Video Article

Tracking Microbial Contamination in Retail Environments Using Fluorescent Powder - A Retail Delicatessen Environment Example

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Abstract

Cross contamination of foodborne pathogens in the retail environment is a significant public health issue contributing to an increased risk for foodborne illness. Ready-to-eat (RTE) processed foods such as deli meats, cheese, and in some cases fresh produce, have been involved in foodborne disease outbreaks due to contamination with pathogens such as *Listeria monocytogenes*. With respect to *L. monocytogenes*, deli slicers are often the main source of cross contamination. The goal of this study was to use a fluorescent compound to simulate bacterial contamination and track this contamination in a retail setting. A mock deli kitchen was designed to simulate the retail environment. Deli meat was inoculated with the fluorescent compound and volunteers were recruited to complete a set of tasks similar to those expected of a food retail employee. The volunteers were instructed to slice, package, and store the meat in a deli refrigerator. The potential cross contamination was tracked in the mock retail environment by swabbing specific areas and measuring the optical density of the swabbed area with a spectrophotometer. The results indicated that the refrigerator (*i.e.* deli case) grip and various areas on the slicer had the highest risk for cross contamination. The results of this study may be used to develop more focused training material for retail employees. In addition, similar methodologies could also be used to track microbial contamination in food production environments (*e.g.* small farms), hospitals, nursing homes, cruise ships, and hotels.

Video Link

The video component of this article can be found at http://www.jove.com/video/51402/

Introduction

Cross contamination of foodborne pathogens in the food preparation environment, especially in the retail section, is a major concern due to an increased risk of foodborne illness from a variety of sources including the meats as well as the vegetables[1-5]. Most often, bacterial pathogens enter the retail environment through contaminated food products[6]. Ready-to-eat foods prepared at the retail level are of particular concern as there is usually no further intervention or treatment (*i.e.* cooking or heating) prior to consumption[7]. Moreover, pathogens present on contaminated RTE food can then be transferred to other food products or food contact surfaces in the retail environment.

While cross contamination can occur within virtually any type of retail food environment, delis are of particular interest specifically because of the association of RTE deli meats with the pathogen *Listeria monocytogenes*[2]. Based on a risk assessment analyses conducted by the U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), contaminated deli meats are likely responsible for 90% of the cases of listeriosis - a rare yet severe human disease caused by *L. monocytogenes* - in the United States[8]. In general, deli meats sliced at retail and food service establishments have been associated with a higher risk of *L. monocytogenes* compared to presliced deli meats in a USDA inspected plant by the manufacturer[9]. This could be because of the increased likelihood of introduction of pathogens by food handlers, incoming raw ingredients, or processed foods that may become contaminated post-treatment[7]. Overall, listeriosis accounts for approximately 28% of the estimated deaths caused by foodborne illness per year in the U.S.

Because of the increased risk of *L. monocytogenes* contamination in deli meats sliced at the retail level, the deli slicer itself has been identified as an important factor for surface transfer of foodborne pathogens in the retail environment[2,10]. As indicated by Sheen[1], slicing is the last processing step prior to consumption; therefore, the deli slicer should be considered a critical control point for both understanding and preventing cross contamination[1].

The overall goal of this study was to develop a methodology to track contamination in the retail deli environment using a fluorescent powder such as Glo Germ. This is a fluorescent compound that has been used to simulate bacterial cross contamination and sanitary practices[11,12]. This study was designed to track pathogen transfer from contaminated meats to the slicer, the surrounding environment, and other deli meats. It is crucial to understand the pathways of contamination to develop both effective interventions and educational material for retail employees and managers. A similar approach may be employed to simulate pathogen transfer in other processing environments (*e.g.* poultry and beef), farms, and hospitals.

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Protocol

1. Standard Curve

1. Weigh 37.5 mg fluorescent powder and dissolve in 6 ml 200 proof ethanol. Make 2-fold serial dilutions of this solution in 200 proof ethanol.
2. Vortex all the samples for 10 sec and measure the absorbance at 370 nm.
3. Calculate the amount of fluorescent powder per 1 ml 200 proof ethanol.
4. Prepare the standard curve (concentration in mg/ml on the y-axis and absorbance at 370 nm on the x-axis) to demonstrate the absorbance based on fluorescent powder concentration.

2. Inoculate Deli Meat (Bologna) with Fluorescent Powder

1. Cut the deli meat into approximately 100 mm thick samples using a clean knife and cutting board. Prepare three samples labeled A, B, and C.
2. Coat sample A evenly with fluorescent powder using a slightly damp, clean sponge. Spread the powder on the meat sample evenly. Do not inoculate samples B and C with the fluorescent powder.
3. Wrap the samples with plastic wrap and label the fluorescent powder coated sample as 'A' and remaining two samples as B and C with tape.
4. Place the samples in the deli case or refrigerator held at 4 °C in the mock kitchen where the experiment will be carried out.

3. Mock Deli Kitchen Preparation

1. Provide clean gloves and 70% ethanol spray in the kitchen.
2. Mount black compact fluorescent lights with 13 W bulbs around the slicer area.
3. Prepare a 5 cm x 5 cm cutout of aluminum foil to serve as a template for swabbing.
4. Prepare 15 ml tubes containing 6 ml 95% ethanol.

4. Video Setup

Mount three video cameras in strategic locations to observe all areas of the mock deli at the same time. Turn on the cameras before simulating the slicer procedure (as explained in Protocol 5) and analyze the results simultaneously (as explained in Protocol 7).

5. Simulating Deli Slicing Procedures to Track Cross Contamination

In order to improve sample size and incorporate variation, perform this study by recruiting participants after approval of protocol by the Institutional Review Board of the respective institution. The participant can be a college student who may or may not have experience working in retail deli environments. Ensure that each participant wears gloves once they enter the deli. Take a "before" picture under black fluorescent light followed by an "after" picture upon completion of study. Provide clear written and verbal instructions to each participant as follows:

1. Go to refrigerator.
2. Remove meat labeled "A".
3. Unwrap meat and save plastic wrap.
4. Place meat on carriage tray of slicer.
5. Secure meat with meat grip.
6. Turn power switch ON.
7. Adjust slicer index knob to "2".
8. Slice and dispense 5 pieces of meat onto deli paper.
9. Turn power switch OFF and release meat grip.
10. Place slices of meat in plastic bag labeled "A".
11. Rewrap meat "A" and return to refrigerator.
12. REPEAT Steps 2-11 with meats labeled "B" and "C".

6. Quantifying Fluorescent Powder

Quantify the fluorescent powder after completion of the mock slicing (Section 5.4) in the deli kitchen. For this do the following:

1. Use the sterile 5 cm x 5 cm aluminum foil template and place it on the areas marked in **Figure 1**.
2. Use a sterile calcium alginate cotton swab soaked in 95% ethanol, swab the area, and place in 6 ml 95% ethanol in a sterile polypropylene tube.
3. Vortex the tube thoroughly and transfer to a glass cuvette to read the absorbance at 370 nm.
4. Refer to the standard curve to determine the amount of fluorescent powder (y) using the following equation: $y = mx + b$ where x is absorbency, m is the slope of the standard curve, and b is the intercept.
5. For normalization of the data, calculate the percent of fluorescent powder per area sampled using the following formula:

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$$\frac{\text{Concentration of fluorescent powder Swab A}}{\text{Sum (Concentration Swab A, Swab B, } \textit{etc.}\text{)}} \times 100$$

7. Video Analysis

Videotape the participants as they complete tasks 5.1-5.12. Sync and save the video recordings to DVD discs so that all angles can be viewed at the same time. Watch the videos to record the number of hand touches (the number of times volunteers touch areas in the mock deli) on various surfaces in the deli environment (4 different researchers will analyze the complete data set independently).

Representative Results

Figure 1 represents the areas of the slicer that were swabbed after the volunteers completed the tasks assigned. The volunteers were videotaped to analyze the average frequency of hand contact on these various surfaces. The frequency of hand contact was analyzed by four different observers and averaged. These results are demonstrated in **Figure 2**. The data shows that the meat grip, deli meat wrapper, deli meat, and deli paper had the highest rates of hand contact (average of 8-14 contacts).

Following this, an analysis was performed to quantitatively study the amount of fluorescent powder on various components of the slicer and deli kitchen after the volunteers completed the actions. Surfaces as such the refrigerator grip, table, meat grip, slicer blade, and various components of the carriage tray were swapped. As seen in **Figure 3**, the results showed that highest level of contamination was found on the refrigerator grip (approximately 25%). The meat grip and back plates also showed high levels of contamination at approximately 12%.

Similar analyses can be used in a variety of different environments to track contamination using a fluorescent compound such as fluorescent powder. This analysis can further help understand key areas that need additional sanitation and aid in creating effective training materials.

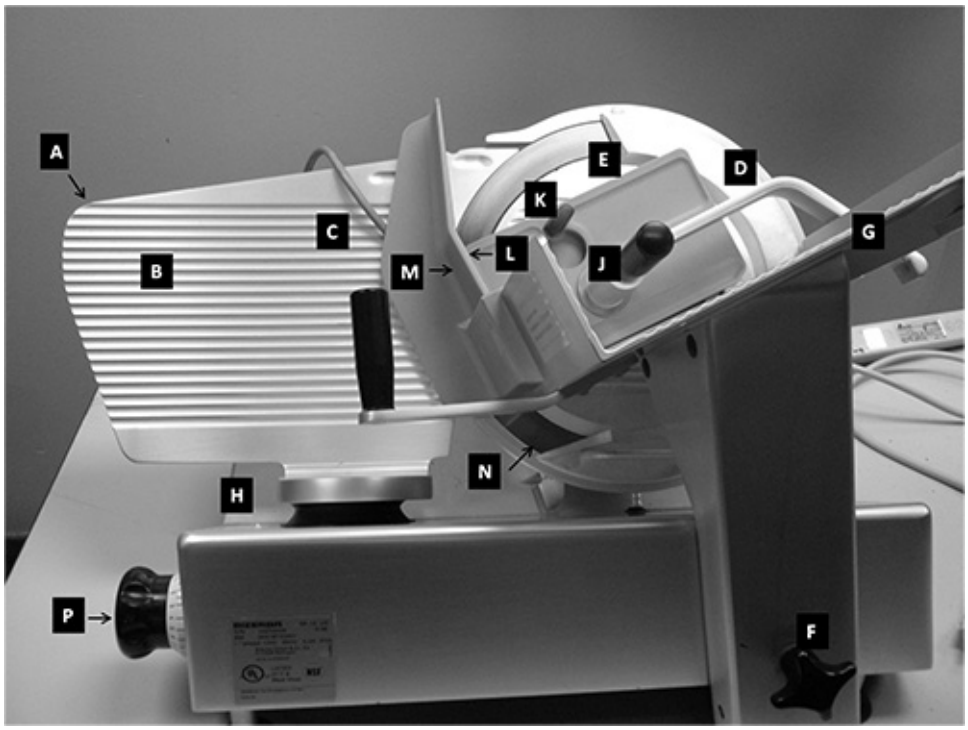

Figure 1. Indicates the various components of the slicer that were swabbed. A, back of slicer; B, back plate; C, back plate; D, blade; E, blade guard; F, carriage handle; G, carriage tray; H, collection area; J, meat grip; K, reverse meat grip; L, inside side wall of carriage tray; M, outside side wall of carriage tray; N, side wall of collection area; P, slicer index knob[12]. Click here to view larger image.

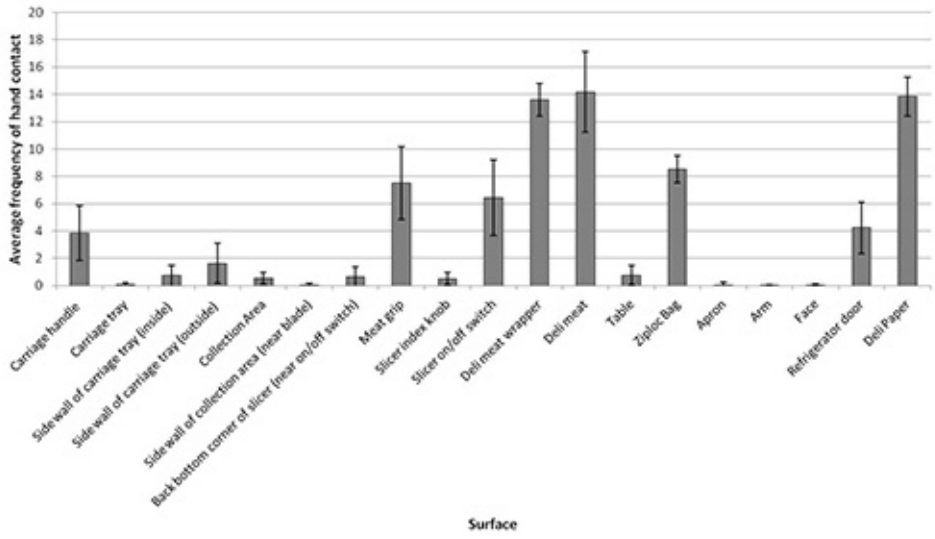

Figure 2. Illustrates the average frequency of hand contact for surfaces in the mock deli kitchen. Eighty two study participants were recorded as the experiment was conducted. Each participant was analyzed thrice by four different observers. Each observer recorded the number of times the participant touched the various surfaces identified in the deli environment. Click here to view larger image.

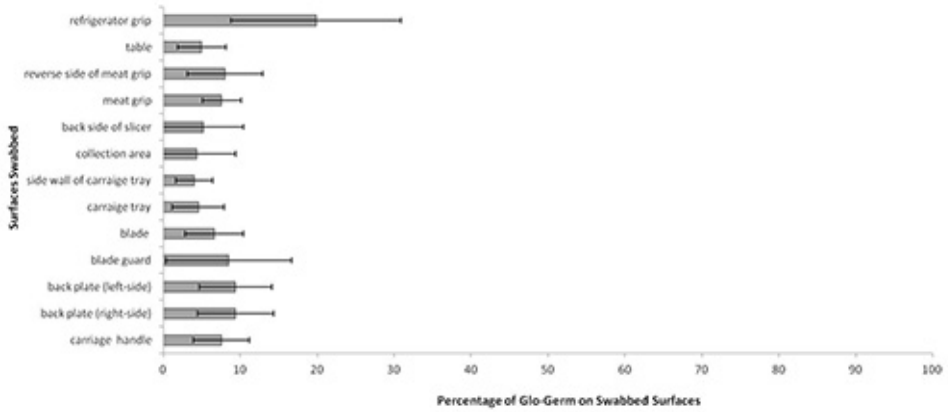

Figure 3. Demonstrates the percentage of fluorescent powder on the 13 surfaces swabbed in the deli kitchen after each participant completes the trial. The surfaces were swabbed using a sterile 5 cm x 5 cm aluminum foil template and a sterile cotton swab. The cotton swab was inserted into a polypropylene tube containing ethanol, vortexed, and analyzed using a spectrophotometer to measure the absorbance at Optical Density (OD) 370 nm. Click here to view larger image.

Discussion

The overall goal of this study was to demonstrate how bacterial cross contamination could occur in a retail deli environment in order to develop strategies to control and reduce the risk of foodborne illness. The retail deli environment has an especially high risk for *L. monocytogenes* contamination. The USDA-FSIS reported that deli meat sliced at the retail store has a seven times higher association with listeriosis that deli meat sliced and packaged at a federally inspected plant[9].

Fluorescent based methods were reported in patents to detect fecal contamination on the surface of carcasses[13,14]. Independent studies have used fluorescent compounds to identify the presence of fecal contamination on meats and cross contamination sources in poultry processing plants[15-17]. Other investigators carried out a study similar to the one reported here. They coated the fluorescent compound on a turkey breast chub and sliced it to determine areas of high contamination[18]. The results showed that the carriage tray, back plate, blade guard, blade, and collection area were contaminated. However, this study did not quantitatively assess the concentration of contamination. The methodology documented in this study quantifies the amount of contamination on trouble areas.

For this study, the investigators used Glo Germ powder - a 5 µm melamine copolymer 183 resin that fluoresces under black light - allowing cross contamination patterns to be seen under fluorescent light for qualitative assessment. However, the high-touch areas can be swabbed and measured at OD_{370} for relative quantification of results. Depending on the type of spectrophotometer and cuvette size, the volume of the test liquid may need to be adjusted.

The observational study involved recording how many times the participants touched a specific area or surface during the study. The video recordings were analyzed thrice by three different observers to ensure consistency. **Figure 2** demonstrates average frequency of hand contact on 20 different surfaces in the deli kitchen (*e.g.* carriage handles, meat grip, apron, face, *etc*.). Overall, the results show that increasing hand contact occurred on the meat drip, deli meat wrapper, deli meats, zip lock bag, refrigerator door, and deli paper. **Figure 3** quantifies the concentration of fluorescent powder in various areas within the mock deli sampled after each participant completed the study. As seen in **Figure 3**, the refrigerator grip is a key "hot-spot" with high contamination and cross contamination risks.

A limitation of this methodology is that fluorescent powder is used to simulate foodborne pathogen *Listeria monocytogenes*. This may not be ideal since the fluorescent powder will not "behave" like a foodborne pathogen. However, the goal of the study is to develop crucial employee training material for effective cleaning in retail kitchens. In addition, this study was performed in a mock deli lab where a pathogenic microorganism cannot be introduced. A critical step in this protocol is to ensure that all the cameras are turned on exactly at the same time. This will ensure that the videos are in sync and aid in data analysis.

This study highlights key areas that should be targeted for cleaning and sanitizing in the deli environment on a consistent basis. The results of this study may be used to develop effective intervention and training material for employees. In addition, this methodology may be used to understand the entry and transfer of pathogens in different environments such as hospitals, hotels, and restaurants.

Disclosures

No conflicts of interest declared.

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