Phase II study of the safety of ready-to-eat foods served in privately owned delicatessens: a confirmatory examination

Abstract

The U.S. Center for Disease Control and Prevention, 2009 reported that each year 76 million cases of foodborne illness occur with over 300,000 people hospitalized and 5,000 deaths. This study identifies key areas for food safety improvement. This study is Phase II of a continuing effort to assess food safety and handling practices of ready-to-eat foods and is designed as a confirmatory evaluation of the Phase I research examining independently owned and operated delicatessen operations, using Escherichia coli, Staphylococcus aureus, and coliforms as indicators to assess food handling and the public’s risk for pathogenic contamination from commonly served ready-to-eat foods. The analysis consisted of a comprehensive strategy of laboratory testing of samples for pathogenic contamination, informal field observation of food handling procedures, and the examination of the most recent health inspection reports for each of the newly selected (n=18) operations visited. The deli turkey, cream cheese and lettuce were tested (matching the original study) using bacteria indicator plates. The results showed widespread levels of contamination. Of the 54 samples tested for: Escherichia coli, 11 showed positive results which are ~20% for E. coli contamination, Staphylococcus aureus, 41 showed positive results which are ~76% for S. aureus contamination, coliforms, 44 showed positive results which are ~82% for coliform contamination. The informal field observations and health report analyses revealed widespread temperature and facility violations and numerous instances of poor food handling. The study offers independent practitioners a strategy designed to improve their health inspections scores, food handling, and mitigation of operator liability. Independent operators traditionally do not enjoy the resources of centralized supervision and expert on-staff training; yet in aggregate they account for significant volume in both dollars and the quantity of product served. This study confirms the results found in an earlier study involving independently owned deli operations and provides important insights into this food niche.

Keywords: food safety, food handling, E. coli, S aureus, Coliforms, public health, ready-to-eat foods, independent delis

Overview of the issue

Traditionally, food safety research, both in the retail and foodservice sectors, has been geared toward chain operations. Studying one company with multiple outlets is efficient and the ability to compare similar like operations sharing a focused concept is appealing. Additionally, it seems intuitive that studying the largest organizations by virtue of their volume and geographical reach would be the most revealing and contribute the largest possible positive impact on food safety. This view is problematic on many levels. A meta-analysis of foodborne illness outbreaks conducted between 1993-1997 conducted by the Center for Disease Control indicated that the highest percentage of outbreaks were associated with independent restaurants, delicatessens, and cafeterias. Other studies have found that large chain operations have distinct food safety advantages in economies of scale, resources, training, and equipment. Phillips et al., further developed this reasoning as they postulated that large chain operators are considered the industry leaders, with corresponding high visibility and increased exposure and liability. Their findings revealed that these large companies have routinely used their financial resources to institute standardized practices and formalize food safety policies and procedures both as a means to protect their public reputation and as the basis for a legal defense strategy. It has been found that by adopting Hazard Analysis and Critical Control Points (HACCP) concepts, chains have developed a critical violation emphasis in their food safety approach as the most viable method of ensuring food safety, or at least minimizing foodborne illness outbreaks. While the numbers of health inspection violations between chain and independent food services have been shown to be similar, the critical violations that can most adversely affect food safety have been shown to be much lower in chain operations. Chain operations have been found to have a statistically significant lower incidence of critical food handling violations, most likely due to the emphasis and resources allocated to controlling critical violations. Conversely, independent operators have been shown to incur higher numbers of critical violations.

Recent research has focused on independent delicatessens and restaurant operations serving ready-to-eat foods and explores the underlying issues of biofilm contamination, facility, equipment, and food handling defects. These studies have shown repeated health code violations most critically time and temperature, the high likelihood of biofilm cross contamination via contact with glass, stainless steel, and most critically with lettuce and the extremely low compliance rate for hand washing guidelines of 7% and 2%.

In addition to formal HACCP systems the other significant advantages enjoyed by the chain operators is their ability to staff locations with trained/certified kitchen managers and fund and...
maintain facilities. Independent food services such as delicatessens do not possess corporate headquarters with highly trained and educated corporate management, consultants, or the ability to recruit hospitality and/or culinary experts. Smaller, independent operators often have little in the way of industry experience or education. In small businesses just maintaining the normally long operating hours and keeping the business functioning can exhaust all of ownerships energy and resources.

Foodborne illness is a significant contributor to human morbidity, mortality and the cost of health care. Ready-to-eat foods can be especially high-risk since they are handled, served, and consumed without the application of antimicrobial processes such as heating prior to consumption. There are many food quality and safety indicator tests such as total plate count, coliforms, fecal coliforms, E. coli, Staphylococcus aureus, Listeria monocytogenes etc. that can be used to test the efficacy of operational food handling. In this study several indicators are used: Coliforms are a gram-negative bacteria commonly cultured by Health departments and regulatory agencies and while not necessarily toxic themselves are commonly used as an indicator of other pathogenic and fecal contamination, E. coli is a normal flora in the lower intestinal tract in humans and other warm-blooded animals and is abundant in human and animal feces. The presence of E. coli is a strong indication of poor quality food, food handling, and hygienic practices. In addition, it can be easily detected by its ability to ferment sugar, Staphylococcus aureus, another key indicator grows in a wide range of temperatures (7 to 48°C), and has low pH, high salt and sugar content (up to 15%) it produces heat stable enterotoxins and is a very common cause of food-borne illness. It is a normal flora in the nose, throat in human and animals. It can cause infection in cuts and other wounds and is easily passed indirectly from food handlers to contaminate ready-to-eat foods, resulting in the high incidence of foodborne illness in the U.S. The sources of food contamination are most often from improper handling of food such as contaminated hand contact defined as improper or infrequent hand washing and glove use/changing, or through airborne pathways such as, coughs, or sneezing. The two indicators used in this study can provide insight on the quality of the food served and the hygiene of food handlers in independent deli operations.

The study involved 18 independently owned delicatessens, which were not part of the original study. Samples were collected and then tested in the lab for pathogenic contamination. The researchers hypothesized that the levels of contamination and the underlying reasons identified would be similar and serve to confirm the results of the original study.

**Literature review**

Among the foods commonly served in delicatessens, Escherichia coli and Staphylococcus aureus, bacteria have been particularly identified and isolated in purchased cooked and processed turkey, lettuce, and cream cheese products. Staphylococcus aureus is a gram-positive bacterium that is ubiquitous on human skin and is found in large amounts in animal and human fecal waste. Some strains of Staphylococcus aureus are capable of producing toxins in food. Escherichia coli is a gram-negative bacterium commonly present in the intestines and fecal waste of both animals and humans. In all cases, the organisms once ingested could produce toxins that are the actual cause of the foodborne illness symptoms that develop from the infection. Typical symptoms are nausea and vomiting with occasional abdominal cramping and diarrhea. Deaths and renal failure, though rare, have occurred amongst people with compromised immune systems such as, the chronically ill, children and the elderly. One serious concern for scientists is that laboratory testing has indicated that S. Aureus is capable of mutating and becoming resistant to antibiotics.

Though studies have indicated that environmental conditions such as irrigation, soil, and farm practices play a large role in food pathogen infection, most of the literature reviewed show clearly that proper food handling and personal hygiene are the critical improvement areas to protect the public. Fecal contamination of food from human handling, cross contamination, time and temperature procedure failures have been shown to be the major sources of pathogenic infection. The FDA has definitively identified fecal coliforms as an indicator of contamination in food. Other studies have specifically linked poor time and temperature processes and controls to food borne illness outbreaks with one study examining temperature and pathogen infection specifically to soft cheese holding and handling. Certainly if proper temperature control of raw and ready to eat foods cannot be maintained, the opportunity for explosive bacterial growth is present. Several studies conducted internationally provide definitive evidence that insufficient decontamination actions are a primary cause of pathogenic contamination. Food handling and personal hygiene research has shown the most common factors in pathogenic contamination for food service operations to be: improperly cleaned or not cleaned knives, faucets, serving utensils, and cutting boards; cross-contamination between use with different products; and bare-handed food contact. Even when gloves are worn they are often unchanged between food and customer contact with the result that the gloves themselves are found over time to be the cause of pathogen transfer. As noted in a study published in the Nursing Standard the simplest way to prevent the spread of disease from these coliforms and salmonella is frequent and thorough hand washing and glove changing.

Seemingly then many cases of food borne disease could be easily prevented through greater emphasis on cleaning: hands, cutting boards, utensils, and any food contact surface; through utilizing gloves and changing them after each new task is completed; and the proper food storage and maintenance of refrigeration temperatures low enough to retard bacterial growth. One problem with the current system of public health policy is the overreliance on inspection and enforcement. The research notes that placing the emphasis on inspection and enforcement can foster a climate of resistance and fear, where the Health Inspector is an outside authority whose function is to catch violators and not an expert resource available to educate and inform.

**Method**

**Sample collections, storage**

The study duplicated the previous study where the food samples were collected from 18 newly selected delicatessens serving prepared and ready-to-eat foods for takeout located in northern New Jersey, USA. Locations of the 18 delicatessens were conveniently picked with the proviso that they were not previously studied, were independently operated, not part of a larger market, store, restaurant or chain, in a close proximity to Montclair State University (less than 30minutes travel to the laboratory) and current health department inspection were on file and available to the investigators.

As indicated in the review of literature, turkey, lettuce, and cream cheese were selected as these foods are common to all the delis under study, are typically used daily, and are handled repeatedly by
multiple workers. The delicatessens were not informed that their food was being tested after purchase. Prior to data collection the field researchers were trained in sample collection procedures. The instructions included: observational keys and note taking, and rigorous sample collection and processing procedures. The observations were restricted to the customer/counter view as the anonymity of the researchers and the project precluded direct access to back of house operations. For each deli, a quarter pound of turkey, side of lettuce, and side of cream cheese (>100g) was purchased individually wrapped either in a bag or a plastic container and then these samples were stored in sealed sterile plastic bags. Temperatures of the samples were taken immediately after purchase using alcohol wipes to sanitize the digital thermometer probes. All of the samples were put on ice to prevent any growth of microorganisms during transport. Upon arrival, sample temperatures and time were again recorded. The samples were processed immediately upon arrival at the lab.

Microorganisms testing

One gram was weighed out from each sample and 9ml of sterile diluents; Butterfield’s phosphate buffer or distilled water was added to homogenize the food for 5 minutes. pH of the samples was measured and titrated with either 1 N NaOH or 1 N HCl to pH between 6.6 and 7.2. Place 3MTMPetrifilm™ E. coli/colliform count plates or 3MTMPetrifilm™ Staphylococcus aureus express count plates on a level surface. On each plate the top film was lifted and 1mL of blended sample was placed onto the center of the bottom film of each plate. With the flat side down, a spreader was placed on the top film over the inoculums and gentle pressure was applied to the spreader to distribute the inoculums over the circular area of the test plate before a gel is formed. Care was taken to not twist or slide the spreader. The sample was allowed to sit and wait a minimum of one minute for the gel to solidify. The plates were placed in the incubator at 37°C with the clear side up in stacks of up to 20. S. aureus plates were allowed to incubate for 24 hours and then colonies were counted. The turkey E. coli plates were allowed to incubate for 24 hours, the lettuce and cream cheese were incubated for 48 hours then the colonies were counted. In order to distinguish and confirm positive vs. negative results the lettuce and cream cheese was seen to be the dark blue colonies, which were easy to count, by human eye. These plates consist of a polyester film, polystyrene foam, transfer adhesive, polypropylene film, guar gum, nutrients, hinge tape, lactose, and pancreatic digest of gelatin. The staph express plates only detected S. aureus. They were incubated for 24 hours at 35°C. The colonies show up as a red-violet color. The ingredients include nutrient media coated on paper with foam retaining dam and film cover sheet.

For each test, positive controls were used. All equipment used was sanitized after each use to make sure there was no cross contamination during the testing.

DNA extraction for PCR-based assay

DNA extraction was performed to test for total coliform using colonies from 3M™ Petrifilms. Colonies were collected from 3M™ Petrifilms and were grown in 3mL of MH media individually overnight at 37°C and constant agitation of 6Xg (Innova 4300, New Brunswick Scientific). 1mL of the sample was pipetted into a 1.5mL of Eppendorf tube aseptically and centrifuged at 9,279Xg for 3 minutes. Resulting pallets were re-suspended into 100µl of distilled water. Samples were boiled on a heat block (Thermolyne Dri-bath- Sybron) at 100°C for 20 minutes and were allowed to cool for 2 minutes. Samples were centrifuged at 23,755Xg for 5 minutes. 5µl of supernatant was used for PCR reaction. A NanoDrop ND-1000 V 3.7 spectrophotometer was used to determine the presence of the DNA prior to performing PCR, by reading at 260nm/280nm absorbance ratio and the concentration (ng/µl) of DNA extraction product. Specific primers were used to target different organisms using PCR based assay. The target organisms, expected amplicon size, and melting temperature (°C) of each primer are shown in Table 1. The reaction mix with primers, DNA and Hot-Start TaqMastermix were prepared and run in an Applied BiosystemsVeriti 96 Well Thermocycler (Life Technologies). All PCR products were visualized through 1% agarose gel electrophoresis with a Hi-Lo DNA Ladder provided by Bionexus, CA. as a marker. In this study PCR based assay was conducted to confirm and support the results obtained using the 3M™ Petrifilms (Table 1).

Table I PCR primers

<table>
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<tr>
<th>Primers</th>
<th>Location</th>
<th>Amplicon size</th>
<th>Reference</th>
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<tr>
<td>Total Coliform</td>
<td>Forward-5'ATGAAGCTGGCTACAGGAAGGCC3'</td>
<td>Lac Z gene of E. coli</td>
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<td></td>
<td>Reverse-5'GGTGTATGCAAGCAAAGAGCGTA3'</td>
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<td>E. coli</td>
<td>Forward-5'AGAGTTGTTGATCGTGGCGTA3'</td>
<td>16S rRNA</td>
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<td></td>
<td>Reverse-5'GGTACCGGTTACGACTT3'</td>
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</table>

Results

The total plate counts of coliform, *Staphylococcus aureus* and *Escherichia coli* from the 18 different delicatessens are shown in Table 2. Coliform was tested to serve as an overall indicator of bacterial contamination and was found in 44 of the 54 samples tested (~82%). The presence of coliforms is a strong indication that inadequate personal hygiene practices are not in place.

Product test results

Microorganism contamination tests show the results from the testing of food samples for the presence of pathogens (Table 2A). The results revealed through testing the turkey sample show: the presence of *E. coli* in 5 of the 18 turkey samples (~28%), *S. aureus* was found in 15 of the 18 samples (~83%), and coliform in 16 of the 18 samples (~89%). The cream cheese samples show: 4 of the 18 samples contained *E. coli* (~22 %), 12 of the 18 samples contained *S. aureus* (~66%), and 15 of the 18 samples revealed coliform (~83%). The lettuce samples show: *E. coli* present in 7 of the 18 samples (~39%), *S. aureus* was present in 14 of the 18 samples (~77%), and coliform was present in 12 of the 18 samples (~72%)

Overall pathogen results

The CFU (Colony Counting Unit) per gram for *E. coli* under 3 is Satisfactory, between 3 to 102 is Marginal and above 102 is Unsatisfactory and Pathogenic strain of *E. coli* should be absent under the category of Potential Hazardous. The summary of the total plate count for *Escherichia coli* is shown in Table 2A. Of the 54 samples tested for *E. coli*, 16 were found to have contamination (~32%). For the turkey sample location 9 with 18-cells/gram and location 18 with 32- cells/gram were marginal. For the cream cheese sample location 3 with 9-cells/gram was marginal and location 9 with 187-cells/gram was unsatisfactory. For the lettuce sample 6 locations recorded marginal results: location 1 with 4-cells/gram, location 2 with 12-cells/gram, location 9 with 9-cells/gram, location 15 with 23-cells/gram, location 16 with 4-cells/gram, and location 17 with 97-cells/gram. Location 14 with 367-cells/gram was unsatisfactory. Locations 4, 5, 6, 7, 8, 12, and 13 had no *Escherichia coli* in any of the samples, and suggested that their food handling practices were effective.

There are 15 out of 18 turkey samples, 12 of 18 cream cheese samples, and 14 out of 18 lettuce samples that tested positive for *Staphylococcus aureus*. Of the 54 samples tested for *Staphylococcus aureus*, 41 were found to have contamination, which is ~76% of the whole. Contaminations of the turkey, cream cheese, and lettuce samples were ~83%, ~77%, ~66% respectively. The CFU (Colon Counting Unit) per gram taken from the FDA Microbiological Quality CFU/gram for *S. aureus* is: under 102 is Satisfactory, range from 102 to 103 is Marginal and from 103 to 104 is Unsatisfactory and above 104 is Hazardous. Location 2 with 462-cells/gram of food, location 14 with 141-cells/gram, and location 16 with 65-cells/gram reported results that are classified as marginal for the turkey samples. The cream cheese samples all fell in the satisfactory range. Location 2 with 354-cells/gram, location 6 with 211-cells/gram, and location 16 with 200-cells/gram reported results classified as marginal for the lettuce sample. The cream cheese contamination had the lowest contamination level with all the samples falling in the satisfactory classification. Location 5, alone had no *Staphylococcus aureus* in any of the samples, and suggested that their food quality and handling practices were effective (Table 2A).

In order to confirm the results from the positive tests results from 3M™ Petrifilms method, PCR based assay was carried out. The specific primers were used to identify the source of contamination using PCR based-assay. The positive result in the 3M plates also indicated positive result in PCR based hence confirmed accuracy of 3M™ Petrifilms method and the consistency of the attained results.

Health reports and informal field observations

None of the 18 delicatessens studied had a health department report without violations, ~100% (Table 2B). The significant findings showed that 14 of the delis studied had been cited for improper cold holding temperature (~78%). Eleven of the reports cited the locations for the sanitation violations of cross contamination of stored foods and unsanitary food contact surfaces ~61%. Only location 17 received no food handling/temperature violations (physical plant violation) (Table 2B).

As the samples were collected there was the opportunity to observe the food handling behavior and processes in the field. In 16 of the 18 locations observed there were food-handling issues such as hand to hair and/or mouth to food contact and barehanded food contact ~89%. In operations where gloves were worn the sample collectors noted that some employees only gloved one hand (n=5) and the gloved hands were never washed or the gloves changed as the employee moved from food handling to cash handling to customer contact (n=8). Cutting boards were either not used, deeply scarred, or discolored (n=3). The observers reported poor food handling resulting in cross contamination between food products, boards, and utensils (n=6) and in two instances the slicer used for the turkey samples had visible debris and in one case the turkey was left out of the refrigerator at room temperature in-between use.

In 17 of the 18 delis studied, the food sample temperatures, taken immediately upon purchase by the investigator, were found to be well above acceptable temperature ranges for the safe storage of refrigerated items ~94%. These findings coincided with temperature-abuse and food handling violations on most of recent health inspection report obtained for each operation studied.

In the course of the two studies ready-to-eat foods from 36 different, privately owned delis were examined. The levels of contamination found were similar. In both cases only 1 in 18 delis were major violation free from their most recent health inspection reports on file. In the Phase 1 study 15 of the 18 samples collected were out of compliance for cold food holding: while in Phase 11 14 of the 18 samples were in violation (~78%). The levels of significant pathogenic contamination (*E. coli* & *S. aureus*) were similar as well (Table 3). The Phase I results showed a ~45% level of *E. coli* contamination versus ~43% in Phase II. Phase I *S. aureus* contamination levels were ~57% versus the Phase II results ~78% (Table 3).

<table>
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<tr>
<th>Site</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>Coliform</th>
<th>Temp (°F)</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>Coliform</th>
<th>Temp (°F)</th>
<th>E. coli</th>
<th>S. aureus</th>
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Table continued...

Table 2B Student observations (Spring 2013)

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<th>Site</th>
<th>Inadequate hand washing</th>
<th>No gloves</th>
<th>Improper single glove use</th>
<th>Inadequate glove changes</th>
<th>Inadequate holding of cold foods*</th>
<th>Cross contamination</th>
<th>Unsanitary food prep surfaces</th>
<th>Unclean establishment</th>
<th>Total violations per deli (8 total)</th>
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</table>

Total delis with violations (18 total) 39% (7) 33% (6) 28% (5) 67% (12) 56% (10) 33% (6) 28% (5) 17% (3)

*Measured temperatures indicate that there was inadequate holding of cold foods at all delicatessens, however improper holding procedures were only observed at the indicated establishments

Discussion

As can be seen from these triangulated results it is obvious that the foods taken from the independent food service operations in this study are more likely to be contaminated than not. Most fecal coliform ingestion produces mild symptoms that often go unreported, thus unrecorded. Therefore, it is possible that conditions noted in this study similarly resulted in unreported illness. As the different pathogenic strains transform and become increasingly resistant, strategies to promote and improve food handling and small operator food safety education is critical.

The fact that 14 of 18 delis had improper cold-holding temperatures and only 1 of the 18 was major violation free in their health inspection reports, results exactly matching the Phase I study, strongly indicates a serious threat to consumers and a failure in public health policy. It seems that some chain operators have used their resources to develop more effective strategies to protect themselves and their customers from food borne disease. Public health departments and policy makers must address, at minimum, the disparity of potential food safety risk between large chains and independent operators. One way would be by following the path already identified by the larger operations to increase food safety, namely managing critical control points. Increasing training and education over inspection and enforcement would be a significant first step in leveling the field. The current system of inspection/violation/citation creates an adversarial relationship that can often accrue between inspectors and operators. Shifting the relationship from inspection to education might give the independent operator an ability to duplicate the chain’s results in inspection scores. While independent foodservice managers in New Jersey and other states are already required to have a food handlers training certification, more advanced level training for all independent food service employees with an emphasis on critical violations seems to be the obvious direction since this strategy of critical violation control and training and certification has proved so effective for chain operators. Temperature control and personal hygiene issues, while the culprits in this study, seem to be the type of problems that are most easily solved and there should be no excuse for not attacking this issue.

Conclusion

The confirmatory study results shows a striking similarity to the earlier study and clearly indicates the poor food handling and lack of food safety knowledge and awareness prevalent in these independently owned delicatessens serving ready-to-eat foods. The study additionally provides us much important information and a solid direction for future research. To further gauge the inherent risk future studies might include more tests for potential pathogen detection such as Salmonella and Listeria etc. Future research should concentrate on reducing the limitations inherent in this investigation. A larger number of operations studied would improve the generalizability of the study results. A larger sample would also allow statistical analysis of differences that might be identified by such mediating factors as dollar volume, number of employees, form of ownership/management, and geographical location. If policy changes were attempted, adding a longitudinal study would allow for the tracking of results gained from the policy adjustments.

Future research designed to develop consistent national and international standards would be of great benefit. Not only for the clarification of tolerance levels regardless of jurisdiction, but to gain a common understanding that would allow researchers everywhere to be using the same metrics in evaluating the safety of ready-to-eat foods across borders.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

References


