PURE Insights

Volume 7 Article 6

2018

Bacterial Abundance and Resistance in Ground Beef Varieties

Savannah Kumar
Western Oregon University, skumar15@mail.wou.edu

Sarah M. Boomer
Western Oregon University, boomers@wou.edu

Bacterial Abundance and Resistance in Ground Beef Varieties

Abstract

Raw ground beef purchased at supermarkets across America have one thing in common: they harbor bacteria, some of which are drug resistant and can be detrimental to public health. To understand the impact of farming and processing practices on the quantity of bacteria and drug resistance, organic and regular beef were assessed using MacConkey media. Bacterial colonies were sorted according to lactose utilization, with positive colonies representing fecal E. coli. Lactose negative colonies were further characterized into one of two groups (fecal Hafnia-like or soil Pseudomonas) using a variety of metabolic tests (oxidase, sulfur, indole). Advanced metabolic testing showed that regular beef contained significantly more fecal E. coli-like bacteria, Hafnia-like bacteria and fecal Providencia-like bacteria than organic beef. Soil Pseudomonas was only isolated from regular beef. This procedure was repeated using MacConkey plates containing commonly used agricultural antibacterial drugs to assess the prevalence and types of drug-resistant bacteria. Bacteria resistant to penicillin, sulfamethazine, cefazolin, or ampicillin were found at significantly higher levels on regular beef than organic. Bacteria resistant to more than one of these drugs were only found on regular beef.

Keywords

Ground beef, antibiotic resistance, E. coli, Pseudomonas, food safety, MacConkey

Cover Page Footnote

I would like to thank the Kenneth M. Walker Undergraduate Research Award for the financial support which provided all culture-based research supplies, antibiotics, and DNA analysis services used for this project. I would also like to thank Western Oregon University for the lab space used to research our topic and Dr. Sarah Boomer for the unwavering support and guidance throughout this process. Finally, I would also like to thank Western Oregon University's Academic Excellence Showcase and The Oregon Academy of Science for the platform on which to share my research.

pure insights

A publication of the Program for Undergraduate Research Experiences at Western Oregon University

Bacterial Abundance and Resistance in Ground Beef Varieties

Savannah Kumar, Western Oregon University
Dr. Sarah Boomer, Western Oregon University
Faculty Sponsor: **Dr. Sarah Boomer**

Raw ground beef purchased at supermarkets across America have one thing in common: they harbor bacteria, some of which are drug resistant and can be detrimental to public health. To understand the impact of farming and processing practices on the quantity of bacteria and drug resistance, organic and regular beef were assessed using MacConkey media. Bacterial colonies were sorted according to lactose utilization, with positive colonies representing fecal E. coli. Lactose negative colonies were further characterized into one of two groups (fecal Hafnia-like or soil Pseudomonas) using a variety of metabolic tests (oxidase, sulfur, indole). Advanced metabolic testing showed that regular beef contained significantly more fecal E. coli-like bacteria, Hafnia-like bacteria and fecal Providencia-like bacteria than organic beef. Soil Pseudomonas was only isolated from regular beef. This procedure was repeated using MacConkey plates containing commonly used agricultural antibacterial drugs to assess the prevalence and types of drug-resistant bacteria. Bacteria resistant to penicillin, sulfamethazine, cefazolin, or ampicillin were found at significantly higher levels on regular beef than organic. Bacteria resistant to more than one of these drugs were only found on regular beef.

Keywords: Ground beef, antibiotic resistance, E. coli, Pseudomonas, food safety, MacConkey

Introduction

Ground beef makes up 60% of all retail beef sales (Close, 2014). With its versatile nature and low price point, it is no surprise that the average American consumes around 53 pounds of ground beef per year (Close, 2014). Although ground beef is popular, it is also problematic because it can harbor bacteria that may be pathogenic and antibiotic resistant (Landers *et al*., 2012, Rock, 2015).

Beef bacteria may originate from the soil (e.g. *Pseudomonas*) or from feces (e.g. *E. coli*, *Hafnia*, *Providencia*, or *Salmonella*). When ground beef is produced, the grinding process increases the surface area of the beef and exposes more of the beef to bacteria. These methods of production increase the ability for it to transmit bacteria to consumers.

Ground beef production in America adheres to Sanitation Standard Operating Procedures, which attempts to reduce the amount of bacteria on the beef (USDA, 2016). The United States Department of Agriculture (USDA) also mandates the testing of beef for the presence of *E.coli*. The number of tests required to be conducted by a facility increases as the volume of ground beef produced at that particular establishment increases (USDA, 2017).

Nevertheless, even when all precautions are followed, bacteria can still be found on both organic beef samples, which come from cows that were not provided antibiotics or hormones, and regular beef samples, which come from cows that may have been provided antibiotics or hormones (Landers *et al*., 2012; USDA, 2015). Matters are further complicated by the use of antibiotics in conventional agricultural methods. A byproduct of this antibiotic use in agriculture is the presence of antibiotic resistant bacteria on agricultural products (Landers *et al*., 2012, Young & Hoffman, 2014). It has recently been demonstrated that foodborne bacteria like *E.coli* or *Salmonella* have the ability to transmit antibiotic resistant infections — such as urinary tract infections, pyelonephritis, bloodstream infections, and diarrheagenic gastrointestinal infections (Landers *et al*., 2012, Young & Hoffman, 2014, Nordstrom *et al*., 2013).

Pathogenic bacteria have been detected in beef for years, including *E.coli, C. perfringens, S. aureus*/MRSA, and *Salmonella* (Rock, 2015, Jackson *et al*., 2013). In 2008, antibiotic resistant *E.coli* was cultured from the feces of feedlot cattle using MacConkey agar amended with tetracycline or ampicillin; these studies concluded that the use of antibiotics increased the prevalence of resistant *E.coli* in the feedlot cattle (Alexander *et al*., 2008). *E.coli* isn't the only bacteria of concern. In 2017, the CDC used DNA-based methods to link an outbreak of *Salmonella* to contaminated ground beef. This outbreak spread to 21 states and affected 106 people (Marshall *et al*., 2018). Researchers have also found greater levels of antibiotic resistant bacteria and multidrug resistant bacteria on conventionally raised beef compared to sustainably raised beef that was either raised with no antibiotics or was organic or grass-fed (Rock, 2015).

With the danger of antibiotic resistant infections ever looming, it is important for modern consumers to understand the relationship between the overall abundance, antibiotic resistant qualities, and multidrug resistant qualities of bacteria present on regular and organic ground beef, so that they can make educated decisions about the products that they are consuming.

The goals of this study are to understand the impact that farming and processing practices have on the quantity and drug resistant nature of ground beef varieties via quantification and categorization of beef bacteria isolates. This study replicates and reflects some earlier work but expands understanding of multi- drug resistance using distinctive drug combinations, as well as examining contamination levels in local beef and grocery products from Oregon.

Material and Methods

Sample Acquisition:

Packages of regular ground beef and USDA certified organic ground beef, which came from cows who were not given added antibiotics or hormones (USDA, 2015), were purchased from local grocery stores in Monmouth, Oregon. In total, 4 different packages of regular beef and 4 different packages of organic beef were analyzed over a period of 2 years (Table 1).

Table 1: Summary of beef samples, replicates, and testing procedures.

Sample, Date	# Plates Evaluated	Performed By	Testing	Categorization Groups
Organic 1, Winter 2017	15[1]	General Microbiology Students	Lactose, Oxidase	*E. coli* *Pseudomonas* Uncertain lac-/ox-
Regular 1, Winter 2017	15[1]			
Organic 2, Winter 2017	36[1]			
Regular 2, Winter 2017	36[1]			
Organic 3, Winter 2017	30[2]	Kumar	Lactose, Oxidase	*E. coli* *Pseudomonas* Uncertain lac-/ox-
Regular 3, Winter 2017	30[2]			
Organic 4, Winter 2018	6[1]	Kumar	Lactose, Oxidase, Sulfur, Indole	*E. coli* *Pseudomonas* *Hafnia* *Providencia*
Regular 4, Winter 2018	6[1]			
Organic 4, Winter 2018	6[3]			
Regular 4, Winter 2018	6[3]			
Organic 4, Winter 2018	5[4]			
Regular 4, Winter 2018	5[4]			

[1] MacConkey plates contained no antibiotics.

[2] MacConkey plates contained a concentration of 50 μg/mL of a tetracycline.

[3] MacConkey plates contained a concentration of 50 μg/mL of a single antibiotic (kanamycin, sulfamethazine, ampicillin, penicillin or cefazolin).

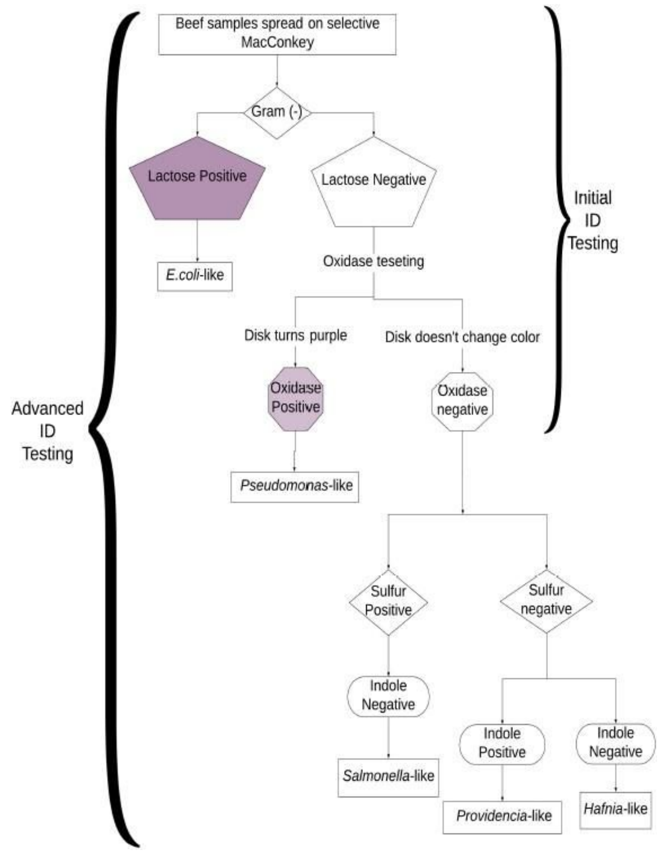
[4] MacConkey plates contained a total concentration of 50 μg/mL of a combination of two antibiotics (penicillin/sulfamethazine or penicillin/cefazolin).

Antibiotic MacConkey Plates:

Bacterial media was prepared using 25 grams of Difco MacConkey agar (Difco, Sparks, MD), 3 grams of additional agar, and 500 mL water. This mixture was autoclaved for 20 minutes, cooled in a 55-60°C water bath for 1 hour and poured into petri dishes. MacConkey agar was used because it is both differential and selective. MacConkey agar is selective in that it only allows Gram negative bacteria to grow. The agar is differential because it turns bacterial colonies that ferment lactose (lac+) purple, and colonies that do not ferment lactose (lac-) white, allowing us to categorize the types of bacteria present on beef samples (Table 1, Figure 1).

For media containing antibiotics, a stock solution of 10 mg/ml was created using antibiotic dissolved in ethanol or water. The dissolved antibiotics were incorporated into the cooled, sterilized liquid MacConkey agar mixture to make a final concentration of 50 μg/mL. The drugs chosen to test for resistance were those used most commonly used in agriculture, including kanamycin, tetracycline, sulfamethazine, ampicillin and penicillin. One more recent drug, cefazolin, was also included because of reported drug resistance in the poultry industry (Millman, 2013). All antibiotics were purchased from Sigma-Aldrich, MO. For assessment purposes, colonies that grew in the presence of the antibiotic were considered to be resistant. Multidrug resistance was assessed using two-drug combinations of penicillin and sulfamethazine (pen/sulf) and penicillin and cefazolin (pen/ceph).

Figure 1: Flow chart used to categorize bacteria



Bacterial Isolation and Testing:

Bacteria were isolated from beef samples by placing 5 grams of thawed beef in water (100 mL). The mixture was then placed on a shaker table for one hour at room temperature. A small amount (0.1 mL) of the beef liquid was spread on MacConkey agar with or without antibiotics. These plates were then incubated at 37 degrees Celsius and checked for growth after 48 hours.

Following isolation, bacteria were categorized by their utilization of lactose. Purple lactose positive (lac+) colonies were defined as *E. coli*-like. White lactose negative (lac-) colonies were further categorized using oxidase testing (BD BBL™ Taxo™ N Discs, Becton and Dickinson and Company, Sparks, MD) to determine if the bacteria produces the enzyme cytochrome oxidase. In our initial assessments, which were carried out by students in General Microbiology, we defined the lac-/ox- colonies as uncertain but resembling *Salmonella*, and the lac-/ox+ colonies as *Pseudomonas*-like. We then performed more advanced testing of lac-/ox- colonies using sulfur indole media (SIM), which tests for the production of sulfide and formation of indole (BD BBL SIM Medium, Becton, Dickinson and Company, Sparks, MD). These tests determined that "uncertain/ *Salmonella*-like" colonies were actually "*Providencia*-like" or "*Hafnia*-like." Testing and determination of which category a bacterial colony belonged to is summarized in Figure 1 and Table 1.

Statistical Analysis:

Regular vs. organic beef bacterial counts were compared using a Microsoft Excel two-tailed unpaired t-tests to assess if the difference in contamination levels and antibiotic resistant colony counts were significant. The initial testing from the spring and winter 2017 were combined into one dataset while antibiotic resistance was assessed using advanced testing and a combination of two datasets and multidrug resistance was assessed using advanced testing and only one dataset. All information on samples, replicates, testing and categorization can be found in Table 1.

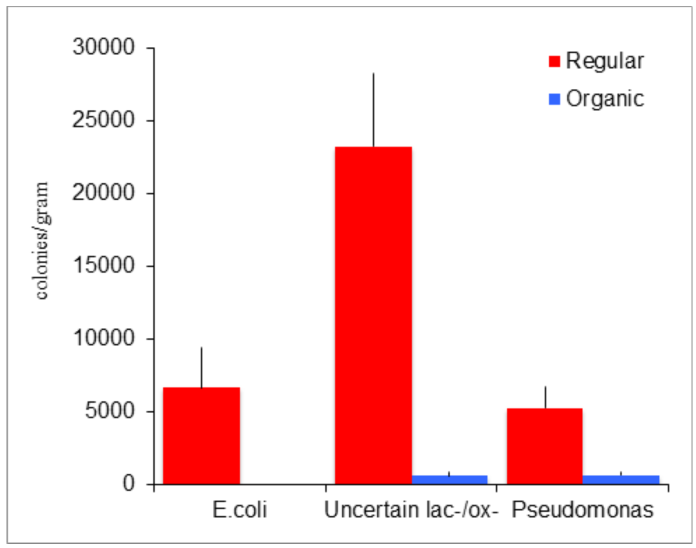
Results

Initial ID Testing Winter and Spring 2017-2018

A portion of this project (winter 2017 through spring 2018) involved General Microbiology (BI 331) students counting and comparing *E.coli*-like, uncertain/ *Salmonella*-like, or *Pseudomonas*-like bacteria from regular vs. organic beef using oxidase testing and lactose utilization results (Figure 1 and Table 1).

Combined data from all classes showed that regular beef had significantly more *E.coli*-like bacteria (6667 colonies/gram) than organic beef (101 colonies/gram)($p = 0.020$; Figure 2). Regular beef had significantly more uncertain lac-/ox- bacteria (23231 colonies/gram) than organic (626 colonies/gram) ($p < 0.0001$; Figure 2). Regular beef had significantly more *Pseudomonas*-like bacteria (5273 colonies/gram) than organic beef (670 colonies/gram) ($p = 0.0025$; Figure 2).

Figure 2: Initial Sample Assessments. Colonies were classified as *E.coli*-like ($p < 0.05$), Uncertain lac-/ox ($p < 0.0001$) or *Pseudomonas*-like ($p < 0.0025$) using the visual lactose phenotype displayed on the MacConkey agar and oxidase testing. Error bars represent standard error of the mean.

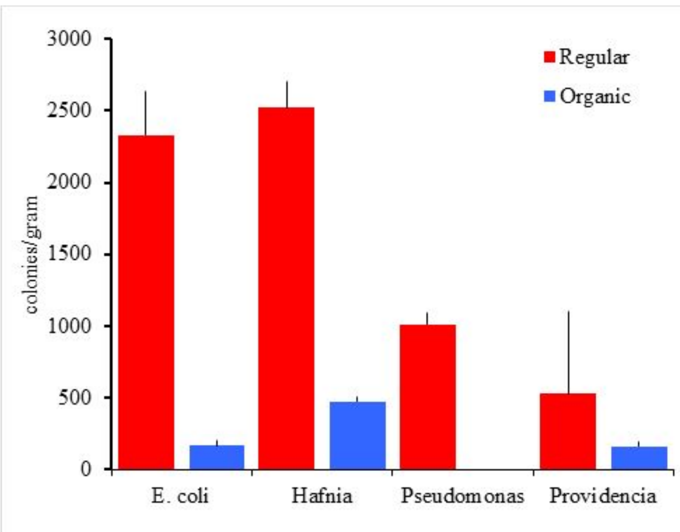


Advanced ID Testing Spring 2018

In order to better characterize lac(-)/ox(-) colonies, we carried out advanced testing using sulfur-indole media (SIM) (Figure 1, Table 1). These results demonstrated that class-defined uncertain lac-/ox- colonies were *Providencia*-like or *Hafnia*-like.

Regular beef contained significantly more *E.coli*-like bacteria (2333 colonies/gram) than organic beef (167 colonies/gram) ($p < 0.0001$; Figure 3). There were also significantly more *Hafnia*-like bacteria on regular beef (2523 colonies/gram) than organic beef (473 colonies/gram) ($p < 0.0001$; Figure 3). Regular beef had significantly more *Pseudomonas*-like bacteria (1010 colonies/gram) than organic beef (0 colonies/gram) ($p < 0.0001$; Figure 3). There was not a significant difference between the levels of *Providencia*-like bacteria on regular beef (525 colonies/gram) when compared to organic beef (159 colonies/gram) ($p = 0.5379$; Figure 3).

Figure 3: Advanced Sample Assessments. Colonies were classified as *E.coli*-like ($p < 0.0001$), *Hafnia*-like ($p < 0.0001$), or *Pseudomonas*-like ($p < 0.0001$), *Providencia*-like ($p = 0.5379$) using the visual lactose phenotype displayed on the MacConkey agar, oxidase testing, and sulfur-indole (SIM) testing. Error bars represent standard error of the mean.



Antibiotic Resistance

Bacteria exhibited sulfamethazine resistance in both regular and organic beef. Regular beef had significantly more resistant *Pseudomonas*-like bacteria (3042 colonies/gram) than organic beef (46 colonies/gram) ($p < 0.0001$; Figure 4). Regular beef also had significantly more resistant *Hafnia*-like bacteria (435 colonies/gram) than organic beef (114 colonies/gram) ($p < 0.0001$; Figure 4). Regular beef had significantly more resistant *E.coli*-like bacteria (2482 colonies/gram) than organic beef (117 colonies/gram) ($p < 0.0001$; Figure 4). Only organic beef contained resistant *Providencia*-like bacteria (24 colonies/gram).

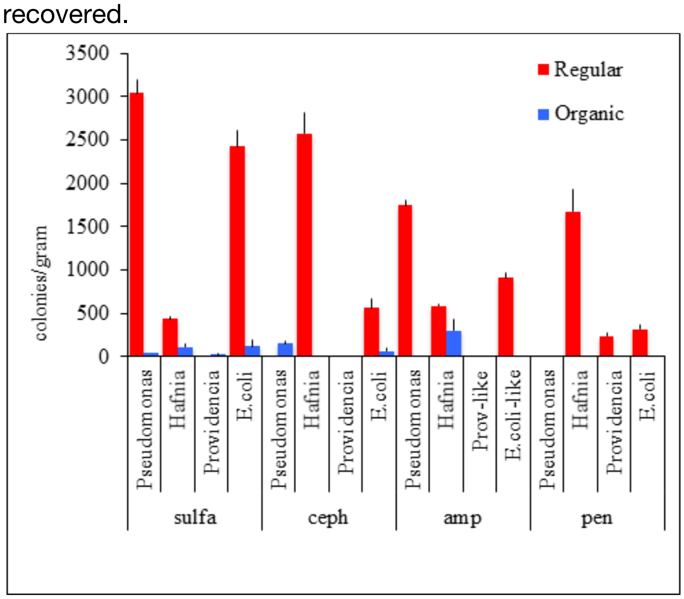
Bacteria exhibited cefazolin resistance in both regular and organic beef. Regular beef had significantly more resistant *E.coli*-like bacteria (563 colonies/gram) than organic beef (113 colonies/gram) ($p = 0.0017$; Figure 4). Organic beef had resistant *Pseudomonas*-like bacteria (150 colonies/gram). Only regular beef had resistant *Hafnia*-like bacteria (2580 colonies/gram). No resistant *Providencia*-like bacteria were found in either regular or organic beef.

Bacteria exhibited penicillin resistance primarily in regular beef. Only regular beef contained resistant *Hafnia*-like bacteria (1675 colonies/gram), resistant *E.coli*-like bacteria (316 colonies/gram), and resistant *Providencia*-like bacteria (240 colonies/gram). There were no resistant *Pseudomonas*-like bacteria in either regular or organic beef.

Bacteria exhibited ampicillin resistance in both regular and organic beef. Only regular beef had resistant *Pseudomonas*-like bacteria (1750 colonies/gram) and resistant *E.coli*-like bacteria (913 colonies/gram). Regular beef contained more resistant *Hafnia*-like bacteria (583 colonies/gram) than organic beef (300 colonies/gram) but the difference was not significant ($p = 0.0559$; Figure 4). No resistant *Providencia*-like bacteria were found in either regular or organic beef.

Of the antibiotics studied, only two drugs, kanamycin and tetracycline, inhibited all bacterial growth in all beef varieties. This suggests that the bacteria found on regular and organic beef samples have yet to develop resistance to kanamycin or tetracycline.

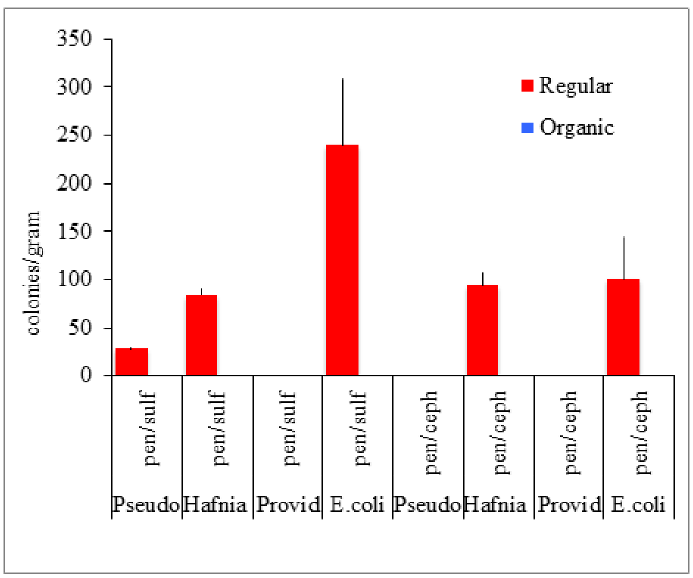
Figure 4: Antibiotic resistant colony counts in the presence of sulfamethazine (sulfa), cefazolin (ceph), ampicillin (amp) and penicillin (pen). Colonies were classified as *E.coli*-like, *Hafnia*-like (Haf-like), or *Pseudomonas*-like (Pseudo-like), *Providencia*-like (Prov-like). Error bars represent standard error of the mean. Kanamycin and tetracyline resistance was not recovered.



Multidrug Resistance

Significantly, no multidrug resistant colonies to combinations of pen/sulf and pen/ceph were found in any organic beef sample (Figure 5). In contrast, multidrug resistant colonies were found across most bacterial categories on regular beef (Figure 5): Regular beef harbored pen/sulf resistant *Pseudomonas*- like colonies (28 colonies/gram), *Hafnia*-like colonies (84 colonies/gram) and *E.coli*-like colonies (240 colonies/gram). Regular beef harbored pen/ceph resistant *Hafnia*-like colonies (94 colonies/gram) and *E-coli*-like colonies (100 colonies/gram).

Figure 5: Multidrug resistant colony counts in the presence of penicillin/sulfamethazine (pen/sulf) or penicillin/cefazolin (pen/ceph) combinations. Colonies were classified as *E.coli*-like, *Hafnia*-like, *Pseudomonas*-like (Pseudo) or *Providencia*-like (Provid) with resistance to pen/ceph or pen/sulf. Error bars represent standard error of the mean.



Discussion

Our study of the abundance and bacterial resistance qualities of beef shows that the samples of regular beef that we assessed had more contamination overall (Figure 3), as well as more antibiotic resistance – both in terms of single drug (Figure 4) and multidrug resistance (Figure 5). While our study represents a limited number of beef samples, our findings are consistent with previous studies on ground beef that reported more antibiotic resistant bacteria, and overall bacterial contamination on beef samples from cows that were fed antibiotics and hormones (Rock, 2015). Taken together with evidence that drug-resistant strains on food can transmit disease to humans (Landers *et al*., 2012, Young & Hoffman, 2014, Nordstrom *et al*., 2013), increased efforts to monitor food contamination and increase awareness about food safety should continue to be a research and government priority.

Initial class findings suggested that there was a significant difference between the levels of all surveyed bacteria, with greater contamination found on regular beef (Figure 2). The presence of *Pseudomonas*-like bacteria and *E.coli*- like bacteria on packaged meats has been documented, with *Pseudomonas* most notably associated with meat spoilage (Ercolini *et al.*, 2009) and *E.coli* most associated with pathogenic foodborne disease (Lim et *al.*, 2010).

In 2018, we made further efforts to identify the lac-/ox- colonies using additional identification tests, and were able to categorize the colonies as *Providencia*-like or *Hafnia*-like (Table 1, Figures 1 and 3), as further confirmed by preliminary DNA-based studies (data not shown). These findings are supported by Consumer Reports studies, which only recovered *Salmonella* in 1% of their samples (Rock, 2015). The presence of *Providencia*-like bacteria on ground beef is supported by Shima *et al.*, 2016, who reported that 68% of beef samples from Thailand contained *Providencia* species. The presence of *Hafnia*- like bacteria on ground beef samples has been supported by Kang *et al.*, 2002, who recovered *Hafnia* from microbially gas-inflated beef packages.

Varying levels of antibiotic resistance were found to most common agricultural antibiotics - sulfamethazine, cefazolin, ampicillin, and penicillin (Figure 4). The general finding of antibiotic resistant bacteria in food-animal products is a well-documented threat to public health (Landers *et al.*, 2012). While our study found no tetracycline resistance, past studies have recovered extensive tetracycline resistance in beef bacteria (Shin *et al*., 2015). The resistant qualities of *E.coli*-like bacteria is well documented, so it is no surprise that we consistently observed *E.coli*-like resistance to sulfamethazine, cefazolin, ampicillin, and penicillin (Klein *et al*., 1998). Our study found that regular beef harbored more antibiotic resistant bacteria than organic beef, consistent with the Consumer Reports study that showed that beef raised with hormones and antibiotics typically harbored more antibiotic resistant bacteria (Rock, 2015).

Multidrug resistance is most commonly associated with the Gram positive bacteria, *Staphylococcus* (Nikaido, 2009). However, pathogenic Gram negative bacteria like *E.coli* and *Pseudomonas* are also developing multidrug resistance and becoming a bigger threat to public health, with certain strains of *Pseudomonas* quickly becoming “pan-resistant,” meaning resistant to all commonly used antibiotics (Nikaido, 2009). Our study utilized 2 two-drug combinations (penicillin/cefazolin and penicillin/sulfamethazine) to assess the quantity of multidrug resistant bacteria. Multidrug resistant bacteria were only recovered from regular beef, suggesting a direct relationship between the use of antibiotics in the production of regular beef and the evolutionary selection of antibiotic resistant bacterial strains present in those cows and beef products (Figure 5). Our findings echo the results of the Consumer Reports study that showed that there was a greater amount of multidrug resistant bacteria on beef samples raised with antibiotics and hormones (Rock, 2015).

The goals of this study were to understand the impact of farming and processing practices on the quantity and drug resistant qualities of organic and regular beef bacteria. Using differential and selective agar, metabolic tests and antibiotics, we have shown that regular beef contains overall greater levels of bacteria, antibiotic resistance, and multidrug resistance than organic beef. Future studies need to be done to determine the effectiveness of other multidrug combinations.

Acknowledgements

I would like to thank the Kenneth M. Walker Undergraduate Research Award for the financial support, which provided all culture-based research supplies, antibiotics, and DNA analysis services used for this project. Finally, I would also like to thank Western Oregon University for the lab space used to research our topic.

References

Alexander, T. W., Yanke, L. J., Topp, E., Olson, M. E., Read, R. R., Morck, D. W., & Mcallister, T. A. (2008). Effect of Subtherapeutic Administration of Antibiotics on the Prevalence of Antibiotic-Resistant Escherichia coli Bacteria in cFeedlot Cattle. *Applied and Environmental Microbiology,74*(14), 4405-4416.

Close, D. “Rabobank: U.S. a 'ground beef' nation.” Last modified February 4th, 2014. Retrieved from https://www.dairyherd.com/article/rabobank-us-ground-beef-nation

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., & Villani, F. (2009). Mesophilic and Psychrotrophic Bacteria from Meat and Their Spoilage Potential In Vitro and in Beef. *Applied and Environmental Microbiology*, *75*(7), 1990–2001.

Jackson, C. R., Davis, J. A., & Barrett, J. B. (2013). Prevalence and Characterization of Methicillin-Resistant Staphylococcus aureus Isolates from Retail Meat and Humans in Georgia. *Journal of Clinical Microbiology, 51*(4), 1199-1207.

Kang, D., Arthur, T. M., & Siragusa, G. R. (2002). Gas Formation in Ground Beef Chubs Due to Hafnia alvei Is Reduced by Multiple Applications of Antimicrobial Interventions to Artificially Inoculated Beef Trim Stock. *Journal of Food Protection*, *65*(10), 1651-1655.

Klein, G., Pack, A., & Reuter, G. (1998). Antibiotic Resistance Patterns of Enterococci and Occurrence of Vancomycin-Resistant Enterococci in Raw Minced Beef and Pork in Germany. *Appl. Environ. Microbiol.* May 1998, *64*(5) 1825-1830

Landers, T. F., Cohen, B., Wittum, T. E., & Larson, E. L. (2012). A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Reports*, *127*(1), 4–22.

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A Brief Overview of *Escherichia coli* O157:H7 and Its Plasmid O157. *Journal of Microbiology and Biotechnology*, *20*(1), 5–14.

Marshall, K. E., Tewell, M., Tecle, S., Leeper, M., Sinatra, J., Kissler, B., . . . Gieraltowski, L. (2018). Protracted Outbreak of Salmonella Newport Infections Linked to Ground Beef: Possible Role of Dairy Cows — 21

States, 2016–2017. *MMWR. Morbidity and Mortality Weekly Report, 67*(15), 443-446.

Millman, J. M., Waits, K., Grande, H., Marks, A. R., Marks, J. C., Price, L. B., & Hungate, B. A. (2013). Prevalence of antibiotic-resistant *E. coli* in retail chicken: Comparing conventional, organic, kosher, and raised without antibiotics. *F1000Research* 2013, 2:155

Nikaido, H. (2009). Multidrug Resistance in Bacteria. *Annual Review of Biochemistry*, *78*(1), 119-146.

Nordstrom, L., Liu, C. M., & Price, L. B. (2013). Foodborne urinary tract infections: A new paradigm for antimicrobial-resistant foodborne illness. *Frontiers in Microbiology, 4*(29).

Rock, A. (2015). "How Safe Is Your Ground Beef?" Last modified December 21st 2015. Retrieved from https://www.consumerreports.org/cro/food/how-safe-is-your-ground-beef

Shima, A., Hinenoya, A., Samosornsuk, W., Samosornsuk, S., Mungkornkaew, N., & Yamasaki, S. (2016). Prevalence of Providencia Strains among Patients with Diarrhea and in Retail Meats in Thailand. *Japanese Journal of Infectious Diseases, 69*(4), 323-325.

Shin, S. W., Shin, M. K., Jung, M., Belaynehe, K. M., & Yoo, H. S. (2015). Prevalence of Antimicrobial Resistance and Transfer of Tetracycline Resistance Genes in Escherichia coli Isolates from Beef Cattle. *Applied and Environmental Microbiology, 81*(16), 5560-5566.

USDA "Meat and Poultry Labeling Terms." Last modified August 10th, 2015. Retrieved from https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/food-labeling/meat-and-poultry-labeling-terms/meat-and-poultry-labeling-terms

USDA "Ground Beef and Food Safety." Last modified February 29th, 2016. Retrieved from https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/ground-beef-and-food-safety/CT_Index

USDA "FSIS Compliance Guideline for Minimizing the Risk of Shiga Toxin-Producing Escherichia coli (STEC) in Raw Beef (including Veal) Processing Operations. (2017)." Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/c1217185-1841-4a29-9e7f-8da6dc26d92c/Compliance-Guideline-STEC-Beef-Processing.pdf?MOD=AJPERES

Young, R. (Writer) & Hoffman, D.E. (Correspondent). (2014).The Trouble With Antibiotics [Television series episode]. In R. Young & A. Szulc (Producer), *Frontline*.