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Microbial Contamination of Grocery Shopping Trolleys and Baskets in West Texas

ABSTRACT

The objective of this study was to identify food safety risk factors associated with supermarket trolleys (grills and handles) and handheld baskets. Indicator microorganisms evaluated were those detected by aerobic plate count (APC), yeast and molds (YM), Enterobacteriaceae (EB), environmental Listeria (EL), coliforms (CF), and E. coli (EC). In addition, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli 0157 and Salmonella sp. were tested for. Trolley grills (n = 36) had EB counts of 2.4×10^2 CFU/cm². Trolley handles (n = 36) had 2.7×10^6 of CF and 5.2 CFU/ cm^2 of YM. The bottom of handheld baskets (n = 25) had 3.5×10^5 CFU/cm² of CF and 5.07 CFU/cm² of EC. S. aureus was found on 96% of the baskets, 50% of the trolley handles (18 out of 36 samples), and 42% of the trolleys' grills. E. coli O157 was identified on 17% of baskets, 3% on trolley grills, and 3% on handles. Salmonella sp. was detected on 16% of baskets and 8% of trolley grills. L. monocytogenes was detected on

17% of the bottoms of handheld baskets but on none of the other samples. These results suggest the need for implementation of sanitation programs to regularly clean trolleys and baskets, as well as for consumer education.

INTRODUCTION

Grocery trolleys and handheld baskets are frequently exposed to a variety of food products known to have an increased risk of pathogen presence, including raw eggs and meat as well as produce. Therefore, environmental contamination with foodborne pathogens in supermarket settings is likely.

Researchers have found the following microorganisms on produce: Listeria sp., L. monocytogenes, E. coli O157, Salmonella, Penicillium sp., enterotoxigenic Staphylococcus spp., Bacillus spp., and yeast and molds. In addition, retail meat and seafood products have been found to harbor the following potential pathogens: Campylobacter spp., Shiga toxin-producing E. coli, and Salmonella (1, 11, 13, 23, 26, 28). If cross-contamination between food products and packaging

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materials occurs, or if any rupture of the wrapping causes leaks, these pathogens could easily be transferred to trolleys, handheld baskets, displays, counters, and registers, as well as to people or to food items. Unwrapped food could come into contact with trolleys and baskets; if these surfaces are contaminated with foodborne pathogens, then food products contacting them are at risk of being cross-contaminated with those pathogens. Some investigations have found undesirable microorganisms, such as potentially pathogenic species of Yersinia, Cronobacter, Klebsiella, Bacillus, Pseudomonas, Shigella, Staphylococcus, and other microorganisms of the family *Enterobacteriaceae* (coliforms and non-coliforms), among other potential pathogens (9, 12, 18), on trolleys. A concern related to contaminated trolleys is human exposure. Several authors have reported exposure of infants to direct and indirect contact to foodborne pathogens, such as Campylobacter and Salmonella, as the result of being placed in or on trolleys in supermarkets and touching the handles (8, 14, 20). As shown in Fig. 1, all of these circumstances may create an unending cycle of cross-contamination among food, humans (children in carts and customers handling carts and baskets), and the contaminated surfaces of the trolleys and baskets.

Ready-to-eat (RTE) foods are currently very popular and widely offered in supermarkets. These products are expected to be consumed without any post-processing treatment designed to reduce the risk of exposure to bacterial pathogens present as the result of contamination at the supermarket. RTE raw produce items are perhaps one of the most vulnerable food categories in terms of microbial contamination risk; if customers place produce

in direct contact with contaminated surfaces instead of using a produce bag, the risk is enhanced. It is important to consider that most produce is not processed with use of lethality treatments, and in many cases is displayed and sold unwrapped, exposed to the environment, and even at room temperature. Produce contamination commonly originates in lack of proper good agricultural practices during harvest and postharvest operations (water quality, human hygiene practices, sanitation procedures, and harvesting practices); however, fruits and vegetables can also become contaminated at the point-of-sale. In fact, that risk has been identified by multiple studies surveying the presence of foodborne pathogens in produce at the point-of-sale (3). Produce has been identified as one of the most common vehicles associated with foodborne illness and outbreaks (6, 10). For example, a *Campylobacter* outbreak that occurred in 1996 was linked to lettuce that had been cross-contaminated with chicken by cutting raw poultry and salad on the same surface without following proper sanitation protocols (7). Because bacterial contamination on trolleys has been recognized as a potential public health hazard, this study aims to determine the degree of contamination at retail grocery stores and to investigate relevant microorganisms associated with foodborne illness present on supermarket trolleys and handheld baskets. The objectives were to: (1) identify the presence of specific foodborne pathogens, such as Salmonella, E. coli O157, L. monocytogenes, and S. aureus, (2) determine the bacterial load of indicator microorganisms, such as those detected by aerobic plate counts, Enterobacteriaceae, E. coli, Coliforms, Yeast and Molds, and (3) identify the areas of highest contamination levels on supermarket trolleys.

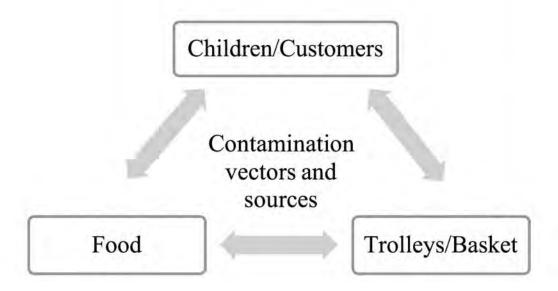


Figure 1. Potential cross-contamination sources of shopping trolleys and handheld baskets.

As important as detecting bacterial pathogens is evaluating the presence and quantification of indicator microorganisms associated with food environments. The occurrence of indicator organisms is typically used as a predictor of the potential presence of pathogens. Therefore, this information can be used to better understand the food safety hazards associated with grocery shopping and hence to establish preventive measures and develop policies to help lower foodborne illness risk to consumers.

MATERIALS AND METHODS

Sample collection

This research consisted of a cross-sectional descriptive study to evaluate microbial contamination hazards associated with grocery shopping in West Texas. A total of 20 supermarkets in 8 different cities were surveyed. Samples (n = 97) were collected from trolley handles, trolley grills, and the bottom of handheld grocery baskets. For sample collection, sterile gloves were worn, and surfaces were swabbed using sterile cellulose sponges pre-moistened with 25-ml buffered peptone water (BPW, World Bioproducts, Illinois, USA. Part EZ-25BPW-CELL). Swabs were passed two times (using both sides of the swab) over the entire area of the surface to be sampled and transported immediately under refrigerated conditions in a cooler with gel packs to the International Center for Food Industry Excellence (ICFIE) lab at Texas Tech University in Lubbock, TX, for testing. Microbial analyses were performed to establish quantitative and qualitative data for indicator microorganisms and foodborne pathogens. Indicator microorganisms investigated in this study were those detected by an aerobic plate count (APC), yeasts and molds (YM), Enterobacteriaceae (EB), environmental *Listeria* (EL), coliforms (CF), and E. coli (EC). Pathogenic microorganisms were Listeria monocytogenes, Staphylococcus aureus, Escherichia coli O157, and Salmonella sp.

Indicator microorganisms

Before microbial analysis was conducted, all swabs were homogenized in a laboratory stomacher for 2 min at 230 rpm. APC microoorganisms, YM, EB, EL, CF, and EC were tested for by use of 3M Petrifilm[™] plates (3M[™] Microbiology, Minnesota, USA), following recommended protocols. After homogenization, 1 ml of the BPW broth from each swab sample was inoculated onto a plate and incubated as follows: 24 h at 35°C for APC, 3 days at 25°C for YM, 24 h at 37°C for EB, and 24 h at 35°C for EC. At the end of the incubation period, colonies were enumerated and reported according to the manufacturer's protocols. For colony enumeration, dilution was considered as 1 ml plated from a 25-ml premoistened swab. The surface area of each type of sample was measured (cm²) and the total number of colonies was divided by the area; therefore, results are presented as CFU/cm². In the case of EL, the BPW was incubated for

1 h at 30°C prior to inoculation of the plates with 3 ml of the sample and plates were then incubated for 28 h at 35°C. Qualitative results (presence or absence) were reported as per the manufacturer's protocol. Colonies from EL-positive plates were characterized for identification of *Listeria* species by use of API *Listeria* strips (bioMérieux, Inc., Durham, NC), following the manufacturer's procedure.

Pathogenic microorganisms

For *S. aureus* detection, the Food and Drug Administration (FDA), Bacteriological Analytical Manual (BAM) protocol was used. An aliquot of 250 µl of the homogenized swab sample was spread plated onto Baird Parker agar containing egg yolk and potassium tellurite (Merck, Darmstadt, Germany) and incubated for 48 h at 37°C. Typical colonies were streaked onto Mannitol Salt Agar (MSA, Becton Dickinson and Company™, Le Pont de Claux, France) for 24 h at 37°C, with a subsequent extended incubation of 24 h to check colony morphology. Typical S. aureus colonies were confirmed by use of ASI™ Staphslide Latex Tests (Arlington Scientific[™], Arlington, USA). The remaining BPW broth from the swab samples was incubated for 24 h at 37°C to proceed with E. coli O157 and Salmonella detection. Detection of E. coli O157 was conducted by transferring 1 ml of the sample into 9 ml of modified tryptic soy broth (mTSB, Neogen® Corporation, Michigan, USA) and incubated for 24 h at 37°C. Immunomagnetic separation (IMS) was performed with anti-O157 beads (Dynabeads®, Invitrogen, Carlsbad, CA) and the automated BeadRetriever™ (Invitrogen, Carlsbad, CA), following the manufacturer's standard protocol. A 50-µl aliquot from the bacteria-bead complex recovered after IMS was inoculated onto Chromagar® O157 plates (CHROMagar®, Paris, France), spread plated, and incubated for 24 h at 37°C. Typical E. coli O157 colonies were confirmed with agglutination tests (Oxoid Ltd., Hants, UK). Salmonella spp. was detected by incubating the remaining BPW broth from the swab sample for 24 h at 37°C. Following incubation, 1 ml was transferred into 9 ml of Rappaport Vassiliadis broth (RV, Neogen® Corporation, Michigan, USA) tube, and 9 ml of Tetrathionate (TT, Hardy Diagnostics ™, California, USA) broth and incubated for 24 h at 42°C and 37°C, respectively. Approximately 10 μl was transferred via loop from each tube, streaked onto Xylose Lysine Tergitol-4 Agar plates (XLT4, Becton Dickinson and Company™, Le Pont de Claux, France), and incubated for 24 h at 37°C. Characteristic Salmonella colonies were confirmed by latex agglutination tests (Wellcolex Colour Salmonella Kit, Remel, San Diego, CA). L. monocytogenes was detected as a part of the characterization method already mentioned for Listeria spp., using API Listeria® strips.

Data analyses

Upon enumeration of indicator organisms, obtained numbers were divided by the area of the surface swabbed to calculate the CFU per cm². Estimated surface areas were: trolley handles, 132 cm²; trolley grill, 2903 cm²; and bottom of handheld baskets, 892 cm². Means, standard deviations, and medians were calculated using Microsoft Excel version 16.25. Mean values were used to analyze findings for each microorganism and surface. With regard to pathogen detection, proportions of each microorganism were calculated by considering the number of positive results relative to the total number of samples analyzed per surface.

RESULTS

Indicator organisms

Microbial indictors provide a general idea of the current hygienic conditions and an indirect measure of potential pathogens present. APC and YM give a measure of the cleanliness of the surfaces, while CB, EB, and CF suggest the possible presence of pathogenic members of *E. coli*, Salmonella, Klebsiella, and Cronobacter, among others. Listeria spp. is typically used as an indicator of the potential presence of *L. monocytogenes*. Tables 1, 2, and 3 show the level at which each indicator microorganism was present on the three surfaces tested. With respect to APC and YM, findings indicate their presence on every sample tested. The greatest APC load was found on trolley handles (1.25×10^2) CFU/cm²), the second greatest on the bottom of handheld baskets $(1.0 \times 10^2 \text{ CFU/cm}^2)$, and the lowest on trolley grills (4.94 CFU/cm²), while YM was at the highest level on the bottom of handheld baskets $(1.0 \times 10^3 \text{ CFU/cm}^2)$ and at much lower levels on trolley handles and grills (5.5 and 1.28 CFU/cm², respectively).

With regard to the group of EB, CF, and EC, the greatest level of contamination was found on the trolley handles (2.72 \times 10⁴, 2.71 \times 10⁶, and 27.73 CFU/cm², respectively), the second greatest on the bottom of handheld baskets (4.67 \times 10³, 3.52 \times 10⁵, and 5.07 CFU/cm², respectively),

and the lowest on trolley grills (2.40×10^2 , 0.27, and 0.03 CFU/cm², respectively).

With regard to environmental *Listeria*, the results are presented as the proportion of samples positive for the genus. A total of 76% (74 out of 96, which comprise the three surfaces) of the tested samples were positive for *Listeria* spp. The highest incidence was observed on the bottom of handheld baskets, with 97% of the samples positive for *Listeria* spp. (24 out of 25), while a total of 69% of trolley grills (25 out of 36 samples) and 69% of trolley handles (25 out of 36 samples) were found to carry *Listeria* spp. Further investigation of the non-pathogenic species revealed that *L. innocua*, *L. grayi*, *L. welshimeri*, *L. seeligeri*, and *L. ivanovii* were present on the surfaces, with a distribution of 53, 4, 26, 7, and 2%, respectively.

With regard to the bacterial load of the studied indicator organisms present on each surface, trolley grills consistently had the lowest concentration of all microbial indicators (APC, YM, EB, CF, and EC), and trolley handles carried the highest microbial concentration of APC, EB, CF, and EC. On the other hand, YM was highest on the bottom of handheld baskets, the surface that also had the highest proportion of samples positive for *Listeria* spp.

Bacterial pathogens

E. coli O157, Salmonella sp., L. monocytogenes, and S. aureus were found on trolleys and grocery baskets (Fig. 2). Trolley handles tested positive only for E. coli O157 and S. aureus, while trolley grills tested positive for both of these pathogens as well as Salmonella. In contrast, the bottom of handheld baskets tested positive for all four pathogens analyzed (E. coli O157, S. aureus, Salmonella, and L. monocytogenes). S. aureus, the most prevalent pathogen, was found at different proportions on all of the surfaces tested. Staphylococcus spp. was found on 81% of the samples (78 out of 96 samples

TABLE 1. Indicator microorganisms on trolley grills*						
	Average** CFU/cm²	Standard deviation	Median CFU/cm ²	Range CFU/cm ²		
Aerobic Plate Count	4.94	6.6	3.5	0.29 - 36.3		
Yeast and Molds	1.28	1.4	0.8	0.1 – 5.61		
Enterobacteriaceae	2.40×10^{2}	1.4×10^{3}	0.03	$0.01 - 8.6 \times 10^3$		
Coliforms	0.27	1.2	0.01	0.01 – 7.28		
Escherichia coli	0.03	0.1	0.00	0.00 – 0.86		

^{*}Sample size n = 36.

Aerobic Plate Count = APC; Yeast and Molds = YM; Enterobacteriaceae = EB; Coliforms = CF; Escherichia coli = EC.

^{**}Value corresponds to the average of microbial concentration of each indicator microorganism, and the CFU/cm² was estimated based on the surface area of the trolley grills.

TABLE 2. Indicator microorganisms on trolley handles*

	Average** CFU/cm²	Standard deviation	Median CFU/cm²	Range CFU/cm ²
Aerobic Plate Count	1.25×10^{2}	4.43×10^{2}	9.0	$1.8 - 1.89 \times 10^3$
Yeast and Molds	5.15	10.8	1.89	0.0 – 62.5
Enterobacteriaceae	2.72 × 10 ⁴	6.9 × 10 ⁴	0.19	$0.19 - 1.9 \times 10^{5}$
Coliforms	2.71×10^{6}	6.9×10^{6}	0.19	$0.10 - 1.9 \times 10^7$
Escherichia coli	27.73	70.5	0.00	$0.00 - 1.99 \times 10^{2}$

^{*}Sample size n = 36.

Aerobic Plate Count = APC; Yeast and Molds = YM; Enterobacteriaceae = EB; Coliforms = CF; Escherichia coli = EC.

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IABLE 3. Indicato	or microorganist	ns on bottom oi	f handheld baskets*

	Average** CFU/cm²	Standard deviation	Median CFU/cm²	Range CFU/cm ²
Aerobic Plate Count	1.07×10^{2}	95.9	73.6	$11.63 - 2.8 \times 10^{2}$
Yeast and Molds	1.0×10^{3}	1.4×10^{3}	13.73	$1.3 - 2.8 \times 10^3$
Enterobacteriaceae	4.67×10^{3}	1.1×10^{4}	1.05	$0.03 - 2.8 \times 10^4$
Coliforms	3.52×10^{5}	9.4 × 10 ⁵	0.70	$0.06 - 2.8 \times 10^6$
Escherichia coli	5.07	11.6	0.07	0.00 - 42.04

^{*}Sample size n = 25.

Aerobic Plate Count = APC; Yeast and Molds = YM; Enterobacteriaceae = EB; Coliforms = CF; Escherichia coli = EC.

tested), but only 58% (56 samples out of 78 *Staphylococcus* spp. positive) were confirmed *S. aureus*, of which the bottom of handheld baskets had the highest level (96%, or 24 out of 25 samples), followed by trolley handles (50%, or 18 out 36 samples), and trolley grills (39%, or 14 out 36 samples). The overall prevalence of *E. coli* O157 was 6.3% (6 out 96 samples), which was found on all three surfaces. The bottom of handheld baskets had the highest prevalence of this pathogen, at 17% (4 out of 24 samples), followed by trolley handles and trolley grills, each with 3% occurrence (1 out of 36). *Salmonella* sp. was found on 7% of the samples (2 out of 96) and was detected on 16% of the handheld baskets (4 out of 25 samples) and 8% of the trolley grills (3 out of 36 samples), but on none of the trolley handles sampled. *L. monocytogenes* was not recovered from trolley grills and

handles, but was found on 17% of handheld basket bottoms (4 out of 24 samples).

DISCUSSION

This study investigated food safety risk factors associated with trolleys and supermarket handheld baskets. These surfaces were tested for the presence and concentration of indicator microorganisms and for the prevalence of the most important foodborne pathogens relevant to public health. Despite concern regarding bacterial contamination of food from the supermarket environment (15, 21, 22), little has been published with regard to surveillance of specific food contact surfaces at the retail level. Supermarket microbiological assessments are frequently performed on food, but information is lacking about bacterial

^{**} Value corresponds to the average of microbial concentration of each indicator microorganism, and the CFU/cm² was estimated based on the surface area of the trolley handle.

^{**}Value corresponds to the average of microbial concentration of each indicator microorganism, and the CFU/cm² was estimated based on the surface area of the handheld basket's bottom.

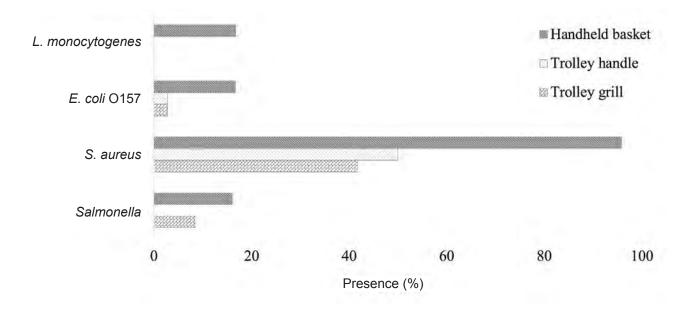


Figure 2. Presence of foodborne pathogens on shopping trolleys and handheld baskets. Data represent the prevalence (%) among all the tested samples (n = 96).

contamination on trolleys and handheld baskets used during grocery shopping. Existing studies on trolley contamination have expressed concerns mostly associated with infants' exposure to pathogens (8, 14, 20); however, it is important to recognize the high potential for cross-contamination of food products if trolleys and baskets carry foodborne pathogens.

The bottom surface of handheld baskets carried every foodborne pathogen tested for (E. coli O157, Salmonella sp., L. monocytogenes, and S. aureus), and also had the highest concentration of indicator microorganisms. The visible uncleanliness of handheld baskets could potentially harbor organisms, favor bacterial multiplication, and encourage biofilm formation. Basket and trolley contamination could be caused by leaking from pre-packaged foods, condensation from cold items, or dripping of water from produce that may contaminate surfaces. Trolleys are often parked outside the stores, where they are exposed to birds, dust, dirt, and weather conditions. Raw products such as produce, meats and seafood are known to carry APC microorganisms, YM, and potential pathogens as reported by Jeddi et al. (2014), who found APC levels between 5.3 and 8.5 log CFU/g; yeast and molds in 100% of their surveyed samples, including minimally processed vegetables and bagged sprouts, and generic E. coli (13). Other authors have reported similar findings. Zhao et al. (2001) found Campylobacter spp., E. coli, and Salmonella in chicken, turkey, pork, and beef obtained from retail stores (28). Samadpour et al. (1994) investigated the occurrence of Shiga toxin-producing *E. coli* in fresh seafood and meats from grocery stores and found a 17% prevalence of virulent species in 294 samples (23). These

examples provide evidence that pathogenic microorganisms can occur on raw foods in supermarket settings, and these may possibly be transferred to trolley and cart surfaces if food packaging is compromised. As mentioned, consumers may place food such as un-bagged produce into direct contact with the bottom of a trolley or handheld basket, making grocery trolleys and baskets potential sources of contamination with foodborne pathogens. Therefore, the risk of foodborne illness attributable to the lack of cleanliness of trolleys and handheld baskets must be considered.

With regard to trolley handles, this study found high bacterial loads of the indicator microorganisms studied (APC, EB, CF, and EC). These high levels could be attributed to cross-contamination with customers' hands, as contaminants can be transferred from hands to trolley and basket surfaces and vice versa (2, 5). It is widely known that hand hygiene plays an important role in food contamination. Proper hygiene and hand washing habits should be practiced during grocery shopping, not only to prevent contamination of hands, but also to avoid becoming a vehicle for transferring microorganisms to other surfaces. As observed during sample collection, different grocery stores appeared to have different cleaning and sanitation standards. Some had greater visible filth and had surrounding areas that required cleaning, while other stores were maintained in much better hygienic and aesthetic condition. These differences were consistent with the numbers obtained for the indicator microorganisms, and explain the large standard deviations observed in the results. Strategies implemented by stores are much needed and include educational and informative communication

to customers, encouraging them to wipe trolley and baskets handles and use produce bags and recommending the use of protective blankets on which to place their infants in shopping carts, among other recommendations.

Trolley grills consistently had lower levels of bacterial concentration, perhaps because of the design and smaller amount of material constituting the surface area. Grills are made of metal wires that do not fully cover the area, so the covered surface area per cm² is much lower than estimated. Trolleys are used when larger amounts of groceries will be purchased, perhaps increasing the chance of using bags to wrap food items. Nonetheless, *E. coli* O157, *Salmonella* spp., and *S. aureus* were found on trolley cart grills, which indicates a possible risk to public health.

This study presents evidence of food safety risks to public health associated with grocery shopping, which is consistent with findings of other publications (9, 19). In general, grocery stores lacked sanitation protocols to properly clean and maintain carts and baskets, a lack that may enhance bacterial pathogen risk, increase the microbial load on these surfaces, and possibly increase biofilm formation (16, 24). Since trolley contamination is emerging as a public health concern, patents now exist to allow for the implementation of washing machines or covers for the trolleys to mitigate these risks (4, 17).

It is recommended that supermarkets implement sanitation operating procedures to baskets and trolleys to help reduce risk. Some retail establishments already have implemented measures by providing sanitizing wipes, and even some states recommend this measure, as is the case of Arkansas through their Health-conscious Shopper Act (25). To protect public health, food processing facilities, as well as grocery stores, should implement proper sanitation protocols. There also appears to be a need to create consumer awareness and education with regard to hand hygiene and use of produce bags to protect unpackaged produce. It will also be important to enforce proper trolley and handheld basket sanitation regulations along with performing microbial surveillance.

Since trolleys and handheld baskets are not considered food contact surfaces under sanitation programs and hygiene practice regulations, little attention is given to this matter. Consequently, risk factor studies associated with retail store environments are scarce. A 2010 risk assessment

(27) conducted by the U.S. Food and Drug Administration (FDA), which collected information from 1998 to 2008, evaluated sanitation conditions in various establishments, including retail stores, over a period of 10 years. Although improvements were observed over time, some of their findings show the need for enhancement of hygiene practices and cleanliness of food contact surfaces at the meat, poultry, and seafood departments. Even though there was a focus on food handling behaviors and hygiene practices, study of trolleys and handheld baskets was excluded, and microbial data were not part of the assessment.

CONCLUSIONS

Findings from this study demonstrate the presence of microbial contamination and foodborne pathogens on trolleys and the bottom surface of handheld baskets. This may suggest a public health risk associated with grocery shopping if food comes into direct contact with these surfaces. The fact that all the foodborne pathogens tested for were found on the surveyed samples should be evidence that public health authorities and private retail stores need to address the situation and take focused measures to mitigate this problem. The high microbial load of Enterobacteriaceae and Coliforms (concentrations up to 6 log CFU/cm²) also need to be further examined. Fruits and vegetables are often placed on trolleys and in handheld baskets without protection; similarly, meat and other foodstuffs may be wrapped in packaging that is not properly sealed at the meat counter or juices from pre-packaged meats may drip and contaminate surfaces. While this study does not correlate its results to foodborne illnesses, the threat must be acknowledged. As food contact surfaces are controlled at retail stores, trolleys and baskets should be included in their standard and mandatory sanitation programs. Retail stores could also encourage customers to use sanitizing wipes to clean their hands and handles prior to and during shopping to use bags to wrap fruits, vegetables, and especially meat and poultry to provide an additional barrier of protection.

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