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

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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 ≥33.9°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 >103 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-stable 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 χ² analysis. The Mann-Whitney
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 = .04). 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.

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

<table>
<thead>
<tr>
<th>Characteristic, location</th>
<th>Proportion of samples with coliform contamination (%)a</th>
<th>Contamination level, median cfu/g of sample</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guadalajara</td>
<td>27/64 (42)</td>
<td>16,000</td>
<td>.2</td>
</tr>
<tr>
<td>Houston</td>
<td>17/67 (25)</td>
<td>22,000</td>
<td></td>
</tr>
<tr>
<td><strong>Cooked vegetables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guadalajara</td>
<td>10/15 (67)</td>
<td>&lt;.001</td>
<td>.01</td>
</tr>
<tr>
<td>Houston</td>
<td>3/30 (10)</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td><strong>Cooked vegetables, served heated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guadalajara</td>
<td>5/6 (83)</td>
<td>&lt;.001</td>
<td>.01</td>
</tr>
<tr>
<td>Houston</td>
<td>2/27 (7.4)</td>
<td>205,000</td>
<td>.37</td>
</tr>
<tr>
<td><strong>Buffet samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guadalajara</td>
<td>8/19 (42)</td>
<td>.22</td>
<td>.37</td>
</tr>
<tr>
<td>Houston</td>
<td>5/21 (24)</td>
<td>32,000</td>
<td></td>
</tr>
<tr>
<td><strong>Cooked buffet samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guadalajara</td>
<td>2/4 (50)</td>
<td>.24</td>
<td>.73</td>
</tr>
<tr>
<td>Houston</td>
<td>1/8 (13)</td>
<td>50,000</td>
<td></td>
</tr>
</tbody>
</table>

* Presence of ≥10⁴ coliform cfu/g of sample.

b 33.9°C–100°C.
The rate of coliform contamination of vegetables from Guadalajara was consistently more common 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 not have 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 enteroga-gregative phenotype, the defining characteristic of enteroaggregative 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

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\begin{array}{|c|c|c|}
\hline
\text{Coliform} & \text{Guadalajara, Mexico} & \text{Houston, Texas} \\
\hline
\text{Enterotoxigenic}^* & 4 & 4 \\
\text{LT toxin producing} & & \\
\text{Citrobacter freundii} & 1 & 0 \\
\text{Enterobacter cloacae} & 0 & 1 \\
\text{Pantoea species} & 1 & 1 \\
\text{ST toxin producing} & & \\
\text{E. cloacae} & 1 & 0 \\
\text{Pseudomonas fluorescens} & 0 & 2 \\
\text{Identification missing} & 1 & 0 \\
\text{Enteroaggregative} & 13 & 11 \\
\text{Klebsiella oxytoca} & 5 & 0 \\
\text{Klebsiella pneumoniae} & 3 & 0 \\
\text{Enterobacter aerogenes} & 1 & 0 \\
\text{Enterobacter amigenus} & 0 & 1 \\
\text{E. cloacae} & 2 & 8 \\
\text{Enterobacter sakazakii} & 0 & 1 \\
\text{Pantoea species} & 0 & 1 \\
\text{Identification missing} & 2 & 0 \\
\text{Both enterotoxigenic}^* \text{ and enteroaggregative} & 10 & 2 \\
\text{LT toxin producing} & & \\
\text{E. cloacae} & 2 & 0 \\
\text{K. oxytoca} & 2 & 0 \\
\text{Serratia fonticola} & 1 & 0 \\
\text{ST toxin producing} & & \\
\text{C. freundii} & 1 & 0 \\
\text{E. cloacae} & 2 & 1 \\
\text{K. oxytoca} & 1 & 0 \\
\text{K. pneumoniae} & 1 & 0 \\
\text{Pantoea species} & 0 & 1 \\
\hline
\end{array}
\]

\textbf{NOTE.} LT, heat labile; ST, heat stable.

* Includes both LT and ST toxin-producing coliforms

Table 2. Microbiological analysis of coliforms isolated from vegetable samples.
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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Potential conflicts of interest. All authors: no conflicts.

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