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Efficacy of antimicrobials for the disinfection of pathogen contaminated green bell pepper and of consumer cleaning methods for the decontamination of knives

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ABSTRACT

While there is strong focus on eliminating pathogens from produce at a commercial level, consumers can employ simple methods to achieve additional pathogen reductions in the domestic kitchen. To determine the ability of antimicrobials to decontaminate peppers, samples of green bell pepper were inoculated with *Salmonella enterica* and *Escherichia coli* O157:H7 and then immersed in 3% (v/v) hydrogen peroxide (H_2O_2), 2.5% (v/v) acetic acid (AA), 70% (v/v) ethyl alcohol (EtOH), or sterile distilled water (SDW). The potential for transfer of pathogens from contaminated peppers to other non-contaminated produce items, and the effect of knife disinfection in preventing this cross contamination, were also tested. Knife disinfection procedures were evaluated by chopping inoculated peppers into 1 cm^2 pieces with kitchen knives. Experimental knives were then treated by either no treatment (control), wiping with a dry sterile cotton towel, rinsing under running warm water for 5 or 10 s, or applying a 1% (v/v) lauryl sulfate-based detergent solution followed by rinsing with warm running water for 10 s. Following disinfection treatment, knives were used to slice cucumbers. Exposure to H_2O_2 for 5 min and EtOH for 1 min resulted in reductions of 1.3 ± 0.3 $\log_{10}$ CFU/cm^2 for both pathogens. A 5 min exposure to AA resulted in a reduction of *S. enterica* of 1.0 ± 0.7 $\log_{10}$ CFU/cm^2 and *E. coli* of 0.7 ± 0.8 $\log_{10}$ CFU/cm^2. No differences ($p \geq 0.05$) were found between numbers of pathogens on knives and numbers of pathogens transferred to cucumber slices, suggesting that organisms remaining on knife surfaces were transferred to cucumbers during slicing. Findings suggest that EtOH and H_2O_2 may be effective antimicrobials for in-home decontamination of peppers, and that use of detergent and warm water is effective for decontamination of implements used during meal preparation.

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1. Introduction

According to the latest data published by the United States Centers for Disease Control and Prevention (CDC), 1034 outbreaks of foodborne disease were reported in 2008 (CDC, 2011). These outbreaks involved 23,152 cases of illness, 1276 hospitalizations, and 22 deaths (CDC, 2011). *Salmonella* was the most common cause of hospitalizations related to foodborne disease outbreaks (62%), followed by Shiga toxin-producing *Escherichia coli* (STEC) causing 17% of the hospitalizations (CDC, 2011). The top commodities associated with these outbreaks of foodborne disease were fruits, nuts, and vine-stalk vegetables (CDC, 2011). Among the outbreaks in 2008 with a known setting where food was consumed, 52% resulted from foods consumed in a restaurant or deli, while 15% resulted from food consumed in a home (CDC, 2011).

Produce can become contaminated with pathogens during production, harvest and processing, as well as at retail outlets, food service establishments and the domestic kitchen (Johnston et al., 2005; Kader, 2002; Lynch et al., 2009). As previously mentioned, numerous outbreaks have resulted from food consumed in a restaurant or food service establishment, or in the domestic environment (CDC, 2011). Furthermore, cross-contamination plays an important role in the transmission of pathogenic microbes, especially when consumers are preparing dishes in the home (de Jong et al., 2008; van Asselt et al., 2008). There are multiple ways in which cross contamination occurs in the kitchen. According to Moore et al. (2007), contact surfaces that are easily cleaned and considered the most hygienic may become an important source of pathogens via cross-contamination, since these surfaces are also more likely to release organisms during food preparation. In the kitchen, microorganisms can diffuse and spread from contaminated foods, such as raw poultry, fish or meat, to the hand and other food contact surfaces such as cutting boards or knives (Gorman et al., 2002). The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food (Kusumaningrum et al., 2003). Therefore, consumers can take action during food preparation in the domestic kitchen setting to reduce the risk of foodborne illness (de Jong et al., 2008).

The use of food antimicrobials has been repeatedly researched and identified as useful in reducing the risk of transmission of produce-

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contaminating enteric bacterial pathogens (Lin et al., 2002; McWatters et al., 2001; McWatters et al., 2002; Samadi et al., 2009; Zhao et al., 2009, 2010). Some of these antimicrobials may be used in the domestic setting to reduce bacterial pathogens during food preparation at home. Acetic acid (AA), hydrogen peroxide (H_2O_2) and ethyl alcohol (EtOH) are listed in Title 21 of the U.S. Code of Federal Regulations (CFR) as generally recognized as safe (GRAS) (21 CFR 184.1005 [AA], 21 CFR 184.1366 [H_2O_2], and 21 CFR 184.1293 [EtOH]). They are common household products available to consumers that have been previously shown to reduce microorganisms on produce. Chang and Fang (2007) reported a 3.0 $\log_{10}$ reduction of *E. coli* O157:H7 on chopped iceberg lettuce after mixing with 5% rice vinegar at 25 °C in sterile plastic bags. Sapers and Jones (2006) reported reductions of up to 1.4 $\log_{10}$ of *E. coli* on dip-inoculated tomatoes after treatment with 1.0% hydrogen peroxide at 20 °C for 15 min. Pinto et al. (2006) reported an approximate 3.0 $\log_{10}$ reduction in *E. coli* on the surfaces of table grapes after a 3 min exposure to 50% EtOH at ambient temperature (25 °C). However, limited additional published scientific information exists regarding the effectiveness of these food antimicrobials against pathogens such as *Salmonella* and *E. coli* O157:H7 for produce surface disinfection (Yang et al., 2009).

The objectives of this study were to determine the efficacy of consumer-available antimicrobials (H_2O_2, AA, EtOH), to reduce *Salmonella enterica* and *E. coli* O157:H7 on surfaces of waxed green bell peppers using procedures likely applied in a domestic kitchen, and to determine the efficacy of cleaning methods at preventing the transfer of pathogens from contaminated knives to a non-contaminated produce item (salad cucumbers) during slicing after chopping inoculated bell peppers.

2. Materials and methods

2.1. Bacterial cultures and inoculum preparation

Produce-recovered isolates of *S. enterica* subsp. *enterica* serovars Montevideo and Poona, *Salmonella* Typhimurium American Type Culture Collection 13311 (ATCC, Manassas, VA, USA), and strains of *E. coli* O157:H7 (designated P41, P8, and E34; beef cattle carcass isolates), resistant to 100 mg/L rifampicin, were obtained from the Center for Food Safety culture collection in the Department of Animal Science (Texas A&M University, College Station, TX, USA). Cultures were maintained on tryptic soy agar (TSA; Becton, Dickinson and Co., Sparks, MD, USA) slants at 5 °C. Working cultures were obtained by transferring a loop of culture from TSA slants to 10 mL of tryptic soy broth (TSB; Becton, Dickinson and Co.) and incubating aerobically without agitation at 35 °C for 24 h. Biochemical identification of pathogens was conducted using Enterotube™ II (Becton, Dickinson and Co.) according to manufacturer instructions. *Salmonella* and *E. coli* O157:H7 were inoculated from TSA slants into 10 mL of TSB and incubated as described. Two milliliters of each TSB culture was inoculated onto 225 cm^2 surfaces of sterile TSA in bottles; strains were inoculated individually into separate bottles. The inoculum was then spread over the medium surface by aseptically adding ~80 sterile glass beads (5 mm diameter) (Thermo-Fisher Scientific, Inc., Waltham, MA, USA). Beads were rotated at a rate of one rotation per second for 10 rotations over the entire surface of the medium (Danyluk et al., 2005). Inoculated bottles were then incubated at 35 °C for 24 h to obtain a bacterial lawn. Lawns were harvested by adding 10 mL of 0.1% (w/v) peptone (Becton, Dickinson and Co.) to each bottle, swirling the glass beads left from the inoculation step to dislodge bacterial cells from agar surfaces, and transferring with a pipette to sterile 15 mL conical centrifuge tubes (Thermo-Fisher Scientific). The suspension then was washed by centrifugation at 2191×*g* in a Jouan B4i centrifuge (Thermo-Fisher Scientific, Inc.) for 15 min at 22 °C. Resulting pellets were suspended in 10 mL of 0.1% peptone and again washed by centrifugation for 15 min at 22 °C; the entire centrifugation and washing procedure was repeated identically twice. After the final cycle, pellets were suspended in 10 mL of 0.1% peptone and 10 mL aliquots of each strain were combined to make a cocktail of *Salmonella* and *E. coli* O157:H7 in a sterile Erlenmeyer flask containing 540 mL 0.1% peptone. This resulted in a concentration of 8.8 ± 0.1 $\log_{10}$ CFU/mL of *Salmonella* and 8.6 ± 0.2 $\log_{10}$ CFU/mL of *E. coli* O157:H7 for the pepper inoculum, confirmed via selective and differential plating on lactose-sulfite-phenol red-rifampicin (LSPR) agar, a medium designed for simultaneous enumeration of rifampicin-resistant *E. coli* and *Salmonella* (Castillo et al., 1998). Survivors were differentiated and enumerated following 24 h of aerobic incubation at 35 °C.

2.2. Preparation of antimicrobials and antimicrobial-appropriate neutralizers

Distilled white vinegar (5.0% acetic acid (w/v); H.J. Heinz Co. Distilled White Vinegar) was diluted (v/v) to achieve 2.5% AA, while 3% H_2O_2 (v/v) (Wal-Mart, Bentonville, AK, USA) was purchased and utilized without subsequent dilution. Absolute ethyl alcohol was diluted (v/v) to achieve 70% EtOH. Antimicrobial-appropriate neutralizers were prepared as described previously (Black et al., 2008). Neutralizer for EtOH and AA-exposed samples was prepared by first making a concentrate consisting of 40 g of lecithin (Sigma-Aldrich Co., St. Louis, MO, USA), 1.25 mL of phosphate buffer stock (Thermo-Fisher Scientific, Inc.) and 280 mL of Polysorbate 80 (Sigma-Aldrich Co.) diluted with sterile distilled water (SDW) to 1.0 L and adjusted to pH 7.2 with 1.0 N NaOH. This concentrate was used to prepare a working neutralizer by adding 100 mL of concentrate to 25 mL of 0.25 M phosphate buffer stock and 1675 mL SDW. The solution was sterilized by autoclaving at 121 °C for 15 min. In the case of H_2O_2-treated samples, a solution consisting of *Micrococcus lysodeikticus*-fermented catalase (Sigma-Aldrich Co.) was prepared in SDW and filter sterilized through a 0.2 μm cellulose acetate membrane filter (Corning Inc., Corning, NY, USA) and added to sterile working neutralizer to a final concentration of 10,000 U/mL. Neutralizer efficacy was validated through preliminary experiments (data not shown).

2.3. Disinfection of pepper surfaces by antimicrobials

Waxed green bell peppers were purchased at a local grocery store and immediately transported to the laboratory. Inoculations were completed by submerging whole peppers in a sterile beaker containing the bacterial cocktail for 1 min, followed by 60 min of drip-drying at room temperature. Inoculation of peppers with bacterial pathogens resulted in 5.8 ± 0.4 $\log_{10}$ CFU/cm^2 *Salmonella* and 5.4 ± 0.5 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 attaching to pepper surfaces. Following inoculation, duplicate identical samples were processed for each combination of antimicrobial treatment and exposure duration; each pepper represented an analytical unit. Each sample was composed of three 10 cm^2 pieces excised with individual sterile scalpels. A sterile template was used to delimitate each 10 cm^2 area. Samples were placed in separate stomacher bags and separated in groups and each group was assigned to treatments consisting of immersion in solutions of 3% H_2O_2 (v/v), 2.5% AA (v/v), 70% EtOH (v/v), or SDW for 0.25, 0.5, 1, 2, 3, 4, or 5 min. After each sample was treated, the samples were immediately submerged for 30 s in an antimicrobial-specific neutralizer (Section 2.2) before being subjected to microbiological analysis.

Microbiological analysis was conducted by adding 99 mL of sterile 0.1% peptone water to each bag with a sample and then pummeling with a Stomacher at 230 rpm for 1 min. The resulting sample suspensions were serially diluted in 0.1% peptone and then plated on LSPR to determine the bacterial load on peppers following antimicrobial treatment. The entire assay was replicated three times identically; duplicate samples were completed for each treatment or control for each replicate (n = 3). The limit of detection of pathogens from pepper surfaces was calculated to be 1 CFU/0.30 cm^2. Two non-