Chapter 15

MICROBIOLOGY AND SANITATION

The Science of Poultry and Meat Processing

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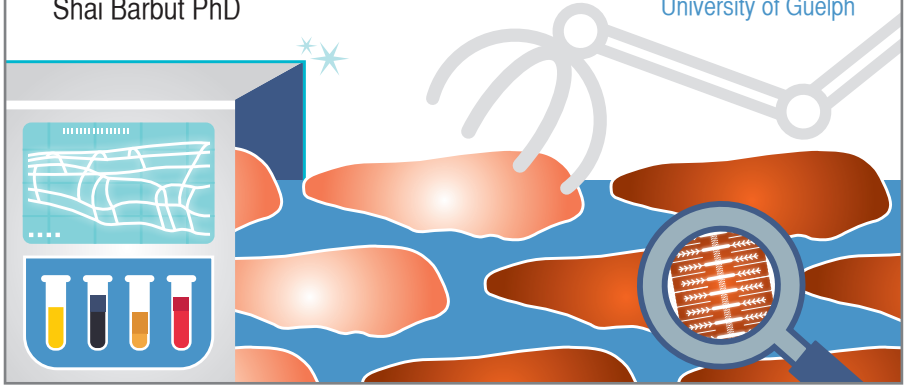
University of Guelph



Chapters

* Topics focussing on poultry. Rest of the chapters are related to both red meat and poultry.

Preface

The aim of The Science of Poultry and Meat Processing book is to provide students and industry personnel with a comprehensive view of the modernized primary poultry meat industry and further processing of both red meat and poultry. An emphasis is placed on basic concepts as well as recent advancements such as automation (e.g. increasing poultry line speed from 3,000 to 13,000 birds per hour over the last 40 years) and food safety (e.g. HACCP in primary and the further processing areas). The book also includes chapters explaining basic muscle biology, protein gelation, heat and mass transfer, microbiology, as well as meat colour and texture to help the reader understand the underlying scientific concepts of meat processing. The Science of Poultry and Meat Processing book is based on over two decades of university teaching experiences, and is designed to be used as a course textbook by students, as well as a resource for professionals working in the food industry. The book is available online, at no cost, to any interested learner. Using this format has also allowed me to include many colour pictures, illustrations and graphs to help the reader.

The book is dedicated to my past and current students who have inspired me to learn more and conduct challenging research projects. I see this as an opportunity to give back to the field that I have received so much from as a student and as a faculty member. Looking back, I have learned a great deal from my MSc and PhD advisor, Dr. A. Maurer, who was the student of Dr. R. Baker - the father of poultry processing in North America. I would also like to thank Dr. H. Swatland with whom I worked for almost 20 years, for the many challenging scientific discussions.

Writing The Science of Poultry and Meat Processing book was a long process, which also included having all chapters peer reviewed. I appreciate the help of my colleagues, but I still take responsibility for any inaccuracy in the book. If you have comments or **suggestions,** I would appreciate hearing from you (sbarbut@uoguelph.ca), as I am planning to revise and update a few chapters on a yearly basis.

I would like to thank the many people who have helped me during the writing process. To Deb Drake who entered all of the material for the book, to Mary Anne Smith who assisted in editing, and to ArtWorks Media for the design and desktop publishing of the book. I greatly appreciate the help of my colleagues who reviewed chapters and provided useful discussions. They include Mark B., Ori B., Sarge B., Gregoy B., Joseph C., Mike D., Hans G., Theo H., Melvin H., Myra H., Walter K., Roland K., Anneke L., Massimo M., Johan M., Erik P., Robert R., Uwe T., Rachel T., Jos V., Keith W., and Richard Z. I would also like to thank my family for their love and support during the entire process.

About the Author

Shai Barbut is a professor in the Department of Food Science at the University of Guelph in Ontario, Canada. He received his MSc and PhD at the University of Wisconsin in meat science and food science. He specializes in primary and further processing of poultry and red meat. His research focuses on factors affecting the quality of meat, as well as protein gelation with an emphasis on structure / function relationships, rheological properties and food safety aspects. He has published over two hundred peer reviewed research papers and is the author of the Poultry Products Processing – An Industry Guide textbook. He is a fellow of the Institute of Food Technologists and has received awards from the Meat Science Association, Poultry Science Association, and the Canadian Institute of Food Science and Technology. He is involved in a number of government committees as well as academic and industrial research projects.

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MICROBIOLOGY AND SANITATION

15.1 Introduction

The overall goal of the food industry is to produce wholesome, nutritious, and tasty food for the consumer. Producing a wholesome, safe food with a reasonable shelf life is a challenge especially when there are so many steps involved in production (e.g., growing farms, processing plants, distribution channels). Today most food is produced far from the consumer and it could be days or weeks before the food is consumed. This presents challenges to all involved in the production chain (e.g., farmer, processor, retailer, food handler), but this is definitely not a new issue (Newell et al., 2010). There is still a big difference between developed and developing countries in terms of dealing with food safety and food borne diseases (e.g., surveillance, budget allocated to deal with the problem). According to the World Health Organization, about 1.8 million people in developing countries die each year as a result of diarrheal diseases related to contaminated food and water. However, food borne diseases are not just a problem of the developing world.

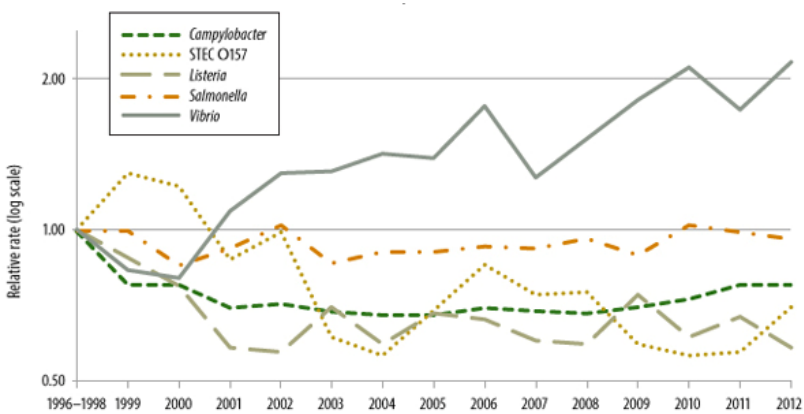

Figure 15.1.1 Relative rates of laboratory-confirmed infections with *Campylobacter*, STEC0157, *Listeria*, *Salmonella*, and *Vibrio* compared with 1996-1998 rates, by year – Foodborne Diseases Active Surveillance Network, United States, 1996-2012. From CDC.

Even in developed countries like the US, roughly 1 in 6 people get sick (~48 million people), 130,000 are hospitalized, and 3,000 die from food borne diseases, with an estimated cost of about $51 billion annually (Scharff, 2011). The good news is that, for most food borne diseases, the incidence is decreasing. Figure 15.1.1 shows the decline in some of the major pathogens seen in North America from 1996 to 2012. The increase in *Vibrio* appears to be large but it should be kept in mind that the 2012 rate per 100,000 inhabitants was 0.41 compared to 14.3 for *Campylobacter*. Data from England and Wales also shows general reduction trends (Fig. 15.1.2).

Figure 15.1.2 Total number of foodborne outbreaks in England and Wales, 1991 – 2011. From Health Protection Report (2012). http://www.hpa.org.uk/hpr/archives/2012/news1812.htm

These data represent a concentrated effort of both government and industry to enhance food safety programs and reduce associated costs (e.g., lost work days and productivity). When discussing food borne diseases it is also important to consider pathogen distribution and illness severity. Figure 15.1.3 shows that in the USA about 60% of the cases are associated with Norovirus outbreaks (note: some are associated with food and some with person to person transmission). In 2008 there were about 5.4 million cases but only 150 deaths (0.050 deaths/100,000 inhabitants) whereas *Salmonella* resulted in about 1.0 million cases and 38 deaths (0.126 deaths/100,000 inhabitants). The symptoms of Norovirus include fever, vomiting, and diarrhea and usually only last for a day or two (sometimes called the 24-hour flu). Mortality is very low and usually occurs due to complications from other diseases.

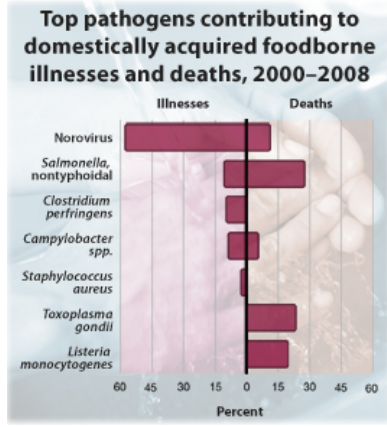

Figure 15.1.3 Top pathogens 2000 – 2008. From CDC. http://www.cdc.gov/Features/dsFoodborneEstimates/dsFoodborneEstimates_270px.jpg

Figure 15.1.4 summarizes the outbreaks and total number of illnesses associated with different food commodities. While meat is a cause of illness, produce (fruits and vegetables) that are grown in or close to the ground represent a higher risk to the consumer. Additionally, often produce is not heat treated prior to eating. This brings to light the importance of looking at the entire production chain (farm to fork) in terms of an integrated prevention approach.

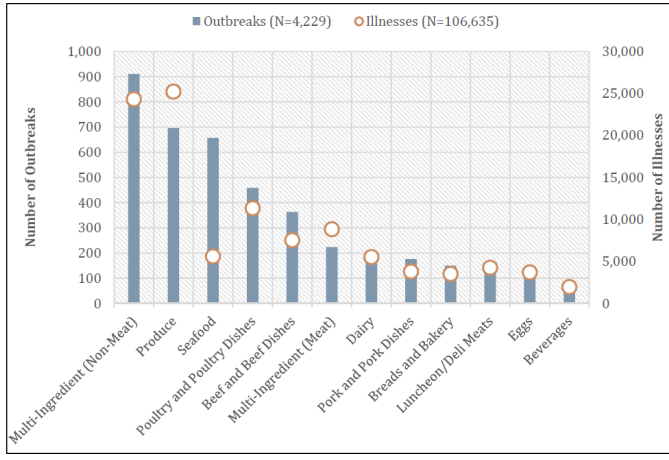

Figure 15.1.4 Outbreaks and illnesses due to food commodities, 2001-2010. From CSPI (2013).

Figure 15.1.5 shows a schematic illustration of the different points along the chain that is applicable for most food products. More information related to this topic and to Figure 15.1.4 is found in the report by Gould et al. (2013), who surveyed food borne diseases in the US from 1998-2008.

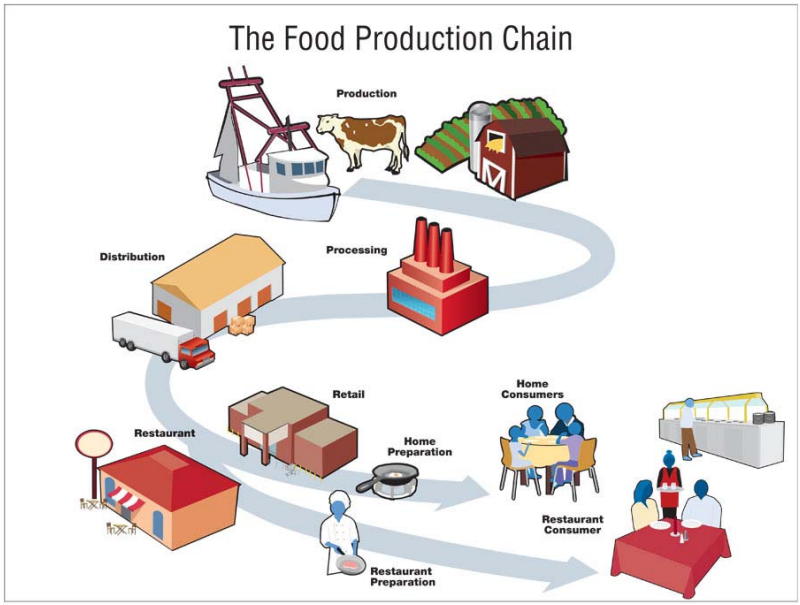

Figure 15.1.5 Food production chain. From CDC. http://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/figure_food_production.html

In the US, incidence of food borne disease has been significantly reduced over the past decade (Fig. 15.1.6) where the number of *Yersinia*, *E. coli* O157, and *Shigella* cases were reduced by 53%, 41%, and 55%, respectively (note: the US has one of the best surveillance programs, which helps to look at overall trends). *Campylobacter*, *Listeria*, and *Salmonella* were reduced by 30%, 26%, and 10%, respectively. These decreases have been the result of implementing better surveillance and reporting (i.e., problems are flagged and dealt with faster), mandatory implementation of specific programs such as Hazard Analysis Critical Control Points (HACCP) and the mandatory Microbial Performance Standards for the meat industry (see Chapters 6 and 12 on HACCP).

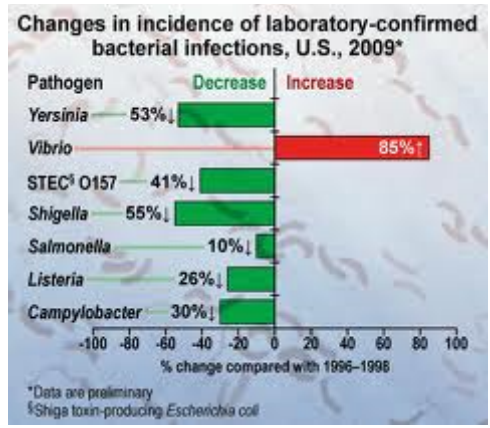

Figure 15.1.6 Changes in bacteria infection 2009. FromCDC. http://www.cdc.gov/Features/dsfoodnet2012/figure1.html

Table 15.1.1 shows the Progress Report for some major pathogens in the US and includes the current infection rate per 100,000 inhabitants and regulatory target values. Most years there is a positive move towards approaching these targets.

Table 15.1.1 Food safety – progress report. From CDC. http://www.cdc.gov/foodnet/data/trends/trends-2012-progress.html

However the table also points out the well-documented problem of under reporting of food borne illnesses, where the cases of patients with relatively mild symptoms are not reported (i.e., they do not seek medical advice) and/or the cause is not fully identified (e.g., doctor sees a patient but no sample is sent to the laboratory). The same is seen in data from Australia (Table 15.1.2) and other countries. The table also allows comparison to the US data presented above. Information from different countries can be found in EFSA (2010) and on other websites. As indicated above, the goal of the food industry is to supply wholesome, nutritious, and safe food.

Table 15.1.2 Estimated incidence of diseases potentially transmitted by food and research summary findings - Australia. From Angulo et al. (2008).

Disease	No. of cases reported nationally	Estimated no. of annual infections[a] (95% credible interval)	Estimated percentage of foodborne diseases (95% credible interval)	Main food vehicles and research findings
Campylobacteriosis	~16,000	223,000 (93,800–362,800)	75 (67–83)	9.6 community cases per case reported to surveillance; infections acquired from chicken (50,000 cases), offal (3500 cases), and pets (8500 cases); low levels of fluoroquinolone resistance among human isolates; molecular typing (*flaA*) improves risk factor identification
Salmonella infection	~8400	48,700 (15,000–91,300)	87 (81–93)	7.6 community cases per case reported to surveillance; *Salmonella* Mississippi (~75 cases annually, 80% of cases in Tasmanian residents; drinking untreated was the water main risk factor, exposure to native birds was important); *Salmonella* Enteritidis (~380 cases annually, ~50 cases locally acquired annually, 75% of cases occurred in Queensland residents, predominantly phage type 26, absent in commercial egg-laying flocks)
Listeria infection	~60	120	98 (92–100)	Host factors were the most important predictor of disease, risky foods were commonly eaten; perinatal case-fatality rate, 25%; nonperinatal case-fatality rate, 25%
Shiga toxin–producing *Escherichia coli* infection	~80	3800 (1000–33,000)	65 (48–82)	High rate in South Australia because of intensive screening of bloody stool samples (2%-4% of bloody stool samples were *stx* positive); predominantly serotype O157; animal exposure was an important predictor of disease

[a] Adjusted for underreporting.

We know that this requires a farm to fork approach (Fig. 15.1.5), which also includes more emphasis on the consumers role in food handling at home (Fig. 15.1.7; see additional discussion below). In this chapter the microbiological issues at the major production/processing points (e.g., growing farm, transportation, processing plant, distribution, and home/restaurant food preparation) are discussed using evidence from several systematic reviews including meta-analyses and meta-regressions of a large number of primary studies. Additionally, you will find a general description of the six major bacteria associated with food borne disease, and discussion on cleaning and sanitary equipment design.

Figure 15.1.7 Logo of the Partnership for Food Safety Education. Safe food handling. www.fightbac.org/safe-food-handling.

15.2 Major Pathogenic Bacteria of Concern in Poultry and Red Meat

Fresh meat is a perishable commodity and therefore should be treated with care. The shelf life of fresh meat, which is related to the growth of spoilage microorganisms, depends on many factors. Among the most important are the initial microbial load (contamination), storage time and temperature, the intrinsic properties of the meat (e.g., pH, nutrient content), and the degree of processing. The latter will be discussed in more detail while focusing on poultry meat; however, the same processing parameters also apply to other meat producing animals and include steps such as evisceration, cutting and chilling. Healthy muscle tissue is basically free of bacteria but it can become contaminated during processing as it comes in contact with microorganisms from the "outside" of the animal (e.g., skin, feathers, hair), the environment (e.g., air, water used for rinsing), and the "inside" of the animal (e.g., intestine). The bacterial load in intestinal contents or in dirt adhering to feathers/skin can be as high as $10^8 – 10^9$ microorganisms per one gram or one ml. Meat sold at the store is definitely not sterile and usually contains $>10^2$ colony forming units (CFU) per gram when it is very fresh. Figure 15.2.1 shows a graph produced in the 1960s that represents the relationship between contamination level and shelf life. In addition to spoilage microorganisms, meat can also be a vehicle for human pathogens that cause food borne disease (e.g., *E. coli*, *Salmonella*, and *Campylobacter*), the cost of which can be very high. Some human pathogens

are carried asymptomatically in the intestine of healthy animals. For example, the prevalence of *Campylobacter jejuni* and *Salmonella typhimurium*, which are not pathogenic to animals such as broilers and pigs, can range from 0 to 100% as reported by Mulder and Schlundt (1999) who summarized numerous studies conducted in different countries. Mead (2000) also reviewed *Salmonella* contamination in fresh poultry meat in Germany, India, the Netherlands, the UK, and the USA between 1990-1994 and indicated levels varying from 4 to 100%. Other examples of common food borne pathogens that can be present in meat include *Clostridium perfringens* (i.e., a common intestinal microorganism), and *Staphylococcus aureus* (i.e., carried on the skin of animals, including humans).

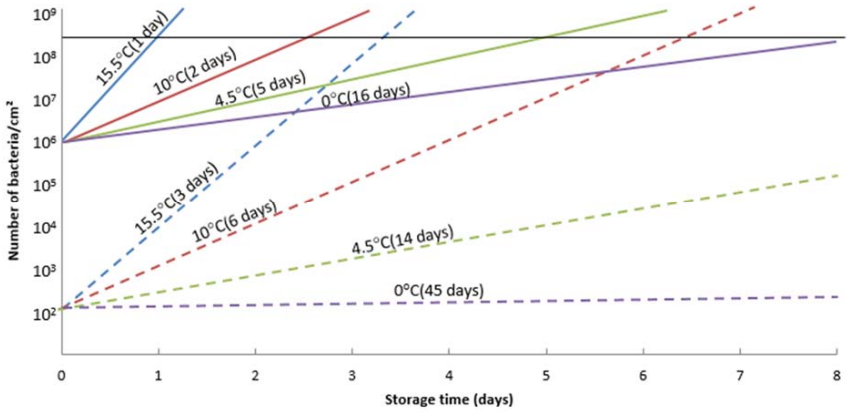

Figure 15.2.1 Effect of storage time, temperature and contamination level on time required for the spoilage of frankfurters (contaminated with high and low levels of psychrophilic bacteria). Spoilage detection (by slime formation) was at a population level of 150 million bacteria/cm^2 of surface area. The high level of contamination was 1 million/cm^2, shown by the solid lines; the low level was 100 bacteria/cm^2, shown by the broken lines. Redrawn from Zottola (1972).

The modern food/meat industry uses different interventions (physical, chemical, and biological) to minimize microbial contamination and multiplication that correspond with the varying modes of action of different bacteria. Food borne diseases are caused by microorganisms that invade the host and/or secrete toxins prior to or after their consumption. Invading microorganisms can cause gastrointestinal disturbances when they stay in the intestines or septicemia and other illnesses when they cross into the blood stream and reside in organs (e.g., *E. coli* O157:H7 in the kidney). It is important to note here that not all gastrointestinal

disturbances are caused by microorganisms, but can also result from overeating, allergic reactions, and chemical poisoning.

As indicated above, pathogenic bacteria can cause:

a. Infection resulting from the ingestion of pathogens that invade and grow inside the human body (e.g., *Campylobacter*, *Salmonella*)
b. Poisoning resulting from the ingestion of toxins. Toxins produced by non-invading microorganisms can be divided into exotoxins, which are secreted into the environment/food by bacteria such as *Clostridium botulinum*, and endotoxins, which are released upon the death of a microorganism

15.2.1 *Campylobacter jejuni*

Campylobacter jejuni is a Gram-negative, rod shaped (≈ 4 μm long and 0.3 μm wide), spiral curved, micro-aerophilic bacterium (Fig. 15.2.1.1) that can cause food borne infection. The infectious dose is fairly small; as few as 500 bacteria can cause illness. Symptoms include headache, fever, diarrhea, severe abdominal pain, and sometimes bloody stools.

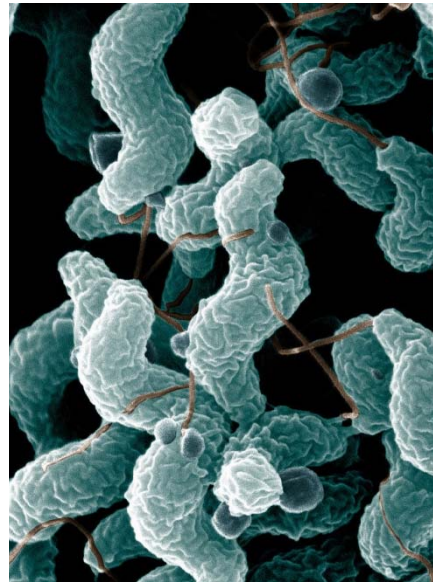

Figure 15.2.1.1 Morphology of *Campylobacter jejuni* and *E. coli* http://commons.wikimedia.org/wiki/file:ars_campylobacter_jejuni.jpg

C. jejuni does not usually multiply at temperatures < 30°C. It is found in the digestive tract of warm blooded animals (poultry, beef, pigs) and also in contaminated water (e.g., by sewage). Cross-contamination of carcasses during processing and handling can be a challenge (Guerin et al., 2010). Data from sporadic cases suggest that handling, preparation, and the consumption of undercooked meat including poultry are particular risk factors. In the US, broiler chicken, Cornish game hen, and, to a lesser extent, turkey meat consumption has been linked to human campylobacteriosis. In most countries, *C. jejuni* is common in poultry meat and up to 100% of flocks may carry it. Counts from poultry skin can exceed 10^4 colony forming units (CFU) per gram, but levels of contamination are often reduced during processing to about 10^1 CFU/g or 10^3 to 10^5 per carcass. In poultry, the bacteria can be isolated from wings, thighs, breast meat, and abdominal cavities, which indicates a general carcass distribution. Some reports show a lower contamination rate for frozen versus chilled poultry as freezing can injure the bacteria. However, other reports suggest this may also be influenced by the method of isolation and whether or not damaged cells were recovered. *C. jejuni* can also be controlled by cooking since it is a heat-sensitive bacterium; normal cooking temperatures are sufficient to destroy it.

Between 2004 and 2008 campylobacteriosis was the most frequently reported zoonotic disease in the European Union and fresh poultry meat was one of the most important reservoirs of human infection (Pasquali et al., 2011). When it comes to live bird/pre-harvest control, the European Food Safety Association (EFSA, 2010) believes that reduction of *Campylobacter* prevalence and load in live poultry is one of the most effective ways of reducing the contamination of foodstuffs and the number of human *Campylobacter* cases. Current pre-harvest strategies available to reduce *Campylobacter* contamination in poultry production include the application of on-farm biosecurity measures, litter decontamination, feed supplementation with compounds that inhibit *Campylobacter*, and drinking water treatment (see additional discussion below about competitive exclusion). Moreover, novel strategies that specifically target *Campylobacter* control at the pre-harvest level include probiotic administration, vaccination, antibiotics, and antimicrobial alternatives (e.g., bacteriophages, bacteriocins).

15.2.2 *Salmonella*

Salmonella is a Gram-negative, facultative-anaerobic, rod shaped bacterium (Fig. 15.2.2.1) that is non-spore forming and found in both warm and cold blooded animals as well as the environment. It is a motile enterobacterium that is about 1 x 2-5 μm in size, with a peritrichous flagellum. *Salmonella* is one of the most commonly cited bacteria in reference to poultry consumption but it is also a concern

in red meat (e.g., pork). As with other bacteria, some strains are pathogenic to humans (e.g., *S. typhi*) but not to other animals. Salmonellosis is the name for the infection that can result from ingestion of any of about 2,600 serovars.

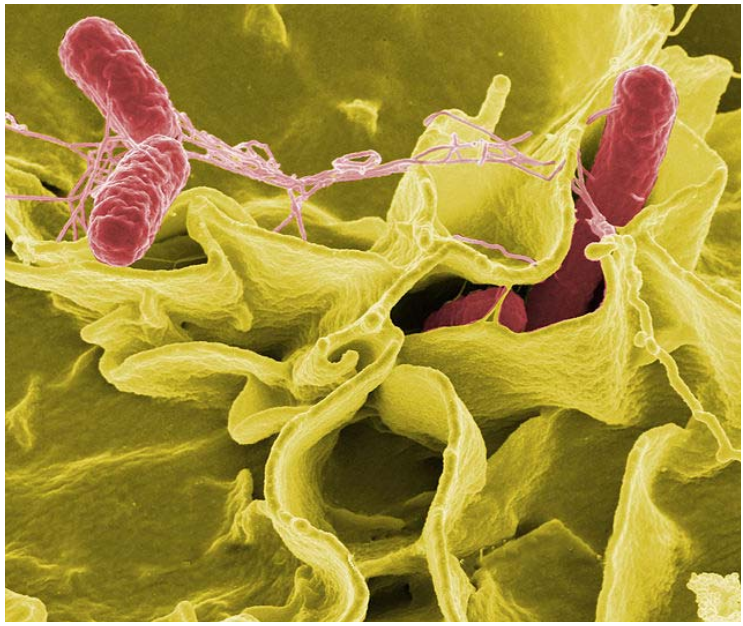

Figure 15.2.2.1 Morphology of *Salmonella*. From Wikipedia. Credit: Rocky Mountain Laboratories, NIAID, NIH. http://en.wikipedia.org/wiki/Bacteria

In the past microbiologists treated each serovar as if it were a separate species; however, significant changes in taxonomy have grouped all *Salmonella* serovars into two species: *S. enterica* and *S. bongori*. These are further divided into six subspecies or groups, most of which are classified under *S. enterica* (Jay et al., 2005). Only some serovars are associated with salmonellosis, which is caused by endotoxins released by bacteria ingested by the host. The common symptoms consist of nausea, vomiting, diarrhea (defense mechanisms of the body to quickly remove an infectious material from the body), fever, and abdominal pain. Symptoms usually appear 6 – 24 hr after consumption of contaminated food. The infectious dose is approximately 10^6 organisms for a healthy adult but would be lower for individuals who are very young (e.g., 10^3 of virulent strains), old, or have a compromised immune system. Mortality from salmonellosis is generally low (see discussion in Section 15.1), with fatalities occurring in infants (< 5 years old), the

elderly, or people who are already affected by other diseases. Because *Salmonella* can be present in animals' digestive tracts without causing symptoms, a major cause of transfer to the meat results from cross-contamination of carcasses during evisceration and chilling. Later, cross-contamination of raw and cooked food can also be a significant problem. *Salmonella* is a fairly heat sensitive bacteria and cooking procedures for meat are designed to sufficiently destroy most *Salmonella* serovars (e.g., 1 min at 65.0°C, 5 min at 62.2°C, 12 min at 60.0°C and 37 min at 57.2°C). Most cooking guidelines for poultry and red meat specify a minimum internal temperature of 70°C, which results in inactivation within a few seconds.

The industry is also working on preventative measures during the growing stage by using procedures such as competitive exclusion (Kerr et al., 2013; see later discussion in the chapter) and vaccination (Bohez et al., 2008), and during processing through procedures such as carcass decontamination with chlorine/acid washes (see further discussion below). Overall, controlling *Salmonella* in the live animal is becoming increasingly important (e.g., antibiotic resistant strains). Domestic poultry may acquire *Salmonella* from three main sources: parents/breeding stocks; the environment (contact with wild birds, mice); and consuming contaminated feed. During hatching, a few contaminated eggs can spread the bacteria. Later, on the farm, birds that carry *Salmonella* shed the organism, which can then be spread via drinking water, feed, or litter. Breeding flocks are routinely scanned for *Salmonella* and are either treated through antibiotics or competitive exclusion or culled when pathogenic strains are detected. Tests are also conducted in some growing farms (e.g., Europe) and problematic flocks are culled.

15.2.3 *Clostridium perfringens*

Clostridium perfringens is a Gram-positive, anaerobic, rod shaped, spore-forming, aerobic bacterium (Fig. 15.2.3.1) that can cause infection in humans. It is found widely in nature in soil, water, and the intestinal tracts of animals and humans. It can produce a variety of toxins and large volumes of gas when exposed to typical human body conditions. The infectious dose is large, $> 10^8$ vegetative cells, for a healthy host. Symptoms are relatively mild and include nausea, diarrhea, occasional vomiting, and abdominal pain that appear within 24 hr of consuming the contaminated food. Time to infection is relatively short because the toxins are already present in the food. Typical epidemic curves for the onset of *C. perfringens* gastroenteritis symptoms show time intervals between 1-24 hr with an average time of 11-13 hr. Variation among individuals can be the result of serving size, other foods consumed, sensitivity of the individual, etc. These times are important for physicians trying to diagnose and set up a treatment plan, as it can take a few days to culture a sample and identify the toxin.

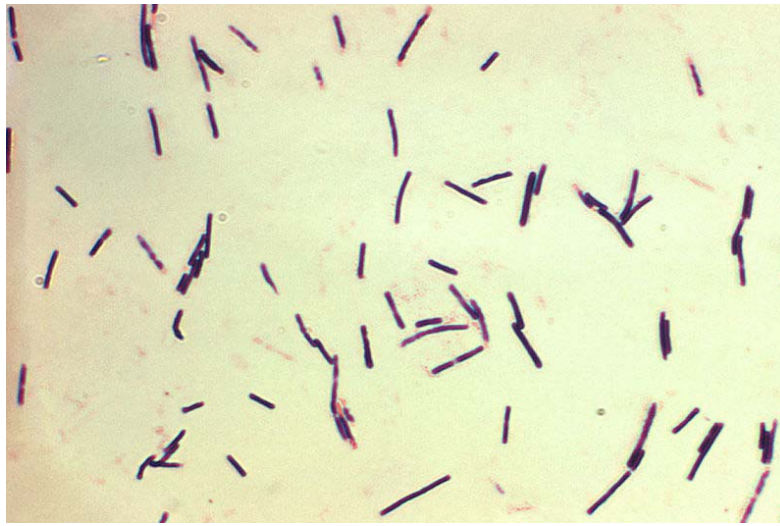

Figure 15.2.3.1 Morphology of *Clostridium perfringens*. From CDC. http://phil.cdc.gov/phil/home.asp

Risk reductions for *C. perfringens* infection focuses on fast chilling of cooked meat and other food products. Adequate refrigeration, especially of leftovers (i.e., best in small containers to achieve rapid cooling), and good sanitation are essential. *C. perfringens* is a special food safety concern because of its ability to form spores. During cooking most non-spore forming microorganisms are destroyed. If the food is cooled slowly this allows *C. perfringens* spores the chance to germinate with little or no competition. When foods are held on a steam table, temperatures should be kept above 60°C. In addition, when leftover foods are reheated, a thorough heating can help destroy the organism and its toxins.

15.2.4 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, non-spore-forming, catalase positive, rod shaped bacterium (Fig. 15.2.4.1). There are 11 species of *Listeria* and 17 serovars that are recognized by the antigen present. The primary pathogenic species, *L. monocytogenes*, is represented by 13 serovars. *Listeria* can produce lactic acid from glucose and other fermentable sugars and tends to be associated with bacteria such as *Lactobacillus* that produce lactate. The nutritional requirements are typical of those of other Gram-positive bacteria and they can grow in many common laboratory media. Although they grow best at pH 6 - 8, some strains, including *L. monocytogenes*, can grow over a pH range of 4.1 - 9.6 (Jay et al., 2005). *Listeria*

can also grow at refrigeration temperatures, with mean minimum growth at temperatures as low as 1°C. This makes it a special challenge for the food industry both in processing environments and in marketing products. The symptoms associated with listeriosis include fever, nausea, headache, vomiting, and, in severe cases, meningitis (bacteria gets into the nervous system), miscarriage in pregnant women, and septicemia. Symptoms usually appear within 1-4 weeks, but may appear more than 10 weeks later depending on the severity of the infection.

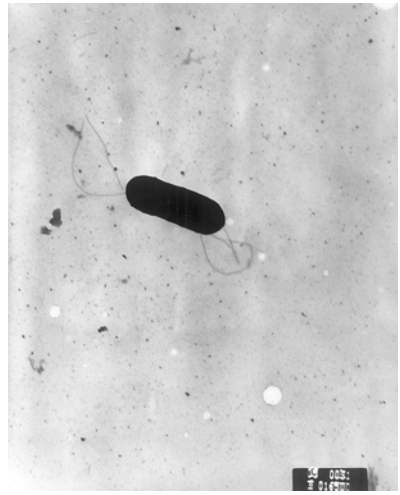

Figure 15.2.4.1 Morphology of *Listeria monocytogenes*. From CDC. http://phil.cdc.gov/phil/home.asp

Overall, *Listeria* is widely distributed in the environment and can be found in animal feces and in decaying organic material in soils, water, and sewage. Several reports have shown that a significant number of samples taken from the shoes of people living in a big city are *Listeria* positive. It is pretty well established that fresh foods of animal or plant origin may contain varying numbers of *L. monocytogenes*. Mead (2000) indicated that about 60% of raw chicken carcasses carry the organism in low numbers. The good news is that the bacteria is heat sensitive and is destroyed by normal cooking procedures (note: some countries require a time x temperature combination to achieve a 9 log reduction of *Listeria* in cooked products). It has been shown that a milk pasteurization protocol of 62.8°C for 30 min or 72°C for 15 sec is adequate to reduce normal population sizes to below detectable levels. In the past, cooked luncheon meat, chicken nuggets, and

cook-chill meats have been implicated in sporadic cases of listeriosis as a result of cross-contamination after cooking. In the US, a large recall of turkey hot dogs occurred after a multistate outbreak of listeriosis. This outbreak later changed the government's approach to food inspection. The bacteria was later isolated and identified (by DNA typing) in patient samples and hot dogs kept in the patients' refrigerators. Finding the outbreak source is an important part of identifying and correcting the problem. It can be a challenge to identify the causative agent, especially when symptoms appear weeks after the original infection took place. Most of us do not remember what we ate last week let alone last month and most opened packages are not stored long.

15.2.5 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, facultative anaerobic, coccus shaped bacterium (Fig. 15.2.5.1) that can cause food poisoning by ingestion of the exotoxins produced in the food prior to consumption. Some of the exotoxins act as enterotoxins in the host and cause an inflammation of the stomach and intestinal lining (called gastroenteritis). Usually, the infectious dose is high (10^5 - 10^6 growing cells) and, as with other food poisonings, the individual's age, state of health, and other illnesses affect the response. Symptoms can include nausea, abdominal cramps, vomiting, and possible diarrhea. The toxins affect the central nervous system and can also act as an antigen that triggers massive immune response, but mortality is low. If mortality does occur, the patient usually has a so-called co-morbidity. The organism is widely spread in nature (including on the skin of humans and other animals) and can be isolated from many healthy individuals. Therefore, food handling by infected people is one of the greatest sources of *Staphylococci* food poisoning and it is one of the most commonly reported food borne diseases in North America. Overall, the microorganism is a poor competitor that favours body temperature. Under favourable conditions, it will multiply to great numbers without significantly altering the flavour, colour, or smell of the food. Toxin production is most rapid around 20°C in foods with fairly neutral pH. However, the microorganism can grow at temperatures between 7 and 45°C. Foods associated with *Staphylococci* food poisoning usually include high protein foods (e.g., dairy, meat, custard and cream filled pastries) and foods that are handled frequently during preparation. While the microorganism is quickly destroyed by heat (e.g., 66°C for 12 min and < 1 min at 100°C), the enterotoxins require severe heat treatment for destruction (e.g., 121°C for 30 min).

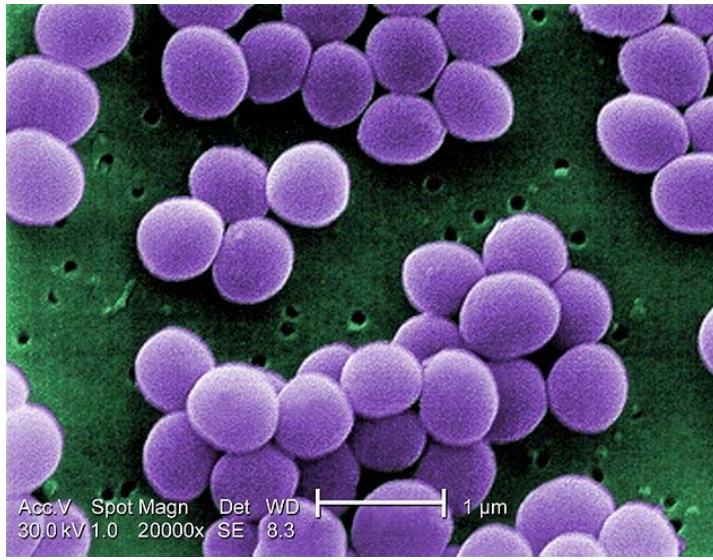

Figure 15.2.5.1 Morphology of *Staphylococcus aureus*.
From CDC. http://phil.cdc.gov/phil/home.asp

15.2.6 *Clostridium botulinum*

Clostridium botulinum is a Gram-positive, rod shaped, spore-forming, anaerobic bacterium (Fig. 15.2.6.1) that can cause food poisoning by a deadly toxin produced in the food during storage. The bacteria form spores that make them resistant to conventional cooking (i.e., in a commercial canning operation a temperature of 121°C is commonly used). *C. botulinum* is found primarily in soil and debris stuck to feathers and skin. The toxin is one of the most potent toxins known and nanogram quantities are extremely dangerous. It affects the central nervous system by blocking the chemical messenger acetylcholine (see Chapter 3) that is used to transfer messages among nerve and muscle fibers. By doing so, the toxin effectively blocks messages from the brain. Initial symptoms include impaired vision, speaking, and breathing as communication between the brain and peripheral organs is interrupted. Respiratory system failure is the first major danger to the victim, and therefore one of the most important initial treatments is breathing assistance where victims are placed in a pressure chamber used to treat diving accidents. The toxin is activated by the enzyme trypsin in the host's digestive system prior to being absorbed into the blood stream. Historically, improperly prepared, low to medium acidity home-canned vegetables, fruits, and meat constituted the largest potential source of botulism. Because the bacteria are

anaerobic, canned and vacuum packaged foods, including meat, are a potential medium for growth. In general, the prevalence of *C. botulinum* spores in meat is fairly low and poisoning from canned or vacuum packaged meats is rare. Severe heat treatments (e.g., 121°C, as used in the canning operation) are not common in meat products so the industry uses nitrite (a chemical) to inactivate the spores. This is an effective way of preventing spore growth in vacuum packed meat products (see Chapter 11). The toxin itself is relatively heat labile (inactivated at 85°C for 15 min, or 100°C for 1 min) while the spores are quite resistant to heat as indicated above. Other means of growth suppression are decreased water activity (< 0.94), low pH (< 4.65), added salt (> 10%), and refrigeration temperatures. Most *C. botulinum* serovars will not grow below 7°C. Canned products that show swelling due to gas produced by microbial activity should always be discarded as they indicate that some microorganisms (potentially including *C. botulinum*) survived the heat treatment.

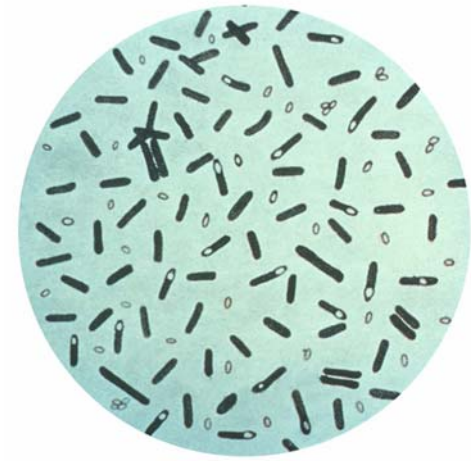

Figure 15.2.6.1 Morphology of *Clostridium botulinum*.
From CDC. http://phil.cdc.gov/phil/home.asp

15.2.7 *Escherichia coli*

Escherichia coli is a Gram-negative, rod shaped facultative anaerobic bacterium (Fig. 15.2.7.1) that can be found in great numbers in the intestinal tract of healthy animals and humans, as well as in soil, water, and on the surfaces of fruits and vegetables. *E. coli* was established as a food borne pathogen in 1971 when cheese imported to the US was found to be contaminated with an entero-hemorrhagic

strain that caused illness in a few hundred people. Prior to that, at least five outbreaks had been reported in other countries, with the earliest being in the UK in 1947. However, evidence suggests that *E. coli* was recognized as a source of infant diarrhea as early as 1700. The six pathogenic strains of *Escherichia* are serologically typed in the same way as other members of the *Enterobacteriaceae* family. Over 200 "O" serovars (which indicate the presence of a somatic antigen) have been recognized. "H" antigenic types, which indicate the presence of a flagellar antigen, are also used for identification (Jay et al., 2005).

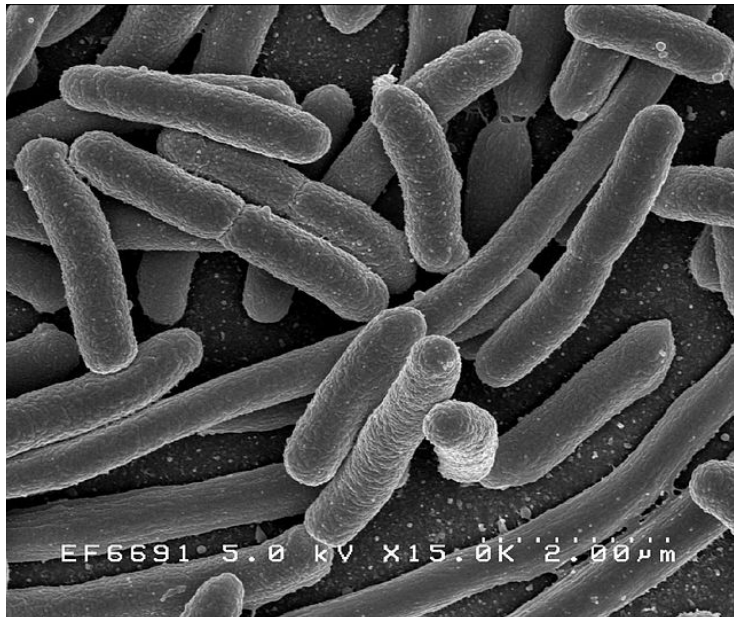

Figure 15.2.7.1 Morphology of *Escherichia coli*. Credit: Rocky Mountain Laboratories. From Wikipedia. http://en.wikipedia.org/wiki/Bacteria

There are four major types of pathogenic *E. coli*, but only the first two types are major causes of foodborne illness in North America:

a. Enterotoxigenic *E. coli* (ETEC) – associated with diarrhea and dehydration. A known cause of "traveller's diarrhea", which usually lasts a few days (note: host specific).
b. Enterohemorrhagic *E. coli* (EHEC) – e.g. *E. coli* O157:H7, where a low number of cells (10^1 – 10^3) can cause infection by penetrating the

intestinal wall and colonizing organs such as the kidney. Symptoms include abdominal pain, bloody diarrhea, vomiting, and in severe cases, kidney failure. It is often associated with undercooked beef and is also known as "Hamburger disease".

c. Enteroinvasive *E. coli* (EIEC) – high dose is needed (10^8 cells) for infection. Symptoms include diarrhea, dehydration, and fever.
d. Enteropathogenic *E. coli* (EPEC) – high dose required and symptoms mainly include diarrhea.

The presence of high numbers of non-pathogenic *E. coli* in fresh meat indicates poor sanitary conditions during its processing and handling. In fact, the presence of *E. coli* is used as an indicator of fecal contamination in drinking water.

15.3 Growing and Live Haul – Microbial Considerations

Farm animals are not raised in a sterile environment and microorganisms can be found on floors, soil, equipment, feed, skin, feathers, and in high numbers in the animal's digestive system. Live animals entering a processing plant carry a natural, diverse microflora that is mostly not pathogenic to humans. The microflora reflects the normal growth of animals on litter floors, exposure to the natural environment, and contact with wildlife (litter beetles, mice, birds). However, animals can also carry several human pathogens such as *Salmonella* and *Campylobacter*. Only occasionally do young poultry show symptoms of *Salmonella* infection; i.e., in most cases they are only healthy carriers of this pathogen (Bilgili, 2010; Jay et al., 2005). In some countries, such as Sweden, comprehensive *Salmonella* eradication programs have been initiated. They include monitoring the birds starting at the grandparent breeder flock level and all the way to the growing farms. Positive breeder stocks are usually eradicated through special indemnity programs while regular positive flocks are either eradicated on the farm or slaughtered, at a processing plant, under special arrangements (e.g., at the end of the day). This is an expensive way to control *Salmonella* and most countries have not adopted all these practices. However, monitoring breeder stocks is a common practice around the world and infected flocks are medicated, vaccinated, or culled.

A newer approach to control *Salmonella* at the farm level is competitive exclusion (Garcia and Brufau, 2010). This approach recognizes that newly hatched chicks are susceptible to *Salmonella* because the hatcheries are strictly sanitized environments. Because chicks have no contact with parent birds, they are slow to develop an intestinal microflora that could successfully compete with ingested

pathogens. Nurmi and Rantala (1973) studied the effect of establishing an adult-type gut microflora in young chicks by orally dosing them with suspensions of anaerobic cultures of gut contents from adult, *Salmonella*-free poultry. The treated chicks became resistant to an oral challenge of about 10^6 *Salmonella* CFU/bird. The protective effect was not significantly influenced by breed, strain, or sex, but depended upon the introduction of viable bacteria, especially anaerobes (Mead, 2000; Garcia and Brufau, 2010). Over the years, attempts have been made to specifically identify and isolate protective bacteria and to develop defined cultures with known composition. However, these isolates were usually less protective than anaerobic cultures of gut content, and tended to lose their protective capability over time. A number of commercial preparations are based on undefined cultures of caecal material. Although these cultures are screened to ensure the absence of avian and human pathogens, the FDA currently does not allow marketing undefined cultures in the US. The preparations are used for newly hatched chicks that are spray inoculated in the hatchery. Birds are wetted on the upper part of the body and later ingest treatment organisms while preening themselves. The treatment can also be applied to older birds/breeding flocks that have been identified as *Salmonella* carriers. Treatment in this case is given after an antibiotic therapy usually delivered via the drinking water. Overall, competitive exclusion must always be combined with good husbandry hygiene because, while the protective flora is becoming established, the birds will remain susceptible to infection. It should be pointed out that the complex mechanism of protection is not fully understood and is likely influenced by factors such as gut pH and Eh, inhibitory substances such as H_2S and bacteriocins, and competition for receptor sites (Mead, 2000; Kerr et al., 2013).

Competitive exclusion is currently used in numerous places and shows no adverse effects on bird health or growth performance. Kerr et al. (2013) performed meta-analysis and meta-regression on 200 studies in this area and reported that a number of competitive exclusion products were effective in reducing *Salmonella* colonization in broilers. The most common route of administration was oral gavage (64% of trials), but spraying chicks at the hatchery was just as effective. Overall, this is a very important concept to help reduce/eliminate the use of antibiotics during livestock growing.

Other factors also play a role in the level of microbial contamination during the growing period. A partial list includes the cleanliness of the barn (e.g., in between flocks, during the growing season), barn conditions (e.g., relative humidity that affects litter drying), contact with wildlife (e.g., bugs, small birds, mice), and feed preparation (e.g., pelleting using heat can inactivate microorganisms). However, it is beyond the scope of this book to cover all of these husbandry factors.

Live haul of birds starts with feed and water withdrawal (see Chapter 4) prior to catching and transporting the birds to a processing plant. The catching operation may be manual or mechanized and the transport crates/cages may be wood (hard to clean), metal, or plastic as described in Chapter 4. Minimizing stress during the loading and unloading operations is also an important step in reducing cross-contamination among birds. Stress during transport is known to cause changes in excretion patterns due to the disturbance of intestinal function. Excretion of pathogens, such as *Campylobacter* and *Salmonella*, will result in increased cross-contamination between birds in the same cage and possibly in the cages below if cage design does not prevent fallout of fecal material while allowing adequate ventilation.

During transportation animals/birds are placed in a new environment that consists of unfamiliar territory as well as different stresses (vibration, noise, wind, lack of food). A detailed description of the effect of factors such as travel time and temperature on live birds is provided in Chapter 4.

Cages should be cleaned and sanitized after each shipment to stop cross-contamination between flocks and farms. Rigby et al. (1980) and others showed that unclean transportation cages (from a previous batch) can transfer *Salmonella* to the next load. Jones et al. (1991) indicated that transportation does not necessarily result in uniformly increasing the frequency of *Campylobacter* contamination throughout the flock. As compared to wooden crates, cleaning and sanitation are much easier when plastic or metal cages are used because the surface is smoother. Modern poultry processing plants usually have an automated system for cleaning and sanitizing the crates. Proper cleaning should include the physical removal of visible dirt (feathers, manure) by scraping or a high pressure water jet, cleaning with chemical detergents, proper rinsing, and a final disinfection step (e.g., chlorine) should only be applied to a clean surface. The effectiveness of the cleaning procedure should be verified (see HACCP; Chapter 6).

15.4 Primary Processing – Microbial Considerations

Overall, the operations inside a poultry processing plant are complex (see Chapter 5) and usually performed at high speed (see Chapter 1). If done inappropriately, these operations can result in a high rate of cross-contamination. Cross-contamination may result from repeated contact with processing equipment, bird to bird contact, use of a common water bath for scalding and chilling, meat handling by employees, and contact with tools such as knives. Good manufacturing practices, hygienic

equipment design (see end of this chapter), and an adequate HACCP program can help reduce cross-contamination and improve the product's safety and shelf life. As in other food processing operations (dairy, fruits and vegetables), people working in the plant should be educated and work to minimize/eliminate contamination. Part of this training is included in the HACCP pre-requisite program (see Chapters 6 and 12), which includes instructions to wear clean clothes, hairnets, and use hand washing stations. It is important that employees recognize that animals arriving at the plant carry microorganisms on their skin (including soil, fecal material attached to hair/feathers) and inside their intestinal tracts (high numbers of 10^8-10^9 CFU/gram) and respiratory systems.

Regulatory guidelines dealing with specific meat pathogens differ among countries, but there has been a general trend of increasing regulations over the years (Barbut and Pronk, 2014; EFSA, 2010). This has required processors to employ more/new intervention strategies and to better understand the whole process. There appears to be more emphasis on physical decontamination methods in Europe while North American countries focus on both chemical (e.g., chlorine) and physical (e.g., hot water) decontamination methods. An example of the evolution of standards is the previous American *Salmonella* standard (USDA-FSIS, 1996), which required prevalence of less than 20% (12 positive out of 51 samples). Later, pathogen reduction data (collected during the USDA national baseline studies) became the driving force for updated performance standards. The new standards (USDA-FSIS, 2011a) include both *Salmonella* and *Campylobacter* (first time) and require prevalence of less than 7.5% (5 positive out of 51 samples) for *Salmonella* and 10.4% for *Campylobacter*.

15.4.1 Unloading

Unloading is the process of moving live birds from the transport cages to the shackle line. This can be done manually or semi-automatically with conscious or unconscious birds (i.e., after gas stunning; see Chapter 8). At this stage, bird stress and/or struggling can extract fecal material which can result in cross-contamination, so it is important to minimize stress.

15.4.2 Stunning and Bleeding

The stunning operation renders the animal unconscious prior to bleeding. Electrical stunning usually results in muscle contraction that can extract fecal material. This obviously depends on the strength of the current (voltage and frequency) used. Gas stunning can also result in convulsions (especially anoxia conditions). In both cases care should be taken to prevent/minimize cross-contamination.

Bleeding results from opening the neck blood vessels mechanically or manually. In both cases, microorganisms can be transferred from the skin and feathers via the knife/blade to the blood stream that, in healthy birds, is virtually free of microorganisms. Even though most blood flows outward, some returning blood can deposit microorganisms in muscles and other organs. Therefore, care should be taken to maintain the cleanliness of the operation. Also, peri-mortem defecation is commonly observed at the end of the bleeding period.

15.4.3 Scalding

Scalding is used to loosen the feathers and facilitate their removal in the next plucking stage. Traditionally, the process is done in a scalding tank that consists of one or a series of water baths at 50-63°C (see soft, medium, and hard scalding in Chapter 5). Newer technologies that use steam (called Aeroscalding) to deliver heat to the feather follicles are also being used. This system eliminates a common bath, which decreases the risk of cross-contamination and significantly reduces water consumption (claimed to be 70% lower). In both water and steam scalding, temperature and time (e.g., 1-3 min) affect the amount of epidermis left on the skin as well as the number of microorganisms present on the skin. Several researchers have reported that scald water in a conventional water bath contains an aerobic bacterial count of about 50,000 microorganisms per ml. Usually, an initial increase is seen at the start of the day, but later the number stabilizes and remains relatively constant throughout the day (Bailey et al., 1987; Young and Northcutt, 2000). Although the external surfaces of the incoming carcasses are contaminated, the number of microorganisms usually stays fairly constant as a result of heat inactivation for some, the introduction of fresh, clean water (requirements depend on the country), the continuous overflow of contaminated scald water, and the use of antimicrobial agents (where permitted). The water bath represents an opportunity for cross-contamination, but usually does not result in a significant difference in the nature or degree of contamination in birds from the same flock. Total bacterial count on broiler skin just after scalding is usually less than 10,000 CFU/cm^2 (Bailey et al., 1987). Some of the heat sensitive bacteria, such as *Salmonella* and *Campylobacter*, are more affected by the scalding process (NACMCF, 1997) and are less likely to be isolated from a hard scald operation. Cason et al. (2000) reviewed 10 studies and found different levels of *Salmonella* contamination in water samples from the scalding tanks; four tanks had none, three had 1-10% positives, two had 20-40%, and one tank had 100%. Companies that design scalders have developed several modifications to improve the situation: a counter-current flow design (clean water flows from the bird's exit point towards the entrance point), a multistage scalding system, and the addition of fresh, clean water for every new bird. A counter-current flow has been shown to be effective

and, together with a multistage scalding system (consisting of several tanks), it was reported to significantly reduce the aerobic counts on broiler carcasses (Cason et al., 1999, 2000). By using a series of three tanks (Fig. 15.4.3.1), the processor can achieve a sequential reduction in bacterial load (Fig. 15.4.3.2). Mean scald water temperatures were 55.8, 55.9 and 56.2°C for tanks 1, 2 and 3, respectively and the mean coliform concentration (8 sampling days after 6 week old broilers were processed for 8 hr) was reduced from 3.4 to 2.0 and 1.2 log10 CFU/ml, respectively. *Salmonella* was isolated from 7 of 8 water samples from tanks 1 and 2, but only 2 of 8 samples from the last tank (3). The average number of *Salmonella* (over 8 testing days) was reduced from tank 1 to 3. Their previous study also demonstrated a successive cleaning effect, where suspended organic and inorganic solids and aerobic counts were reduced from the first to the last tank (5.12 to 1.04 g/L and 4.61 to 3.85 log10 CFU/ml, respectively; Cason et al. 1999).

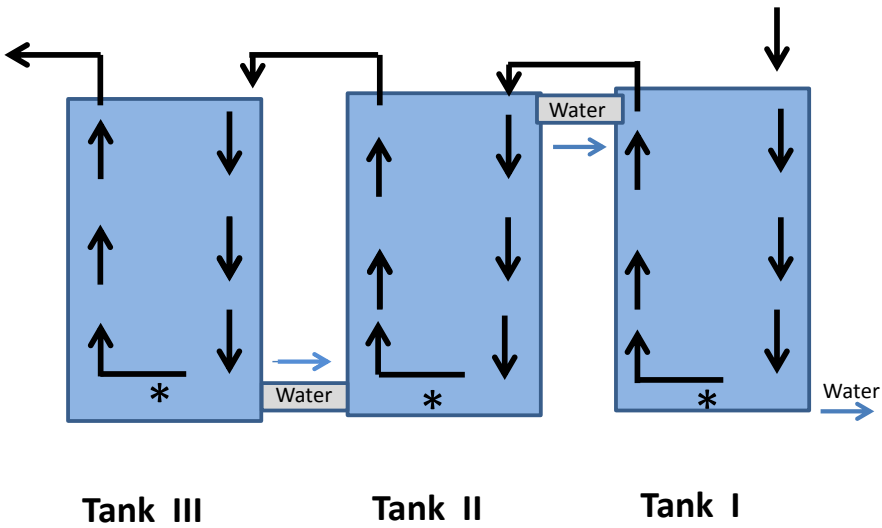

Figure 15.4.3.1 Diagram of the three-tank, two-pass, counterflow scalder as seen from above. Movement of broiler carcasses through the scalder is shown by the large arrows. Potable water is added to tank 3 and flows by gravity through tank 2 and tank 1. The sampling point in each tank is marked with an asterisk. From Cason et al. (2000).

James et al. (1992) suggested an addition to the counter-current flow tank in the form of a post-scald hot water rinse cabinet (240 mL of 60°C water spray at 40 psi/bird). The water is then collected and sent to the scalder. They showed a reduction

in aerobic and *Enterobacteriaceae* counts at the pre-chill station (after scalding) over their 7-day study period. However, the percentage of *Salmonella*-positive carcasses increased slightly due to cross-contamination in the scalder. Overall, they reported that scalding resulted in a lower microbial load in both the modified and baseline processes. Psychrotrophic bacteria, those that prefer cooler temperatures, are commonly present on the skin, feathers, and feet of live birds. The most common genera include *Achromobacter*, *Corynebacterium*, and *Flavobacterium*. Their numbers usually decrease after scalding.

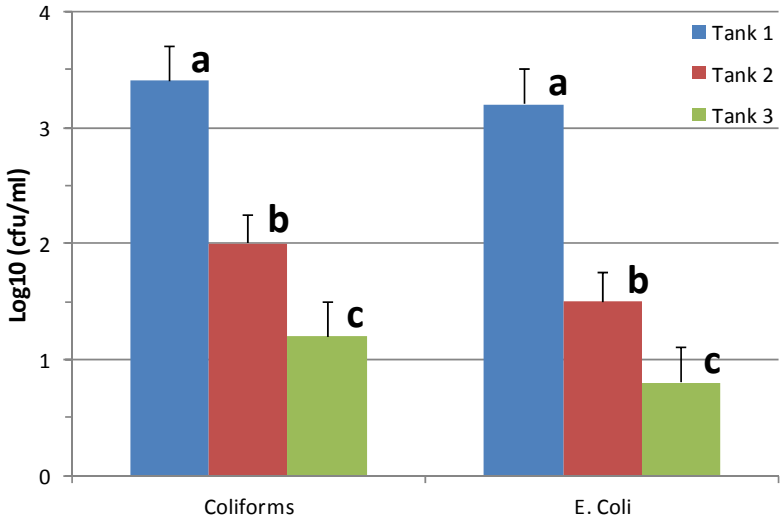

Figure 15.4.3.2 Mean counts (log10[CFU/ml] ± SD) of coliforms and *E. coli* in water samples from a three-tank, counterflow scalder operating in a broiler processing plant. Bars labelled with different letters are significantly different ($P < 0.05$, $n = 8$ for each bar). Adapted from Cason et al. (2000).

Sometimes there is also a potential for scald water to enter the trachea, which might contaminate the lungs. Such contamination is decreased when the Kosher cut is used, the bleed time exceeds 2 min, and the birds are electrically stunned (Bailey et al., 1987).

Several reports, but not all, indicate that high scald temperatures (> 58°C) reduce shelf life of the bird. This may be associated with the degree to which the epidermal layer is removed as higher temperatures can remove more of the cuticle

layer during subsequent mechanical feather picking (rubbing action of the fingers). On the other hand, semi-scalding (around 52°C) does not damage the cuticle (see Chapter 3 for the different skin layers). It is possible that cuticle removal improves the skin's suitability as a substrate for spoilage microorganisms (e.g., *Pseudomonas*). When a hard scald (> 58°C) is used, the skin must be kept moist to prevent discolouration. Therefore, processors usually use water rather than air chilling for these birds. When soft-scalding (52-54°C) is used, the skin can dry more without discolouration and therefore air chilling can be used.

15.4.4 Feather Removal (Picking)

The picking operation is used to remove/rub off the feathers. It is usually a fully automated process. Manual and batch-type (i.e., carcasses are placed in a rotating drum, see Chapter 4) operations are not commonly used in large processing plants, but can represent the same microbiological challenges as with automated lines. One problem is potential cross-contamination caused by the rubber fingers (a few thousand in a fast speed line; see photo in Chapter 5) that contact each passing bird. The conditions within the plucker (high humidity and warm temperature) are also favourable for some microbial growth. Mead and Scott (1994) colonized the defeathering equipment with a marker microorganism and showed that the level of cross-contamination increased with each subsequent carcass. Worn or cracked rubber fingers can allow bacteria to penetrate below the surface, where they are more protected against cleaning and sanitizing compounds. It has been reported that plucking can result in higher numbers of non-psychrotrophic bacteria and pathogens (NACMCF, 1997). *S. aureus* has been identified as one of the bacteria that best flourish under these conditions (Mead, 2000). This is of interest because this bacterium is not noted for its ability to compete with other microorganisms. Nevertheless, it has been shown to persist in defeathering equipment for months when routine cleaning is not effective. Pathogens may be transferred to the birds and enter into the feather follicles as the feathers are removed and before the openings are closed. Clouser et al. (1995) observed significant *Salmonella* cross-contamination during conventional defeathering in a turkey operation when a series of four pickers was used (after a 58°C scalding operation for 1.3 min). When they studied another plant that used a steam-spray scalding and defeathering operation they found no significant increase in *Salmonella* level before and after defeathering. Turkeys with a high initial bacterial load (> 10^4 CFU) showed a load reduction between bleeding and chilling. Carcasses with lower counts (< 10^4 CFU) were cleaner initially but did not show lower total counts at the end of the process.

Most, if not all, poultry operations position a spray washer to rinse the carcass just after plucking. This helps remove loose feathers, debris, and some bacteria

present in the water film on the bird's surface. The concept of removing bacteria and maintaining a water film (i.e., not allowing the skin surface to dry) before bacteria have a chance to attach to the skin is getting a lot of attention today from academics and industry personnel. A constant water spray (chlorinated or not) during the defeathering operation has also been shown to help reduce or prevent bacteria from colonizing the equipment. However, caution must be exercised when a water spray is used, as aerosol droplets from the high speed finger rotation can transmit bacteria to other plant locations. Therefore, most plants use covers around the equipment to minimize aerosol particles and also reduce noise. In any case, this step should be given attention and should be well contained. Many processors today position the equipment in a separate room (e.g., build walls around the pluckers).

In waterfowl operations (e.g., duck, geese) feather removal is more complicated. The birds are scalded at 60°C, plucked by machine, and then immersed in molten wax at 90°C to trap the small feathers. To speed up cooling and harden the wax, the birds are immersed in cold water and then the cold wax is stripped from the birds by hand or machine. The wax is later melted, filtered, and reused. Mead (2000) reported that the high temperature treatment appears to have a beneficial effect on the microbial quality of the final product, as water-chilled ducks treated with wax usually carry very low numbers of coliform bacteria on their skin.

15.4.5 Evisceration

The evisceration process consists of opening the abdominal cavity and removing the digestive system, heart, and lungs. In small plants, the operation may be carried out manually while in high volume plants the entire process is automated (see Chapter 5) by using equipment to perform individual tasks (e.g., vent cutting, opening, drawing). Automated equipment is used to perform labour intensive, repetitive motions in a fast and efficient manner. In both manual and automated operations there is potential for contamination if the digestive tract is ruptured and contents leak on the equipment and/or other carcasses. Ruptures may occur due to an incorrect cut by an employee (e.g., manual operation), poor equipment adjustment, or the condition of the birds (e.g., time of feed withdrawal affects the fullness of the gut, disease conditions; see Chapter 4). As indicated in Chapters 1 and 5, modern processing lines handle over 13,000 broilers per hour and the evisceration process is performed quickly, so adjustment of machinery can be an issue. Investing in high quality equipment designed to minimize cross-contamination (e.g., application of a vacuum to the cloaca while automatically pulling it out) is very important and offers a great return on investment. A well adjusted, automated system can help minimize potential bird-to-bird cross-contamination problems. There is also focus

today on in-line continuous cleaning where equipment is commonly fitted with a cleaning-in-place (CIP) system with or without the application of a sanitizer. Similarly, in manual operations employees should wash their hands often and/or dip their knives in hot water.

The opening process usually includes three steps. The first is cutting out the cloaca (known as vent cutting) without separating the attached large intestine. In an automated process this is done using a cylindrical rotary blade (in some cases vacuum and pressure are applied to the area in order to empty the distal end). The next step is opening the abdominal cavity. Care should be taken to prevent rupturing the intestine and spilling the gut content, since 1 mL may contain up to 10^9 CFU (i.e., a small volume can result in a high contamination level). Height adjustment of the equipment is required when the size of the bird changes (e.g., new flock arrives) and/or when high variation among birds within the same flock is expected. The equipment must be monitored and adjusted on a continuous basis. After the body cavity is opened, a scoop is used to draw out the intestines, giblets, heart, and lungs. The pack is either left attached to the carcass or separated from the carcass and hung on a separate line for inspection (see Chapter 5). The latter system was introduced about 20 years ago to improve microbial quality by reducing cross-contamination (i.e., the intestines no longer attached to the carcass). Another improvement in reducing cross-contamination was the introduction of automated carcass transfer systems (e.g., from the defeathering line to the evisceration line, and later also to the chilling line; see Chapter 5). This reduces handling/touching by the plant's employees, and cross-contamination as a result of carcass accumulation (dumping) at the transfer point.

15.4.6 Cropping

Crop removal also presents a potential contamination point. Hargis et al. (1995) indicated that there is an 80 times greater risk of carcass contamination from crop rupturing than from removing the intestinal tract and caecum. They also mentioned that *Salmonella* is more readily extracted from the crop than the caecum. Fifty-two percent of the 500 bird samples were positive for *Salmonella* in the crop, but only 15% were positive in the caecum. They later reported similar trends for *Campylobacter* (60% vs. 4%, respectively) and emphasized that special care should be taken when the crop is removed. Therefore this processing station should be maintained clean (e.g., by continuous spray washing) to minimize cross contamination among birds.

15.4.7 Washing and Other Interventions

Carcasses are often rinsed after the evisceration process to clear away any debris, loose tissue, residual blood, and some microorganisms (Notermans et al., 1980; EFSA, 2010). This is done with low/high pressure nozzles and inside/outside bird washers. Over the past few decades, various antimicrobial rinses have also been suggested (see below) as demand for lower microbial counts increased (USDA-FSIS, 1996; USDA-FSIS, 2011a and b; Barbut and Pronk, 2014). Chlorine, for example, has been used in spray water at a concentration of 20-50 ppm (Mead, 2000). As indicated earlier, the use of chlorine in processing water is not permitted in some countries (e.g., Europe). In the case of water, there are usually no regulations as to the amount or pressure used in the washer. Overall, the use of washers throughout primary processing has been shown to be effective in removing unattached microorganisms (Notermans et al., 1980). As indicated before, this is important in maintaining the water film on the skin as well as washing off some of the bacteria. Various pieces of equipment have been designed to perform outside and/or inside washes of poultry carcasses (see Chapter 5). Some of the old washers consist of a set of showerheads, while the newer washers are designed to wash the outside of the bird from top to bottom (e.g., washers are positioned at different heights along the line). Other inside/outside washers include a shaft equipped with spray nozzles that can be lowered into the body cavity and spray in a way that ensures effective cleaning. The water (with or without an antimicrobial agent) is then either drained from the crop opening (rotating shaft can exit from the crop) or the carcass is tilted to drain the wash water. This does not completely remove all microorganisms because some bacterial attachment to the skin/inner cavity membranes has usually already occurred.

Various groups of antimicrobial agents have been studied over the years. Loretz et al. (2010) reviewed different intervention strategies (physical, chemical, biological) to help decontaminate poultry carcasses. A list of strategies is provided in Table 15.4.7.1. Physical interventions include water-based treatments, irradiation, ultrasound, air chilling, and freezing (note: operational principles of irradiation, ultrasound, and freezing are discussed in Chapter 11). Among these methods, hot water, steam, electrolyzed water (EW), and irradiation have been shown to effectively reduce bacterial loads. Log reductions obtained by hot water, steam, and EW ranged from 0.9 to 2.1, 2.3 to 3.8, and 1.1 to 2.3, respectively. However, it should be noted that very hot water or steam might adversely impact carcass appearance. Chemical interventions are primarily comprised of organic acid, chlorine, and phosphate based treatments. Loretz et al. (2010) indicated that acetic and lactic acid, acidified sodium chlorite, and trisodium phosphate yielded log reductions in the range from 1.0 to 2.2. Organic matter can reduce

the antimicrobial activity of some chemicals such as chlorine. They also reviewed combination treatments, which have been used to enhance microbial decontamination (Table 15.4.7.2; additional tables are provided in their review). Furthermore, biological interventions (e.g. bacteriophages) constitute promising alternatives but further investigation is required. Loretz et al.(2010) cautioned that although the interventions reduced bacterial load to some extent, decontamination treatments must always be considered as part of an integral food safety system. Later, Bruckner et al. (2012) also provided a meta-analysis and review of the effectiveness of different interventions (e.g., chlorine, acids, phosphates, electrolyzed water, cetylpyridinum chloride, sodium bisulfate) on reducing *Salmonella* prevalence.

Table 15.4.7.1 Decontamination treatments for poultry carcasses. From Loretz et al. (2010)

Physical	Chemical	Biological and Combination
• Water-based treatments ▪ Water ▪ Steam ▪ Pressurized water ▪ Electrolyzed water ▪ Ozonated water ▪ Irradiation ▪ Ultrasound ▪ Air chilling ▪ Freezing	• Organic acids ▪ Acetic acid ▪ Lactic acid ▪ Citric acid • Chlorine-based treatments ▪ Chlorine and chlorine dioxide ▪ Hypochlorite, sodium hypochlorite, and sodium chlorite ▪ Acidified sodium chlorite ▪ Cetylpyridinium chloride ▪ Mono chloramine • Phosphate-based treatments ▪ Trisodium phosphate ▪ Other phosphate-based compounds • Other chemical treatments ▪ Hydrogen peroxide	• Antibacterial activity of biological decontamination treatments • Phages • Antibacterial activity of combined decontamination treatments

Today meat processing plants use a combination of interventions (Hurdle technology) to achieve safe products (as discussed at the end of this chapter). Examples of studies that include combinations of chemical and physical treatments are provided below. Bautista et al. (1997) examined the effect of three groups of antimicrobial agents (chlorine 0-50 ppm; tripolyphosphate 0-20%; lactic acid 0-8%) applied at pressures ranging from 40 to 90 psi (using a laboratory-type inside/outside bird washer) to clean contaminated turkey carcasses.

Table 15.4.7.2 Examples of antibacterial activity of selected combinations of chemical treatments on the surface of poultry carcasses and parts. Adapted from Loretz et al. (2010).

Combination	Microorganism	Reduction (log CFU)	Application[b]	Reference
Chlorine + acetic acid	Aerobic bacteria Coliforms *Escherichia coli* *Salmonella Typhimurium*	1.4 ml^{-1} 1.4 ml^{-1} 1.4 ml^{-1} 2.0 ml^{-1}	IM/SP	1
Chlorine + trisodium phosphate	Aerobic bacteria Coliforms *Escherichia coli* *Salmonella Typhimurium*	1.4 ml^{-1} 1.7 ml^{-1} 1.7 ml^{-1} 2.0 ml^{-1}	IM/SP	1
Lactic acid + potassium sorbate	Aerobic bacteria	0.7–1.2 g^{-1}	IM	2
Lactic acid + sodium benzoate	Aerobic bacteria	1.7–1.8 g^{-1}	IM	2
Lauric acid + potassium hydroxide	Aerobic bacteria *Escherichia coli* *Clostridium perfringens* Staphylococci	2.0 ml^{-1a} >3.4 ml^{-1a} >2.3 ml^{-1a} 2.6 ml^{-1a}	R	3
Levulinic acid + sodium dodecyl sulfate	Aerobic bacteria *Salmonella Enteritidis*	>7.0 g^{-1a} 7.0 g^{-1a}	IM	4
Salmide® + EDTA	*Salmonella Typhimurium*	1.7–2.7 ml^{-1}	IM	5
Salmide® + sodium lauryl sulfate	*Salmonella Typhimurium*	1.2–1.7 ml^{-1}	IM	5
Salmide® + trisodium phosphate	*Salmonella Typhimurium*	3.0 ml^{-1}	IM	5
Tripotassium phosphate + lauric acid	Aerobic bacteria *Escherichia coli* Enterococci *Campylobacter jejuni* *Pseudomonas* Staphylococci	1.5 ml^{-1a} 1.1 ml^{-1a} 1.3 ml^{-1a} 2.7 ml^{-1a} 1.3 ml^{-1a} 1.7 ml^{-1a}	R	6
Tripotassium phosphate + myristic acid	Aerobic bacteria *Escherichia coli* Enterococci *Campylobacter jejuni* *Pseudomonas* Staphylococci	1.1 ml^{-1a} 0.6 ml^{-1a} 1.4 ml^{-1a} 1.4 ml^{-1a} 1.2 ml^{-1a} 0.3 ml^{-1a}	R	6

[a] Highest reduction obtained.
[b] IM, immersion; SP, spraying; R, rinsing.
1. Fabrizio et al. (2002); 2. Ismail et al. (2001); 3. Hinton and Cason (2008); 4. Zhao et al. (2009); 5. Mullerat et al. (1994); 6. Hinton and Ingram (2005)

The results indicated that 4.25% lactic acid was the best in reducing the total microbial load as well as the coliform load, and that pressures above 40 psi did not

show a marked effect. Tripolyphosphate and chlorine were not as effective and did not show a significant improvement compared to a water spray rinse. Bautista et al. (1997) reported some discolouration (bleaching) when 4.25% lactic acid was used during a 10 sec rinse; however, this was not a problem after water chilling the carcasses. Mead and Scott (1994) reported that a post-evisceration spray of 20 ppm chlorine to birds inoculated with a "marker" strain of *E. coli* during the defeathering process did not reduce the proportion of carcasses that acquired the "marker" nor the number of organisms being transferred. However, they mentioned that while chlorine had little direct effect on carcass contamination, it did control bacterial buildup on equipment and destroyed spoilage bacteria present in the water supply.

Tamblyn and Conner (1997) examined the bactericidal effect of acetic, citric, lactic, malic, mandelic, propionic, and tartaric acids (concentrations of 0.5, 1, 2, 4, and 6%) on *S. typhimurium* loosely or firmly attached to broiled skin at different temperatures. They compared three application methods and, similar to Bautista et al. (1997), found that the greatest reduction was achieved by lactic acid at their "scalding application" (2 min at 50°C), followed by "chiller application" (60 min at 0°C) and post-process dip (15 sec at 23°C). However, use of a $\geq$ 4% acid in a scalding or chiller tank might be cost-prohibitive due to the large volume of water required in the tank. Therefore, some chiller manufacturers have recently developed smaller post-chiller finishing or dip tanks where a high concentration of antimicrobial agent can be maintained (see illustration in Chapter 5). This development can be seen today in several plants in North America.

15.4.8 Chilling

The chilling operation is very important in suppressing microbial growth (both pathogens and spoilage microflora) and is mandatory in most countries around the world. The time to reach a specific deep muscle temperature is often recommended (e.g., in the USA 4, 6, and 8 hr to reach 4.4°C in carcasses weighing < 1.8, 1.8 to 3.6 or > 3.6 kg. Note: in the past it was mandated in the US but now only recommended). As indicated in Chapter 5, meat can be chilled by water or air. Selection of a chilling medium is based on water availability, cost (to buy fresh water and treat waste water), energy cost, market demand, etc. There are also hybrid systems where both air and water are used (e.g., water chill for 10 min followed by air chill). Water chill systems are more popular in America whereas air systems are more popular in Europe. Both systems are used to reduce carcass temperature from ~ 40°C to < 10°C. Most air chilling operations employ a mist or water spray to facilitate chilling and to prevent drying during the operation (which usually takes more than an hour).

Various research groups have compared the microbial quality of poultry chilled by each system but there is no consensus as to which system is better; studies have shown conflicting results or no effect. James et al. (2006) reviewed the results of a few dozen research studies and wrote, "many people believe that there is some clear microbiological based reason behind the selection of air chilling. However, the published data do not appear to support this belief, and if anything, point to a microbial advantage of immersion systems". An earlier comprehensive comparison of the two systems was provided by Mead et al. (1993). They examined five plants (two water and three air) and showed that total microbial counts on carcasses were similar or lower after water chilling (Table 15.4.8.1). Levels of *Pseudomonas* spp. after chilling were relatively high in all processes for both broilers and turkeys.

Table 15.4.8.1 Effect of processing plant (5 different chicken plants; except #2 - turkeys) and water chilling method on *Pseudomonas* spp. contamination of neck skin. Modified from Mead et al. (1993).

Plant	#1		#2		#3		#4		#5	
Chilling method	**Water**		**Air**		**Water**		**Air**		**Air**	
After:										
• bleeding	2.3[1]	(8)[2]	2.5	(8)	< 2.0	(4)	< 2.0	(8)	< 2.0	(5)
• scalding	< 2.0	(3)	< 2.4	(6)	< 2.0	(1)	< 2.0	(1)	< 2.0	(0)
• defeathering	2.5	(13)	3.2	(15)	< 2.0	(1)	2.3	(8)	< 2.0	(3)
• evisceration	3.0	(15)	3.4	(15)	2.4	(11)	< 2.1	(6)	< 2.1	(6)
• washing	2.7	(12)	2.7	(15)	2.2	(13)	< 2.1	(7)	< 2.2	(7)
• chilling	3.3	(15)	3.9	(15)	< 2.0	(2)	2.6	(9)	3.2	(15)
• packaging	3.9	(15)	4.0	(15)	N/A	N/A	2.9	(13)	3.5	(15)

[1] Mean (log_{10}) CFU/g of neck skin.
[2] Number of samples positive out of 15, by direct plating. N/A, not available.

Some research has shown that the number of microorganisms in a water chiller also depends on the amount of water overflow (expressed as the amount of water used per 1 kg of carcass mass). Bailey et al. (1987) reported that an overflow of about 2:1 resulted in a skin microbial count reduction of 60-95%. This demonstrates that plain water can be used to reduce microbial load. However, researchers have pointed out that small populations of pathogens, present prior to chilling, may be distributed to other carcasses through the water bath. Several studies have shown no increase in *Salmonella* as a result of immersion chilling, while others have found higher incidences. Busta et al. (1973) studied chill water samples from 3 turkey processing plants and found *C. perfringens* in 53%, *S. aureus* in 22%, *Salmonella* in 17.6%, and coliforms in 100%. The incidence of these organisms on turkey skin

did not significantly differ before and after chilling (*C. perfringens* 87% before and 83% after; *S. aureus* 71% before and 67% after; *Salmonella* 28% before and 24% after; coliforms 100% before and after chilling). Waldroup et al. (1993) and Waldroup (1996) reported that the incidence of *Salmonella* spp. increased by 20% from pre-chill to post-chill and *Campylobacter* spp. by 5%. In a well-controlled chilling system, there is usually a net reduction in bacteria due to washing and chemicals (where permitted) that minimize cross-contamination. Figure 15.4.8.1 shows that microbial load plateaus about 2-3 h into the day (similar to what is observed in a scalding tank). Microbial loads on chilled carcasses obtained after 3 h were similar to those obtained at the end of an 8 hr shift. The addition of chemicals to an immersion water chiller can help control the microbial loads. Different forms of chlorine are commonly used and legal limits vary by country (0-50 ppm).

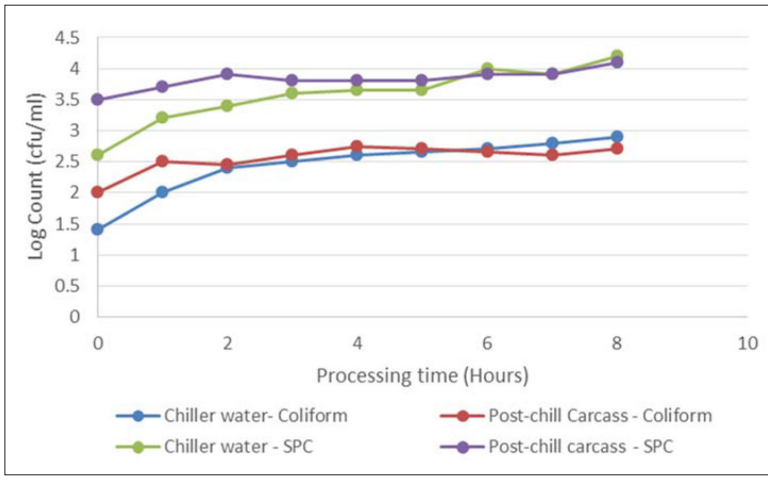

Figure 15.4.8.1 Standard plate count (SPC) and coliform populations (log CFU/mL) as a function of time of day in chiller water and on post-chilled carcasses. For each point, n = 5. Redrawn from Blank and Powell (1995).

A level of 20-50 ppm can help control microorganisms, but concentrations of 300 to 400 ppm would be required for a complete eradication of a pathogen such as *Salmonella*. Such high concentrations are not feasible as they would strongly affect the smell of the meat and bleach the skin. A lower active chlorine level in the chiller can help maintain a manageable level of microorganisms, but it should be frequently monitored as its concentration is reduced when chlorine reacts with organic material. Waldroup et al. (1992) reported that 5 ppm active/free chlorine in the chiller overflow was beneficial in reducing the microbial load on commercially

processed broilers. The authors also examined other modifications (carcass wash, counter current flow) that were suggested by the US National Broiler Council and approved by the Food Safety and Inspection Service. All modifications assisted in reducing microbial counts on processed broilers. Hydrogen peroxide is an example of another effective antimicrobial agent that can be used to control the number of microorganisms. However, in order to reduce microbial load by 95%, concentrations in excess of 6,000 ppm were required. Again, such levels are not feasible as they cause bleaching and blotting problems. The use of various acids, such as acetic acid, can assist in reducing *Enterobacteriaceae* and others (Tamblyn and Conner, 1997). This is similar to the idea previously discussed in the spray washing operation. In such a case, a smaller rinsing cabinet positioned before the chiller can allow the use of a higher concentration of acids/chlorine/phosphate in a more economical way, since the volume required per carcass is relatively low. However, as indicated above, a low level of chemical control does help in maintaining a large chilling water system at a manageable microbial load.

Air chilling operations have been shown to reduce some groups of microorganisms but the effect depends on the system used (e.g., dry versus a continuous water spray system). Demirok et al. (2013) evaluated three commercial systems: water, no spray air, and an in-line combination which included water immersion and air chilling. Water immersion showed the greatest reduction of *Salmonella* (40%) and *Campylobacter* (43%) due to the washing effect and the presence of chlorine. There was no significant difference in shelf life between methods. The water system showed the highest added yield (6.5%), followed by the combination system (1.9%) and the dry air system (-1.1%). However, breast meat was significantly tenderer for the air and combination systems. There were no other sensory differences in the quality of breast filets and drum meat among the systems. Allen et al. (2000) evaluated five commercial air chilling systems and one water chiller (conventional, counter-flow, three-stage unit with about 45 ppm chlorine). The residence time in the air chillers varied according to carcass size, staff break times, line stoppages, and whether or not carcasses were stored in the chiller overnight. The results of the microbial reduction are presented in Figure 15.4.8.2. The air chillers ran at a nominal 3°C and chlorinated water (50 ppm) was used in the sprays in the second, third and fourth systems (exact operating conditions are described in the paper). Overall, the results shown in Figure 15.4.8.2 indicate that the design and mode of operation of the air chiller strongly influences the residual microbial level on the skin. When a completely dry process was used (the sixth system) microbial numbers were reduced approximately ten-fold in the body cavity. The use of water sprays tended to increase the microbial level in the cavity, while heavy spraying with non-chlorinated water substantially increased the numbers of *Pseudomonas* spp. The results also confirmed that water immersion chilling can provide a washing effect to reduce microbial contamination of

carcasses, although initially the numbers of *Pseudomonas* spp. tended to increase. Sanchez et al. (1999) reported that air and water chilling resulted in similar counts of psychrotrophs and generic *E. coli*, but air chilling showed higher total aerobic and coliform counts. Incidence of *Salmonella* was about 20% lower in air chilled birds. Their results show that cross-contamination can occur in air chilling when a water spray is used. However, some skin drying, as a result of air chilling, can reduce certain groups of bacteria.

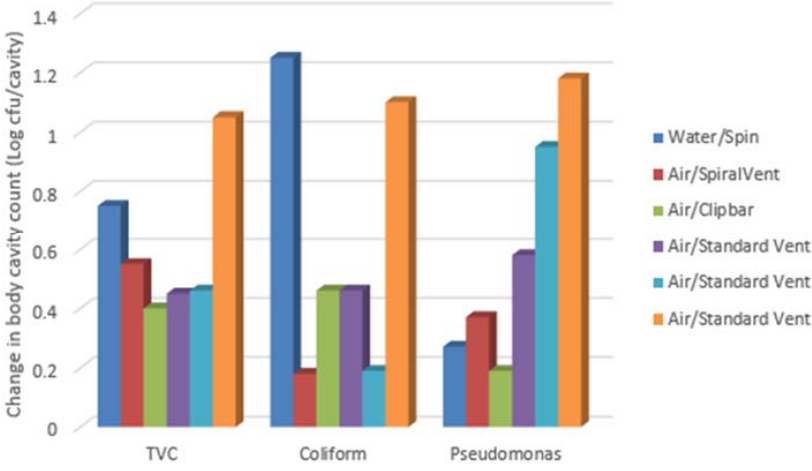

Figure 15.4.8.2 Microbial quality of chicken carcasses chilled by air or water; TVA = total viable count. Redrawn from Allen et al. (2000).

Recently, there has been a considerable increase in US poultry processing facilities that employ post-chiller antimicrobial interventions (Nagel et al., 2013). This is advantageous because post-chill antimicrobial intervention introduces an additional intervention or hurdle for pathogens. The smaller (400 – 600 gal) post-chill immersion tank resembles a traditional chiller but has a minimal footprint and results in a shorter dwell time (generally 30 s) with a higher concentration of antimicrobials. Primary chillers, which hold 20,000 to 50,000 gal (dwell time of 1.5–2.0 h), are less efficient and cost-effective. Additionally, because organic load may reduce the efficacy of these antimicrobials (e.g., chlorine), post-chillers can increase the efficacy of some antimicrobials. Post-chill tanks have now been installed in many plants. Nagel et al. (2013) studied them in order to control *Salmonella* and *Campylobacter* counts (Fig. 15.4.8.3) as per US government guidelines. The authors evaluated five post-chill water treatments consisting of 40

ppm total chlorine, 400 ppm or 1,000 ppm peracetic acid (PAA), and 1,000 or 5,000 ppm lysozyme. Treatment with 400 or 1,000 ppm PAA was most effective ($P \leq 0.05$) in reducing populations of *Salmonella* and *Campylobacter* as compared to the chlorine treatment at 40 ppm and lysozyme treatments at 1,000 and 5,000 ppm, as well as the water treatment and the positive control. Intervention strategies such as post-chill decontamination tanks have provided an alternative approach for pathogen reduction during poultry processing.

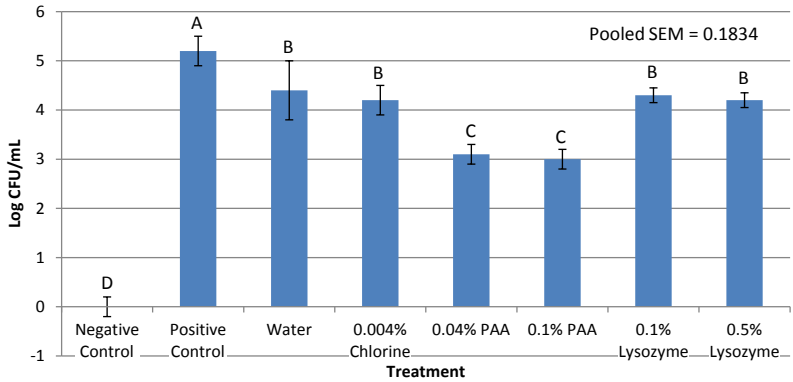

Figure 15.4.8.3 *Salmonella Typhimurium* recovered from inoculated carcasses (n = 160) treated with various antimicrobials in a post-chill immersion tank reported as mean log colony-forming units of *S. Typhimurium* per sample for each treatment group. PAA= peracetic acid. a–d Means with no common letter differ significantly ($P \leq 0.05$). From Nagel et al. (2013).

Antimicrobials that are currently approved for use in poultry applications are described in the FSIS Directive 7120.1 Revision 9 (USDA-FSIS, 2011b). In the US, chlorine has historically been used to prevent cross-contamination in immersion chilling systems and throughout the poultry processing plant. However, the efficacy of chlorine for bacterial reduction decreases with increasing pH and organic load (Nagel et al., 2013).

In recent years, peracetic acid (PAA), a combination of acetic acid and hydrogen peroxide, has replaced chlorine as the industry standard for antimicrobial application during poultry processing. This antimicrobial is effective due to its combined acidic and oxidizing properties. For antimicrobial applications in poultry, the maximum allowable concentration is 2000 ppm in a post-chill dip (USDA-FSIS, 2011b). Overall, validation of antimicrobials under commercial settings is extremely important because efficacy is affected by factors such as temperature, contact time, concentration, and coverage.

15.4.9 Hurdle Concept – Primary Processing

Reducing the number of microorganisms in meat requires a multifaceted approach. The importance of monitoring the health of parental flocks, growth conditions on the farm, transportation, and steps to prevent cross-contamination in the processing plant have already been highlighted. The concept is based on combining several approaches or 'hurdles' that pathogens have to overcome if they are to stay alive and active in a given food product. The hurdles can include high acidity, heat processing, salt addition, cold storage, etc. Below, combinations of different interventions within the primary processing plant are discussed.

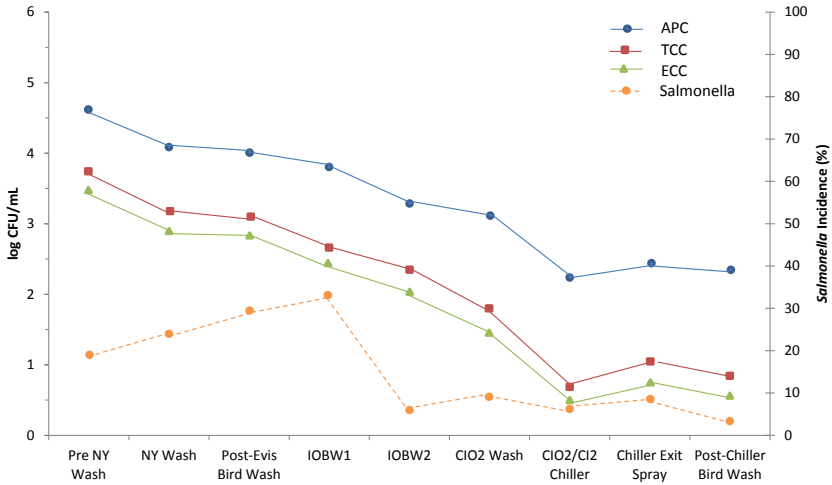

Figure 15.4.9.1 Microbial populations (log CFU per milliliter, mean ± SD) and Salmonella incidence (%) of carcasses following multiple interventions applied in sequence along the evisceration (slaughter) line at poultry plant A. The first point represents the stage before the first intervention, and every point thereafter represents the populations after the specific intervention. NY Wash, New York wash; Post-Evis, postevisceration wash; IOBW1, inside-out- side bird wash 1; IOBW2, inside-outside bird wash 2; ClO_2, chlorine dioxide wash; ClO_2-Cl_2, chlorine dioxide wash plus chlorine chiller. From Stopforth et al. (2007).

Stopforth et al. (2007) investigated the efficacy of individual and multiple sequential interventions to decrease microbial load. Figure 15.4.9.1 shows aerobic plate counts (APC), total coliform counts (TCC), *E. coli* counts (ECC), and *Salmonella* incidence on poultry carcasses processed at one of the plants (identified as Plant A; they studied and reported data for three different plants).

This plant processed 140 birds per min and included the following interventions: New York wash (spray application of 20 – 50 ppm chlorinated water following defeathering), post-evisceration wash (spray application of 20 – 50 ppm Cl_2), inside/outside bird wash 1 and 2 (IOBW consisting of 20 – 50 ppm Cl_2 following neck removal), chlorine dioxide spray application immediately before chilling (ClO_2 prepared by acidifying 500 – 1,200 ppm sodium chlorite with citric acid at pH 2.7), chlorinated chiller (using 20 – 50 ppm Cl_2; chiller operated at pH 6.5 – 7.0 according to the facility's HACCP plan), chiller exit spray (with 20 – 50 ppm Cl_2), and a post-chiller spray (with 20 – 50 ppm Cl_2) immediately following carcass sizing. Observations were made over 5 days with 15 samples taken each day before and after each intervention step. Results from all three plants showed that the majority of individual interventions significantly ($P < 0.5$) reduced microbial populations on or in carcasses, carcass parts, and processing water. Reductions in APC, TCC, and ECC due to individual interventions ranged from 0 to 1.2, 0 to 1.2, and 0 to 0.8 log CFU/ml, respectively. Individual interventions reduced *Salmonella* incidence by 0 to 100% depending on the process type and product. Sequential interventions also resulted in significant reductions ($P < 0.05$) in APC, TCC, ECC, and *Salmonella* incidence of 2.4, 2.8, and 2.9 log CFU/ml and 79%, respectively, at plant A. The other two plants had 6 and 3 intervention steps, respectively (figures not shown). At plant B the corresponding reductions were 1.8, 1.7, and 1.6 log CFU/ml and 91%. At plant C they were 0.8, 1.1, and 0.9 log CFU/ml and 40%. The authors concluded the results validated the poultry processing interventions and provided a source of information to help the industry in its selection of antimicrobial strategies.

Gill et al. (2006) also examined the effectiveness of different steps in a large poultry processing plant. The plant processed 1.3 – 1.6 kg broilers using a 90 sec scald (at 58 ± 1°C) and immersion chilling in chlorinated water. About half of the birds were packed and shipped without further processing, and those remaining were portioned, deboned, or marinated and tumbled in brine. The results presented in Table 15.4.9.1 are part of a larger study designed to look at the effectiveness of different intervention steps as well as evaluate the effects on specific groups of bacteria. The study was done to validate HACCP steps (see also Chapter 6) as it is currently recommended that HACCP systems be developed on the basis of objective assessments of hazards and risks associated with each individual intervention. Subjective judgments can be uncertain as the relationship between fecal or other visible carcass contamination and microbial meat condition is not consistent (Gill, 2004). Similar operations at different plants can have very different effects on the microbial conditions of the products. Gill et al. (2006) obtained samples from carcasses by excising a strip of skin measuring approximately 5 x 2 cm from randomly selected sites on each carcass (sampling procedure shown in Fig. 15.4.9.2) or by rinsing the carcass portion.

Table 15.4.9.1 Statistics for sets of 25 coliform and aerobic counts (CFU/cm^2) recovered from chicken carcasses or portions of such carcasses, at various stages of processing at a poultry packing plant. Adapted from Gill et al. (2006).

Product	Stage of processing	Statistics – Coliforms				Statistics – Aerobic			
		$\bar{\times}$	s	log A	N	$\bar{\times}$	s	log A	N
Carcasses	Before second wash	1.74A	0.79	2.45	3.63	3.53BCD	0.86	4.37	6.12
	After second wash	1.53A	0.84	2.35	3.9	3.19CDE	0.68	3.72	5.22
	After evisceration	1.79A	0.77	2.47	3.89	3.08DE	0.96	4.13	5.53
	Before third wash	1.39AB	0.76	2.05[a]	3.56	2.77E	0.46	3.02	4.39
	Before cooling	1.25ABC	0.77	1.93	3.3	2.94DE	0.52	3.24	4.68
	After cooling	0.17D	0.73	0.78	2.1	2.66E	0.89	3.58[a]	5.49
Skin-on thighs	Before packing	0.80C	0.44	1.03	2.36	3.73BC	0.6	4.14	5.53
Boneless breasts	Before tumbling	0.85BC	0.31	0.96[a]	2.35	4.51A	0.43	4.72	6.17
	After tumbling with brine	0.67CD	0.25	0.74	2.13	4.01AB	0.37	4.16	5.55

$\bar{\times}$, mean log; s, standard deviation; log A, log mean; N, log of the total number recovered from 25 samples. Mean logs with the same letter are not significantly different ($P > 0.05$).
[a] Set of log counts is not normally distributed ($P < 0.05$).

As indicated in the table, each value represents an average of 25 samples per sampling point where 5 samples were collected on each of 5 days (previously shown to obtain representative results; Gill, 2004). The log mean numbers of aerobes, coliforms, *E. coli*, and presumptive *Staphylococci* plus *Listeria* on carcasses after scalding and plucking were about 4.4, 2.5, 2.2, and 1.4 log CFU/cm^2, respectively. The numbers of bacteria on eviscerated carcasses were similar. After a series of operations for removing the crop, lungs, kidneys, and neck, the numbers of aerobes were about 1 log unit less than on the eviscerated carcasses, but the numbers of other bacteria were not substantially reduced. After water chilling, the numbers of coliforms and *E. coli* were about 1 log unit less and the numbers of presumptive *Staphylococci* plus *Listeria* were about 0.5 log unit less than the numbers on dressed carcasses, but the numbers of aerobes were not reduced.

When further looking at deboning and marinating, the numbers of aerobes were 1 log unit higher on boneless breasts and 0.5 log units higher on skin-on thighs and breasts that had been tumbled with brine than on cooled carcasses.

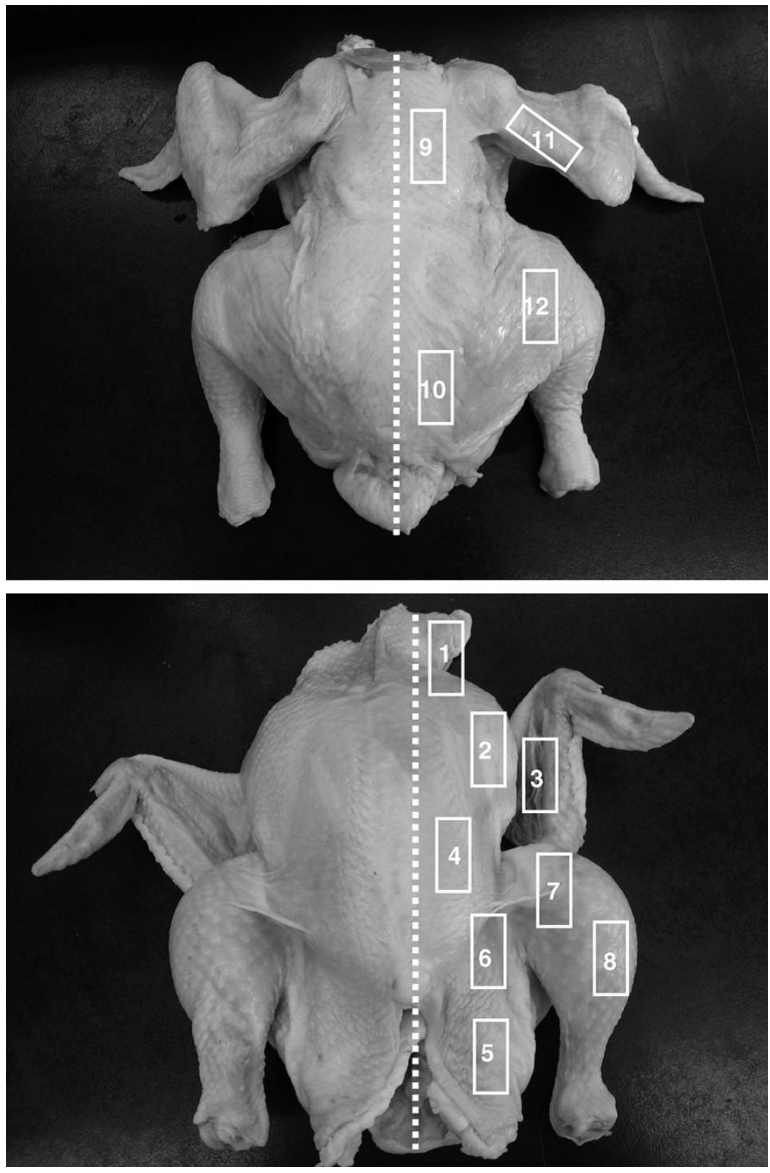

Figure 15.4.9.2 Pictures used for identification of sites from which to obtain samples of skin from chicken carcasses. From Gill et al. (2006).

The presumptive *Staphylococci* plus *Listeria* were 0.5 log unit more on thighs than on cooled carcasses. This is probably the result of the extra meat handling as will be discussed in the next section.

Guerin et al. (2010) reviewed changes in *Campylobacter* prevalence during processing. They looked at information from 8 electronic databases using key words for "*Campylobacter*", "chicken", and "processing" and identified 1,734 unique citations. Thirty-two studies described prevalence at more than one stage during processing and were included in the review. Of the studies that described the prevalence of *Campylobacter* on carcasses before and after specific stages of processing, the chilling stage had the greatest number of studies (9), followed by washing (6), defeathering (4), scalding (2), and evisceration (1). Studies that sampled before and after scalding or chilling, or both, showed that the prevalence of *Campylobacter* generally decreased immediately after a certain stage (scalding: 20 to 40% decrease; chilling: 100% decrease to 26% increase). However, the prevalence of *Campylobacter* increased after defeathering (10 to 72%) and evisceration (15%). The prevalence after washing was inconsistent among studies (23% decrease to 13% increase). Eleven studies reported the concentration of *Campylobacter*, as well as, or instead of, the prevalence. Studies that sampled before and after specific stages of processing showed that the concentration of *Campylobacter* decreased after scalding (minimum decrease of 1.3 CFU/g, maximum decrease of 2.9 CFU/g), evisceration (0.3 CFU/g), washing (0.3 – 1.1 CFU/g), and chilling (minimum 0.2 CFU/g, maximum 1.7 CFU/carcass) and increased after defeathering (minimum 0.4 CFU/g, maximum 2.9 CFU/mL). Guerin et al. (2010) indicated that "more data are needed to better understand the magnitude and mechanism by which the prevalence and concentration of *Campylobacter* changes during processing. This understanding should help researchers and program developers identify the most likely points in processing to implement effective control efforts".

Bruckner et al. (2012) published a large scale review (six databases searched) and meta-analysis of published material related to the application of carcass spray and dip treatments to reduce *Salmonella* prevalence and concentration on broiler chickens. Visual evaluation of the forest plots indicated overall reduction trends for six spray treatments:

a. trisodium phosphate (n=48 trials)
b. acidic electrolyzed oxidizing water (n=2)
c. cetylpyridinium chloride (n=43)
d. lactic acid (n=24)
e. sodium bisulfate (n=11)
f. potable water (n=36)

Note: references for these antimicrobials and others can be found in Bruckner et al. (2012). The authors indicated moderate to considerable heterogeneity between studies and methodological problems within the studies including a lack of research conducted under commercial conditions (i.e., precluding the full benefits of robust meta analysis). The review by Loretz et al. (2010), mentioned earlier in the chapter, also contains a section on combining interventions at different steps (Table 15.4.7.2).

15.5 Secondary Processing

Fresh poultry is commonly sold as whole carcasses, cut-up parts, minced meat (see Chapter 9), or as a fully cooked product (see Chapter 13). The meat can be packaged in individual bags, trays wrapped in polyethylene, or in bulk without individual wrapping (see Chapter 11). The secondary processes involve manipulation of the product (e.g., portioning, de-skinning, marinating, tumbling, cooking), which can also affect its microbial quality. Extra handling by people and machines (e.g., ground meat can be handled 10 – 12 times) can increase microbial load and/or change its composition. For example, bacteria from the surface are transferred to the deep tissue while grinding or injecting the meat. If ingredients such as carbohydrates are added they are used right away as a simple energy source for microbial growth (e.g., added to help fermentation in certain meat products). Extended handling and storage times also play a role in the shelf life of the product (see Fig. 15.2.1; relationship between initial bacterial load and storage temperature) as well as potential cross-contamination (Fig. 15.1.7). On the other hand, several secondary processing treatments (e.g. cooking) can lower the microbial load and help destroy pathogens.

15.5.1 Cutting and Portioning

Portioning the carcass involves extra handling and exposure to more surfaces (e.g., cutting boards, containers, blades installed on automated deboning equipment; see Chapter 6). An operation such as skinning poultry portions has been reported to increase aerobic counts in a high speed processing line (Table 15.4.9.1). As indicated above, ground meat is commonly handled 10 – 12 times, which results in a shorter shelf life compared to intact muscle pieces (e.g., 3-5 days versus 1-2 weeks).

15.5.2 Storage Shelf Life

The shelf life of fresh poultry depends on the initial microbial population (number and type), storage temperature, pH, additives, and other factors. Temperature fluctuations in the cold chain are particularly important. Bruckner et al. (2012) reported on this topic in the case of fresh poultry and pork meat (Fig. 15.5.2.1) and showed similar results for 4°C storage condition (note; this is similar to results published in 1972 shown in Figure 15.2.1). When temperature was increased for a certain period of time to 7 and 15°C, shelf life was reduced (Table 15.5.2.1). Overall, the authors indicated that fresh poultry and pork showed similar spoilage patterns under dynamic temperature conditions with a remarkable reduction in shelf life when short temperature upshifts occurred at the beginning of storage (reductions were up to 2 days or over 30% shorter). As expected, scenarios with shifts to 15°C led to greater reductions than 7°C for both meats.

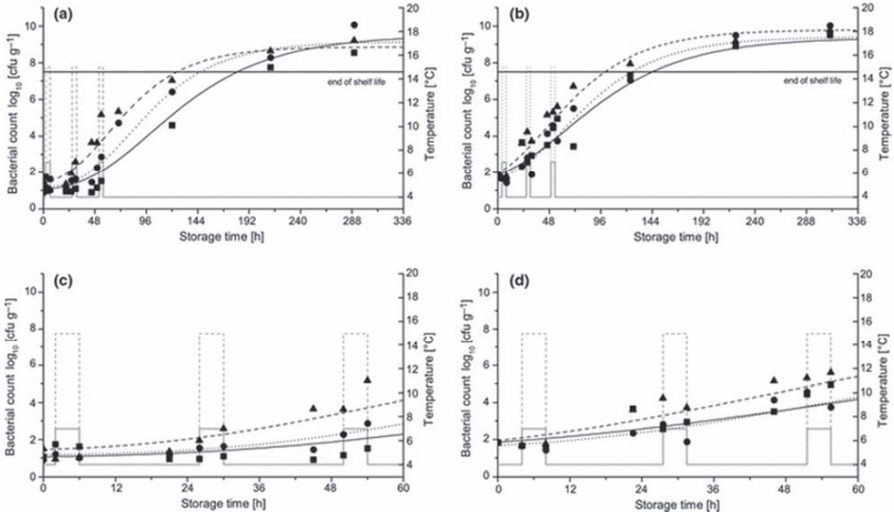

Figure 15.5.2.1 Growth of *Pseudomonas spp.* in trial B fitted with the Gompertz model on pork (left) and poultry (right), (a, b): during the complete storage, (c, d); during the first 60 h of storage; (■ —) scenario B0 at 4°C constant, (● ····) scenario B1 with shifts to 7°C, (▲− −) scenario B2 with shifts to 15°C (solid grey line; temperature profile B1, dashed grey line; temperature profile B2).
From Bruckner et.al (2012).

An early report (Ayres et al., 1950) on the effect of storage temperature on shelf life of fresh, eviscerated, and cut up poultry indicated shelf lives of 15-18 days when stored at 0°C, 6-8 days at 4.4°C, and 2-3 days at 10.6°C. Later studies indicated similar trends where meat stored at 10°C spoiled about twice as fast as at 5°C and three times as fast at 15°C (Cox et al., 1998). The type of spoilage flora that develops on eviscerated chicken is influenced by storage temperature. Barnes and Thornley (1966) noted that the predominant species found on freshly processed broiler carcasses were initially mesophilic such as micrococci, Gram-positive rods, and flavobacteria (50, 14 and 15 different strains, respectively). When the meat was held at 1°C, however, the number of detectable strains decreased to three. In that case, psychrotrophic strains of *Pseudomonas* eventually dominated the culture (the number of detected strains increased from 2 to 70) and caused spoilage. Since the publication of Barnes and Thornley's (1966) original article, *P. putrefaciens* (the principle spoilage bacteria on fresh poultry) has been reclassified as *Alteromonas putrefaciens*. This bacterium is present at relatively low numbers on carcasses immediately after processing (no strains detected; i.e., probably due to below detection level of the method used) but went up to 19, 4, and 4 when the storage temperature was held at 1, 10, and 15°C respectively. In living birds, this bacterium is found on the feathers and feet. Later, during processing, it can be isolated from chill tank water but is rarely found in the intestines. If the meat is held at 10°C, *Pseudomonas*, *Acinetobacter*, and *Enterobacteriaceae* spp. multiply fairly rapidly. At 15°C, *Acinetobacter* and *Enterobacteriaceae* spp. will dominate the microbiota because their optimal growth temperatures are higher than that of *Pseudomonas*.

Pooni and Mead (1984) have also indicated that bacteria isolated from temperature-abused fresh poultry are different from those isolated from meat held at an appropriate storage temperature (< 5°C). At 20-22°C, 70% of the bacterial population consisted of Proteus species (mesophilic) and only 20% were *Pseudomonas* (psychrotrophs).

Although spoilage bacteria grow at refrigeration temperatures, their growth rate is slower at lower temperatures. Most mesophilic bacteria will survive but will not multiply at refrigeration temperatures. The generation time of a mesophilic bacteria such as *E. coli* has been reported to be 0, 0, 20, 6, 2.2, 1.2, 0.7 and 0.4 hr at temperatures of -2, 1, 5, 10, 15, 20, 25 and 30°C, respectively (note: the lag phase time at 10 and 5°C might exceed 60 and 215 hr respectively; USDA, 2015).

Table 15.5.2.1 Calculated shelf life times and shelf life reductions for fresh pork and fresh poultry in different dynamic storage trials. From Bruckner et al. (2012).

			Pork			**Poultry**		
Storage Trial	Scenario[a]	Number of Shifts	Shelf life[b] (h)	Shelf life reduction[c] (h)	Shelf life reduction (%)	Shelf life (h)	Shelf life reduction (h)	Shelf life reduction (%)
Continuous temperature abuse during storage (trial A)								
Trial A	A0	0	148.6	–	–	140.0	–	–
	A1	4	144.2	4.4	3.0	130.5	9.5	6.8
	A2	4	126.5	22.1	14.9	122.4	7.6	12.6
Temperature abuse in the beginning of storage (trial B, C and D)								
Trial B	B0	0	180.9	–	–	138.4	–	–
	B1	3	146.6	34.3	19.0	125.0	13.4	9.7
	B2	3	124.7	56.2	31.1	100.0	38.4	27.7
Trial C	C0	0	169.1	–	–	140.2	–	–
	C1	2	157.5	11.6	6.9	133.5	6.7	4.8
	C2	2	121.1	48.0	28.4	106.7	33.5	23.9
Trial D	D0	0	138.9	–	–	133.5	–	–
	D1	1	124.0	14.9	10.7	122.1	11.4	8.5
	D2	1	103.5	35.4	25.5	102.9	30.6	22.9

Shelf life was estimated from time point zero of the laboratory investigations, which means 24 hr after slaughtering.

[a]Scenarios: A0 – control at 4°C; A1 - four shifts for 4hr from 4 to 7°C; A2 - four continuous shifts for 4hr from 4 to 15°C; B0 – control (no shifts, at 4°C constant); B1 – three shifts for 4hr from 4 to 7°C; B2 - three continuous shifts for 4hr from 4 to 15°C; C0 – control (no shifts, at 4°C constant); C1 – two shifts for 6hr from 4 to 7°C; C2 - two shifts for 6hr from 4 to 15°C; D0 – control (no shifts, at 4°C constant); D1 – one shift for 12 hr from 4 to 7°C; D2 – one shift for 12 hr from 4 to 15°C.

[b]Evaluated by count of *Pseudomonas* spp.: End of shelf life: 7.5 $\log_{10}$ cfu g^{-1}.

[c]In relation to shelf life at 4°C (Scenario 0 in each trial).

When spoilage microorganisms grow on poultry meat they produce by-products such as slime (a protective carbohydrate secretion) and off-odour molecules. The number of bacteria required to cause a noticeable change on the surface of the meat (e.g., appearance of slime) is estimated at about 10^6 to 10^8 CFU/cm^2. At the beginning of the storage period, a small psychrotroph population would mainly utilize glucose or other simple sugars as an energy source. The by-products of glucose metabolism do not contribute substantially to detectable spoilage. However, as glucose is depleted the bacteria switch to other compounds, such as amino acids, which cause the formation of odourous by-products (Pooni and Mead, 1984). Various spoilage signs have been reported, usually starting with the

appearance of small, translucent dots (i.e., microbial colonies) on the cut surfaces of skin/meat. Initially, the colonies appear as tiny water droplets, but later they grow, become opaque, and finally create a uniform, sticky, or slimy layer. At this stage, the meat usually develops an offensive ammonia odour or the so called "dirty dishrag" odour. Coloured colonies (e.g., gray, yellow, brown) are usually associated with a specific spoilage microorganism (e.g., pigmented *Pseudomonas*). In order to obtain meaningful bacterial counts, an appropriate incubation temperature should be used (e.g., 2-5°C is used to encourage psychrotroph growth and prevent the growth of mesophilic bacteria). In general, agar plates should be incubated at or near the temperature at which the product is stored. Enumeration of mesophilic bacteria can be more difficult than that of psychrotrophs because some psychrotrophs will also grow at elevated temperatures. For example, *Pseudomonas* and *Aerobacter* are capable of growth between 0 and 30°C; however, at 35°C they will be inhibited. Knowing the maximum growth temperature of the different psychrotrophs is important in determining the incubation temperature required to enumerate mesophilic bacteria in a mixed population.

Frozen storage is used to extend the shelf life of food products by weeks or months. At freezing temperatures water is unavailable to the microorganisms and most cannot grow (see also preservation discussion in Chapter 11). Poultry meat freezes at -1 to -2°C because of its salt and mineral content, which suppresses the freezing point. During freezing, a portion of the microbial population is killed or sub-lethally injured. Bacterial survival after thawing can range from 1-100% but is commonly around 50%. Survival depends on factors such as the food composition (e.g., high versus low fat content), freezing rate, and microorganism type (e.g., *Campylobacter* is more sensitive than *E. coli*; *S. aureus* is more tolerant of freezing and becomes significant during the thawing process). Slow freezing destroys more microorganisms than fast freezing because it forms intra- and extracellular osmotic gradients throughout the cell that damage its structure. During fast freezing, no/less such gradient is formed (Jay et al., 2005; Cepeda et al., 2013) and this process can actually be used to preserve bacteria for medical or food applications (e.g. very rapid freezing using liquid nitrogen at around -190°C can be used to save cells for later use in a starter culture). The effect of freezing on the shelf life of thawed chicken has been studied by various researchers and most reports indicate no major differences in shelf life after the meat has been thawed (Sauter, 1987).

15.5.3 Cooking

Cooking meat products in industrial facilities has become a common practice around the world (see also Chapters 1 and 11) as a result of consumer demand for convenience and extended shelf lives, as heating (commonly to 68-74°C)

inactivates pathogens and many spoilage bacteria. When a heating step is applied, the processor must adhere to strict food safety procedures (e.g., a minimum end point cooking temperature followed by a predetermined chilling rate). Destruction of the major groups of spoilage microorganisms and pathogens is discussed below. Over the past decade, post-cooking contamination of ready to eat (RTE) meat products with pathogens such as *Listeria* has been a topic of concern for both governments and their citizens (FSIS, 1999a and b; Borchert, 1999; Sofos, 2010). Overall, *Listeria* will be destroyed at 70°C, but post-cooking contamination problems have been associated with people and equipment (e.g., slicing cooked meat) recontaminating the product, the ability of *Listeria* to survive at low temperatures, and its widespread presence within our environment. Most large companies use specific measures to reduce listeriosis risk such as positive airflow in slicing areas, chemical additives, post-packaging heat, or high pressure treatments.

It should be remembered that cooking to 68-74°C does not sterilize the product, as would be the case in a canning operation where the product is heated to 121°C to kill all spore forming microorganisms. In pasteurized products spoilage microorganisms are still present and they will spoil/degrade the product over time. To illustrate what processors of cooked meat products are facing, a few examples of common problems and microorganisms involved in spoilage are provided below:

a. Gas production without a bad odour has been reported of *Leuconostoc* growth in vacuum packed, fully cooked meats such as chunked and formed turkey/pork ham, frankfurters, cooked sausages, and summer sausages (Ray and Bhunia, 2007). The pH of these products is usually about 5.0-6.0 and tests show the predominant presence of lactic acid bacteria. *Leuconostoc carnosum* and *L. mesenteroides* are likely responsible for the CO_2 production that causes package bloating. It is assumed that this problem is associated with post-heat contamination (e.g., slicing, packaging, handling operations).
b. Off odours and gas production in cooked, vacuum packed, refrigerated meats are usually associated with *Clostridium* spp. Gases, including H_2S, have been reported due to the growth of *Clostridium* isolated from the product (Ray and Bhunia, 2007), where some isolates show typical terminal spores. In one case, purge accumulating after about 3 weeks of cold storage was also due to a large number of *Leuconostoc* (10^8/ml of the purge) growing in the package. Ammonia off odours in cooked, vacuum packed, turkey breast rolls with gas and purge accumulation was reported to be due to high numbers (10^8/ml) of Gram-negative *Serratia*

liquifaciens and Gram-positive *Leuconostoc mesenteroids* (Ray and Bhunia, 2007). It was suspected that the product was contaminated post-cooking and the pH did not decrease significantly because of the alkaline phosphate used in the product. In that case, *Leuconostoc* produced gas and then *Serratia* spp. metabolized proteins (deamination), which released ammonia. Some products also showed pink discolouration that could be due to reduction of metmyoglobin (see Chapter 17). This is an example of "succession growth" where one group of microorganisms paves the way for the next group (e.g., this concept is also used to describe the steps in making sauerkraut).

c. Gray discolouration (spots, patches) was reported in stored turkey luncheon meat slices after 2-3 days of aerobic refrigerated storage (Ray and Bhunia, 2007). The bacterium responsible was a *Lactobacillus* strain that produced H_2O_2, which could oxidize myoglobin to produce a gray colour. Under vacuum conditions this strain will not produce H_2O_2.
d. Yellow spots/discolouration in vacuum packed cooked luncheon meat. The colour usually develops after 3-4 weeks of storage at 4-5°C. A picture of the product can be seen in Chapter 17 (see colour defects). The microorganism responsible has been identified as *Enterococcus faecium* ssp. *casseliflavus*, which survived a 71.1°C cooking temperature for 20 min.

15.5.4 Hurdle Concept – Secondary Processing

The ability to extend the shelf life of cooked meat products depends on combining different factors that control microbial growth (both spoilage and pathogenic microorganisms). This is called the hurdle concept/technology, where the combination of several antimicrobial tactics, each at a relatively low level, can substantially increase the shelf life. For example, the long shelf life of a hot dog (guaranteed by many manufacturers for 30-70 days) is due to the combination of a relatively low salt concentration (about 2%), ingredients that reduce the pH (e.g., lactate), cooking to 72°C (which eliminates pathogens, but not all spoilage microorganisms), vacuum packing, and refrigeration at ≈ 2°C. Removing even one of these measures (e.g., refrigeration) can result in a catastrophic effect on shelf life and food safety. Table 15.5.4.1 shows examples of the commonly employed technologies/ingredients that are used in combination to increase the safety and shelf life of food products (see also Chapter 11).

Table 15.5.4.1 Potential hurdle steps to improve safety and extend the shelf life of a cooked meat product. See text for more details.

Hurdle	Notes
Physical	
Temperature	• Keep raw meat and other perishable items at low temperature. • Cook meat to appropriate temperature to destroy pathogens and most spoilage microorganisms.
Clean Environment	• Maintain the cleanest possible environment and equipment.
Irradiation	• Use (where permitted) to inactivate microorganisms.
Drying	• Use to reduce water activity.
Chemical	
Nitrite	• Used at 100-200 ppm, in some products, to inactivate *C. botulinum* (i.e., in products heated to 70-75°C spores cannot be destroyed by heat).
Salt	• Commonly used at 1.5-3.0% to help suppress certain groups of microorganisms.
Lactate	• Added to reduce pH and suppress certain groups of microorganisms (Glass et al., 2002). In fermented products, live lactic acid bacteria are used.
Storage and Distribution	
	• Modified atmosphere (Genigeorgis, 1985). • Low temperature package kept intact in storage coolers, trucks, retail stores.

15.6 Cleaning/Sanitation and Equipment Design

Government regulations require that food processing plants be kept clean. Maintaining a clean food processing operation, on a continuous basis, is not an easy task as raw materials are coming in all the time from different locations (local /international suppliers). In addition, certain operations can result in contamination and/or cross-contamination (e.g., evisceration, deskinning, and defeathering). In order to achieve a clean operation, management needs good planning, adequate equipment design, employee commitment (i.e., production staff, maintenance, sanitation crew), knowledge of available cleaning compounds, and an adequate supply of clean water.

Aside from government regulations, there are obvious reasons for maintaining good sanitation:

a. The company reputation is on the line each time a consumer buys a product. Gaining brand loyalty is a time consuming and expensive process that can be lost as a result of a single food poisoning incident. It is also important to remember that in our competitive global economy, switching from one brand to another is easy for the average consumer (e.g., switching might only require moving a few steps along the display cooler in a store).
b. Lawsuits are becoming a major issue where consumers who suffer from using/eating a defective product seek financial compensation. Legal bills, compensation awards, and bad publicity can result in big financial losses, high insurance premiums, and bankruptcy.
c. Fresh meat and processed products are perishable ingredients that will quickly spoil without adequate sanitation and storage.
d. Avoiding recalls, either mandatory or voluntary, can save the company bad publicity and expenses. National and international recalls can be extremely complex, expensive, and difficult to conduct.

15.6.1 Cleaning a Meat Processing Plant

This section is intended to provide an overview of the important aspects of cleaning a food processing plant to help the reader realize the complexity of the process when so many raw materials, people, and services (e.g., water supply, electricity) are entering and leaving the plant. For an extensive review of the topic the reader should consult special text books written on this topic such as the one by Marriott and Gravani (2006). The methods and chemicals used for cleaning a food processing plant are based on the soil material present and sanitizer rotation required. Meat plants mainly deal with protein and fat under wet environments and, therefore, alkaline solutions are the most common cleaning solutions used. Today, a great number of cleaning compounds are available on the market. Some are based on alkali compounds (e.g., phosphates, carbonates, silicates), some on acids (e.g., citric acid, phosphoric acid), and some on synthetic detergents (e.g., anionic, cationic, nonionic base). In the meat industry today, the common cleaning solutions are often based on an alkaline solution, with about 1.5% sodium hydroxide. This is used to saponify the fat and hydrolyze the protein deposits. Various synthetic detergents are also used by the meat industry to remove meat deposits, fat, and dirt. After allowing adequate contact time at the right temperature, the solution (with/without a foaming agent) is washed away with water. Later, the remaining scale/

mineral deposits can be removed with a weak/strong acid. Another approach to cleaning involves the use of enzymes, where a solution containing proteases (i.e., to break down protein deposits) is used in a mild alkaline solution that saponifies the fat deposits. Because enzymes would be inactivated at high pH and temperatures, corrosion problems are minimized. However, enzyme solutions are not as popular for general use because they are still more expensive and also present a higher risk to the people using them.

When designing a cleaning procedure it is important to follow logical steps in order to minimize the costly use of chemicals, time, and heated water. Common cleaning procedures include:

a. Physical removal of soils from surfaces. This step is usually done manually (e.g., scrapers to remove meat chunks) to help reduce soil loads and later save on cleaning compounds and wastewater treatment.
b. High pressure water to rinse away the soil. The water temperature should be below 55°C to prevent cooking the meat on the surface. Note: in some plants, high pressure is not used in order to reduce aerosols.
c. Washing with an alkaline solution or a synthetic detergent to loosen the soil deposits. It is important to allow sufficient time for the chemical reaction(s) to occur. A contact time of 6-12 min and a cleaning solution temperature of 50-55°C is usually recommended. If vertical surfaces are cleaned, a foaming agent is used to keep the compounds in close contact with the surface. As indicated above, an enzyme solution can also be applied. When enzyme solutions are used, the water temperature should be lowered to prevent enzyme denaturation.
d. Rinsing with clean water to remove loose soil and alkaline or detergent solutions.
e. Washing with an acid to remove scale deposits. Mineral deposits (appear as a rusty or whitish scale) are not removed by an alkaline solution and therefore acids (e.g., phosphoric, hydrochloric, or organic acids such as citric, gluconic) are used.
f. Inspecting (visual, microbial) all equipment surfaces to ensure removal of all soil and cleaning compounds. Section 15.6.2 discusses equipment design and good drainage from surfaces.
g. Application of sanitizing agents. It is essential to apply this step only after all the equipment has been thoroughly cleaned. Otherwise the sanitizer would not be in close contact with the surface and its activity is diminished. A chlorine solution (100-200 ppm), iodine (20-30 ppm) or quaternary ammonium solution (150-200 ppm) are commonly used.

h. Washing/rinsing the sanitizer is a step that depends on the chemical used. Some sanitizers will react and become neutralized (e.g., chlorine), others have a prolonged residual effect and can be left on the equipment (e.g., quaternary ammonium), and still others need to be rinsed (e.g., iodine).
i. In cases where corrosion is a problem, oil is sprayed on sensitive areas/ equipment. Unless it is a food-grade, the oil is removed before the next processing shift starts.

Continuous cleaning using a cleaning-in-place (CIP) method is also used for some moving belts and other pieces of equipment. Another application of CIP is in a closed system such as a smokehouse at the end of the operation where a system is used to dispense heavy duty detergents that can be used to effectively remove soil deposits without exposing employees to harsh chemicals. This is an example of using automation to effectively clean a challenging area (e.g., a slippery stainless steel floor) exposed to soil deposits that are difficult to clean (e.g., smoke). In any case, today CIP systems are fairly limited to specific areas in a meat processing plant.

15.6.2 Hygienic Design of Processing Equipment

Equipment design can play a key role in minimizing microbial problems in a food processing plant. Recently, more emphasis has been given to designs that reduce cross-contamination by eliminating microbial growth niches and avoiding potential transfer points (e.g., product contact surfaces). The former refers to niches that are not easily accessible to cleaning and sanitation and can harbor microorganisms. The exterior of non-product contact surfaces (floors, walls) should also be arranged to prevent harboring bacteria, pests, etc. The food industry uses a lot of conveyer belts to transport raw and cooked food products (Fig. 15.6.2.1). Good hygienic design is therefore essential to ensure the highest level of food safety while reducing time, effort and cost of cleaning while providing economic benefits. A revised European guideline (EHEDG, 2014) is used here to illustrate the importance of the topic and provide an industry recognized source of information. Overall the document provides guidance specifically for the hygienic design of conveyor belts and is supplementary to the general requirements and standards for hygienic equipment design. The guidance applies where the foodstuff is in direct contact with the conveyor and also in those areas where there is a hygienic risk from indirect contamination. The major components of conveyors described in the document include: friction driven conveyers, positively driven conveyers, modular belts, metal and wire belts, round- and V-profile belts, frames, belt support systems, lateral guides for belts, drive stations, motors, and accessories. An example of improving the sprockets used for a positive belt drive

is shown in Figure 15.6.2.2, which made it much easier to clean and eliminate the risk of meat/food trapped in between the teeth.

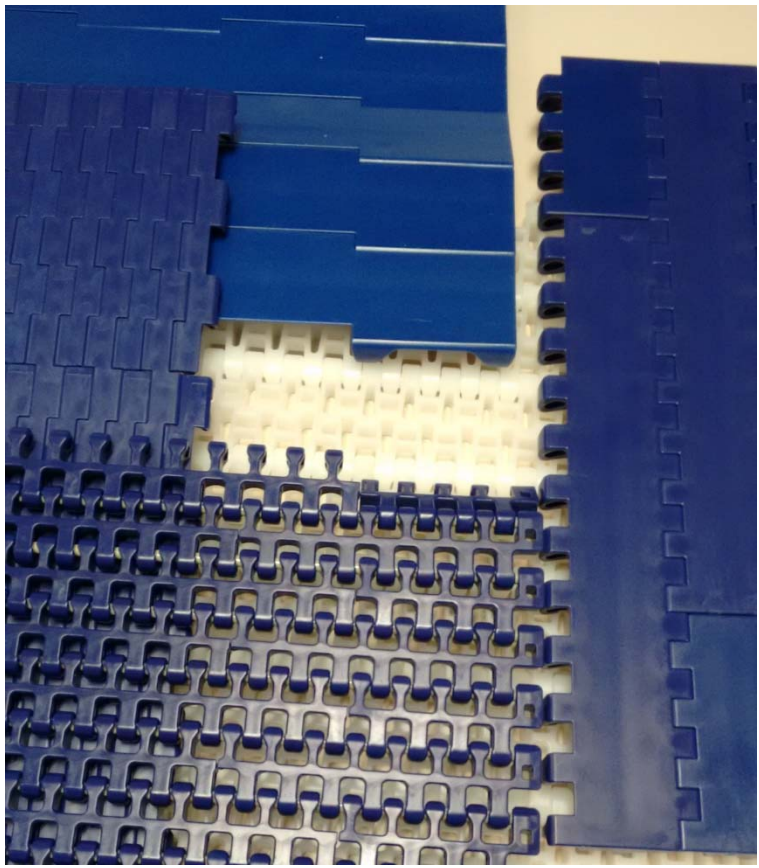

Figure 15.6.2.1 Examples of plastic conveyor belt designs used for moving fresh and cooked meats. Photo by S. Barbut.

The meat industry has only recently started to emphasize equipment design whereas the dairy industry developed its 3M sanitation standards for equipment much earlier.

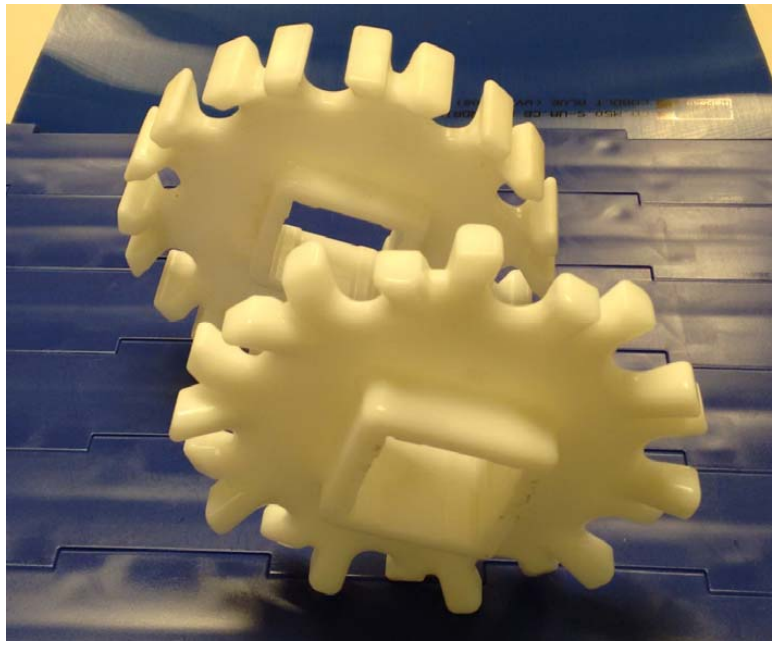

Figure 15.6.2.2 Improvement in sprockets for belt drive (front wheel with rounded teeth). Photo by S. Barbut.

Today, guidelines for hygienic design are based on different international standards (Bilgili, 2006):

a. Hygiene Requirements for the Design of Meat and Poultry Processing Equipment (American National Standard ANSI/NSF/3A 14159-1)
b. Hygiene Requirements for the Design of Hand Tools used in Meat and Poultry Processing (American National Standard ANSI/NSF/3A 14159-2)
c. Assessment for Cleanability of Belting Materials used in Meat and Poultry Processing Equipment (American National Standard ANSI/NSF/3A 14159-3)
d. 3-A Sanitary Standards
e. National Sanitation Foundation International Standards (NSF International)
f. European Norms for Food Processing Machinery
g. International Organization for Standardization (ISO)

Ten principles of sanitary equipment design were developed by the American Meat Institute (AMI, 2003) to guide new equipment design and/or modify existing equipment. The list also includes a checklist attached to each principle (see below), which allows processors to conduct an audit based on the assigned points. In such an audit the equipment must be used in the processing line for a 90-day period, disassembled to its normal daily level, and evaluated visually and microbiologically. Full points are assigned to satisfactory items, half points are assigned to marginal items, and no points are given to unsatisfactory items. An overall score of 1000 is considered acceptable whereas a score of < 1000 needs improvement. The ten design principles include:

a. Cleanable To A Microbiological Level

Food equipment must be constructed to ensure effective and efficient cleaning of the equipment over its life span (100 points total; as measured post-installation):

1. The equipment should be designed as to prevent bacterial ingress, survival, growth and reproduction on both product and non-product contact surfaces (20 points).
2. All surfaces are cleanable as measured by less than one colony-forming unit (CFU) per 25 square cm, less than one CFU per 10 ml when the item is rinsed, acceptable RLU (device specific) when measured by residual adenosine triphosphate, and/or negative for residual protein or carbohydrate when using swabs (20 points).
3. All surfaces are accessible for mechanical cleaning and treatment to prevent biofilms (20 points).
4. When requested, data is available to demonstrate that soiled equipment is cleanable as indicated above, by an individual using the cleaning protocol provided by the supplier (20 points).
5. Surfaces are clean visually and to the touch, and pass operational inspections using sight, touch, and smell (20 points).

b. Made Of Compatible Materials

Construction materials used for equipment must be completely compatible with the product, environment, cleaning, and sanitizing chemicals and the methods of cleaning and sanitation (100 points total).

1. Product contact surfaces are made with materials that are corrosion resistant, non-toxic, and non-absorbent as approved in NSF/ANBSI/3A 141159-1 (10 points).

2. In general, stainless steel shall be AISI 300 series or better (10 points).
3. Composites and plastics remain intact without changes in shape, structure, and function through cleaning and sanitation (10 points).
4. Plated, painted, and coated surfaces are not used for food contact surfaces or for surfaces above the product zone areas (10 points).
5. Coatings and plating must remain intact (10 points).
6. Cloth back belts are not used (10 points).
7. Materials such as wood, enamelware, uncoated aluminium, uncoated anodized aluminium, and others per NSF/ANSI/3A 14159-1 are not used (10 points).
8. Metals are compatible with one another (10 points).
9. Seals and O-rings are designed to minimize product contact (10 points).
10. Materials used in construction are compatible with the product, the environmental conditions to which they will be exposed, as well as cleaning methods and chemicals (10 points).

c. Accessible For Inspection, Maintenance, Cleaning and Sanitation

All parts of the equipment shall be readily accessible for inspection, maintenance, cleaning and sanitation without the use of tools (150 points total). See examples in Figure 15.6.2.3.

1. All surfaces in the product zone are readily accessible for cleaning and inspection (15 points).
2. Product zone components with inaccessible surfaces can be disassembled without tools and easily (15 points).
3. Where access or disassembly is not possible, the entire unit is cleaned by clean-in-place (CIP) or clean-out-of-place (COP) methods (10 points).
4. Parts remain attached or hung on the equipment for easy cleaning and to prevent damage and loss. Separate part carts are supplied as an alternative (5 points).
5. Machinery and chain guards drain away from product zones and are easily removed (15 points).
6. Product catch pans or drip pans are easily removable for cleanup, so they are not lost or separated from the equipment (10 points).
7. All belting is easily removable or the belt tension is removed easily without tools so the surfaces underneath can be cleaned (15 points).
8. All surfaces in non-product zones shall be readily accessible for cleaning and inspection (15 points).
9. Installation will maintain a 46 cm floor clearance for any product contact areas or conveyor travel paths. Equipment design provides 31 cm of clearance to the floor (15 points).

10. Equipment is located 77 cm from overhead structures and 92 cm from the nearest stationary object (15 points).
11. All air, vacuum, and product hoses and their assemblies on the equipment are easily removable for soaking and sanitizing (10 points).
12. All air, vacuum, and product hoses are transparent or opaque, and meet product contact surface guidelines (10 points).

a)

b)

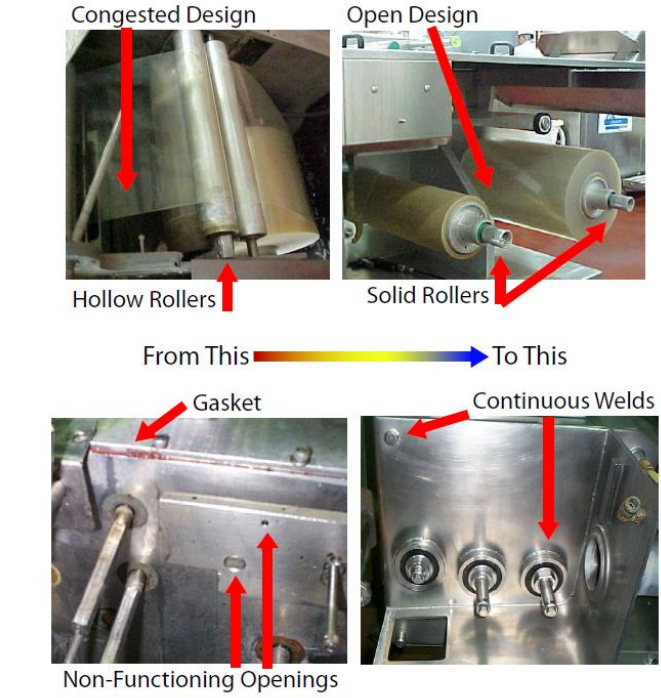

Figure 15.6.2.3 Principles of design. Pictures demonstrating potential problems and corrections related to equipment design: (a) showing the importance of using compatible materials, as related to 'Principle b' described in the text; (b) showing a potential problem with a hollow area that can trap food, as related to 'Principle c'; (c) showing how to improve on enclosure spaces needed for maintenance according to 'Principle e'. Courtesy of AMI (2014).

d. No Product Or Liquid Collection

Equipment should be self-draining to assure that liquid, which can harbour and promote the growth of bacteria, does not accumulate, pool or condense on the equipment (total 100 points).

1. All surfaces should be designed to eliminate water pooling and to be self-draining (10 points).
2. Round framework is used for horizontal members where possible (20 points).
3. Where square or rectangular bases are used, the flat surface is turned 45 degrees to horizontal where possible (10 points).
4. All open surfaces are made of sufficient strength to prevent warping and subsequent pooling of water (10 points).
5. Moisture does not drip, drain or draw into product zones (15 points).
6. Belt tension is adequate throughout operations to prevent water from pooling on the belts (15 points).
7. Dead spaces are eliminated (15 points).
8. Materials used in the construction are non-absorbent (15 points).

e. Hollow Areas Should Be Hermetically Sealed

Hollow areas of equipment, such as frames and rollers must be eliminated whenever possible or permanently sealed. Bolts, studs, mounting plates, brackets, junction boxes, nameplates, end caps, sleeves, and other such items should be continuously welded to the surface, not attached via drilled and tapped holes (150 points total).

1. All rotating members, such as drive sprockets or belt pulleys, are to be solid or filled with dye and fully sealed with continuous welds (30 points).
2. All stationary hollow tube construction, such as frame members or blade spacers, are fully sealed with continuous welds to prevent interior contamination (30 points).
3. There are no fastener penetrations into hollow tube construction (30 points).
4. Threaded leg adjustments are internal and do not penetrate the tube frame members (30 points).
5. Name plates and tags are minimized. When attached, plates and tags are continuously welded. Rivet- or screw-attached plates (often sealed with caulk) are absent (30 points).

f. No Niches

Equipment parts should be free of niches such as pits, cracks, corrosion, recesses, open seams, gaps, lap seams, protruding ledges, inside threads, bolt rivets and dead ends (150 points total).

1. Surface texture of a product contact surface shall not exceed 32 microns, except as described in NSF/ANSI/3A 14159-1 (10 points).
2. Surface texture on a non-product contact surface shall not exceed 125 microns (10 points).
3. Internal corners and angles shall have a smooth and continuous radius of at least 3 mm (angles < 35 degrees) (10 points).
4. No lap joints (10 points).
5. Hermetically sealed spacers are used to allow for space between two adjoining pieces to permit mechanical action during cleaning (10 points).
6. Caulking is not used (10 points).
7. All joints and welds are flush and free of pits, cracks, and corrosion (10 points).
8. All welds are continuous, smooth and polished (10 points).
9. Sleeved assemblies (bushings, sprockets, and bearings) are no longer than 1.5 inches or are disassembled for cleaning (10 points).
10. Press and shrink fits are not used (10 points).
11. Fasteners are not used in or above product zone (10 points).
12. Fasteners that are product contact surfaces must utilize the ACME 60-degree stub thread (10 points).
13. If fasteners are necessary, they do not have exposed threads and have a positive locking method to prevent falling or vibrating off the machine (10 points).
14. Belt scrapers do not have lap joints and are removed without tools (10 points).
15. Belt supports are constructed from single pieces of material (10 points).

g. Sanitary Operational Performance

During normal operations, the equipment must perform so it does not contribute to unsanitary conditions or the harbourage and growth of bacteria (100 points total).

1. Buttons on control panels are easily cleaned and sanitized during operations (15 points).
2. All compressed air used for blowing on the product or contact surfaces is filtered to a minimum of a 0.3 micron level and dried to prevent the formation of moisture in the piping system (15 points).

3. No bearings are present in the product contact zone areas (15 points).
4. A separation exists between the product contact and non-product contact areas to prevent cross-contamination during operation (15 points).
5. All surfaces near the product contact zone areas are designated as if they were product contact zone areas (15 points).
6. Product contact surfaces are made to prevent accumulation of product residue during operation (15 points).
7. Shafts passing through a product zone shall have an air gap to prevent product contamination (10 points).

h. Hygienic Design Of Maintenance Enclosures

Maintenance enclosures and human machine interfaces such as push buttons, valve handles, switches, and touch screens, must be designed to ensure food product, water or product liquid does not penetrate or accumulate in or on the enclosure or interface. Also, physical design of the enclosures should be sloped or pitched to avoid use as storage area (50 points total).

1. Drives, chain guards, electrical control boxes, and bearings are not located over open product zones (10 points).
2. Control and junction boxes are fastened to the frame in a manner consistent with the sanitary design principles (10 points).
3. Utility supply lines and pipes are separated to prevent catch points and allow for cleaning (5 points).
4. Utility lines are 31 cm above the floor and cleanable (5 points).
5. Conduit and supply lines are not routed above product contact areas (10 points).
6. Maintenance enclosures in direct wash-down areas must be able to be exposed to water and chemicals used in cleaning and sanitation (10 points).

i. Hygienic Compatibility With Other Plant Systems

Equipment design should ensure hygienic compatibility with other equipment and systems, such as electrical, hydraulics, steam, air and water (50 points total).

1. Exhaust systems have welded seams with adequate access for cleaning and inspection (10 points).
2. Vertical duct sections have a drain to prevent drainage from flowing back into the equipment (10 points).
3. Separate exhausts are supplied for raw and ready-to-eat product zones (10 points).

4. CIP systems are designed, installed, and validated using a recognized third-party in sections of duct work that are not easily cleaned through access openings (10 points).
5. Equipment is designed to meet criteria of waste water infrastructure capability to assure no backups of drainage lines result under normal operation (10 points).

j. Validate Cleaning And Sanitation Protocols

Procedures for cleaning and sanitation must be clearly written, designed and proven effective and efficient. Chemicals recommended for cleaning and sanitation must be compatible with the equipment and the manufacturing environment (50 points total).

1. Cleaning and sanitizing are considered in the design process (10 points).
2. Cleaning protocols must be safe, practical, effective, and efficient (10 points).
3. Cleaning and sanitation protocols are developed by the manufacturer, validated by a third-party, and provided in a training manual that is easy to read and understood by cleaning and sanitation employees (10 points).
4. Equipment design and materials are capable of withstanding standard cleanup procedures. Equipment materials have been reviewed with Materials Safety Data Sheets for the cleaning and sanitizing chemicals to assure compatibility (10 points).
5. All belts should withstand heating to 71°C for up to 30 minutes (10 points).

Design emphasis is placed not only on efficiency and safety, but also on hygiene. The latter has become a non-competitive issue (AMI, 2003, 2014) and information is shared among equipment manufacturers and processors.

In conclusion, it is important to understand that meat is a perishable food item because it contains all nutrients required for microbial growth and its pH (5.5-6.5) is not inhibitory to most spoilage and pathogenic microorganisms. The extensive fabrication, handling (ground meat can be handled 10-12 times before it gets to the consumer), and distribution of meat can increase exposure to microbial contamination. Living, healthy muscle is essentially free of microorganisms but after slaughter the natural defense mechanisms no longer function. During slaughter, the blades that cut through the skin can transfer microorganisms into the bloodstream. Because blood circulation is not immediately stopped, this can distribute microorganisms throughout the carcass. It is important to realize

that 1 g of soil (dirt or manure) attached to the skin or feathers can contain 1 billion microorganisms. Evisceration or removal of the digestive tract is another significant potential point of contamination. The digestive tract harbours high numbers of microorganisms (e.g., 100 million microorganisms/g) and if it is ruptured and its contents spilled on the carcass, high contamination levels are expected. Other potential contamination sources can come from people handling the meat, air coming into the plant (or moved from the primary to the secondary processing area), water used to rinse the carcasses/equipment, and insects getting into the plant. All surfaces in contact with the meat should be periodically cleaned and disinfected (e.g., a common practice for a manual cutting operation is dipping knives in a > 80°C water bath) and employee hygiene should be enforced at all times. This includes measures such as hair nets (mandatory in most food processing plants), clean gloves, aprons and coats, removal of jewelry, and mandatory hand washing before starting work. In some specialized operations, such as packaging of cooked products, employees might be required to cover their nose and mouth with a mask to minimize the spread of microorganisms. This can be another important measure to reduce the risk of pathogens and also increase the shelf life of the product. In such an operation, air filters for incoming air are usually installed as well as keeping a positive air pressure within the room in order to prevent suction of air from other areas of the plant. Today there is also more emphasis on consumer education where cooking instruction labels and raw meat handling stickers appear on meat packages. All of these measures are integral in achieving the larger goal of supplying the customer wholesome and safe food.

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