence and proliferation of these organisms (IFSAC, 2015). Additionally, on-going surveillance performed by the Foodborne Diseases Active Surveillance Network (FoodNet) identified *Salmonella* and *Campylobacter* bacteria present on raw poultry as leading causes of foodborne disease and illness (CDC, 2015).

Further highlighting poultry as a source of food borne illnesses, Batz et al., (2012) used attribution and epidemiology studies to determine estimates of the number of illnesses, hospitalizations and deaths as well as cost and quality-adjusted life year (QALY) estimates associated with common pathogen-commodity pairs. From these calculations, it was determined that the *Campylobacter*-poultry pair ranked number one and that *Salmonella*-poultry pair ranked number four in the top 50 pathogen-commodity pairs (Batz et al., 2012). Additionally, in attribution analyses obtained from outbreak data, Painter et al., (2013) determined that poultry commodities caused more deaths from foodborne infections than any other commodity. Numerous researchers, industry and regulatory agencies agree that poultry commodities have an inherent amount of risk to cause food borne disease, (Cox et al., 2011), USDA-FSIS agencies have been charged with reducing the risks associated with consumption of these products so that any risks can be minimized or eliminated (USDA, 1996).

Food safety has long been recognized by government bodies as a public health concern, and as the amount of information that has been disseminated to the public has increased, due to the increased numbers of media outlets and sources of public information, the attention the subject receives from the general population has intensified in recent years (Durrant, 1995; Rutsaert et al., 2013; Ricke et al., 2015; Torrence, 2003). As outbreaks have occurred, the identification and attribution of certain commodities as causative agents in certain types of food borne illnesses have become widely accepted as sources of disease and infection (Batz et al., 2012; 2014). As *Campylobacter* and *Salmonella* infections are routinely identified as being caused by the consumption of raw poultry products (Batz et al., 2012) or from other foods contaminated by raw poultry products (Buncic and Sofos, 2012), the recognition that this commodity has some inherent risk in its raw form have only increased as the reliance on poultry as a protein food source has increased (Barbut, 2002; Cox et al., 2011; Durrant, 1995; Foley et al., 2011).

The increase in poultry consumption can likely be attributed to many factors, including the ever-growing human global population as well as the social and dietary shifts that have occurred in large segments of this growing population (Chen and Wang, 2003; Durrant, 1995; Nychas 2008; Kearney 2010; Rask and Rask 2011). In return, increased demand for poultry products has caused exponential growth in the U.S. poultry industry (Luján-Rhenals et al., 2017), and over the last two decades, production rates have had an average increase of 2.4% per year (Zhuang and Moore, 2015). This has resulted in a 23.7% increase in the number of pounds of poultry being inspected in the last two decades (Smith, 2014). The commodity's increased demand along with the increased rate (13.0%) of campylobacteriosis infections when 2014 rates were compared to 2006 to 2008 incidences, as well as the relatively steady rate of salmonellosis infections (CDC,

2015) have led regulators to evaluate policies and procedures related to the production and processing of this commodity (USDA, 2014).

2.5 Poultry Processing: History of Inspection and Regulatory Compliance

There is evidence to suggest the sale of unwholesome or contaminated meat was not an accepted practice as far back as the 12th century, actual legislation did not come about until the industrialization age evolved in European countries in the 19th century (Bilgili, 2010). Generally, the early days of large scale poultry production would likely be considered rudimentary and profit driven with limited focus on the food safety aspects of the processes (Sinclair, 1906). With the publication of Sinclair's "The Jungle" (1906), many aspects of the poor conditions in slaughterhouses and meat packing became public knowledge, which led to an increased focus toward food safety and animal welfare for these operations.

Over the next 50 years, voluntary inspection programs and regulations increased and in 1957, mandatory inspection for interstate poultry products was required under the "Poultry Products Inspection Act" (United States Code, 2014). Under this act, antemortem and postmortem inspections were established, as well as inspections related to the general processing operations, sanitation procedures and labeling requirements. These laws covered interstate products, but inspections for intrastate poultry products did not become mandatory until the 1968, under the "Wholesome Poultry Act" which required all states to maintain standards and inspection programs equivalent to those required for the federal programs (United States Code, 2014).

The antemortem, postmortem, operations, sanitation, and labeling inspections continued to be carried out under the authority of USDA-FSIS, with little change in policy or associated procedures even as the industry's size and the complexity of the processing systems had evolved.

Debates about the values of extensive visual/organoleptic inspection of every bird/carcass became commonplace within the industry and in response, USDA-FSIS charged the National Research Council (NRC) with performing an independent evaluation of the current policies and programs. In 1987, the NRC concluded that risk-based inspection could be utilized to address any public health concerns within the poultry processing industry (NRC, 1987). This gave USDA-FSIS the authority to vary inspection polices in processing plants based on that facility's history of compliance to the standards, product type(s) and personnel commitment, but also determined whether the establishment of more scientific based inspections would be needed to further improve the systems (NRC, 1987). In 1996, with implementation of the Pathogen Reduction and Hazard Analysis Critical Control Point (PR-HACCP) system's final rule, facilities were charged with establishing HACCP plans to address any specific hazards associated with poultry processing systems as well as meet performance standards established to reduce pathogens associated with the products (USDA, 1996).

Based on the information gathered by researchers and regulatory bodies it has been concluded that while a processor should work to ensure that all food produced is safe for human consumption, complete elimination of all microbiological hazards may not be possible as targeted pathogens are not able to be eliminated in a raw poultry processing system (Buncic and Sofos, 2012; Selgas et al., 1993; USDA, 1996). Ongoing evaluations performed by USDA-FSIS have been able to establish prevalence levels of targeted pathogenic organisms and other indicator organisms within the production process of young broilers, and these levels have been used to establish qualification measurements to determine how a processor is performing (USDA, 1999; 2004; 2009).

The baseline evaluation performed in 1994 (prior to PR-HACCP final rule) concluded that levels of APC microorganisms on raw poultry after chilling and prior to any further processing, were typically in the range of 3.0 to 4.0 log (USDA, 1994). Generic *E. coli* was found in 99.6% of the samples, with the majority (85.0%) of these samples having less than or equal to 100 cfu/mL. For those samples positive for *Campylobacter* (88.2%) or *Salmonella* (20.0%), the majority (87.3%) that were positive for *Salmonella* had less than 0.30 MPN/mL of that organism and the majority (61.3%) that were positive for *Campylobacter* had less than 30.0 MPN/mL of that organism. With these findings, the agency concluded that although the pathogens were present, the levels were low enough that proper cooking and handling could render any contaminated products safe to consume (USDA, 1994).

In a follow-up baseline evaluation performed in 1999 to 2000 (after the implementation of PR-HACCP final rule), young chickens were evaluated for *Salmonella* and generic *E. coli* prevalence (USDA, 1999). *Escherichia coli* was detected in 95.3% of the samples, with the majority (93.5%) of those samples having less than or equal to 100 cfu/mL and *Salmonella* was detected in only 8.7% of the samples (USDA, 1999). These findings reveal a reduction in *E. coli* and *Salmonella* levels when compared to the results from the 1994 baseline, but also highlighted that while improvements in processing have been able to reduce the populations of pathogenic organisms present on raw poultry, complete elimination of pathogenic and spoilage organisms from this commodity may not be possible (USDA, 1999).

Baseline evaluations performed in more recent years have expanded testing to include other microorganisms to better understand the microbiological profile of poultry carcasses processed in the US (USDA, 2007). Along with determining the prevalence of *Salmonella* and *E. coli* levels on post-chill carcasses, the sampling also included testing for *Campylobacter*, APC and

coliform microorganisms from both rehang (post-picker, pre-evisceration) and post-chill carcasses (USDA, 2007). Carcasses collected from post-chill locations, were 97.07% positive for APC, 57.40% positive for *Enterobacteriaceae*, 47.82% positive for coliforms and 38.66% positive for *E. coli*. When comparing *Salmonella* and *Campylobacter* incidence on carcasses from re-hang to post-chill carcasses, levels were reduced by 35.51% and 60.70%, respectively. Overall, prevalence levels for *Salmonella* and *Campylobacter* on post-chill carcasses were determined to be 7.5% with a standard error of 0.43% and 46.7% with a standard error of 0.87%, respectively.

By determining prevalence levels of *Salmonella* and *Campylobacter* of post-chill poultry carcasses, agencies were provided the opportunity to establish set points by which to measure changes over time (USDA, 2007). These prevalence levels and associated changes are used for the development of risk calculations and associated risk management practices, which help determine policies and procedural changes that may be necessary to ensure that risk to human health is mitigated (USDA, 2007). Comparisons of baseline samplings performed in 1994, 1999, and 2007 (USDA, 1994; 1999; 2007) have shown that while there was a 11.85% reduction (20.0% reduced to 8.15%) in *Salmonella* prevalence on post-chill poultry carcasses, the number of illnesses caused by *Salmonella* has remained relatively constant, and the number of illness caused by *Campylobacter* have increased by 13% since 2008 (CDC, 2015). While raw poultry is not the only commodity that poses a risk of causing salmonellosis and campylobacteriosis, it has been linked to 145 *Salmonella* and 6 *Campylobacter* outbreaks between 1998 and 2008, or 49.9% of the 309 outbreaks identified as being caused by bacterial etiological agent from poultry products (MMWR, 2013). Furthermore, at 18.9%, poultry was the commodity most commonly implicated in *Salmonella* illnesses reported to The Foodborne Disease Outbreak Surveillance

System (FoodNet) between 1998 and 2008 (MMWR, 2013) and estimation models utilizing FoodNet data from 2000 through 2008, reported that 1.0 million of the 9.4 million annual food borne illnesses could be attributed to poultry (Scallan et al., 2011).

2.6 Poultry Processing: Good Manufacturing Practices and HACCP

Commercial operations for processing poultry carcasses for human food consumption requires an extensive use of equipment and automation in order for the process to remain efficient and cost-effective (Barbut, 2002; Erdtsieck, 1995; St. Cyr, 2003). To help maintain efficiencies and produce final products with the desired level of quality, establishments operate under a systemic set of principles typically referred to as good hygiene practices (GHP) or good manufacturing practices (GMP) (Bolton et al., 2014; Escudero-Gilete et al., 2013; St. Cyr, 2003). These practices dictate certain requirements from personnel and equipment within a processing system to create standardized operations and universal practices to ensure that factors negatively effecting the microbiological profile of the product, are removed or controlled, while also continuously working to reduce the microbiological load present (Belluco et al., 2016; Bolton et al., 2014; Chen and Wang, 2003; St. Cyr, 2003). Specifically, within a poultry processing operation, GMP practices serve to promote the use of good hygiene practices and intervention systems so that the risks of cross-contamination between individual carcasses and multiple flocks are reduced or mitigated (Barbut, 2002; Bolton et al., 2014, Buncic and Sofos, 2012; Escudero-Gilete et al., 2013). These systems work in conjunction to provide a multi-hurdle system that works to continuously reduce or eliminate populations of pathogenic and/or undesirable microorganisms by the time the bird has been processed into its final product (Barbut, 2002; Buncic and Sofos, 2012). Any stage of the process where personnel and/or equipment are handling the birds, carcasses, parts or final products, or where the birds, carcasses, parts, or final

products are allowed to comingle have the potential to represent a possible point of cross-contamination (Barbut, 2002; Buncic and Sofos, 2012; Cunningham, 2012; Hayama and Tsutsui, 2011; Mead, 1995) and subsequently, considerable emphasis is placed on maintaining and improving GMP related to equipment design, wash systems and process flows to help mitigate these risks (Buncic and Sofos, 2012; Simonsen, 1995).

Along with a prerequisite GMP program, a processor is also required to have a HACCP program which measures, as well as prevents or eliminates hazards and identifies Critical Control Points (CCP) which may pose a potential risk to the product (Bolton et al., 2014; Curtis, 2005: Heggum et al., 2015). A HACCP program provides a systematic approach to the monitoring and corrective action system by which the process is verified to be operating as intended (Gould, 1990; Heggum et al., 2015; Hui et al., 2003; Simonsen et al., 1987). While there are many factors that must be considered when determining how to assess the effectiveness of a process and control of that process, at a minimum, assessments will include frequent monitoring and data collection (Hui et al., 2003; Simonsen et al., 1987; Tompkin, 1990). One aspect of data collection results in the collection of samples from the process in review and for a poultry processing facility, this would include the collection of carcasses and/or associated parts and organs (Simonsen et al., 1987; Tompkin, 1990).

In order to determine if control systems are functioning as intended, FSIS personnel visually inspect as well as perform microbiological verification testing on applicable stages of the production process (slaughter, first processing, and further processing) to determine prevalence levels of target organisms, *Salmonella* and *Campylobacter* (USDA, 2014; 2015; 2016). The data is utilized to categorize processing facilities in terms of process control. Processing facilities that have a history of operating 50% below the regulatory standard performance levels in the last 3

months (in a 52 week moving window), are considered to have a system under consistent process control and fall under the title of Category 1 (USDA, 2016). A facility that is currently operating at the regulatory standard performance levels, but had been 50% over the performance standard level in the last 3 months (in a 52-week moving window), is considered to a have a system under variable process control, or Category 2 (USDA, 2016). A facility that has exceeded the regulatory standard performance in the last 3 months (in a 52-week moving window), is considered to have a highly variable process control, or Category 3 (USDA, 2016).

2.7 Poultry Processing: Modernization of Poultry Slaughter Inspection

To help facilitate the reduction of *Campylobacter* and *Salmonella* in poultry products, updated inspection programs have been outlined in the Federal Register (FR) under the title, Modernization of Poultry Slaughter Inspection (USDA, 2014). Under this final rule, the New Poultry Inspection Program (NPIP) requires processors to perform assessments on carcasses and parts and identify any quality defects prior to on-line inspections, allowing more time for offline, process control inspections (USDA, 2014). With these rules, the online carcass inspector (CI) would be allowed more time to effectively inspect carcasses or parts since evaluations had already been performed by plant personnel (USDA, 2014). Additionally, an offline verification inspector (VI) is required to evaluate all procedures related to sanitation, HACCP and process controls for compliance and ensure that the facility is managing its operation such that it meets all regulatory demands (USDA, 2014).

Under NPIP, establishments are also required to assess and implement strategies to prevent carcasses with visible fecal material from entering the chiller (USDA, 2014). These strategies, including both on-line reprocessing and off-line reprocessing, must include detailed procedures and policies and be incorporated into the facility's HACCP systems (USDA, 2014). Assessment

of these procedures will include (at a minimum) microbial testing of carcasses just prior to entering the chiller (pre-chill) and carcasses exiting the chiller (post-chill), along with other verification activities to ensure that the programs are performing as intended (USDA, 2014). In the past, assessment of enteric microorganisms present within a poultry slaughter process were based on the mandated enumeration of generic *E. coli* organisms (USDA, 1996), but under NPIP, an establishment will now be able to choose the microorganism(s) by which to assess process control (USDA, 2014). Additionally, under NPIP, the FSIS is removing specific time and temperature chilling requirements and instead, establishments will be required to address chilling requirements in associated HACCP systems (USDA, 2014).

2.8 Poultry Processing: New Performance Standards for Non-Ready to Eat Comminuted Chicken and Chicken Parts

During 2011, widespread salmonellosis outbreaks in the U.S. were attributed to the consumption of comminuted turkey products which prompted federal investigations into the production processes of both comminuted turkey and chicken products (USDA, 2012). After performing investigations of the processes and the producers of the implicated products, findings prompted USDA-FSIS to require establishments producing comminuted poultry product to reassess HACCP programs in regards to *Salmonella* control (USDA, 2012). At this time, establishments were also notified that the categorical performance labeling (Category 1, 2, 3) used for ground poultry could also be applied to comminuted product as previous performance standards were based solely on compliance or non-compliance (USDA, 2012). The agency also indicated that on-going assessments of these product types would likely result in changes in the performance standards for *Salmonella* and *Campylobacter* (USDA, 2012).

In February 2016, USDA-FSIS published implementation schedules in which performance standards and verification activities for non-ready to eat (non-RTE) comminuted poultry and for chicken parts would be instituted (USDA, 2016). To achieve a 30% reduction of human salmonellosis and campylobacteriosis infections caused by poultry products, performance standards of non-RTE comminuted chicken samples would be assessed on a 52-sample window with allowable prevalence levels of 25% and 1.9% for *Salmonella* and *Campylobacter,* respectively. Likewise, for a 52-sample window, chicken parts were given allowable prevalence levels of 15.4% and 7.7%, for *Salmonella* and *Campylobacter*, respectively. Furthermore, the agency would begin to post results of a facility's compliance performance, as well as perform risk assessments and food safety audits as necessary (USDA, 2016).

2.9 Microbiology of Processed Poultry

The colonization and proliferation of several pathogenic and spoilage-related microorganisms within the GIT of birds (both wild and domestic) are readily recognized as being problematic to the poultry industry (Bailey, 1988; Bolder, 1998; Buncic and Sofos, 2012; Cunningham, 2012; Dunkley et al., 2009; Horrocks et al., 2009; Newell and Davison, 2003; Rabsch et al., 2003). While typically not problematic to the health of the birds, the introduction of these microorganisms into the processing system can have a profound effect on the microbiological quality of the final product (Buncic and Sofos, 2012). As previously discussed, the intricacies involved in the poultry production process can lead to problems of cross contamination (Bauermeister et al., 2008; Bolder, 1997; Davies and Wray, 1994; Olsen et al., 2003;) and dissemination of microorganisms through the entire processing system (Buncic and Sofos, 2012; Parveen et al., 2007).

The necessity to ensure that the slaughter and production processes, and any associated interventions are operating as intended as well as verifying that the facility is operating within the standards required by regulatory bodies mandates the need to perform microbiological evaluations of various stages within a commercial broiler operation (Chipley, 1987; Cox and Pavic, 2009; Lillard, 1990; González-Miret et al., 2001; Oakley et al., 2013). Microbiological evaluations are typically performed by analyzing samples from a variety of matrices using culturing methods or molecular techniques or a combination of both (Oakley et al., 2013). Samples collected within the live poultry production stage will obviously differ from the samples collected within the poultry slaughter and processing stages. Furthermore, sample collections performed as part of a verification program will typically be analyzed for one or more target organisms, while sample collections performed as part of a whole or segmented process evaluation will routinely be analyzed for target organisms and other indicator microorganisms (Cox and Pavic, 2009; Martins and Germano, 2008; Todd, 2004).

2.9.1 Pathogenic Organisms in Poultry Processing

The *Campylobacter* genus is a diverse genus comprised of Gram-negative, curved, rod-shaped bacteria with a distinct spiral or curved shape and a distinct spiral or corkscrew-like motility (Barbut, 2002; Bolton, 2015; Corry and Line, 2015; Park, 2002; Sahin et al., 2003). Of all the species, the thermophilic organisms, *Campylobacter coli* and *Campylobacter jejuni,* are the most important species in relation to food safety (Corry and Line, 2015; Sahin et al., 2003). Campylobacteriosis infection in humans is typically characterized by self-limiting diarrhea, cramping and fever, but in some patients with compromised immune systems or other contributing factors, more severe symptoms such as septicemia, arthritis, or Guillain–Barré syndromes may develop (Altekruse et al., 1999; Janssen et al., 2008). *Campylobacter* is

commonly found in the GIT of warm blooded animals and can often be isolated from contaminated water and sewage (Barbut, 2002; Corry and Line, 2015; Kaneene and Potter, 2003; Sahin et al., 2003). Generally, this genus survives and proliferates only under somewhat limited temperature and pH ranges and has relatively specific nutrient and oxygen requirements (Corry and Line, 2015; Park, 2002; Wesley, 2009).

Identifying the routes of transmission and colonization of human enteric pathogens of commercial broiler flocks has been studied extensively for many years (Oakley et al, 2013; Olsen et al., 2003). As *Campylobacter* has been identified as one of the most common causes of foodborne illness in the United States (Altekruse et al., 1999), and the colonization of *Campylobacter* organisms commonly occurs in poultry (Kaneene and Potter, 2003), there is an obvious connection between the consumption of poultry meat and an increased risk in campylobacteriosis infection (Altekruse et al., 2009; Horrocks et al., 2009). As *Campylobacter* is a commensal organism of many animals and birds, as well as insects (Hald et al., 2004; Horrocks et al., 2009), and has been isolated from water sources used for commercial farms (Altekruse et al., 2009), horizontal transmission of a commercial flock would appear to be a predominant mode of transmission (Herman et al., 2003; Newell and Fearnley, 2003). Horizontal transmission of *Campylobacter* infection has been demonstrated to some extent, but direct linkage of genotypes in the environment to those genotypes that have colonized poultry in a commercial setting is still lacking (Stern et al., 2001). This lack of association between the genotypes in the environment to the genotypes colonizing the birds may be due to the susceptibility of *Campylobacter* to competitive exclusion and environmental conditions, resulting in a low-level prevalence of *Campylobacter* genotypes within the environment (Davies, 2005). There is experimental evidence to suggest that vertical or pseudo-vertical transmission

can occur either via oviduct colonization or from microorganisms penetrating the egg shell, but evidence that this leads to infected chicks at hatch is limited and subject to debate (Davies, 2005; Kaneene and Potter, 2003; Newell and Fearnley, 2003). Regardless of the route of transmission, high colonization rates of a flock after an initial inoculation are likely due to the host's susceptibility to the organism as well as shedding of the bacteria (Kaneene and Potter, 2003).

Salmonella is a Gram-negative, facultative, bacilli microorganism (Galis et al., 2013) comprised of over 2500 serovars, divided into two species: *S. enterica* and *S. bongori* (Foley et al., 2013). *S. enterica* subspecies *enterica* comprises the majority of serovars that have the potential to be pathogenic to birds and mammals (Brenner et al., 2000; Foley et al., 2013). Commonly found in the GIT of animals, birds, and humans, *Salmonella* spp. are excreted with feces, and transferred to water, insects and other animals in the environment (Barbut, 2002; Buncic and Sofos, 2012). For those serovars known to cause infection or illness in humans, it is generally understood that the leading source of meat and poultry contamination is from gastrointestinal contents, feathers, hair, skin and debris on the surface of the animals being transferred to carcasses and equipment during processing (Barbut, 2002; Buncic and Sofos, 2012; Carrasco et al., 2012; Rabsch et al., 2003).

After meat has become contaminated with *Salmonella*, limiting the proliferation and/or eliminating the bacteria is required in order to ensure that the meat consumed will not cause harm when ingested (Barbut, 2002; Carrasco et al., 2012). However, certain characteristics of some serovars of *Salmonella* may hinder the effectiveness of intervention measures (Calhoun and Kwon, 2010; Jarvis et al., 2016). Some *Salmonella* serovars are more readily capable of adhering to both processing equipment and poultry skin (Chia et al., 2009; Marin et al., 2009; Tamblyn and Conner, 1997) thereby becoming embedded within niches or biofilms that limit the

microorganisms contact with any given intervention (Diez-Garcia et al., 2012; Marin et al., 2009; Schonewille et al., 2012; Steenackers et al., 2012). Under stress, some *Salmonella* microorganisms will undergo genetic mutations or genetic transfers that enable some strains to survive and proliferate (Cosby et al., 2015). As the genus can survive and thrive in a wide range of refrigeration temperatures (Barbut, 2002), there is a greater risk of microorganisms multiplying during storage to reach levels that could cause illness if the contaminated product is consumed (Pradhan et al., 2012). Additionally, some *Salmonella* serovars can proliferate over a wide range of pH levels and can be relatively heat resistant, thereby necessitating cooking to prescribed timing and temperature requirements (Barbut, 2002; Jarvis et al., 2016). Assigning specific time and temperature requirements must consider the different serovars that could be present, as well as the specific food matrix, and variability of the cooking equipment (Jarvis et al., 2016). Also of concern, is the risk of cross-contamination of other food products during processing and food preparation, exemplifying the necessity to maintain "dirty" or "raw" and "clean" or "fully cooked" divisions within both commercial processing, retail and within the consumer's homes (Barbut, 2002; Buncic and Sofos, 2012; Carrasco et al., 2012; Cosby et al., 2015; Mead, 1995; Zweifel and Stephan, 2012).

2.9.2 Spoilage Organisms in Poultry Processing

Fresh poultry has a relatively short shelf-life of 4 to 5 days (Bolton, et al., 2014 Fernández-Pan et al., 2014) and in the absence of intervention or control measures during processing and subsequent storage, spoilage microorganisms may proliferate and compromise product quality (Hinton et al., 2004; Veerkamp, 1995). Microbiological spoilage of poultry typically results in a 7.0 to 8.0 $\log_{10}$ per cm dominance of psychrotrophic bacteria, namely *Pseudomonas*, which are aerobic, Gram-negative, rod shaped microorganisms that can grow and

multiply at relatively low temperatures (20 to 30 C˚) (Hinton et al., 2004; Lopez et al., 2015; Mohareb et al., 2015; Oyarzabal and Hussain, 2010; Russell, 1996). The growth and multiplication of these microorganism adversely affect product by producing by products that lead to the proliferation of foul odors, textural changes and rancidity in the product (de Boer et al., 2015; Remenant et al., 2015). The prolificacy of *Pseudomonas* in fresh poultry products is largely due to specific phenotypic properties of many of the species including proteolytic, lipolytic, saccharolytic, and biosurfactant mechanisms which allow certain species to persist and thrive in refrigerated poultry products (Lopez et al., 2015; Morales et al., 2016; Remenant et al., 2015)

2.9.3 Indicator Organisms in Poultry Processing

While specific pathogens of concern in a poultry processing environment would be those known to cause human illness, there are several reasons why those organisms may not be the most logical organisms to quantify (Cason et al., 1997). As pathogenic bacteria such as *Salmonella* and *Campylobacter* generally occur in low numbers and are not uniformly distributed on the carcass or within the processing system, enumeration of these organisms from poultry samples would require extensive sampling to ensure complete absence from the product or processing system (Cason et al., 1997, Oyarzabal and Hussain, 2010; Schaffner and Smith-Simpson, 2014). Instead, efficacy studies are usually performed by enumerating other organisms also known to colonize the GIT of poultry (Cason et al., 1997). For example, *E.coli* and other coliforms have proven to be relatively easy to identify and quantify while also occurring at high enough levels to be used as process control indicators (Altekruse et al., 2009). However, the appropriate choice of indicator organisms continues to be debated as there is data that suggests little correlation exists between indicator organisms such as aerobic plate count (APC),

Enterobacteriaceae, and *Pseudomonas* and the hygienic quality of the process and associated sanitary dress of the carcasses produced (Cason et al., 1997; Milios et al., 2014). Additionally, bacterial culturing may have limited value in process evaluations as some microorganisms may become dormant and not be culturable in routine microbiological evaluations (Ayrapetyan and Oliver, 2016; Hutchison et al., 2006; Oakley et al., 2013). A microorganism's state of viable but not culturable (VBNC) may be induced under environmental stresses which could allow for enhanced resistance to interventions, while still maintaining virulent properties (Ayrapetyan and Oliver, 2016; Kassem et al., 2013; Saucier, 2016; Trevors, 2011).

2.9.4 Epidemiology of Pathogenic Bacteria in Poultry Processing

Campylobacter colonization of poultry can occur via a variety of sources, but contaminated water is thought to be a major contributing factor (Hazeleger et al., 1998; Kaneene and Potter, 2003: Newell and Fearnley, 2003). Water sources on a commercial poultry farm are typically subject to some sort of treatment in order to reduce the likelihood of bacterial contaminants (Newell and Fearnley, 2003), but contamination can still occur due to a myriad of factors, including transmission by wild animals or insects, introduction of water from untreated water sources (groundwater), and lack of hygiene/biosecurity measures by workers/farmers when working in more than one poultry house or when other animals are present on the farm (Davies, 2005; Kaneene and Potter, 2003). Once ingested, *Campylobacter* is known to colonize the GIT, although the epithelial cells in the ceca and intestines may be more susceptible to colonization than others areas of the GIT (Bolton et al., 2015; Byrd et al., 2001; Hargis et al., 1995; Newell and Fearnley, 2003). Campylobacter colonization of the ceca can occur relatively quickly (within 24 hours) after infection, which is likely due to the helical cell shape and flagella which propel cells through the gastrointestinal environment (Bolton et al., 2014). Stress responses

allowing for adaptation of the environment within the GIT, including chemotaxis movement toward more favorable environments also aid in *Campylobacter* survival and persistence within this environment (Bolton et al., 2014) Poultry meat likely becomes contaminated during processing as the microorganisms from the feathers, skin, fecal and ingesta of infected carcasses, are disseminated during the production process by aerosolization, equipment reuse, and co-mingling of carcasses in scalder and chiller waters (Berrang et al., 2000; Buncic and Sofos, 2012; Hargis et al., 1995).

The processing of an infected flock can lead to the spread of the *Campylobacter* throughout the processing system, which can lead to the spread of the microorganism(s) to other carcasses being processed on the same day or subsequent days if cleaning and sanitation processes are not adequate to remove all microorganisms from the processing equipment (García-Sánchez et al., 2017; Peyrat et al., 2008). There is also evidence to suggest that certain physiological adaptations of *Campylobacter* aid movement toward favorable substrates so viability of the microorganism in stress environments is enhanced (Bolton et al., 2014; Hazeleger et al., 1998).

Broiler poultry flocks are typically infected with *Salmonella* by one or a combination of sources: laying hens and breeding stock, direct or indirect contact with infected animals (wild or domestic), insects or contaminated water, and contaminated feed products (Barbut 2002; Davies, 2005; Dorea et al., 2010; Simonsen, 1995). After ingestion and subsequent infection, the microorganisms migrate to the lower parts of the GIT, and are excreted with fecal material (Mead, 1995), although *Salmonella* has also been observed in the livers, gallbladders and spleens of infected poultry (Cox et al., 2007).

It is understood that the *Salmonella* infection status of the breeder flock providing the hatching eggs plays an important role in determining the presence and persistence of *Salmonella*

infections in the chicks hatched from those eggs (Davies and Wray, 1994; Galis et al., 2013; Wesley, 2009). Commercial operations usually make every attempt to ensure that the eggs received for hatchery operations are from low prevalence or *Salmonella*-free breeding stock (Davies, 2005), but when eggs from an infected flock are received, hatchery cleaning and sanitation should be sufficient enough to ensure that any *Salmonella* present is at low enough levels to not pose a threat to future flocks (Davies and Wray, 1994). However, improper or poor air ventilation as well as improper or poor cleaning and antimicrobial procedures in the environment within commercial laying and hatching operations can easily compound opportunities for cross-contamination (Davies and Wray, 1994).

2.9.5 Epidemiology of Spoilage Bacteria in Poultry Processing

Relative abundance of psychrophilic bacteria, especially species belonging to the *Shewenella*, *Moraxella, Acinetobacter* and *Pseudomona*s genera, on refrigerated raw poultry increases when compared to poultry products directly after processing (Barnes, 1972; Hinton et al., 2004; González-Miret et al., 2001; Russell, 1996). This is not necessarily surprising as these organisms thrive and proliferate in temperatures between -2°C and 32°C (Barnes, 1972; Bautista and Tortorello, 2014; Oyarzabal and Hussain, 2010). The isolation of psychrophilic microorganisms from live production areas (including feet and feathers of live poultry) and processing environments, indicate their presence and proliferation on raw poultry is a cross-contamination and temperature control issue (Hinton et al., 2004; Mead, 1995; Nychas et al., 2008; Zhang et al., 2017). Furthermore, generation time of certain psychrophilic microorganisms can be 30% shorter than other microorganisms, so once established, proliferation can occur at a rate that leads psychrophilic microorganisms to dominate in the spoiled product (Oyarzabal and Hussain, 2010; Russell, 1996). These microorganisms metabolize a variety of energy sources present

within the processed meat to produce end products that cause adverse sensory attributes including off-odors, discoloration and slime (Bautista and Tortorello, 2014; Nychas et al., 2008; Russell 1996).

Spoilage of fresh poultry may also be influenced by the presence and proliferation of yeasts (Hinton et al., 2002). While typically outnumbered on frozen or chilled poultry by psychrophilic bacteria, the metabolic activities of yeasts could prove to be detrimental to the lipids and proteins within the product as well as provide materials that could be used as substrates for other microorganisms thereby aiding in the establishment and proliferation of those microorganisms (Hinton et al., 2002).

2.9.6 Microbiological Evaluation of Multi-Hurdle Processing Systems

Process evaluations in the form of microbiological analysis or process biomapping, are an integral part of any food safety program for a poultry processor and may be utilized for both troubleshooting exercises (Belluco et al., 2016; Heggum et al., 2015), process performance indicators (González-Miret et al., 2001; Sampers et al., 2010), as well as categorical labeling of individual facilities reported by governmental agencies (USDA, 2016). Applying microbial evaluations to a multi-hurdle poultry processing system, along the farm to fork continuum, will require the use a variety of sampling methodologies and analyses (Cox et al., 2011; Sampers et al. 2010). Considerable debate on how to perform sampling within the live production environment (Davies and Wray, 1996) and slaughter systems has resulted in the evaluations and comparisons of various sampling methods (Capita et al., 2004; Carrique-Mas and Davies, 2008; Izat et al., 1989; Russell et al., 1997). The differences between the results from research studies, regulatory and industry sampling may differ due to the sample types being collected (live production versus finished product) as well as by program specification (USDA-FSIS regulatory

programs versus industry), researchers' preferences, and even lack of uniformity within the samples (Corry et al., 2007a).

2.9.7 Sampling Methods for Evaluating Multi-Hurdle Poultry Processing Systems

Environmental sampling within the live production areas provides insight into the efficacies of intervention measures put into place to mitigate cross-contamination between flocks (Davies and Wray, 1996; Slader et al., 2002; Stern et al., 1995). With the vast space and numerous types of equipment involved with live production areas, efficient, yet effective sampling is important to understand true incidence rates of pathogenic bacteria as well as the effectiveness of any intervention treatments utilized within this environment (Davies and Wray, 1996). Several types of sampling techniques may therefore be used to sample one broiler house (Carrique-Mas and Davies, 2008; Davies and Wray, 1996; Lahellec and Colin, 1985). The resulting different types of samples will inherently have different culturing sensitivities which may lead to artificially skewed recovery levels of target organisms during microbiological evaluations (Carrique-Mas and Davies, 2008; Davies and Wray, 1996; Lahellec and Colin, 1985). In evaluations performed within a poultry house by Davies and Wray (1996), environmental swabbing of feed hoppers and nesting areas, yielded higher incident rates of *Salmonella* when compared to litter from the same houses. However, Lahellec and Colin (1985) compared *Salmonella* incidence from collected litter samples to water retrieved from the drinker systems, and found that the litter samples demonstrated higher prevalence rates of *Salmonella* compared to the water samples, 27.1% compared to 17.9%, respectively.

As a bird or carcass within the processing system will not have a homogenous microbiological population, and sampling of both the internal and external portions of the carcass are necessary to perform accurate microbiological assessments, common sampling methods have

utilized tissue swabs, nitrocellulose membrane transfers, excisions followed by blending, and WBC rinses (Izat et al., 1989; Russell et al., 1997; Sarlin et al., 1998). Comparisons of these methods have shown that not all methods will result in comparable or unequivocal evaluations, especially when the target organism(s) are present in low numbers (Izat et al., 1989; Russell et al., 1997). Additionally, depending on the frequency of sampling, destructive sampling (excision and blending) is usually unfavorable as the excision could lead to damaged product which could prove to increase the overall cost of the evaluations (Russell et al., 1997). Whole bird carcass rinses or parts rinses have proven to be informative by providing an overall evaluation of the whole carcass (Sarlin et al., 1998), but may be limiting in determining which area(s) of the carcass are the major contributors to the microbial counts (Smith et al., 2007).

Process evaluations may also be performed by capturing water or solutions used during the processing system, or by surface sampling of equipment within the processes being evaluated (Jackson, 2014; Sampers et al., 2010). Examples would be an evaluation involving the sampling of scald water to help determine the effect that the scalding process (Cason et al., 2000) has on the treated carcasses or monitoring the surface of equipment within the process to determine risk points related to cross-contamination (Sampers et al., 2010). While samples of these types do provide information regarding the microbial loads in the water and associated equipment, and can help to highlight possible routes of cross-contamination, the information gained from these sample types could be limited if the microbiological impact on the product is not monitored extensively as well (Cason et al., 2000).

2.9.8 Culture Dependent Analyses of Multi-Hurdle Poultry Processing Systems

Due to the complexity of poultry processing systems coupled with the diverse microbial ecology of the various stages, culture dependent evaluations will typically involve microbial

enumeration analyses for identification of specific spoilage, pathogens, or indicator microorganisms (Milios et al., 2014; Oyarzabal and Hussain, 2010). Quantifiable determination of microbial loads for poultry processing samples and associated materials can be performed a variety of ways, including serial dilutions of homogenized samples followed by enumeration on specified media, as well as MPN analyses in which serial dilutions are replicated and values are determined from the number of replicates that are positive for the target organism (Corry et al., 2007a; Oyarzabal and Hussain, 2010). For samples that may have a low number of the target organisms, prevalence levels can also be determined by enrichment and incubation followed by testing for the presence or absence of target organisms utilizing PCR methods or other selective methods (Corry et al., 2007a; Oakley et al., 2013; Oyarzabal and Hussain, 2010).

As *Salmonella* and *Campylobacter* have different physiological characteristics and metabolic requirements, samples requiring analysis of both *Salmonella* and *Campylobacter*, are divided into two samples. While measures to ensure homogeneity in the original sample can be utilized, the division of the sample, nevertheless results in the analyses being performed on two different, albeit closely related, samples. These sampling schemes can be further complicated by the fact that only aliquots of the original sample are used for analysis, and if the pathogens are present in low numbers, then there could be a reduced likelihood of the either of the samples containing the target pathogens (Simmons et al., 2003).

Regardless of the sample type or analysis methods, culturing limitations can arise due to the differences in selectivity of media used, incubation atmospheres and temperatures, especially when samples that typically contain a wide range of microorganisms are subjected to specific requirements to select for target microorganisms (Deusch et al., 2015; Oakley et al., 2012; Ricke et al., 2015). Due to the low pH and anaerobic conditions of poultry GIT, it is hypothesized that

only 20 to 50% of the microorganisms present within the GIT of poultry have been cultured (Oakley et al., 2012; Patterson and Burkholder, 2003; Ricke and Pillai, 1999; Wei et al., 2013). This could apply limitations to understanding shifts and changes of the microbial ecology of the poultry processing and dressing process as identification of all the microbiological members in the consortia will be limited to those cultured microorganisms (Ricke et al., 2015). Selectively culturing enriched samples containing a wide variety of microorganisms can be problematic if the enrichment, and subsequent culturing media are not selective enough to successfully isolate the target organisms or if selectivity is such that some serotypes are inadvertently selected for over others (Chon et al., 2012; Gorski, 2012; Oakley et al., 2012). However, selectivity must also be balanced with nutrient density while being respectful of injured cells as the unsuccessful resuscitation of these microorganisms may prove to severely limit the culturing potential within any given sample (Ayrapetyan and Oliver, 2016; Juste et al., 2008). Furthermore, while a number of culturing methods utilize a very basic growth media, the ingredients or concentrations of the ingredients as well as the atmosphere used to prepare the agar and culture the microorganisms, may substantially differ from the natural environment in which the microorganisms exist which could prove to be relatively toxic and limit the success of culturing methods (Connon and Giovannoni, 2002; Deusch et al., 2015; Ricke and Pillai, 1999). Disparities also arise when multiple strains of the same serotypes are present in a cultured aliquot, as metabolic rates and growth characteristics may differ from strain to strain in any given medium (Gorski, 2012).

2.9.9 Microbiome Mapping of Multi-Hurdle Poultry Processing Systems

The abundant number of microorganisms represented in a poultry processing system are derived from a variety of internal and external sources (Handley et al., 2015; Zhang et al., 2017).

Some microorganisms may be introduced at the farm, while others are inherently part of the processing facility, and still others are derived solely from the carcasses being processed (Handley et al., 2015). Additionally, the pressures exerted on the microorganisms during each step of the process, can influence proliferation or lack of proliferation, as well as dispersion within the processing system (Arnold and Silvers, 2000; Oakley et al., 2013; Todd et al., 2009). Identifying the microorganisms along with the genetic diversity, or microbiome (Dumas et al., 2011; Roto et al., 2015: Waite and Taylor, 2015) in a poultry processing system (from farm to fork) can be informative for food safety quality assurance planning as the genotypic identification and classification of human pathogens in the process may help further identify more prevention strategies that can work to eliminate these specific pathogens in the final product (Diaz-Sanchez et al., 2013; Kim et al., 2017; Oakley et al., 2014; Parveen, 2007; Rouger et al., 2017; Vongkamjan and Wiedmann, 2014). Identifying the microbiome of poultry processing can also aid in identifying areas of improvement related to the monitoring, enumeration and reduction of spoilage organisms within the process (de Boer et al., 2015; Ercolini, 2013; Mayo et al., 2014; Remenant et al., 2015). Furthermore, ecological analysis of the processing system can result in a microbiological process map which can provide a visual assessment of the specific microorganisms present, point of introduction and how dispersion through the processing system occurs, which can be informative in mitigating risks that may be prove to be detrimental to the quality and wholesomeness of the final product (Bokulich et al., 2015; Rouger et al., 2017).

The development and refinement of molecular identification techniques, offers a more advanced technology to better understand process control, the microbiological ecology of any given organism or system, as well as changes or shifts in microbial populations that may have

previously been studied by using culturing methods or molecular techniques targeting specific organisms (Deusch et al., 2015; Dumas et al., 2011; Ercolini, 2013; Kim et al., 2011; Leonard et al., 2015; Remenant et al., 2015; Ricke et al., 2015; Ricke and Pillai, 1999; Samarajeewa et al., 2015). Initial molecular subtyping or sequencing methods would have utilized Sanger sequencing techniques which resulted in fragmented DNA strands that could be separated based on size (Diaz-Sanchez et al; 2013; Hall, 2007). While Sanger sequencing allows for an increased sequence read length (up to 900 bp) the number of bp per run are limited, hindering the application to large sample sets outside of genome centers (Hall, 2007; Morozova and Marra, 2008; Wei et al., 2013). To overcome some of the limitations of Sanger sequencing, various next-generation sequencing (NGS) technologies have been developed, in which the amount of labor and expense involved in obtaining sequences as well as the analyzing and identification of sequences has been enormously reduced (Diaz-Sanchez et al., 2013; Ghanbari et al., 2015; Hellberg et al., 2013; Jarvis et al., 2015; Wei et al., 2013). The advancements have included an increase in the output of sequences per run so that as many as 1 trillion bp can be sequenced, where the Sanger method has a limited capacity of only producing thousands of bp per run (Foster et al., 2012; Wei et al., 2013). Relying on PCR technology to amplify genetic material (eliminating the need to perform culturing and isolation techniques) as well as applying barcodes to individual samples (multiplexinig), a single sequence run is capable of analyzing several sample sets at one time (Foster et al., 2012; Parameswaran et al., 2007). Additionally, due to the commercialization of this technology, the cost has been dramatically reduced, allowing for use in a broad range of applications (Ghanbari et al., 2015; Hellberg et al., 2013; Waite and Taylor, 2015; Voelkerding et al., 2009; Wei et al., 2013).

The application of NGS technologies to microbiome map poultry processing is in the beginning stages, but the possibilities of applying the technologies in this manner are likely to be of great importance to quality and food safety concerns within the poultry industry (Oakley et al., 2013; Kim et al., 2017; Park et al., 2016; Ricke et al., 2015; Rothrock et al., 2016; Wei et al., 2013). One NGS platform currently being utilized with poultry processing research is the Illumina MiSeq platform based on the amplification and identification of the highly conserved regions (V3-V4) of the 16S rRNA gene in bacterial cells (Gall-David et al., 2017; Kim et al., 2017; Park et al., 2016).

Utilizing NGS to evaluate the effects of prebiotic feed amendments on gastorintestinal microbiomes of poultry have provided a glimpse into specific microbiota changes that would have been previously studied with culturing or qPCR methods (Deusch et al., 2015; Park et al., 2016; Wei et al., 2013). Understanding these microbiota shifts and the subsequent effects exerted on the host, could help to provide more complete explanations about the effects of specific diet components (Deusch et al., 2015; Wei et al., 2013), specifically in regards to any effects on known foodborne pathogens (Park et al., 2016). In a study where pasture raised poultry were divided into three groups and fed either a control diet, or one of two diets supplemented with brewer's yeast as a probiotic, the resulting ceca sequencing data showed no statistically significant differences in the relative abundance of *Campylobacter* OTUs between the two yeast supplemented diets (1.43% and 1.78%). Conversely, the relative abundance of *Campylobacter* OTUs in the ceca samples from the control group were significantly lower, at 0.17%, than either of the supplemented diets (Park et al., 2016). Quantitative PCR analysis confirmed these findings, demonstrating that the birds fed the test diets had similar $\log_{10}$ counts of *Campylobacter* (3.85 ± 0.17 and 3.71 ± 0.17, Test Diet 1 and Test Diet 2, respectively), and

those values were smaller than the $\log_{10}$ counts (3.34 ± 0.16) observed in the birds fed the control diet (Park et al., 2016). Direct plating of samples on *Campylobacter* selective agar, Campy-Cefex, was also performed and although $\log_{10}$ values of the enumerated counts could not be directly compared to relative abundance values obtained with sequencing analysis, the researchers site previous research in which background growth on Campy-Cefex plates was not adequately inhibited and ascertain that this could reduce the accuracy of this enumeration method (Park et al., 2016).

During poultry processing, understanding the microbiome shifts that occur within each step could be informative in understanding the routes of cross-contamination for both pathogenic and spoilage microorganisms (Rothrock et al., 2016). As chiller and scalder tanks have been identified as points where cross-contamination can occur, understanding how these processing waters may shift during a processing day, could be informative to the processor so that risk of cross-contamination is reduced or mitigated (Rothrock et al., 2016). When scalder waters from three different days of processing were evaluated at a commercial poultry processing facility, the pooled samples showed a significant shift in microbiota from water collected at the start of the processing day compared to waters from the middle and end of the processing days (Rothrock et al., 2016). The scald water collected at the beginning of the processing day represented 8 different phyla operational taxonomic units (OTUs), with approximately 25% of the sequences belonging to the phyla *Firmicutes,* while samples collected from the middle and end of the processing days resulted in *Firmicutes* representing 99.7% and 95.5% of the OTUs, respectively. This is not too surprising as the GIT of poultry has been identified as being dominated by *Firmicutes* (Waite and Taylor, 2015) but could prove to be informative because many species in this phylum, belonging to the genus *Lactobacillus,* have been identified as influential in the

development of spoilage in poultry products (Remenant et al., 2015). Also, identified within these scalder samples was the thermophilic genus *Anoxybacillus,* which was also present in the chiller water samples *(*Rothrock et al., 2016*).* The presence of *Anoxybacillus* in chiller waters could likely be attributed to scalder waters being carried by processed carcasses entering the chiller throughout the processing day (Rothrock, et al., 2016). *Anoxybacillus* has been recently isolated from wastewater systems affiliated with poultry processing facilities, so the microorganism's presence could be utilized to help understand how and why certain microorganisms become established and are able to persist in this environment (Rothrock, et al., 2016).

In research performed by Kim et al., (2017), NGS analysis and direct plating culturing methods demonstrated that the prevalence of *Campylobacter* populations on WBC were higher than *Salmonella* levels, which was similar to findings previously published by other researchers. While direct plating enumeration counts and OTU relative abundances cannot be directly compared, inferences about the population levels can be made as there were no OTUs identified as *Salmonella* while *Campylobacter* OTUs represented 1.6% of the OTUs present. These findings are also supported by comparing the results of enumeration performed by direct plating, which demonstrated mean *Campylobacter* levels at 3.75 log10 cfu/WBC, while *Salmonella* could only be detected after enrichment steps due to a low number of cells being present within the samples (Kim et al., 2017). Sequence analysis of the pooled colonies obtained on Campy-Cefex agar plates demonstrated variable selectivity levels in regards to *Campylobacter* OTUs. At the phyla level, OTUs were predominately (62.76% ± 4.30) *Proteobacteria*. This would be expected as the agar is selective for *Campylobacter* microorganisms, but when the relative abundances were evaluated at the genus level, there were other OTUs present belonging to

several other genera, including *Oscillospira*, *Acinetobacter*, *Enterococcus*, *Bacillus*, *Paenibacillus*, *Sporanaerobacter*, *Lactobacillus*, and *Clostridium*, at relative abundance levels ranging from 12.70% to 1.02% (Kim et al., 2017). This may indicate that the accuracy achieved with the selectivity of this particular agar plate could be compromised by the presence of background microorganisms, and while enumerated counts utilizing this agar could provide information in terms of relative population levels, the analysis could be hindered when used with samples containing a wide range of microorganisms.

Applying NGS to samples from several steps within the farm to fork continuum of poultry processing, has shown to be informative in showing not only the reductions in food pathogen microorganisms along the continuum, but also unique microbiota communities during each stage of poultry processing that had not been previously identified (Oakley et al., 2013). Review of relative abundance values of *Campylobacter* OTUs from fecal and litter samples demonstrated that *Campylobacter* was present at levels equal to 1.0 to 3.0 $\log_{10}$ gene copies per ng DNA compared to carcass rinse and retail carcass weep samples where *Campylobacter* OTUs were reduced to less than 0.75 $\log_{10}$ gene copies per ng DNA (Oakley et al., 2013). Core microbiota analysis of samples from each stage of the process demonstrated that each stage of the process was represented by a unique community microbiome and that microbiome overlap between the stages was limited (Oakley et al., 2013). These differences could prove to be informative in developing strategies to prevent the establishment and proliferation of food borne pathogens within the poultry processing system (Oakley et al., 2013).

Recent published research has established that the application of NGS to any step within the poultry process can provide valuable information related to understanding microbial and genetic shifts occurring throughout the continuum (Kim et al., 2017; Oakely et al., 2013; Park et

al., 2016; Rothrock et al., 2016). For example, establishing a microbiome map of the poultry production process, from the farm to fork, including feed and feed ingredients, the equipment and environment in the processing facility, as well as the microbiome shifts during shelf-life storage of a product could help to highlight the problematic areas within the process. Furthermore, a microbiome map could highlight possible sanitary design issues, foodborne pathogen harborage areas, points of cross-contamination, and intervention inefficiencies. The identification of poultry processing plant specific microbiomes may allow a deeper understanding of microbiological differences between facilities, which could help to categorize facility and regional variances so that applied interventions and practices could be targeted toward specific microbiomes. Additionally, microbiome analysis of processed poultry could aid in identifying more suitable indicator organisms that could be used to assess and manage food safety and quality assurance interventions within any processing facility.

In an initial step in understanding how microbial populations might differ in the earliest stages of poultry processing, including scalding and feather removal, microbiome analysis was performed in the current study on WBC rinsates collected at three different commercial poultry processing facilities. The samples collected represented three different age and types of commercial broilers, including Cornish broilers, mid-size broilers (4 to 6 lbs or 1814 to 2722 g) and large size broilers (6 to 8 lbs, or 2722 to 3629 g). Understanding from previous research that microbial shifts within the rearing environment and subsequently the GIT of poultry, occur throughout the life cycle of commercial broilers, the three different broilers represented three different ages of poultry. While the processing steps of the three different aged broilers are similar, the different microbial populations present could inherently differ, and understanding the differences could aid in process improvements of food safety and quality assurance programs.

2.10 References

Alali, W. Q., C. L. Hofacre, G. F. Mathis, G. Faltys, S. C. Ricke, and M. P. Doyle. 2013. Effect of non-pharmaceutical compounds on shedding and colonization of *Salmonella enterica* serovar Heidelberg in broilers. Food Control 31:125-128. doi http://dx.doi.org/10.1016/j.foodcont.2012.10.001

Allen, V. M., M. H. Hinton, D. B. Tinker, C. Gibson, G. C. Mead, and C. M. Wathes. 2003a. Microbial cross-contamination by airborne dispersion and contagion during defeathering of poultry. Br. Poult. Sci. 44:567-576.

Allen, V. M., D. B. Tinker, M. H. Hinton, and C. M. Wathes. 2003b. Dispersal of micro-organisms in commercial defeathering systems. Br. Poult. Sci. 44:53-59.

Altekruse, S. F., M. E. Berrang, H. Marks, B. Patel, W. K. Shaw, P. Saini, P. A. Bennett, and J. S. Bailey. 2009. Enumeration of *Escherichia coli* cells on chicken carcasses as a potential measure of microbial process control in a random selection of slaughter establishments in the United States. Appl. Environ. Microbiol. 75:3522-3527. doi 10.1128/AEM.02685-08

Anderson, R. C., R. B. Harvey, J. A. Byrd, T. R. Callaway, K. J. Genovese, T. S. Edrington, Y. S. Jung, J. L. McReynolds, and D. J. Nisbet. 2005. Novel preharvest strategies involving the use of experimental chlorate preparations and nitro-based compounds to prevent colonization of food-producing animals by foodborne pathogens. Poult. Sci. 84:649-654.

Arnold, J. W., and S. Silvers. 2000. Comparison of poultry processing equipment surfaces for susceptibility to bacterial attachment and biofilm formation. Poult. Sci. 79:1215-1221.

Arritt, F. M., J. D. Eifert, M. D. Pierson, and S. S. Sumner. 2002. Efficacy of antimicrobials against *Campylobacter jejuni* on chicken breast skin. J Appl Poult Res 11:358-366. doi 10.1093/japr/11.4.358

Aviagen, 2014. Ross 308 Broiler Nutrition Specfications. Accessed at: http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Handbook-2014i-EN.pdf

Ayrapetyan, M., and J. D. Oliver. 2016. The viable but non-culturable state and its relevance in food safety. Curr Opin Food Sci 8:127-133. doi http://dx.doi.org/10.1016/j.cofs.2016.04.010

Bailey, J. S., A. D. Shakelford, N. A. Cox, and J. E. Thomson. 1986. Chlorine spray washing to reduce bacterial contamination of poultry processing equipment. Poult. Sci. 65:1120-1123.

Bailey, J. S. 1988. Integrated colonization control of *Salmonella* in poultry. Poult. Sci. 67:928-932.

Barbut, S. 2002. Poultry Products Processing: An Industry Guide. CRC Press, Boco Raton, FL.

Barnes, E. M. 1972. Food poisoning and spoilage bacteria in poultry processing. Vet. Rec. 90:720-722.

Bashor, M. P., P. A. Curtis, K. M. Keener, B. W. Sheldon, S. Kathariou, and J. A. Osborne. 2004. Effects of carcass washers on *Campylobacter* contamination in large broiler processing plants. Poult. Sci. 83:1232-1239.

Batz, M. B., S. Hoffmann, and J. G. Morris, Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J. Food Prot. 75:1278-1291. doi 10.4315/0362-028x.jfp-11-418

Batz, M., S. Hoffmann, and J. G. Morris, Jr. 2014. Disease-outcome trees, EQ-5D scores, and estimated annual losses of quality-adjusted life years (QALYs) for 14 foodborne pathogens in the United States. Foodborne Pathog. Dis. 11:395-402. doi 10.1089/fpd.2013.1658

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008a. The microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. Poult. Sci. 87: 2390-2398.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008b. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. J. Food Prot. 71:1119-1122.

Bautista, D. A., and M. L. Tortorello. 2014. Spoilage problems: problems caused by bacteria. Pages 465-470 in Encyclopedia of Food Microbiology. ed. C. A. Batt. Academic Press, Oxford, U.K.

Belluco, S., L. Barco, A. Roccato, and A. Ricci. 2016. *Escherichia coli* and *Enterobacteriaceae* counts on poultry carcasses along the slaughterline: A systematic review and meta-analysis. Food Control 60:269-280. doi http://dx.doi.org/10.1016/j.foodcont.2015.07.033

Berrang, M. E., J. K. Northcutt, and J. A. Dickens. 2004. The contribution of airborne contamination to *Campylobacter* counts on defeathered broiler carcasses. J Appl Poult Res 13:1-4.

Berrang, M. E., and J. S. Bailey. 2009. On-line brush and spray washers to lower numbers of *Campylobacter* and *Escherichia coli* and presence of *Salmonella* on broiler carcasses during processing. J Appl Poult Res 18:74-78. doi 10.3382/japr.2008-00067

Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. J. Food Prot. 64:2063-2066.

Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. Poult. Sci.79:286-290. doi 10.1093/ps/79.2.286

Bilgili, S. F., D. E. Conner, J. L. Pinion, and K. C. Tamblyn. 1998. Broiler skin color as affected by organic acids: influence of concentration and method of application. Poult. Sci. 77:752-757. doi 10.1093/ps/77.5.752

Bilgili, S. F., A. L. Waldroup, D. Zelenka, and J. E. Marion. 2002. Visible ingesta on prechill carcasses does not affect the microbiological quality of broiler carcasses after immersion chilling. J Appl Poult Res 11:233-238. doi 10.1093/japr/11.3.233

Bilgili, S. F. 2010. Poultry meat inspection and grading. Pages 67-99 in Poultry Meat Processing, eds C. M. Owens, C. Z. Alvarado, and A. R. Sams. CRC Press, Boca Raton, FL.

Blankenship, L. C., N. A. Cox, S. E. Craven, A. J. Mercuri, and R. L. Wilson. 1975. Comparison of the microbiological quality of inspection-passed and fecal contamination-condemned broiler carcasses. J. Food Sci. 40:1236-1238.

Bokulich, N. A., J. Bergsveinson, B. Ziola, and D. A. Mills. 2015. Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. Elife 4. doi 10.7554/elife.04634

Bolder, N. M. 1997. Decontamination of meat and poultry carcasses. Trends Food Sci Technol 8:221-227. doi http://dx.doi.org/10.1016/S0924-2244(97)01040-6.

Bolder, N. M. 1998. The microbiology of the slaughter and processing of poultry. Pages 158-173 in The Microbiology of Meat and Poultry, eds A. Davies and R. Board. Blackie Academic and Professional, London, UK.

Bolton, D. J. 2015. *Campylobacter* virulence and survival factors. Food Microbiol. 48:99-108. doi http://dx.doi.org/10.1016/j.fm.2014.11.017

Bolton, D. J., H. Meredith, D. Walsh, and D. A. McDowell. 2014. The effect of chemical treatments in laboratory and broiler plant studies on the microbial status and shelf-life of poultry. Food Control 36:230-237. doi http://dx.doi.org/10.1016/j.foodcont.2013.08.027

Bowker, B. C., H. Zhuang, and R. J. Buhr. 2014. Impact of carcass scalding and chilling on muscle proteins and meat quality of broiler breast fillets. LWT - Food Sci Technol 59:156-162. doi http://dx.doi.org/10.1016/j.lwt.2014.05.008

Breen, P. J., C. M. Compadre, E. K. Fifer, H. Salari, D. C. Serbus, and D. L. Lattin. 1995. Quaternary ammonium compounds inhibit and reduce the attachment of viable *Salmonella* Typhimurium to poultry tissues. J. Food Sci. 60:1191-1196. doi 10.1111/j.1365-2621.1995.tb04553.x

Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* Nomenclature. J. Clin. Microbiol. 38:2465-2467.

Brown, M. H. 2000. Validation and verification of HACCP plans. Pages 231-272 in HACCP in the Meat Industry. ed M. Brown. Woodhead Publishing, Cambridge, England.

Buhr, R. J., J. M. Walker, D. V. Bourassa, A. B. Caudill, B. H. Kiepper, and H. Zhuang. 2014. Impact of broiler processing scalding and chilling profiles on carcass and breast meat yield. Poult. Sci. 93:1534-1541.

Buncic, S., and J. Sofos. 2012. Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. Food Res. Int. 45:641-655. doi http://dx.doi.org/10.1016/j.foodres.2011.10.018

Capita, R., M. Prieto, and C. Alonso-Calleja. 2004. Sampling methods for microbiological analysis of red meat and poultry carcasses. J. Food Prot. 67:1303-1308.

Carrasco, E., A. Morales-Rueda, and R. M. García-Gimeno. 2012. Cross-contamination and recontamination by *Salmonella* in foods: A review. Food Res. Int. 45:545-556. doi http://dx.doi.org/10.1016/j.foodres.2011.11.004

Carrique-Mas, J., and R. Davies. 2008. Sampling and bacteriological detection of *Salmonella* in poultry and poultry premises: a review. Rev. - Off. Int. Epizoot. 27:665-677.

Cason, J. A., J. S. Bailey, N. J. Stern, A. D. Whittemoore, and N. A. Cox. 1997. Relationship between aerobic bacteria, *Salmonellae*, and *Campylobacter* on broiler carcasses. Poult. Sci. 76:1037-1041.

Cason, J. A., A. Hinton, Jr., and K. D. Ingram. 2000. Coliform, *Escherichia coli*, and *Salmonellae* concentrations in a multiple-tank, counterflow poultry scalder. J. Food Prot. 63:1184-1188.

Cason, J., and A. Hinton Jr. 2006. Coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* in a counterflow poultry scalder with a dip tank. Int. J. Poult. Sci. 5:846-849.

CDC. 2013. Surveillance for Foodborne Disease Outbreaks — United States, 1998–2008. Morbidity and Mortality Weekly Report.

CDC. 2015. Morbidity and Mortality Weekly Report. Pages 495-499 in Preliminary Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food-Foodborne Active Surveillance Network, 10 U.S. Sites, 2006-2014.

Chambers, J. R., and J. Gong. 2011. The intestinal microbiota and its modulation for *Salmonella* control in chickens. Food Res. Int. 44:3149-3159. doi http://dx.doi.org/10.1016/j.foodres.2011.08.017

Chao, K., M. S. Kim, and D. E. Chan. 2014. Control interface and tracking control system for automated poultry inspection. Computer Standards & Interfaces 36:271-277. doi http://dx.doi.org/10.1016/j.csi.2011.03.006

Chaveerach, P., D. A. Keuzenkamp, L. J. Lipman, and F. Van Knapen. 2004. Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, gut microflora and histological cell changes. Poult. Sci. 83:330-334.

Chen, T. C., and P. L. T. Wang. 2003. Poultry processing, product sanitation, and HACCP. Pages 515-541 in Food Plant Sanitation, eds. Y. H. Hui, B. L. Bruinsma, J. R. Gorham, W. K. Nip, P. S. Tong, and P. Ventresca. Marcel Dekker, Inc., New York, NY.

Chia, T. W. R., R. M. Goulter, T. McMeekin, G. A. Dykes, and N. Fegan. 2009. Attachment of different *Salmonella* serovars to materials commonly used in a poultry processing plant. Food Microbiol. 26:853-859. doi http://dx.doi.org/10.1016/j.fm.2009.05.012

Chipley, J.R, 1987. Standard and experimental methods of identification and enumeration. Pages 43-192 in The Microbiology of Poultry Products, eds. F.E. Cunningham and N.A. Cox. Academic Press, Orlando, FL.

Clouser, C. S., S. Doores, M. G. Mast, and S. J. Knabel. 1995a. The role of defeathering in the contamination of turkey skin by *Salmonella* species and *Listeria monocytogenes*. Poult. Sci. 74:723-731.

Clouser, C. S., S. J. Knabel, M. G. Mast, and S. Doores. 1995b. Effect of type of defeathering system on *Salmonella* cross-contamination during commercial processing. Poult. Sci. 74:732-741.

Connon, S. A., and S. J. Giovannoni. 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl. Environ. Microbiol. 68:3878-3885. doi 10.1128/AEM.68.8.3878-3885.2002

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poult. Sci. 78:45-49.

Corry, J. E., B. Jarvis, S. Passmore, and A. Hedges. 2007a. A critical review of measurement uncertainty in the enumeration of food micro-organisms. Food Microbiol. 24:230-253. doi 10.1016/j.fm.2006.05.003

Corry, J. E. L., S. J. James, G. Purnell, C. S. Barbedo-Pinto, Y. Chochois, M. Howell, and C. James. 2007b. Surface pasteurisation of chicken carcasses using hot water. J Food Eng 79:913-919. doi http://dx.doi.org/10.1016/j.jfoodeng.2006.03.018

Corry, J. E. L., and J. E. Line. 2015. *Campylobacter*. Pages 365-373 in Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, DC.

Cosby, D. E., N. A. Cox, M. A. Harrison, J. L. Wilson, R. J. Buhr, and P. J. Fedorka-Cray. 2015. *Salmonella* and antimicrobial resistance in broilers: A review. J Appl Poult Res 24:408-426.

Cowen, P., D. E. Hansen, C. L. Hofacre, E. J. Noga, D. G. Pyburn, and A. G. Wondwossen. 2003. Food animal industry activities in food safety. Pages 19-26 in Microbial Food Safety in Animal Agriculture: Current Topics, eds. M. E. Torrence, and R. E. Isaacson. Iowa State Press, Ames, IA.

Cox, J. M., and A. Pavic. 2010. Advances in enteropathogen control in poultry production. J. Appl. Microbiol. 108:745-755. doi 10.1111/j.1365-2672.2009.04456.x

Cox, N. A., J. A. Cason, and L. J. Richardson. 2011. Minimization of *Salmonella* contamination on raw poultry. Annu Rev Food Sci Technol 2:75-95. doi doi:10.1146/annurev-food-022510-133715

Cox, N., L. Richardson, R. Buhr, J. Northcutt, J. Bailey, P. Cray, and K. Hiett. 2007. Recovery of *Campylobacter* and *Salmonella* serovars from the spleen, liver and gallbladder, and ceca of six-and eight-week-old commercial broilers. J Appl Poult Res 16:477-480

Cunningham, F. 2012. The Microbiology of Poultry Meat Products. Elsevier Science, Oxford, U.K.

Davies, R. H., and C. Wray. 1994. An approach to reduction of *Salmonella* infection in broiler chicken flocks through intensive sampling and identification of cross-contamination hazards in commercial hatcheries. Int. J. Food Microbiol. 24:147-160.

Davies, R. H., and C. Wray. 1996a. Determination of an effective sampling regime to detect *Salmonella* e*nteritidis* in the environment of poultry units. Vet. Microbiol. 50:117-127. doi http://dx.doi.org/10.1016/0378-1135(96)00031-4

Davies, R. H., and C. Wray. 1996b. Persistence of *Salmonella enteritidis* in poultry units and poultry food. Br. Poult. Sci. 37:589-596. doi 10.1080/00071669608417889

Davies, R. H. 2005. Pathogen populations on poultry farms. Pages 101-152 in Food Safety Control in the Poultry Industry, ed. G. C. Mead. CRC Press. Boca Raton, FL

de Boer, P., M. Caspers, J.-W. Sanders, R. Kemperman, J. Wijman, G. Lommerse, G. Roeselers, R. Montijn, T. Abee, and R. Kort. 2015. Amplicon sequencing for the quantification of spoilage microbiota in complex foods including bacterial spores. Microbiome 3:1-13. doi 10.1186/s40168-015-0096-3

Deusch, S., B. Tilocca, A. Camarinha-Silva, and J. Seifert. 2015. News in livestock research — use of omics-technologies to study the microbiota in the gastrointestinal tract of farm animals. Comput Struct Biotechnol J 13:55-63. doi http://dx.doi.org/10.1016/j.csbj.2014.12.005

DHEW. 1979. Healthy People, The surgeon general's report on health promotion and disease prevention. Public Health Service, Washington, D.C.

Díez-García, M., R. Capita, and C. Alonso-Calleja. 2012. Influence of serotype on the growth kinetics and the ability to form biofilms of *Salmonella* isolates from poultry. Food Microbiol. 31:173-180. doi http://dx.doi.org/10.1016/j.fm.2012.03.012

Diaz-Sanchez, S., I. Hanning, S. Pendleton, and D. D'Souza. 2013. Next-generation sequencing: the future of molecular genetics in poultry production and food safety. Poult. Sci. 92:562-572. doi 10.3382/ps.2012-02741

Dickens, J. A., and A. D. Whittemore. 1994. The effect of acetic acid and air injection on appearance, moisture pick-up, microbiological quality, and *Salmonella* incidence on processed poultry carcasses. Poult. Sci. 73:582-586.

Dickens, J. A., and A. D. Whittemore. 1995. The effects of extended chilling times with acetic acid on the temperature and microbiological quality of processed poultry carcasses. Poult. Sci. 74:1044-1048. doi 10.3382/ps.0741044

Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbiological quality of broiler carcasses prior to evisceration. Poult. Sci. 76:657-660. doi 10.1093/ps/76.4.657

Dickens, J. A., B. G. Lyon, A. D. Whittemore, and C. E. Lyon. 1994. The effect of an acetic acid dip on carcass appearance, microbiological quality, and cooked breast meat texture and flavor. Poult. Sci. 73:576-581. doi 10.3382/ps.0730576

Dórea, F. C., D. J. Cole, C. Hofacre, K. Zamperini, D. Mathis, M. P. Doyle, M. D. Lee, and J. J. Maurer. 2010. Effect of *Salmonella* vaccination of breeder chickens on contamination of broiler chicken carcasses in integrated poultry operations. Appl. Environ. Microbiol. 76:7820-7825.

Dumas, M. D., S. W. Polson, D. Ritter, J. Ravel, J. Gelb, R. Morgan, and K. E. Wommack. 2011. Impacts of poultry house environment on poultry litter bacterial community composition. PLoS ONE 6(9): e24785. doi:10.1371/journal.pone.0024785.

Dunkley, K. D., T. R. Callaway, V. I. Chalova, J. L. McReynolds, M. E. Hume, C. S. Dunkley, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2009. Foodborne *Salmonella* ecology in the avian gastrointestinal tract. Anaerobe 15:26-35. doi 10.1016/j.anaerobe.2008.05.007

Durrant, R. J. 1995. Production, processing and marketing of poultry meat. Pages 189-220 in World Animal Science C: Production System Approach-Poultry Production. ed. P. Hunton. Elsevier, Amsterdam, Netherlands.

Ercolini, D. 2013. High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. Appl. Environ. Microbiol. 79: 3148–3155. http://doi.org/10.1128/AEM.00256-13

Erdtsieck, E. 1995. Quality requirements in the modern poultry industry. Pages 1-30 in Processing of Poultry, ed. G. C. Mead. Chapman and Hall, Bristol, UK

Escudero-Gilete, M. L., M. L. González-Miret, and F. J. Heredia. 2014. Application of multivariate statistical analysis to quality control systems. Relevance of the stages in poultry meat production. Food Control 40:243-249. doi http://dx.doi.org/10.1016/j.foodcont.2013.12.004

Fabrizio, K. A., R. R. Sharma, A. Demirci, and C. N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. Poult. Sci. 81:1598-1605. doi 10.1093/ps/81.10.1598

Fernández-Pan, I., X. Carrión-Granda, J.I. Mate. 2014. Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillets. Food Control 36: 69-75.

Fletcher, D. L., E. W. Craig, and J. W. Arnold. 1997. An evaluation of on-line “reprocessing” on visual contamination and microbiological quality of broilers. J Appl Poult Res 6:436-442. doi 10.1093/japr/6.4.436

Foley, S. L., T. J. Johnson, S. C. Ricke, R. Nayak, and J. Danzeisen. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiol. Mol. Biol. Rev. 77:582-607. doi 10.1128/mmbr.00015-13

Foley, S. L., R. Nayak, I. B. Hanning, T. J. Johnson, J. Han, and S. C. Ricke. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Appl. Environ. Microbiol. 77:4273-4279. doi 10.1128/AEM.00598-11

Foster, J. A., J. Bunge, J. A. Gilbert, and J. H. Moore. 2012. Measuring the microbiome: perspectives on advances in DNA-based techniques for exploring microbial life. Brief. Bioinformatics 13:420-429.

Gabriel, I., M. Lessire, S. Mallet, and J. F. Guillot. 2006. Microflora of the digestive tract: critical factors and consequences for poultry. Worlds Poult Sci J 62:499-511.

Galis, A. M., C. Marcq, D. Marlier, D. Portetelle, I. Van, Y. Beckers, and A. Théwis. 2013. Control of *Salmonella* contamination of shell eggs—preharvest and postharvest methods: a review. Compr Rev Food Sci Food Saf 12:155-182. doi 10.1111/1541-4337.12007

Gall-David, S., V. Meuric, G. Benzoni, S. Valière, A. Guyonvarch, J. Minet, M. Bonnaure-Mallet, and F. Barloy-Hubler. 2017. Effect of zeolite on small intestine microbiota of broiler chickens: A case study. Food Nutr Sci. 8: 163-188.

García-Sánchez, L., B. Melero, I. Jaime, M.-L. Hänninen, M. Rossi, and J. Rovira. 2017. *Campylobacter jejuni* survival in a poultry processing plant environment. Food Microbiol. 65:185-192. doi http://dx.doi.org/10.1016/j.fm.2017.02.009

Ghanbari, M., W. Kneifel, and K. J. Domig. 2015. A new view of the fish gut microbiome: Advances from next-generation sequencing. Aquaculture 448:464-475. doi http://dx.doi.org/10.1016/j.aquaculture.2015.06.033

Giombelli, A., D. Hammerschmitt, M. F. Cerutti, E. Chiarini, M. Landgraf, B. D. G. M. Franco, and M. T. Destro. 2015. High pressure spray with water shows similar efficiency to trimming in controlling microorganisms on poultry carcasses. Poult. Sci. 94:2589-2595

González-Miret, M. L., M. T. Coello, S. Alonso, and F. J. Heredia. 2001. Validation of parameters in HACCP verification using univariate and multivariate statistics. Application to the final phases of poultry meat production. Food Control 12:261-268. doi http://dx.doi.org/10.1016/S0956-7135(01)00007-X

Gorski, L. 2012. Selective enrichment media bias the types of *Salmonella enterica* strains isolated from mixed strain cultures and complex enrichment broths. PLoS ONE 7(4): e34722. doi:10.1371/journal.pone.0034722

Gragg, S. E., and M. M. Brashears. 2014. Microbiological safety of meat: Hurdle technology. Pages 345-347 in Encyclopedia of Meat Sciences. Academic Press, Oxford, UK.

Grau, F. H. 1986. Microbial ccology of meat and poultry. Pages 1-47 in Advances in Meat Research : Meat and Poultry Microbiology. eds. A. M. Pearson and T. R. Dutson. AVI Publishing, Westport, CT.

Gregory, N. G. 1995. Stunning and Slaughter. Pages 31-63 in Processing of Poultry, ed. G. C. Mead. Chapman and Hall, Bristol, UK

Hald, B., S. Henrik, B. Dang Duong, K. Pedersen, J. Dybdahl, J.B. Jespersen, and M. Madsen. 2004. Flies and *Campylobacter* infection of broiler flocks. Emerging Infect. Dis. 10:1490. doi 10.3201/eid1008.040129

Hall, N. 2007. Advanced sequencing technologies and their wider impact in microbiology. J. Exp. Biol. 210:1518-1525. doi 10.1242/jeb.001370

Handley, J. A., Z. Shi, S. H. Park, T. M. Dawoud, Y. M. Kwon, and S. C. Ricke. 2015. *Salmonella* and the potential role for methods to develop microbial process indicators on chicken carcasses. Pages 81-104 in Food Safety. ed. S.C. Ricke, J.R. Donaldson, C.A. Phillips. Academic Press, San Diego, CA

Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. Poult. Sci. 74:1548-1552.

Hayama, Y., T. Yamamoto, F. Kasuga, and T. Tsutsui. 2011. Simulation model for *Campylobacter* cross-contamination during poultry processing at slaughterhouses. Zoonoses Public Health 58:399-406. doi 10.1111/j.1863-2378.2010.01385.x

Hazeleger, W. C., J. A. Wouters, F. M. Rombouts, and T. Abee. 1998. Physiological activity of *Campylobacter* jejuni far below the minimal growth temperature. Appl. Environ. Microbiol. 64:3917-3922.

Heggum, C., J. J. G. Vallejos, O. B. Njie, and A. O. Adegboye. 2015. Application of the moving window approach in the verification of the performance of food safety management systems. Food Control 58:17-22. doi http://dx.doi.org/10.1016/j.foodcont.2015.02.024

Hellberg, R. S., K. G. Martin, A. L. Keys, C. J. Haney, Y. Shen, and R. D. Smiley. 2013. 16S rRNA partial gene sequencing for the differentiation and molecular subtyping of *Listeria* species. Food Microbiol. 36:231-240. doi http://dx.doi.org/10.1016/j.fm.2013.06.001

Herman, L., M. Heyndrickx, K. Grijspeerdt, D. Vandekerchove, I. Rollier, and L. De Zutter. 2003. Routes for *Campylobacter* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. Epidemiol. Infect. 131(3), 1169–1180.

Hermans, D., A. Martel, A. Garmyn, M. Verlinden, M. Heyndrickx, I. Gantois, F. Haesebrouck, and F. Pasmans. 2012. Application of medium-chain fatty acids in drinking water increases *Campylobacter jejuni* colonization threshold in broiler chicks. Poult. Sci. 91:1733-1738. doi 10.3382/ps.2011-02106

Hinton, A., J. A. Cason, and K. D. Ingram. 2002. Enumeration and identification of yeasts associated with commercial poultry processing and spoilage of refrigerated broiler carcasses. J. Food Prot. 65:993-998.

Hinton Jr, A., J. A. Cason, and K. D. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. Int. J. Food Microbiol. 91:155-165. doi http://dx.doi.org/10.1016/S0168-1605(03)00377-5

Horrocks, S. M., R. C. Anderson, D. J. Nisbet, and S. C. Ricke. 2009. Incidence and ecology of *Campylobacter jejuni* and *coli* in animals. Anaerobe 15:18-25. doi http://dx.doi.org/10.1016/j.anaerobe.2008.09.001

Hui, Y. H., W. K. Nip, and J. R. Gorham. 2003. The FDA's GMPs, HACCP, and the Food Code. Pages 31-50 in Food Plant Sanitation, eds. Y. H. Hui, B. L. Bruinsma, J. R. Gorham, W. K. Nip, P. S. Tong, and P. Ventresca. Marcel Dekker, Inc., New York, NY

Humphrey, T. J., D. G. Lanning, and D. Beresford. 1981. The effect of pH adjustment on the microbiology of chicken scald-tank water with particular reference to the death rate of *Salmonellas.* J. Appl. Bacteriol. 51:517-527.

Hutchison, M. L., L. D. Walters, G. C. Mead, M. Howell, and V. M. Allen. 2006. An assessment of sampling methods and microbiological hygiene indicators for process verification in poultry slaughterhouses. J. Food Prot. 69:145-153.

IFSAC. 2015. Foodborne Illness Source Attribution Estimates for *Salmonella*, *Escherichia coli* O157, *Listeria*, and *Campylobacter* using Outbreak Surveillance Data. Accessed Oct. 2016.

Izat, A. L., M. Colberg, C. D. Driggers, and R. A. Thomas. 1989. Effects of sampling method and feed withdrawal period on recovery of microorganisms from poultry carcasses. J. Food Prot. 52:480-483.

Jackson, T. 2014. Management of microbiological hazards: role of testing as verification. Pages 889-917 in Food Safety Management. eds, Y. Motarjemi and H. Lelieveld. Academic Press, San Diego, CA.

James, C., C. Vincent, T. I. de Andrade Lima, and S. J. James. 2006. The primary chilling of poultry carcasses, a review. Int J Refrig 29:847-862. doi http://dx.doi.org/10.1016/j.ijrefrig.2005.08.003

Jarvis, K. G., J. R. White, C. J. Grim, L. Ewing, A. R. Ottesen, J. J. Beaubrun, J. B. Pettengill, E. Brown, and D. E. Hanes. 2015. Cilantro microbiome before and after nonselective pre-enrichment for *Salmonella* using 16S rRNA and metagenomic sequencing. BMC Microbiol. 15:160. doi 10.1186/s12866-015-0497-2

Jarvis, N. A., C. A. O'Bryan, T. M. Dawoud, S. H. Park, Y. M. Kwon, P. G. Crandall, and S. C. Ricke. 2016. An overview of *Salmonella* thermal destruction during food processing and preparation. Food Control 68:280-290. doi http://dx.doi.org/10.1016/j.foodcont.2016.04.006

Jiménez, S. M., M. S. Salsi, M. C. Tiburzi, and M. E. Pirovani. 2002. A comparison between broiler chicken carcasses with and without visible faecal contamination during the slaughtering process on hazard identification of *Salmonella* spp. J. Appl. Microbiol. 93:593-598.

Justé, A., B. P. H. J. Thomma, and B. Lievens. 2008. Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. Food Microbiol. 25:745-761. doi http://dx.doi.org/10.1016/j.fm.2008.04.009

Kaneene, J. B., and R. C. Potter. 2003. Epidemiology of *Campylobacter* spp. in animals. Pages 175-181 in Microbial Food Safety in Animal Agriculture, Current Topics, eds. M. E. Torrence, and R. E. Isaacson. Iowa State Press, Blackwell Publishing, Ames, IA.

Kassem, I. I., K. Chandrashekhar, and G. Rajashekara. 2013. Of energy and survival incognito: a relationship between viable but non-culturable cells formation and inorganic polyphosphate and formate metabolism in *Campylobacter jejuni*. Front Microbiol 4:183.

Kearney, J. 2010. Food consumption trends and drivers. Philos Trans R Soc Lond B Biol Sci. 365:2793-2807. doi 10.1098/rstb.2010.0149

Keener, K. M., M. P. Bashor, P. A. Curtis, B. W. Sheldon, and S. Kathariou. 2004. Comprehensive review of *Campylobacter* and poultry processing. Comprehensive Reviews in Food Sscience and Food Safety 3:105-116. doi 10.1111/j.1541-4337.2004.tb00060.x

Kemp, G.K., M. L. Aldrich, M. L. Guerra, and K. R. Schneider. 2001. Continuous online processing of fecal- and ingesta-contaminated poultry carcasses using an acidified sodium chlorite antimicrobial intervention. J. Food Prot. 64:807-812

Kim, J.-W., and S. Doores. 1993. Attachment of *Salmonella* Typhimurium to skins of turkey that had been defeathered through three different systems: scanning electron microscopic examination. J. Food Prot. 56:395-400.

Kim, J.-W., S. J. Knabel, and S. Doores. 1993a. Penetration of *Salmonella* Typhimurium into turkey skin. J. Food Prot. 56:292-296.

Kim, J.-W., M. F. Slavik, C. L. Griffis, and J. T. Walker. 1993b. Attachment of *Salmonella* Typhimurium to skins of chicken scalded at various temperatures. J. Food Prot. 56:661-671.

Kim, M., M. Morrison, and Z. Yu. 2011. Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. J. Microbiol. Methods 84:81-87. doi http://dx.doi.org/10.1016/j.mimet.2010.10.020

Kim, S.A., S. H. Park, S. I. Lee, C. M. Owens-Hanning, and S. C. Ricke. 2017. Assessment of chicken carcass microbiome responses during processing in the presence of commercial antimicrobials using a next generation sequencing approach. Scientific Reports 7:43354. doi 10.1038/srep43354

Korver, D. R. 2012. Implications of changing immune function through nutrition in poultry. Anim. Feed Sci. Technol. 173:54-64. doi http://dx.doi.org/10.1016/j.anifeedsci.2011.12.019

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, and C. M. Owens. 2013. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult. Sci. 92:811-819.

Lahellec, C., and P. Colin. 1985. Relationship between serotypes of *Salmonellae* from hatcheries and rearing farms and those from processed poultry carcases. Br. Poult. Sci. 26:179-186.

Leonard, S. R., M. K. Mammel, D. W. Lacher, and C. A. Elkins. 2015. Application of metagenomic sequencing to food safety: detection of shiga toxin-producing *Escherichia coli* on fresh bagged spinach. Appl. Environ. Microbiol. 81:8183-8191. doi 10.1128/AEM.02601-15

Liljebjelke, K. A., C. L. Hofacre, T. Liu, D. G. White, S. Ayers, S. Young, and J. J. Maurer. 2005. Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. Foodborne Pathog. Dis. 2:90-102. doi 10.1089/fpd.2005.2.90

Lillard, H. S. 1980. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. Poult. Sci. 59:1761-1766. doi 10.3382/ps.0591761

Lillard, H. S., S. E. Craven, A. D. Shackelford, L. C. Blankenship, and J. A. Dickens. 1987. Effect of acetic acid on the microbiological quality of scalded picked and unpicked broiler carcasses. J. Food Prot. 50:112-114.

Lianou, A., K. P. Koutsoumanis, and J. N. Sofos. 2012. Organic acids and other chemical treatments for microbial decontamination of food. Pages 592-664 in Microbial Decontamination in the Food Industry: Novel Methods and Applications. Woodhead Publishing, Cambridge, UK.

Lopez, M., M. Carvalho, D. Gouvêa, L. Batalha, I. Neves, and R. Mendonça. 2015. Isolation and characterization of lytic bacteriophages as an alternative to prevent *Pseudomonas* spp in poultry industry. MOJ Food Process Technol 1:00018.

Luján-Rhenals, D., R. Morawicki, E. Van Loo, and S. Ricke. 2017. Waste management and emissions in poultry processing. pages 411-431 in Achieving Sustainable Production of Poultry Meat. Volume 1: Safety, Quality and Sustainability. ed. S.C. Ricke. Burleigh Dodds Science Publishing Limited, Cambridge, U.K.

Lyon, C. E., C. M. Papa, and J. R. L. Wilson. 1991. Effect of feed withdrawal on yields, muscle pH, and texture of broiler breast meat. Poult. Sci. 70:1020-1025.

Mainali, C., G. Gensler, M. McFall, R. King, R. Irwin, and A. Senthilselvan. 2009. Evaluation of associations between feed withdrawal and other management factors with *Salmonella* contamination of broiler chickens at slaughter in Alberta. J. Food Prot. 72:2202-2207.

Marin, C., A. Hernandiz, and M. Lainez. 2009. Biofilm development capacity of *Salmonell*a strains isolated in poultry risk factors and their resistance against disinfectants. Poult. Sci. 88:424-431.

Maurer, A. J. 2003. Poultry: chicken. Pages 4680-4686 in Encyclopedia of Food Sciences and Nutrition. Academic Press, Oxford, UK.

Martins, E. A., and P. M. L. Germano. 2008. Microbiological indicators for the assessment of performance in the hazard analysis and critical control points (HACCP) system in meat lasagna production. Food Control 19:764-771. doi http://dx.doi.org/10.1016/j.foodcont.2007.08.001

Mayo, B., C. T. Rachid, A. Alegria, A. M. Leite, R. S. Peixoto, and S. Delgado. 2014. Impact of next generation sequencing techniques in food microbiology. Curr. Genomics 15:293-309. doi 10.2174/1389202915666140616233211

McKee, S. R., J. C. Townsend, and S. F. Bilgili. 2008. Use of a scald additive to reduce levels of *Salmonella* Typhimurium during poultry processing. Poult. Sci. 87.8: 1672-77.

Mead, G.C. 1995. Hygiene problems and control of process contamination. Pages 183-220 in Processing of Poultry, ed. G.C. Mead. Chapman and Hall, Bristol, UK

Milios, K. T., E. H. Drosinos, and P. E. Zoiopoulos. 2014. Food safety management system validation and verification in meat industry: carcass sampling methods for microbiological hygiene criteria – a review. Food Control 43:74-81. doi http://dx.doi.org/10.1016/j.foodcont.2014.02.041

Miranda, D. J. A., S. L. Vieira, A. Favero, C. R. Angel, C. Stefanello, and E. T. Nogueira. 2015. Performance and meat production of broiler chickens fed diets formulated at different crude protein levels supplemented or not with L-valine and L-isoleucine. Anim. Feed Sci. Technol. 206:39-47. doi http://dx.doi.org/10.1016/j.anifeedsci.2015.04.018

Mohareb, F., M. Iriondo, A. I. Doulgeraki, A. Van Hoek, H. Aarts, M. Cauchi, and G.-J. E. Nychas. 2015. Identification of meat spoilage gene biomarkers in *Pseudomonas putida* using gene profiling. Food Control 57:152-160. doi http://dx.doi.org/10.1016/j.foodcont.2015.04.007

Mohamed, T., S. Zhao, D. G. White, and S. Parveen. 2014. Molecular characterization of antibiotic resistant *Salmonella* Typhimurium and *Salmonella* Kentucky isolated from pre- and post-chill whole broilers carcasses. Food Microbiol. 38:6-15. doi 10.1016/j.fm.2013.08.002

Morales, P. A., J. S. Aguirre, M. R. Troncoso, and G. O. Figueroa. 2016. Phenotypic and genotypic characterization of *Pseudomonas* spp. present in spoiled poultry fillets sold in retail settings. LWT - Food Science and Technology 73:609-614. doi http://dx.doi.org/10.1016/j.lwt.2016.06.064

Morozova, O., and M. A. Marra. 2008. Applications of next-generation sequencing technologies in functional genomics. Genomics 92:255-264. doi http://dx.doi.org/10.1016/j.ygeno.2008.07.001

Mulder, R., 1999. Hygiene during transport, slaughter and processing. Proc. Poultry Meat Science. Poult. Sci. Symposium Series. Oxfordshire, UK.

Munther, D., X. Sun, Y. Xiao, S. Tang, H. Shimozako, J. Wu, B. A. Smith, and A. Fazil. 2016. Modeling cross-contamination during poultry processing: Dynamics in the chiller tank. Food Control 59:271-281. doi http://dx.doi.org/10.1016/j.foodcont.2015.05.007

Nagel, G. M., L. J. Bauermeister, C. L. Bratcher, M. Singh, and S. R. McKee. 2013. *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. Int. J. Food Microbiol. 165:281-286.

Nde, C. W., J. M. McEvoy, J. S. Sherwood, and C. M. Logue. 2007. Cross contamination of turkey carcasses by *Salmonella* species during defeathering. Poult. Sci. 86:162-167. doi 10.1093/ps/86.1.162

Newell, D. G., and H. C. Davison. 2003. *Campylobacter*: Control and Prevention. Pages 211-220 in Microbial Food Safety in Animal Agriculture: Current Topics, ed. M. E. I. Torrence. Iowa State Press, Ames, IA.

Newell, D. G., K. T. Elvers, D. Dopfer, I. Hansson, P. Jones, S. James, J. Gittins, N. J. Stern, R. Davies, I. Connerton, D. Pearson, G. Salvat, and V. M. Allen. 2011. Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. Appl. Environ. Microbiol. 77:8605-8614. doi 10.1128/AEM.01090-10

Newell, D. G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69:4343-4351.doi: 10.1128/aem.69.8.4343–4351.2003

Northcutt, J. K., M. E. Berrang, J. A. Dickens, D. L. Fletcher, and N. A. Cox. 2003. Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, *Campylobacter*,

Escherichia coli and *Salmonella* on carcasses before and after immersion chilling. Poult. Sci. 82:169-173. doi 10.1093/ps/82.1.169

Northcutt, J. K., D. P. Smith, M. T. Musgrove, K. D. Ingram, and A. Hinton. 2005. Microbiological impact of spray washing broiler carcasses using different chlorine concentrations and water temperatures. Poult. Sci. 84:1648-1652. doi 10.1093/ps/84.10.1648

Northcutt, J. K., J. A. Cason, D. P. Smith, R. J. Buhr, and D. L. Fletcher. 2006. Broiler carcass bacterial counts after immersion chilling using either a low or high volume of water. Poult. Sci. 85:1802-1806. doi 10.1093/ps/85.10.1802

Northcutt, J. K., J. A. Cason, K. D. Ingram, D. P. Smith, R. J. Buhr, and D. L. Fletcher. 2008. Recovery of bacteria from broiler carcasses after immersion chilling in different volumes of water, Part 2. Poult. Sci. 87:573-576

Northcutt, J.K., and R.J. Buhr. 2010. Preslaughter factors affecting poultry meat quality. Pages 5-24 in Poultry Meat Processing, eds. C. M. Owens, C. Z. Alvarado, and A. R. Sams. CRC Press, Boca Raton, FL.

NRC. 1987. Poultry Inspection: The Basis for a Risk-Assessment Approach, Report of the Committee on Public Health Risk Assessment of Poultry Inspection Programs. ed. Board, Food and Nutrition. Natl Acad Press, Washington, DC.

NRC, Subcommitte of Poultry Nutrition. 1994. Nutrient requirements of chicken. Pages 19-34 in Nutrient Requirements of Poultry. Natl Acad Press, Washington, DC.

Nychas, G.-J. E., P. N. Skandamis, C. C. Tassou, and K. P. Koutsoumanis. 2008. Meat spoilage during distribution. Meat Science 78:77-89. doi http://dx.doi.org/10.1016/j.meatsci.2007.06.020

Oakley, B. B., H. S. Lillehoj, M. H. Kogut, W. K. Kim, J. J. Maurer, A. Pedroso, M. D. Lee, S. R. Collett, T. J. Johnson, and N. A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiology Letters 360:100-112. doi 10.1111/1574-6968.12608

Oakley, B. B., C. A. Morales, J. Line, M. E. Berrang, R. J. Meinersmann, G. E. Tillman, M. G. Wise, G. R. Siragusa, K. L. Hiett, and B. S. Seal. 2013. The poultry-associated microbiome: Network analysis and farm-to-fork characterizations. PLoS ONE 8:e57190. doi 10.1371/journal.pone.0057190

Oakley, B. B., C. A. Morales, J. E. Line, B. S. Seal, and K. L. Hiett. 2012. Application of high-throughput sequencing to measure the performance of commonly used selective cultivation methods for the foodborne pathogen *Campylobacter*. FEMS Microbiol Ecol. 79:327-336. doi 10.1111/j.1574-6941.2011.01219.x

Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acetic acid on the death rates at 52°C of *Salmonella* Newport, *Salmonella* Typhimurium and *Campylobacter jejuni* in poultry scald water. J. Food Prot. 49:500-503.

Olsen, J. E., D. J. Brown, M. Madsen, and M. Bisgaard. 2003. Cross-contamination with *Salmonella* on a broiler slaughterhouse line demonstrated by use of epidemiological markers. J. Appl. Microbiol. 94:826-835.

Oviedo-Rondón, E.O. 2009. Molecular methods to evaluate effects of feed additives and nutrients in poultry gut microflora. Revista Brasileira de Zootecnia. 38:209-25.

Oyarzabal, O. A. 2005. Reduction of *Campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: A review from the United States perspective. J. Food Prot. 68:1752-1760.

Oyarzabal, O. A., and S. K. Hussain. 2010. Microbial analytical methodology for processed poultry products. Pages 527-544 in Handbook of Poultry Science and Technology, Volume 2: Secondary Processing. eds. I. Guerrero-Legarreta, and Y. H. Hui. Wiley and Sons, Inc., Hoboken, N.J.

Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerging Infect. Dis. 19:407-415.

Pan, D., and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5:108-119. doi 10.4161/gmic.26945

Park, S. F. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int. J. Food Microbiol. 74:177-188. doi http://dx.doi.org/10.1016/S0168-1605(01)00678-X

Park, S. H., S. I. Lee, and S. C. Ricke. 2016. Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an Illumina MiSeq platform. PLoS ONE 11:e0151944. doi 10.1371/journal.pone.0151944

Parry, R. T. 1995. Technological developments in pre-slaughter handling and processing. Pages 65-102 in Processing of Poultry, ed. G. C. Mead. Chapman and Hall, Bristol, UK

Parameswaran, P., R. Jalili, L. Tao, S. Shokralla, B. Gharizadeh, M. Ronaghi, and A. Z. Fire. 2007. A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. Nucleic Acids Res. 35. doi 10.1093/nar/gkm760

Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and antimicrobial resistance of *Salmonella* recovered from processed poultry. J. Food Prot. 70:2466-2472.

Patterson, J., and K. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

Petrak, T., Z. Kalodera, P. Novaković, and L. Gumhalter Karolyi. 1999. Bacteriological comparison of parallel and counter flow water chilling of poultry meat. Meat Sci. 53:269-271. doi http://dx.doi.org/10.1016/S0309-1740(99)00066-2

Peyrat, M., C. Soumet, P. Maris, and P. Sanders. 2008. Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. Int. J. Food Microbiol. 124:188-194.

Rabsch, W., C. Altier, H. Tschape, and A. J. Baumler. 2003. Foodborne *Salmonella* infections. Pages 97-107 in Microbial Food Safety in Animal Agriculture, Current Topics, eds. M. E. Torrence, and R. E. Isaacson. Iowa State Press, Blackwell Publishing, Ames, IA.

Rajan, K., Z. Shi, and S. C. Ricke. 2017. Current aspects of *Salmonella* contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. Critical Reviews in Microbiology 43:370-392. doi 10.1080/1040841X.2016.1223600

Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak, M. E. Hume, D. E. Corrier, DeLoach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult. Sci. 76:654-656. doi 10.1093/ps/76.4.654

Rask, K. J., and N. Rask. 2011. Economic development and food production–consumption balance: A growing global challenge. Food Policy 36:186-196. doi http://dx.doi.org/10.1016/j.foodpol.2010.11.015

Ravindran, V. 2014. Nutrition of meat animals: Poultry. Pages 463-70 in Encyclopedia of Meat Sciences, eds. C. Devine. and M. Dikeman. Elsevier Science, Oxford, UK.

Remenant, B., E. Jaffrès, X. Dousset, M.-F. Pilet, and M. Zagorec. 2015. Bacterial spoilers of food: Behavior, fitness and functional properties. Food Microbiol. 45, Part A:45-53. doi http://dx.doi.org/10.1016/j.fm.2014.03.009

Ricke, S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632-639.

Ricke, S.C., T. M. Dawoud, and Y. M. Kwon. 2015. Application of molecular methods for traceability of foodborne pathogens in food safety systems. Pages 37-63 in Food Safety. ed. S.C. Ricke, J.R. Donaldson, C.A. Phillips. Academic Press, San Diego, CA

Ricke, S. C., and S. D. Pillai. 1999. Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. Crit. Rev. Microbiol. 25:19-38. doi 10.1080/10408419991299176

Rinttilä, T., and J. Apajalahti. 2013. Intestinal microbiota and metabolites: Implications for broiler chicken health and performance. J Appl Poult Res 22:647-658.

Rivera-Perez, W., E. Barquero-Calvo, and R. Zamora-Sanabria. 2014. *Salmonella* contamination risk points in broiler carcasses during slaughter line processing. J. Food Prot. 77:2031-2034. doi 10.4315/0362-028X.JFP-14-052

Rothrock, M. J., A. Locatelli, T. C. Glenn, J. C. Thomas, A. C. Caudill, B. H. Kiepper, and K. L. Hiett. 2016. Assessing the microbiomes of scalder and chiller tank waters throughout a typical commercial poultry processing day. Poult. Sci. 95:2372-2382 doi 10.3382/ps/pew234

Roto, S. M., P. M. Rubinelli, and S. C. Ricke. 2015. An introduction to the avian gut microbiota and the effects of yeast-based prebiotic-type compounds as potential feed additives. Front Vet Sci 2:28. doi 10.3389/fvets.2015.00028

Rouger, A., B. Remenant, H. Prévost, and M. Zagorec. 2017. A method to isolate bacterial communities and characterize ecosystems from food products: Validation and utilization in as a reproducible chicken meat model. Int. J. Food Microbiol. 247:38-47. doi http://dx.doi.org/10.1016/j.ijfoodmicro.2016.04.028

Roush, W., D. Boykin, and S. Branton. 2004. Optimization of phase feeding of starter, grower, and finisher diets for male broilers by mixture experimental design: Forty-eight-day production period. Poult. Sci. 83:1264-1275.

Russell, S. M., D. L. Fletcher, and N. A. Cox. 1996. Spoilage bacteria of fresh broiler chicken carcasses. Poult. Sci. 74:2041-2047

Russell, S. M., and J. M. Walker. 1997. The effect of evisceration on visible contamination and the microbiological profile of fresh broiler chicken carcasses using the Nu-Tech evisceration system or the conventional streamlined inspection system. Poult. Sci 76:780-784. doi 10.1093/ps/76.5.780

Russell, S. M., N. A. Cox, and J. S. Bailey. 1997. Sampling poultry carcasses and parts to determine bacterial levels. J Appl Poult Res 6:234-237. doi 10.1093/japr/6.2.234

Russell, S. M., and S. P. Axtell. 2005. Monochloramine versus sodium hypochlorite as antimicrobial agents for reducing populations of bacteria on broiler chicken carcasses. J. Food Prot. 68:758-763.

Rutsaert, P., Á. Regan, Z. Pieniak, Á. McConnon, A. Moss, P. Wall, and W. Verbeke. 2013. The use of social media in food risk and benefit communication. Trends Food Sci Technol 30:84-91. doi http://dx.doi.org/10.1016/j.tifs.2012.10.006

Sahin, O., Q. Zhang, and T. Y. Morishita. 2003. Detection of *Campylobacter*. Pages 183-193 in Microbial Food Safety in Animal Agriculture, Current Topics, eds. M. E. Torrence, and R. E. Isaacson. Iowa State Press, Blackwell Publishing, Ames, IA.

Samarajeewa, A. D., A. Hammad, L. Masson, I. U. H. Khan, R. Scroggins, and L. A. Beaudette. 2015. Comparative assessment of next-generation sequencing, denaturing gradient gel electrophoresis, clonal restriction fragment length polymorphism and cloning-sequencing as methods for characterizing commercial microbial consortia. J. Microbiol. Methods 108:103-111. doi http://dx.doi.org/10.1016/j.mimet.2014.11.013

Sampers, I., L. Jacxsens, P. A. Luning, W. J. Marcelis, A. Dumoulin, and M. Uyttendaele. 2010. Performance of food safety management systems in poultry meat preparation processing plants in relation to *Campylobacter* spp. contamination. J. Food Prot. 73:1447-1457.

Sams, A. R. 1999. Meat quality during processing. Poult. Sci. 78:798-803. doi 10.1093/ps/78.5.798

Sams, A.R., and C.Z. Alvarado. 2010. Introduction to poultry meat processing. Pages 1-3 in Poultry Meat Processing, eds. C. M. Owens, C. Z. Alvarado, and A. R. Sams. CRC Press, Boca Raton, FL.

Sams, A.R., and S.R. McKee. 2010. First processing: slaughter through chilling. Pages 25-49 in Poultry Meat Processing, eds. C.M. Owens, C.Z. Alvarado and Alan R. Sams. CRC Press, Boca Raton, FL.

Sarlin, L. L., E. T. Barnhart, D. J. Caldwell, R. W. Moore, J. A. Byrd, D. Y. Caldwell, D. E. Corrier, Deloach, and B. M. Hargis. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. Poult. Sci. 77:1253-1257. doi 10.1093/ps/77.8.1253

Saucier, L. 2016. Microbial spoilage, quality and safety within the context of meat sustainability. Meat Sci. 120:78-84. doi http://dx.doi.org/10.1016/j.meatsci.2016.04.027

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. Emerging Infect. Dis. 17:7-15.

Selgas, D., M. Luisa Marín, C. Pin, and C. Casas. 1993. Attachment of bacteria to meat surfaces: A review. Meat Sci. 34:265-273. doi http://dx.doi.org/10.1016/0309-1740(93)90076-T

Shackelford, A. D. 1988. Modifications of processing methods to control *Salmonella* in poultry. Poult. Sci. 67:933-935. doi 10.3382/ps.0670933

Schaffner, D. W., and S. Smith-Simpson. 2014. Microbiological analysis: Indicator organisms in meat. Pages 301-305 in Encyclopedia of Meat Sciences (Second Edition) Academic Press, Oxford, UK.

Schilling, M. W., Y. Vizzier-Thaxton, and C. Z. Alvarado. 2014. Slaughter-line operation: poultry. Pages 303-308 in Encyclopedia of Meat Sciences, eds. C. Devine and M. Dikeman. Academic Press, Oxford, UK.

Schonewille, E., L. L. Nesse, R. Hauck, D. Windhorst, H. M. Hafez, and L. K. Vestby. 2012. Biofilm building capacity of *Salmonella enterica* strains from the poultry farm environment. FEMS Immunol. Med. Microbiol 65:360-365.

Sergeant, M. J., C. Constantinidou, T. A. Cogan, M. R. Bedford, C. W. Penn, and M. J. Pallen. 2014. Extensive microbial and functional diversity within the chicken cecal microbiome. PLoS ONE 9:e91941

Simmons, M., D. L. Fletcher, M. E. Berrang, and J. A. Cason. 2003. Comparison of sampling methods for the detection of *Salmonella* on whole broiler carcasses purchased from retail outlets. J. Food Prot. 66:1768-1770.

Simonsen, B. 1995. Microbiological criteria for poultry products. Pages 221-250 in Processing of Poultry, ed. G.C. Mead. Chapman and Hall, Bristol, UK

Simonsen, B., F. L. Bryan, J. H. B. Christian, T. A. Roberts, R. B. Tompkin, and J. H. Silliker. 1987. Prevention and control of food-borne salmonellosis through application of hazard analysis critical control point (HACCP). Int. J. Food Microbiol. 4:227-247. doi http://dx.doi.org/10.1016/0168-1605(87)90040-7

Slader, J., G. Domingue, F. Jørgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. Appl. Environ. Microbiol. 68:713-719. doi 10.1128/AEM.68.2.713-719.2002

Smith, J., S. Corkran, S. R. McKee, S. F. Bilgili, and M. Singh. 2015. Evaluation of post-chill applications of antimicrobials against *Campylobacter jejuni* on poultry carcasses. J Appl Poult Res 24:451-456. doi 10.3382/japr/pfv046

Smith, D., J. Cason, D. Fletcher, and J. Hannah. 2007. Evaluation of carcass scraping to enumerate bacteria on prechill broiler carcasses. Poult. Sci. 86:1436-1439.

Smith, D. P. 2014. Poultry processing and products. Pages 549-566 in Food Processing: Principles and Applications. eds. S. Clark, S. Jung, and B. Lamsa. John Wiley & Sons, Hoboken, N.J.

Sofos, J. N., G-J. Flick, C. O'Bryan, S.C. Ricke, and P.G. Crandal. 2013. Meat, poultry, and seafood. Pages 111-167 in Food Microbiology: Fundamentals and Frontiers, eds. M. P. Doyle, and R. L. Buchanan. ASM Press, Washington, DC. doi:10.1128/9781555818463.ch6

Stanley, D., S. Denmen, R. Hughes, M. Geier, and H. Chen. 2014. Microbiota of the chicken gastrointestinal tract: Influence on health, productivity and disease. Appl. Microbiol. Biotechnol. 98:4301-4310.

Steenackers, H., K. Hermans, J. Vanderleyden, and S. C. J. De Keersmaecker. 2012. *Salmonella* biofilms: An overview on occurrence, structure, regulation and eradication. Food Res. Int. 45:502-531. doi http://dx.doi.org/10.1016/j.foodres.2011.01.038

Stefani, L. M., R. G. Backes, G. A. Faria, C. P. Biffi, J. M. de Almeida, H. K. da Silva, G. B. das Neves, and A. Langaro. 2014. Trimming and washing poultry carcass to reduce microbial contamination: A comparative study. Poult. Sci. 93:3119-3122.

Stern, N. J., M. R. S. Clavero, J. S. Bailey, N. A. Cox, and M. C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. Poult. Sci. 74:937-941.

Stern, N. J., P. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. in selected U.S. poultry production and processing operations. J. Food Prot. 64:1705-1710.

Stopforth, J. D., R. O'Connor, M. Lopes, B. Kottapalli, W. E. Hill, and M. Samadpour. 2007. Validation of individual and multiple-sequential interventions for reduction of microbial populations during processing of poultry carcasses and parts. J. Food Prot. 70:1393-1401.

St. Cyr, A. J. 2003. Food plant inspections. Pages 51-60 in Food Plant Sanitation, eds. Y. H. Hui, B. L. Bruinsma, J. R. Gorham, W. K. Nip, P. S. Tong, and P. Ventresca. Marcel Dekker, Inc., New York, NY.

Svihus, B., H. Hetland, M. Choct, and F. Sundby. 2002. Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. Br. Poult. Sci. 43:662-668. doi 10.1080/0007166021000025037

Thomas, C. J., and T. A. McMeekin. 1980. Contamination of broiler carcass skin during commercial processing procedures: An electron microscopic study. Appl. Environ. Microbiol. 40:133-144.

Thomas, C. J., and T. A. McMeekin. 1982. Effect of water immersion on the microtopography of the skin of chicken carcasses. J. Sci. Food Agric. 33:549-554. doi 10.1002/jsfa.2740330609

Thomas, C. J., T. A. McMeekin, and J. T. Patterson. 1987. Prevention of microbial contamination in the poultry processing plant. Pages 163-179 in Elimination of Pathogenic Organisms from Meat and Poultry: Proceedings of the International Symposium: Prevention of Contamination, and Decontamination in the Meat Industry, ed. F. M. J. Smulders. Elsevier Science Publishers, New York, NY.

Todd, E. C. D. 2004. Microbiological safety standards and public health goals to reduce foodborne disease. Meat Sci. 66:33-43. doi http://dx.doi.org/10.1016/S0309-1740(03)00023-8

Todd, E. C. D., J. D. Greig, C. A. Bartleson, and B. S. Michaels. 2009. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. J. Food Prot. 72:202-219.

Tompkin, R. B. 1990. The Use of HACCP in the production of meat and poultry products. J. Food Prot. 53:795-803. doi 10.4315/0362-028x-53.9.795

Torrence, M. E. 2003. U.S. federal activities, initiatives, and research in food safety. Pages 3-9 in Microbial Food Safety in Animal Agriculture, eds. M. E. Torrence, and R. E. Isaacson. Iowa State Press, Ames, IA.

Trevisan, R. B., V. S. Nakagi, P. T. Bravo, and D. E. Faria. 2014. Feeding programs and their effects on broiler performance and economic indexes. J Appl Poult Res 23:593-604.

Trevors, J. T. 2011. Viable but non-culturable (VBNC) bacteria: Gene expression in planktonic and biofilm cells. J. Microbiol. Methods 86:266-273. doi http://dx.doi.org/10.1016/j.mimet.2011.04.018

USDA, FSIS. 1994. The nationwide broiler chicken microbiological baseline data collection program. Accessed January 2016. http://www.fsis.usda.gov/wps/wcm/connect/9db350d5-ff76-4193-adcc-9287794301a3/broilers_1994-1995.pdf?MOD=AJPERES

USDA, FSIS. 1996. Pathogen reduction; hazard analysis critical control point (HACCP) systems, final rule. 9CFR Part 301. Fed. Regist. 61: 38805-38989.

USDA, FSIS. 1999. The nationwide young chicken microbiological baseline data collection program. Accessed January 2016. http://www.fsis.usda.gov/wps/wcm/connect/5fc8968b-cba1-4a38-a36d-9924b8deb21d/Baseline_Data_Young_Chicken.pdf?MOD=AJPERES.

USDA, FSIS. 2007. The nationwide microbiological baseline data collection program: young chicken survey. Accessed January 2016. http://www.fsis.usda.gov/wps/wcm/connect/deab6607-f081-41a4-90bf-8928d7167a71/Baseline_Data_Young_Chicken_2007-2008.pdf?MOD=AJPERES.

USDA, FSIS. 2012. HACCP plan reassessment for not-ready-to-eat comminuted poultry products and related agency verification procedures. 9 CFR Parts 417. Fed. Regist. 77: 72686- 72691.

USDA, FSIS. 2014. Modernization of poultry slaughter inspection, final rule. 9 CFR Parts 381 and 500. Fed. Regist. 79: 49566- 49637.

USDA, FSIS. 2015. Changes to the *Salmonella* and *Campylobacter* verification testing program: proposed performance standards for *Salmonella* and *Campylobacter* in not-ready-to-eat comminuted chicken and turkey products and raw chicken parts and related agency verification procedures and other changes to agency sampling. Fed Regist 80: 3940-3950.

USDA, FSIS. 2016. New performance standards for *Salmonella* and *Campylobacter* in not-ready-to-eat comminuted chicken and turkey products and raw chicken parts and changes to related agency verification procedures: response to comments and announcement of implementation schedule. Fed. Regist. 81: 7285- 7300.

United States Code. 2014. Title 21 - Food and drugs. Chapter 10: Poultry and poultry products inspection. U.S. Government Publishing Office.

U.S. Department of Health and Human Services. 1986. The 1990 health objectives for the nation: a midcourse review. Public Health Service: Office of Disease Prevention and Health Promotion. Rockville, MD.

U.S. Department of Health and Human Services. 1991. Healthy people 2000: national health promotion and disease prevention objectives and full report, with commentary. Public Health Service: Office of Disease Prevention and Health Promotion. Rockville, MD.

U.S. Department of Health and Human Services. 2000. Healthy people 2010: Understanding and improving health. 2nd ed. U.S. Government Printing Office, Washington, DC.

Wabeck, C. J., D. V. Schwall, G. M. Evancho, J. G. Heck, and A. B. Rogers. 1968. *Salmonella* and total count reduction in poultry treated with sodium hypochlorite solutions. Poult. Sci. 47:1090-1094. doi 10.3382/ps.0471090

Wabeck, C. J. 1972. Feed and water withdrawal time relationship to processing yield and potential fecal contamination of broilers. Poult. Sci. 51:1119-1121. doi 10.3382/ps.0511119

Waite, D., and M. Taylor. 2015. Exploring the avian gut microbiota: Current trends and future directions. Front Microbiol 6: 673. doi 10.3389/fmicb.2015.00673

Waldroup, A., N. Imel, and B. Rathgeber. 1993. Microbiological aspects of counter current scalding. J Appl Poult Res 2:203-207.

Wei, S., M. Morrison, and Z. Yu. 2013. Bacterial census of poultry intestinal microbiome. Poult. Sci. 92:671-683. doi 10.3382/ps.2012-02822

Wesley, I. V. 2009. Food safety issues and the microbiology of poultry. Pages 169-185 in Microbiologically Safe Foods, eds. N. Heredia, I. Wesley, and S. Garcia. Wiley & Sons, Inc., Hoboken, NJ.

Veerkamp, C. H. 1995. Chilling, freezing and thawing. Pages 103-125 in Processing of Poultry, ed. G. C. Mead. Chapman and Hall, Bristol, UK

Voelkerding, K. V., S. A. Dames, and J. D. Durtschi. 2009. Next-generation sequencing: from basic research to diagnostics. Clin. Chem. 55:641-658.

Vongkamjan, K., and M. Wiedmann. 2014. Starting from the bench--prevention and control of foodborne and zoonotic diseases. Prev. Vet. Med. 118:189-195. doi 10.1016/j.prevetmed.2014.11.004

Yang, H., Y. Li, and M. G. Johnson. 2001. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. J. Food Prot. 64:770-776.

Yang, Z., L. Yanbin, and M. Slavik. 1998. Use of antimicrobial spray applied with an inside–outside birdwasher to reduce bacterial contamination on prechilled chicken carcasses. J. Food Prot. 61:829-832. doi 10.4315/0362-028x-61.7.829

Yegani, M., and D. R. Korver. 2008. Factors affecting intestinal health in poultry. Poult. Sci. 87:2052-2063. doi 10.3382/ps.2008-00091

Zhang, L., J. Y. Jeong, K. K. Janardhanan, E. T. Ryser, and I. Kang. 2011. Microbiological quality of water immersion-chilled and air-chilled broilers. J. Food Prot. 74:1531-1535. doi 10.4315/0362-028X.JFP-11-032

Zhang, Q., J. Cao, and L. Cheong. 2017. Spoilage microorganisms in poultry products. Pages 101-112 in Food Spoilage Microorganisms, eds. Y. Wang, W. Zhang, and L. Fu. CRC Press, Boca Raton, FL. CRC Press.

Zhuang, R., and T. Moore. 2015. Factors influencing US poultry exports. IFAMR. 18:13-26.

Zorlugenc, B., and F. K. Zorlugenc. 2012. Ozone in food preservation. Pages 231-245 in Progress in Food Preservation, eds. Bhat, R., Karim Alias, A., Paliyath, G. Wiley-Blackwell, Oxford, UK.

Zweifel, C., D. Althaus, and R. Stephan. 2015. Effects of slaughter operations on the microbiological contamination of broiler carcasses in three abattoirs. Food Control 51:37-42. doi http://dx.doi.org/10.1016/j.foodcont.2014.11.002

Zweifel, C., and R. Stephan. 2012. Microbial decontamination of poultry carcasses. Pages 60-95 in Microbial Decontamination in the Food Industry. Woodhead Publishing, Oxford, UK.

3 Microbiome Comparisons of Poultry Carcass Samples at Pre-Evisceration Sites Within Commercial Processing Facilities

Co-Authors: Kristina M. Feye, Si Hong Park, Sun Ae Kim, and Steven C. Ricke.

Poultry processing systems are a complex network of equipment and automation systems that require a proactive approach to monitoring in order to protect the food supply. Process oversight requires the use of multi-hurdle intervention systems to ensure that any undesirable microorganisms are reduced or eliminated by the time the carcasses are processed into final products. In the present study, whole bird carcass rinses (WBCR) collected at the post-scalder and post-picker locations from three different poultry processing facilities (Plant A: mid-weight broiler processing, B: large-weight broiler processing, C: young broiler (Cornish) processing) were subjected to next generation sequencing (NGS) and microbial quantification using direct plating methods to assess the microbial populations present during these stages of the poultry process. The quantification of aerobic plate counts (APC) and Enterobacteriaceae (EB) demonstrated that reductions for these microbial classes were not consistent between the two sampling locations for all facilities, but did not provide a clear picture of what microorganism(s) may be affecting those shifts. With the utilization of NGS, a more complete characterization of the microbial communities present including microorganisms that would not have been identified with the employed direct plating methodologies were identified. Although the foodborne pathogens typically associated with raw poultry, *Salmonella* and *Campylobacter*, were not identified, sequence analysis performed by Quantitative Insights of Microbiology Ecology (QIIME) indicated shifts of *Erwinia*, *Serratia* and *Arcobacter*, which are microorganisms closely related to *Salmonella* and *Campylobacter*. Additionally, the presence of *Chryseobacterium* and *Pseudomonas* at both sampling locations and at all three facilities provides evidence that these

microorganisms could potentially be utilized to assess the performance of multi-hurdle intervention systems.

3.1 Introduction

The complexity of commercial processing of poultry requires an efficient and extensive network of equipment, automation; and oversight to maintain quality and food safety standards (Chao et al., 2014; Handley et al., 2015; Zweifel et al., 2015). The industry employs a wide range of policies and procedures to control and monitor fecal and ingesta contamination. Upon arrival to the abattoir, live chickens will be dirty, with microorganisms and environmental contamination on their feet, feathers and skin. Furthermore, the nature of slaughter liberates microorganisms from the alimentary tract onto the surface of the carcass and abattoir (Grau, 1986; Handley et al., 2015; Oakley et al., 2014; Rinttilä and Apajalahti, 2013). The dispersal of some microorganisms, namely *Salmonella* and *Campylobacter*, in poultry processing is a constant concern to the industry due to the risk of food borne disease that can be caused by consuming raw poultry (Batz, et al., 2012; Buncic and Sofos, 2012).

The scalding and feather removal stages in commercial poultry processing has been identified as one of the first steps within the process with the potential to contaminate poultry carcasses (Allen et al., 2003a,b; Berrang et al., 2001; Buncic and Sofos, 2012; Dickens and Whittemore, 1997). Contamination events have been identified via microbiological culturing techniques including enumeration of indicator microorganism(s) and/or pathogenic microorganism(s), as well as qualitative molecular methods (Corry et al., 2007; Oakley et al., 2013; Oyarzabal and Hussain, 2010). However, the advancements of NGS has allowed for a more comprehensive characterization of the ecology of microbiota on poultry carcasses within the evisceration and chilling stages of poultry processing (Handley et al., 2018; Kim et al., 2017;

De Cesare et al., 2018). Analysis of the microbiome profiles of the poultry carcasses sampled at the latter stages of the process have provided information about the efficacy of multi-hurdle interventions, as well as identified both indicator and pathogenic microorganisms impacting food safety and quality (Handley et al., 2018). With this knowledge, it would suffice that the earlier stages of the process, including scalding and feather removal could also benefit from microbiome analysis utilizing NGS technologies. If the microbial communities from these processing stages at different plants are better understood, there could be a possibility to tailor the employment of different antimicrobials based on microbial communities present at the facility level.

This data serves to provide insight into the microbial ecology present within the early stages of poultry processing utilizing both traditional methods and un-restrained-NGS microbiome sequencing. A comparison of traditional microbiological and molecular screens with the unrestrained, culture-free NGS microbiome analysis will determine if weaknesses exist in current methods for the detection of pathogens and indicator organisms. With the application of NGS, a deeper understanding of these shifts will enable the poultry industry to identify new microbiome patterns and indicator organisms, which has the potential to elicit more specific intervention measures and improve food safety.

3.2 Poultry Processing Plant Selection and Operations

Sixty whole bird carcass rinses in total were collected from 3 different commercial poultry plants (n=20) located in the southern geographical region of the United States during the summer months (May to June) of 2015. At each plant, 10 whole bird carcasses were collected from the process line at the post-scalder and post-picker locations. The post-scalder and post-picker locations were chosen for collection sites as these process points represent the early stages of processing and theoretically, carcass rinses performed at these stages would have the highest

CFU/mL microbial counts in terms of APC and EB. To limit variation in data, collections at each processing facility were limited to carcasses from the same flock/lot. Sample collection occurred at three different processing facilities, each equipped to process three different classes of broilers. Each class of broiler represented different stages of the grow out period: Cornish hens weighing 2 to 4 lbs. (907 to 1,814 g), mid-weight broilers weighing 4 to 6 lbs. (1,814 to 2,722 g), and larger broilers weighing 6 to 8 lbs. (2,722 to 3,629 g). The poultry processing facilities were chosen to provide microbial data related to bird age, as well as data related to facility specificity as the equipment and operations used are typically tailored to process carcasses of specific sizes as well as the final food product produced.

The commercial processing systems at each facility had similar scalding and picker operations. All facilities had scalder tanks equipped with a counter-flow water influx in which fresh water was added to the final tank. Tank overflow was released from the initial tank, which is where the organic load would be expected to be the highest. This allows carcasses to move through progressively cleaner water in the scalder tanks. The water temperature and immersion time varied slightly for each facility due to the size of the bird and its end consumer use. Plant A and B operated a soft scald system in which carcasses were immersed in a 52 to 57°C scalding tank for 180 seconds. Plant C also operated a soft scald system, however as the carcasses were smaller, the dwell time was slightly reduced to between 90 to 100 seconds. After scalding, the carcasses moved into the feather removal or picking machines where rubber fingers or protrusions applied pressure to pull and remove feathers from the follicles. The operations of the picking machines were similar for all three facilities, in which the carcasses were subjected to 3 banks (each 0.91 meter in length) of rubber fingers. Total picking time averaged 180 s for each carcass.

3.3 Sample Collection

Conforming to USDA protocols (USDA, 2013), whole carcasses were randomly selected from the operating lines at the designated sampling points (post-scalder and post-picker) and placed in sterile rinse bags. Carcasses were selected in quick succession at both locations and samples were representative of the same lot/flock. After collection, 400 mL of Butterfield's Phosphate Buffer solution were poured over the surface of the carcass and the rinse bag was folded close. The carcasses were rinsed for 1 minute in an arcing motion to ensure that rinsate could move along the carcass surface. Collected rinsate was transferred to the original container and these containers were placed on ice in a cooler and then transported to the testing laboratory.

3.4 APC and Enterobacteriaceae Analyses

Upon receipt at the testing laboratory, 1.0 mL aliquots of rinsates and associated serial dilutions were enumerated for APC and EB counts with 3M™ APC or EB count petrifilm™ (3M™, St. Paul, MN, USA). The gelling and nutrient components of 3M™ Petrifilm™ allow for the analysis and enumeration of a wide range of microorganisms. Analyses for APC counts were performed per Official Methods of Analysis (OMA) 990.12 published by the International Association of Official Analytical Chemists (AOAC) and analysis for EB counts were performed according OMA 2003.01 with a modified incubation temperature (35±1°C) per Compendium of Methods for the Microbiological Examination of Foods recommendations (Kornacki, et al., 2015).

3.5 16S rDNA Microbiome Sequencing

Aliquots (50 mL) of the rinsates were centrifuged (Sorvall Lynx 6000, Thermo Fisher Scientific, Langenselbold, Germany) for 15 min at 8,000•g, to pellet the bacterial cells. Genomic DNA extraction of the formed pellets were performed using a QIAamp DNA Stool Mini Kit (Qiagen,

Valencia, CA, USA) following the kit's standard protocol except for reducing the elution volume to 50 µL. The DNA concentration and purity were measured for each extraction using a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). Aliquots were stored -20°C until further analysis could be performed.

The sequencing pipeline is as per Kozich et al. (2013) and Park et al. (2016). The individual rinsate sample DNA was diluted to 10 ng/µL and an Illumina MiSeq Library was prepared, and targeted the V4 region of 16S rRNA using dual-indexed primers via Eppendorf Mastercycler pro S (Eppendorf, Westbury, NY, USA). Confirmation of amplicon presence and relative size was conducted via a 1% agarose gel. The library was normalized via SequalPrep™ Normalization kit (Life Technology) as per the manufacturer's instructions, except that the final elution volume was modified to 15 µl. The library was pooled and assessed for purity and concentration was evaluated via quantitative polymerase chain reaction (qPCR) using the standard protocol from the KAPA Library Quantification Kit (Kapa Biosystems, Woburn, MA, USA). The qPCR reactions were performed on an Eppendorf Mastercycler EP Gradient S (Eppendorf, Westbury, NY, USA) (R^2=0.999; Efficiency=0.97). The amplicon size and concentration was further confirmed by Agilent 2100 Bioanalyzer System (Agilent, Santa Clara, CA, USA). Amplicons were diluted to 4 nM and combined with prepared PhiX Control v3 (5%, v/v) (Illumina, San Diego, CA, USA) and sequenced. Sequencing was carried out as per the Illumina MiSeq v2 (500 cycle) Reagent Cartridge (Illumina) instructions.

3.6 QIIME Sequence Data Processing and Analysis

The sequence output (FASTA files) was downloaded from the Illumina Biospace Website and the following protocol is based on Park et al. (2016). The analysis of the sequence reads was performed using Quantitative Insights into Microbial Ecology (QIIME) pipeline version 1.9.0,

which provides analysis of the sequence, classification of Operational Taxonomic Units (OTUs), and species diversity and richness of the reads. Sequences were assigned to taxonomic levels based on 97% identity levels to those found on the Greengenes 16S rRNA gene database and chimeric sequences were discarded. Samples for each facility (Plant A, B and C) were normalized to a fixed read depth based on the lowest number of sequences achieved for that sample set. Associated alpha and beta diversity measurements were obtained from QIIME package using these read depths. Taxonomic tables of sequences were limited to sequences present at relative abundances greater than or equal to 0.5% within that facility's sample set.

3.7 Statistical Analysis

Enumerated counts (CFU/mL) obtained from 3M™ petrifilm™ analyses for each carcass were $\log_{10}$ transformed. The $\log_{10}$ transformed average values were compared for mean differences in microbial populations between post-scalder and post-picker sample collections within each facility, and used to provide numerical values for comparisons among the three processing facilities. The log transformed count data were evaluated using an analysis of variance (ANOVA) in SAS (Statistical Analysis Software, Cary, NC), where collection site was treated as a main effect and location means were separated using Duncan's multiple range test. Statistical significance is determined at $p<0.05$.

Alpha and beta diversity calculations were used to analyze the resulting OTU sequences. Alpha diversity measurements including Chao 1 indices and OTU rarefaction curves, provided information about the OTU richness and diversity of individual samples within each population. Beta diversity measurements including both weighted and unweighted UniFrac diversity plots of principal coordinate analysis (PCoA) (Lozupone and Knight, 2005) were used to evaluate OTU diversity between the post-scalder and post-picker populations. Utilizing these diversity

measurements, the microbiomes were analyzed to determine what differences existed in sampling locations (post-scalder and post-picker) for each of the facilities.

3.8 Results: APC and Enterobacteriaceae Load Quantitation

Average bacterial counts of APC and EB on chicken carcass rinsates from post-scalder and post-picker at three different processing plants are presented in Figure 1. The average log APC counts of rinsates from the post-picker location at Plant A and Plant B were 0.53 log CFU/mL and 0.61 log CFU/mL lower, respectively, and significantly different ($p \leq 0.05$) than the rinsates collected from the post-scalder locations (Figures 1A and 1C). In contrast, there was no significant difference in APC levels between post-scalder and post-picker location at plant C (Figure 1E). At Plant C, EB counts for the post-picker rinsates were 0.84 log CFU/mL higher than rinsates from the post-scalder location (Figure 1F). While the increase in EB counts between the two sampling locations at Plant C was significantly different ($p \leq 0.05$), there were no significant differences in EB levels from plant A and B (Figures 1B and 1D).

3.9 Results: Microbiome Analysis

Alpha and beta diversity are important tools that are utilized as a component of microbiome analysis to assess the diversity of sequencing depth (alpha) and the compositional variety between samples (beta). Normalization of the OTU sequences that were analyzed per facility, ensured that the depth of sequences was equal and allowed for alpha diversity measurements via Chao1 and OTU rarefaction curves (Figures 2 and 3, respectively). Overall, the samples collected at the post-picker site were generally less diverse in terms of OTU richness when compared to the samples collected at the post-scalder locations. The largest difference between the two sampling locations was observed at Plant C, while the smallest difference was observed at Plant B. Observed OTU rarefactions indicated there were lower numbers of OTUs observed

in samples collected at the post-picker locations when compared to those samples collected at the post-scalder locations. The largest number of OTUs for both sampling locations was observed at Plant A.

Weighted and unweighted principal coordinated analysis (PCoA) UniFrac plots generated by the beta diversity analysis from the three plants are presented in Figure 4. Beta diversity measurements from Plant A demonstrated clustering based on location for both the weighted and unweighted PCoA plots. Comparing the two PCoA plots, the communities appear to have mostly different compositions, although clustering is somewhat reduced in the weighted plots which suggest the populations are slightly more similar than what was depicted by the unweighted measurements. Conversely, for Plants B and C, the weighted PCoA plots do not demonstrate distinct separation between the two communities, whereas the unweighted PCoA plot does demonstrate clustering based on sample location, although the separation is slightly more pronounced at Plant B.

The major bacterial phyla represented in the pooled rinsates are presented in Figure 5, and the major genera identified within the pooled rinsates from each facility are presented in Figures 6-8. Samples collected at Plant A at the post-scalder location had the largest number of OTUs present with most the OTUs being derived from three major phyla groups*: Firmicutes, Proteobacteria,* and *Bacteroidetes*. At the post-picker location for Plant A, no OTUs represented the phyla *Firmicutes*, but there was a large increase in relative abundance of phyla *Proteobacteria* (47.50% to 74.65%), and a small increase in phyla *Bacteroidetes* (16.19% to 18.89%). The most abundant sequences (29.74%) within the phyla *Proteobacteria* belong to the *Pseudomonadaceae*, and 24.73% are identified to genus level as *Pseudomonas*, and 4.74% are identified to species level as *Pseudomonas viridiflava* (Figure 6). There was also an increase in OTUs representing

the phyla *Bacteroidetes*, and furthermore, post-picker samples appeared to be more diverse as there were five OTUs identified (*Flavobacterium*, *Chryseobacterium*, *Sphingobacterium*, *Dysgonomonas* and *Bacteroides*) while only three OTUs were identified (*Flavobacterium*, *Chryseobacterium*, and *Bacteroides*) in post-scalder samples (Figure 6).

Plant B relative abundance values of OTUs were like Plant A, with the exception that OTUs belonging to phyla *Firmicutes* were still present at the post-picker location, albeit at low levels (1.31%) (Figure 7). This is interesting as the relative abundance levels (16.41%) of *Firmicutes* at the post-scalder location were lower than those observed within Plant A levels (29.85%). Like Plant A, the relative abundance levels of *Proteobacteria* at the post-picker location (83.12%) were higher than the relative abundance levels of *Proteobacteria* at the post-scalder location (59.47%). For both sampling locations, the most abundant sequences from the phyla of *Proteobacteria* were from *Pseudomonadaceae* (31.67% and 52.76%, post- scalder and post-picker, respectively), although none of the sequences are identified past the genus level at the post-scalder site, and only one species was identified at the post-picker site (*Pseudomonas veronii*) (Figure 7).

At Plant B, there was an increase in relative abundance values of OTUs identified as belonging to *Enterobacteriaceae* between the two sampling locations (1.96% and 3.04%, post- scalder and post-picker, respectively). At the post-picker location, the *Enterobacteriaceae* was represented by *Serratia* (0.95%) and an unidentified sequence (2.09%). At the post-scalder location, none of the sequences were representative of *Campylobacteraceae*, while 5.59% of the OTUs present at the post-picker location could be attributed to *Campylobacteraceae*, identified as *Arcobacter*. While there was an increase in *Proteobacteria* OTUs between the two sampling locations, the relative abundance level of phyla *Bacteroidetes* decreased (19.32% and 12.96%, post-scalder and

post-picker, respectively), although the genera diversity between the two sites appears to be similar as the same OTUs were present in both locations (*Flavobacterium, Chryseobacterium, Pedobacter*, and *Sphingobacterium*).

Many of the OTUs obtained from both sampling locations at Plant C represent microorganisms belonging to phyla *Proteobacteria* and *Bacteroidetes,* although there were a small percentage of members of phyla *Firmicutes* and *Actinobacteria* present at the post-scalder location. The relative abundance of *Proteobacteria* OTUs at each sampling location increased from 60.75% to 72.19%. The most abundant OTUs from the *Proteobacteria* phyla were identified as *Stenotrophomonas* (15.96%) at the post-scalder location, and *Pseudomonas* (27.87%) at the post-picker location (Figure 8). Like the other plants, there was an increase in the relative abundance of OTUs belonging to *Enterobacteriaceae* (represented by genus *Erwinia*) between the two sampling locations (1.92% and 6.07%, post-scalder and post-picker, respectively). At Plants A and B, there was a decrease in *Bacteroidetes* OTUs, between the two sampling locations (35.09% and 23.96%, post-scalder and post-picker, respectively), as well as a decrease in the genera diversity as only two of the three genera present at the post-scalder location (*Chryseobacterium*, *Pedobacter*, and *Sphingobacterium*) were present at the post-picker location (*Chryseobacterium* and *Sphingobacterium*).

3.10 Discussion

Traditional microbiological methods have been employed to detect pathogen and indicator organism carriage and load on rinsates. Interestingly, the same methods have also detected the differences in the growth conditions, transport and processing facility environments, and bird age at processing (Davies, 2005; Davies and Wray, 1994; Grau, 1986; Kim et al., 2017; Lyon et al., 1991; Oakley et al., 2014; Rinttilä and Apajalahti, 2013; Schilling et al., 2014; Zweifel and

Stephan, 2012). This research uses these methods as well as microbiota data to assess and quantify differences between microbial loads on different sized carcasses before and after feather removal (picking) to potentially provide a more robust microbiological profile of these process stages at three different processing facilities.

The age of the broiler, and the specificities of equipment and operations within the processing facility undoubtedly play roles in creating microbial ecology diversity. Understanding how the microbiomes are established as well as how they differ between facilities could be used by processors to tailor intervention strategies on a plant by plant basis. Further, the identification and monitoring of closely related microorganisms belonging to the same family as the target pathogen(s) can provide a more robust indication of the risk of those pathogens in the process or system being evaluated. Particularly, for this data set, shifts of phyla *Proteobacteria*, which encompass OTUs from *Enterobacteriaceae* and *Campylobacteraceae*, could be used to determine how these process steps influence the presence or persistence of those microorganisms that can cause food borne illnesses, namely *Salmonella* and *Campylobacter*. Determining the most inclusive indicator microorganism(s) is dependent on several factors including whether the microorganism(s) are adequate in terms of their response to the environment and conditions that the target pathogen(s) encounter (Sinclair et al., 2012). Response of microorganisms in any given environment is controlled by the genetic makeup, and taxonomically related microorganisms will typically respond similarly (Sinclair et al., 2012), so close evaluation of related microorganism(s) present within a system could be informative when microbiologically evaluating a process or system. At Plant A, OTU composition of samples collected at the post-scalder site demonstrated a relatively large proportion of the phyla group *Proteobacteria* (47.50%), although only 0.81% of the OTUs represented *Enterobacteriaceae*, of which

Salmonella belong. The *Enterobacteriaceae* present were instead identified as belonging to the genus *Erwinia* (Figure 6). Similar results from plants B and C also indicated increases as well as changes in OTU composition of phyla *Proteobacteria* abundancies between the two sampling locations. At Plant B, OTUs representing *Enterobacteriaceae* at the post-scalder location (unidentified OTU) were different than those identified at the post-picker location (unidentified OTU and *Serratia*) and at Plant C, *Enterobacteriaceae* OTUs present were identified as genus *Erwinia*. None of the sequences represented OTUs from *Campylobacteraceae*, of which *Campylobacter* belong, although 5.59% of the sequences identified at the post-picker location at Plant B were identified as genus *Arcobacter*. *Arcobacter*, previously identified as "aerotolerant Campylobacters" (Phillips, 2002), can grow at colder temperatures when compared to *Campylobacter* sp., and have also been isolated from human feces in patients presenting with intestinal distress (Atabay et al, 1998; Kabeya et al., 2004). *Arcobacter* has been isolated at various rates in broiler meat, fecal and cloacal sampling (Kabeya et al., 2004), but the rates of isolation in poultry carcasses compared to those obtained from caecal sampling indicate that carcass contamination is a function of the processing environment and not necessarily due to infection present within the entire flock (Driessche and Houf, 2007; Phillips, 2001). As the processing steps evaluated in this study occur prior to any evisceration steps, the presence of microorganisms belonging to *Enterobacteriaceae* indicate that the enteric microorganisms are present within the scald waters and associated equipment surfaces and could likely be attributed to expulsion of the viscera as well as external contamination of the bird's feet and feathers. The persistence of these microorganisms in the environment could also be a factor of their abilities to adhere to various surfaces including stainless steel which could allow for their survival in process waters (Assanta et al, 2002; Driessche and Houf, 2007).

The presence and increase in relative abundance of the phyla *Bacteroidetes* within this sample set may also be an indication of the effect the processing environment has on possible cross contamination events within these stages. Members of this phyla, have been identified as major constituents in the microbial consortium of the lower GIT of poultry (Han et al., 2016; Oakley et al., 2016). Similar to findings by Handley et al., (2018), the presence of *Chryseobacterium* at both post-scalder and post-picker sites, which occur prior to an evisceration steps, indicate that this microorganism could be indicative of cross-contamination due to presence of fecal material and soil on the feathers and feet of processed carcasses. Isolation and identification of this class of microorganisms could therefore be useful in evaluating processing systems to better understand the impact of fecal material, soil, and feather contamination as well as provide information regarding the extent of cross contamination events that may occur between flocks.
It is well documented that species belonging to Pseudomonadaceae are often isolated from raw and spoiled poultry products (Deusch et al., 2008; Nychas et al., 2008). Pseudomonadaceae are introduced into the processing environment on the feet, feathers, dirt and debris of the birds being processed, and the associated sterile muscle meat produced becomes contaminated with these spoilage microorganisms via equipment, water and aerosol production in the processing environment (Firildak et al., 2015; Geornaras et al., 1999; Rouger et al., 2017). Understanding the microbiome of these poultry process stages can help to determine shifts in Pseudomonadaceae populations including when the microorganisms are introduced, dispersal routes, as well as response to sanitation practices and environment changes. While some of these shifts may be identified by direct plating techniques, including 3M™ petrifilm™, the increase of spoilage organisms represented by Pseudomonadaceae may not be specifically identified due to the selectivity induced by the mesophilic incubation temperatures used for the petrifilm analyses

(Barnes, 1972; Oyarzabal and Hussain, 2010). Moreover, as biofilm formation of some *Salmonella* species has shown to be enhanced in the presence of *Pseudomonas*, understanding the effect of environment changes on Pseudomonadaceae could aid in understanding the effects that those changes may also exert on *Salmonella* (Habimana et al., 2010). Specifically, in this data set, the dominance of species from the *Pseudomonadaceae* could represent an opportunity to address interventions/measures that could better control the presence and proliferation of both spoilage and pathogenic microorganisms that may be present. The detection of *Pseudomonas* within different stages of the processing system serves to illustrate that these microorganisms could also be used as indicators of process performance as suggested previously by Handley et al. (2018).

3.11 Conclusion

This study is a unique approach to the application of microbiome analysis of the earliest, and microbial diverse stages (scalding and feather removal) within a commercial poultry processing system. Moreover, it highlights how diversity can differ among facilities, and that this diversity may change depending on the facility's equipment designs and operational standards as well as the age of the birds. Although, *Salmonella* and *Campylobacter* OTUs were not identified, the identification of closely related genera including *Erwinia*, *Serratia*, and *Arcobacter* could indicate that these stages could be further evaluated for possible improvements in terms of reducing *Enterobacteriaceae* microorganisms, which could help to reduce incidence of these microorganisms in later stages of the processing systems. Specifically, the presence of genus *Erwinia* at both post-scalder and post-picker locations at Plants A and C could indicate that these microorganisms could be used as predictors of *Enterobacteriaceae* prevalence and persistence in the environment which could provide information about potential carcass and flock cross-

contamination events. Additionally, the differences in OTU populations between post-scalder and post-picker locations for all three plants could indicate that the sources of the OTUs differed between the two locations, and specifically increases in diversity on post-picker carcasses could be attributed to the processing environment being influenced by other carcasses or flocks being processed on the same day. While this is somewhat expected, microbiome analysis provides a deeper understanding of the complete microbiome present, and provides specific identities of microorganisms to help determine the extent of the microbial cross contamination between these two stages within the process. Repeating this experiment and considering flock or shift variations could be used to help identify and evaluate the risks within these and other processing stages.

3.12 References

Ahmet, A., G. Firildak, and E. Goren. 2015. Chicken carcasses bacterial concentration at poultry slaughtering facilities. Asian J Biol Life Sci. 8:16-29.

Allen, V. M., M. H. Hinton, D. B. Tinker, C. Gibson, G. C. Mead, and C. M. Wathes. 2003a. Microbial cross-contamination by airborne dispersion and contagion during defeathering of poultry. Br. Poult. Sci. 44:567-576.

Allen, V. M., D. B. Tinker, M. H. Hinton, and C. M. Wathes. 2003b. Dispersal of microorganisms in commercial defeathering systems. Br. Poult. Sci. 44:53-59.

Assanta, M.A., D. Roy, M.J. Lemay, and D. Montpetit. 2002. Attachment of Arcobacter butzleri, a new waterborne pathogen, to water distribution pipe surfaces. J. Food Prot. 65: 1240-1247.

Atabay, H.I., J.E.L Corry, and S.L.W On. 1998. Diversity and prevalence of *Arcobacter* spp. in broiler chickens. J Appl Environ Microbiol. 84: 1007-1016. doi:10.1046/j.13652672.1998.00437.x

Barnes, E. M. 1972. Food poisoning and spoilage bacteria in poultry processing. Vet. Rec. 90:720-722.

Batz, M. B., S. Hoffmann, and J. G. Morris, Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J. Food Prot. 75:1278-1291. doi 10.4315/0362028x.jfp-11-418

Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. J. Food Prot. 64:2063-2066.

Buncic, S., and J. Sofos. 2012. Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. Food Res. Int. 45:641-655. doi http://dx.doi.org/10.1016/j.foodres.2011.10.018

Chao, K., M. S. Kim, and D. E. Chan. 2014. Control interface and tracking control system for automated poultry inspection. Computer Standards & Interfaces 36:271-277. doi http://dx.doi.org/10.1016/j.csi.2011.03.006

Corry, J. E., B. Jarvis, S. Passmore, and A. Hedges. 2007. A critical review of measurement uncertainty in the enumeration of food micro-organisms. Food Microbiol. 24:230-253. doi 10.1016/j.fm.2006.05.003

Davies, R. H. 2005. Pathogen populations on poultry farms. Pages 101-152 in Food Safety Control in the Poultry Industry, ed. G. C. Mead. CRC Press, Boca Raton, FL

Davies, R. H., and C. Wray. 1994. An approach to reduction of *Salmonella* infection in broiler chicken flocks through intensive sampling and identification of cross-contamination hazards in commercial hatcheries. Int. J. Food Microbiol. 24:147-160.

De Cesare, A., F. Palma, A. Lucchi, F. Pasquali, and G. Manfreda. 2018. Microbiological profile of chicken carcasses: A comparative analysis using shotgun metagenomic sequencing. Ital J Food Saf. 7:6923.

Deusch, S., B. Tilocca, A. Camarinha-Silva, and J. Seifert. 2015. News in livestock research — use of omics-technologies to study the microbiota in the gastrointestinal tract of farm animals. Comput Struct Biotechnol J 13:55-63. doi http://dx.doi.org/10.1016/j.csbj.2014.12.005

Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbiological quality of broiler carcasses prior to evisceration. Poult. Sci. 76:657-660. doi 10.1093/ps/76.4.657

Driessche, E.V. and K. Houf. 2007. Discrepancy between the occurrence of arcobacter in chickens and broiler carcass contamination. Poult. Sci. 86: 744-751.

Geornaras, I., N. F. Kunene, A. V. Holy, and J. W. Hastings. 1999. Amplified fragment length polymorphism fingerprinting of pseudomonas strains from a poultry processing plant. Appl. Environ. Microbiol. 65: 3828–3833.

Grau, F. H. 1986. Microbial ccology of meat and poultry. Pages 1-47 in Advances in Meat Research : Meat and Poultry Microbiology. eds. A. M. Pearson and T. R. Dutson. AVI Publishing, Westport, CT.

Habimana, O., T. Møretrø1, S. Langsrud, L. K. Vestby, L. L. Nesse, and E. V. Heir. 2010. Micro ecosystems from feed industry surfaces: a survival and biofilm study of Salmonella versus host resident flora strains. BMC Vet. Res. 6:48Han, G. G., E. B. Kim, J. Lee, J.Y. Lee, G. Jin, J. Park, C.-S. Huh, I.-K. Kwon, D. Y. Kil, Y.-J. Choi, and C. Kong. 2016. Relationship between the microbiota in different sections of the gastrointestinal tract, and the body weight of broiler chickens. Springerplus 5:911. doi 10.1186/s40064-016-2604-8

Handley, J. A., Z. Shi, S. H. Park, T. M. Dawoud, Y. M. Kwon, and S. C. Ricke. 2015. *Salmonella* and the potential role for methods to develop microbial process indicators on chicken carcasses. Pages 81-104 in Food Safety. ed. S.C. Ricke, J.R. Donaldson, C.A. Phillips. Academic Press, San Diego.

Handley, J.A., S.H. Park, S.A. Kim, and S. C. Ricke. 2018. Microbiome profiles of commercial broilers through evisceration and immersion chilling during poultry slaughter and the identfication of potential indicator microorganisms. Front. Microbiol. 9:345.

Kabeya, H., S. Maruyama, Y. Morita, T. Ohsuga, S. Ozawa, Y. Kobayashi, M. Abe, Y. Katsube, and T. Mikami. 2004. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. Int J Food Microbiol. 90: 303-308.

Kim, S.A., S.H. Park, S. I. Lee, C. M. Owens-Hanning, and S. C. Ricke. 2017. Assessment of chicken carcass microbiome responses during processing in the presence of commercial antimicrobials using a next generation sequencing approach. Scientific Reports 7:43354. doi 10.1038/srep43354

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112-5120. doi 10.1128/aem.01043-13

Lyon, C. E., C. M. Papa, and J. R. L. Wilson. 1991. Effect of feed withdrawal on yields, muscle pH, and texture of broiler breast meat. Poult. Sci. 70:1020-1025.

Nychas, G.-J. E., P. N. Skandamis, C. C. Tassou, and K. P. Koutsoumanis. 2008. Meat spoilage during distribution. Meat Science 78:77-89. doi http://dx.doi.org/10.1016/j.meatsci.2007.06.020

Oakley, B. B., C. A. Morales, J. Line, M. E. Berrang, R. J. Meinersmann, G. E. Tillman, M. G. Wise, G. R. Siragusa, K. L. Hiett, and B. S. Seal. 2013. The poultry-associated microbiome: Network analysis and farm-to-fork characterizations. PLoS ONE 8:e57190. doi 10.1371/journal.pone.0057190

Oakley, B. B., H. S. Lillehoj, M. H. Kogut, W. K. Kim, J. J. Maurer, A. Pedroso, M. D. Lee, S. R. Collett, T. J. Johnson, and N. A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100-112. doi 10.1111/1574-6968.12608

Oakley, B. B., and M. H. Kogut. 2016. Spatial and temporal changes in the broiler chicken cecal and fecal microbiomes and correlations of bacterial taxa with cytokine gene expression. Front Vet Sci 3:11. doi 10.3389/fvets.2016.00011

Oyarzabal, O. A., and S. K. Hussain. 2010. Microbial analytical methodology for processed poultry products. Pages 527-544 in Handbook of Poultry Science and Technology, Volume 2: Secondary Processing. eds. I. Guerrero-Legarreta, and Y. H. Hui. Wiley and Sons, Inc., Hoboken, N.J.

Park, S. H., S. I. Lee, and S. C. Ricke. 2016. Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an Illumina Miseq platform. PLoS One 11:e0151944. doi 10.1371/journal.pone.0151944

Phillips, C.A. 2001. *Arcobacter* spp in food: isolation, identification and control. Trends Food Sci Technol. 12: 263-275.

Rinttilä, T., and J. Apajalahti. 2013. Intestinal microbiota and metabolites: implications for broiler chicken health and performance. J Appl Poult Res 22:647-658.

Rouger, A., O. Tresse, and M. Zagorec. 2017. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms. 5:50.

Schilling, M. W., Y. Vizzier-Thaxton, and C. Z. Alvarado. 2014. Slaughter-line operation: poultry. Pages 303-308 in Encyclopedia of Meat Sciences, eds. C. Devine and M. Dikeman. Academic Press, Oxford, UK.

Sinclair, R. G., J. B. Rose, S. A. Hashsham, C.P. Gerba, and C. N. Haas CN. 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Appl. Environ. Microbiol. 78(6): 1969-77.

USDA, FSIS. 2013. *Salmonella* and *Campylobacter* verification program for raw meat and poultry products. FSIS Directive, 10,250.1. Washington, D.C. Accessed February 24, 2017.

Zweifel, C., D. Althaus, and R. Stephan. 2015. Effects of slaughter operations on the microbiological contamination of broiler carcasses in three abattoirs. Food Control 51:3742. doi http://dx.doi.org/10.1016/j.foodcont.2014.11.002

Zweifel, C., and R. Stephan. 2012. Microbial decontamination of poultry carcasses. Pages 60-95 in Microbial Decontamination in the Food Industry. Woodhead Publishing, Oxford, UK.

3.13 Figures

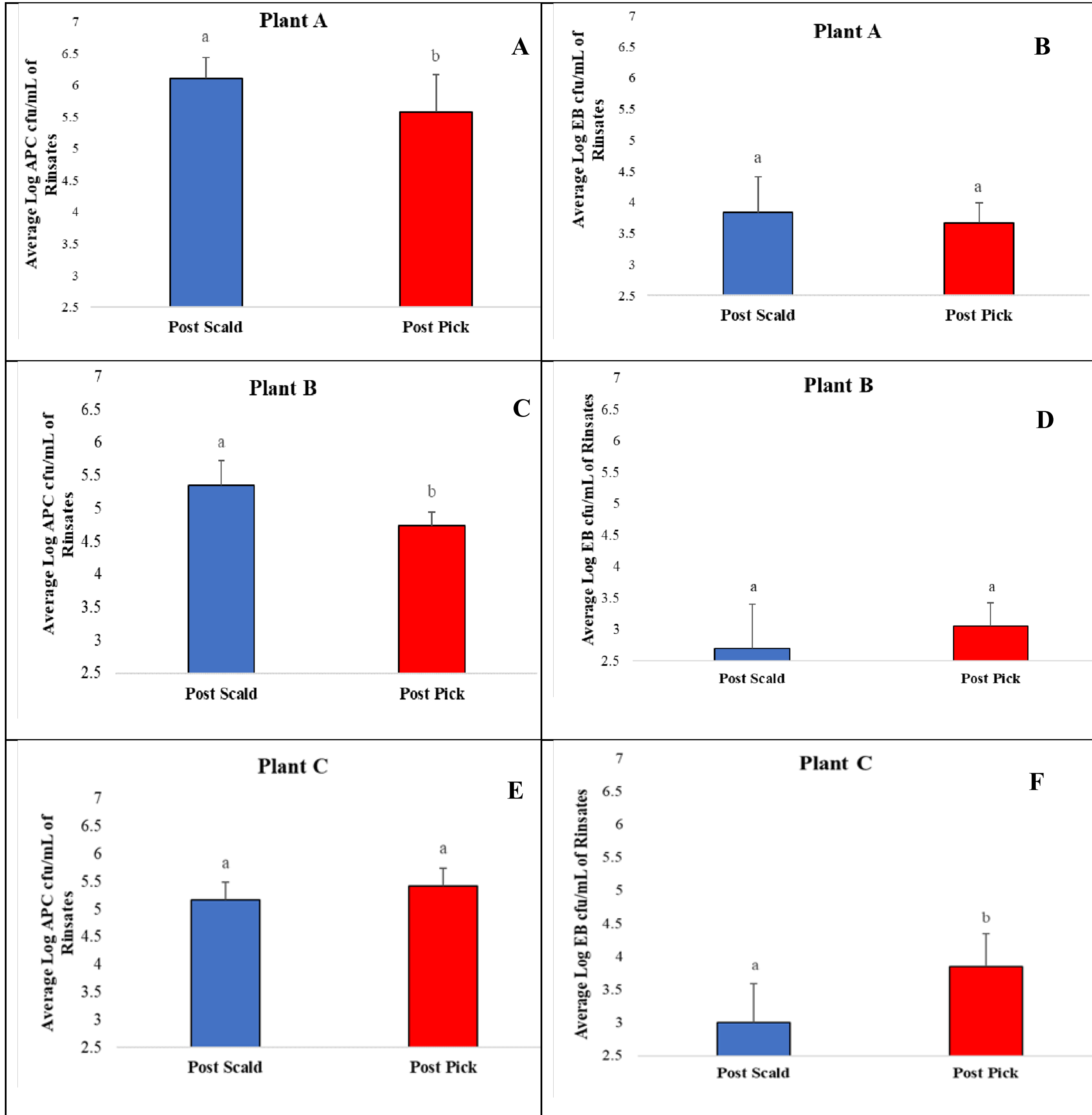

Figure 1 | Average Log APC **(A,C,E)** and EB **(B,D,F)** counts of rinsates collected at post-scalder and post-picker location for Plants A, B, and C. [ab]Log values denoted with different letters were significantly different between the post-scalder and post-picker collection sites at $P \leq 0.05$.

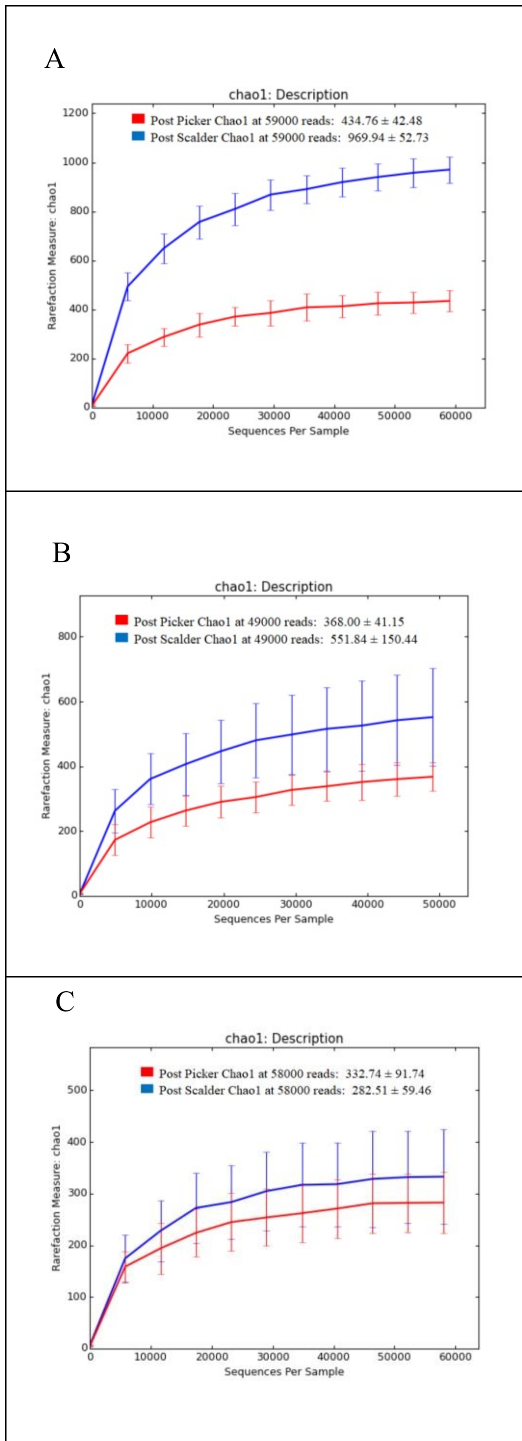

Figure 2 | Chao 1 alpha diversity metrics for sampling locations within each facility. **(A)** represents richness measurements for Plant A OTUs. **(B)** represents richness measurements for Plant B OTUs. **(C)** represents richness measurements for Plant C OTUs.

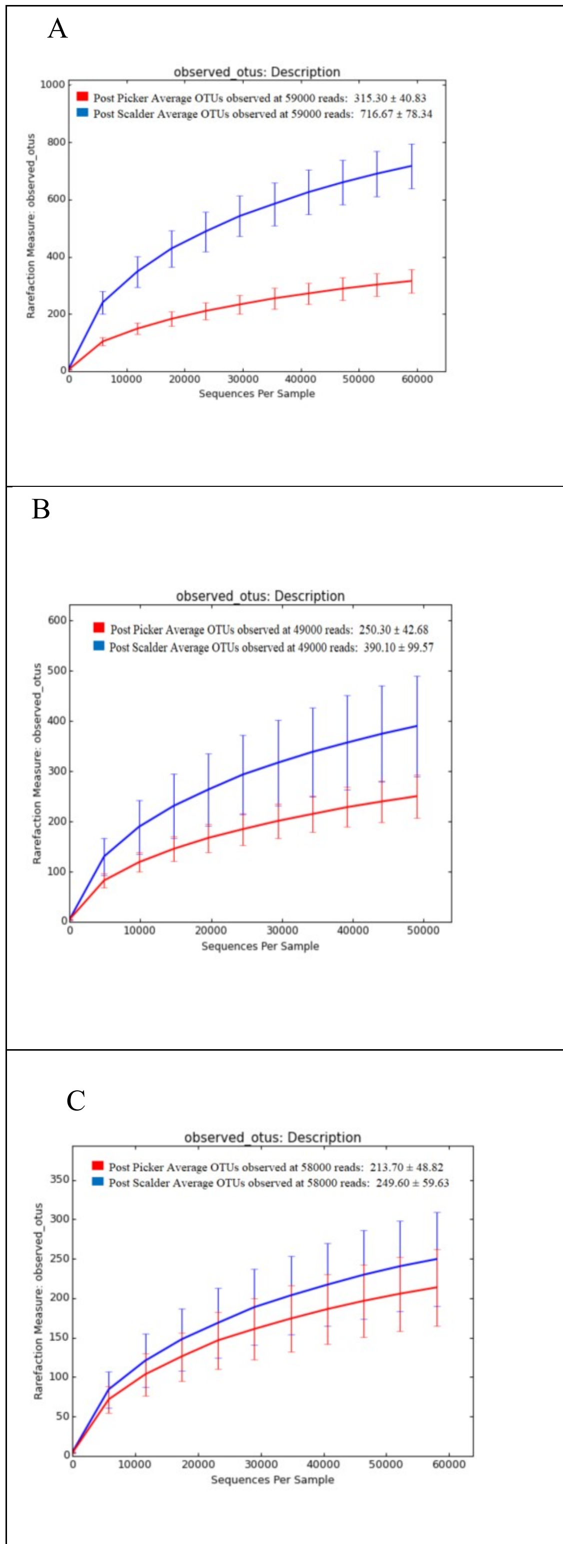

Figure 3 | OTU rarefaction curves depicting number of OTUs versus sequence depth for sampling locations within each facility. (A) represents Plant A observed OTUs. (B) represents Plant B observed OTUs. (C) represents Plant C observed OTUs.

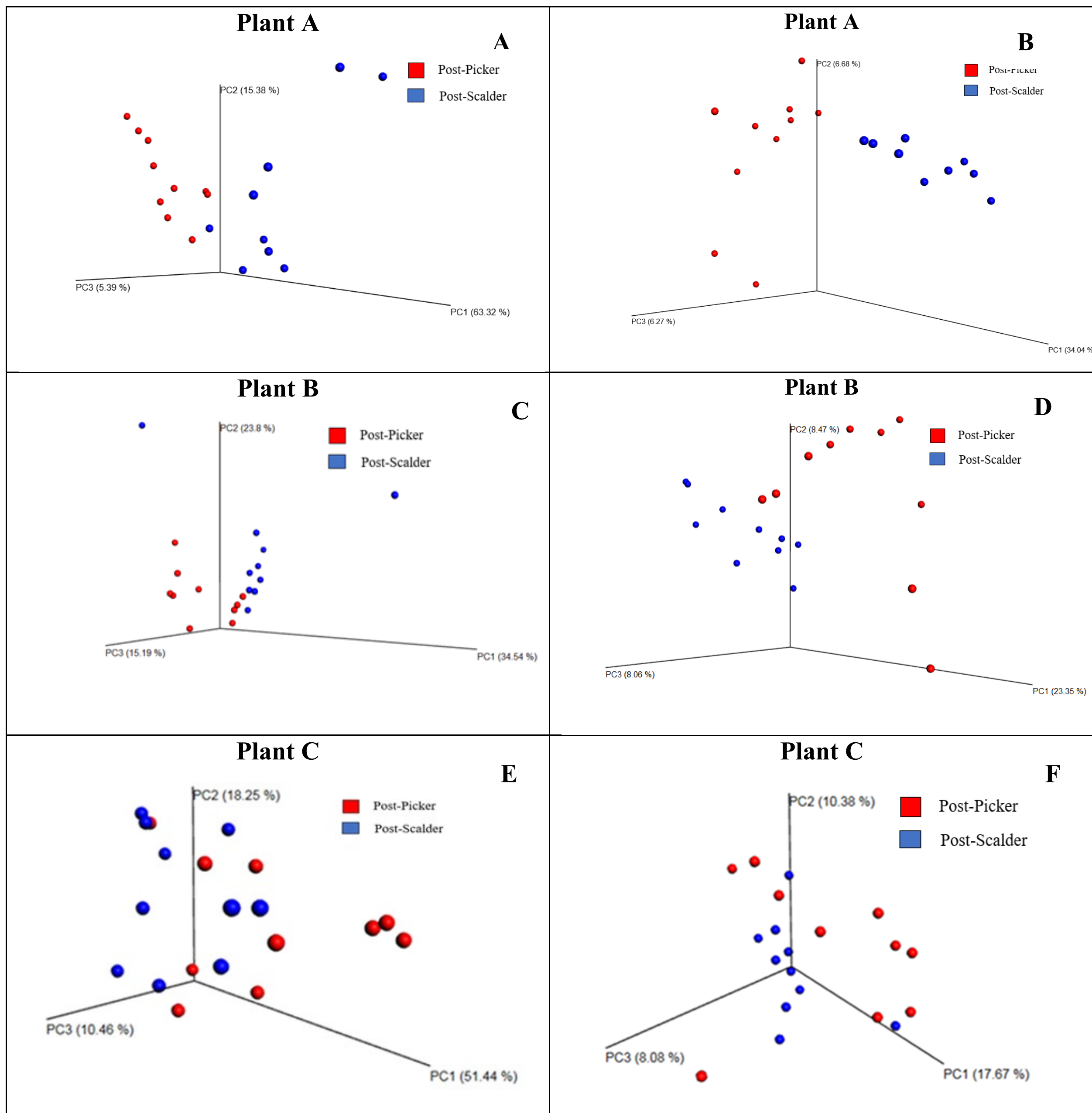

Figure 4 | Weighted and Unweighted UniFrac plots depicting OTU diversity between microbiome populations of rinsates collected at post-scalder and post-picker locations. Weighted plots consider the relative abundance values of the OTUs present, and unweighted plots are based on the number of unique OTUs. Weighted UniFrac plots for Plant A, B, and C are represented in left column **(A, C, and E)**, respectively. Unweighted UniFrac plots for Plants A, B, and C are represented in right column **(B, D, and F)**, respectively.

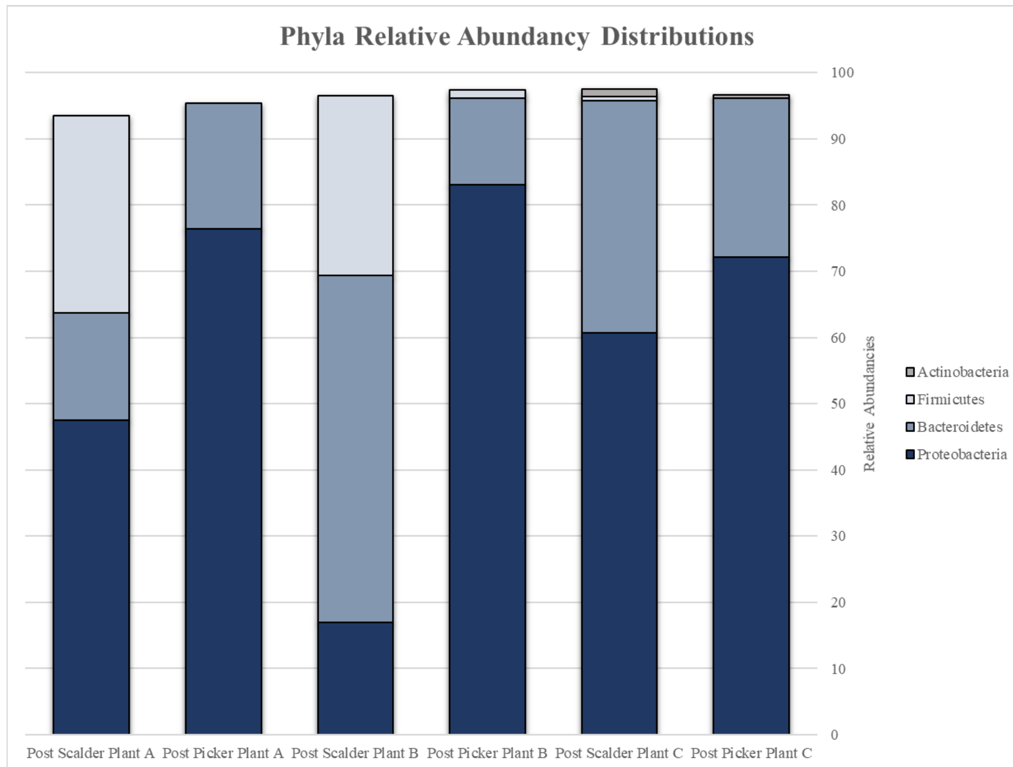

Figure 5 | Relative abundancies of phyla represented by OTUs present at post-scalder and postpicker sites for Plant A, B, and C. OTUs at Plants A and B were representative of *Proteobacteria*, *Bacteroidetes*, and *Firmicute*s. OTUs at Plant C were representative of *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*.

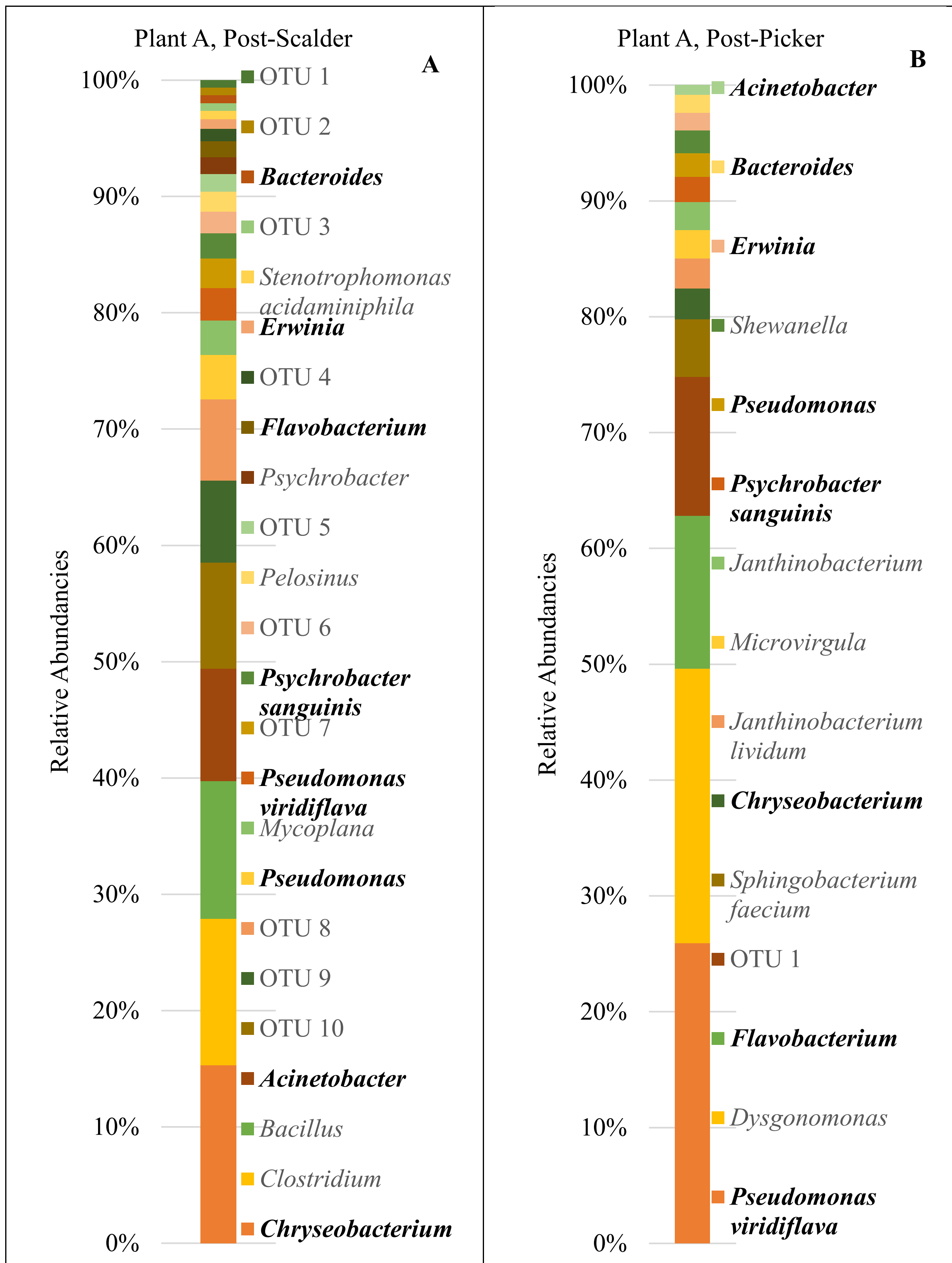

Figure 6 | Relative abundancies of OTUs at Plant A. (A) Post-scalder collection site. (B) Post-picker collection site. OTUs in bold were present at both sampling locations.

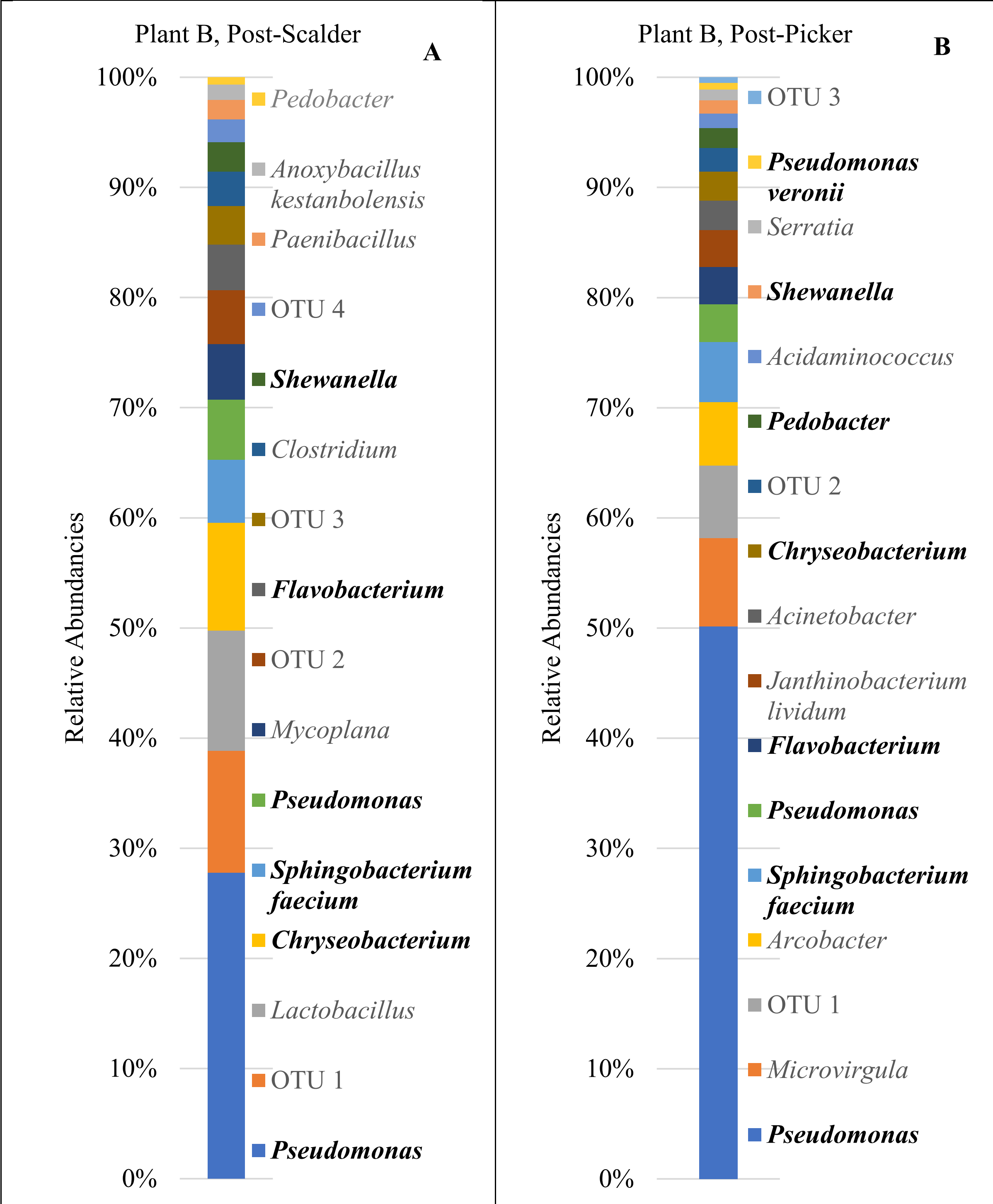

Figure 7 | Relative abundancies of OTUs at Plant B. (A) Post-scalder collection site. (B) Post-picker collection site. OTUs in bold were present at both sampling locations.

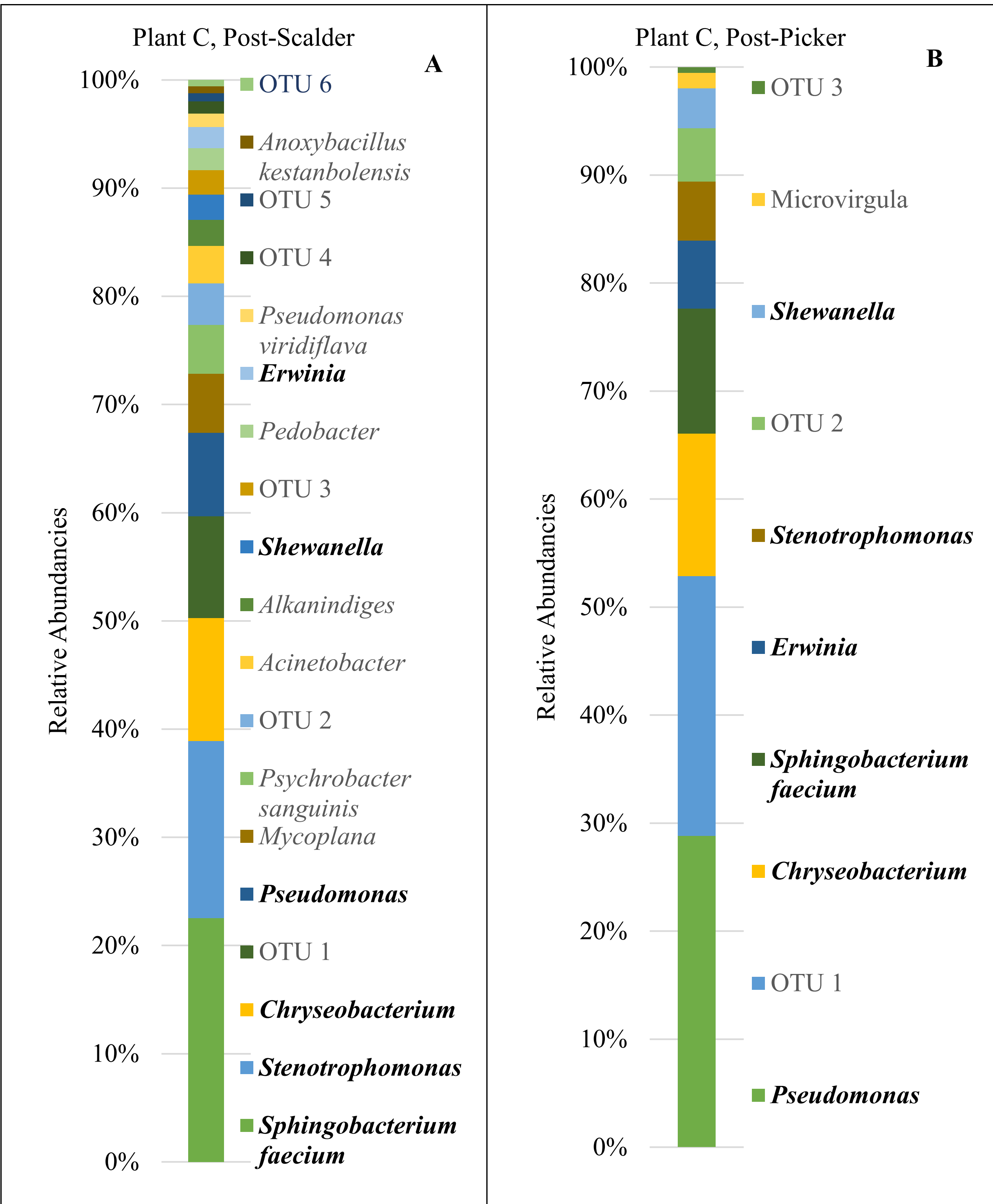

Figure 8 | Relative abundancies of OTUs at Plant C. (A) Post-scalder collection site. (B) Post-picker collection site. OTUs in bold were present at both sampling locations.

4 Elucidating Microbiome Shifts using 16S and Culture Based Microbiological Analyses of Whole Bird Carcass Rinses Collected with nBPW and BPW from a Commercial Broiler Processing Facility

The poultry industry and associated regulatory bodies use whole bird carcass (WBC) rinsates to evaluate different stages of broiler processing systems for the prevalence of food-borne pathogens, including *Salmonella* spp. and *Campylobacter* spp. Within industry and research groups, the similar samples collections are enumerated to determine *E. coli*, *Enterobacteriaceae* (EB), and Aerobic Plate Count (APC) microorganisms. Analysis of these indicator microorganisms provides numerical data that can be used to demonstrate the effects of specific process control steps where low occurrences of target pathogens hinder the exclusive use of prevalence data. In 2016, USDA FSIS established guidelines which altered the Buffered Peptone Water (BPW) rinsate material to include additional compounds that would better neutralize residual processing aids and allow for better recovery of sublethal injured *Salmonella* organisms. While the compounds added were identified as aiding in the recovery of *Salmonella* spp., specific data to understand how the new rinsate, neutralizing Buffered Peptone Water (nBPW), effected the recovery of other microorganisms (including *Campylobacter* spp. and indicator microorganisms) was not initially available. This study was conducted to determine how the rinse solutions (BPW or nBPW) used in WBC sample collections could influence microbiome compositions of collected rinsates and in turn, how those compositions could influence results obtained from culturing methodologies. Paired carcasses exiting a peroxyacetic acid (PAA) finishing chiller were rinsed in 400 mL of either nBPW or BPW and resulting rinsates were analyzed for both prevalence levels of *Salmonella* spp. and *Campylobacter* spp. as well as count levels of APC and EB. Genomic DNA was extracted from the rinsates, petrifilm,

agar plates and enrichment media and amplified based on V4 region of 16S rRNA gene sequences, and resulting sequences were multiplexed, pooled and sequenced using MiSeq® v2 Reagent cartridge on the Illumina MiSeq®. Raw data sequences were analyzed using QIIME2.2018.11 and taxonomic analyses including alpha, beta and ± compositional analyses were performed using MicrobiomeAnalyst. Cultured microbiological methods indicated significant differences ($P \leq 0.05$) in APC levels of the two rinsates, but no significant differences in EB, *Salmonella* spp. or *Campylobacter* spp. prevalence. In contrast, beta diversity analysis of the microbiome compositions of the two rinsate types and associated matrices used to assess those levels of microorganisms revealed a marked difference ($P < 0.05$) at the operational taxonomic unit (OTU) level.

4.1 Introduction

Raw poultry carcasses and parts are commonly implicated as contributing sources of food borne-disease caused by *Salmonella* and *Campylobacter* (Antunes et al., 2016; Bryan and Doyle, 1995; Painter et al., 2013; Skarp et al., 2016; Tompkin, 1990). Commensal within the gastrointestinal tract (GIT) of broilers, the human pathogens are often liberated from the feathers, feet, crop, and GIT of processed carcasses (Josefsen et al., 2015; Roccato et al., 2016). To aid in control of these microorganisms and limit the amount of contamination and cross-contamination events, multi-hurdle interventions including the use of carcass and equipment washes, product chilling, and the application of antimicrobials are commonly employed (Dogan et al., 2019; Stopforth et al., 2007; Wideman et al., 2016). To measure antimicrobial efficacy of applied control steps, whole bird carcass (WBC) rinses are collected at sites that represent pre-intervention(s) and post-intervention(s) locations within the processing system and evaluated for target pathogenic microorganisms as well as indicator and spoilage microorganisms (Milios et

al., 2014; Saini et al., 2011). The microbiological evaluations of these rinses provide both processors and regulatory bodies quantifiable information that can be used to assess the performance of specific processing steps and interventions within a poultry harvest system (Milios et al., 2014; Stopforth et al., 2007).

With the implementation of the final rule "Pathogen Reduction and Hazard Analysis Critical Control Point" (PR-HACCP) in 1996, carcasses from commercial broiler processing establishments were subject to periodic sampling and microbiological analysis by United States Department of Agriculture-Food Safety and Inspection Services (USDA-FSIS) (USDA, 1996). The results provided information related to the prevalence of *Salmonella* in post-chill broilers which was used to determine if an establishment's processing system was "in control" and capable of reducing microbial loads on processed carcasses. In 2011, USDA-FSIS also began concurrently analyzing samples for prevalence of *Campylobacter* spp. The application of this final rule was in response to increased cases of Campylobacteriosis and the need to evaluate commercial processing systems for levels of *Campylobacter* spp. as well as determine an applied system's ability to reduce and control *Campylobacter* spp. (USDA, 2011).

As more stringent standards were placed on processors to reduce both *Salmonella* spp. and *Campylobacter* spp. levels in poultry carcasses and parts, the implementation of novel interventions systems and antimicrobials became standard practice (Smith et al., 2015). Some changes included the addition of a post-chill chemical spray or dip, with a short dwell time as well as antimicrobial solutions with different modes of actions and chemical properties not previously applied to poultry processing systems (Gamble et al., 2016; Smith et al., 2015). The changes in intervention systems and chemical applications introduced opportunities to further evaluate the materials and methods used to perform sample collections in commercial poultry

processing. In the case of WBC rinses, the use of a buffered peptone water (BPW) rinsate had been used to aid in the recovery of *Salmonella* from collected samples, as it provided a non-selective nutrient source with a built-in phosphate buffering system which helped to maintain neutral pH levels (Baylis et al., 2000). However, as the industry had evolved and introduced new interventions and post-chill applications, research by Gamble et al., (2016), indicated that use of BPW alone may not be wholly suitable to mitigate the antimicrobial carryover that may occur during WBC rinse sample collection. Further, the presence of a non-neutralized antimicrobial in the rinsate could hinder the recovery of some microorganisms, namely *Salmonella*, from the collected rinsates and lead to false-negative results (Gamble et al., 2016).

In response to the identified limitations of BPW as a resuscitative medium, in 2016 USDA-FSIS began using a specialized neutralizing buffered peptone water (nBPW) to analyze WBC.   The modified BPW solution included sodium thiosulfate, sodium bicarbonate, and soy lecithin to provide more effective neutralization of a broader spectrum of antimicrobials and further aid in recovery of *Salmonella* from collected rinsates (Gamble et al., 2017). Specifically, the addition of sodium thiosulfate aids in the neutralizing antimicrobial properties of chlorine and iodine, sodium bicarbonate increases the acid neutralizing capacity of the solution and soy lecithin neutralizes the effects of quaternary ammonia compounds.

While the application of nBPW had been identified as aiding in the recovery of *Salmonella* from poultry processing samples, the application of these materials to address the recovery of *Campylobacter* spp. had not been specifically evaluated prior to the introduction of the new rinsate. Additionally, processors and researchers often concurrently analyze collected rinsates for both *Salmonella* spp. and *Campylobacter* spp. as well as indicator organisms, including APC, EB, and *E. coli*. (Bourassa et al., 2019, Yu et al.,). As *Salmonella* spp. and

Campylobacter spp. may be present at low levels and not necessarily homogenously dispersed within the process system, the use of APC and other indicator organisms are often used in conjunction with verification programs to monitor both sanitary dress measures and applied process controls (Boroussa et al. 2019; Yu et al.).

In this study, microbiome analysis was used to better understand the effect of rinse solutions on the recovery of *Salmonella* spp., *Campylobacter* spp., as well as indicator microorganisms using traditional, industry-specific, culturing methodologies typically applied to samples collected within a poultry processing system. Specifically, compositional analysis of the microbiomes present in nBPW and BPW rinsates that were collected after carcasses exited a finishing chiller equipped with PAA at a commercial broiler processing facility. Additionally, the microbiomes present within *Salmonella* spp. and *Campylobacter* spp. enrichment broths, as well as bacterial growth from CCPM agar plates used to ascertain *Campylobacter* spp. prevalence, and corresponding 3M™ Petrifilm™ used to obtain indicator counts of APC and EB were compared. Elucidating the microbiome compositions of the different rinsates and materials from associated culturing methodologies provides an improved understanding of how the rinsate alters results of microbiological analyses which can help explain why conflicting results may result if different rinsate solutions are utilized.

4.2 Sample Collection

Sample collection occurred within a USDA-inspected, commercial facility, processing approximately 240 birds per min using two evisceration lines which fed one chilling system. Samples were collected after exiting a 20 to 30 second finishing chiller containing 800 to 1000 ppm peroxyacetic acid (PAA) of a Cornish hen broiler commercial processing facility on two different days. To ensure that the carcasses collected were representative of the same flock, and

subjected to similar processing conditions, all collections occurred in quick succession. During the first day of collection, eighteen total WBC were collected, and the first and last pair of samples were collected within 30 minutes of each other. During the second day of collection, 80 total WBC were collected, and the first and last pair of samples were collected within 1.5 hours of each other.

Carcasses were collected in pairs using a new pair of sterile gloves for each carcass. Collected carcasses were hung by legs on shackles and allowed to drip for 1 minute and then placed in sterile rinse bags (Fisher Scientific, Pittsburgh, PA, USA). For each pair of WBC collected, 400 mL of either nBPW (CultureMediaConcepts, Millennium Life Sciences, Inc., Anaheim, California) or BPW (Becton, Dickinson and Company, Sparks, MD, USA) was poured over the surface and interior cavity of the individual carcass. Conforming to USDA collection practices (USDA, 2013), the carcasses were rinsed for one minute in an arcing motion to ensure that rinsate could move along the carcass surface and cavity. At the end of one minute rinse, the rinsate was transferred to the original container and the carcass was returned to the processing line. After collection, all rinses were placed on ice in a cooler and transported to the testing laboratory for same day analysis.

4.3 Microbiological Analysis

On the same day of collection, rinsates and associated serial dilutions were enumerated for APC counts and EB counts with 3M™ APC petrifilm™ and 3M™ *Enterobacteriaceae* petrifilm™ (3M™, St. Paul, MN, United States) per Official Methods of Analysis (OMA) 990.12 and 2003.01, respectively, published by the International Association of Official Analytical Chemists (AOAC). In brief, 1.0 mL aliquots of original rinse material and prepared serial dilutions were pipetted onto the surface of petrifilm which includes both gelling and

nutrient agents for the detection of APC or EB microorganisms, dependent on the class of petrifilm used. Incubation and subsequent enumeration of petrifilm occurred per prescribed published protocols except for incubation temperature of the EB petrifilm which was altered to align with USDA-FSIS Microbiological Laboratory Guidebook (MLG) Chapter 3.02 (USDA, 2015), in which EB detection for meat and poultry products are typically incubated at 35 ± 1 °C. In addition to EB count data, the presence or absence of EB typical growth from direct plating analysis was used to determine EB prevalence within the sample sets.

To determine prevalence of *Campylobacter* spp., 30 mL aliquots of rinsates were enriched in 30 mL 2X blood-free Bolton broth (ThermoFisher, Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 42 ± 1 °C under microaerophilic conditions (85% nitrogen, 10% carbon dioxide, and 5% oxygen) for 48 ± 2 h per guidelines in Chapter 41.04 of USDA-FSIS MLG (USDA, 2016). After incubation, presence of *Campylobacter* spp. was determined by inoculating a R & F® *Campylobacter* jejuni/*C. coli* Chromogenic Plating Medium (CCPM; R & F Products, Downers Grove, IL, USA) agar plate with 10 µL of incubated sample. Inoculated plates were incubated at 42 ± 1 °C under microaerophilic conditions for 48 ± 2 h per guidelines from manufacturer. After incubation, plates were evaluated for presence of typical *Campylobacter* spp. colonies which appear pink or salmon in color.

To determine prevalence of *Salmonella*, 30 mL aliquots of rinsates were enriched in 30 mL of BPW (Becton, Dickinson and Company, Sparks, MD, USA) and incubated at 35 ± 1 °C for 20 to 24 h. Incubated samples were analyzed for *Salmonella* prevalence utilizing Hygiena© BAX® (Hygeina, LLC, Camarillo, California, USA) PCR based testing system and *Salmonella* assay kit per guidelines provided in OMA 2003.09 AOAC.

4.4 DNA Extraction and Library Preparation

Aliquots (80 mL) of the original nBPW and BPW rinses, as well as 40 mL aliquots of *Salmonella* spp. and *Campylobacter* spp. incubated enrichments (BPW and 2X blood-free Bolton broth) were centrifuged (Sorvall Lynx 6000, Thermo Fisher Scientific, Langenselbold, Germany) for 15 min at 8,000 g to achieve initial pellet material. Pellets were re-suspended in room temperature PBS at volumes equal to 2X the approximate size of the pellet (Oxoid Microbiology Products, Thermo Scientific, Wilmington, DE, United States) centrifuged for 15 min at 8,000 g. This wash step was repeated 2 times and after the final wash, the remaining pellet was re-suspended in 500 µL PBS and placed in -80 °C freezer.

Genomic DNA extraction of the pelleted material from the rinses, BPW and Bolton broth suspensions were performed using a QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA). In brief, 1.0 mL of supplied InhibitEx buffer was added to the 500 µL pellet suspensions and vortex mixed continuously for 1.0 minute or until the sample appeared thoroughly homogenized. The resulting mixture was heated for 5 min at 70 °C to lyse cells and after heating, the lysed mixture was vortex mixed for 15 s and then centrifuged (Sorvall Legend Micro 21, Thermo Fisher Scientific) for 1 minute to pellet particles. Further steps were performed as indicated in the manufacturer's standard protocol except doubling the volume of proteinase K used and using DNAse/RNAse free water (Fisher Scientific) at a reduced volume of 30 µL for the final eluent.

Prior to extraction, petrifilm plates were held at -20 °C to limit further changes in bacteria counts and growth. The gelled matrices of the petrifilm were harvested using a sterile loop and the material was placed into a 2 mL microcentrifuge. Samples were immediately extracted using DNeasy Blood and Tissue Kit (Qiagen) following the standard protocol except adding 400 µL

(instead of 180 μL) enzymatic lysis buffer, and incubating mixtures at 56°C for up to 60 min as needed to achieve homogenous mixture of gelled matrices. Low level DNA concentrations, likely due to limited EB growth on most of the samples, from EB petrifilm precluded those samples from being further evaluated.

Agar plates used to evaluate *Campylobacter* spp. prevalence were held at refrigerated temperatures (0 to 4 °C) until the surface of the agar plate could be harvested. The surfaces of the CCPM agar plates were harvested by sweeping a sterile loop across the entire surface of the agar plate, and then collected material was deposited into a microcentrifuge containing 200 μL of PBS. The mixture was vortex mixed thoroughly for 1 min and then centrifuged for 1 min at 17,000 g to pellet the material. The supernatant was removed and 500 μL of PBS was added to the pellet for a wash step. To wash the pellet, the mixture was vortex mixed for 1 min and then centrifuged again for 1 min at 17,000 g to pellet the material. The final pellet was re-suspended in 200 μL of PBS and DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen) following the same steps as outlined for the petrifilm samples.

The extracted genomic DNA from the two days of collection were sequenced at different times but steps for multiplexing and amplification of gene libraries were similar for both sample sets. In short, targeted 16S rRNA gene libraries from all samples were constructed by multiplexing and amplifying the V4 region of the 16S rRNA gene sequences using mixtures of AccuPrime™ Pfx (Thermo Scientific) and forward and reverse primer sets which included dual indices as described in Kozich et al., (2013). Resulting amplicons were normalized using SequealPrep™ Normalization Plate Kit (Thermo Scientific) to achieve similar nucleic acid concentrations in each sample. Normalized samples were pooled and sequenced using MiSeq®

v2 (500 cycle) Reagent cartridge on the Illumina MiSeq platform (Illumina, San Francisco, CA, USA), per the manufacturer's instructions.

4.5 Analysis of Sequences

Illumina sequence data was retrieved from BaseSpace Hub Account (Illumina) and raw sequence reads were assigned to taxonomic features or operational taxonomic units (OTUs) based on analysis performed using the "moving pictures" tutorial available within the Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline version 2019.7 (Bolyen et al., 2018). Quality filtering steps including the removal of any phiX reads and chimeric sequences were performed separately on each of the two sequence runs, following the DADA2 plug-in procedures (Bolyen et al., 2018). Reads were trimmed to remove lower quality regions based on Phred scores by viewing the "Interactive Quality Plot" tab in the demux.qzv files of each run and trimming reads where base call quality scores averaged less than 30. After filtering and normalization of the separate runs, the runs were merged and resulting features were assigned to taxa using trained classifier "Silva 132 99% OTUs" with an applied confidence level of 95%. To analyze the *Campylobacter* spp. enrichment and *Campylobacter* plate extraction sample sets, feature tables were filtered to exclude *Campylobacter* spp. OTUs so that the other features would be amplified and composition differences could be better evaluated. The rinsates, petrifilm, and *Salmonella* enrichments sample sets were analyzed using the complete feature tables (including *Campylobacter* spp.).

After taxonomic assignments were made, MicrobiomeAnalyst (Dhariwal et al., 2017) was used to evaluate taxonomy compositional and statistical analyses including alpha and beta diversity of the different sample sets. Using the Marker Data Profile (MDP) module within MicrobiomeAnalyst, 140,611,144 sequences representing 455 samples and 643 different features

were analyzed to determine differences between microbiome compositions of the rinsates (nBPW and BPW) and the materials obtained from microbiological enumeration and prevalence testing. Sequences obtained from the rinsates, petrifilm, *Salmonella* spp. and *Campylobacter* spp. enrichments were analyzed separately using the sample editor feature so that overall compositional shifts due to the use of nBPW or BPW on those samples sets could be easily delineated. For each separate analysis, features were filtered using default parameters set up within the "feature filter" section of the MDP module. In short, features identified as rare or low abundancies (≤ 4 counts in ≤ 20% of the samples), and features identified in the samples as present at a rate of 10% in all samples based on inter-quantile range (IQR) scores were limited from downstream analyses. Reads were normalized based on total sum scaling (TSS), in which the number of feature reads were divided by the total number of reads obtained.

4.6 Statistical Analyses

Count values from cultured microbiological analyses were Log_{10} transformed and averaged for each rinsate type. Count data was analyzed using analysis of variance (ANOVA) and prevalence data determined from enriched samples was analyzed using a chi-squared test with $P < 0.05$ considered significant.

Alpha diversities of the individual samples within the two populations (nBPW and BPW samples) was evaluated using the average number of observed OTUs per sample and the Shannon index which is a measurement of the diversity and evenness of OTUs within a sample. Calculated observed OTUs and Shannon diversity metrics of paired nBPW and BPW samples (original rinsates, enrichment broths, growth from agar and petrifilm plates), were evaluated using the Mann-Whitney U test to determine statistical differences ($P < 0.05$) between microbiome compositions of each of those populations. Beta diversity including Bray-Curtis

dissimilarity metrics visualized using Principle Coordinates of Analysis (PCoA) and analyzed using analysis of group similarities (ANOSIM) to determine population composition differences between each of the paired sample sets. Linear discriminant analysis (LDA) effect size (LEfSe) was used to determine statistical significant differences ($P \leq 0.05$) in specific features within paired populations as well as identify and quantify differentially abundant OTUs (LDA scores ≥ 2.0) between the two sample types.

4.7 Results

The only significant differences ($P < 0.05$) in microbiological analyses of the BPW and nBPW rinsates were the resulting average Log_{10} APC CFU/mL counts, in which the BPW counts were 0.39 Log_{10} CFU/mL higher compared to the nBPW rinsates (Table 1). Average EB counts and EB prevalence levels for the two rinsates were not significantly different as both BPW and nBPW average counts for EB were 0.27 Log10 CFU/mL, and prevalence levels were 77.55% and 73.47% for BPW and nBPW, respectively. Like the observed EB prevalence levels, *Salmonella* spp. and *Campylobacter* spp. prevalence levels were not significantly different ($P < 0.05$) although levels for both were higher in nBPW rinsates compared to BPW rinsates (48.98%, and 91.84% for *Salmonella* spp. and *Campylobacter* spp. in nBPW rinsates compared to 32.65%, and 79.59% in BPW rinsates, respectively).

Microbiome analysis of the collected rinsates revealed the presence of 45 different OTUs with 20 of those OTUs were present at relative abundancies greater than or equal to 1% in at least one of the rinsate types (nBPW or BPW) (Figure 1). The relative abundance values for the observed OTUs in both nBPW and BPW rinsates indicated that the major constituents of both microbiomes belonged to the genus Pseudomonas; with those OTUs representing 29.22% and 28.14% of the total number of identified OTUs for BPW and nBPW rinsates, respectively. The

second most abundant OTU in the BPW rinsates was Escherichia_Shigella at 17.18%, while the second most abundant OTU in the nBPW rinsates belonged to Psychrobacter at 15.19%. Comparisons of alpha diversities of nBPW and BPW rinsates did not reveal significant differences ($P < 0.05$) of either the observed OTUs or Shannon indices of the two microbiome compositions (Table 2). Overall, the diversity of the populations appears to be very similar in regards to the number of OTUs per sample, with the BPW rinsates comprised of 38.09 ± 14.94 OTUs and the nBPW rinsates comprised of 37.78 ± 16.40 OTUs. Similarly, the distributions of OTUs within the individual samples of the two rinsates were not significantly different, and Shannon indices for the microbiome compositions of the rinsates were 1.98 ± 0.88 for the nBPW and 1.72 ± 1.03 for BPW rinsates. In contrast, the calculated Bray-Curtis dissimilarity beta diversity metric did indicate a significant difference ($P \leq 0.05$) in overall diversity between the two populations (Figure 2a). LEfSe analysis highlighted 16 OTUs that were differentially abundant ($P < 0.05$) within the two populations (Figure 6a). Of those 16, 13 were differentially more abundant in the BPW rinsate microbiome, with the most differentially abundant OTU identified as *Escherichia_Shigella*. Of the three OTUs that were underabundant in the BPW rinsate, the most differentially abundant was an OTU belonging to the genus *Clostridium*.

The microbiome compositions obtained from the associated 3M™ APC Petrifilm™ indicated a significant difference ($P < 0.05$) in the number of observed OTUs represented. The growth obtained from the BPW rinsates was more microbially diverse than the nBPW rinsates although the average number of observed OTUs per sample for the two groups were relatively similar at 11.86 ± 2.27 and 10.58 ± 2.16, for BPW and nBPW, respectively (Table 2). Shannon indices of the two populations were also statistically different ($P < 0.05$) as distribution of OTUs within the samples was more even for the BPW samples than the nBPW samples (1.20 ± 0.38

and 0.89 ± 0.42, for BPW and nBPW, respectively). Likewise, beta diversity measured by Bray-Curtis dissimilarity and compared using ANOSIM for the two APC populations indicated significant differences ($P < 0.05$, $R=0.4731$) (Figure 2c).

Of the ten different OTUs or features identified from the APC petrifilm, *Escherichia_Shigella* was the most prevalent in both populations (53.93% and 34.85%, nBPW and BPW, respectively) (Figure 3). The second most abundant feature for nBPW was identified as *Staphylococcus* while the second most abundant feature for BPW was identified as *Streptococcus*. While the largest proportion of OTUs within each of the populations belonged to the same feature (*Escherichia_Shigella*), LEfSe analysis of the two microbiomes indicated that there were 7 differentially abundant OTUs, with 5 of those being more abundant in the APC growth obtained from the BPW rinsates (Figure 6d). The two features that were underabundant in the APC growth obtained from the BPW rinsates, were *Escherichia_Shigella* and *Pseudomonas*. This is line with the alpha and beta diversity metrics previously discussed, in which there was a higher overall diversity in BPW samples, even though the most abundant feature for both populations was identified as *Escherichia_Shigella*.

Review of microbiome compositions of the *Salmonella* spp. enrichment samples, revealed that calculated alpha indices were not significantly different ($P < 0.05$) for either calculated metrics (Table 2). The average number of OTUs per sample were similar for both enrichment types, with an average of 12.26 ± 2.51 and 11.53 ± 2.17 OTUs represented in the nBPW and BPW based enrichments, respectively. Similarly, the calculated Shannon indices used to measure diversity within individual samples were similar at 1.19 ± 0.38 and 1.19 ± 0.27 for nBPW and BPW samples, respectively. In contrast, beta diversity Bray-Curtis metrics of the

two populations indicated that the populations were significantly ($P < 0.05$) dissimilar. (Figure 2b).

Seventeen different OTUs were represented in the microbiome compositions of the *Salmonella* spp. enrichments, with 15 OTUs present at relative abundance levels greater than or equal to 1% in at least of one of the enrichment types (Figure 4). The most abundant feature within the BPW enrichment was an OTU belonging to Aeromonadales, representing 39.65% of the sequences. In contrast, the most abundant feature within the nBPW enrichments was Escherichia_Shigella, representing 43.33% of the sequences. LEfSe analysis identified 11 differentially abundant ($P < 0.05$) features represented within the microbiomes of the two enrichments (Figure 6b). Of the 11 differentially abundant OTUs, 7 of those, including *Lactococcus*, Planococcaceae_OTU1, Enterococcaceae_OTU1, *Fusobacterium*, *Streptococcus*, *Enterococcus*, and *Escherichia_Shigella* were differentially underabundant in the BPW enrichments. Features differentially more abundant in the BPW enrichments included Aeromonadales_OTU1, Burkholderiaceae_OTU2, *Acinetobacter*, and *Aeromonas*.

Metrics for alpha diversities did not indicate significant differences for either observed OTUs or Shannon diversity indexes (Table 2) in the *Campylobacter* spp. enrichments prepared from BPW and nBPW rinsates. In contrast, Bray-Curtis beta diversity indices did indicate a significant difference ($P < 0.05$, $R=0.1344$) in the compositions of the two enrichment matrices (Figure 2d). Eleven different features were present at relative abundance levels greater than or equal to 1.0% in *Campylobacter* spp. enrichment matrices prepared from the two rinsate types (Figures 5a and 5b). Of those, the most abundant feature identified in the BPW enrichments was *Lactobacillus* (92.48%), while this same feature was only abundant at 1.70% in the nBPW enrichments. The most abundant feature identified in the nBPW was *Escherichia_Shigella*

(72.18%), while the same feature was only abundant at 2.31% in the BPW enrichments. LEfSe analysis indicated 4 different OTUs were differentially abundant in the BPW enrichment matrices, with *Staphylococcus* being more abundant and *Escherichia_Shigella*, Burkholderiaceae_OTU2, Aeromonadales_OTU1 being underabundant.

In contrast to the alpha metrics observed with the *Campylobacter* spp. enrichments, the alpha diversity metrics for the CCPM extractions prepared from the two different rinsates were significantly different ($P < 0.05$) with the nBPW samples exhibiting a higher average number of observed OTUs (3.83 ± 0.92) compared to the BPW samples (3.23 ± 1.07) (Figure 2). Similarly, Shannon diversities of the individual samples within the each of the extractions were also significantly different ($P < 0.05$). Overall, the two rinsates were represented by significantly different ($P < 0.05$, $R=0.1384$) populations as observed by the PCoA plot of the beta diversity metric (Figure 2e). After features identified as *Campylobacter* spp. were removed, six different features were present in the extractions collected from the surfaces of the CCPM agar plates. Of those features, the most abundant feature was identified as *Escherichia_Shigella*, representing 60.28% of the sequences in the BPW samples and 55.93% sequences in the nBPW samples (Figure 5). The second most abundant feature in BPW samples was *Enterococcus* (18.80%), while the second most abundant feature in the nBPW samples was *Staphylococcus* (19.74%). LEfSe analysis indicated only one differentially abundant feature was present within the extractions; with Staphylococcus being underabundant in the BPW extractions.

4.8 Discussion

Microbiome analysis of nBPW and BPW rinsates and associated materials used to ascertain pathogen and indicator microorganism levels revealed diversities in microbial populations that were not fully recognized by the applied culturing methodologies. At the OTU

or feature level, it would be expected that the samples within each group of rinsates would be similar in terms of overall diversity as the rinse solution is acting as a “rinse” of the surface of the carcass, and in theory should not impart any selectivity in regards to what microorganisms are recovered from the carcasses being sampled. This was confirmed with the alpha indices measured, which did not indicate that there were any significant differences in the number of observed OTUS or the distribution of those features within the samples. In contrast, the PCoA plot and associated Bray-Curtis beta index did indicate a significant difference ($P \leq 0.05$) between the two populations, however, these differences appear to be due to a few outlying samples representative of both rinsate types. If the beta diversity was more significantly different (lower P-value) or if one rinsate type appeared to be more divergent away from the average population, it could indicate that the rinsate choice could alter microorganism recovery even at collection which in turn would most assuredly affect the detection of both pathogen microorganisms as well as indicator organisms. Additionally, as the diversity of the microbiome compositions for each rinsate were compromised of the same top 6 OTUs (*Pseudomonas, Escherichia_Shigella, Psychrobacter, Acinetobacter*, *Ruminococcaceae*_OTU11, and *Campylobacter*), it appears that either rinsate can recovery microorganisms typically found on the surfaces of processed carcasses and neither rinsate exerts any selective pressures on the microorganisms that may be present.

Salmonella spp. prevalence levels determined by culturing methods of the two types of collected rinsates were not significantly different ($P<0.05$), but beta diversity metrics used to evaluate differences at the OTU or feature level did indicate there were differences in the overall diversity of the two enrichments. While *Salmonella* spp. was not specifically identified, the presence of the *Escherichia_Shigella* OTU is often presumed to be indicative of *Salmonella* spp.

in these sample types, due to the close phylogenetic relationship of the three species which may hinder the ability of 16S rDNA sequencing to fully delineate sequences as belonging to genera *Salmonella, Escherichia,* or *Shigella* (Ceuppens et al., 2017; Grim et al., 2017*)*. Thus, the higher relative abundancy of *Escherichia_Shigella* OTUs (43.33%) in nBPW enrichments does indicate there could be improved detection of *Salmonella* spp. in nBPW rinses of WBC collected at a post-PAA finishing chiller location. This is further demonstrated by the lower relative abundance (22.34%)of the *Escherichia_Shigella* OTU in the *Salmonella* spp. enrichments prepared from BPW rinses. The presence and higher relative abundancies of other OTUs representing additional enteric microorganisms including *Enterobacteriaceae*_OTU1 (15.55%), *Enterococcus* (11.16%), and Enterococcaceae_OTU1 (1.97%) also indicate that the recovery of *Enterobacteriaceae* could be positively influenced when nBPW is used. While these features represented approximately 28.68% of the sequences in the nBPW *Salmonella* spp. enrichments, the same features only represented 9.35% of the sequences in the enrichments prepared with BPW. Coinciding with the higher abundancies of *Enterobacteriaceae* OTU present in the nBPW enrichments, there was a greater proportion of non-enteric OTUs belonging to the order Aeromonadales (39.65% and 8.73%, in BPW and nBPW prepared enrichments, respectively) and a member of the family Burkholderiaceae (15.77% and 0.97%, in BPW and nBPW prepared enrichments, respectively) in the BPW enrichments, which are not typically indicative of enteric or fecal contamination associated with processing poultry.

After the implementation of nBPW by USDA-FSIS in 2016, it became apparent that microbiological methods that use 3M™Petrifilm™ or other direct plating materials may have been unintentionally affected by the rinse solution as the initial pH of the rinsate was slightly higher (pH 7.7 +/- 0.5 at 25ºC.) when compared to BPW (pH 7.2 +/- 0.2 at 25 ºC.) solutions

(Anonymous, 1996; 2018). Microbiome analysis as well as APC indicator counts of this sample set confirm that the pH level of the rinse solution may alter recovery as selectivity may be introduced due to a microorganisms' tolerance to pH fluctuations or pH levels outside of neutral ranges. Within this sample set, the only samples that had significantly different results for both the microbiological culturing methods as well as the microbiome analyses, were the APC Petrifilm™ associated with evaluating Log_{10} APC CFU/mL indicator levels. Overall, the BPW rinsates recovered a higher level of APC, 1.60 Log_{10} CFU/mL compared to 1.21 Log_{10} CFU/mL for the nBPW. Review of the abundancies of the major features indicated that the major difference in relative abundancies between the two populations was a lower percentage of *Escherichia_Shigella* (34.85%) in the APC counts recovered from the BPW rinsates, compared to 53.93% in the APC counts recovered from the nBPW rinsates. In turn, APC counts from the BPW rinsates had a higher proportion of *Streptococcus* (20.64%) compared to 4.61% in the nBPW rinsates. As *Streptococcus* is a lactic acid bacteria, it is typically favored in slightly acidic environments, so this shift could be possibly be attributed to the pH levels of the collected rinsates. Results from randomly selected samples within each sample group, indicated an average pH range of 6-6.5 pH for the BPW rinses and an average pH range of 7-7.5 for the nBPW rinses (crude measurements using pH strips were performed; data not shown). It appears that within this sample set, the nBPW solutions provided a more robust buffering capacity so that collected rinsates were more likely to remain at a neutral pH level. This would be especially important to consider for collections made to evaluate acid or acid blend interventions, in which buffering the pH of collected samples is important to ensure microorganism recovery. However, in the case of this sample set it is important to note that while the nBPW solution effectively buffered the collected rinsate, the same effects could have likely been achieved by sampling carcasses with

BPW and then pH adjusting the sample to a more neutral pH level after collection, as theoretically, if the pH of sample is within the neutral range, residual acids could be considered effectively neutralized. In addition, if a facility is only using acid interventions, allowing for adequate drip time prior to rinsing the carcass may prove to be just as effective as neutralizing the sample after collection and BPW could be considered an effective rinse solution as long as the sample is at a neutral pH range after collection. Additionally, while the built-in buffering capacity of the nBPW solution is convenient, since the initial starting pH of the solution used is slightly higher than the BPW, the use of nBPW solutions may still require pH adjustment to reach neutral ranges especially at collection sites prior to the application of acids, especially for those methods that may be more sensitive to pH, i.e. petrifilm or direct plating methodologies. In fact, samples collections by industry and research personnel who may be collecting WBC rinses to determine sanitary dress indications, perform process control evaluations or verify HACCP programs may not always need to use nBPW for all collections sites and in some cases, the use of nBPW for these collections could add an extra neutralization step.

Review of industry-wide *Salmonella* spp. and *Campylobacter* spp. prevalence trends of broiler carcasses after the implementation of nBPW identified a significant ($P < 0.05$) proportional increase (0.02 to 0.08) in *Salmonella*-positive samples within the commercial broiler processing sector while the *Campylobacter* spp. prevalence levels in carcasses remained constant at 0.027 (Williams et al., 2018). The increase in *Salmonella* spp. prevalence provided meaningful data in support of the theory that ineffective neutralization of antimicrobials in collected rinsates may have resulted in lower detection rates of *Salmonella* spp. in some samples, but also highlighted the need to further evaluate the impact of rinsate solution on recovery of *Campylobacter* spp. as expectations would be that effective neutralization of residual sanitizer

would have an impact on *Campylobacter* spp. prevalence levels as well (Williams et al., 2018). While the initial implementation of nBPW did not specifically address whether results in *Campylobacter* spp. prevalence would be affected, the continued concern of *Campylobacter* spp. food borne diseases and implication of poultry products, and therefore the need to accurately estimate *Campylobacter* spp. levels on WBC collected from commercial broiler processing systems, continues to spur interest in better understanding the effect of rinse solution on these sample collections and the recovery of *Campylobacter* spp. (Berrang et al., 2018). In research performed by Bourassa et al., (2019), in which a multi-hurdle poultry process system with PAA interventions was evaluated from pre-chill to post finishing chiller using both rinse solutions, overall prevalence levels of *Campylobacter* spp. within the processing system were lower in nBPW rinsates compared to BPW rinsates, with nBPW rinsates being only 55% positive while BPW rinsates were 70% positive. In contrast, in this study, there was not a significant difference in *Campylobacter spp.* prevalence levels, and overall, prevalence was higher in nBPW samples.

Analyzing the microbiome of the different rinse solutions, and associated materials from culturing methodologies pinpoints shifts in OTUs which can help explain the difference in results obtained from different data sets. It is generally understood that to ensure that the samples collected from a poultry processing system are a true reflection of the microbial loads present, including sublethal injured microorganisms, it is necessary to either perform carcass collections using a resuscitative medium or supplement rinsates with a resuscitative medium prior to microbiological evaluations. Up to this point, most of the research in understanding how to best recovery sub-lethally injured cells in WBC rinses has been focused on recovery of *Salmonella* spp. While introducing materials and methods in sample collections to aid in the recovery of injured *Salmonella* spp. is necessary to ensure that informative data is obtained for

regulatory bodies, industry and research personnel, failure to ensure the materials and methods are adequate to aid in recovery of other target pathogens, namely *Campylobacter* spp., as well as indicator microorganisms, including APC and EB, may result in skewed data not completely indicative of the system being analyzed. Further, the application of one rinse material to all processors, all sampling locations, without consideration for the antimicrobial intervention could introduce conflicting results between processors, regulatory bodies and researchers.

4.9 References

Anonymous. 1996. Instructions for Use: Buffered Peptone Water and Neutralizing Buffered Peptone. Hardy Diagnostics, 1430 West McCoy Lane, Santa Maria, CA 93455, USA

Anonymous. 2018. White Paper: Compatibility of 3M™ Petrifilm™ Plates with Neutralizing Buffered Peptone Water. 3M™ Food Safety, St. Paul, MN 55144-1000, USA

Antunes, P., J. Mourão, J. Campos, and L. Peixe. 2016. Salmonellosis: the role of poultry meat, Clin Microbiol Infect, 22(2), 110-121.

Baylis, C.L., S. MacPhee, and R.P. Betts. 2000. Comparison of two commercial preparations of buffered peptone water for the recovery and growth of *Salmonella* bacteria from foods. J Appl Microbiol, 89: 501-510.

Berrang, M. E., G. R. Gamble, J.A. Hinton, and J.J. Johnston. 2018. Neutralization of residual antimicrobial processing chemicals in broiler carcass rinse for improved detection of *Campylobacter*. J Appl Poult Res, 27(3), 299-303.

Bourassa, D. V., J.L. Lapidus, A.E. Kennedy-Smith, and A. Morey. 2019. Efficacy of neutralizing buffered peptone water for recovery of *Salmonella*, *Campylobacter*, and *Enterobacteriaceae* from broiler carcasses at various points along a commercial immersion chilling process with peroxyacetic acid. Poult Sci, 98(1): 393-397.

Bolyen E. R., J.R. Rideout, M.R. Dillon, N.A. Bokulich. C. Abnet, G.A. Al-Ghalith, H. Alexander, E.J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J.E., Bisanz, K. Bittinger A. Brejnrod A, C.J. Brislawn C.T. Brown, B.J. Callahan, A.M. Caraballo-Rodríguez, J. Chase, E. Cope, R. Da Silva, C. Diener, P.C. Dorrestein, G.M. Douglas, D.M. Durall, C. Duvallet, C.F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S.M. Gibbons, D.L Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. Huttley G, S. Janssen, A. K. Jarmusch, L. Jiang, B. Kaehler, K.B. Kang, C.R. Keefe, P. Keim, S.T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M.G. Langille, J. Lee, R. Ley, Y. Liu, E. Loftfield, C. Lozupone, M. Maher M, C. Marotz, B.D. Martin, D. McDonald, L.J. McIver, A.V. Melnik, J.L. Metcalf, S.C. Morgan, J. Morton, A.T. Naimey, J.A. Navas-Molina, L.F. Nothias, S.B. Orchanian, T. Pearson, S.L. Peoples, D. Petras, M.L. Preuss, E. Pruesse, L.B. Rasmussen, A. Rivers, M.S. Robeson II, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S.J. Song, J.R. Spear, A.D. Swafford L. R. Thompson, P.J. Torres, P. Trinh, A. Tripathi, P.J. Turnbaugh, S. Ul-Hasan, J.J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K.C. Weber, C.H. Williamson, A.D. Willis, Z.Z. Xu, J.R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, J.G. Caporaso. 2018. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. Nat. Biotechnol. 37(8): 852-857.

Bryan, F.L. and M.P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter* jejuni in raw poultry. J. Food Prot., 58: 326-344.

Ceuppensa, S., D. D. Coninck, N. Bottledoorn, F.V. Nieuwerburgh, and M. Uyttendaelea. 2017. Microbial community profiling of fresh basil and pitfalls in taxonomic assignment of enterobacterial pathogenic species based upon 16S rRNA amplicon sequencing. International Journal of Food Microbiology. 257: 148–156.

Dhariwal, A., J. Chong, S. Habib, I. King, L.B. Agellon, and J. Xia. 2017. MicrobiomeAnalyst - a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res, 45: 180-188.

Dogan, O. B., J. Clarke, F. Mattos, and B. Wang. 2019. A quantitative microbial risk assessment model of *Campylobacter* in broiler chickens: Evaluating processing interventions. Food Control, 100: 97-110.

Gamble, G. R., M.E. Berrang, R. J. Buhr, A. Hinton Jr., D.V. Bourassa, J. J. Johnston, K.D. Ingram, E.S. Adams, and P. W. Feldner. 2016. Effect of simulated sanitizer carry-over on recovery of *Salmonella* from broiler carcass rinsates. J. Food Prot., 79: 710-714.

Gamble, G., E. M.E. Berrang, R. J. Buhr, A. Hinton Jr, D. V. Bourassa, K.D. Ingram, and J. J. Johnston. 2017. Neutralization of bactericidal activity related to antimicrobial carryover in broiler carcass rinse samples. Journal of Food Protection, 80, 685-691. doi:10.4315/0362-028X.JFP-16-412

Gibbons, S.M., C. Duvallet, and E.J. Alm. 2018. Correcting for batch effects in case-control microbiome studies. PLoS Comput. Biol., 14: 4, e1006102. doi:10.1371/journal.pcbi.1006102

Grim, C.J., N. Daquigan, T. S. Lusk Pfefer, A R. Ottesen, J. R. White, and K.G. Jarvis. 2017. High-resolution microbiome profiling for detection and tracking of *Salmonella enterica.* Front. Microbiol. 8:1587.

Josefsen, M. H., A. K. Bhunia, E. O. Engvall, M. S. R. Fachmann, and J. Hoorfar. 2015. Monitoring *Campylobacter* in the poultry production chain — from culture to genes and beyond. J. Microbiol. Methods, 112: 118-125.

Milios, K. T., E. H. Drosinos, and P.E. Zoiopoulos. 2014. Food safety management system validation and verification in meat industry: Carcass sampling methods for microbiological hygiene criteria – A review. Food Control, 43: 73-80.

Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerging Infectious Diseases, 19(3), 407-415. https://dx.doi.org/10.3201/eid1903.111866.

Roccato, A., M. Macin, L. Barco, V. Cibin, K. Antonello, F. Cocola, and A. Ricci. 2018. Usefulness of indicator bacteria as potential marker of *Campylobacter* contamination in broiler carcasses. Int J Food Microbiol, 276: 63–70.

Saini, P.K., H.M. Marks, M.S. Dreyfuss, P. Evans, L.V. Cook, and U. Dessai. 2011. Indicator organisms in meat and poultry slaughter operations: their potential use in process control and the role of emerging technologies. J. Food Prot., 74: 1387-1394

Skarp, C. P. A., M. L. Hänninen, and H. I. K. Rautelin. 2016. Campylobacteriosis: The role of poultry meat. Clin Microbiol Infect, 22: 103-109.

Smith, J., S. Corkran, S.R. McKee, S.F. Bilgili, and M. Singh. 2015. Evaluation of post-chill applications of antimicrobials against *Campylobacter jejuni* on poultry carcasses. J. Appl. Poult. Res. 24: 451–456.

Stephens, P. J., and B. M. Mackey. 2003. In Corry J. E. L., Curtis G. D. W. and Baird R. M.(Eds.), *Chapter 2 recovery of stressed microorganisms* Elsevier. doi:https://doi.org/10.1016/S0079-6352(03)80005-X

Tompkin, R.B. 1990. The Use of HACCP in the Production of Meat and Poultry Products. J. Food Prot., 53(9): 795-803.

USDA, FSIS. 1996. Food Safety and Inspection Service Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. Fed Regist. 61:38805–55.

USDA, FSIS. 2013. *Salmonella* and *Campylobacter* verification program for raw meat and poultry products. FSIS Directive, 10,250.1. Washington, D.C. Accessed April 14, 2019.

USDA, FSIS. 2015. Microbiology Laboratory Guidebook Quantitative Analysis of Bacteria in Foods as Sanitary Indicators. MLG 3.02. Athens, GA. https://www.fsis.usda.gov/wps/wcm/connect/f2162091-af72-4888-997b-718d6592bcc9/MLG-3.pdf?MOD=AJPERES. Accessed November 14, 2019.

USDA, FSIS. 2016. Microbiology Laboratory Guidebook: Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product Samples. MLG 41.04. Athens, GA. https://www.fsis.usda.gov/wps/wcm/connect/0273bc3d-2363-45b3-befb-1190c25f3c8b/MLG-41.pdf?MOD=AJPERES. Accessed April 14, 2019.

Wideman, N., M. Bailey, S. F. Bilgili, H. Thippareddi, L. Wang, C. and M. Singh. 2016. Evaluating best practices for *Campylobacter* and *Salmonella* reduction in poultry processing plants. Poult Sci, 95(2), 306-315.

Williams, M.S., E.D. Ebel, S.A. Hretz, and N. J. Golden. 2018. Adoption of Neutralizing buffered peptone water coincides with changes in apparent prevalence of *Salmonella* and *Campylobacter* of broiler rinse samples. J. Food Prot., 81(11): 1851–1863.

Yu, Zhongjia, M. F. Peruzya, C. Dumolinc, M. Joossensc, and K. Houf. 2019. Assessment of food microbiological indicators applied on poultry carcasses by culture combined MALDI-TOF MS identification and 16S rRNA amplicon sequencing. Food Microbiol., 82: 53–61.

4.10 Tables and Figures

Table 1 | Average Log10 APC and EB CFU/mL counts for each rinsate type. EB prevalence data based on detection by direct plating. Salmonella spp. and Campylobacter spp. prevalence levels based on enrichment data obtained for each rinsate type. Values (ab) denoted in same columns with different subscripts were significantly different (P < 0.05).

RINSATE TYPE	Log_{10} APC CFU/mL	Log_{10} EB CFU/mL	EB Prevalence	*Salmonella* spp. Prevalence	*Campylobacter* spp. Prevalence
nBPW	1.21^a	0.27	77.55%	48.98%	91.84%
BPW	1.60^b	0.27	73.47%	32.65%	79.59%

Table 2 | Alpha diversity metrics: Average OTUs and Shannon indices.

Sample Type	Number of Samples	Average Number of Observed OTUs per Sample	P-value	Mann-Whitney	Shannon	P-value	Mann-Whitney
nBPW Rinsate	n=49	37.78 ± 16.40	0.74	1083	1.72 ± 1.03	0.28	1274
BPW Rinsate	n=46	38.0 9 ± 14.94			1.98 ± 0.88		
nBPW *Salmonella* spp. Enrichment	n=47	12.26 ± 2.51	0.08	838	1.19 ± 0.38	0.85	1033
BPW *Salmonella* spp. Enrichment	n=45	11.53 ± 2.17			1.19 ± 0.27		
nBPW *Campylobacter* spp. Enrichment	n=17	8.00 ± 2.83	0.07	98	1.22 ± 0.34	0.83	160
BPW *Campylobacter* spp. Enrichment	n=18	6.17 ± 2.36			1.17 ± 0.57		
nBPW CCPM Extractions	n=37	3.83 ± 0.92	<0.02	496	1.03 ± 0.24	<0.05	480
BPW CCPM Extractions	n=39	3.23 ± 1.07			0.84 ± 0.33		
nBPW APC Petrifilm	n=48	10.58 ± 2.16	<0.05	1504	0.89 ± 0.42	<0.05	1652
BPW APC Petrifilm	n=49	11.86 ± 2.27			1.20 ± 0.38		

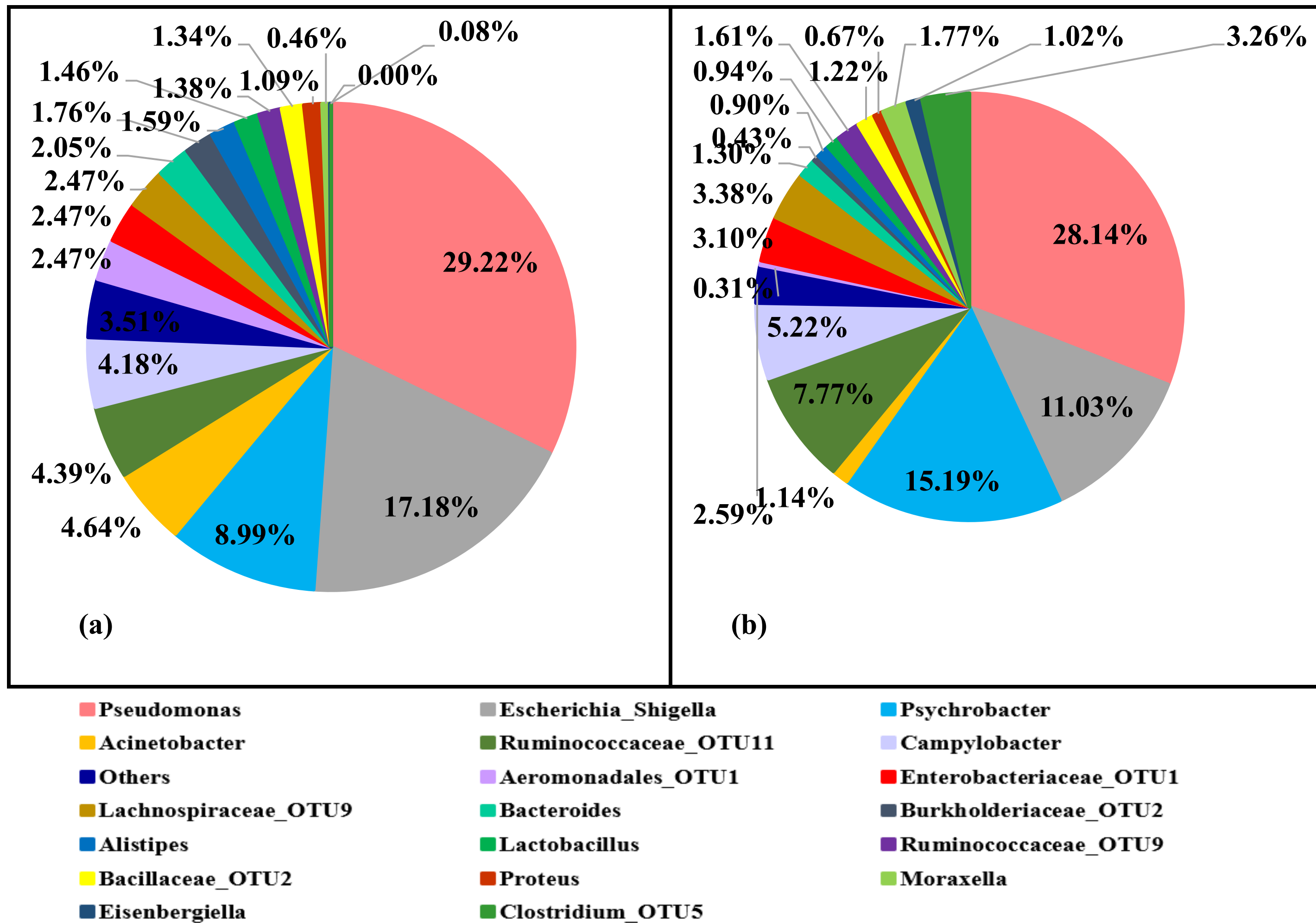

Figure 1 | (a). OTU composition of most abundant sequences in BPW rinsates. **(b).** OTU Composition of most abundant sequences in nBPW rinsates.

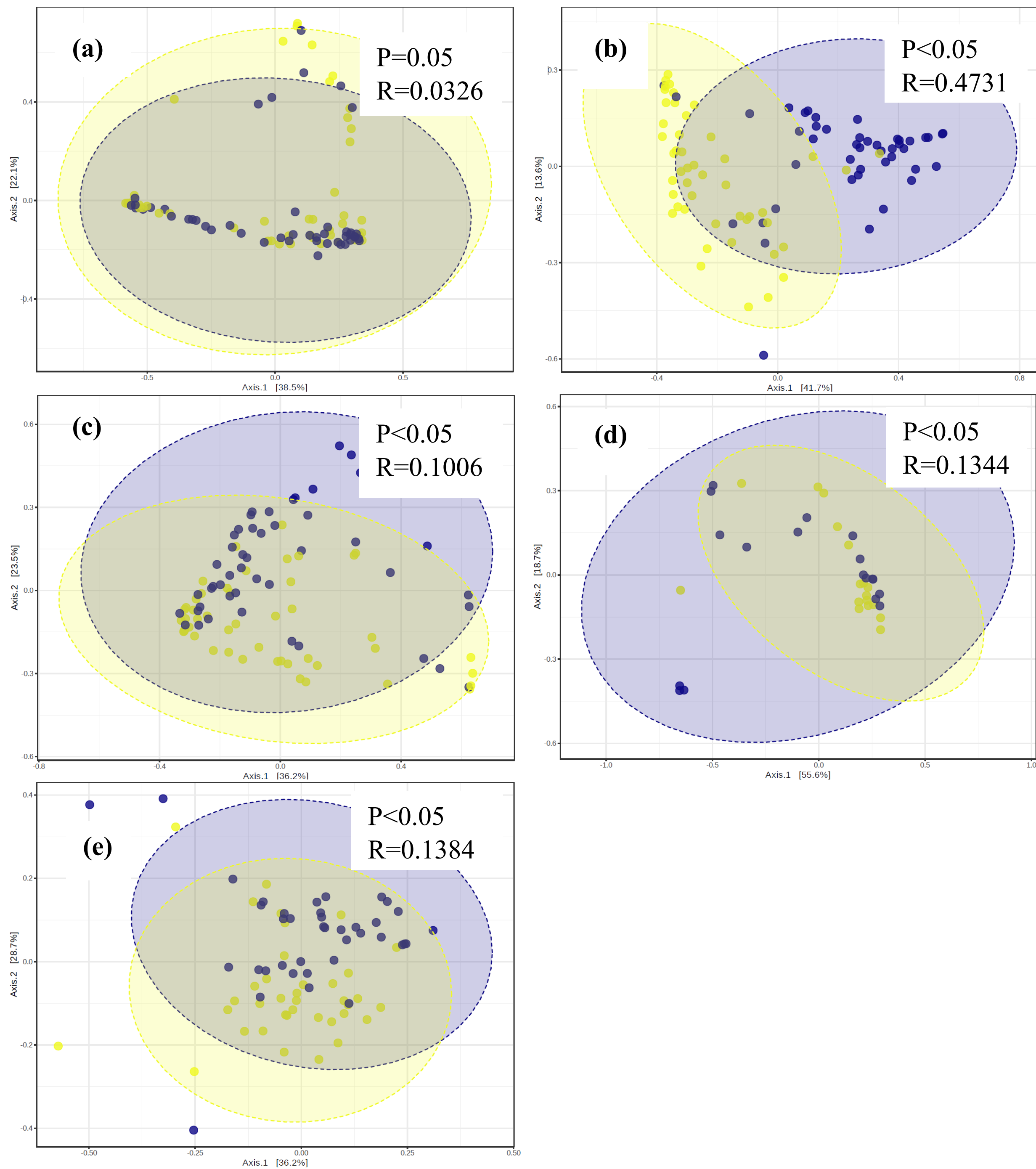

Figure 2 | Beta diversity: Principle Coordinates of Analysis and Bray-Curtis Dissimilarity evaluated with ANOSIM. Purple shaded eclipses and dots represent BPW samples and yellow shaded eclipses and dots represent nBPW samples. **(a).** Rinsates **(b).** *Salmonella* spp. enrichments. **(c).** APC extractions**. (d).** *Campylobacter* spp. enrichments **(e).** CCPM extractions.

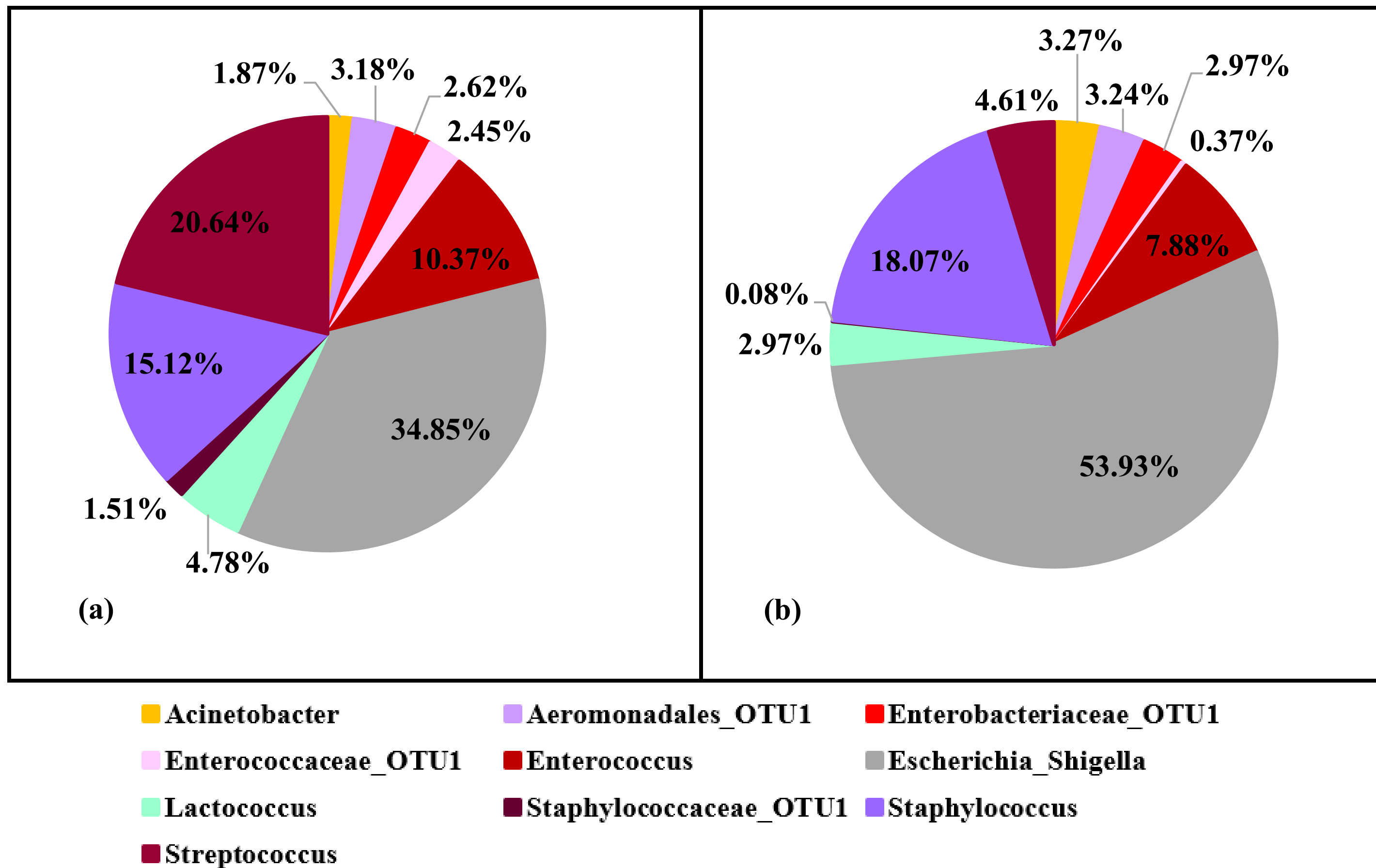

Figure 3 | (a) OTU composition of most abundant sequences of extracted gel from 3M™ APC Petrifilm™ prepared from BPW rinsates. **(b)** OTU composition of most abundant sequences of extracted gel from 3M™ APC Petrifilm™ prepared from nBPW rinsates**.**

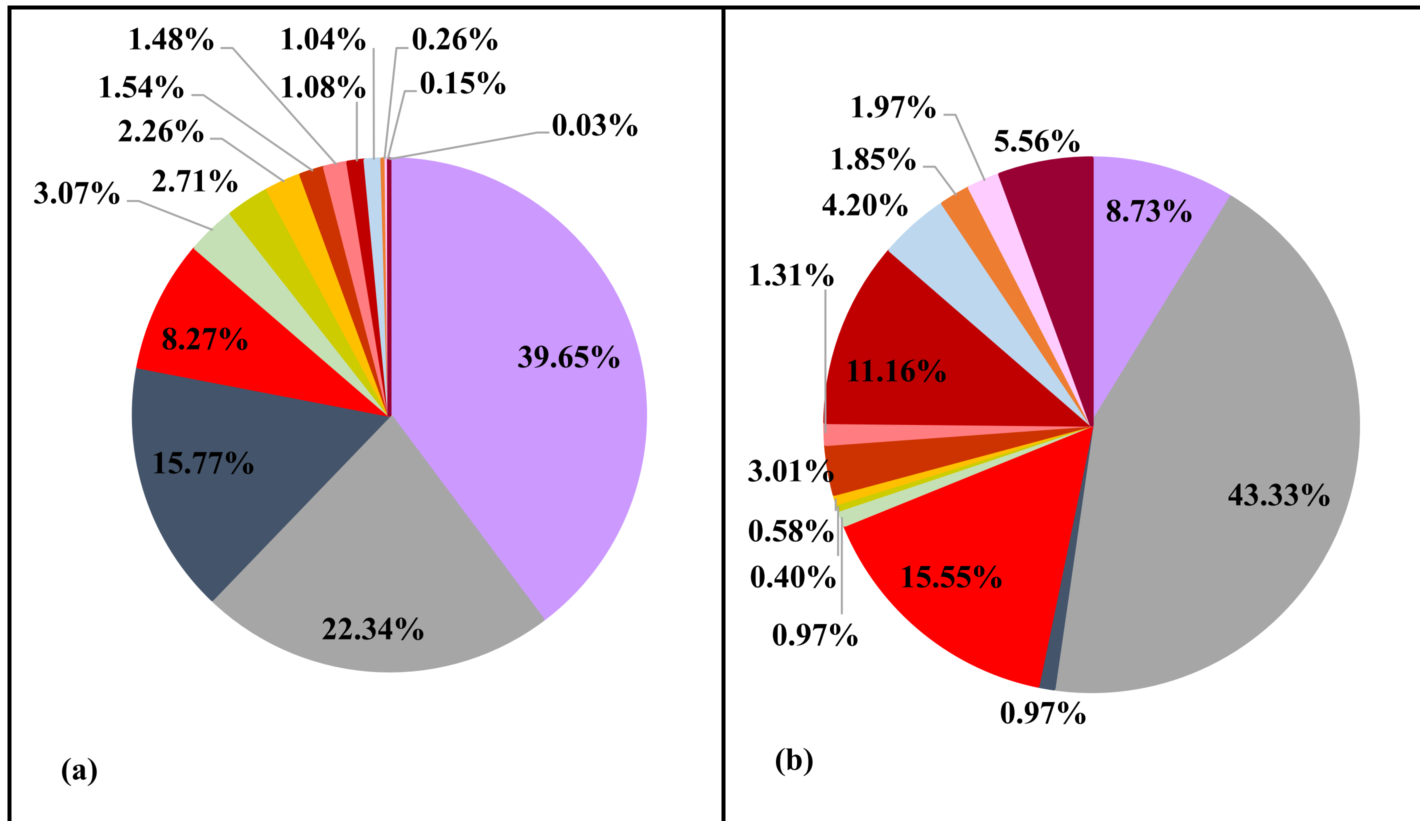

Figure 4 | (a) OTU composition of most abundant sequences in *Salmonella* spp. enrichments prepared from BPW rinsates. **(b)** OTU Composition of most abundant sequences in *Salmonella* spp. enrichments prepared from nBPW rinsates.

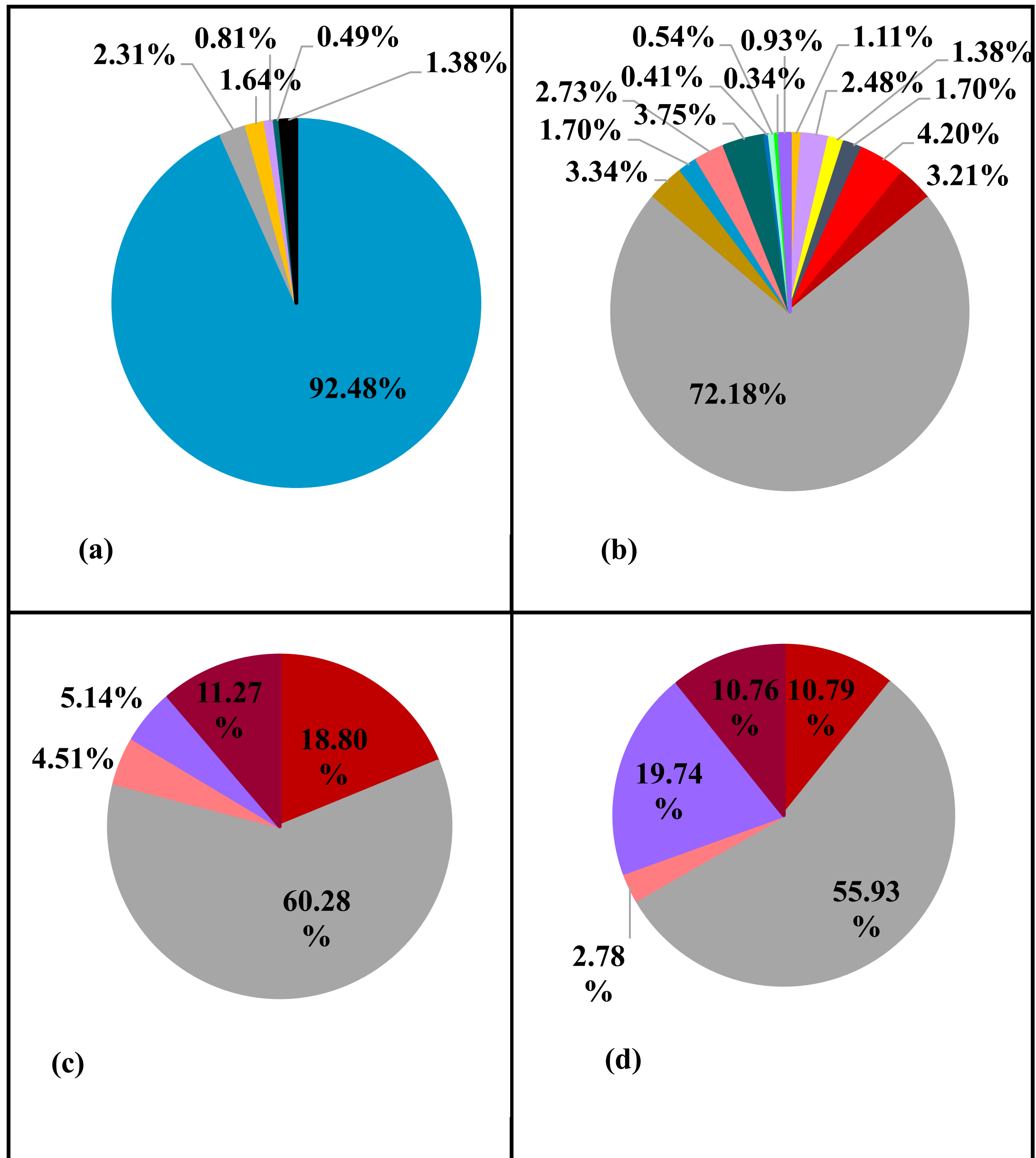

Figure 5 | (a) OTU composition of most abundant sequences in *Campylobacter* spp. enrichments prepared from BPW rinsates. **(b)** OTU Composition of most abundant sequences in *Campylobacter* spp. enrichments prepared from nBPW rinsates. **(c)** OTU composition of most abundant sequences from CCPM agar plates prepared from BPW rinsates. **(d)** OTU composition of most abundant sequences from CCPM agar plates prepared from nBPW rinsates.

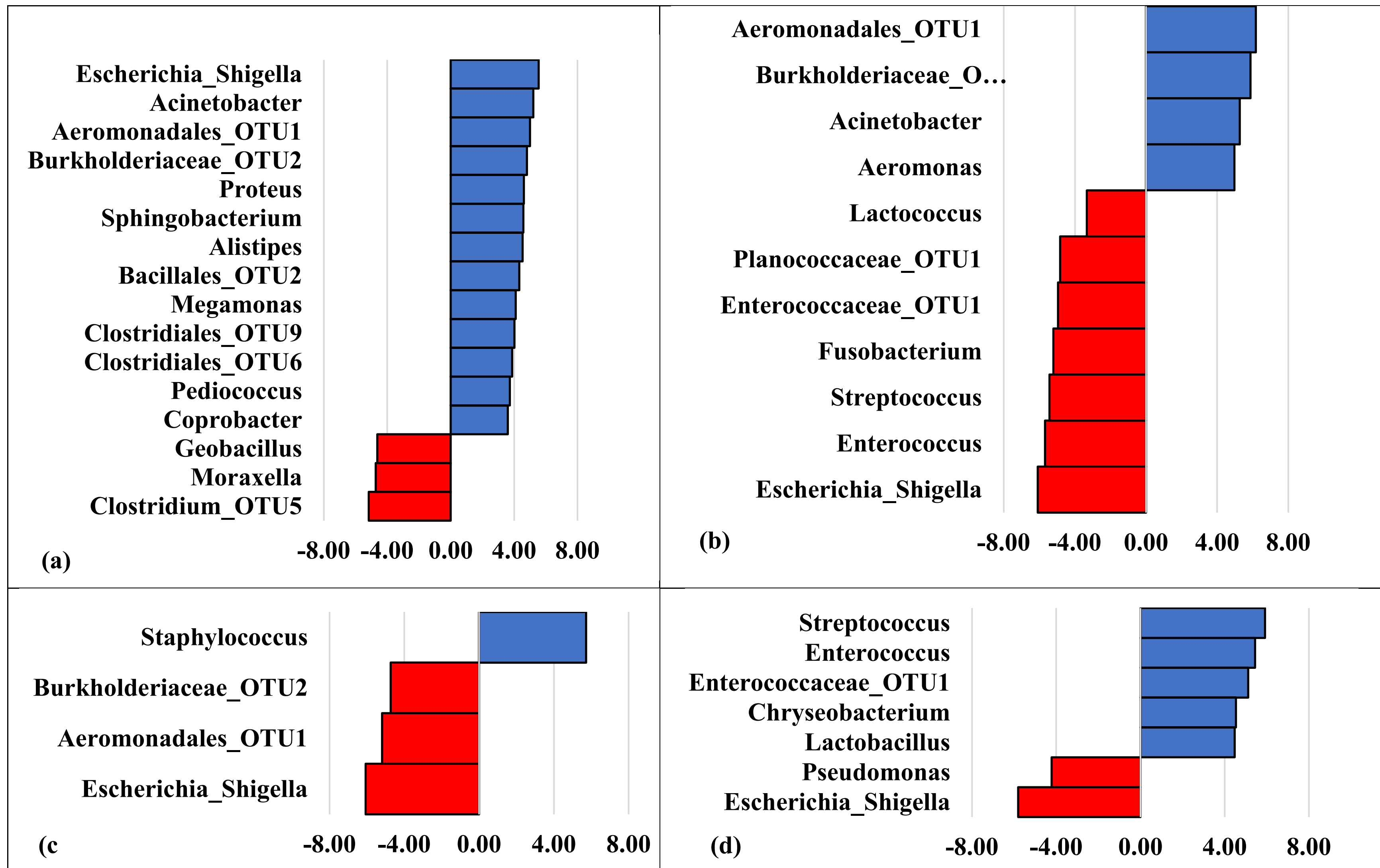

Figure 6 | Histograms of significantly ($P < 0.05$) differentially abundant OTUs determined by LEfSe analysis (blue OTUs overabundant in BPW rinsates, red OTUs underabundant in BPW rinsates). **(a).** Collected rinsates. **(b).** *Salmonella* spp. enrichments. **(c).** *Campylobacter* spp. enrichments. **(d).** APC Petrifilm™ extractions.

5 Conclusion

Mitigating and controlling the microbiological food safety and quality risks associated with raw poultry products requires the use of extensive process control and multiple hurdle interventions within commercial broiler harvest and processing systems. Verification of applied controls are typically obtained by documentation and monitoring of operations, along with microbiological sampling. Microbiological sampling is accomplished using identified, industry-specific methodologies which exploit the culturability of those microorganisms typically found in the associated environment. The concurrent application of non-culture based microbiota characterization using 16s rDNA sequencing to these processing steps provides an improved understanding of the microbiological shifts related that may alter the risks of food-safety concerns as well as assure the quality of associated poultry products. Microbiota characterization of poultry processing systems can be considered an informative molecular microbiological tool that researchers, industry and regulatory personnel can apply to inform decisions within poultry processing systems.

6 Appendix

6.1 Letter from Frontiers in Microbiology, allowing reuse of previously published materials, regarding article “Comparison of 16S rDNA Next Sequencing of microbiome communities from post-scalder and post-picker stages in three different commercial poultry plants processing three classes of broilers”

From: Phillip Achike (Frontiers Application Support) [mailto:support@frontiersin.org]
Sent: Thursday, January 30, 2020 2:32 AM
To: Wages, Jennifer <Jennifer.Wages@tyson.com>
Subject: [EXTERNAL] - [Frontiers Zendesk] Re: Publishing article in PhD Disseration

-- Please type your reply above this line --

Your request (324602) has been solved. To reopen this request, reply to this email.

Phillip Achike (Frontiers Application Support)

Jan 30, 09:32 CET

Dear Jennifer,

Thank you for your email. Reuse of published article is permitted provided that the authors and original source are appropriately credited and that no third-party licenses apply (please see the citation on the article on-line page).Frontiers does not provide any formal permissions for reuse.
Should you have other concerns, please feel free to contact us.

Best regards,

Phillip Achike

Senior Application Support Analyst

Jennifer Wages

Jan 30, 01:34 CET

It does not appear that there are any concerns with including a previously published article as a chapter in my PhD dissertation, provided I indicate that it was originally printed in Frontiers. Is this correct? Is there anything else to address prior to including that article in my dissertation?

Thanks for you help,
Jennifer

6.2 Email correspondence with Tyson Legal and FSQA departments regarding publishing "Comparison of 16S rDNA Next Sequencing of microbiome communities from post-scalder and post-picker stages in three different commercial poultry plants processing three classes of broilers"

Jennifer Wages, Sr. Manager Science and Innovation, FSQA Prepared Foods
Cell: 479-381-1059

From: Bedell, Mary Lynn
Sent: Monday, September 17, 2018 2:44 PM
To: Griffino, Alison <Alison.Griffino@tyson.com>; Wages, Jennifer <Jennifer.Wages@tyson.com>
Subject: Fwd: Peer Reviewed Documents/Publications

Jennifer,

I reviewed and had no issues from a legal perspective but would like Alison to take a look and make sure there are no concerns regarding exposure of Tyson trade secrets.

Mary Lynn

Sent from my iPad

Begin forwarded message:

From: "Wages, Jennifer" <Jennifer.Wages@tyson.com>
Date: September 12, 2018 at 7:44:48 AM CDT
To: "Bedell, Mary Lynn" <MaryLynn.Bedell@tyson.com>, "Adams, Jacquelyn" <jacquelyn.adams@tyson.com>
Subject: RE: Peer Reviewed Documents/Publications

Hi Mary Lynn,
Thanks for your help. I have recently finished a manuscript while working on my PhD dissertation. We used some samples that were being evaluated at our lab, and then did some additional analysis through the U of A. My focus has been on microbiome evaluations of poultry processing systems using next generation sequencing. The methodologies and applications to poultry processing are not new or novel, but have not necessarily been applied to the pre-evisceration stages of the system and those results compared to traditional microbiological methods. I've attached the draft manuscript for your review. Please let me know if you have any questions or concerns.
Thank you.