Research Note

Prevalence of Foodborne Pathogens in Grilled Chicken from Street Vendors and Retail Outlets in Reynosa, Tamaulipas, Mexico


1Departamento de Biología Molecular y Bioingeniería, Unidad Académica Multidisciplinaria Reynosa-Aztlan, Universidad Autónoma de Tamaulipas Reynosa, Tamaulipas, México; and 2Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo Léon, Monterrey, Nuevo León, México

ABSTRACT

We analyzed a total of 70 grilled chicken samples bought randomly from street vendors and retail outlets in the city of Reynosa, Mexico, to determine the prevalence of Escherichia coli (Shiga toxin producing and enterotoxin producing), Salmonella spp., Staphylococcus aureus, Listeria spp., and Campylobacter spp. using microbiological methods and PCR detection of bacterial sequences. Of the 70 samples, 27 (38.5%) were from retail outlets and 43 (61.4%) from street vendors. All specimens were negative by both microbiological and molecular methods for Listeria monocytogenes. Shiga toxin 2 of Shiga toxin–producing E. coli, Lt of enterotoxin-producing E. coli, and st enterotoxin, and all were negative for Salmonella spp. and Campylobacter jejuni by PCR. Of the samples studied, 49 (70%) had undetectable levels of the foodborne pathogens studied with the methods used. In the remaining 21 (30%) specimens, at least one pathogen was isolated or detected, with E. coli being the pathogen most frequently isolated and with two samples bearing the hlyA gene. We found no statistical difference in bacterial prevalence between retail and street vendor samples. The presence of pathogens in grilled chicken is an important public health risk because of the great demand for and daily consumption of this product in this region.

Foodborne diseases are an increasing public health problem worldwide. The World Health Organization has recorded every year the seven main foodborne pathogens, Campylobacter jejuni, Clostridium perfringens, Shiga toxin–producing Escherichia coli (STEC) (also referred to as enterohemorrhagic Escherichia coli), Listeria monocytogenes, Salmonella spp., Staphylococcus aureus, and Toxoplasma gondii, which cause up to 12.3 million infections in the United States. In 2007, 4,859 cases of foodborne diseases per 100,000 population were reported. Of these, 2,347 were outbreaks of diarrheal illness and food poisoning caused by Salmonella spp., E. coli, and S. aureus (7, 18, 19). Also, outbreaks of human listeriosis have been associated with contaminated food in several countries (5, 6, 8).

Campylobacter spp. and Salmonella spp. can contaminate food anywhere along the food production process (10), but undercooked poultry is the most important source of infection (3, 22, 26). Several studies have been carried out on raw food sold by street vendors and in retail outlets (13, 16, 17, 25), but there are few studies that include cooked foods, especially in Mexico (2). In our region and all over northeastern Mexico, grilled chicken, bought from both street vendors and retail outlets, is frequently consumed.

Surveillance of foodborne disease is a key element for planning, implementing, and evaluating public health policies to prevent these diseases (18). Based on this premise, the objective of this study was to determine the presence of Salmonella spp., S. aureus, Listeria monocytogenes, Campylobacter jejuni, STEC, and enterotoxin-producing E. coli (ETEC) in grilled chicken from both street vendors and retail outlets in the city of Reynosa, Mexico.

MATERIALS AND METHODS

Samples. Seventy grilled chicken samples were obtained from randomly selected street vendors and retail outlets during a 2-month period (July and August 2009) in the border city of Reynosa, Tamaulipas, Mexico. The average environmental temperature during that period was 39°C. The specimens were immediately transported on ice to the laboratory and processed within an hour.

Preenrichment. Twenty-five grams of each specimen was placed in a plastic bag with 200 ml of buffered peptone (Bioxon, BD, Cuatitlán-Izcalli, Mexico). Samples were shaken manually for 3 min, and 50 ml of this suspension was added to 50 ml of 2× Trypticase soy broth (Bioxon, BD) for E. coli, Salmonella spp., S. aureus, and Listeria or thioglycolate supplemented with 5% sheep blood broth for Campylobacter. The media were incubated for 24 h at 37°C.

Culture. For E. coli, the preenrichment step was followed by a 24-h, 37°C subculture in MacConkey and eosin-methylene blue agar plates (Bioxon, BD). Typical colonies were subcultured in 10 ml of Trypticase soy broth and incubated for 24 h at 37°C. For Salmonella spp., the preenrichment broth was a 24-h, 37°C

* Author for correspondence. Tel: +52 899 9213340; Fax: +52 899 9213340; E-mail: vbocanegr@yahoo.com.
subculture on selenite broth (Difco, BD Diagnostics, Le Pont de Clais, France) and on Salmonella-Shigella agar plates (Difco). For the isolation of *S. aureus*, ~10 μl of preenrichment culture was subcultured in Vogel-Johnson agar plates with 1% tellurite (Difco). For the isolation of *Listeria*, a similar procedure was performed with the use of Oxford agar plates (Difco). Presumptive colonies of *S. aureus*, *E. coli*, *Salmonella* spp., and *Listeria* spp. were identified by Gram staining and traditional biochemical tests. For *Campylobacter*, the preenrichment broth was inoculated onto Skirrow’s agar (Difco) and incubated for 48 h at 42 °C in an atmosphere of 10% CO₂. All preenrichments were used for both culture and molecular assays.

**PCR assays.** Specific primers for the partial amplification of conserved genes of *Salmonella* spp. (invA), *S. aureus* (nuc), *L. monocytogenes* (hemolysin gene), *C. jejuni* (peb1), STEC (Shiga toxin 1 and 2 genes), enterohemorrhagic *E. coli* (hlyA), and ETEC (*lt* and *st* enterotoxin genes) were used for molecular detection of foodborne pathogens by PCR. PCRs were performed using both previously published and newly designed primers (Table 1).

TABLE 1. Primers used for PCR and gene amplified or expected product

<table>
<thead>
<tr>
<th>Species/subtype</th>
<th>Gene or product</th>
<th>Primer (5’–3’)</th>
<th>Product size (bp)/primer alignment temp (°C)</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>hlyA</td>
<td>O157-3, GTAGGGAACGGAACAGAG</td>
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<tr>
<td></td>
<td></td>
<td>O157-4, AAGCTCGGTGCTGCTGAA</td>
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<tr>
<td><em>E. coli</em> (STEC)</td>
<td>Shiga toxin 1</td>
<td>Stx-1a, GAAGATCGTCGATCTAAGCTCATA</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Srx-1b, AGCTGACGCTAATTAATATAGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> (STEC)</td>
<td>Shiga toxin 2</td>
<td>Stx-2a, TTAAACACACCCCAACGGGCAGT</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Stx-2b, GCTCTGGATGACATCTGCTGTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> (ETEC)</td>
<td><em>lt</em> enterotoxin</td>
<td>EcoLT-1, GAGACCGGTATTACGAAACAC</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>EcoLT-2, GAGGTCATGTAATCCAGA</td>
<td></td>
<td></td>
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<tr>
<td><em>E. coli</em> (ETEC)</td>
<td><em>st</em> enterotoxin</td>
<td>St-1, ATTTTCCTCTGTTATTTGTC</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>St-2, CACCCTGACAGGAGGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td><em>invA</em></td>
<td>Sal-3, TATCCGCACTGCAGGAC</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Sal-4, TCCGACCGTCACAGGAAC</td>
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<td></td>
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<tr>
<td><em>S. aureus</em></td>
<td><em>nuc</em></td>
<td>SA-1, GCGATTGTAGGTTGATGCTGTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SA-2, CAAGCTGACTGAACTAACG</td>
<td></td>
<td></td>
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<tr>
<td>*L. monocytogenes</td>
<td>Hemolysin</td>
<td>LM-1, CGGAGGTCGCCGAAGATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LM-2, CCTCGAGATTGACAGTTT</td>
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<tr>
<td><em>C. jejuni</em></td>
<td><em>peb1</em></td>
<td>27F-5’, TCCGTTGCATGAATGGGCTG</td>
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<tr>
<td></td>
<td></td>
<td>27R-5’, AGTATCTGCTATAGCAACCGT</td>
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</tbody>
</table>

Both microbiological and molecular methods, and all were negative for *Salmonella* spp. and *C. jejuni* by PCR. Among the samples studied, 49 (70%) had undetectable levels of all foodborne pathogens studied with the methods used, and in the remaining 21 (30%) specimens, at least one pathogen was isolated or detected (Table 2).

Specimens positive for any of the pathogens evaluated were more frequent in retail outlets (12 of 27) than from street vendors (9 of 43) (*P* = 0.068). There was a higher frequency of *E. coli* in samples from retail outlets (10 of 27) than from street vendors (5 of 43) (*P* = 0.011), with two samples positive for *E. coli* bearing hlyA. In contrast, *S. aureus* was isolated in six samples, with four being from street vendors (*P* = 0.64). Furthermore, *Salmonella* organisms were isolated in two specimens from street vendors. None of the samples were positive for *L. monocytogenes*.

In four samples, two different bacterial species were detected from both a retail outlet (1, 3.7%) and street vendors (3, 6.97%) (Table 2).

**RESULTS**

All specimens were negative for *Listeria monocytogenes*, STEC Shiga toxin 2, ETEC *lt*, and *st* enterotoxin by
bacteria was present in three (6.97%) of the samples from street vendors and in one (3.7%) from retail outlets. Contamination with two or more types of bacteria was more common with street vendors because of exposure to dust and the environmental temperature, since sampling occurred during the summer months, when the temperature reaches 38 to 40°C (12).

\textit{E. coli} was detected in retail outlets in 10 (37%) of 27 samples and for street vendors in 5 (11.6%) of 43 samples, suggesting that contamination with bacteria that are not among the usual flora of raw chicken occurs because of manipulation of the finished product. Of the samples analyzed, two produced \textit{stx}_1, one from retail vendors and one from a street vendor; none had \textit{stx}_2. Also, two samples from retail vendors had \textit{hlyA}, which is a characteristic indicator of the O157:H7 strain.

Higher levels of prevalence of \textit{S. aureus} and \textit{Salmonella} were detected in grilled chicken from street vendors. The presence of these pathogens has been associated with inadequate manipulation of cooked food in the case of \textit{S. aureus} and to undercooked food in the case of \textit{Salmonella} spp. This may be the cause of our positive samples (26). Regarding these two pathogens, we found discrepancies in our results, since we detected \textit{Salmonella} only in culture but not by PCR, and two samples that were positive for \textit{S. aureus} in culture were negative by PCR, which could be attributed to the presence of PCR inhibitors in those particular samples.

The presence of \textit{Listeria} spp. is considered an indicator of contamination by \textit{L. monocytogenes} since it can colonize the same environmental setting (14). In our study, the presence of \textit{L. monocytogenes} was not detected by culture or PCR. In this study, we did not analyze raw chicken, so we cannot assume that the cooking temperature eliminates \textit{Listeria} spp. from grilled chicken.

The range of contamination with \textit{Campylobacter} spp. in raw products varies widely, with values as high as 100% (26) but most values lower than 60.9% (4). In our study, \textit{Campylobacter jejuni} was found in only 1 (3.7%) of 27 samples from retail vendors and none from street vendors. This is slightly greater than the prevalence found in another study of roasted chicken from Mexico that reported no bacterial contamination (2) but less than the prevalence reported by Quiñones-Ramírez et al. (27%), which was also a study of cooked chicken from Mexico (20). The sample that was positive for \textit{C. jejuni} was also positive for \textit{E. coli}. The lower prevalence of this pathogen in our samples supports the fact that cooking of the grilled chicken was nearly satisfactory.

Our study indicates that grilled chicken sold retail in Reynosa, Tamaulipas, Mexico, is frequently contaminated with ETEC, \textit{Salmonella} spp., \textit{S. aureus}, \textit{Listeria} spp., and \textit{Campylobacter} spp., with \textit{E. coli} predominating. Surprisingly, there was a higher prevalence of pathogens in samples from retail outlets than from street vendors. One would suspect that the lack of adequate installations together with environmental exposure of the product from street vendors would contribute to an opposite result.

Interestingly, an unexpectedly high prevalence of \textit{st} positive \textit{E. coli} was observed, although a low prevalence was reported in raw poultry products in other studies (3, 11). This indicates that bacterial contamination occurs because of manipulation of the finished product. The presence of the pathogens found in grilled chicken is an important public health risk. The implementation of sanitary measures oriented toward reducing bacterial contamination of meat products is essential.

### REFERENCES


