Current Issues Related to Meatborne Pathogenic Bacteria

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Introduction

Food animals may be infected, contaminated or be asymptomatic carriers of pathogenic microorganisms and together with the environment they serve as sources of contamination for carcasses during the slaughtering process and for meat products during processing, storage and handling, or for water and other foods through contaminated manure (Sofos, 2002a). Foodborne microbial hazards have a devastating impact on human suffering because they are estimated to cause approximately 76 million cases of illness, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead *et al.*, 1999). It is estimated that bacterial agents are responsible for only 30% of the total foodborne illnesses; however, 72% of total deaths are due to consumption of foods contaminated with bacteria (Mead *et al.*, 1999). The United States National Health Objectives for 2010 aim at reducing the incidence of illness caused mainly by four foodborne pathogens, namely *Campylobacter*, *Salmonella*, *Escherichia coli* O157:H7 and Listeria monocytogenes, to 12.3, 6.8, 1.0, and 0.25 cases per 100,000 population, respectively (DHHS, 2000). According to the latest (2002) surveillance data (CDC, 2003), *Salmonella* are responsible for causing the highest total number of cases of gastrointestinal illness among bacteria; however, despite the high incidence of illness, the case-fatality rate is <0.05%. *Campylobacter* is responsible for the second highest total number of gastrointestinal illnesses and like *Salmonella*, it has a case-fatality rate of <0.05% (CDC, 2003). Although *E. coli* O157:H7 has a much lower rate of incidence compared to *Salmonella* and *Campylobacter*, this organism has a higher case-fatality rate (0.1%). Compared to the abovementioned pathogens, *L. monocytogenes* has the lowest rate of incidence but a significantly higher (approximately 20%) fatality rate (Mead *et al.*, 1999). Thus, there is a need to control pathogenic microorganisms in animals and their products in order to enhance the safety of our meat supply.

An outbreak of *E. coli* O157:H7 in the western United States in 1992-1993 was attributed to consumption of undercooked ground beef patties and led to development of illness in several hundred people and four deaths (Bell *et al.*, 1994). This highly publicized outbreak may be considered as the beginning of intensified public scrutiny on food safety that has led to major developments, including the complete change of the United States meat inspection system, which was in place since the early 1900s. The new United States Meat and Poultry Inspection Regulation was published in 1996 (FSIS, 1996) and requires federally inspected meat and poultry plants: (1) to establish sanitation standard operating procedures to serve as a foundation in meat processing; (2) to implement the hazard analysis critical control point (HACCP) system of process control (NACMCF, 1998); and, (3) to apply performance criteria in the form of microbial testing for *Escherichia coli* counts and *Salmonella* incidence as criteria of HACCP verification and pathogen reduction, respectively (FSIS, 1996; Sofos, 2002a; Sofos and Smith, 1998; Sofos *et al.*, 1999). Furthermore, publicity over food safety issues has led to the establishment of national food safety initiatives such as: (1) the United States National Food Safety Initiative and associated programs or activities such as the FoodNet® and PulseNet® foodborne illness surveillance networks; (2) the FightBac® and Thermy® educational programs; (3) emphasis on risk assessment studies and evaluations; and, (4) an increase in federal funding for food safety research and education issues (http://www.foodsafety.gov).

The following sections provide brief information on general characteristics of the bacterial pathogens of most concern in recent years (Bacon and Sofos, 2003), brief summaries of current research activities and interventions to control bacterial pathogens in meat products, and an introduction to certain concerns associated with efforts to control pathogens.

Characteristics of Bacterial Pathogens

Escherichia coli O157:H7: *Escherichia coli* are mostly harmless natural colonizers of the gastrointestinal tract of humans and other warm-blooded animals; however, pathogenic *E. coli* strains exist and are associated with syndromes

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of diarrheal illness (Bacon and Sofos, 2003). Strains producing Shiga-like toxins (SLT), also known as verotoxins (VT), are associated with hemorrhagic colitis and hemolytic uremic syndrome in humans and are regarded as enterohemorrhagic *E. coli* (EHEC). The predominant EHEC serotype associated with foodborne illness is *E. coli* O157:H7. *Escherichia coli* O157:H7 are gram-negative, facultatively anaerobic, nonspore-forming rods that are mostly motile, and grow at temperatures ranging from 7 to 46°C, with an optimum between 35 and 40ºC. *Escherichia coli* O157:H7 require a water activity (aw) of at least 0.95 and are able to grow in the presence of 6.5% sodium chloride. Although they grow best at pH 6.0 to 7.0, they can also grow at pH 4.4 to 9.0 and, unlike most foodborne pathogenic bacteria, they are tolerant to acidic environments. Illness associated with *E. coli* O157:H7 results through fecal-oral transmission by contaminated hands or consumption of contaminated foods or water. Between 1993 and 1998, most (72%) of the *E. coli* O157:H7 outbreaks were foodborne and of the foods implicated in the outbreaks, beef was responsible for 45% of the cases and 90% of the time the beef product was ground. Following ingestion (>101 cells) and a 3 to 9 day incubation period, *E. coli* O157:H7, can cause a wide range of symptoms including mild or severe bloody diarrhea (hemorrhagic colitis), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Bacon and Sofos, 2003).

Listeria monocytogenes: They are nonspore-forming, aerobic, microaerophilic or facultatively anaerobic, gram-positive rods that are motile by means of peritrichous flagella (Bacon and Sofos, 2003). This organism is ubiquitous in the environment and is harbored in approximately 11 to 52% of animals. *Listeria monocytogenes* has become a concern to the industry as it has been isolated from an extensive range of meat plant environments including floors, drains, condensed and standing water, and food residues on processing equipment. It is notable that *L. monocytogenes* forms resistant biofilms on equipment surfaces under conditions of limited nutrient availability. *Listeria monocytogenes* have also been isolated from <1-70% of whole and processed red meats, up to 60% of ready-to-eat poultry and 80 to 90% of raw or processed poultry. *Listeria monocytogenes* is psychrotrophic and can grow at temperatures as low as –0.4°C and up to 45°C (optimum of 30 to 37°C). Growth of *L. monocytogenes* occurs in environments of pH 4.4 to 9.4, at aw levels above 0.92, and survives at sodium chloride levels of up to 30%. Listeriosis is mainly an infection of the central nervous system (meningitis and meningoencephalitis), bacteremia and resulting in stillbirth, fetal death or spontaneous abortion in pregnant woman. The infectious dose of listeriosis is speculated to be as low as 100 cells/g, and the illness has an incubation period of a few days to 2 to 3 months (Bacon and Sofos, 2003).

Salmonella: They are gram-negative, facultatively anaerobic, nonspore-forming rods. The only two species recognized are *S. enterica*, possessing six subspecies, and *S. bongori*. There are approximately 2,600 *Salmonella* serotypes of which *S. Typhimurium* and *S. Enteritidis* are the most prevalent in the U.S. (Bacon and Sofos, 2003). *Salmonella* can grow at temperatures as low as 5.2 and as high as 46.2°C, pH values of 3.8 to 9.5, and at aw levels above 0.93. The primary reservoir for *Salmonella* is the intestinal tract of infected hosts or carriers, where cells are subsequently sloughed and excreted in the feces. Nontyphoidal *Salmonella* strains usually cause gastroenteritis after an incubation period of 5 h to 5 days resulting in diarrhea, nausea, mild fever, chills, vomiting and abdominal cramping. Infectious doses of *salmonellae* may range from as low as 100 to 103, depending on the serotype, vehicle of transmission and on the individual's immune system (Bacon and Sofos, 2003).

Campylobacter jejuni: They are gram-negative nonspore-forming rods that are slender and curved, which along with the single, polar flagellum located at one or both ends contributes to the organism's characteristic "corkscrew-type" motility (Bacon and Sofos, 2003). The bacterium is microaerophilic, growing best in environments with 2.0 to 5.0% oxygen and 5.0 to 10.0% carbon dioxide, while growth is inhibited in the presence of 21% oxygen. The temperature and pH ranges for growth of *C. jejuni* are 30 to 45°C (optimum 37 to 42ºC) and 4.9 and 8.0 (optimum 6.5 to 7.5), respectively. *Campylobacter jejuni* are sensitive to salinity (>0.5% sodium chloride), drying (require aw above 0.912), freezing, heat and acidic conditions (< pH 5.0). Campylobacteriosis may result from as few as 500 viable cells and infection typically requires 2 to 10 days before onset of gastroenteritis-associated symptoms. Infection typically involves acute colitis combined with fever, malaise, abdominal pain, headache, watery or sticky diarrhea with minor traces of blood (occult), inflammation of the lamina propria, and crypt abscesses. Infection may result in further sequelae, the most severe including the acute paralytic disease of the peripheral nervous system known as Guillain-Barre syndrome and Reiter's syndrome (autoimmune disease caused by infection) (Bacon and Sofos, 2003).

Other Meatborne Bacterial Pathogens: Several other bacterial pathogens are associated with meat and poultry products but their contribution to foodborne disease has been overshadowed by the impact of the above-mentioned four pathogens in recent years. They include *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens*, and *Bacillus cereus*. For more information on these and other pathogens see Bacon and Sofos (2003).

Current Approaches to Bacterial Pathogen Control in Meat Products

The increasing prevalence of pathogens, such as *E. coli* O157:H7, on animals before slaughter in recent years (Elder *et al.*, 2000) necessitates employment of interventions for their control. In its efforts to promote control of the incidence of *E. coli* O157:H7 and other pathogens in meat, the FSIS/USDA has been enforcing a zero tolerance policy for

visible soil on carcasses during slaughter and has declared *E. coli* O157:H7 an adulterant in fresh ground beef and other non-intact fresh beef cuts (http://www.fsis.usda.gov). Testing of fresh beef for this pathogen has resulted in several, highly publicized, product recalls from the marketplace. All segments, including, regulators, educators, consumers, health authorities, research scientists and the industry agree that efforts should be made to reduce incidence and eliminate or control pathogenic bacteria at all stages of the food chain (Sofos, 2002a). The producers of food animals in the United States have contributed to the overall effort of improving food safety by supporting development and applying quality assurance programs and by financially supporting, through their associations, research and development studies on microbial reduction and pathogen control interventions applied during animal slaughter and meat processing. The meat processing industry has also undertaken initiatives and efforts to comply with consumer demands for food safety, customer specifications or criteria, and regulatory requirements included in the new meat and poultry inspection regulations (FSIS, 1996). The target of the meat processing industry has been to improve operations through implementation of HACCP programs, and employment of various carcass decontamination or pathogen control interventions.

A variety of processes have been developed with the objective of reducing contamination on carcasses. Decontamination processes include animal cleaning, chemical dehairing at slaughter, spot-cleaning of carcasses before evisceration by knife-trimming or steam and vacuum, spraying, rinsing, deluging or dipping of carcasses before evisceration and/or before chilling with water or chemical solutions (e.g., organic acids, acidified sodium chlorite, peroxyacetic acid-based products, trisodium phosphate, etc.) or steam. These interventions are applied at various concentrations or intensities, pressures (2-20 bar), temperatures (15-80°C) and for different lengths of time (5-20 sec), individually or in sequential combinations. Decontamination interventions are used extensively in the United States and they are integrated into food safety management systems, such as HACCP, which, as indicated, is required by regulation (Bacon *et al.*, 2000; Smulders and Greer, 1998; Sofos and Smith, 1998). Most processors of fresh meat in the United States may employ more than one decontamination intervention, in sequence, and this "multiple hurdle" approach to decontamination should result in microbiologically cleaner carcasses and may assist plants in meeting regulatory requirements (Bacon *et al.*, 2000; Sofos *et al.*, 1999). Thus, application of decontamination processes should contribute to the enhancement of product safety, provided that chilling, cutting, processing, storage, distribution and preparation for consumption are also performed properly and under hygienic conditions. It is important to realize, however, that control or management of food safety risks should be based on an integrated effort and approach that addresses all sectors, from the producer through the packer, processor, distributor, retailer, food service worker and consumer. Reduction of pathogen prevalence on animals pre-harvest may lead to a reduced probability that errors occurring in subsequent parts of the food chain will lead to foodborne illness. Additional interventions to help in enhancing food safety or to eliminate pathogens in ready-to-eat meat products are applied during processing and include heating, chilling, freezing, drying, fermentation, use of chemicals as acidulants or antimicrobials, packaging, proper storage and distribution, and appropriate handling and preparation for consumption. Indeed, food safety assurance involves activities and responsibilities throughout the food chain.

Recently the FSIS/USDA (http://www.fsis.usda.gov) has issued directives, notices and guidances for meat operations to consider *E. coli* O157:H7 a food safety hazard that is reasonably likely to occur in fresh beef. Thus, they should re-evaluate their HACCP plans and establish plant-validated control measures (FSIS Directive 10,010.1/February 1, 1998; FSIS Notice 44-02/November 4, 2002; Proposed FSIS Directives in Federal Register October 7, 2002/Volume 67, Number 194, Pages 62-325-62334; FSIS Guidance for Minimizing the Risk of *Escherichia coli* O157:H7 and *Salmonella* in Beef Slaughter Operations; FSIS Guidance for Beef Grinders and Suppliers of Boneless Beef and Trim Products).

In addition to *E. coli* O157:H7, *L. monocytogenes* has become a major concern for the meat processing industry worldwide. Following a listeriosis outbreak in the United States in 1998-1999, which caused 21 deaths and at least 100 illnesses in 14 states due to consumption of post-processing contaminated hot dogs and luncheon meats (CDC, 1999), *L. monocytogenes* has re-emerged as a meat-borne pathogen of concern in the United States. Another listeriosis outbreak in 2002 caused 50 illnesses, 7 deaths and 3 miscarriages in 8 northeastern states of the United States and was associated with consumption of contamination ready-to-eat poultry products (CDC, 2002). These fatal outbreaks and the frequent and highly publicized recalls of meat products potentially contaminated with the pathogen have alerted the industry, public health authorities and researchers to develop and establish effective measures and procedures to maintain product safety and increase consumer confidence in ready-to-eat meat products (Bernard and Scott, 1999; Tompkin, 2002; Tompkin *et al.*, 1999). Results of our studies have shown that use of modified marinades in the form of multiple hurdles are effective in enhancing destruction of pathogenic bacteria during drying of meat products as well as post-drying contaminants during product (i.e., beef jerky) storage (Calicioglu *et al.*, 2002a,b, 2003). Inclusion of antimicrobials (acetates, diacetates, lactates, benzoates, sorbates, glucono-delta-lactone, and their combinations at reduced concentrations) in the formulation or their application as dipping solutions after product slicing and before packaging were found effective in controlling *L. monocytogenes* in ready-to-eat cured meat products contaminated after cooking (Bedie *et al.*, 2001; Samelis *et al.*, 2001a, 2002a).

For ready-to-eat meat and poultry products, the FSIS/USDA has proposed the following performance criteria: (1) a 6.5 log reduction of *Salmonella* during processing of ready-to-eat meat products; (2) a 7.0 log reduction of *Salmonella* during processing of ready-to-eat poultry products; (3) a 5.0 log reduction of *E. coli* O157:H7 during processing of fermented meat products containing beef; (4) no more than 1.0 log growth of *Clostridium perfringens* and no growth of *Clostridium botulinum* during cooling of all ready-to-eat meat products (hhtp://www.fsis.usda.gov). Relative to control of *L. monocytogenes* in ready-to-eat meat and poultry products, the USDA/FSIS has published a directive (10,240.3/December 9, 2002) titled Microbial Sampling of Ready-To-Eat (RTE) Products for the FSIS Verification Testing Program.

Potential Concerns Associated with Bacterial Pathogen Control Approaches

As they have done in the past, microorganisms continue to evolve and their large genetic variability and short generation times increase their potential for survival in less than favorable environments. The emergence of resistant bacteria as a result of the ubiquity of antimicrobials in their environment, has led to public health concerns centered around increased morbidity and mortality associated with failing antimicrobial treatments (Bacon *et al.*, 2002). In addition to the emergence of antibiotic resistance, common foodborne pathogenic bacteria have demonstrated resistance and cross-protective capabilities to food preservation stresses as well as increased virulence. The induction of bacterial resistance to environmental stresses such as temperature and pH extremes involves the production of "protective" shock proteins, some of which possess cross-protective capabilities, or the ability to confer protection to more than one type of stress. Recent and continuing research efforts in our laboratory have focused on potential food safety risks and critical control points concerning stress-adapted pathogens (Samelis *et al.*, 2001b,c, 2002b,c; Stopforth *et al.*, 2002, 2003). It was shown that *E. coli* O157:H7 has greater potential to survive in organic acid (more so in acetic than lactic acid) beef decontamination runoff fluids (washings) compared to *L. monocytogenes* and *S. Typhimurium*, even with moderate previous acid-adaptation (Samelis *et al.*, 2001b). Acid-adaptation was shown to enhance survival of *E. coli* O157:H7 for up to 14 d in mixtures of both acetic and lactic acid (2%) washings mixed with water washings at ratios of 1:1, 1:9 or 1:99 (Samelis *et al.*, 2002b). In addition, it was shown that acid-adaptation of *E. coli* O157:H7 negatively influenced the pathogen's ability to readapt to a sudden shift to higher pH (6.5 to 7.5) conditions of beef water washings (Samelis *et al.*, 2002b). Results have demonstrated that acid decontamination interventions may alter the microbial ecology of meat plant environments (Samelis *et al.*, 2002b; Stopforth *et al.*, 2003), selecting for the growth and attachment to equipment surfaces of the natural meat flora and may enhance the survival of attached pathogens following long-term stressing (Stopforth *et al.*, 2003). In general, exposure to sublethal stress during food processing may result in stress-hardened pathogens surviving more readily subsequent antimicrobial treatment applications aimed at improving microbiological food quality, potentially resulting in persistent microbiological populations possessing elevated virulence factor expression (Samelis and Sofos, 2003; Sheridan and McDowell, 1998; Sofos, 2002b). Overall, however, interventions for decontamination of carcasses are useful because they reduce levels of contamination and allow plants to meet regulatory performance criteria and standards as well as contractual specifications for product contamination. Evidence indicates that these interventions cause major reductions in prevalence of pathogens such as *E. coli* O157:H7 (Elder *et al.*, 2000). It should be noted, however, that these interventions are generally instantaneous or of short intensity and inadequate for complete microbial inactivation or removal. Issues such as those associated with microbial penetration in muscle tissues, biofilm formation, bacterial sublethal injury, alteration of metabolic activity potentially resulting in development of stress adaptation and cross protection, and changes in meat and plant environment microbial association need to be considered. As we develop knowledge to better understand these concerns, we will be able to select and apply intervention treatments of optimum intensity and in a sequence that maximize antimicrobial effects.

Overall, the microbiological status of the products that reach the consumers, either as raw meat or processed products, will depend on exposure to contamination and its control during all steps of the food production, processing, distribution, storage, retailing and preparation for consumption chain. Proper application of the processes described above will yield products that should be safe for consumption following proper cooking and/or serving.

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