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Antibiotic Resistant *E. coli* in Chicken

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Abstract:

To understand the quality of grocery store bought chicken, this research determined if chicken samples from four commonly purchased brands were contaminated with antibiotic resistant Escherichia coli (E. coli). Chicken was placed into enrichment culture, samples were inoculated on plates with antibiotics and growth was measured. Results revealed that Gold'n Plump (antibiotic free) chicken had the least amount of antibiotic resistant bacteria followed by Whole Foods (antibiotic free), and Cub and Market Pantry (both conventionally raised) had the largest. Additionally, most chicken contained ampicillin resistant bacteria and gentamicin sensitive bacteria. A Kirby-Bauer assay was then performed, followed by the use of matrix assisted laser desorption/ionization and time of flight mass spectrometry (MALDI-TOF MS) technology. The Kirby-Bauer assay revealed that Market Pantry had bacteria resistant to the highest average number of drug classes. The MALDI-TOF MS reported that the majority of the bacteria in the chicken was E. coli. Given the results, it can be concluded that Market Pantry contained the highest amount of antibiotic resistant E. coli, and Whole Foods and Gold'n Plump contained the least.

Introduction

Antibiotics treat bacterial infections in humans and animals. Antibiotic resistance is a growing concern and judicious use of our valuable medications must be enforced to minimize it. Antibiotic resistance can result from spontaneous mutation or horizontal gene transfer in bacteria. Non-judicious use of antibiotics such as incorrect or widespread use can support antibiotic resistant bacteria, selecting for resistant genes that are already present in the population by removing non-resistant competitors. This can make it more difficult to find new and effective medications inexpensive enough for use by the general public (Bax and Griffin 2012). A potential source of antibiotic resistant bacteria is the animal industry. It is a common practice to feed high levels of antibiotics to animals for short durations to treat disease, which may contribute to resistance. It is also common to use subtherapeutic levels to promote growth and prevent disease.

Using antibiotic plates, a Kirby-Bauer assay and MALDI-TOF MS technology, grocery store chicken (half antibiotic free, half conventionally raised) was tested to look for the presence of the antibiotic resistant coliform E. coli. Theoretically, antibiotic free brands would not contain resistant bacteria and conventionally raised brands would. However, bacteria can transfer antibiotic resistance through conjugation, which can allow for the exchange of plasmids that carry resistance; therefore, bacteria do not have to be previously exposed to to antibiotics to become resistant. The bacteria present on the antibiotic free chicken could have easily acquired resistance from bacteria present on fomites, animal handlers or other sources. Analysis of the results obtained also indicated food quality. Meat containing coliforms, which are bacteria that live in the intestines, is potentially contaminated with fecal matter and could host pathogens that are transferred through the fecal oral route. Eating and handling meat, contacting animals, or contacting contaminated animal stool are ways antibiotic resistance can spread to humans (CDC 2017a).

Materials and Methods

To determine if chicken harbors antibiotic-resistant coliforms, MacConkey Agar (MAC) was selected as the broth to be inoculated. Coliforms are gram-negative, and the bile salts and crystal violet in MAC broth select against gram-positive bacteria. Chicken legs were selected from packages of Gold'n Plump, Whole Foods, Market Pantry and Cub chicken. Each leg was placed in a double Ziploc bag to prevent leakage of the broth. Approximately 50 ml of MAC broth was poured into the double-bag containing the chicken leg, which was then sealed tightly and gently massaged. The double-bag was then sealed inside of a small plastic box. Since non-fecal coliform has an ideal growth temperature range of 35-37°C, the chicken/broth was incubated at 44°C for 24 hours to select specifically for E. coli (fecal coliform) and kill other coliforms.

Three microfuge tubes each received 900 microliters of diluent, and a serial dilution was performed. Once the serial dilution was complete, 100 microliters from each of the three microfuge tubes were plated onto four MAC antibiotic plates (ampicillin, ceftriaxone, gentamicin, and tetracycline), and the 12 plates were incubated overnight at 37°C.

Next, the antibiotic plates were examined for coliforms (dark pink, lactose fermenting colonies), or tan colonies likely indicating Salmonella or Pseudomonas. Class results are summarized in Fig. 1. An isolation streak was then performed using resistant coliform colonies from Ampicillin and Ceftriaxone plates.

Under a biosafety cabinet, a saline suspension was made from the isolation streaks referenced above using a plastic disposable loop. Streaking was then performed on a large Mueller Hinton (MH) plate. The MH plate is commonly used in Kirby-Bauer assays because it is non-selective and non-differential. Once inoculated with saline suspension of the isolate, each MH plate was stamped with the antibiotics in Table 1. Results from the assay are represented in Fig. 2. From the same saline suspensions for the two chosen isolates, isolation streaks were prepared on blood agar plates (BAP) and incubated at 37°C overnight. BAP were used because they typically yield more accurate MALDI-TOF-MS results.

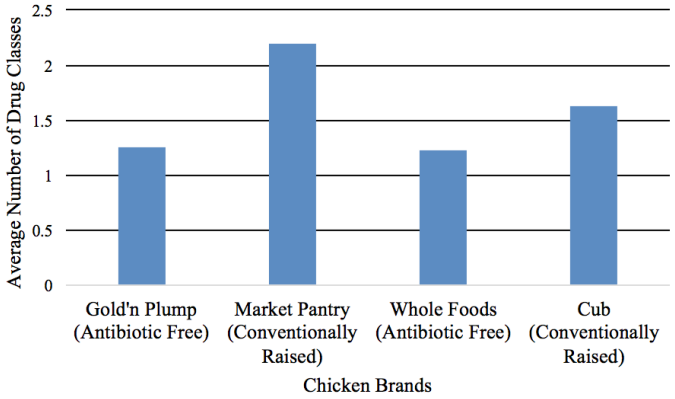

Figure 2. Antibiotic Resistance in Chicken. The graph displays the average number of drug classes an isolate in each chicken brand was resistant to. Twenty samples were Gold'n Plump, 20 samples were Market Pantry, 18 samples were Whole Foods and 16 samples were Cub.

Results

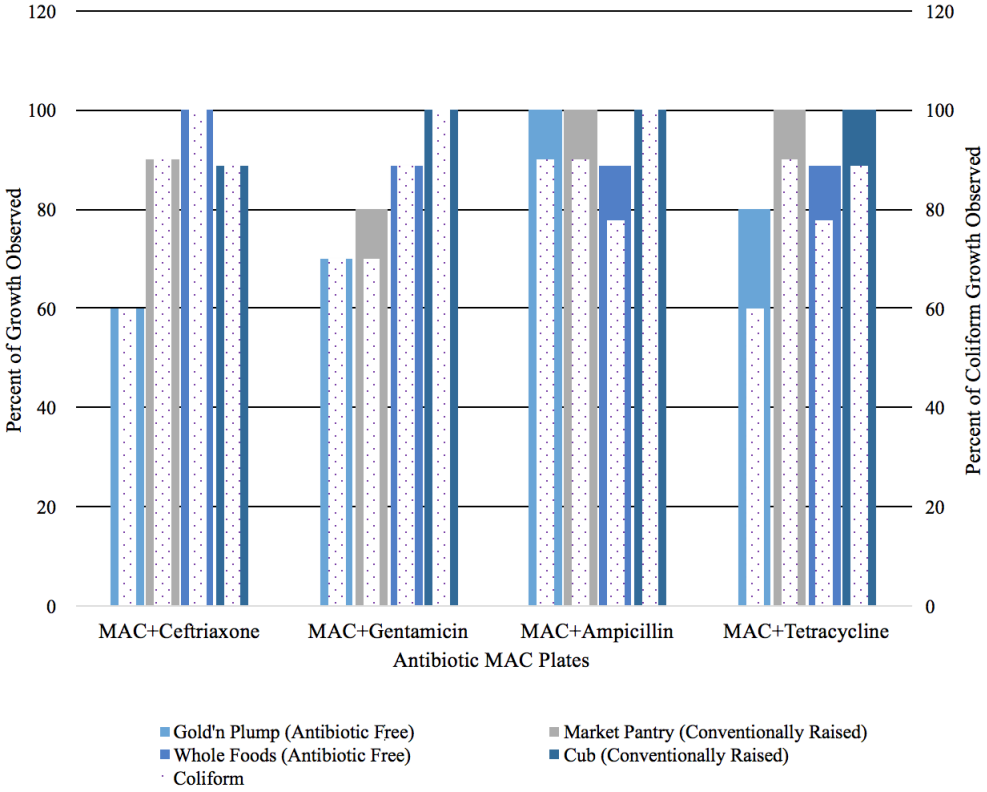

Figure 1. Amount of Bacteria and Coliform Growth From Chicken on Antibiotic Plates. The graph displays percent of total bacterial and coliform growth observed on chicken once bacterial samples from chicken were plated on antibiotic MacConkey (MAC) plates. Percentages were obtained from observing growth on ten Gold'n Plump and Market Pantry samples, and nine Whole Foods and Cub samples. The speckling, representing coliform growth, corresponds to the chicken sample it's superimposed onto.

Table 1. The Antibiotics Used In The Kirby-Bauer Assay and Corresponding Drug Class

Antibiotics Used in the Kirby-Bauer Assay	Drug Class
Sulfamethoxazole-Trimethoprim	Sulfonamides
Amoxicillin/Clavulanic Acid	Beta Lactams
Ciprofloxacin	Fluoroquinolones
Nitrofurantoin	Nitrofurans
Levofloxacin	Fluoroquinolones
Ampicillin	Beta Lactams
Tetracycline	Tetracyclines
Ceftriaxone	Beta Lactams
Gentamicin	Aminoglycosides

Discussion

Fig. 1. shows that samples from Gold'n Plump contained the least bacterial growth on the MAC plates; therefore, it contained the least amount of resistant bacteria as would be predicted since it is antibiotic free. Fig. 1. reveals that samples taken from both conventionally raised chicken brands contained the largest amount of growth, with Cub exceeding Market Pantry. Samples from antibiotic free chicken were more susceptible to antibiotics while samples from conventionally raised chicken contained a greater amount of antibiotic resistant bacteria. Fig. 1. also suggests most chicken contained ampicillin resistant bacteria with least resistance to gentamicin. In contrast, a similar study found that beef raised without antibiotics had a similar amount of antibiotic resistant bacteria as beef raised conventionally, indicating that a reduction in antibiotic use in food production may not reduce antibiotic resistance as much as thought (Vikram et al. 2017).

Table 1. states that ampicillin, the drug that most of the the bacteria in the chicken samples are resistant to, is a beta lactam. Therefore, the bacteria may be resistant to other forms of beta-lactam antibiotics, which inhibit cell wall synthesis. Additionally, since the bacteria was least resistant to gentamicin, Table 1. can be used to conclude that perhaps the bacteria is sensitive to other aminoglycosides.

Fig. 2. reveals that bacteria in Market Pantry and Cub (both conventionally raised) were resistant to the highest average number of drug classes compared to Gold'n Plump and Whole Foods (both antibiotic free). Fig. 2. also indicates that conventional chicken is more likely to contain antibiotic resistant bacteria in comparison to chicken raised without antibiotics. Despite being antibiotic free, bacteria from Gold'n Plump and Whole Foods also had multidrug resistance. This was likely achieved through random mutation or conjugation. In fact, plasmid transfer rates through conjugation are highest in the absence of antibiotics, which might explain the multidrug resistance seen in the samples from chicken raised without antibiotics (Handel et al. 2015).

Table 2. reveals the majority of the bacteria in the chicken was E. coli. Additionally, Table 2. reveals that Gold'n Plump had the most variety in the bacterial species it contained. The primary bacteria found in the chicken were coliform, suggesting possible fecal matter contamination.

This research indicates that chicken raised conventionally contains a greater amount of antibiotic resistant bacteria than chicken raised antibiotic free, suggesting the use of antibiotics in food production contributes to antibiotic resistant bacteria. Our relationship with antibiotics invites us to reach out to subject experts to understand ways in which we can limit their use. To stop the spread of antibiotic resistant bacteria in meat products, the CDC recommends consumers wash hands and cooking surfaces/utensils before and after handling raw meat, separate raw meat and fish from other food products, cook meat to the right temperature and refrigerate promptly (CDC 2017b). Although consumers may be aware of the proper practices when handling raw meat, a systematic review of qualitative research has shown that consumers are generally unconcerned about food safety (Young and Waddell 2016). Additionally, general knowledge about these practices alone is unlikely to change habits. The systematic review also suggests that relevant social pressure from healthcare professionals and others may impact behavior. This research asserts the need for education by food safety and medical professionals on how to handle raw meat in order to stop the spread of antibiotic resistant bacteria (Young and Waddell 2016).

In conclusion, our data indicate that conventionally raised chicken contains higher levels of antibiotic resistant bacteria, specifically the coliform E. coli. These findings suggest that antibiotic use in agriculture should be regulated more carefully.

Bacteria	Cub	Whole Foods	Market Pantry	Gold'n Plump
Escherichia coli	100 %	95.83 %	88.89 %	55.26 %
Citrobacter braakii	0 %	0 %	5.56 %	10.53 %
Citrobacter freundii	0 %	0 %	5.56 %	0 %
Escherichia hermannii	0 %	0 %	0 %	5.26 %
Acinetobacter baumannii	0 %	0 %	0 %	5.26 %
Low Value/No Data	0 %	4.17 %	0 %	23.68 %

Table 2. Bacterial Identity In Chicken, Based On Enteropluri Tube and MALDI-TOF MS

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