Research Note

Presence of Shiga Toxin–Producing Escherichia coli, Enteroinvasive E. coli, Enteropathogenic E. coli, and Enterotoxigenic E. coli on Tomatoes from Public Markets in Mexico

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ABSTRACT

Diarrheagenic Escherichia coli pathotypes (DEP) are important foodborne pathogens in various countries, including Mexico. However, no data exist on the presence of DEP on fresh tomatoes (Solanum lycopericum) from Mexico. The frequency of fecal coliforms (FC), E. coli, and DEP were determined for two tomato varieties. One hundred samples of a saladette tomato variety and 100 samples of a red round tomato variety were collected from public markets in Pachuca, Mexico. Each tomato sample consisted of four whole tomatoes. For the 100 saladette samples, coliform bacterial, FC, E. coli, and DEP were identified in 100, 70, 60, and 10% of samples, respectively. For the 100 red round samples, coliform bacterial, FC, E. coli, and DEP were identified in 100, 75, 65, and 11% of samples, respectively. Identified DEP included Shiga toxin–producing E. coli (STEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC), and enterotoxigenic E. coli (ETEC). STEC were isolated from 6% of saladette samples and 5% of red round samples. ETEC were isolated from 3% of saladette samples and 4% of red round samples. EPEC were isolated from 2% of saladette samples and 3% of red round samples, and EIEC were isolated from 1% of saladette samples. Both STEC and ETEC were identified in two saladette samples and 1 red round sample. E. coli O157:H7 was not detected in any STEC-positive samples.

Tomato (Solanum lycopericum) is a major commercial crop in Mexico, accounting for over 1,872,481 tons (1.8 × 10^9 kg) of production in 2011. Of this crop, 879,693 tons (8.8 × 10^8 kg) were the saladette type and 254,890 tons (2.5 × 10^8 kg) were the red round type (26). These two tomato types are the most commonly consumed in a raw state (e.g., in green salads) in Mexico and in other countries. From 2005 to 2006 at least four multistate outbreaks of Salmonella infection were associated with fresh tomatoes (4). However, no tomato-associated outbreaks of caused by the diarrheagenic Escherichia coli pathotypes (DEP) enterotoxigenic E. coli (EPEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), enteroaggregative E. coli (EAEC), diffuse adherent E. coli, Shiga toxin–producing E. coli (STEC), and enteroaggregative-hemorrhagic E. coli have been reported. However, a recent outbreak of foodborne illness in several European countries that was caused by enterotoxigenic-hemorrhagic E. coli in sprouts (1) highlights the importance of screening for DEP in fresh vegetables such as tomatoes.

DEP are foodborne pathogens (12) and are classified according to their virulence traits. In Mexico, DEP from foods or people with acute diarrhea are not routinely tested by the Health Secretariat. However, identifying the presence of DEP in foods in Mexico is vital because these bacteria have been associated with diarrheal illness in both Mexican children (9) and visitors to the country (23). EPEC, ETEC, EIEC, and STEC are important causes of diarrhea in developed countries (such as México) and in visitors from regions where DEP are not endemic (8, 9, 19, 22).

EPEC is a human pathogen responsible for outbreaks of diarrhea in both developing and developed countries (19). EPEC strains cause diarrhea through localized destruction of the intestinal brush border and distortion of the apical enterocyte membrane. Lesions result in a reduction in the absorptive capacity of the intestinal mucosa with consequent disruption of the electrolyte balance and diarrhea (19). EPEC strains have been isolated from a variety of animal species, such as cattle, goats, sheep, chickens, pigeons, and gulls (7).
ETEC is the leading cause of weanling diarrhea in developing countries and of traveler’s diarrhea around the world. ETEC strains cause diarrhea through the action of two enterotoxins: one that is heat labile and one that is heat stable. ETEC strains may express one heat-labile toxin only, one heat-stable toxin only, or both types of enterotoxins (19). Pigs and cattle are the predominant reservoirs of ETEC (18).

EIEC strains are biochemically, genetically, and pathogenetically related closely to Shigella spp. and cause an invasive, dysenteric form of diarrhea in humans (19). Pathogenicity of EIEC is primarily due its ability to invade and destroy colonic tissue. The invasion phenotype is encoded by a high-molecular-weight plasmid (19). Humans are a major reservoir for EIEC (12).

STEC strains are human pathogens associated with illness. Some strains can cause hemorrhagic colitis, which in some cases may progress to hemolytic uremic syndrome (19). Serotype O157:H7 STEC strains are most frequently associated with illness in the United States, causing approximately 63,000 illnesses per year, whereas non-O157 STEC strains are responsible for approximately 113,000 infections annually (25). STEC strains cause illness through the action of a family of cytotoxic Shiga toxins (Stx). The Stx family consists of two major, immunologically non-cross-reactive groups, Stx1 and Stx2, encoded by the genes stx1 and stx2, respectively. A single STEC strain may express stx1 only, stx2 only, or both genes or even multiple forms of stx2. Other genes involved in pathogenesis include those for type III secretion, intimin (eaeA), translocated intimin receptor, and enterohemolysin (19). STEC strains are mostly commensal bacteria in animals, with a high potential for foodborne transmission to humans (2). Ruminants, primarily cattle, are the predominant reservoir of STEC (2). Foodborne bacteria are usually transmitted by contaminated food. Fecal contamination of food and drinking water is the major route of infection of pathogenic bacteria for humans (13).

Although consumer demand for tomatoes continues to grow, no data exist on the presence of DEP on fresh tomatoes from Mexico or other countries. The objective of this study was to measure the prevalence of fecal coliforms (FC), E. coli, and ETEC, EPEC, EIEC, STEC, and EAEC on saladette and red round tomato types.

MATERIALS AND METHODS

Tomato samples. The city of Pachuca, Hidalgo State, Mexico, has 13 public markets, but only 4 of these are sizable. Samples of fresh tomatoes (saladette and red round types) were collected from 5 vegetable retailers in each of these 4 large markets (total of 20 retailers) during a 10-week study period in the summer. Samples were purchased every 2 weeks at each retailer, resulting in 20 samples per day and a total of 100 saladette and 100 red round samples for the entire study period. Saladette samples were tested after red round samples. At the time of purchase, tomatoes were in plastic containers stored at room temperature. Each tomato sample consisted of four ripe whole tomatoes, with an average weight of 600 g for the saladette sample (~150 g per tomato) and 800 g for the red round sample (~200 g per tomato). Tomatoes of each type had similar shape, weight, and color. Samples were placed in sterilized plastic bags and held in a cooler with frozen gel packs for transport to the laboratory. All samples were analyzed no more than 1 h after purchase.

Samples preparation. One liter of sterilized peptone water (0.1%; Bioxon, Mexico City, Mexico) was added to each tomato sample. Samples were rubbed manually for 90 s while in the sealed bag. The rubbed tomatoes were then removed from the bag, the bag was pummelled in a stomacher for 1 min, and sample dilutions with 0.1% sterilized peptone water were prepared for microorganism counts. Samples were analyzed for the presence of FC, E. coli, ETEC, EPEC, EIEC, STEC, and EAEC.

Microbiological analyses. FC and E. coli were analyzed by the most-probable-number (MPN) procedure following the method described in the U.S. Food and Drug Administration Bacteriological Analytical Manual (29). Each serial dilution (1 ml) of the sample homogenates in 0.1% sterile peptone water was inoculated into nine tubes containing lactose broth (Bioxon) (three tubes from dilution 10⁻¹, three tubes from dilution 10⁻², and three tubes from dilution 10⁻³) and into Durham tubes. After incubation at 37°C for 48 h, one loopful of positive culture suspension (determined by turbidity and gas production) was transferred to tubes containing Fluorocult brilliant green 2%–bile lactose broth (Merck, Darmstadt, Germany). After incubation at 44.5 ± 0.2°C for 24 to 48 h, tubes positive for growth and gas production were considered FC positive. For determining the presence of E. coli specifically, FC-positive tubes were used to identify indole formation. All tubes positive for indole and gas production were streaked onto eosin methylene blue agar (EMB; Bionox). Two or three presumptive E. coli colonies were selected from the EMB plates and biochemically characterized using the IMViC (indole, methyl red, Voges-Proskauer, and citrate) test (29). Biochemical confirmation of presumptive E. coli isolates was done with the API 20E test (bioMérieux, Hazelwood, MO). All E. coli isolates formed typical colonial forms. FC and E. coli levels were calculated following the MPN method in the Bacteriological Analytical Manual (29). All confirmed E. coli strains were streaked on tryptic soy agar (TSA; Bionox) slants, incubated at 37°C for 24 h, and maintained at 3 to 5°C until they were used for the PCR.

Multiplex PCRs for DEP locus identification. All confirmed E. coli isolates were analyzed with two multiplex PCR assays. PCR A was used to identify the following loci (15) encoding thermostable and thermolabile enterotoxins (st; lt) for ETEC, intimin (eaeA) and bundle-forming pilus (bfp) for EPEC, Stx1 and Stx2 (stx1, stx2) for STEC, and invasion-associated loci (ial) for EIEC. PCR B was used to identify three EAEC plasmidborne virulence genes encoding the master regulator (aggR), dispersin (aap), and autotransporter Tol C (aatA) (5). Both multiplex PCRs were conducted exactly as described by López-Saucedo et al. (15) and Cerna et al. (5). E. coli strains stored on TSA slants were streaked on TSA plates and incubated at 37°C for 24 h. After boiling for 1 min and then freezing at 5°C, one colony of each strain from the TSA plates was suspended in 1 ml of sterilized and deionized water. All bacterial lysates were analyzed first with PCR A and then with PCR B.

For PCR A, each PCR tube contained 23 μl of reaction mix (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2 mM MgCl₂; 100 μg/ml gelatin; 5% glycerol [vol/vol], 200 μM concentrations of each deoxynucleoside triphosphate, and 0.5 U/23 μl Taq polymerase [Invitrogen, Carlsbad, CA]), 2 μl of bacterial lysate, and a mixture of 14 primers (Invitrogen): 5’-GGC GAC AGA TTA TAC CGT GC-3’, R [reverse]: 5’-CGG TCT CTA TAT TCC
TABLE 1. Populations of fecal coliforms (FC), E. coli, and diarrheagenic E. coli pathotypes (DEP) on saladette and red round tomato samples

<table>
<thead>
<tr>
<th>Tomato type</th>
<th>Microorganism</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saladette</td>
<td>FC</td>
<td>&lt;3</td>
<td>4.4 × 10^4</td>
<td>2.75 × 10^5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>&lt;3</td>
<td>9.0 × 10^2</td>
<td>2.75 × 10^5</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>DEP</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>1.15 × 10^5</td>
<td>10</td>
</tr>
<tr>
<td>Red round</td>
<td>FC</td>
<td>&lt;3</td>
<td>1.6 × 10^4</td>
<td>2.75 × 10^5</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>&lt;3</td>
<td>1.7 × 10^3</td>
<td>2.75 × 10^5</td>
<td>65</td>
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<tr>
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<td>DEP</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>1.15 × 10^5</td>
<td>11</td>
</tr>
</tbody>
</table>

Microbiological analysis revealed E. coli in 70% of saladette samples and 75% of red round samples. DEP were isolated from 10% of saladette and 11% of red round samples (Table 1). Populations of FC and E. coli were between <3 and 2.75 × 10^5 MPN per tomato for the saladette and red round samples. DEP levels were <3 to 1.1 × 10^5 MPN per tomato for both types (Table 1).

High FC levels are related to poor hygiene but not necessarily to the presence of fecal matter, which is frequently found on raw vegetables (10, 17, 24) including tomatoes (30). Potential reasons for high levels of FC in tomatoes include extensive exposure to contamination during harvest, transport, processing, and marketing.

The Kruskal-Wallis test indicated no significant difference (P > 0.05) in FC counts between the two types of tomato. However, red round tomatoes had significantly higher E. coli counts (P < 0.05) than did the saladette tomatoes.

For many years, FC and E. coli have been used as indicators of fecal contamination of foods. In numerous studies, FC levels have been correlated with the presence of E. coli. However, the value of the FC assay as a fecal contamination indicator is nullified when bacteria of nonfecal origin are the principal microbes detected by the assay. FC are not effective fecal indicators in fresh vegetables (21), particularly because common nonfecal coliforms such as Klebsiella and Enterobacter can grow under thermotolerant test conditions (20). Considered the best indicator, E. coli is probably the most commonly used microbial indicator of fecal contamination in foods. Microbiological criteria have been regularly established for E. coli, but disagreement still exists as to whether its presence is necessarily associated with the presence of fecal matter or pathogenic bacteria.

Identified DEP were STEC, EIEC, EPEC, and ETEC (Table 2); the STEC pathotype was most common in both tomato types. The STEC pathotype was detected in six saladette samples and five red round samples, the ETEC pathotype was detected in three saladette samples and four red round samples, the EPEC pathotype was detected in two saladette samples and three red round samples, and the EIEC pathotype was detected only in one saladette sample.
In the positive samples, STEC levels were $9.0 \times 10^2$ to 1.1
$\times 10^5$ MPN per tomato in the saladettes and $9.0 \times 10^2$
$\times 1.0 \times 10^4$ MPN per tomato in the red rounds; ETEC levels
were $9.0 \times 10^2$ to $7.0 \times 10^3$ MPN per tomato in both the
saladettes and the red rounds; and EPEC levels were $9.0 \times 10^2$
$\times 7.0 \times 10^3$ MPN per tomato in the saladettes and $9.0$
$\times 10^2$ to $1.1 \times 10^5$ MPN per tomato in the red rounds. The
EIEC level was $1.0 \times 10^4$ MPN per tomato in the saladettes,
but no EIEC was detected in red rounds. The STEC and ETEC
pathotypes were isolated from the same two saladette samples
and the same red round samples.

More STEC isolates from these tomatoes expressed the
$stx_1$ locus than the $stx_2$ locus (Table 2). Of the STEC-
positive saladette tomatoes, the $stx_1$ locus was identified
from four samples and $stx_2$ was identified from two samples.
Of the five STEC strains isolated from the red round
samples, three had only the $stx_1$ locus, and two had only the
$stx_2$ locus (Table 2). The pathogenesis of STEC is linked to
different virulence factors such as $stx_1$ and $stx_2$, intimin,
and enterohemolysin (19). It have been reported that $Stx1$ and
$Stx2$ cause different degrees and types of tissue damage
(16); $Stx2$ is more toxic than $Stx1$ to human renal
endothelial cells (16). No O157 and H7 antigens were
detected in any STEC-positive tomato samples. Similar
results showing the lack of O157 and H7 antigens in STEC
strains isolated in Mexico have been reported (9). STEC
strains have been identified from raw foods in Mexico such
as vegetable salads (3) and carrot juice (27).

ETEC, EPEC, and EIEC strains also were found in
these tomato samples. ETEC represent is a major cause of
diarrhea in developing countries and in visitors from regions
where ETEC is not endemic (traveler’s diarrhea) (19). These
pathogens usually are transmitted by contaminated food.
The ETEC pathotype has been isolated from raw vegetable
salads (3) and carrot juice (27). EPEC is a leading cause of
diarrhea in developed countries (9, 28). Typical EPEC
strains contain both $eaeA$ and $bfp$, but atypical EPEC strains
contain only $eaeA$. In industrialized countries, typical EPEC
infections have decreased and atypical $E. coli$ infections
seem to have increased in recent years (11). Unlike typical
EPEC strains, which are found only in humans, atypical
EPEC strains have been isolated from a variety of animal
species (7). Humans are a major reservoir for $EIEC$ (12, 14).
EIEC strains have been isolated from ready-to-eat vegetable
salads (3), mung bean sprouts (6), and chili sauces (15) in
Mexico.

To the best of our knowledge, this report is the first
concerning STEC, ETEC, EPEC, and EIEC prevalence in
tomatoes in Mexico. The microbiological quality and DEP
prevalence analyses were applied to tomatoes acquired in
the city of Pachuca, Hidalgo, an urban area of more than
500,000 inhabitants who receive most of their vegetables
(including tomatoes) from local producers. The results
indicate that tomatoes in this area represent a potential risk
of foodborne illness to the local population and visitors.
Most of the tested tomatoes were contaminated with fecal
indicators, and some carried pathogenic bacteria such as
DEP. Multiple sources of pathogenic microorganisms can
affect tomatoes during packaging, distribution, and
marketing. Once incorporated into prepared foods, tomatoes can
act as sources of cross-contamination with pathogenic
microorganism. Prevention and mitigation of the risk
associated with DEP requires incorporation and consistent
application of good agricultural practices and good
manufacturing practices throughout the tomato production
process, from crop to harvest to retailer. Proper tomato
handling and processing practices must be promoted and
implemented by both tomato growers and consumers. In the
home, much of the risk can be mitigated through proper
handling and food safety practices, such as thorough
washing and disinfection, prevention of contamination
during preparation, and proper storage and discarding of
leftover foods. Although the sample size analyzed here was
small, we did detect DEP on tomatoes, indicating that these
vegetables represent a potential health risk.

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