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Incidence of Bacterial Enteropathogens in Foods from Mexico

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We examined food consumption patterns of U.S. students temporarily living in Guadalajara, Mexico. Consumption of foods prepared in Mexican homes was associated with an increased risk of acquisition of diarrhea. Foods from commercial sources and private Mexican homes in Guadalajara were subsequently examined for contamination with coliforms, fecal coliforms, and bacterial enteropathogens. For comparison, selected restaurant foods were obtained in Houston, Tex. Food obtained from Mexican homes showed generally higher counts of coliforms and fecal coliforms than those obtained from commercial sources in Mexico and Houston. The foods in Mexico, both from homes and commercial sources, commonly contained *Escherichia coli* and occasionally enterotoxigenic *E. coli*. Foods in Houston were not contaminated with *E. coli* or enterotoxigenic *E. coli*. *Salmonella* (17 isolates), *Shigella* (4 isolates), and *Aeromonas hydrophila* (1 isolate) were found only in the foods obtained from Mexican homes. Enterotoxigenic non-*E. coli Enterobacteriaceae* was recovered with approximately equal frequency from all food sources.

Travelers' diarrhea is a major inconvenience to visitors arriving in developing countries from more industrialized areas. This disease may be an important factor inhibiting tourism to developing countries. In visitors to Mexico approximately 80% of diarrhea is of bacterial origin (8), with enterotoxigenic *Escherichia coli* (ETEC) being the most important organism. Epidemiological evidence has implicated food as an important vector of enteropathogenic bacteria (11, 21). Several authors have reported low levels of enterotoxigenic organisms in foods in various countries, including Sweden (3), the United States (18), and the Philippines (10). Jiwa et al. (14) reported that enterotoxigenic organisms of many genera were found in food and water in an Ethiopian community. These authors were unable to recover ETEC from foods. The clinical significance of enterotoxigenic non-*E. coli* remains unclear.

In 1975, we initiated studies of acute travelers' diarrhea which have continued to the present time (5–9, 11, 21). The subjects under study are young adults from the United States studying in Mexico. Epidemiological evidence in this setting suggested that patterns of food consumption were predictive of diarrhea occurrence (11, 21). In an effort to further define the role of food as a source of travelers' diarrhea, we initiated the present studies. The studies reported here were carried out with three main objectives: (i) to determine whether a relationship exists between eating food prepared in Mexican homes and acquisition of diarrhea by U.S. students in Mexico; (ii) to investigate the incidence of coliform and fecal coliform bacteria in commercially and home-prepared Mexican food; and (iii) to determine the incidence of diarrheagenic and enterotoxigenic bacteria in these foods.

MATERIALS AND METHODS

Consumption of food in Mexican homes and incidence of gastroenteritis. A total of 137 U.S. students attending summer school in Guadalajara, Mexico, during the summer of 1980 were enrolled as subjects in a study of antibiotic prophylaxis against travelers' diarrhea. Each subject was enrolled within 48 h of arrival in Mexico. Each subject kept a daily diary and recorded information including gastrointestinal symptoms and location of food consumption over a 3-week period. An analysis of food consumption patterns was undertaken to determine whether there was an association with diarrhea rates. Definition of diarrhea and other details of this study have been published previously (6). A second study was carried out in 1982 after the first study linked consumption of food in Mexican homes with the occurrence of diarrhea. Twenty-four students living with six Mexican families were asked to complete a questionnaire on a weekly basis seeking information about the occurrence and timing of diarrheal illness. These students furnished foods from the homes one to three times a week for microbiological study to allow a comparison to be made of diarrhea outbreaks and levels of food contamination. These students were followed for 4 weeks.

Determination of coliform and fecal coliform contami-

nation of foods. Commercially prepared foods were purchased at various public establishments in Guadalajara during the summer of 1981. These included a supermarket, a university cafeteria, five vendors in a street market, and nine restaurants. Samples were placed in sterile bags (Whirl-Pak; Scientific Products, Houston, Tex.) for transport to the Guadalajara laboratory. Samples were generally processed immediately, but a few were refrigerated overnight. In all cases, samples were processed within 18 h of collection. In the laboratory, 20 to 25 g of food was weighed into a sterile bag (Whirl-Pak). Sufficient sterile phosphate-buffered saline was added to give a 1:10 dilution. The sample was homogenized with a Stomacher 400 blender (Dynatech, Alexandria, Va.) for 30 s to 1 min. The resulting suspension was used to prepare serial 1:10 dilutions in phosphate-buffered saline. These dilutions were used to inoculate triplicate tubes of lauryl sulfate tryptose broth (Difco Laboratories, Detroit, Mich.), each containing an inverted gas tube. These tubes were incubated at 35°C for 24 h. Tubes showing gas production or effervescence were recorded as positive. Negative tubes were reincubated for a further 24 h at 35°C. Results were then interpreted by using tables of most probable number (MPN), and the index of coliforms per gram of food sample was obtained. Positive lauryl sulfate tryptose broth tubes were subcultured to *E. coli* broth (Difco) tubes, each with an inverted gas tube. After incubation at 44.5°C for 24 h, tubes showing gas production or effervescence were recorded as positive. MPN tables were used to obtain the index of fecal coliforms per gram of original food sample. These methods are in accordance with recommended procedures for enumeration of coliforms and fecal coliforms in foods (2).

The original serial dilutions in phosphate-buffered saline were streaked to tryptose phosphate bile yeast extract agar (TPBY), which was recommended by Mehlman and Romero (15) to recover coliforms with minimal loss of virulence characteristics. After streaking, the TPBY plates were incubated at 35°C for 2 h to resuscitate stressed cells and then incubated at 44.5°C for a further 22 h. Up to 10 typical colonies per food sample were picked without further screening and inoculated into peptone agar stabs. Stabs were incubated at 35°C overnight and then stored at room temperature for transport to Houston. In the laboratory in Houston, isolates were checked for purity and identified by using rapid biochemical test systems (API 10S and API 20E; Analytab Products, Plainview, N.Y.) to determine enteric pathogens. This procedure was also followed for foods obtained from two Houston restaurants serving Mexican-type foods.

Home-prepared foods. Students living with six Mexican families brought home-prepared food samples to the laboratory in plastic sandwich-type bags. Foods constituting the meal from a single home were combined to constitute a sample of 25 g or less total weight. The combined samples were placed in sterile bags (Whirl-Pak), homogenized, and diluted as described above. Serial dilutions were plated on MacConkey agar and incubated overnight at 37°C for enumeration of enteric bacteria. MacConkey and TPBY plates were inoculated for each dilution and incubated at 44.5°C to enumerate fecal coliforms. Numbers of bacteria obtained at 37°C were recorded as total enteric counts, and lactose-negative colonies were selected from these plates to isolate *Salmonella* and *Shigella* spp. Colonies resembling *E. coli* were selected from the 44.5°C MacConkey plates and the 44.5°C TPBY plates and inoculated into peptone stabs. These colonies were tested for indole production by the method of Anderson and Baird-Parker (1) and were also grown overnight in sulfite indole motility medium (Difco) at 37°C. Indole-positive colonies were tentatively identified as *E. coli*. The identity of all isolates was confirmed by API 10S and API 20E testing.

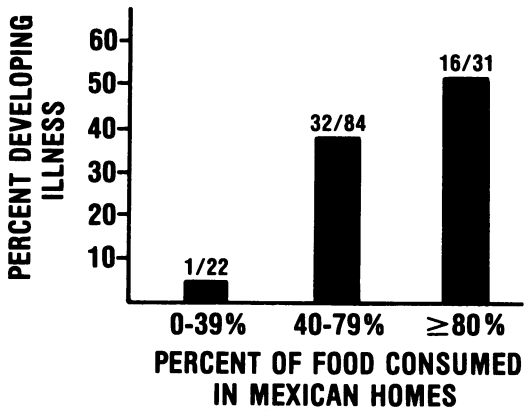

FIG. 1. Association between eating in Mexican homes and acquisition of diarrhea. Figures above bars are number of individuals ill over number of individuals in category.

Testing for production of enterotoxins. Production of heat-stable enterotoxin was examined by the suckling mouse assay described by Dean et al. (4) and modified by Giannella (13). Intestinal weight to body weight ratios above 0.083 were considered positive. Heat-labile enterotoxin was assayed by the Y-1 adrenal cell method described by Sack and Sack (17).

RESULTS

Epidemiology study. The location of food consumption, expressed as the percentage of food consumed in Mexican homes, and acquisition of diarrhea are shown in Fig. 1. The percentage of students developing illness increased with the proportion of food consumed in private Mexican homes. Eight students who prepared all their meals in their own apartments reported no occurrence of diarrhea.

Microbiological studies of food. The rates of contamination of commercially prepared foods from Guadalajara and Houston are shown in Table 1. All foods examined from the supermarket, cafeteria, and street vendors in Mexico were contaminated with coliforms. Of 15 samples, 11 were contaminated with fecal coliforms. Two supermarket foods (fresh shrimp and potato salad) contained ETEC. Despite relatively high contamination with coliforms and fecal coliforms, cafeteria foods yielded no enterotoxin-producing organisms. All five samples from

TABLE 1. Contamination levels of commercially prepared foods by coliforms, fecal coliforms, and enterotoxigenic bacteria

Source	No. of foods	MPN coliforms/g		MPN fecal coliforms/g		No. of foods containing enterotoxigenic organisms (%)
		Range	Mean	Range	Mean	
Guadalajara						
Supermarket	5	3–11,000	3,349	0–1,500	529	2 (40)
Cafeteria	5	4–4,600	1,108	0–4,600	940	0
Street vendors	5	460–1,100	844	0–1,100	232	5 (100)
Restaurants	11	0–1,100	828	0–1,100	487	3 (27)
Houston restaurants	12	0–1,100	561	0–1,100	189	2 (17)

the street vendors produced non-*E. coli* enterotoxigenic bacteria. Foods from the street vendors included watermelon and fruit salad as well as three heated items (a taco, pigs foot with rice, and a rice and vegetable mixture). Enterotoxigenic bacteria isolated included *Enterobacter cloacae, Klebsiella pneumoniae*, and *Klebsiella oxytoca*. Restaurant foods from Guadalajara were generally more contaminated with coliforms and fecal coliforms than similar foods from Houston. Three food samples from Guadalajara (hamburger garnish, grapefruit juice, and picante sauce) contained enterotoxigenic bacteria. These included *E. coli* and *E. cloacae*. Two samples from a Mexican restaurant in Houston (pico de gallo and salad) contained enterotoxigenic bacteria, including *Enterobacter aerogenes, K. pneumoniae*, and *Citrobacter freundii*. No recognized pathogens such as ETEC, salmonellae, or shigellae were isolated from foods in Houston.

The total enteric and fecal coliform counts obtained from meals prepared in Mexican homes are shown in Table 2. This table includes homes from which complete student questionnaires were obtained for the 4-week period of the study. Thirty additional meals were obtained from six homes for which diarrhea incidence data were not available. These meals were used in the isolation of enteropathogens. When the $\log_{10}$ of the mean fecal coliform counts were correlated with the incidence of illness, a correlation coefficient of 0.67 was obtained.

A total of 162 bacterial isolates were obtained from commercially prepared foods in Mexico (Table 3). These included 40 *E. coli*, of which 4 (10%) were enterotoxin producers. Fourteen other isolates, including *E. aerogenes, E. cloacae, Enterobacter agglomerans, K. pneumoniae* and *K. oxytoca*, were also enterotoxigenic. Of these 14 enterotoxigenic isolates, 3 could not be identified by the API 20E system. Of 72 *E. coli*, 6 (8%) isolated from home-prepared foods were enterotoxigenic. Twenty-two other enteric pathogens were also isolated from food prepared by Mexican families. These included 17 *Salmonella* sp., 4 *Shigella* sp., and one *Aeromonas hydrophila*. It was of interest that three isolates of *Salmonella*, from two homes, were positive in the heat-stable enterotoxin assay. We are currently characterizing these strains further.

DISCUSSION

Our previous studies on food consumption patterns carried out in rural Mexico (Cholula, Puebla) demonstrated an increased risk of acquiring diarrhea among students who ate a majority of their meals in public restaurants or a school cafeteria, compared with those preparing their own meals in apartments (21). The current study was an extention of this earlier observation. The U.S. students in urban Guadalajara commonly lived in the homes of Mexican families. We found that there was a direct relationship of acquisition of diarrhea with eating in

TABLE 2. Correlation of illness with coliform and fecal coliform contamination of foods prepared in private Mexican homes

House no.	No. of meals sampled	Total enteric bacteria/g		Fecal coliforms/g		% Illness
		Range	Mean	Range	Mean	
1	7	$80–2 \times 10^6$	3×10^5	$0–4 \times 10^2$	1×10^2	10
2	8	$40–8 \times 10^6$	3×10^4	$0–8 \times 10^4$	1×10^4	20
3	11	$20–4 \times 10^6$	8×10^5	$0–1 \times 10^5$	2×10^4	24
4	9	$80–8 \times 10^5$	2×10^5	$30–1 \times 10^4$	2×10^3	27
5	8	$60–3 \times 10^3$	1×10^3	$0–2 \times 10^3$	5×10^2	28
6	9	$3 \times 10^2–2 \times 10^5$	8×10^4	$0–7 \times 10^5$	8×10^4	35

TABLE 3. Isolation of enteric pathogens and enterotoxigenic organisms from commercially prepared and home-prepared foods

Source	No. of isolates	No. of *E. coli*	No. of ETEC	No. of enterotoxigenic non-*E. coli*	No. of other pathogens
Commercial food, Guadalajara	162	40	4[a]	14[b]	0
Home-prepared food, Guadalajara	353	72	6[a]	9[c]	22[d]
Commercial food, Houston	30	0	0	7[a]	0

[a] Heat-stable enterotoxin only.
[b] Heat-stable enterotoxin only, 11; heat-labile enterotoxin only, 2; heat-stable and heat-labile enterotoxins, 1.
[c] Heat-stable enterotoxin only, 6; heat-labile enterotoxin only, 1; heat-stable and heat-labile enterotoxins, 2.
[d] 17 *Salmonella* isolates, 4 *Shigella* isolates, 1 *A. hydrophila* isolate.

Mexican homes. In the previous and current studies, we found that the students from the United States who prepared most of their meals in their own apartments remained free of illness throughout the 4 weeks that they were followed. In the present study, those who generally consumed food at public restaurants experienced diarrhea with rates intermediate between the groups who ate their meals with Mexican families and those consuming foods in their own apartments.

The microbiological survey reported here strongly supports the epidemiological studies. Food obtained from Mexican homes showed the highest bacterial counts. These foods showed counts of total enteric bacteria and fecal coliforms at levels considerably higher than those obtained from commercial sources in Mexico and Houston. *E. coli* and ETEC strains were recovered from foods in Mexico but not from those obtained in Houston. Repeated exposure to ETEC in food consumed among Mexicans undoubtedly explains the high geometric mean titer of antitoxin antibody found (12) and explains the resistance to clinical ETEC infection among Mexican persons (7). The rarity of ETEC in the United States explains the remarkable susceptibility of persons living in the United States to infection by these bacteria. When strains do find their way into food or water in the United States, large outbreaks of infection occur (16, 20).

The present study revealed that enterotoxigenic non-*E. coli* strains of *Enterobacteriaceae* could commonly be found in food sampled in commercial establishments in Mexico or in the United States and in food obtained in the homes of Mexicans. Finding these strains from all sources casts some doubt on their importance as common causes of disease, although *K. pneumoniae* and *Enterobacter* sp. (3) have been implicated occasionally in food-borne outbreaks of gastroenteritis.

The widespread occurrence of fecal contamination in Mexican foods makes it difficult to develop clear recommendations for persons as they travel from low- to high-risk areas. One recent uncontrolled study indicated that it is hard to avoid illness by merely watching what one eats (19). However, self-preparation of meals should be a useful means of minimizing illness for select persons at risk. Also, we feel that when foods are consumed in public eating establishments in these areas, it would be prudent to follow traditional advice and select foods which are steaming hot, can be peeled, are bottled, or are dry (bread or tortillas) and to avoid any foods remaining at room temperature for periods of time, including items served buffet style, leafy green vegetables, or hot sauces left on table tops. We are currently investigating the microbiological validity of these concepts.

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