Alternatives to Antibiotics: Chemical and Physical Antimicrobial Interventions and Foodborne Pathogen Response

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ABSTRACT Successful control of foodborne pathogens requires placement of chemical and physical hurdles in the preharvest and postharvest food production sectors. Pathogens may also encounter indigenous antimicrobials in foods including certain botanical compounds that have historically been used for flavor enhancement as well as preservation. Chemical additives have traditionally included organic acids to control microbial contamination in foods and feeds. However, there is some concern that continuous application of certain chemical antimicrobials can lead to a buildup of microbial resistance. This creates problems if foodborne pathogens survive and develop resistance to a variety of environmental stressors encountered in pre- and postharvest animal production. To expand the diversity of potential antimicrobials that have practical application to food animal production requires exploring the interaction between the food matrix and foodborne pathogens. There is potential for isolating antimicrobial compounds that exhibit mechanisms unrelated to conventional antimicrobial compounds. However, understanding the potential for novel antimicrobial compounds in foods and feeds will require the physiological examination of foodborne pathogen response under experimental conditions comparable to the environment where the pathogen is most likely to occur. Research on foodborne *Salmonella* pathogenesis is extensive and should provide a model for detailed examination of the factors that influence antimicrobial effectiveness. Analysis of pathogen response to antimicrobials could yield clues for optimizing hurdle technologies to more effectively exploit vulnerabilities of *Salmonella* and other foodborne pathogens when administering antimicrobials during food and feed production.

(*Key words*: antimicrobial, natural compound, foodborne pathogen, virulence, *Salmonella* spp.)

2005 Poultry Science 84:667–675

INTRODUCTION

In the United States, pathogen contamination of foods by organisms such as *Salmonella, Escherichia coli* O157:H7, *Staphylococcus aureus, Clostridium, Camplyobacter,* and *Listeria monocytogenes* is responsible for over 76 million cases of foodborne illness and 323,000 hospitalizations at a cost of approximately $7 to 10 billion annually (Mead et al., 1999). Estimates are that salmonellosis alone results in 1.4 million cases, 500 deaths, and medical costs and lost productivity ranging from approximately $0.5 to 2.3 billion (Frenzen et al., 1999; CDC, 2003). Common vehicles of *Salmonella* and *Campylobacter* transmission on poultry are carcasses arriving at the processing plants or carcasses that become cross-contaminated with intestinal contents during processing (Cox et al., 1981; Connor et al., 2001). The deaths of children in the Pacific Northwest in 1993 resulting from the consumption of undercooked ground beef contaminated with *E. coli* O157:H7 (Mermelstein, 1993) prompted the USDA-FSIS (1993) to require that physical contaminants be removed (zero tolerance) from beef carcasses. Additionally, they have amended cooking regulations for all meat and poultry products (USDA-FSIS 1999; CFR, 2002). These changes are the result of implementing a more science-based approach to inspection using hazard analysis and critical control point (HACCP) principles to monitor and verify safe processing conditions (USDA-FSIS, 1996).

In ready-to-eat (RTE) products, cross-contamination or recontamination by pathogens in the processing plant (e.g., human handling, contaminated processing equipment) generally are the major concerns (Borch and Arinder, 2002). Recontamination, in fact, can result in a more serious problem for decontamination than untreated products, especially with spore-forming microbes such as *Clostridium* or cold-tolerant, psyhcrotrophic bacteria such as *Listeria monocytogenes*, because of a lack of competing microflora (e.g., lactic acid bacteria). Listeriosis acquired during consumption of RTE products represents

Received for publication August 13, 2004.
Accepted for publication September 17, 2004.
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Abbreviation Key: RT = reverse transcription; RTE = ready-to-eat.

a serious public health concern because of the high mortality rates associated with the illness. Potential economic losses due to outbreaks and recalls of products due to *L. monocytogenes* contamination have been publicized (CDC, 1999, 2002; Mead et al., 1999). Of particular concern was an outbreak associated with precooked turkey deli meat that caused 53 illnesses, 8 deaths, 3 miscarriages, and the recall of 27 million pounds of product with a month-long plant shutdown (CDC, 2002). Subsequent potential temperature abuse of meat and poultry products obviously exacerbates the problem, and even proper cooking may not eliminate illness-causing toxins produced by organisms such as *Staphylococcus aureus* or *Clostridium botulinum.*

CLASSIFICATION OF ANTIMICROBIALS

Food preservation has an extensive historical record of discovery and application for preventing food deterioration and extending the shelf life of perishable food ingredients. Food preservation approaches can be categorized as physical, chemical, or biological. Physical methods include drying, cold storage, freezing, and modified atmospheric packaging (Farkas, 2001). The list of chemical additives developed over the years is rather extensive, but traditional antimicrobials have included organic acids and esters, various salt compounds, and specific compounds such as lysozyme (Davidson, 2001). Naturally occurring compounds used for food preservation include those of animal origin, such as lactoperoxidase, and various iron-binding proteins and compounds derived from plants, such as spices, oils, and phenolics (Davidson, 2001). Biological systems, which will not be discussed in this review, that have been developed or have potential food preservation properties include controlled acidification of foods by lactic acid bacteria that produce organic acids from carbohydrate fermentation (Montville et al., 2001). Bacteriocins and bacteriophages have recently become of greater interest due to their potential utility for targeting specific pathogens (Campbell, 2003; Joerger, 2003; Weld et al., 2004). Most of the work on antimicrobials has been conducted with foods to be used for human consumption. In principle, many of the same strategies and concepts would also be applicable for animal feeds. However, given the available information, most of this review will be focused on the concepts established for antimicrobial amendments in human foods.

PHYSICAL AND CHEMICAL INTERVENTION STRATEGIES

In response to demands from consumers for safer meat and poultry products and implementation of government regulations, numerous studies testing possible interventions have been conducted, particularly over the last 10 yr. A wide variety of approaches to sanitize meat and poultry products after harvesting (Conner et al., 2001; Mermelstein, 2001; Huffman, 2002; White, 2002) have been developed and include, in part, cold and hot water rinses; steam pasteurization or steam vacuum treatment; trimming; a variety of chemical rinses including chlorine or chlorine dioxide, ozonated or electrolyzed water, trisodium phosphate, or acidified calcium sulfate; organic acid rinses (e.g., lactic, acetic) with or without surfactants; and γ-irradiation or electron beam irradiation. In addition, antimicrobial compounds may be added to many RTE products, including sodium or potassium lactate, sodium diacetate, sodium citrate, and a variety of antioxidant compounds that also exhibit antimicrobial properties. Not surprisingly, the combination of more than one intervention treatment often has been found to produce a greater antimicrobial effect than any single treatment, often working synergistically. The latter has been referred to as hurdle technology.

As discussed previously, processing has been implicated as a major source of *Salmonella* and *Campylobacter* cross-contamination of broiler carcasses. Consequently, research has focused on effective methods to substantially decrease contamination during the final stages of processing (Thomson et al., 1979; Davidson et al., 1985; Lillard, 1987; James et al., 1992). Several methods for chemical and mechanical decontamination of carcasses have been tested and reported in the literature. Cox et al. (1974b) reported a 1-log reduction in total counts of surface bacteria on broiler breast skin from carcasses that had been immersed in 60°C water for 1 min, a 2-log reduction using 71°C water, and a 0.5-log reduction using water below 60°C. Carcasses receiving 60°C water treatment or higher exhibited a partially cooked appearance. In a subsequent study, Morrison and Fleet (1985) found that carcasses immersed in 60°C water (no treatment) for 10 min had 100-fold reductions in *Salmonella* contamination. Additionally, by incorporating 200 ppm chlorine or 2.5% potassium sorbate into the 60°C immersion water, significantly greater reductions were noted than with water alone.

Chemical decontamination methods also have been examined for meat and poultry carcasses. The most commonly used are chlorine, chlorine dioxide, acidified sodium chlorite, electrolyzed water, ozone, trisodium phosphate and cetylpyridinium chloride. The latter has been evaluated in several studies (Breen et al., 1997; Pohlman et al., 2002a,b; Ransom et al., 2003) producing significant reduction of pathogens and was recently approved for food use by the USDA. The gaseous antimicrobials (chlorine, chlorine dioxide, ozone, acidified sodium chlorite, which generate an oxy-halogen) usually are applied as an aqueous solution and generally have resulted in approximately 2- to 4-log reduction of pathogens depending on concentration, temperature of application, and contact time (Mullerat et al., 1995; Reagan et al., 1996; Castillo et al., 1999; Koseki et al., 2001; Park et al., 2002; Pohlman et al., 2002a,b; Stivarius et al., 2002; Trachoo and Frank, 2002; Ransom et al., 2003). The effects tend to be transient, providing no extended bactericidal or bacteristatic effect after treatment. The primary reason is that these compounds are readily reactive with unsaturated bonds, thus quickly removing them from solution and

negating further action against bacterial cells. Trisodium phosphate, on the other hand, is an alkaline salt solution that can leave residual reactive hydroxyl radicals on the treated product and suppress further growth (Dorsa et al., 1998; Ramirez et al., 2001; Pohlman et al., 2002a,b,c). It generally has been found to improve the color of the meat product treated. The treatment also generates large amounts of phosphates, which can be environmentally harsh and cause problems for disposal.

Organic acid treatments have also been reported to significantly reduce bacterial contamination of broiler skin (Cox et al., 1974a; Mulder et al., 1987; Hwang and Beuchat, 1995; Marshall, 2003; Dubal et al., 2004). Cox et al. (1974a) reported a 1.21 mean log reduction in bacterial counts on broiler thigh skin that had been immersed in a 5% succinic acid treatment at 60°C for 3 min. Skin treated with 5% succinic acid at 24°C for 3 min had a mean log reduction in bacteria of only 0.78. In this study, both the heated and unheated succinic acid treatments resulted in discoloration and deterioration of carcass quality. In a subsequent study, Hwang and Beuchat (1995) reported that a lactic acid and sodium benzoate solution enhanced the rate of inactivation of *Salmonella* on chicken skin during refrigerated storage. In addition, Mulder et al. (1987) found lactic acid (1%) and hydrogen peroxide (0.5%) resulted in 4-log reductions in *Salmonella* on experimentally inoculated carcasses or in naturally occurring *Salmonella* infections. Although many reported treatments have been effective in decreasing bacterial contamination, many of these procedures used heated water or organic acid immersion treatments, which result in discoloration and deterioration of the carcass quality. In addition, some of the organic acids tested were not generally recognized as safe (GRAS) and, thus, could not be applied commercially. Therefore, other than addition of chlorine to chill immersion tanks and carcass sprayers (a process that becomes ineffective in the presence of large amounts of organic material), there is no single decontamination method predominantly used in commercial poultry processing.

As another example, acidified calcium sulfate (sold by Mionix Corp., Roseville, CA under the trade name Safe2O) has been found to be a very effective beef and poultry carcass washing agent (Huffman, 2002) as well as a rinsing agent for RTE meats with considerable residual listericidal or listeristatic activity (Keeton et al., 2002). According to Mionix, this material disables the proton pumps in the bacterial membrane and thus attacks bacteria in a different fashion than other organic acids (e.g., lactic). However, only these few studies have been reported. The potential use of these treatments as part of a multiple hurdle approach requires further investigations.

NATURAL ANTIMICROBIALS

Many derived-plant products (e.g., spices, fruit preparations, vegetable preparations or extracts) have been used for centuries for the preservation and extension of the shelf life of foods (Davidson, 1993; Billing and Sherman, 1998; Smid and Gorris, 1999; Cutter, 2000; McCarthy et al., 2001; Islam et al., 2002; Draughon, 2004). In addition, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHA/BHT), and benzoic acid may be added in restricted amounts to some foods (Gailani and Fung, 1984; Raccach, 1984). The majority of antioxidant activity ascribed to various fruit or vegetable preparations has been suggested to be due to the content of phenolics containing reactive hydroxyl groups (e.g., catechins, gallic acid, cholinergenic acid) neocholinergic acid and other compounds but may also involve anthocyanins, carotenoids, and flavenoids (Guo et al., 1997; Donovan et al., 1998; Stacewicz-Sapuntzakis et al., 2001). These generally are used as food additives rather than surface treatments and are generally recognized as safe ingredients. Most provide only a 1- to 2-log reduction of pathogens, but they seem to be synergistic with heat and acid treatments because they affect different aspects of cell structure and metabolism. In addition, many of these compounds may provide an added health benefit in the diet (often referred to as nutricuticals or functional food ingredients). Recent work at the Agricultural Research Service's Jean Mayer Human Nutrition Center on Aging at Tufts University (Boston, MA) has suggested that foods with a high oxygen radical absorbance capacity (ORAC) value (Cao et al., 1995) may reduce the risk of diseases of aging including atheroschlerosis, diabetes, and cancer.

The combination of 2 or more antimicrobial intervention treatments in lower doses may act in a synergistic manner. By attack on multiple fronts, pathogens effectively may be reduced while food quality is maintained using minimal individual treatments (Huffman, 2002; Samelis et al., 2002; Sommers, 2002; Nam and Ahn, 2003). For example, sources of natural antioxidants (spice extracts, plum preparations, and other sources) have been shown to have antimicrobial activities as well as antioxidant properties that also tend to reduce meat and poultry lipid oxidation (rancidity; Pokorny, 1999; Nam and Ahn, 2003). However, by themselves, they generally are not effective enough to meet the current standards of zero pathogen tolerance. In conjunction with other interventions (e.g., organic acids, hot water, or steam treatment) they may not only become more effective for pathogen reduction but also serve to decrease the negative impact on the quality of meat and poultry products resulting in more shelf-stable products. Additionally, antioxidants attack pathogens by different mechanisms than heat or acids, thus making them a potentially complementary treatment.

MICROBIAL RESPONSE TO ANTIMICROBIALS

It is relatively difficult to develop general concepts on optimal intervention strategies for limiting foodborne pathogens on foods and feeds. A general problem encountered with the majority of the antimicrobial intervention procedures is that most pathogens that survive initial treatment will activate protective mechanisms in response to the stressor. In addition, most of these mechanisms

are general enough that cross-tolerance to other forms of stress are set in motion, which may, in addition, increase the virulence (infectivity) of the surviving organisms (Kwon and Ricke, 1998a,b; Gahan and Hill, 1999; Durant et al., 2000a,b,c,d; Kwon et al., 2000; Jay, 2000; Marshall, 2003; Ricke, 2003; Rowbury, 2003; Yousef and Juneja, 2003; Bayles, 2004).

A possible practical outcome for food processing is that the number of cells needed to initiate infection is decreased. *Salmonella* spp. usually requires an infectious dose of approximately 10^5 cells, but acid-induced resistance responses could lower the infectious dose to 10 to 100 cells (Marshall, 2003). Thus, even a 2- to 3-log reduction of pathogen by an accepted intervention procedure may leave behind survivors that have been rendered even more infective. Heat treatment followed by acid can have the same effect. On the positive side, microorganisms generally are able only to cross-tolerate stressors that affect the cells in the same general manner. An intervention that acts by a different mechanism and exerts different stresses may become more effective when used in conjunction with something that already has compromised the stress response capabilities of a bacterial cell. Thus, pathogens previously stressed by heat or acid may tend to become more susceptible to destruction by antioxidants (Raccach, 1984). The following sections describe the examination and consequences of these responses using foodborne *Salmonella* as the example.

SALMONELLA AS A MODEL ORGANISM FOR EXAMINING FOODBORNE PATHOGEN RESPONSE TO HURDLE TECHNOLOGY

Foodborne salmonellae represent one of the better-understood and studied pathogens for designing effective intervention steps based on mechanisms that the organism can potentially express. Given the breadth of knowledge on *Salmonella* genetics and physiology, it has become possible to study the stress and virulence characteristics of this organism under antimicrobial intervention conditions. *Salmonella* is commonly found in the gastrointestinal tracts of animals and has been commonly associated with foods such as raw meat, poultry, eggs, and dairy products. *Salmonella* is usually thought to be spread by the fecal-oral route. In the commercial broiler industry, with a common turnover rate of 42 d, inadequate decontamination can lead to several possible contamination sites, such as litter, air, and feed (Humphrey, 2000). Birds that are also raised in close proximity to other birds increase the likelihood of horizontal transfer. Because *Salmonella* has several opportunities to be introduced to the host, there is an increased risk of *Salmonella* reaching the consumer. Given the widespread environmental dissemination of foodborne *Salmonella* spp., it is not unreasonable to speculate that salmonellae have several physiological systems for survival under a variety of different suboptimal growth conditions. Consequently, it represents an ideal foodborne pathogen model with which to examine the relative efficacy of antimicrobial compounds as single additives or in combination with other hurdle intervention technologies. In the following sections, *Salmonella* pathogenesis and methods for studying virulence expression are described.

Salmonella Mechanisms of Pathogenesis

To invade the host, *Salmonella* must undergo an infectious disease cycle. The conventional infectious cycle consists of entry of the pathogen, establishment and multiplication, avoidance of host defenses, and finally damage and exit (Donnenberg, 2000). *Salmonella* undergoes all of these steps when it invades a host. Due to the prevalence of *Salmonella* contamination of many different food products, it can easily gain access to and colonize the intestinal system of the host. *Salmonella* has 5 different pathogenicity islands that encode the spectrum of the virulence genes used for invasion and evasion of host defense mechanisms. Pathogenicity islands is a term used to describe a set of genes encoding for virulence that are located on a particular loci in the bacterial genome but are absent from nonvirulent strains of the same species (Donnenberg, 2000).

The clinical beginning of a *Salmonella* infection lies in the initial contact of *Salmonella* to the epithelium of the gastrointestinal system of the particular host, such as humans or chickens. The bacteria that have made it this far have had to overcome such hazards such as low pH in the gastrointestinal system as well as any antimicrobial or physical barriers or both. It is believed that the appendages observed only when in contact with the epithelial cells help mediate this survival, as they are no longer observed after entry (Ginnochio et al., 1994). The next challenge facing the invading bacteria involves colonization of the intestine through attachment (Lucas and Lee, 2000). The *Salmonella* cells must also be able to undergo proliferation as well as evade any further barriers, such as pH or acid shock that may try to hamper their colonization (Lucas and Lee, 2000). The preferred entry point for *Salmonella* is the Peyer's patches in the distal ileum (Jones, 1997).

Once the *Salmonella* have been taken up by the cell, they rely on other mechanisms for survival. In the cell, *Salmonella* has evolved ways to circumvent targeting by the phagosome-lysosome fusion pathway. *Salmonella* Typhimurium has been shown to require an acidic pH to induce replication and survival within the cells (Rathman et al., 1996). It is believed to take up residence in the membrane-bound vacuole of phagocytic and nonphagocytic cells (Finlay and Falkow, 1997). When *Salmonella* enters the vacuole, the presence of lysosomal glycoproteins and removal of the surface marker assists with changes to the vacuole (Finlay and Falkow, 1997). The type III secretion system causes subsequent neutrophil (heterophils in birds) and fluid accretion in the ileum (Zhang et al., 2003). The neutrophil addition causes necrosis of the surrounding tissue and ultimately diarrhea, thereby bringing about the symptoms of disease (Zhang et al., 2003).

FOODBORNE *SALMONELLA* PATHOGENESIS AND EXPRESSION OF THE *hilA* VIRULENCE GENE

The *hilA* (hyperinvasive locus) gene is a transcriptional activator encoded on *Salmonella* pathogenicity island 1 that is part of the OmpR/ToxR family (Bajaj et al., 1995). Determination of hyperinvasive mutants occurred through insertions of constitutive neopromoters into the S. Typhimurium genome (Lee et al., 1992). The *hilA* gene encodes for a protein that is believed to be between 531 to 553 amino acids in length (Bajaj et al., 1995, 1996; Darwin and Miller, 1999; Lucas et al., 2000; Lostroh and Lee, 2001; Lucas and Lee, 2001; Baxter et al., 2003; Boddicker et al., 2003). According to Bajaj et al. (1995, 1996) HilA may be a binding protein for DNA. The *hilA* gene is also thought to be a requirement for *Salmonella* invasion due to its transcriptional properties. The *hilA* gene provides a key step in invasion gene regulation, whereas *Salmonella* mounts an infection against its host (Bajaj et al., 1995) and is required for the access of *Salmonella* into epithelial cells (Lee et al., 1992). The expression of *hilA* and other invasion genes is dependent on environmental signals, oxygen levels, osmolarity, pH, and growth phase (Galán and Curtiss, 1989; Ernst et al., 1990; Lee and Falkow, 1990; Schiemann and Shope, 1991; Lee et al., 1992; Behlau and Miller, 1993; MacBeth and Lee, 1993; Jones et al., 1994; Pegues et al., 1995; Bajaj et al., 1996; Gunn et al., 1996; Johnston et al., 1996; Véscovi et al., 1996; LeClerc et al., 1998; Rakeman et al., 1999; Fahlen et al., 2000, 2001; Boddicker et al., 2003).

MOLECULAR METHODS FOR STUDYING VIRULENCE GENE RESPONSE

Tissue culture and in vivo genetic screening methods have answered many questions on *Salmonella* pathogenesis mechanisms and have led to identification of several key genes. However, these approaches do not lend themselves easily to more routine application or screening of a wide variety of environmental factors that *Salmonella* and other foodborne pathogens may encounter during food processing. Specific genetic constructs of *Salmonella* with key virulence gene promoter sites combined or fused with structural genes encoding for synthesis of enzymes, such as β-galactosidase, have proven to have utility for screening different incubation conditions in laboratories. Creation of these fusions allows quantification of virulence gene expression as a simple enzyme assay. Construction of *hilALacZY Salmonella* fusion strains has been used to study and elucidate many of the factors responsible for induction of these key regulatory components (Bajaj et al., 1995, 1996). However, in situ application of these fusion strains is less feasible as these strains are no longer capable of synthesizing functional virulence genes. In addition, for some fusion strains such as the β-galactosidase constructs, there is a risk that lactose using nonsalmonellae background microflora would also be capable of synthesizing β-galactosidase enzyme and, thus, masking the amount synthesized in vivo by the fusion strain. Consequently, for effective in vivo examination of individual virulence gene expression, more direct quantitative measurement of gene response is required. Ideally, monitoring instantaneous fluctuations of individual genes as they are induced would provide a more realistic picture of foodborne pathogen response during exposure to food and feed processing conditions and possible antimicrobial amendments.

Synthesis of messenger RNA (mRNA) after gene induction represents a fairly accurate window to immediate response by the bacterium. Currently, there are several techniques used to evaluate the amount of mRNA expression including Northern blotting, cDNA arrays, in situ hybridization, RNase protection assays, and reverse transcription (RT) PCR (Giulietti et al., 2001). Reverse transcription along with PCR has proven to be a highly accurate method to quantify gene expression (Noonan et al., 1990; Horikoshi et al., 1992). Of these methods RT-PCR is the method of quantification that is the most discerning in theory and application (Giulietti et al., 2001). Real time RT-PCR is rapidly becoming a popular method for determining mRNA expression (Orlando et al., 1998; Bustin, 2000; Pierson et al., 2003) due to its capacity to use 1000-fold less RNA than other known methods (Hashimoto et al., 2004).

The RT-PCR measures buildup of the product during the exponential phase of the reaction, which can be observed on an amplification plot (Giulietti et al., 2001). Results of real time RT-PCR can be observed while the amplification plot itself is being generated without further detection techniques. Consequently, detection of PCR products avoids the use of agarose gel electrophoresis and ethidium bromide staining, which can be less precise. SYBR Green is a fluorescent dye with a high affinity for double-stranded DNA that shows its fluorescence when bound to double stranded cDNA (Dhar et al., 2002; Li et al., 2003). Accumulation of fluorescence is read at the end of each cycle via the target amplification. The threshold cycle values are determined by the cycle at which the fluoresecent emission rises above the threshold (Guiletti et al., 2001). Because SYBR Green is not sequence specific, melting curves can be used to analyze different products. With this method primer, dimers can be distinguished by their lower melting temperature (Tm) (Ririe et al., 1997; Wittwer et al., 2001). The Ct (cycle at which the amplification crosses a specified threshold) decreases as the amount of target increases (Guiletti et al., 2001).

An important requirement for quantifying gene response with RT-PCR is the inclusion of measurement of other gene responses, which are expressed at a constant quantitative level and can be used as a comparative baseline. A method designed for determining relative quantification is the comparative Ct method. This method does not require the construction of cDNA plasmids nor does it depend on techniques in which the variations in transcription efficiency cannot be controlled. The comparative Ct method is determined by normalizing the target to an RNA standard gene, which is subsequently compared

with an untreated sample or control. By using an RNA standard gene, minor variations in amount of RNA used or differences in RT efficiency can be accounted for. Recently, such approaches have been used to determine *Salmonella* response in water samples and have led to a precise evaluation of metabolic activity under the conditions typically encountered in water (Fey et al., 2004). Application of RT-PCR techniques will allow for the simultaneous monitoring of gene expression of any of the key genes at any time during exposure to food or feed processing conditions. Key areas for application could include measurement during thermal treatments as well as after carcass rinses and exposure to antimicrobial compounds. It can be envisioned that such approaches may help to identify indicator genes that could be used to potentially isolate step(s) within a food or feed processing system that may stimulate increases in virulence expression that might become problematic.

CONCLUSIONS

Natural components in foods and feeds represent additional hurdle technologies that may complement or act synergistically with traditional intervention approaches. Certainly there is need to continue to identify such compounds to provide alternatives to some of the more overused antimicrobial compounds currently available. In addition, some of these natural compounds may exhibit antimicrobial properties that differ mechanistically from other antimicrobials being used. This becomes important given the capabilities of most pathogens to not only produce mechanisms of resistance to various compounds but for at least some of these mechanisms, once expressed, to provide cross-protection to other intervention methods. Consequently, it becomes important to be able to genetically screen pathogens for virulence capacity and to systematically determine which environmental factors are involved in stimulation under typical food and feed processing conditions. Foodborne *Salmonella*, in addition to being one of the more troublesome and resourceful pathogens, has also served as a fairly useful model for gaining a better understanding of not only host-pathogen relationships but regulation of virulence genes under different environmental conditions. Given the capabilities of quantifying gene response at the mRNA synthesis level, application of such approaches in the future should lead to more detailed scenarios of pathogen responses during all aspects of food and feed processing. Generation of these detailed analyses should yield clues for optimizing hurdle technologies to better exploit vulnerabilities of foodborne pathogens during food and feed processing and application of antimicrobials.

ACKNOWLEDGMENTS

This review was supported by Hatch grant H8311 administered by the Texas Agricultural Experiment Station USDA-NRI grant 2001-02675 and FSIS-C-14-2004.

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