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Contamination of knives and graters by bacterial foodborne pathogens during slicing and grating of produce

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ABSTRACT

Poor hygiene and improper food preparation practices in consumers' homes have previously been demonstrated as contributing to foodborne diseases. To address potential cross-contamination by kitchen utensils in the home, a series of studies was conducted to determine the extent to which the use of a knife or grater on fresh produce would lead to the utensil's contamination with *Escherichia coli* O157:H7 or *Salmonella enterica*. When shredding inoculated carrots (ca. 5.3 log CFU/carrot), all graters became contaminated and the number of *E. coli* O157:H7 present on the utensil was significantly greater than *Salmonella* ($p < 0.05$). Contamination of knives after slicing inoculated produce (4.9–5.4 log CFU/produce item) could only be detected by enrichment culture. After slicing tomatoes, honeydew melons, strawberries, cucumbers, and cantaloupes, the average prevalence of knife contamination by the two pathogens was 43%, 17%, 15%, 7%, and 3%, respectively. No significant increase in the incidence or level of contamination occurred on the utensils when residues were present ($p > 0.05$); however, subsequent contamination of 7 produce items processed with the contaminated utensils did occur. These results highlight the necessity of proper sanitization of these utensils when used in preparation of raw produce.

1. Introduction

Microbial cross-contamination is a term that refers to the transfer, direct or indirect, of microorganisms (bacteria, virus, parasites, or fungi) from a contaminated item to a non-contaminated item (Minnesota Dept. Health, 2007). In the case of food, cross-contamination of foodborne pathogens is a major concern as it increases the risk for humans to ingest contaminated food and become ill. Unfortunately, many consumers have little awareness that domestic food contact surfaces and utensils are considered a contributing factor to transmission and the occurrence of sporadic foodborne disease (Bloomfield, 2003; Pérez-Rodríguez et al., 2008). Evidence to support such a role is based on epidemiological data from Europe, North America, Australia, and New Zealand that infers that a substantial proportion of foodborne disease is attributable to improper food preparation practices in consumers' homes (Redmond and Griffith, 2003).

A number of sources may contribute to the entry of foodborne pathogens into consumers' kitchens including contaminated food, consumer's hands that have been improperly washed and sanitized (Fischer et al., 2007), and domestic pets (Overgaauw et al., 2009; Stull et al., 2013). Subsequent contact of these sources with other kitchen surfaces has been documented to transfer these pathogens to those surfaces. A few examples include: counter surfaces (Gorman et al., 2002), refrigerator handles (Gorman et al., 2002), kitchen faucets (Kishimoto et al., 2004), dish cloths (Gorman et al., 2002; Macías-Rodríguez et al., 2013), cutting boards (Chai et al., 2008; Luber et al., 2006; Wachtel et al., 2003; Zhao et al., 1998) and knives (Kishimoto et al., 2004; Luber et al., 2006). A number of factors contribute to the dynamics of pathogen transference and these have been divided into extrinsic (environmental) and intrinsic factors in a review article by Pérez-Rodríguez et al. (2008). In that publication, some of the extrinsic factors included the surface properties of both the donating and receiving objects, level of moisture present on their surfaces, relative humidity in the atmosphere, contact pressure between the two contacting surfaces, and the time of that contact. Intrinsic factors that were identified included the pathogenic agent's characteristics (surface structures,

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presence of exopolysaccharides) and the pathogen's contamination level.

In conducting cross-contamination studies from pathogen-contaminated foods to food contact surfaces, a number of studies have examined these interactions by applying a defined pressure between the food product and coupons (small flat pieces) of the subject material (Escudero et al., 2012; Kusumaningrum et al., 2003). Although such scenarios are designed to represent typical contact pressures encountered during kitchen operations and provide more consistent results, simulations of actual kitchen practices should provide more realistic data for inclusion in risk assessment models. Using such an approach, several studies have examined the transfer of pathogens from contaminated chicken to lettuce, cucumbers, or fruit salad via the interim contamination of the cutting board and knife (Luber et al., 2006; Ravishankar et al., 2010; Verhoeff-Bakkenes et al., 2008; Zhao et al., 1998). Based on these results, guidance documents advocate that consumers use separate cutting boards for preparing meat and produce (US HHS, 2014). Adherence to this guideline would therefore minimize risk of cross-contamination in the kitchen, but it does not eliminate it. Raw produce itself may be contaminated either during pre- or postharvest operations (Doyle and Erickson, 2008) and hence, could lead to cross-contamination and foodborne illness when kitchen utensils used in their preparation are subsequently used on other items that would be consumed raw. To illustrate this point, immediately after cutting raw vegetables (mung bean sprouts, Indian pennywort, and winged bean) that were naturally contaminated with *Campylobacter jejuni*, transfer rates of the pathogen to a cucumber cut on the same board ranged from 22.6 to 73.3% (Chai et al., 2008). Similarly, *Escherichia coli* O157:H7 inoculated on lettuce at either a high (6 log CFU/g) or low (4 log CFU/g) dose was transferred to a sterile knife and then to uninoculated lettuce at a level of 4 log CFU/g and 2 log CFU/g, respectively (Poimenidou et al., 2012). In that same study, however, the level of pathogen transferred to cabbage was much less than it was to lettuce exemplifying that the properties of the target vegetable influence the magnitude of pathogen transfer.

Cleaning of knives and boards after individual operations would be an advantageous practice to minimize cross-contamination of diverse food items; however, Jevšnik et al. (2008) reported that 66% of consumers (n = 1030) did not wash knives before reuse. Unfortunately, it would appear that improvements in that practice have not occurred since that study was conducted as Kennedy et al. (2011) reported that 50% of their consumers (n = 60) only rinsed the contaminated knife with water and another 20% did not wash the contaminated knife at all. Regardless of these disappointing statistics, there is also the realization that cleaning knives is not a guarantee that all contamination will be removed. When six *E. coli* O157:H7 and *Salmonella*-contaminated knife blades (3.6–3.7 log/cm^2) were wiped with a dry sterile cotton towel, both pathogens were present after this operation (2.4–3.0 log/cm^2) and contamination of cucumbers sliced with these wiped knives also occurred (1.9–2.6 log CFU/cm^2) (Perez et al., 2012). Furthermore, wiping knives with a sponge hydrated with soap (1% lauryl sulfate-based detergent) and rinsing the knives with warm running water for 10 s before slicing cucumbers still led to contamination of 50% of those cucumbers (0.1–0.2 log/cm^2). Given these results and consumers' propensity to leave knives dirty, transfer of pathogens on knives should be conducted in the presence of surface residues. In studies where residue was considered as a variable, cell attachment differed depending on the nature of the residue (Whitehead et al., 2010).

In an effort to gain a deeper understanding of the range of pathogen transfer that occurs between ready-to-eat produce items and common kitchen utensils, two different approaches were taken in this study. In the first approach, the prevalence and level of contamination incurred on clean and soiled knives and graters used to process an assortment of *Salmonella*- or *E. coli* O157:H7-contaminated produce items was evaluated. In the second approach, the extent of transfer of these pathogens to uncontaminated produce items was assessed when knives and graters that had been contaminated from processing contaminated produce were used. Since a fundamental assumption to conducting such a study entails that conditions for extraction of viable pathogens from utensils and food be optimal, a preliminary study was also performed whereby extraction solution formulations and physical methods to dislodge *Salmonella* from utensils were compared.

2. Materials and methods

2.1. Source and handling of produce prior to experiment

Cantaloupe, carrots, cucumbers, honeydew melons, strawberries, and tomatoes were purchased from local supermarkets and stored for 1–5 days in a walk-in cooler (4 °C) before using them in experiments. At the time of their use in experiments, all produce was still deemed to be in a “fresh” state.

2.2. Kitchen utensil description and maintenance

Two stainless steel utensils were selected as common items in consumers' kitchens that would be applied to fresh produce items. They were a steak knife (J.A. Henckels, Hawthorne, NY; blade size of 12.5 cm in length and 1.8 cm width at the base) and a flat grater (Bed, Bath & Beyond, Union, New Jersey; size of 22.0 cm in length, 11.0 cm in width) that had its rubber handles removed. Utensils were used multiple times throughout the study. To sterilize the utensils between uses, knives and graters were autoclaved (121 °C, 15 min) to ensure that resident pathogens that might have lodged in cracks or crevices on the items during a previous trial were inactivated. To remove rust spots that occurred on knives after autoclaving, knives were brushed with a scouring pad.

2.3. Pathogen source, cultivation, and enumeration of inocula

E. coli O157:H7 (alfalfa sprout outbreak F4546 isolate, human fecal ATCC 43888 isolate, cattle E0122 isolate, 2006 spinach outbreak K3995 isolate, and 2006 Taco Bell outbreak K4492 isolate) and *Salmonella enterica* isolates (*S.* Enteritidis isolates ME-18, H4639, and H3353 of unknown origin and *S.* Newport beef 11590-K isolate) were obtained from the culture collection housed at the University of Georgia, Center for Food Safety (Griffin, GA). The method described by Ma et al. (2011) had been used to label all isolates with a green fluorescent protein (GFP) plasmid containing an ampicillin-resistant marker. The resulting transformed colonies emitted bright green fluorescence when viewed under a handheld UV light (365 nm).

Cultivation of pathogens involved thawing frozen individual isolates of each pathogen, streaking onto TSA-Amp plates containing tryptic soy agar (Acumedia, Lansing, MI) and 100 μg/ml ampicillin (Roche Diagnostics, Indianapolis, IN), and incubating at 37 °C for 24 h. Individual colonies from these plates were then transferred into10 ml tryptic soy broth containing 100 μg/ml of ampicillin (TSB-Amp). This suspension was incubated for 18–24 h at 37 °C before harvesting the bacteria by centrifugation (4050 × g, 15 min, 4 °C), washing the pelleted cells three times with 0.1% peptone water, and suspending in sterile deionized water (SDW) to achieve an optical density of 0.5 at 630 nm that was equivalent to ca. 10^8 CFU/ml. Equal volumes from the four-to five-strain isolates were combined to obtain separate *E. coli* O157:H7