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Article

Quantifying coliform bacteria in ground beef to evaluate food safety guidelines

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SUMMARY

Ground beef, while common in the American diet, can cause many bacterial-related illnesses, including *Escherichia coli* infection and death, when eaten undercooked. The Centers for Disease Control and Prevention (CDC) recommends cooking ground beef to well-done, 160°F, to prevent foodborne illness. However, only around 46% of American adults prefer their burgers well-done or medium-well, so many Americans are at risk. We tested ground beef samples to determine whether the CDC's recommendations are necessary, or merely a precaution, by quantifying the amount of coliform bacteria, an indicator organism that is correlated to individual species of disease-causing bacteria. Additionally, we tested if lower ground beef prices were an indicator of higher coliform contamination. We hypothesized that rare beef would have more coliform bacteria than well-done, and that more expensive beef would have less coliform bacteria. We prepared four brands of ground beef at three temperatures (raw, 130°F, 160°F). We homogenized samples with PBS, centrifuged the samples, and dispensed the supernatant onto film plates which simultaneously detect *E. coli* and coliforms after 24 hours of incubation. We counted colonies, and calculated colony forming units (CFUs) per gram of beef used to prepare the supernatant. We did not find any conclusive relationship between the meat price and the quantity of coliform bacteria. However, all raw samples contained coliform bacteria colonies, and half of the medium-rare samples (130°F) contained at least one coliform bacteria sample. No samples cooked to 160°F grew coliform bacteria or *E. coli*. Thus, the CDC's guidelines for should be followed to avoid foodborne illness exposure from ground beef.

INTRODUCTION

To prevent foodborne illness, the Centers for Disease Control and Prevention (CDC) recommends cooking ground beef to well-done, at 160°F (1). However, only around 46% of American adults prefer their burgers well-done or medium-well, putting many Americans at risk for becoming ill (2). Although the United States Department of Agriculture (USDA) has regulations to ensure the safety of meats for consumption, such as slaughter facility hygiene standards and post-processing temperature handling requirements, foodborne illness accounts for approximately 128,000 hospitalizations and 3,000 deaths per year in the United States (3,4). About 10.4–14.1% of foodborne illnesses in the United States from 1999–2017 were caused by food products from animals, such as cattle (5). A rare strain of *Escherichia coli* that lives in animal intestines, *E. coli* O157:H7, is estimated to cause 73,000 cases of infection and 60 deaths per year in the US (6). The primary cause of infection for this strain of *E. coli* is hypothesized to be the consumption of undercooked ground beef (6).

Ground beef is particularly likely to be contaminated by bacteria compared to other forms of beef because the meat from several different animals is frequently combined when making ground beef (1). During the evisceration step of the slaughter process, meat can become contaminated with *E. coli* and other bacteria if the animal's gastrointestinal tract is perforated (7). Furthermore, grinding tools can become contaminated and transfer bacteria from the surface throughout the meat (7).

One way to assess the danger of ground beef is to quantify the amount of coliform bacteria, which typically reside in animal intestines and are passed through animal feces (8). Coliform bacteria are a group of rod-shaped, gram-negative, non-spore-forming bacteria that produce gas and acids during lactose fermentation, and multiply at temperatures between 40 and 140°F (7). Although not all coliform bacteria are necessarily disease-causing, *E. coli* are a subgroup of coliforms which can cause illness (7). In the US, the critical limit, or the acceptable maximum quantity, for total coliform is 1000 colony forming units per gram (CFU/g) and the critical limit for *E. coli* is 500 CFU/g (8, 9).

In this study, we investigated differences in coliform bacteria growth on beef cooked to different temperatures and from beef sold at different prices. We hypothesized that more expensive beef would have fewer coliform bacteria than less expensive beef, thinking that a higher price would indicate a more careful slaughter process with lower instances of gastrointestinal tract perforation. We hypothesized that undercooked beef (raw or cooked medium-rare at 130°F) has more coliform bacteria than beef cooked well-done at 160°F, as recommended by the CDC. In order to test this, we compared four ground beef brands: Signature Farms, Snake River Farms, Diablo Foods, and Foodmaxx. We tested the meat for coliform bacteria when raw and after being cooked to 130°F and 160°F.

Ultimately, we found that all raw samples contained coliform bacteria colonies, suggesting that contamination occurred at some point during the meat processing. We also detected one *E. coli* CFU in one of our raw samples from Signature Farms. The amount of coliform bacteria detected

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from samples cooked to 130°F was reduced for all brands except Signature farms, and no coliforms were detected in either Snake River Farms samples cooked to that temperature. Most significantly, all samples had zero bacterial colonies when cooked to the recommended temperature of 160°F, which aligns with the CDC recommended temperature. There isn't a clear connection between price and coliform contamination in our results, since the lowest price did not have the most contamination but the most expensive brand did have substantially fewer coliforms when raw. Our findings warrant future research regarding the source of ground beef contamination, and the steps that can be taken to reduce contamination.

RESULTS

To test which factors affect coliform bacteria growth, we obtained ground beef samples from a range of price points and cooked to different temperatures. We tested four different brands of ground beef with varying prices: Signature Farms ($9.49/lb), Snake River Farms ($9.99/lb), Diablo Foods ($9.49/lb), and Foodmaxx ($4.99/lb). We took samples for raw beef from both the edge and middle of the package, and from the edge and middle of the patty for cooked beef. We hypothesized that more expensive beef would have fewer coliform bacteria than less expensive beef, and predicted undercooked beef would have more coliform bacteria than thoroughly cooked beef. We also predicted that there would be a difference in the amount of coliform bacteria between the edge and middle of the patty for medium-rare beef, since the edges of the patty would spend more time at a higher temperature during cooking due to direct contact with the pan.

We tested the beef when it was raw, at 130°F, and at 160°F by homogenizing it with sterile phosphate buffered saline (PBS), centrifuging samples and incubating the supernatant on Petrifilm plates that detect both coliforms and *E. coli*. After 24 hours of incubation, the Petrifilm plates revealed clear differences between samples cooked at different temperatures. All raw ground beef samples contained coliform bacteria, half of the medium-rare samples contained coliform bacteria, and none of the well-done samples contained coliform bacteria (**Figure 1**). Only one raw sample showed specific *E. coli* growth with the Signature Farms sample having one colony (data not shown). This supports our hypothesis, as coliform bacteria colonies decreased as the beef was cooked (**Figure 2**). The control, a plate incubated with only PBS, contained no coliform bacteria (**Figure 2**). There was a substantial decrease in coliform bacteria from raw beef to beef cooked to 130°F. Four medium-rare samples had zero coliform colonies, and all but one of the medium rare plates were under the 1000 CFU/g critical limit (**Figure 2**). Our data demonstrates how cooking beef to medium rare is less likely that raw beef to contain coliform bacteria, and thus potentially disease-causing *E. coli*, but it is still not sufficient to kill all potentially harmful bacteria.

The four brands that were used varied in price from $4.99/lb to $9.99/lb. Every brand sold ground beef with some coliform bacteria when raw. The most expensive brand, Snake River Farms, had approximately 10x fewer coliform colonies when raw and no bacteria colonies were found on the Snake River Farms samples at 130°F (**Figure 2**). All other brands had some coliform colonies when cooked to 130°F (**Figure 2**). Snake River Farms was the most expensive brand tested and had the fewest coliform bacteria colonies, which supports our hypothesis that more expensive beef has fewer coliform bacteria. However, the other three brands did not follow this trend, as the cheapest brand did not have the most coliform bacteria (**Figure 1**).

Five of eight raw samples, had a coliform count over the 1000 CFU/g critical limit including both samples from the FoodMaxx and Diablo Foods brands and the Signature Farms sample taken from the middle of the sample (**Figure 2**) (9). One of the eight medium-rare samples, the Signature Farms sample from the middle of the cooked patty, also had a coliform count of over 1000 CFU/g (**Figure 2**). Because only one gram of ground beef sample was used to make the solutions incubated on each plate, we deduced that these samples were over the critical limit measured in CFU/g.

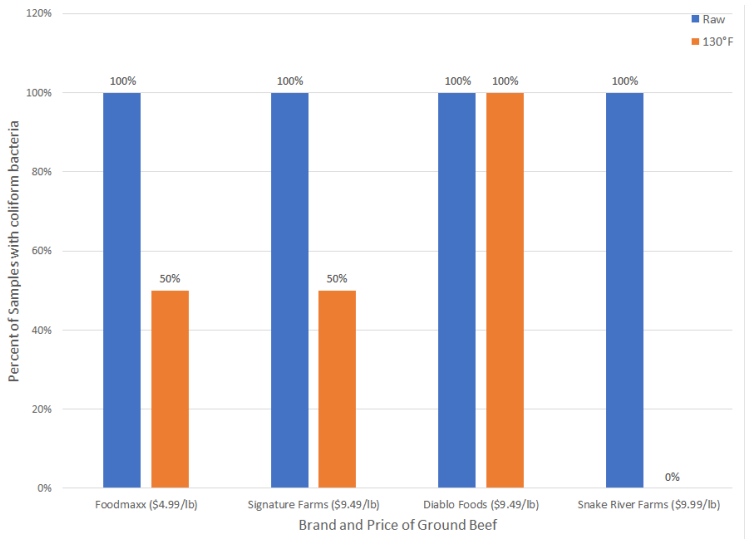

Figure 1: Percent of samples prepared raw or medium-rare with at least one coliform bacteria colony among four beef brands at different prices. Percent of beef samples with at least one bacteria colony among different brands when raw or cooked to medium rare (130°F). Bacteria were cultured and counted after 24 hours of incubation at 37°C.

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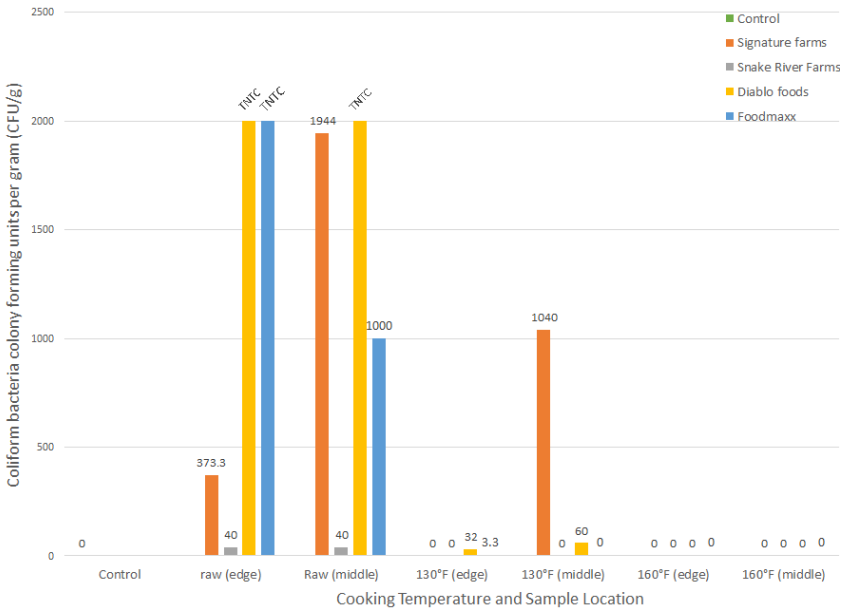

Figure 2: Coliform bacteria colony forming units from all ground beef samples obtained from four ground beef brands. Bacterial contamination as measured by colony forming units (CFU)/g of ground beef samples that were raw or cooked (to 130°F or 160°F). Bacteria were cultured and counted after 24 hours of incubation at 37°C and calculated per g of meat used to prepare the solution plated. TNTC, too numerous to count.

Coliform contamination did vary between samples taken from the center or edge of the patty for medium-rare samples, with more colony forming units detected in samples from the middle of the patty for the Signature Farms and Diablo foods samples. The Foodmaxx sample had some coliform from the edge of the medium-rare patty, calculated to 3.3 CFU/g, but did not have any coliforms detected from the center of the patty (**Figure 2**).

DISCUSSION

The presence of coliform bacteria in all raw beef samples indicates that the meat was likely contaminated during the slaughter and packaging process. Coliform bacteria originate in animal intestines and fecal matter but not muscle tissue, so the presence of coliforms in packaged meat indicates that bacteria was transferred from the gastrointestinal tract to the finished meat product (9). This is relevant because coliforms are a broad group of bacteria that includes pathogenic bacteria species like *Enterobacter* spp. and *E. coli* O157:H7 (10). Monitoring coliforms is a standard method microbiologists use for evaluating both food and drinking water system safety, since testing for individual pathogenic species is too complex to use for routine testing and may not represent all possible pathogenic bacteria (11). The presence of coliform bacteria in some brands of ground beef at 130°F indicates that cooking ground beef to medium rare does not necessarily kill all bacteria. Therefore, our results indicate the importance of cooking ground beef fully to 160°F as recommended by the CDC.

The results of our study are aligned with other recent research, such as a 2020 study of ground beef from samples in Lebanon, which showed 98% of raw beef samples were contaminated by fecal coliforms, and 76% were contaminated by *E. coli* specifically (9). Risk assessments of ground beef consumption produced by the USDA acknowledge the pathway for microbial contamination of ground beef explicitly and acknowledge food handling practices like refrigeration and cooking temperatures as a necessity with our current meat production and processing practices (7).

Although the plates used in this study are capable of detecting both coliform and *E. coli* bacteria, one limitation in the *E. coli* analysis was that the Petrifilm plates used could not differentiate the disease-causing O157:H7 strain of *E. coli* from other strains that may not cause foodborne illness. Future experiments should attempt to determine the strain of *E. coli*, through DNA sequencing or other molecular means. Another limitation was in the design of our protocol for homogenizing and plating our samples. We did not anticipate that more thoroughly cooked meats would absorb more PBS, so we were unable to obtain 1 mL of supernatant for all samples. To adjust, we used as much supernatant as we could obtain, either 0.25 mL or 0.5 mL and used the dilution factor to calculate CFU/g for those samples. Our study also only included grocery store meats, and did not include animals raised in different environments, like pasture-raised animals. This is a limitation because meat ground from a single animal may have less coliform contamination due to differences in the bacteria in the gastrointestinal tract or the overall health between different animals. This question arose due to the low coliform contamination of the Snake River Farms meat, which claimed to be “minimally processed” on the package, but that statement was not further defined. Future experiments should also look into coliform bacteria and *E. coli* in meats ground by local farms that process and grind beef from individual animals.

Future experiments could also explore the impact of packaging on the contamination process. The samples with the most coliform bacteria contamination were the Diablo Foods samples, which may be due to the way the meat was displayed in open refrigerated air. When it was purchased, the

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meat was packaged in wax paper. In contrast, Snake River Farms used vacuum-sealed plastic packaging. Researching the optimal way to package ground beef to reduce bacterial contamination may provide a simple method to reduce colony counts.

Nevertheless, the presence of coliform bacteria in all raw samples and in some beef samples cooked to 130°F demonstrates the importance of cooking ground beef well done (160°F). As shown by the lack of growth in all plates with samples cooked to 160°F, this temperature kills coliform bacteria. While not all coliform bacteria cause illness, their presence indicates the conditions for disease-causing bacteria growth are met. Based on the results of this study, ground beef cooked to 160°F is much less likely to contain coliform bacteria, and therefore disease-causing *E. coli* than meat cooked to 130°F. Thus, our results support the CDC's recommendation to cook all ground beef to well-done.

MATERIALS AND METHODS

Sample Preparation

Four packages of ground beef were purchased from the following brands: Signature Farms, Snake River Farms, Diablo Foods, and Foodmaxx. For each brand, a 1.0 g raw sample was taken from both the edge and middle of the container. Then, A one quarter-pound patty was made using ground beef from both the center and edge of the package. Each patty of ground beef was cooked in its own disinfected frying pan with no cooking oils or seasonings. The internal temperature was determined by an electric food thermometer (KitchenAid, KQ904). When the beef reached 130°F and again at 160°F, two 1g samples were taken: one at the edge of the patty and one at the center. Thus, each brand of ground beef had a total of 6 samples analyzed. All meat samples were stored in sanitized plastic containers and refrigerated at 2 to 8°C before plating.

Plating Samples

Each 1.0 g sample was combined with 1–2 mL of sterile 1X PBS and mashed in standard marble mortar and pestles, sanitized by soaking in 10% bleach and washed with soap and water before air drying. Then, the sample was centrifuged for 2 minutes at 3,500 rpm. The supernatant was transferred to a 3M Petrifilm E. coli/Coliform Count Plate (Flinn Scientific, Catalog #FB1173). The amount of sample liquid varied from 0.25-1 mL per plate. A control plate with 1 mL of 1X PBS was made. All plates were incubated for 24 hours at 37°C.

Plate Analysis

The Petrifilm plates detect and dye coliform bacteria colonies and *E. coli* bacteria. Coliform bacteria are dyed red due to colonies producing an acid on Violet Red Bile with lactose (VRBL) agar and *E. coli* bacteria are dyed blue due to an indicator of glucuronidase activity, typical *of E. coli* (12). A dissecting microscope was used at 1X magnification to count red colonies with associated gas bubbles as coliform bacteria, according to the manufacturer instructions (12). However, when there were large numbers of colonies, the number of individual colonies was approximated by multiplying the number of colonies in 1 cm^3 by the number of grid squares with similar numbers of colonies, as outlined by the manufacturer (12). Colonies with an uncountable number of colonies such as a bacterial lawn, many gas bubbles, or a deepening of the gel color from red to purple-blue were labeled as "too numerous to count" (TNTC) as directed by the plate manufacturer's package insert (12). For plates grown with less than 1 mL of supernatant, CFU/g was calculated.

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