

Coliform Contamination of Vegetables Obtained from Popular Restaurants in Guadalajara, Mexico, and Houston, Texas

Hoonmo L. Koo,^{1,2} Zhi-Dong Jiang,² Eric Brown,² Coralith Garcia,⁵ Huibin Qi,² and Herbert L. DuPont^{1,2,3,4}

¹Baylor College of Medicine, ²School of Public Health and ³Medical School, University of Texas Health Science Center at Houston, and ⁴St. Luke's Episcopal Hospital, Houston, Texas; and ⁵Cayetano Heredia University, Lima, Peru

Food is the primary vehicle of transmission for traveler's diarrhea. We evaluated coliform contamination of vegetables from popular restaurants in Guadalajara, Mexico, and Houston, Texas. Contamination of vegetables in Guadalajara restaurants was widespread. Prevention of traveler's diarrhea by avoidance of "high-risk" foods may be unsuccessful, because contamination of foods may occur regardless of how they are prepared.

Traveler's diarrhea affects ~40% of persons who travel from developed nations to developing regions of the world. This disease continues to be the most common health problem encountered by travelers to developing nations. Food is the important known vehicle of transmission of traveler's diarrhea in developing countries [1–3].

Traditionally, travelers are counseled to "boil it, cook it, peel it, or forget it." Kozicki et al. [4] demonstrated that the more international travelers deviated from these traditional dietary recommendations, the greater the incidence of diarrhea among these travelers. Unfortunately, this strategy of cautious selection of "safer" foods for prevention of traveler's diarrhea has had limited success in preventing diarrhea [5]. We currently are performing a series of studies to determine whether "safer" foods can be identified. An earlier study focused on the safety

of hot sauces in Mexico and the United States [1]. In the current study, we looked at vegetables purchased in public restaurants to see whether preparation methods determined relative safety. We selected vegetables for study because they are often served uncooked and are a known vehicle of traveler's diarrhea [6]. We used total coliform counts as the measurement of contamination, because this likely reflects overall hygienic quality, in which pathogens are generally present in lower counts [3, 7].

Methods. Sixty-four vegetable samples were collected from 18 independently owned, popular restaurants in Guadalajara, Mexico, and 67 vegetable samples were collected from 32 restaurants in Houston, Texas; all samples were collected from an evening meal during the summer of 2006. The temperature of each food item was obtained with a digital thermometer (Pyrex Products) immediately upon receipt of the food at the table. The food item was either classified as cooked or noncooked on the basis of appearance at the time of table service. If a cooked food sample was found to have a temperature $\geq 33.9^{\circ}\text{C}$, it was considered to be a cooked item, served heated. In the restaurant, an aliquot of the study item (~15 g) was placed in a sterile plastic bag and stored in an insulated thermos containing wet ice.

The samples were refrigerated at 4°C overnight until processing the next morning in our laboratories in Guadalajara or Houston. The food samples were diluted with sterile water in a 1:10 ratio, placed in sterile Whirl-Pak bags (American Scientific Products), and homogenized using a Stomacher 400 blender (Dynatech Laboratories). The food items were studied for enteric pathogens by use of published methods [8]. Total coliform counts were determined by performing serial 2-fold dilutions of food sample suspensions on MacConkey agar, which was incubated at 37°C overnight. Coliforms were considered to be gram-negative, facultative anaerobic bacteria that fermented lactose [7]. Coliform contamination was defined as the detection of $>10^4$ coliform cfu/g of sample [9]. Five *Escherichia coli*-like colonies were selected from MacConkey agar plates of the vegetable samples, transferred to peptone stabs for storage, and transported to the Houston laboratory. Biochemical identification of these *E. coli*-like colonies was performed with API 20 tests (bioMérieux). *E. coli*-like organisms were tested by PCR for production of enterotoxigenic *E. coli* heat-labile and heat-stable toxin [10] and by the HEp-2 cell adherence assay for enteroaggregative *E. coli* [11].

Statistically significant differences between groups were evaluated with Fisher's exact test or χ^2 analysis. The Mann-Whitney

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Reprints or correspondence: Dr. Herbert L. DuPont, School of Public Health, University of Texas–Houston, 1200 Herman Pressler, RAS E-733, Houston, TX 77030 (Herbert.L.DuPont@uth.tmc.edu).

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U test was used to compare the median number of coliform colony-forming units per gram of sample.

Results. Twenty-seven (42%) of 64 vegetable samples from Guadalajara were contaminated with coliforms, compared with 17 (25%) of 67 vegetable samples from Houston ($P = .04$). The median coliform count for foods from Mexico was 16,000 cfu/g of vegetable sample, compared with 22,000 cfu/g of sample for the vegetables from Houston ($P = .20$) (table 1). For cooked vegetables, 10 (67%) of 15 samples from Guadalajara were contaminated with coliforms, compared with 3 (10%) of 30 samples from Houston ($P < .001$). The median coliform count of cooked vegetables from Mexico was 48,000 cfu/g of sample, compared with 50 cfu/g of sample for cooked vegetables from Houston ($P = .01$).

When cooked vegetables were served heated, the rate of contamination ($P < .001$) and the median coliform count ($P = .01$) of cooked vegetables from Guadalajara were significantly higher than those for cooked vegetables from Houston (table 1). However, no statistically significant difference in the rate of contamination ($P = .55$) or level of contamination ($P = .12$) was noted when cooked vegetables were served at room temperature for the 2 locations (data not shown).

Eight (42%) of 19 vegetable samples obtained from Guadalajara buffets had coliform contamination, compared with 5 (24%) of 21 vegetable samples from buffets in Houston ($P = .22$). The median coliform counts for vegetable samples collected from buffets in the 2 cities were not significantly different ($P = .37$). There was also no statistically significant difference in the frequency of contamination ($P = .24$) or the

level of contamination ($P = .73$) of cooked vegetables from buffets in the 2 sites.

Microbiological studies of the coliform bacteria recovered from vegetable samples in Guadalajara demonstrated that 4 of 27 samples were positive for enterotoxigenic coliforms (table 2). The HEp-2 cell adherence assay revealed that 13 of 27 coliform bacterial strains recovered from Mexican samples displayed an enteroaggregative phenotype. Coliform strains recovered from 10 of 27 Guadalajara food items were found to be both enterotoxigenic and enteroaggregative. For the contaminated Houston vegetables, coliforms from 4 of 17 samples were enterotoxigenic, and 11 of 17 were enteroaggregative. Coliforms with both enterotoxigenic and enteroaggregative properties were recovered from 2 of 17 Houston samples. Biochemical testing was subsequently performed for further identification of the coliforms. The most common enterotoxigenic bacteria isolated from the 2 sites were *Enterobacter cloacae*, *Klebsiella oxytoca*, *Pantoea* species, and *Pseudomonas fluorescens*. The most common enteroaggregative bacteria identified were *E. cloacae*, *K. oxytoca*, and *Klebsiella pneumoniae*. No *E. coli* or *Shigella*, *Salmonella*, *Campylobacter*, or *Vibrio* species were identified from the vegetable samples from either city.

Discussion. Food is known to be the main vehicle of transmission of enteropathogens that cause traveler's diarrhea in developing regions of the world. The popular tourist destination Guadalajara is known to have high rates of traveler's diarrhea [11, 12]. We studied vegetable samples from popular restaurants in Guadalajara and Houston, using the presence of

Table 1. Comparison of coliform contamination of vegetable samples from Guadalajara, Mexico, and Houston, Texas.

Characteristic, location	Proportion of samples with coliform contamination (%) ^a	P	Contamination level, median cfu/g of sample	P
All samples				
Guadalajara	27/64 (42)	.04	16,000	.2
Houston	17/67 (25)		22,000	
Cooked vegetables				
Guadalajara	10/15 (67)	<.001	48,000	.01
Houston	3/30 (10)		50	
Cooked vegetables, served heated ^b				
Guadalajara	5/6 (83)	<.001	205,000	.01
Houston	2/27 (7.4)		0	
Buffet samples				
Guadalajara	8/19 (42)	.22	32,000	.37
Houston	5/21 (24)		77,000	
Cooked buffet samples				
Guadalajara	2/4 (50)	.24	50,000	.73
Houston	1/8 (13)		78,000	

^a Presence of $\geq 10^4$ coliform cfu/g of sample.

^b 33.9°C–100°C.

Table 2. Microbiological analysis of coliforms isolated from vegetable samples.

Coliform	Guadalajara, Mexico (n = 27)	Houston, Texas (n = 17)
Enterotoxigenic ^a	4	4
LT toxin producing		
<i>Citrobacter freundii</i>	1	0
<i>Enterobacter cloacae</i>	0	1
<i>Pantoea</i> species	1	1
ST toxin producing		
<i>E. cloacae</i>	1	0
<i>Pseudomonas fluorescens</i>	0	2
Identification missing	1	0
Enteroggregative	13	11
<i>Klebsiella oxytoca</i>	5	0
<i>Klebsiella pneumoniae</i>	3	0
<i>Enterobacter aerogenes</i>	1	0
<i>Enterobacter amnigenus</i>	0	1
<i>E. cloacae</i>	2	8
<i>Enterobacter sakazakii</i>	0	1
<i>Pantoea</i> species	0	1
Identification missing	2	0
Both enterotoxigenic ^a and enteroggregative	10	2
LT toxin producing		
<i>E. cloacae</i>	2	0
<i>K. oxytoca</i>	2	0
<i>Serratia fonticola</i>	1	0
ST toxin producing		
<i>C. freundii</i>	1	0
<i>E. cloacae</i>	2	1
<i>K. oxytoca</i>	1	0
<i>K. pneumoniae</i>	1	0
<i>Pantoea</i> species	0	1

NOTE. LT, heat labile; ST, heat stable.

^a Includes both LT and ST toxin-producing coliforms

coliforms as a measure of food contamination. Heavy growth of coliforms is indicative of poor food handling and processing and is likely to be associated with transmission of enteric pathogens [3, 7].

Coliform contamination of vegetables was consistently more common in Guadalajara restaurants than in Houston restaurants for the various methods of food preparation studied. Interestingly, neither cooking nor serving cooked foods hot appeared to offer any protection from coliform contamination or to make the foods “safer” in Guadalajara restaurants. In our study, the rates of coliform contamination of vegetables from Guadalajara were significantly higher than those for vegetables from Houston, regardless of whether vegetables were cooked or cooked and served hot. It is possible that the handling of the food items after they had been cooked contributed to contamination of the foods.

Although there was a trend toward more frequent contamination of buffet vegetables in Guadalajara restaurants than in

Houston restaurants, no statistically significant differences in the rates or levels of contamination between the 2 sites were noted. However, this difference in contamination between the 2 cities for buffet vegetables may have not been observed because of increased contamination of our Houston control samples, which had higher levels of coliform detection in the buffet setting than in the nonbuffet setting (data not shown). Statistically significant differences may also have been recognized if a greater number of samples were tested.

There are several notable findings with the microbiological analysis of the coliforms. We originally assumed, as many other previous studies have done [1, 13, 14], that lactose-fermenting bacteria isolated from MacConkey agar would be *E. coli*. Well-known *E. coli* virulence properties—including the presence of enterotoxins (heat-labile and heat-stable toxins) and enteroggregative phenotype, the defining characteristic of enteroggregative *E. coli* [15]—can be found with other non-*E. coli* coliforms found in food. These results emphasize the importance of proper identification of bacteria, through biochemical testing or other means, because non-*E. coli* bacteria not only appear morphologically similar to *E. coli* but also have virulence properties similar to those of *E. coli*.

Toxin production by other non-*E. coli* bacteria has been well described elsewhere [3, 16]. However, there are very few reports regarding non-*E. coli* bacteria that display the unique property of adherence to HEp-2 cells in a “stacked-brick” appearance, including *Aeromonas* species [17]. From our studies, it appears that *Enterobacter* and *Klebsiella* species can bind in a similar fashion. We have isolated *Enterobacter* and *Klebsiella* species from human diarrheal stool samples that also display this aggregative phenotype (authors’ unpublished data).

Study limitations include a lack of detection of established enteric pathogens associated with traveler’s diarrhea, use of coliform detection as a marker of contamination (rather than testing specifically for fecal coliforms or *E. coli*), and lack of clinical correlation with identification of the contaminated vegetables. Of interest, *K. oxytoca* has recently been described as a cause of antibiotic-associated hemorrhagic colitis [18]. We plan to pursue future studies to determine whether our vegetable isolates are potential pathogens. Finally, these findings may not be generalizable to other food groups because of naturally high levels of colonization of vegetables with Enterobacteriaceae [9]. However, we believe that our results fit with the results of other studies that we have conducted, adding vegetables as a potential high-risk item in developing regions such as Mexico and showing that safety cannot be assured by the type of vegetable obtained from a commercial eating establishment.

This study provides insights as to why prevention of traveler’s diarrhea by avoidance of “high-risk” foods has, thus far, been unsuccessful [5]. Our results indicate that contamination of

foods is widespread in developing countries, such as Mexico, making selection of “safer” foods difficult. Although it is easy to assume that one is more likely to avoid contaminated foods by choosing cooked foods over uncooked foods, our study indicates that this belief may not hold true. Tourists may be exposed to contaminated vegetables regardless of how they select food items in a developing region such as Mexico. The difficulty of avoiding contaminated foods even with prudent food selection provides further support for the use of chemoprophylaxis in certain populations of travelers, as a means of reducing diarrhea during travel to high-risk regions.

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