Cross-Contamination Risks in Reusable Grocery Shopping Bags and Potential Risks to Patrons: A Model Applied to Leafy Greens *E. coli* O157:H7

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Abstract

Reusable polypropylene-grocery shopping bags have increased in popularity as a method to reduce environmental impact of plastic one-use bags. Although not heavily studied, previous work has shown generic *Escherichia coli* (*E. coli*) in eight percent of used-reusable bags tested. It is important to better understand the potential reusable bags pose in cross-contamination of foodborne pathogens. First, this study focused on patron use of reusable grocery bags. Second, this study considered the potential of pathogen transfer from contaminated leafy greens to a reusable bag and survival on a reusable bag. In order to explore practices carried out by patrons with their reusable bags, 107 patrons participated in a convenience sample. Leafy greens were inoculated with 10^5 CFU/ml tetracycline and chloramphenicol resistant *E. coli* O157:H7. Inoculated and non-inoculated leafy greens were placed in reusable bags (21 bags per treatment) and 30-minute transport was simulated using a large sample mixer. The microbial load of the reusable bags was measured and all bags were stored at 21°C. Three bags per treatment were tested on Day 1, 3, 5, 8, 10, 12, and 14. Bags were sampled in five locations (10 x 10 cm), one on the bottom and four at varying levels on the side-panels of the bag, using wet swabs. Reusable bags were measured for aerobic plate count, coliform, and *E. coli* O157:H7. Two-hours after transport simulation and on Day 3, 10^3 CFU/ml tetracycline and chloramphenicol resistant *E. coli* O157:H7 was recovered. The microbial loads from Day 3 to Day 14 were minimal, 10^1 CFU/ml *E. coli* O157:H7 was consistently recovered. Counts were similar regardless of sampling location on the bag but were highest on the bottom location. Non-pathogenic microorganisms were recovered at low concentrations. It is important to consider potential pathogens available for transfer to reusable bags and further to food products that come in contact with contaminated bags. The data generated helps with the development of a better model for assessing microbial movement within reusable bags. The results of this study convey the potential for survival and cross-contamination of foodborne pathogens on reusable bags.

Introduction

Shiga toxin-producing *E. coli* (STEC*s) are estimated to cause 63,000 illnesses, 2,100 hospitalizations, and 20 deaths in the United States annually (Scallan et al., 2011). Food sources of *E. coli* O157:H7, thought to be the most common of these pathogens include beef products, leafy greens, unpasteurized milk and cheese, unpasteurized apple juice/cider, sprouts, fruits, and nuts. The U.S. Food and Drug Administration (FDA) reports that 28 foodborne illness outbreaks were associated with the consumption of leafy greens from 1996 to 2008 (FDA, July 2009).

Because of its complexity, cross-contamination is likely underreported and the potential as source of pathogens is greater than epidemiological data implies (Clayton and Griffith, 2004; Redmond et al., 2004 Griffiths et al., 2002 and Chapman et al., 2011)). Reusable grocery shopping bags have increased in popularity as a method to reduce environmental impact of plastic one-use bags. Williams and colleagues (2011) found that 8% of sampled bags contained generic *E. coli* in a study of 60 reusable bags acquired from shoppers in San Francisco Bay area, greater Los Angeles, and Tucson, Arizona Identifying the likelihood that reusable bags can pose as a vehicle for pathogen transmission is important. The aims of this study were to (1) to better understand patron use of reusable bags through self-reported handling, storage, and cleaning practices and (2) investigate the potential of pathogen transfer and survival from contaminated leafy greens to a reusable bag.

Materials & Methods

Survey of handling, storage, and cleaning of reusable grocery bags by patrons

Surveys (n=107) were collected at three different sites (grocery store, farmers' market, and university community). Users were asked about reusable grocery bag use; average length of transport (after purchase and prior to use or storage) of reusable grocery bags after food purchase; cleaning of reusable grocery bags; and storage of reusable grocery bags. The aim of the structured question on length of transport was incorporated to provide a length of time for food contamination exposure of the reusable bags during cross-contamination testing.

***Cross-contamination of reusable grocery bags with Leafy Greens E. coli* O157:H7**

To assess the potential for cross-contamination and survival, spinach greens were purchased from a local grocery store and held at 4°C for 24 hours prior to treatment (Delaquis et al., 2002). Inoculated leafy greens (25 g/bag) were treated with 2.5 milliliters of 10^5 CFU/ml tetracycline and chloramphenicol resistant *E. coli* O157:H7 in Lennox Broth (LB) based on the methods established by Neal et al. (2012). Non-inoculated leafy greens (25 g/bag) were treated with 2.5 ml LB to provide equal nutrients as compared to inoculated leafy greens. Inoculated and non-inoculated leafy greens were placed in reusable bags (21 bags per treatment). Once leafy greens were placed in the bags, a 30-minute transport (based on the mean transport time reported from self-reported surveys) was simulated using a large sample mixer. Reusable bags were measured for aerobic bacteria, coliform, and tetracycline and chloramphenicol resistant *E. coli* O157:H7

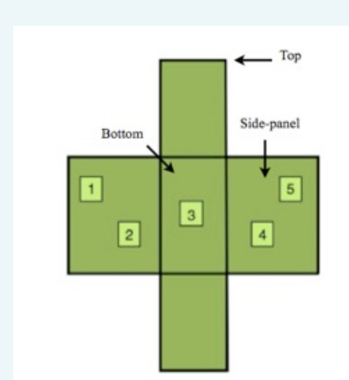

Figure 1: Swab locations on reusable bag (bags were cut along seems and laid flat)

Bags were sampled in five locations (10 x 10 cm), one on the bottom and four at varying levels on the side-panels of the bag using wet swabs (Figure 1). The microbial load of three new reusable bags was tested to provide the microbial baseline prior to the transportation simulation. To investigate initial transfer, the microbial load of the reusable bags was measured two-hours after simulation and all bags were stored at 21°C. Each treatment was tested in triplicate. To investigate survival, the microbial load of the reusable bags were sampled on Day 1, 3, 5, 8, 10, 12, and 14.

Results

Survey of handling, storage, and cleaning of reusable grocery bags by patrons

Results indicate that 34.69% of patrons surveyed have attempted to clean their reusable bags and only 5% of those patrons claimed to do it on a regular basis. Responses from the patrons who claimed to have cleaned their bags used methods of hand cleaning (7%) and machine washing (93%).

Most patrons expressed hesitation and lacked an explanation of a potential reaction to how they would handle a situation of meat drippings or contamination. Most (72%) of the patrons surveyed do not separate or specify type of food product placed into each bag.

***Cross-contamination of reusable grocery bags with Leafy Greens E. coli* O157:H7**

Figure 6 displays the persistence of aerobic bacteria, coliform, and tetracycline and chloramphenicol resistant *E. coli* O157:H7 on the bags exposed to inoculated and non-inoculated leafy greens. The aerobic bacteria and coliform counts of both the bags exposed to inoculated leafy greens and bags exposed to non-inoculated leafy greens were similar during each testing phase.

Microbial Counts of Reusable Bags w/ tetracycline and chloramphenicol resistant *E. coli O157:H7*

	Aerobic Bacteria	Generic Coliform	E. coli O157:H7 (tet + chlora)
2 hours	1.77E+04	1.33E+03	1.23E+03
Day 1	1.65E+04	8.71E+02	6.53E+02
Day 3	1.34E+02	9.21E+01	6.17E+01
Day 5	1.34E+02	6.73E+01	4.27E+01
Day 8	3.56E+01	TFTC	TFTC
Day 10	TFTC	TFTC	TFTC
Day 12	TFTC	TFTC	TFTC
Day 14	TFTC	TFTC	TFTC

Microbial Counts of Reusable Bags w/out tetracycline and chloramphenicol resistant *E. coli O157:H7*

	Aerobic Bacteria	Generic Coliform	E. coli O157:H7 (tet + chlora)
2 hours	7.37E+02	TFTC	TFTC
Day 1	4.51E+02	TFTC	TFTC
Day 3	2.30E+01	TFTC	TFTC
Day 5	1.74E+01	TFTC	TFTC
Day 8	1.56E+01	TFTC	TFTC
Day 10	TFTC	TFTC	TFTC
Day 12	TFTC	TFTC	TFTC
Day 14	TFTC	TFTC	TFTC

Figure 6: Persistence of bacteria on reusable grocery bags due to leafy greens (TFTC: too few to count)

Discussion

Survey of handling, storage, and cleaning of reusable grocery bags by patrons

Collecting patron reusable grocery bag practices was important for better understanding the potential risk factors for pathogen transmission with reusable grocery bags as a vehicle. High variability in responses was seen in responses pertaining to the number of reusable grocery bags, bag material, frequency of use, and length of travel time for food products. The 34.69% of patrons who claimed to have cleaned their reusable bags was unexpected based on the lower numbers reported by Williams et al. survey responses (2011). The low number of patrons who knew risk reduction actions for meat juice spills on a reusable bag and the low number of patrons who separate food products based on type also displays a potential lacking of food safety knowledge and behaviors by the surveyed patrons.

Based on the data from the transport and storage simulation, the persistence of *E. coli* O157:H7 in the reusable grocery bags was low. In regards to initial transfer, a two-log reduction of *E. coli* O157:H7 was seen between the inoculated leafy greens and the reusable bags two-hours after the simulation of the inoculated leaves. The reduction may be due to lack of transfer from the leaves to the bags and lack of nutrients on the bags. This simulation provides information only on the transfer of *E. coli* O157:H7 with leafy greens as the vehicle to unused reusable bags. By Day 8, the *E. coli* O157:H7 was undetectable by swabbing of the reusable bags.

Given the lack of nutrients on the unused bags, this data cannot be transferred to all reusable grocery bags and the persistence of *E. coli* O157:H7. Even after inoculating the leafy greens at a higher concentration than most testing of contaminated leafy greens has shown and allowing contaminated leafy greens direct contact with the reusable grocery bags (Arthur et al., 2007; Mukherjee et al., 2004; and L.M. Johnston et al., 2005), *E. coli* O157:H7 did not grow or persist on the tested bags after Day 8.

Considering the low mean infectious dose of *E. coli* O157:H7 has been shown to be between 1 – 100 colony forming units (Paton et al.), even the smallest amount of *E. coli* O157:H7 persistence has the potential to cause illness. While this study shows a lack of *E. coli* O157:H7 persistence on new reusable bags, this study did not look at the addition of nutrients or moisture to growth or survival.

Also, the transfer of pathogens from a reusable grocery bag should be simulated to determine the potential for cross-contamination from a reusable bag contaminated to a food product. In the only other study published about the microbial safety of reusable bags, Williams and colleagues (2011) tested used reusable bags for presence of E. coli and spiked new bags with *Salmonella* to look at growth. The study by Williams and colleagues (2011) only looked at presence and growth of pathogens, not transfer. Presence and growth of bacteria does not convey transfer. The most important factor to look for with pathogens in reusable grocery bags is cross-contamination to ready-to-eat foods.

It is important to consider potential pathogens available for transfer to reusable bags and further food products. The data generated helps with the development of a better model for assessing microbial movement within reusable bags. Even though the concentration of pathogens transferred was low, the results of this study convey the potential for survival and cross-contamination of foodborne pathogens on reusable bags.

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