How We Analyzed Ground Meat for Harmful Bacteria

SAMPLING

A pre-retrieval assessment of grocery stores was conducted in metro market areas of the 10 standard federal regions, to determine product availability within each region.

Based on the pre-retrieval survey, a CR statistician assigned a three-digit code to each product and selected approximately 27-50 samples to be purchased for each region. Samples were purchased from 36 different stores. A total of 351 samples were purchased, which include 110 turkey, 75 chicken, 119 beef, and 47 pork.

For each meat type, major brands and private labels were purchased, including store brands and products wrapped in clear film. We included 52 ground beef brands, 20 ground chicken brands, 26 ground pork brands, and 24 ground turkey brands.

Shopping took place over a 4-week period, from December 2021 to January 2022 and testing took place in January and February 2022.

At each retail location, samples were purchased in their primary packaging and immediately placed in a cooler with ice packs. They were transported to the sampling laboratory and stored refrigerated until they were ready to ship to the testing laboratory. All samples were labeled with their corresponding 3-digit code along with other sample attributes used to track the product throughout the project.

All samples were shipped to a single analytical laboratory overnight with ice-packs. Upon receipt, the temperature of all samples was noted, and verified to be within the acceptable temperature range. The label and other information in each sample was cross-checked against the spreadsheet provided by CR, and further documented by taking a photograph of each sample label.

MICROBIAL PATHOGEN ASSESSMENT AND CONFIRMATION

The ground meat samples (beef, turkey, chicken, and pork) were weighed and divided into appropriate portions depending on the microbiological analysis procedure. Generic *E. coli* testing was performed using 50 grams of each sample (Microfilm TCEc). Microfilms were incubated at 35°C for 24 +/- 2 hours. Only colonies with dark blue to violet color in the microfilm were counted and reported.

For ground beef and ground pork samples, 325 grams were processed and analyzed for the presence of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* using a method based on the USDA/FSIS Microbiology Laboratory Guidebook, Chapters 4 and 5, and AOAC-RI PTM 100701, *E. coli* O157, Stx-producing *E. coli* (STEC) with Intimin and *Salmonella* Test System. All meat portions were enriched with 975 ml of pre-warmed mTSB and incubated at 42°C +/- 2°C for 15 hours. All presumptive positive samples were confirmed using the laboratory's internal methodology and MLG 4.11 methods, respectively.

For ground turkey and ground chicken, (325 grams were processed and analyzed for *Salmonella* and *Campylobacter.* Meat portions were enriched with 1,950 mL of BPW (1:6 sample: media volume ratio). After homogenizing, 30 ml of the medium was transferred to another sterile bag containing 30 ml of 2X BF-BEB media and incubated at 42°C for 48 hours for enriching *Campylobacter* spp. (MLG 41.07, Appendix 2.04). The remaining enrichment was incubated at 35°C +/- 2°C for 20-22 hours for *Salmonella* analysis. After the specified incubation time, each enrichment was tested for the presence of *Salmonella* and *Campylobacter* using a multiplex PCR based method. Cultural confirmation was performed following a method based on USDA/FSIS method(MLG 4.07 (*Salmonella*) and MLG41.07 (*Campylobacter*).

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All confirmed isolates were re-streaked on the appropriate culture medium to confirm purity, and the genome was sequenced using Whole Genome Sequencing (WGS) and analyzed for antibiotic resistance genes.

WHOLE GENOME SEQUENCING & ANTIBIOTIC RESISTANCE ANALYSIS

The genomic library of each confirmed isolate was prepared using the Nextera XT DNA sample preparation kit (Illumina, CA). WGS was performed using the MiSeq desktop sequencer (Illumina) loaded with a paired-end 2 × 250 cycle MiSeq reagent kit version 3. The raw shotgun reads were then assembled using the SPAdes assembler (https://cab.spbu.ru/software/spades/). From the whole genome assembly, the presence or absence of various antibiotic resistance genes was determined using SRST2 software. (https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-014-0090-6).