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# Cross-Contamination of Lettuce (*Lactuca sativa* L.) with *Escherichia coli* O157:H7 via Contaminated Ground Beef†

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## ABSTRACT

A lettuce outbreak strain of *E. coli* O157:H7 was used to quantitate the pathogen's survival in ground beef and its transfer to hands, cutting board surfaces, and lettuce. Overnight storage of inoculated beef at 4°C resulted in no pathogen growth, while room-temperature storage allowed multiplication. Hamburger patty formation allowed the transfer of bacteria to hands. Contaminated fingers subsequently transferred the pathogen to lettuce during handling. *E. coli* was transferred from hamburgers to cutting board surfaces; overnight storage of boards decreased the numbers of recoverable pathogens by ~1 log CFU. A 15-s water rinse failed to remove significant numbers of pathogens from cutting boards whether it was applied immediately after contamination or following overnight room-temperature storage. Three lettuce leaves were successively applied to a single contaminated cutting board area both immediately after contamination and after overnight room-temperature storage of contaminated boards. Another set of leaves was pressed onto boards immediately following contamination and was then stored overnight at 4°C before pathogen enumeration. The numbers of pathogens transferred to the first pressed leaves were larger than those transferred to the second or third leaves. There were no significant differences in the numbers of pathogens recovered from leaves pressed immediately after contamination whether pathogens were enumerated immediately or following overnight storage at 4°C. However, fewer pathogens were transferred to leaves pressed to boards stored overnight at room temperature prior to contact with lettuce. Twenty-five lettuce pieces were successively pressed onto one area on a board containing $1.25 \times 10^2$ CFU of *E. coli*. Pathogens were transferred to 46% of the leaves, including the 25th exposed leaf.

Postharvest contamination and growth of bacterial pathogens on produce can occur via several mechanisms, including contamination of transport equipment, processing wash water, or ice; temperature abuse during storage or packaging; and cross-contamination from other foods *(5)*. In recent years, several *Escherichia coli* O157:H7 outbreaks have been caused by the consumption of lettuce or salad contaminated after harvest. Although farm-to-fork food safety practices have received much attention recently, outbreaks caused by poor adherence to these standards continue to occur.

Thirty Boy Scouts were infected with *E. coli* O157:H7 in 1995 after consuming lettuce that had been stored with raw beef *(12)*. A restaurant salad bar was the source of a 1999 *E. coli* O157:H7 outbreak that sickened 46 people. The pathogen was thought to have been transferred to salad via preparation by an infected food handler *(11)*. In March 1999, 72 people fell ill during an *E. coli* O157:H7 outbreak in Kearney, Nebr. Case patients were infected with the pathogen via contaminated iceberg lettuce at a restaurant salad bar. Epidemiologic analysis implicated a sick restaurant employee and improper storage procedures in the outbreak *(16)*.

Previously, we simulated the lettuce contamination and storage conditions found in the Nebraska restaurant to determine the extent of possible pathogen cross-contamination and growth during chopping, washing, and storage *(18)*. We noted the lack of information in the literature on the potential for the growth of *E. coli* O157:H7 in ground beef as well as the transfer of the organism from hamburger patties to produce via common food-handling practices. Here, we extended our earlier studies to include possible cross-contamination via handling of ground beef and transfer to lettuce from human handling and cutting boards.

Previous studies by others have focused on various aspects of cross-contamination, including the role of gloves and cutting boards in the promotion or inhibition of pathogen attachment and growth. Miller et al. *(13)* found that the levels of attachment of beef bacterial microbiota to polyethylene and wooden cutting boards were similar. In this study, chemical cleaners removed similar numbers of bacteria relative to water alone. Ak et al. *(1)* showed that fewer *E. coli* O157:H7 cells were recovered from wooden boards than from plastic boards; the extent of this difference increased with incubation time. The pathogen was recovered from plastic cutting boards after 12 h, and bacteria

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multiplied on plastic cutting board surfaces when applied in filter-sterilized chicken juice. High humidity was necessary for the persistence and multiplication of bacteria on plastic cutting boards *(2).*

Transfer rates among hands, foods (including chicken and lettuce), and kitchen surfaces were studied by Chen et al. *(7),* who used a nonpathogenic indicator, *Enterobacter aerogenes,* to provide data for risk management efforts. These investigators also examined transfer rates from food to both gloved and bare hands. Although gloves provided a barrier to bacterial transfer, polyethylene gloves were permeable to bacteria *(14).*

Here, we used a relevant lettuce outbreak strain of *E. coli* O157:H7 to quantitate bacterial survival in ground beef and subsequent transfer of bacteria to hands and cutting board surfaces. We also investigated the cross-contamination of iceberg lettuce by contaminated hands and cutting boards and the potential for produce contamination after repeated contact with cutting boards.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** *E. coli* O157:H7 strain F6460 was used throughout this study. This strain, originally isolated from patients during a 1999 lettuce outbreak linked to a Nebraska restaurant, was obtained from Timothy Barrett (Centers for Disease Control, Atlanta, Ga.) and Thomas Safranek (Nebraska Health and Human Services System, Lincoln, Nebr.). The (nonpathogenic) *E. coli* normal flora strain 97A-5984 was obtained from Sharon Abbott and Michael Janda (California State Department of Health Services, Microbial Diseases Laboratory, Berkeley, Calif.). Nalidixic acid–resistant strains F6460 $Nal^R$ and 97A-5984 $Nal^R$ were isolated as described previously *(18).* Bacteria were stored at −80°C in Luria-Bertani (LB) broth (Difco Laboratories, Detroit, Mich.) containing 25% (vol/vol) glycerol. *E. coli* O157:H7 strains were grown on LB agar plates or in LB broth at 37°C. When appropriate, nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) was added to achieve a final concentration of 20 μg/ml.

***E. coli* O157:H7 cross-contamination experiments.** All cross-contamination experiments were performed three or four times; samples were tested in triplicate unless otherwise indicated.

**Transfer of *E. coli* O157:H7 from contaminated ground beef to fingers via human handling.** *E. coli* strains F6460 $Nal^R$ and 97A-5984 $Nal^R$ were grown overnight (for 12 to 16 h) at 37°C in LB broth containing 20 μg of nalidixic acid per ml. Tenfold serial dilutions of bacteria were prepared with phosphate-buffered saline (PBS) and held in test tubes for 2 days at room temperature (ca. 25°C) to starve the bacteria, thus simulating a realistic inoculum. Fresh 80 and 93% lean ground beef samples and iceberg lettuce (*Lactuca sativa* L.) were obtained from a local supermarket. Both the ground beef and the lettuce were stored at 4°C and used within 12 to 16 h of purchase. Ground beef was divided into 0.25-lb (113-g) samples and inoculated with 2.2 ml of the starved *E. coli* O157:H7 culture. Bacteria were mixed into the ground beef by hand for 1 min with the use of latex gloves (Evolution One, Microflex Medical Corp., Reno, Nev.). The gloved fingers of each hand were gently pressed onto LB agar plates containing nalidixic acid, which were incubated overnight at 37°C before the enumeration of bacteria. Gloves were changed between samples.

Alternatively, ground beef samples were inoculated with *E. coli* normal flora strain 97A-5984 $Nal^R$ and mixed with gloved or bare hands as described above. Contaminated fingers were pressed onto LB agar containing nalidixic acid and incubated overnight at 37°C before the enumeration of bacteria. Gloves were changed after each sample, and bare hands were washed thoroughly with a 50% solution of Ultra Joy concentrated dishwashing detergent (Proctor and Gamble, Cincinnati, Ohio) and warm tap water between samples.

***E. coli* O157:H7 growth and survival in ground beef.** Ground beef was inoculated with *E. coli* O157:H7 strain F6460 $Nal^R$ as described above and divided into halves, with each half weighing ca. 56.5 g. One 5-g sample from the first inoculated half was analyzed immediately, and a 5-g sample from the second half was analyzed following overnight storage (16 h) at 4°C or at room temperature (ca. 25°C). The remainder of each half patty was reserved for cutting board transfer experiments (see below). Ground beef samples were placed in a Whirl-Pak bag (Nasco, Fort Atkinson, Wis.) containing 25 ml of sterile peptone water and homogenized in a Stomacher 80 Biomaster (Seward Limited, London, UK) for 1 min at high speed. Homogenates were filtered through sterile glass wool, and duplicate samples were spiral plated (Wasp II Spiral Plater, DW Scientific, West Yorkshire, UK) onto LB agar plates containing nalidixic acid. After overnight (12- to 16-h) incubation at 37°C, colonies were enumerated with an automated plate counter (Protoc, Synoptics, Cambridge, UK).

**Transfer of *E. coli* O157:H7 from contaminated fingers to lettuce via human handling.** Ground beef was divided and hand mixed with *E. coli* O157:H7 strain F6460 $Nal^R$ for 1 min with the use of latex gloves as described above. A 7-g sample of lettuce was then handled with contaminated gloved fingers for 15 s. Gloves were changed after the handling of each sample. Lettuce samples were homogenized in 35 ml of sterile peptone water and plated on selective medium, and the associated pathogens were enumerated as described above.

**Transfer of *E. coli* O157:H7 from contaminated hamburger patties to cutting boards and subsequent rinsing of boards.** Ground beef was hand mixed with *E. coli* O157:H7 strain F6460 $Nal^R$ for 1 min with the use of latex gloves and divided as described above. The inoculated beef was pressed firmly onto a sterile polyethylene cutting board (America Cooks, Robinson Knife Co., Buffalo, N.Y.) for ca. 5 to 10 s to form hamburger patties ca. 8 cm in diameter and removed. Cutting boards were tested for *E. coli* cross-contamination immediately or after overnight storage (16 h, uncovered) at room temperature (ca. 25°C). A sterile cotton swab was dipped into 1 ml of sterile PBS, rubbed for 10 s over the entire cutting board area that had been in contact with the contaminated hamburger, and then vortexed in PBS. The swab was then pressed along the edge of the tube to remove most of the remaining PBS. Duplicate cutting board swab samples were plated onto LB agar containing nalidixic acid, incubated, and enumerated as described above.

A cutting board rinsing experiment was carried out by inoculating ground beef and forming hamburger patties on cutting boards as described above. Contaminated boards were rinsed for 15 s with warm (ca. 35°C) tap water at a medium flow rate of ca. 4 liters/min. Water was allowed to hit the board ca. 10 cm above the contaminated spot to rinse off associated bacteria. Pathogens remaining on cutting boards following the water rinse were enumerated as described above.

**Transfer of *E. coli* O157:H7 from contaminated cutting boards to successive lettuce pieces.** Ground beef was inoculated with *E. coli* O157:H7 strain F6460 $Nal^R$, and hamburger patties

were formed on sterile cutting boards as described above. The outer leaves of commercial iceberg lettuce heads were removed; the inner leaves were cut into 4-g pieces (ca. 8 by 8 cm) with a sterile knife and firmly pressed by hand onto the contaminated areas of cutting board surfaces for 5 s. The pressure used to press lettuce onto the cutting boards was about the pressure that a food handler would exert to hold lettuce heads while chopping and was sufficient to deform the lettuce such that the lettuce made full contact with the board surface. Three pieces of lettuce were pressed in succession onto the same contaminated spot and processed immediately (for both nonstored cutting boards and cutting boards stored overnight at room temperature). An additional set of three lettuce pieces was immediately pressed onto contaminated cutting boards and then stored overnight at 4°C in sealed Ziploc bags (S. C. Johnson and Son, Inc., Racine, Wis.) before processing. Lettuce pieces were placed in Whirl-Pak bags containing 20 ml of sterile peptone water, homogenized, and plated, and bacteria were enumerated as described above.

**Transfer of *E. coli* O157:H7 from contaminated cutting boards to 25 lettuce pieces in succession.** Ground beef was inoculated with *E. coli* O157:H7 strain F6460 $Nal^R$, and hamburger patties were formed on sterile cutting boards as described above. Twenty-five iceberg lettuce pieces (4 by 4 cm) were firmly pressed in succession for 5 s onto the same contaminated spot on the cutting board. Five 1-$cm^2$ pieces were cut from the center of each pressed lettuce piece with a razor (to facilitate the placement of lettuce into the test tube). All five pieces cut from the same pressed lettuce sample were placed into a single test tube containing LB broth supplemented with nalidixic acid, and the tubes were incubated on a roller drum overnight at 37°C to select for the growth of *E. coli* O157:H7 strain F6460 $Nal^R$ (25 tubes for each set of 25 leaves pressed against a single contaminated spot on the cutting board). Three bacterial cultures from each set of leaves positive for growth in selective media were confirmed to be *E. coli* O157:H7 cultures by polymerase chain reaction (PCR) analysis with primers specific for *eae (8),* the gene encoding the intimin outer-membrane protein *(4, 10).*

**Analysis of ground beef normal flora.** For each trial, two 25-g samples of each ground beef type (80% lean or 93% lean) were placed in sterile 400-ml filter bags (Spiral Biotech, Norwood, Mass.) and diluted 1:10 (Dilumat3, AES Laboratories, Combourg, France) in 0.1% sterile peptone (Difco). Each sample was homogenized in a Stomacher 400 (Interscience, Switzerland) at high speed for 2 min. Serial dilutions were made, spiral plated in duplicate onto plate count agar (Difco), and incubated at 30°C for 24 h. After incubation, five colonies were picked from each plate, restreaked for isolation onto plate count agar, and incubated at 30°C for 24 hr. A MacFarland standard was prepared from the isolated colonies on each of the plates, and a corresponding oxidase test (for gram-negative organisms) or catalase test (for gram-positive organisms) was performed. Each isolated organism was inoculated into a test card and loaded into the Vitek 32 apparatus (bioMerieux, Inc., Hazelwood, Mo.) for identification according to the manufacturer's instructions. PCR analysis was performed on three colonies identified as *Yersinia* spp. but undefined at the species level. PCR reactions were carried out with primers specific for the 16s rRNA and *ail* genes. Further analysis was conducted with the Biolog MicroLog (Release 4.0, Biolog Microlog, Hayward, Calif.) system according to the manufacturer's instructions. Three *E. coli* colonies were analyzed by PCR with primers specific for *eae (8).*

**Fat and moisture analysis of ground beef.** Approximately 4 g of ground beef was placed in a Whatman no. 2 filter paper packet. The packet was oven dried overnight at 100°C (Napco no. 5861, Precision Scientific, Chicago, Ill.) to determine the percentage of moisture (=[wet weight − dry weight]/wet weight × 100). Dried samples were then extracted with petroleum ether (certified ACS, Fisher Scientific, Fair Lawn, N.J.) in a Soxhlet apparatus (Corning, Inc., Acton, Mass.) *(3).* Samples were subjected to six ether flushes (ca. 12 to 16 h total extraction time), air dried to evaporate ether vapors, and then oven dried at 100°C for 5 h to remove traces of moisture. The percentage of fat was calculated as (dry weight − extracted weight)/wet weight × 100. Six replicates were tested for each sample.

**Statistics.** Data were analyzed with the use of the General Linear Model procedure of the Statistical Analysis System (Version 7, SAS, Cary, N.C.) *(15).* Treatments were arranged in a randomized complete block design, with $X$ and $Y$ being used as main effects. Means were separated with Duncan's multiple-range test, and correlations between variables were determined with Pearson's correlation factors. Differences were considered significant at $P \leq 0.05$.

## RESULTS

**Pathogen contamination and temperature abuse of ground beef result in subsequent produce contamination via human handling.** To determine the effect of temperature abuse on *E. coli* O157:H7 growth in contaminated ground beef and on the potential for the subsequent transfer of bacteria to produce via human handling, we carried out cross-contamination studies. Fresh commercial 80 and 93% lean ground beef and iceberg lettuce were used for these studies. To begin these studies, we analyzed commercial ground beef for fat and moisture content, as well as for the possible presence of bacterial pathogens, including *E. coli* O157:H7. Fat and moisture analysis of ground beef sampled on 6 days revealed means of 64.15% ± 1.98% moisture and 82.36% ± 1.64% lean tissue for ground beef labeled 80% lean and means of 73.06% ± 1.83% moisture and 92.47% ± 0.91% lean tissue for ground beef labeled 93% lean (data not shown). The normal microfloras of the ground beef were characterized with the use of a Vitek 32 apparatus. The identification of 79 colonies isolated from 80% lean beef revealed 62% *Pseudomonas* spp., 14% *Streptococcus* spp., 7% *Staphylococcus* spp., 5% *Yersinia intermedia,* 4% *Enterococcus* spp., 3% *Yersinia* spp., 2% *Aeromonas* spp., 1% *E. coli,* and 1% *Alcaligenes* spp. Seventy-five colonies isolated from 93% lean ground beef were characterized as 67% *Pseudomonas* spp., 23% *Streptococcus* spp., 4% *Yersinia* spp., 2% *Staphylococcus* spp., 1% *Listeria* spp., 1% *Aeromonas* spp., and 1% *Corynebacterium* spp. PCR analysis was conducted for three colonies identified as *Yersinia* but not defined at the species level. Results obtained with primers specific for the 16s rRNA and *ail* (attachment invasion locus) genes *(20)* suggested that these colonies were not pathogenic *Yersinia enterocolitica* colonies. One of these colonies was confirmed to be a *Yersinia frederiksenii* colony with the Biolog MicroLog system. Three *E. coli* colonies tested negative for the presence of the *eae* gene by PCR analysis *(8),* suggesting that these colonies were not *E. coli* O157:H7 colonies.

TABLE 1. *Growth and survival of* E. coli *O157:H7 strain F6460 Nal$^R$ in ground beef*[a]

| Initial inoculum level (CFU/g of ground beef) | % leanness of beef | Incubation temperature | *E. coli* O157:H7 count (CFU/g of ground beef) | |
|---|---|---|---|---|
| | | | 0 h | 16 h |
| $4 \times 10^5$ | 93 | 4°C | $2.2 (\pm 0.1) \times 10^5$ | $2.5 (\pm 0.8) \times 10^5$ |
| | 80 | 4°C | $2.5 (\pm 0.3) \times 10^5$ | $3.0 (\pm 0.6) \times 10^5$ |
| $6 \times 10^4$ | 93 | RT | $4.3 (\pm 2.9) \times 10^5$ | $4.8 (\pm 0.7) \times 10^7$ |
| | 80 | RT | $1.7 (\pm 0.6) \times 10^5$ | $3.9 (\pm 0.4) \times 10^8$ |
| $4 \times 10^4$ | 93 | 4°C | $2.3 (\pm 0.6) \times 10^4$ | $2.9 (\pm 0.3) \times 10^4$ |
| | 80 | 4°C | $2.8 (\pm 0.7) \times 10^4$ | $3.4 (\pm 0.4) \times 10^4$ |
| $6 \times 10^2$ | 93 | RT | $5.8 (\pm 4.3) \times 10^2$ | $9.2 (\pm 5.8) \times 10^4$ |
| | 80 | RT | $1.8 (\pm 0.4) \times 10^3$ | $1.2 (\pm 0.3) \times 10^5$ |

[a] Ground beef (93 or 80% lean) was inoculated with strain F6460 Nal$^R$ and formed into patties. Beef was sampled immediately (0 h) and after storage for 16 h at 4°C or room temperature (RT), and pathogens were then enumerated. Three samples were analyzed for each treatment; means and standard errors of the mean are given. The limit of detection was 120 CFU/g of ground beef.

Ground beef patties (113 g [ca. 0.25 lb]) were inoculated with *E. coli* O157:H7 outbreak strain F6460 Nal$^R$ and stored for 12 to 16 h at 4°C or at room temperature (ca. 28°C). Storage at 4°C resulted in no significant pathogen growth at two bacterial inoculation levels and two different beef fat levels (Table 1) ($P > 0.05$ for 93 versus 80% lean beef and for 0 versus 16 h). However, overnight storage at room temperature allowed significant bacterial multiplication in all samples tested (Table 1) ($P = 0.0056$ for 0 versus 16 h). Ground beef inoculated with $6 \times 10^4$ CFU/g displayed 2.04 and 3.38 log units of pathogen growth after 16 h of storage at room temperature in 93 and 80% lean beef, respectively. A lower inoculum level ($6 \times 10^2$ CFU/g) resulted in 2.20 and 1.84 log units of growth in 93 and 80% lean beef, respectively, under the same storage conditions (Table 1).

TABLE 2. *Transfer of* E. coli *from contaminated ground beef to fingers via human handling*[a]

| Initial inoculum level (CFU/g of ground beef) | *E. coli* strain | % leanness of beef | Level of *E. coli* recovered (CFU/10 fingers) |
|---|---|---|---|
| $4 \times 10^4$ | F6460 Nal$^R$ | 93 | $6.2 (\pm 0.2) \times 10^2$ |
| | F6460 Nal$^R$ | 80 | $5.1 (\pm 0.8) \times 10^2$ |
| $4 \times 10^3$ | F6460 Nal$^R$ | 93 | $2.5 (\pm 0.2) \times 10^2$ |
| | F6460 Nal$^R$ | 80 | $2.0 (\pm 0.6) \times 10^2$ |
| $7 \times 10^4$ | 97A-5984 Nal$^R$ | 93 | $4.2 (\pm 0.2) \times 10^2$ |
| | 97A-5984 Nal$^R$ | 80 | $6.2 (\pm 0.7) \times 10^2$ |
| $7 \times 10^3$ | 97A-5984 Nal$^R$ | 93 | $5.0 (\pm 0.1) \times 10^2$ |
| | 97A-5984 Nal$^R$ | 80 | $4.4 (\pm 0.1) \times 10^2$ |

[a] Ground beef (93 or 80% lean) was inoculate with bacteria and formed into patties. Hands covered with latex gloves were used to inoculate beef with *E. coli* O157:H7 strain F6460 Nal$^R$, and bare hands were used to inoculate beef with the (nonpathogenic) normal human flora strain 97A-5984 Nal$^R$. Bacteria were then recovered from the 10 contaminated fingers of the two inoculating hands and quantitated. Three samples were analyzed for each treatment; means and standard errors of the mean are given. The limit of detection was 1 CFU per 10 fingers.

To determine the capacity of *E. coli* O157:H7 for transfer from contaminated beef to produce via human handling, we first quantitated bacteria associated with gloved and nongloved hands after the manual formation of a hamburger patty from contaminated ground beef for 1 min. Lettuce was then subjected to handling with contaminated hands. The handling of ground beef containing bacteria at levels of $4 \times 10^4$ and $4 \times 10^3$ CFU/g resulted in averages of 2.76 and 2.35 log units of *E. coli* O157:H7 associated with gloved fingers, respectively (Table 2). There was no significant difference between levels of pathogen transfer for 93 and 80% lean beef ($P = 0.910$). Similar levels of transfer to gloved fingers (data not shown) and bare hands (Table 2) were seen for *E. coli* normal flora strain 97A-5984 Nal$^R$. Again, beef fat content did not affect this transfer rate ($P = 0.1635$ for 93 versus 80% lean beef). Human handling of lettuce with contaminated gloved fingers for ca. 15 s resulted in the transfer of ca. 10% *E. coli* O157:H7 from contaminated ground beef to lettuce for the inoculum level $9.6 \times 10^4$ CFU/g and in the transfer of ca. 1% *E. coli* O157:H7 from contaminated ground beef to lettuce for the inoculum level $9.6 \times 10^3$ CFU/g (Table 3). Beef fat content did not significantly affect this transfer rate overall ($P = 0.5500$ for 93 versus 80% lean beef for all samples tested).

**Pathogens transferred to cutting boards and produce persist despite storage and repeated contact.** Contact of hamburger patties containing $4 \times 10^5$ CFU of *E. coli* O157:H7 per g with plastic cutting board surfaces for 5 to 10 s resulted in significant transfer of bacteria to the board surfaces (with an average transfer level of ca. $7 \times 10^3$ CFU/50 cm$^2$) (Fig. 1A). Overnight storage of contaminated boards at room temperature (ca. 25°C) significantly decreased the numbers of recoverable pathogens on cutting boards by an average of 1 log CFU in all cases (Fig. 1A) ($P = 0.0036$ for 0 versus 16 h of storage). However, numbers of bacteria cultured from cutting boards remained at levels sufficient to cause human disease (an average of 7.09

TABLE 3. *Transfer of* E. coli *O157:H7 from contaminated gloved fingers to lettuce*[a]

| Initial inoculum level (CFU/g of ground beef) | % leanness of beef | Level of *E. coli* O157:H7 recovered (CFU/g of lettuce) |
|---|---|---|
| $9.6 \times 10^4$ | 93 | $1.0 (\pm 0.3) \times 10^4$ |
| | 80 | $1.3 (\pm 0.3) \times 10^4$ |
| $9.6 \times 10^3$ | 93 | $5.9 (\pm 1.3) \times 10^1$ |
| | 80 | $9.4 (\pm 0.4) \times 10^1$ |

[a] Ground beef was hand mixed with *E. coli* O157:H7 strain F6460 $Nal^R$ with the use of latex gloves. Commercial iceberg lettuce was handled for 15 s with contaminated gloved fingers, and the pathogens associated with the lettuce were then quantitated. Four samples were analyzed for each treatment; means and standard errors of the mean are given. The limit of detection was 120 CFU/g of lettuce.

$\times 10^2$ CFU for the dose $4 \times 10^5$ CFU and an average of $3.55 \times 10^1$ for the dose $4 \times 10^4$ CFU).

The number of pathogens dissociated from cutting boards after a 15-s rinse with warm (35°C) water was not significantly different from the number dissociated from nonrinsed boards (Fig. 1B) ($P = 0.0550$ for all samples tested over time). A rinse applied immediately after bacterial contamination resulted in an average 0.23-log decrease in the number of pathogens associated with cutting boards, with an average of 39.8% of the pathogens being removed. Inoculation of cutting boards followed by 16 h of storage prior to rinsing resulted in the disassociation of an average of 0.39 log units (58.6%) of *E. coli*. Again, the numbers of pathogens remaining on cutting board surfaces following 16 h of storage and a warm-water rinse were sufficient to cause disease *(9, 17)*.

To quantitate the transfer of *E. coli* O157:H7 from contaminated cutting boards to lettuce, three leaves were pressed in succession onto a single contaminated area of the board immediately after the boards were exposed to tainted beef patties and after room-temperature (ca. 25°C) storage of contaminated boards for 16 h. An additional set of leaves was pressed onto boards immediately after contamination by beef patties and stored overnight at 4°C before the enumeration of pathogens. The difference between the numbers of pathogens transferred to the first pressed leaves and the numbers transferred to third leaves was, on average, 0.47 log CFU/g of lettuce (Fig. 2A and 2B). The total number of pathogens recovered from the three lettuce leaves combined was similar to the numbers of pathogens swabbed from contaminated boards (Fig. 2A and 2B versus Fig. 1A for the inoculum level $4 \times 10^5$ CFU). We recovered $2.05 \times 10^3$ CFU of pathogens from three lettuce leaves applied in succession to a 50-$cm^2$ area on the board containing $8.03 \times 10^3$ CFU. After overnight incubation, $9.53 \times 10^2$ CFU was cultured from cutting board swabs, while three pressed leaves were found to carry $4.67 \times 10^2$ total CFU (93% lean beef). Similar results were obtained when 80% lean beef was used to contaminate cutting boards (data not shown). Overall, the numbers of pathogens transferred to the first pressed leaves were significantly larger than the numbers transferred to the second and third leaves (Fig. 2A and 2B) ($P = 0.0018$ for the numbers of pathogens associated with first leaves versus the numbers associated with second or third leaves under all conditions tested). However, there was no significant difference in the numbers of pathogens transferred to second leaves and the numbers transferred to third leaves ($P > 0.05$). There was no significant difference between the numbers of pathogens recovered from leaves pressed immediately after contamination and enumerated immediately and the numbers re-

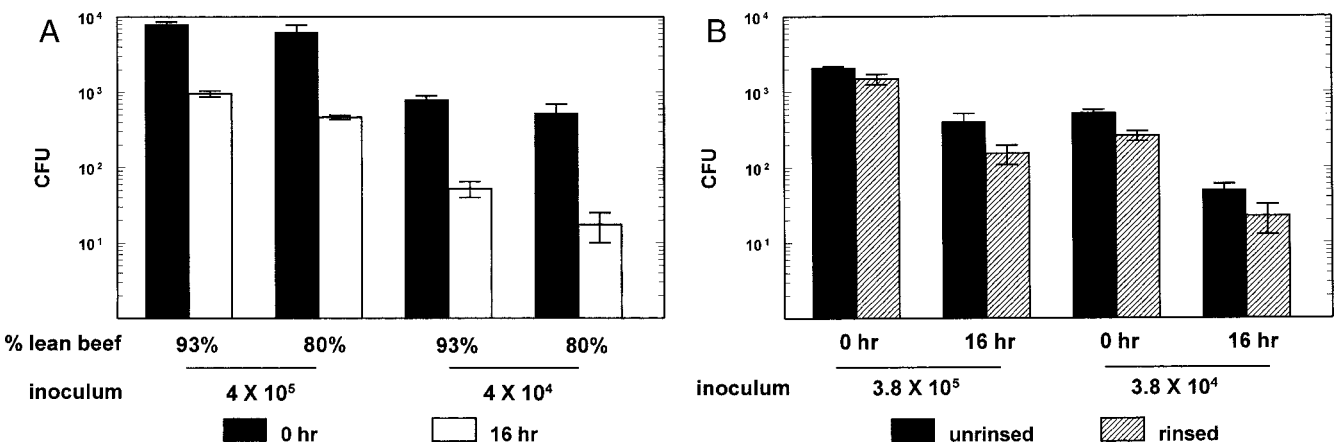


FIGURE 1. *Transfer of* E. coli *O157:H7 from contaminated ground beef patties to cutting boards via human handling and subsequent rinsing of boards. Ground beef (93 or 80% lean) was inoculated with* E. coli *O157:H7 strain F6460 $Nal^R$; inoculum levels (CFU/g of ground beef) are indicated. Hamburger patties were formed and gently pressed onto polyethylene cutting boards. The values shown on the* y *axis represent the total numbers of* E. coli *CFU recovered from a ~50-$cm^2$ area of the cutting board contaminated by the inoculated beef patties. (A) Pathogens associated with boards were quantitated immediately (solid bars) and after storage at room temperature (ca. 25°C) for 16 h (open bars). (B) For 93% lean beef only, pathogens associated with boards were enumerated immediately (solid bars, time indicated), after a 16-h storage period (solid bars, time indicated), and after a 15-s rinse with warm tap water (ca. 35°C) (cross-hatched bars, time indicated). Three or four samples were tested for each datum point; the datum points shown represent means and standard errors of the means. The limit of detection was 10 CFU.*

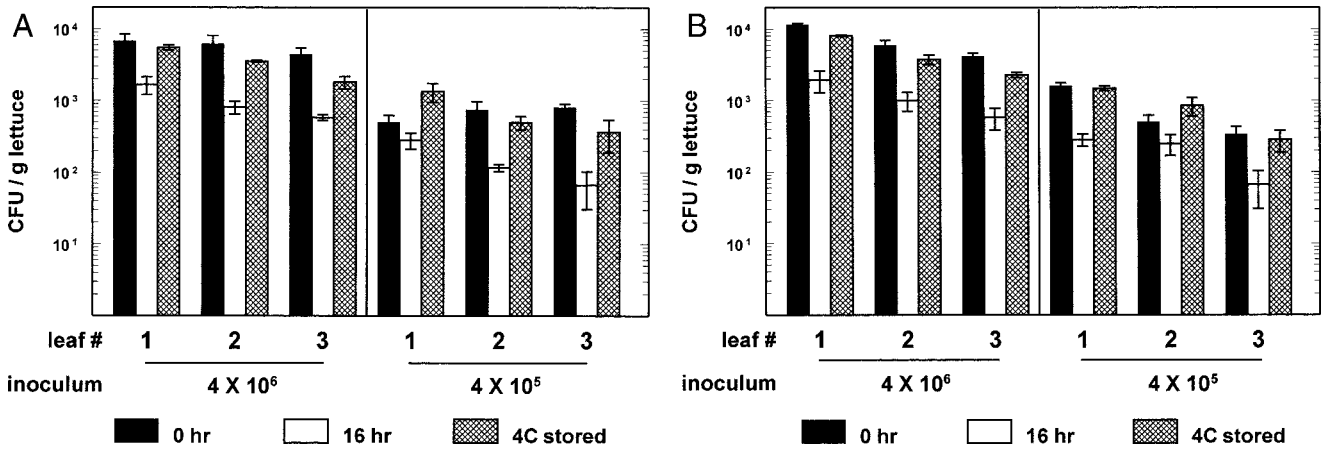


FIGURE 2. *Transfer of* E. coli *O157:H7 from contaminated cutting boards to lettuce pieces pressed to the boards in succession. (A) Ninety-three percent lean and (B) 80% lean ground beef was inoculated with* E. coli *O157:H7 strain F6460 Nal$^R$; inoculum levels (CFU/g of ground beef) are indicated. Hamburger patties were formed and gently pressed onto polyethylene cutting boards. Three lettuce leaves were pressed in succession onto one contaminated area of the board immediately (solid bars) and after storage of the boards at room temperature (ca. 25°C) for 16 h (open bars). Another set of three lettuce pieces were pressed to board surfaces immediately after beef contamination and analyzed for the enumeration of pathogens following overnight storage of the lettuce in bags at 4°C (cross-hatched bars). Three samples were tested for each datum point; the datum points shown represent means and standard errors of the means. The limit of detection was 120 CFU/g of lettuce.*

covered following overnight storage at 4°C ($P > 0.05$). However, significantly smaller numbers of pathogens were transferred to leaves pressed to boards stored at room temperature (ca. 28°C) for 16 h prior to contact with lettuce ($P < 0.0001$). The beef fat content did not significantly affect the numbers of pathogens transferred to lettuce leaves ($P = 0.4894$).

To determine the incidence of pathogen transfer after repeated contact with a contaminated cutting board surface, 25 lettuce pieces were pressed in succession onto a single contaminated spot on the board. Individual lettuce pieces were then cultured in separate tubes containing a selective medium to test for the presence of the pathogen. An inoculum level of $9.8 \times 10^2$ CFU on a 50-cm$^2$ area of the cutting boards (determined by swabbing) resulted in an average transfer incidence of 90%, with the 23rd and 25th lettuce leaves becoming contaminated in two different experiments (Table 4). The transfer of pathogens was more sporadic at an inoculum level of $1.25 \times 10^2$ CFU. At this dose, an average of 46% of the leaves were contaminated with *E. coli* O157:H7; the 25th leaf was among these contaminated leaves in one experiment. Most lettuce leaves remained uncontaminated at the lowest dose tested ($1.75 \times 10^1$ CFU), although the 11th leaf tested positive in two trials.

For each experiment, three contaminated lettuce cultures testing positive for the growth of a Nal strain in LB broth were subjected to further PCR analysis with probes specific for *eae (8)* to verify that the cultures were those of strain F6460 Nal (16 samples altogether, with 1 sample tested for an inoculum level of $1.75 \times 10^1$ CFU/g [trial 2]). All lettuce pieces testing positive for growth in nalidixic acid amplified DNA fragments of the appropriate sizes for the *eae* primers used, suggesting that these strains were indeed *E. coli* O157:H7 strains (data not shown). No DNA was amplified from the negative controls (lettuce pressed to uninoculated spots on cutting boards) when these *eae* primers were used.

## DISCUSSION

In this study, we showed that small numbers of *E. coli* O157:H7 can be transferred from contaminated plastic cutting boards to cut lettuce pieces even after successive pieces of lettuce have come in contact with the chopping surface. Although the method used to determine bacterial numbers on cutting boards, which involves the use of a cotton swab, underestimates pathogen numbers, it is remarkable that pathogens were transferred to the 11th leaf exposed to a contaminated cutting board containing $1.75 \times 10^1$ CFU on a 50-cm$^2$ area in two independent experiments. In addition, 46% of lettuce leaves, including the 25th exposed leaf, were cross-contaminated with the pathogen, when an inoculum level of $1.25 \times 10^2$ CFU was used. Because the infectious dose of *E. coli* O157:H7 may be <50 organisms *(9, 17),* these observations stress the need for strict attention to food safety practices when potentially contaminated foods are handled coincidentally with foods to be eaten raw.

We observed that room-temperature storage of contaminated cutting boards decreased the number of recoverable pathogens by an average of 1 log CFU. Although the pathogens may have entered a viable but nonculturable state that prevented recovery *(6)* or may have adhered strongly (possibly forming biofilms) to prevent recovery, at this juncture we have no indication that either of these situations occurred. It is possible that these organisms simply died. In contrast to our results, Ak et al. *(1)* observed overnight multiplication of *E. coli* O157:H7 when the pathogen was applied to plastic cutting boards. In their study, however, pathogens were suspended in filter-sterilized raw chicken juice, which undoubtedly provided nutrients to support the

TABLE 4. *Transfer of* E. coli *O157:H7 from cutting boards to 25 successive lettuce pieces*

| Leaf no. | Result for inoculum level (CFU on a 50-cm$^2$ area of a cutting board surface exposed to lettuce)[a] | | | | | |
|---|---|---|---|---|---|---|
| | 9.8 (±0.8) × $10^2$ | 9.8 (±0.8) × $10^2$ | 1.25 (±0.6) × $10^2$ | 1.25 (±0.6) × $10^2$ | 1.75 (±0.4) × $10^1$ | 1.75 (±0.4) × $10^1$ |
| 1 | + | + | + | + | + | − |
| 2 | + | + | − | + | + | − |
| 3 | + | + | + | + | + | − |
| 4 | + | + | + | + | − | − |
| 5 | + | + | + | + | − | − |
| 6 | + | + | + | + | − | − |
| 7 | + | + | + | − | − | − |
| 8 | + | + | − | − | − | − |
| 9 | + | + | + | − | − | − |
| 10 | + | + | + | − | − | − |
| 11 | + | − | + | − | + | + |
| 12 | + | + | + | + | − | − |
| 13 | − | + | − | + | − | − |
| 14 | + | + | − | + | − | − |
| 15 | + | + | + | − | − | − |
| 16 | + | − | − | + | − | − |
| 17 | + | + | − | − | − | − |
| 18 | + | + | + | − | − | − |
| 19 | + | + | − | − | − | − |
| 20 | + | + | − | − | − | − |
| 21 | + | + | − | − | − | − |
| 22 | + | + | − | − | − | − |
| 23 | + | + | − | − | − | − |
| 24 | − | + | − | − | − | − |
| 25 | − | + | + | − | − | − |
| % positive | 88 | 92 | 52 | 40 | 16 | 4 |

[a] Contaminated hamburger patties were pressed onto polyethylene cutting boards. Twenty-five lettuce pieces in succession were then pressed onto a single contaminated spot on the board, and five 1-cm$^2$ sections of each leaf were cultured in LB Nal broth overnight to determine whether the pathogen was present. A plus sign indicates that LB Nal cultures were positive for bacterial growth, and a minus sign indicates that no growth was observed following overnight culture. Each inoculum was tested four times; data shown are representative of two experiments per dose. Hamburger patties inoculated with $1.9 \times 10^5$ CFU of *E. coli* O157:H7 strain F6460 Nal$^R$ per g contained $1.31 \times 10^5$ CFU/g and resulted in the transfer of $9.8 \times 10^2$ CFU to a ~50-cm$^2$ area on the board (as determined by swabbing). Patties inoculated with $1.9 \times 10^4$ CFU of *E. coli* O157:H7 strain F6460 Nal$^R$ per g contained $1.16 \times 10^4$ CFU/g and resulted in the transfer of $1.25 \times 10^2$ CFU to the board. Patties inoculated with $1.9 \times 10^3$ CFU of *E. coli* O157:H7 strain F6460 Nal$^R$ per g contained $3.28 \times 10^3$ CFU/g and resulted in the transfer of $1.75 \times 10^1$ CFU to the board. Lettuce pieces exposed to uncontaminated cutting boards tested negative for growth in LB broth supplemented with nalidixic acid.

growth of the organisms. In addition, these authors found both the persistence and the multiplication of bacteria on plastic cutting boards to depend on the maintenance of high humidity *(2).* Our experiments were conducted with starved stationary-phase pathogens in a standard room atmosphere, with only typical hamburger patty residue remaining on the boards to simulate real-world cross-contamination conditions. Regardless, the capacity for the organism to withstand starvation, cross-contamination, overnight desiccation, and subsequent culture is noteworthy.

Following bacterial cross-contamination of plastic cutting boards or gloved hands, subsequent contamination of foods in contact with these surfaces occurred readily. In addition, significant numbers of pathogens remained on these boards after a 15-s warm-water rinse. This finding contrasts with the observations of Miller et al. *(13),* who reported the efficient removal of ground beef microbiota from cutting boards with water alone. Given that our experiments included beef microflora as well as *E. coli* O157:H7, the human pathogen may be better equipped for attachment to boards than the normal beef microflora is. We previously showed the inefficacy of water alone and highly chlorinated water in the removal of *E. coli* from cut leaf lettuce and lettuce seedlings *(18, 19).* We are currently investigating the molecular mechanisms through which this attachment occurs.

In conclusion, it is apparent that adherence to strict food safety practices during food handling is necessary for the prevention of pathogen cross-contamination from contaminated foods to kitchen equipment or other foods that may be eaten raw. Given the difficulty with which pathogens are removed from equipment surfaces and raw produce, adherence to good manufacturing practices and hazard analysis critical control point principles during produc-

tion and processing, along with consumer and food-handler education, are critical to the maintenance of a wholesome food supply.

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