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Metagenomic Description of Preenrichment and Postenrichment of Recalled Chapati Atta Flour Using a Shotgun Sequencing Approach

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ABSTRACT The bacterial microbiome of flour recalled for possible *Escherichia coli* O121 contamination was characterized before (hour 0) and after (hour 24) enrichment using shotgun sequencing. At hour 0, *Staphylococcus* (46.8 to 66.5%) and *Pantoea* (12.6 to 21.0%) bacteria were dominant. At hour 24, *Enterobacter* (28.7 to 70.9%) and *Klebsiella* (25.6 to 68.6%) bacteria dominated, and *Escherichia coli* ranged from 0.3 to 17.9%.

In 2016, wheat flour contaminated with *Escherichia coli* O121:H19 and O26:H11 was linked to an outbreak of 63 illnesses, 17 hospitalizations, and 1 instance of hemolytic-uremic syndrome (1, 2). In 2017, two additional flour-related outbreaks of *E. coli* O121 were linked to a total of 39 illnesses across 7 provinces in Canada (3, 4).

An integral component of source tracking for foodborne pathogens relies on the recovery of pathogens by enrichment (growing the actual cells). Typically, this process can take up to 5 days to arrive at an individual isolate. One useful approach to expediting source attribution has been the use of shotgun sequencing of enrichment media during the enrichment process. Here, we describe hour 0 (pre) and hour 24 (post) enrichment for *E. coli* recovery protocols applied to the samples from the same lots of flour that were recalled for potential contamination. Overnight enrichment in modified buffered peptone water with pyruvate (mBPWp) (with an acriflavin-cefsulodin-vancomycin supplement to kill fungal species) is the first step in the recovery of *Stx*-producing *E. coli* (STEC) and O157:H7 from a variety of food matrices (5). The efficiency of this medium for recovering non-O157 STEC from flour is poorly understood due to the infrequent occurrence of *E. coli* flour contamination.

A 20-lb bag of chapati atta flour, which was included in a voluntary recall for possible *E. coli* O121 contamination, was purchased in Maryland. Seven subsamples of 25 g each from the recalled flour were placed in Whirl-pak bags and enriched following the protocol of the FDA's Bacteriological Analytical Manual (5). A volume of 225 ml mBPWp broth was added to each bag, and bags were incubated at 37°C for 5 h. An acriflavin-cefsulodin-vancomycin supplement was then added, and the samples were incubated at 42°C overnight. A volume of 1 ml was retrieved from each bag before and after incubation. Two control subsamples of the flour (25 g each) were placed in Whirl-pak bags with 225 ml Butterfield's phosphate buffer, and 1 ml was retrieved from each bag, which were not incubated. All 1-ml aliquots were stored at −80°C prior to DNA extraction via the Qiagen blood and tissue kit. All DNA samples were stored at −80°C until sequenced. Libraries were prepared using the TruSeq Nano DNA HT kit (Illumina) and shotgun sequenced using the NextSeq 500 system.

Samples enriched using mBPWp broth were dominated by bacteria belonging to the genera *Enterobacter* (28.7 to 70.9%) and *Klebsiella* (25.6 to 68.6%). Unenriched samples in the mBPWp and Butterfield's broths were composed of bacteria belonging

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to the genera *Staphylococcus* (46.8 to 66.5%), *Pseudomonas* (6.7 to 20.9%), and *Pantoea* (12.6 to 21.0%). Generic *E. coli* was observed in all postenrichment samples (0.3 to 17.94%) but not in any of the preenrichment samples.

Traditionally, foodborne illnesses caused by *E. coli* O157:H7 and other STEC strains have been linked to the consumption of ground beef, leafy greens, sprouts, and juice (6). However, recent outbreaks have involved novel low-moisture vehicles (e.g., flour). This work provides some of the first metagenomic profiling of enriched and unenriched flour microbiota. Subsequent work will focus on improved strain-level characterization of *E. coli* identified in the flour matrix in an effort to improve recovery precision and source attribution for outbreaks involving *E. coli* O157:H7 and non-O157 STEC strains in atypical food matrices.

Accession number(s). All data have been deposited in NCBI GenBank under Sequence Read Archive numbers SRR6426147 through SRR6426162 (BioProject number PRJNA417782).

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