CURRENT AND FUTURE TECHNOLOGIES FOR THE DECONTAMINATION OF CARCASSES AND FRESH MEAT

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Abstract:

The objective of this review is to describe current methods and technologies used to decontaminate food animal carcasses in the United States and describe new technologies and methods that are under development. Bacterial reduction during the conversion of muscle to meat has always been an important challenge for the meat processing industry due to the impact on product safety and quality. More intense microbiological testing and improved microbiological methods have led to a greater awareness by industry and government about the levels of pathogenic bacteria on meat carcasses and in meat products. This increased awareness has spurred research and development on new technologies implemented sequentially in the process, aimed at reducing and eliminating bacteria on carcasses and meat products.

Introduction:

The harvest of livestock and the subsequent processing of raw meat products from livestock is a process that will consistently produce safe meat products for the consuming public provided the meat is handled safely and is properly cooked prior to consumption. However, history has shown that bacterial pathogens may evade even the best efforts to eliminate them by industry, government, and consumers. This may lead to adverse regulatory implications for the firm, or more importantly, may lead to foodborne illness in certain persons who may consume those products without a proper heat treatment prior to consumption.

Historically, skeletal muscle from healthy animals has been considered sterile prior to slaughter, with the exception of the lymph nodes (Romans, Costello, Carlson, Greaser, and Jones, 1994). There have been reports of bacteria identified in animal tissues (Ingram and Dainty, 1971; Zagaevskii, 1973; Johnson, Doyle, Cassens, and Schoeni, 1988). While intrinsic bacteria may rarely be present in low levels in muscle tissues, this is not the most common source of contamination. Extrinsic factors are by far the greatest contributor to carcass and meat contamination. Bacteria may come in contact with the meat carcass during steps in the harvest stage where external surfaces of the carcass become exposed to potential sources of contamination. Meat carcasses may become contaminated from fecal material, paunch contents, and the hide according to Lahr (1996). Additional sources of cross-contamination exist in the slaughter process, such as processing tools and equipment, structural components of the facility, human contact, and carcass-to-carcass contact. Fortunately, the majority of microflora transferred to carcass surfaces, while aesthetically undesirable, are non-pathogenic (IFT, 2002).

Decontamination techniques for carcasses are targeted at reducing or eliminating bacteria that may be human pathogens as well as those that may cause meat spoilage. According to Kotula and Kotula (2000) the bacteria of most concern for meat spoilage include *Pseudomonas*, *Acinetobacter/Moraxella, Aeromonas, Alteromonas putrefaciens, Lactobacillus*, and *Brochothrix thermosphacta*. The pathogenic bacteria of most concern include *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter*, *Clostridium botulinum, Clostridium perfringens, Staphylococcus aureus, Aeromonas hydrophila,* and *Bacillus cereus*. Generally, conditions created by decontamination methods that lead to the reduction of overall levels of bacteria as measured by total aerobic plate counts or total coliforms, provide some indication of the potential effects on pathogens of concern. However, since this does not hold true in all cases, validation studies conducted in laboratory settings have specifically measured reductions of artificially inoculated bacterial pathogens.

Meat processors strive to produce raw products that have low levels of bacteria on the surface and no pathogenic bacteria; however, the process is not conducted in a sterile environment and contamination is unavoidable, and occasionally pathogenic microorganisms may come in contact with the surface of the meat carcass. Routine slaughter practices have evolved over the years to reduce the likelihood of inadvertent microbial contamination. This evolution has led to the adoption of the hurdle technology approach to microbial carcass interventions. Routine activities, such as sanitary hide removal and rapid carcass chilling, have been combined with a series of technologies, both physical and chemical in nature, to achieve a lower likelihood of carcass contamination, or to prevent growth of bacteria if present. Leistner (2002) describes the principles of hurdle technology and states that if the initial microbial load is substantially reduced as a result of carcass decontamination procedures, fewer microorganisms are present, which are then more easily inhibited in subsequent processing steps. The effectiveness of hurdle technology during the slaughter process has been demonstrated experimentally for beef decontamination technologies under controlled conditions by Graves Delmore, Sofos, Schmidt and Smith (1998), and Castillo, Lucia, Goodsen, Savell, and Acuff (1998). The concept of hurdle technology for beef carcass decontamination has also been validated to be effective in field observations in beef processing facilities (Bacon, Belk, Sofos, Clayton, Reagan, and Smith, 2000a).

Significant research in recent years has been focused on the potential for control or reduction of microorganisms, including pathogens, in livestock prior to slaughter. Numerous challenges exist in validating and implementing microbial intervention strategies at the farm level, however, if livestock could arrive at the slaughter facility with lower prevalence and/or lower quantitative levels of certain microorganisms, the effectiveness of subsequent intervention strategies in the slaughter process could be enhanced. It would seem appropriate that if successful intervention strategies could be identified and proven practical in field settings, the concept of "hurdle technology" as described by Leistner (2002) could be extended to the pre-harvest stage.

The remainder of this manuscript will provide an overview of the occurrence of bacterial contamination on meat carcasses, and of current and future technologies that may have the ability to reduce bacterial levels. The information provided describes methods and technologies that are common in the United States since those are the systems with which the author is most familiar. Technologies and practices may differ in other parts of the world.

Prevalence of Bacteria on Meat Carcasses:

Numerous studies have been conducted that provide insight on the percentage of carcasses or lots of product that can be shown through testing to be positive for pathogenic bacteria. The U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) conducted a nationwide microbiological baseline study to assess the levels of bacteria on meat product categories. USDA-FSIS (1994, 1996b, 1996c, 1996d) sampled 2,000 steer/heifer, 2100 cow/bull, 2,100 pig carcasses and 563 ground beef samples over a period from October 1992 to March 1996 (Table 1). Aerobic plate count at 35oC, total coliforms, *E. coli* (biotype I), *C. perfringens* and *S. aureus*, are reported as colony forming units (CFU) per square centimeter (cm^2) of surface area analyzed. The pathogens *L. monocytogenes*, *C. jejuni/coli*, *E. coli* O157:H7, and *Salmonella*, because they require enrichment, are reported as most probable number (MPN) per square

centimeter of surface area analyzed. The minimum detection limit for the MPN method was 0.03 MPN/cm^2. Nearly 100% of meat categories tested had detectable levels of aerobic bacteria at the time of sampling. The geometric means ranged from 47 cfu/cm^2 for steer and heifer carcasses to 7,900 cfu/g for ground beef. Coliform prevalence ranged from 16% of samples for steers and heifers to 92% of samples for ground beef. Prevalence and quantitative levels for the pathogenic bacteria measured in this survey were rather low. *Escherichia coli* O157:H7 was not detected in cow and bull carcasses, pork carcasses or ground beef samples, and was only detected in 0.2% of steer and heifer carcasses. By contrast, prevalence of *Staphylococcus aureus* ranged from 45% in steer and heifer carcass samples to 30% in ground beef samples.

The USDA-FSIS conducts routine testing for *Escherichia coli* O157:H7 in ground beef from federally inspected facilities and in distribution at retail supermarkets. Since the advent of this surveillance program in 1995, there have been over 44,000 samples collected. During the fiscal year 2000, the percentage positive rate was .86% and in 2001 the rate was .87%. Additional data on important pathogenic bacteria on beef is available from USDA through the results of the USDA school lunch ground beef-purchasing program (USDA, 2002). For the purchasing period 2001 – 2002, 1,491 samples were collected and 1.01% and 3.96% of samples were positive for *Escherichia coli* O157:H7 and *Salmonella*, respectively.

Since the collection of the USDA-FSIS microbiological baseline data, there have been other published reports on prevalence that have indicated higher levels of certain organisms on cattle and meat carcasses. Most notably was the publication of researchers at the USDA Agricultural Research Service (ARS) (Elder, Keen, Siragusa, Barkocy-Gallagher, Koohmaraie, and Lagreid, 2000) which indicated levels of *Escherichia coli* O157 on beef cattle and carcasses were higher than previously reported. In this study cattle feces, hides, and carcasses at three points in the slaughter process were sampled at four major midwestern packing plants. These authors reported that 1.8% of carcass samples taken post-processing (similar to the point in the process of the USDA baseline survey) were positive for *Escherichia coli* O157 and 16.7% of the lots tested had at least one positive carcass. This study also analyzed carcass samples pre-evisceration and post-evisceration (prior to antimicrobial treatment) and revealed 43.4% of samples and 86.7% of the lots were positive pre-evisceration, and 17.8% of samples and 56.7% of lots tested positive post-evisceration. These authors attribute the greater prevalence of *Escherichia coli* O157 in this study to improved sampling methods and analytical techniques. An important conclusion by these authors was that current in-plant processing practices appear to reduce the level of carcass contamination with *Escherichia coli* O157. Bacon, et al. (2000a) drew similar conclusions in an evaluation of eight commercial beef slaughter facilities when they reported substantial reductions (14.7% to 1.9%) in *Salmonella* spp. from the beef carcass hide to the post-intervention, chilled carcass. Other bacterial measurements including total aerobic plate count, total coliforms and *Escherichia coli* Biotype I, showed similar reductions. Bacon, Belk, Sofos, and Smith (2000b) reported on a separate field evaluation of twelve beef slaughter facilities and reported that the incidence of *Escherichia coli* O157:H7 was 3.6%, 0.4%, and 0.0% for samples collected from beef hides, from carcasses prior to washing, and from carcass sides following final decontamination, respectively.

These studies provide support that levels of total bacteria and pathogenic bacteria are reduced in the slaughter process, when one takes into consideration the levels of bacteria in or on livestock as they are presented for slaughter. However, the systems in place today do not provide for complete elimination of spoilage bacteria or pathogens. The following sections will examine technologies that have been studied to reduce bacterial contamination.

Pre-harvest Reduction of Bacteria on Livestock:

Effects of diet:

Significant research has been done on the effects of manipulating feed ingredients and/or practices (dietary modification) on the shedding of pathogenic bacteria, especially on the shedding of *Escherichia coli* O157:H7. Diez-Gonzales, Callaway, Kizoulis, and Russell (1998) gained significant notoriety when their work published in Science showed that a brief period of feeding hay to cattle that had previously been fed a grain diet, significantly reduced the shedding of acid-resistant *Escherichia coli*. In contrast, Tkalcic et al. (2000) demonstrated that calves fed either a high-roughage diet or high-concentrate diet did not differ in levels of *Escherichia coli* O157:H7 or in length of shedding of *Escherichia coli* O157:H7. Buchko, Holley, Olsen, Gannon, and Veira (2000) evaluated effects of three different grain diets on shedding of an inoculated strain of *Escherichia coli* O157:H7. These authors reported that while levels of a marker strain of *Escherichia coli* shed by cattle on all diets did not differ, there were significantly fewer animals shedding the organism when on the diet of 85% cracked corn versus the diet of 85% barley. This continues to be an area of active research, however, to date; there have been no proven methods of simple dietary modification that effectively reduces pathogenic bacteria in livestock destined for slaughter. Proposals aimed at dietary modification must be balanced with the practical applications of commercial livestock feeding operations. Research on the addition of compounds to feed, water, or feeding environment have shown promise in pilot studies and are described below.

Competitive Exclusion:

An Expert Panel Report on Emerging Microbiological Food Safety (IFT, 2002) states that significant effort has been focused recently on interventions in live animals. On-farm technologies such as feeding probiotics are under development. The panel states that decreasing the level of *Escherichia coli* O157:H7 in cattle would significantly decrease the potential for food and water contamination. Similar statements could be made concerning other pathogens and other livestock species, including poultry.

One potential option that has been extensively studied is the concept of utilizing the benefits of certain "good" bacteria to prevent or reduce colonization of pathogens in the gut of food animals. This concept has been described as "competitive exclusion". A competitive exclusion product with the trade name Preempt™ gained U.S. Food and Drug Administration approval in 1998. This product is a mixture of 29 unique bacteria commonly found in the gastrointestinal tract of mature, healthy chickens. When young chicks are treated shortly after hatching the prevalence of birds positive for *Salmonella* drops significantly (Hume, Corrier, Nisbet, and DeLoach, 1998).

Selected probiotic bacteria administered to cattle prior to exposure to *Escherichia coli* O157:H7 was shown to reduce the level of carriage of *Escherichia coli* O157:H7 in beef calves by Zhao, Doyle, Harmon, Brown, Mueller, and Parks (1998). Murinda, Roberts, and Wilson (1996) have studied colicins, which are proteins produced by strains of *Escherichia coli*, that exert specific inhibitory activity against closely related strains. These researchers screened 24 colicin-producing strains for inhibitory activity against 27 strains of pathogenic *Escherichia coli*, including 11 strains of serotype O157:H7. In these in vitro studies, one specific colicin, Colicin E2, inhibited all 11 strains of *Escherichia coli* O157:H7. These authors concluded that potential exists for colicins to control pathogenic strains of *Escherichia coli* including *Escherichia coli* O157:H7.

Currently, there are other studies underway in the U.S. that have shown promising results for probiotic bacterial treatment. The potential for a feed ingredient to prevent colonization of *Escherichia coli* O157:H7 in beef cattle has been demonstrated under controlled experimental conditions but has yet to be proven under actual field conditions.

Drinking water treatment:

Water has been shown to be a primary reservoir of *Escherichia coli* O157:H7 in the pre-harvest environment (LeJeune, Besser, and Hancock, 2001). Treatment of livestock drinking water may prove an effective control point in the pre-harvest environment. LeJeune et al. (2001) demonstrated that *Escherichia coli* O157:H7 could survive for up to 245 days in the sediment of a simulated water trough. The primary treatment of municipal water supplies in the U.S. is chlorination at a minimum level of 0.2 ppm of free chlorine. A survey of municipal water supplies in the U.S. in 1992 indicated that the median level of chlorine residual in the water system prior to the point of first use was 1.1 ppm with an exposure time of 45 minutes. Rice, Clark, and Johnson (1999) reported that chlorine at 1.1 ppm free chlorine would inactivate 4 log cfu/ml of seven different strains of *Escherichia coli* O157:H7 within 1 min. Doyle (2002) has demonstrated similar results, however his lab has identified one strain of *Escherichia coli* O157:H7 that is particularly tolerant to the chlorine treatment, suggesting that strain variability may exist with regards to chlorine treatment. While standard municipal water treatment would appear to be effective against even the more tolerant strains of *Escherichia coli* O157:H7, the effects of large quantities of organic materials in drinking water troughs on the farm or in the feedlot would likely negate the effectiveness of this treatment. Other methods of treatment of drinking water are being evaluated, as this route seems to provide a logical point for exerting control in the pre-harvest system.

Chlorate administration:

Researchers at the USDA –ARS in College Station, Texas (Anderson et al. 2001a, Anderson et al. 2001b) have reported that the oral administration of sodium chlorate reduced intestinal concentrations of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in experimentally infected pigs and wild type *Escherichia coli* concentrations in non-challenged pigs. Anderson et al. (2001a, 2001b) hypothesize that the mechanism within *Escherichia coli* and *Salmonella* that reduces nitrate to nitrite using respiratory nitrate reductase activity will also reduce chlorate to cytotoxic chlorite, which can destroy the bacterial cell. Additional field trials are underway to determine if this pre-harvest treatment has potential application.

Other pre-harvest technologies:

Research is ongoing in several key areas to find practical methods to reduce bacteria in the pre-harvest stage. The search for a vaccine for cattle that would potentially immunize cattle against intestinal colonization of *Escherichia coli* O157:H7 is on-going and several research labs have active programs in this area. A large field trial is underway in Canada (Potter, 2000) to determine if preliminary results on a potential cattle vaccine for *Escherichia coli* O157:H7 have promise. Several research groups are exploring the possibility of administration of bacteriophage, active against targeted pathogenic bacteria, as a means of control. Preliminary data have shown mixed results with this approach.

Post-Harvest Decontamination Techniques:

Dickson and Anderson (1992) compiled a review of the then-current decontamination techniques studied on fresh meat and concluded that a decontamination step during the slaughtering process can reduce contamination and possibly contribute to improvement of shelf-life and safety and should be an essential part of the slaughtering/dressing process. The USDA-FSIS (1996a) has also recognized that a decontamination step should be a part of the slaughter dressing process in guidance materials. In general, washing and sanitizing agents have been effective in reducing bacterial populations and presence of pathogens on carcasses. The following sections cover some of the more widely researched intervention strategies.

Chemical dehairing

Bowling and Clayton (1992) describe a method for dehairing beef cattle using a chemical treatment. The process of chemical dehairing is described as three bacteriostatic/bactericidal steps: application of sodium sulfide; use of hydrogen peroxide; and rinsing with lactic acid. Chemical dehairing was studied in a commercial beef slaughter facility by Schnell et al. (1995). The process was applied immediately post exsanguination on beef steers and the microbiological profile was compared to normally processed cattle. While these authors were able to demonstrate a reduction in visible contaminants in cattle that had been subject to chemical dehairing versus cattle processed conventionally, aerobic plate counts were not significantly different between the two treatments. Castillo, Dickson, Clayton, Lucia, and Acuff (1998) reported that chemical treatment of bovine skin could reduce counts of pathogenic bacteria that had been artificially inoculated. Graves-Delmore, Sofos, Schmidt, Smith (1997) also reported reduction in pathogenic bacteria on beef hides after treatment with a chemical process.

Hot water rinse:

USDA–FSIS (1996a) acknowledges that significant scientific evidence exists to conclude that hot water (>74° C) will produce a sanitizing effect on carcasses. Specific research reports find varied levels of effectiveness from hot water, which is likely due to study differences in bacterial attachment time, sampling of lean vs. fat tissues, and factors related to the microorganisms that were assayed. Kelly, Dempster, and McLoughlin (1981) measured significant decreases of greater than 1.0 $\log_{10}/cm^2$ in aerobic plate counts on lamb carcasses sprayed with hot water at temperatures of 80°C or greater. Barkate, Acuff, Lucia, and Hale (1993) reported that when the surface temperature of beef carcasses was raised to 82°C for about 10 seconds using hot water sprays (95°C), significant reduction in aerobic plate counts was observed. When hot water was applied prior to final carcass wash the mean reduction was 1.3 $\log_{10}cm^2$ while the mean reduction for carcasses treated after the final wash was 0.8 $\log_{10}cm^2$. Gorman, Sofos, Morgan, Schmidt, and Smith (1995) demonstrated spray washing was more effective at removing visible fecal contamination and *Escherichia coli* on beef tissue when the pressure and temperature of the water in the spray was increased from 2.76 to 18.89 bar and from 16°C and 35°C to 74°C, respectively. Acuff, Castillo, and Savell (1996) reported significant reductions in total coliforms, thermotolerant coliforms, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 when beef carcasses were sprayed at 95°C at 24 psi for 5 sec at a distance of 12.5 cm. In summary, the data would indicate that hot water wash applications to carcasses have been experimentally validated to reduce bacterial counts by 1- to 3-$\log_{10}$ cycles. To effectively implement hot water as a decontaminant step, validation and ongoing verification activities must account for water temperature, water pressure, carcass coverage, and dwell time. There has been concern expressed by industry concerning the effect of hot water on carcass discoloration. This has been addressed by several researchers and in a review by Castillo, Hardin, Acuff, and Dickson (2002) reported that washing carcasses with water at temperatures greater that 80°C did not produce permanent discoloration of the carcass surface. In summary, hot water treatment (>74°C) of beef carcasses is widely practiced in the industry.

Ellebracht, Castillo, Lucia, Miller, and Acuff (1999) reported on the effects of using hot water and lactic acid to reduce pathogens on beef trimmings prior to grinding. These authors reported that hot water treatment of 95°C for 3 sec reduced *Escherichia coli* O157:H7 and *Salmonella* Typhimurium by 0.5 $\log_{10}$CFU/g and 0.7 $\log_{10}$CFU/g, respectively. The authors are quick to point out that the system described is not approved by USDA – FSIS because trimmings are not be allowed to gain water weight during the treatment, and in this treatment scenario the trimmings experienced a 1.31% increase in weight due to the hot water treatment.

Steam Pasteurization:

A logical extension of the work described with hot water washes is the use of condensing steam as a means to accomplish thermal destruction of bacteria on the surface of meat carcasses. Phebus, Nutsch, and Schafer (1996) described the use of steam pasteurization and demonstrated that it is efficacious for lowering microbiological counts on beef tissues. Nutsch et al. (1998) evaluated a steam pasteurization system in a commercial beef processing facility and found significant reductions in total aerobic plate counts and *Escherichia coli* counts at five separate anatomical locations on the carcasses. A recent report addressed the theory that carcass surfaces that had been previously exposed to a steam treatment may be more susceptible to bacterial attachment if re-contaminated (Warriner, Eveleigh, Goodman, Betts, Gonzales, and Waites, 2001). These authors concluded that steam pasteurization treatment does not promote or inhibit bacterial reattachment on beef carcass surfaces. Regarding the effects of this steam pasteurization on meat color, Phebus et al. (1996) reported that steam pasteurization treatment exposure times of <15 sec resulted in an initial meat surface color graying immediately after treatment, but after a standard 24 hour chilling period, acceptable color returned. USDA-FSIS (1996a) permits the use of steam for carcass decontamination. The commercialization of the steam pasteurization system has been successful and the systems are in use in many large beef slaughter facilities in the U.S.

Steam Vacuum

A variation on the use of whole carcass steam pasteurization has been described by Dorsa (1996). Steam (or hot water) is sprayed on a beef carcass followed by vacuuming, which has the combined effect of removing and/or inactivating surface contamination. The hand-held device includes a vacuum wand with a hot water spray nozzle, which delivers water at approximately 82 – 88°C to the carcass surface, as well as the vacuum unit. Steam vacuuming is approved for use by USDA-FSIS as a substitute for knife trimming for removing fecal and ingesta contamination when such contamination is less that 2.54 cm at its greatest dimension. Kochevar, Sofos, Bolin, Regan, and Smith, (1997) demonstrated that two different steam / hot water vacuum systems reduced aerobic plate counts and total coliform counts by 1.1 to 2.3 and 1.2 to 2.2 $\log_{10}$ CFU/ cm^2, respectively, from initial levels of 4.6 to 5.1 and 2.9 to 3.2 $\log_{10}$ CFU/cm^2. Dorsa, Cutter, Siragusa, and Koohmaraie (1996) reported reductions in aerobic plate counts, total coliform counts and *Escherichia coli* counts of 6.2, 5.0 and 4.8 $\log_{10}$CFU/cm^2, respectively to 3.2, 1.0 and 0.8 $\log_{10}$CFU/cm^2 when beef short plates were artificially inoculated. Steam vacuuming has gained wide acceptance by industry as an effective tool for spot decontamination on the slaughter floor prior to final inspection and chilling. A more recent publication has evaluated the possibility of utilizing steam vacuum after carcass chilling to further enhance removal of small diameter contaminated surfaces (Bacon, Sofos, Belk, and Smith 2002b). The authors reported that the use of steam vacuum on a chilled beef surface tissue did not appear to provide an effective means of removing inoculated *Salmonella* organisms. Bacterial attachment time and the formation of biofilms on the surface were offered as possible explanations for the ineffectiveness of the steam vacuum in this study.

Chemical rinses

The most frequently used chemical decontaminants are solutions of organic acids (Belk, 2001). Baird Parker (1980) states that the undissociated molecule is likely responsible for the antimicrobial properties of organic acids. Booth (1985) describes the effect as being related to the accumulation of undissociated weak acids in the cytoplasm of the cell. If intracellular pH is higher than the pKa of the acid, the protonated acid will dissociate, releasing a proton thus acidifying the cytoplasm of the microorganism. USDA-FSIS (1996a) has approved the use of organic acid solutions such as acetic, lactic and citric acids at concentrations of 1.5 - 2.5%.

Organic acids are typically applied as a rinse to the entire surface of the carcass. Castillo et al. (2002) in a book chapter on reduction of microbial contaminants on carcasses, provide a comprehensive review of organic acid sprays. Of the organic acids evaluated in the literature, acetic and lactic acids have been most widely accepted as carcass decontamination rinses. Additionally, it has become widely accepted that the effectiveness of organic acids is best achieved shortly after hide removal, when the carcass is still warm. Numerous studies have reported on the effects of organic acids on general bacterial populations as well as certain pathogenic organisms (Snijders, van Lotestijn, Mossel, and Smulders, 1985; Dickson and Anderson 1992; Hardin, Acuff, Lucia, Oman, and Savell, 1995; Dorsa, 1996; Castillo, Lucia, Mercado and Acuff, 2001). Snijders et al. (1985) reported on several studies on the use of lactic acid on various species including beef, veal, and pork and at various points in the process. These authors reported that lactic acid treatment, when applied early post-mortem to the hot carcass surface, could reduce aerobic plate count by 1.5 log cycles. Hardin et al. (1995) reported that a beef carcass wash followed by a 2% acid spray was more effective than either trimming or washing with water alone, in the reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. These studies were conducted within 45 minutes post-exanguination while the carcass was still warm. In contrast, Castillo et al. (2001) attempted to obtain reductions in aerobic plate count, coliforms and *Escherichia coli* on cold carcass surfaces using a lactic acid solution (4%) heated to 55°C. This study was conducted in a commercial facility and the data indicate that the cold carcass application was effective when lactic acid was applied at higher temperatures. The authors believed that this data confirms the effectiveness of being able to use a lactic acid treatment downstream from the slaughter floor on chilled carcasses and primals.

Calicioglu, Kaspar, Buege, and Luchansky (2002) conducted a study that included an evaluation of the effect of pre-spraying a nonionic surfactant, Tween 20, prior to addition of lactic acid spray to beef carcasses. These authors reported average total reductions of *Escherichia coli* O157:H7 of 2.0, 3.1, and 3.4 $\log_{10}$ cfu/cm^2 for carcass cuts treated with sterile water, with lactic acid, and with lactic acid in combination with sodium benzoate, respectively, when the carcass had been pre-treated with Tween 20 (5% vol/vol). The authors postulate that Tween 20 may loosen or prevent attachment of bacteria with its surfactant and hydrophobic effects, thus making the cells more vulnerable to the effect of lactic acid.

A recently approved proprietary compound from Mionix has shown promising results in recent trials (Teat, 2002). $Safe_2O^{TM}$-Beef Carcass Wash is low-pH proprietary blend of GRAS components including acidic calcium sulfate and lactic acid. This carcass rinse treatment was compared to a "current practice" chlorine rinse on the neck and shoulder area of beef carcasses post-chill. The carcasses treated with the proprietary compound had an average of 0.7 $\log_{10}$CFU for aerobic bacteria compared with an average of 3.4 $\log_{10}$CFU on chlorine treated carcasses. Seventy three percent of the carcasses treated with this compound had non-detectable aerobic plate counts, while in comparison; none of the chlorine treated carcasses had non-detectable levels of bacteria.

Castillo, Lucia, Kemp, and Acuff (1999) examined the effectiveness of citric acid-activated acidified sodium chlorite spray (CASC) for reducing inoculated *Escherichia coli* O157:H7 on beef carcasses. The compound was applied at room temperature, to surfaces that had been inoculated with *Escherichia coli* O157:H7 at 5.5 $\log_{10}$CFU/cm^2 and a reduction of 4.5 log cycles was observed. CASC effectively reduced the amount of pathogens spread to areas beyond the initial contaminated area of cuts to levels close to or below the counting method detection limit (0.5 $\log_{10}$CFU/cm^2). While reductions were achieved, 22% to 50% of the artificially inoculated carcasses treated by CASC still yielded countable *Escherichia coli* O157:H7 colonies.

Several researchers have studied the effect of organic acids on sensory properties, primarily meat color. Bell, Marshall and Anderson (1986) reported that treating beef with 1.2% v/v acetic acid for 1 min did not result in discoloration, however, a solution of 0.6% lactic acid for 10 min resulted in significant discoloration when compared with untreated controls. Goddard, Mikel, Conner, and Jones (1996) treated beef strip loins with a mixture of lactic and acetic acid and found not differences in meat color, fat color, or odor when compared to untreated controls.

The use of organic acids must be considered with some degree of caution, in light of recent research indicating that acid adaptation of *Escherichia coli* O157:H7 and other pathogens may occur in dilute decontamination fluids in meat packing plants. Samelis, Sofos, Kendall, and Smith, (2002) reported that a previously adapted *Escherichia coli* O157:H7 strain survived for extended periods (up to 14 days) in acid containing waste fluids from meat decontamination. Furthermore, the authors point out that survival may increase when acetic acid rather than lactic acid is used for carcass decontamination.

Kim and Slavik (1996) provided evidence that cetylpyridinium chloride (CPC) was effective in reducing the numbers of *Salmonella* on poultry skin. Compared with controls, CPC spraying reduced the numbers of *Salmonella* by 0.9 to 1.7 $\log_{10}$ units. Similar results were obtained when the poultry skin was immersed (1.0 to 1.6 $\log_{10}$ unit reductions). These authors concluded that CPC was effective at reducing *Salmonella* and on the basis of the amount of CPC used, immersion appears to be more cost-effective than spraying CPC on poultry skin. Cutter et al. (2000) reported that spray-washing (862 kPa, 15 s, 35°C) beef adipose surfaces with a 1% (wt/vol) solution of CPC reduced 5 to 6 $\log_{10}$ CFU/cm^2 of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium to virtually undetectable levels (0 $\log_{10}$ CFU/cm^2). However, residual CPC levels following any of the treatments were considered excessive for human consumption (Cutter *et al.*, 2000). USDA-FSIS approval for use of CPC on meat carcasses has not been granted for CPC at the time of this writing.

Lactoferrrin:

Naidu and Bidlack (1998) describe a group of compounds classified as microbial blocking agents, and suggest that lactoferrin, an iron binding protein, has the potential to be an antimicrobial in foods. Lactoferrin can be found naturally in milk, saliva, tears, seminal fluids, mucins and the secondary granules of neutrophils (Naidu, 2002). This compound can be extracted in commercial quantities from skim milk or whey. Naidu (2002) describes a patented process that results in "activated lactoferrin"; a compound that has received generally recognized as safe (GRAS) status from the U.S. Food and Drug Administration and has recently been accepted by the USDA-FSIS for use on fresh beef. Naidu (2002) describes the potential for this compound as a spray treatment on carcasses or on chilled primal cuts as a microbial blocking agent that can interfere with adhesion / colonization, cause detachment of live or dead microorganisms from biological surfaces, deter microbial growth, and neutralize the activity of endotoxins. The author states that activated lactoferrin has demonstrated activity against an array of bacterial pathogens including *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter*, as well as some meat spoilage organisms including *Pseudomonas* spp. and *Klebsiella* spp. Inoculation trails have been conducted on beef tissue in pilot plant settings with promising results. The recent USDA-FSIS approval paves the way for in-plant testing of this promising technology.

Combined treatments – "Hurdle Technology":

Intuitively one would expect that if more than one microbial decontamination treatment be applied to carcasses along a processing line, that the combined bacterial reduction effect might be greater than the effect of any one treatment alone. In fact, several studies have attempted to quantify the synergistic effect of applying multiple technologies in the slaughter process.

Hardin et al. (1995) reported that washing with 35°C water, followed by a carcass rinse with an organic acid (lactic or acetic) was more effective than single treatments of knife trimming or water washing, at reducing inoculated levels of *Salmonella* Typhimurium and *Escherichia coli* O157:H7. Bacon et al. (2000a) evaluated multiple-sequential decontamination interventions in eight commercial beef slaughter facilities, including steam vacuuming, pre-evisceration carcass washing, pre-evisceration organic acid solution rinsing, hot water carcass washing, post-evisceration final carcass washing, and post-evisceration organic acid solution rinsing. The results of this trial indicated progressively lower mean values for aerobic plate counts, total coliforms and *Escherichia coli* counts as the carcasses moved through various stages of the slaughter process. *Escherichia coli* counts were reduced from a range of 2.6 to 5.3 $\log_{10}$ CFU/100 cm^2 to a range of 1.0 to 3.0 $\log_{10}$ CFU/100 cm^2 after final intervention treatment. After carcass chilling (24 – 36 h), *Escherichia coli* counts were reduced further to 1.3 to 0.9 $\log_{10}$ CFU/100 cm^2. These authors concluded that multiple decontamination processes, as applied in actual plant settings, resulted in significant improvements in microbiological quality. Elder *et al.* (2000) also provides data to support the effectiveness of in-plant application of multiple decontamination technologies. These authors reported that 43.4% of lots sampled pre-evisceration were positive for *E. coli* O157, however after multiple carcass decontamination methods on the slaughter floor, only 1.9% of the lots remained positive post-processing.

Summary:

The conversion of muscle to meat during the slaughter process creates unique food safety and product quality challenges for industry, government, and the research community. Significant resources have been invested to compare, validate, and implement carcass decontamination technologies. Adherence to good manufacturing practices and good hygiene practices within the slaughter facility provides the foundation upon which intervention technologies can be most effective. Methods to reduce the prevalence and/or levels of pathogens on livestock entering the slaughter facility are still under development, but may at some point in the future provide another step in the process whereby microbial intervention may be effective. Methods for carcass decontamination in use today, such as steam vacuuming, carcass washing with hot water or steam, application of antimicrobial chemicals, and combinations of these technologies have been widely researched and have been proven effective at reducing bacterial levels. Implementation of these technologies has led to significant improvements in bacterial profiles of fresh meat. However, evidence exists that raw meat products continue to have a low level of contamination with enteric bacteria, which may include pathogens of concern to human health. This fact requires that industry must continue to seek new and better methods of implementing existing technologies, while at the same time support research to find new and promising ways to continually enhance the safety of fresh meat products.

Table 1. USDA-FSIS Microbiological Baseline Prevalence and Quantitative Levels for Carcasses and Ground Beef.

Microorganism	Units of measure	Steers/ Heifers[a]	Cows/Bulls[b]	Pork[c]	Ground Beef[d]
Indicator Bacteria					
Aerobic Plate Count (@35°C)	%, (cfu/g or cm^2)	99 (475)	100 (1,130)	100 (4,900)	100 (7,900)
Total Coliform	%, (cfu/g or cm^2)	16 (35)	32 (40)	45 (77)	92 (96)
Escherichia coli (biotype 1)	%, (cfu/g or cm^2)	8 (35)	16 (33)	31 (76)	76 (54)
Pathogenic bacteria					
Campylobacter jejuni/coli	%, (mpn/g or cm^2)	4 (0.1)	1 (0.1)	31 (0.1)	0.002 (ND[e])
Escherichia coli O157:H7	%, (mpn/g or cm^2)	0.2 (0.6)	0 (0)	0 (0)	0 (ND)
Salmonella spp.	%, (mpn/g or cm^2)	1 (0.1)	3 (0.3)	9 (0.2)	8 (0.05)
Clostridium perfringens	%, (cfu/g or cm^2)	3 (45)	8 (47)	10 (71)	53 (67)
Staphylococcus aureus	%, (cfu/g or cm^2)	4 (24)	8 (25)	16 (84)	30 (31)
Listeria monocytogenes	%, (mpn/g or cm^2)	4 (0.2)	11 (0.3)	7 (0.3)	12 (3)

[a] 2,000 samples from October 1992 – September 1993. Based on a excision sample of 60 cm^2
[b] 2,100 samples from December 1993 – November 1994. Based on a excision sample of 60 cm^2
[c] 2,100 samples from April 1995 – March 1996. Based on a excision sample of 60 cm^2
[d] 563 samples from August 1993 – March 1994. Based on 25 gram sample.
[e] ND – not detected.
Source: adapted from USDA-FSIS Nationwide Microbiological Baseline Surveys.

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