Antibiotic Susceptibility of non-pathogenic *Escherichia coli* from meat and produce available in the Chobe region of Botswana

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Introduction & Background

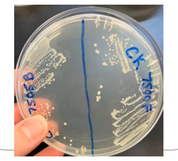
Antibiotic resistant bacteria have been rising in numbers, leading to a food safety problem due to the use of antibiotics to prevent disease, or promote growth, in feed animals. It is reported that 66% of antibiotics produced worldwide are being used on healthy animals, in preparation for food production. Foodborne antibiotic resistance is an important topic to understand since the resistant bacteria can be passed through the food chain into the gut microbiome of humans, and through our feces, then back into the environment.

Objective: This research aimed to investigate the susceptibility of *Escherichia coli* isolated from produce and meats available for purchase in the Chobe region of Botswana, against different antibiotics. The goal is to determine the presence, or absence, of a pattern of antibiotic resistance among produce and meats contaminated by non-pathogenic *E. coli*.

Methods

Day 1

- Bacterial cultivation – *E. coli* isolates were streaked for isolation onto 100 mm tryptic soy agar (TSA) petri plates and incubated at 35° Celsius for 20-22 hours.
- *E. coli* ATCC 25922 strain was used for quality control.



Day 2

- Antibiotic resistance testing by disk diffusion – using the Kirby-Bauer susceptibility protocol, one isolated colony was picked and mixed with PBS, then compared to a 0.5 McFarland standard. A lawn was streaked onto a 150mm Mueller Hinton Agar plate, then 12 antibiotics were distributed evenly around the plate. Incubated at 35° Celsius for 18-20 hours.

Day 3

- The zones of inhibition surrounding the antibiotic disks were measured and each isolate was characterized as susceptible (S), resistant (R), or intermediate (I), according to the CLSI guidelines.

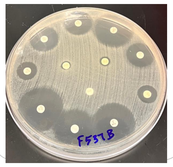


Table 1: Twelve antibiotics tested on *E. coli* isolates with abbreviations, class, concentrations, and susceptibility standards assigned by CLSI standards.

Antibiotic	Abbrev.	AB Class	Concentration	Susceptible Standard (mm)	Intermediate Standard (mm)	Resistant Standard (mm)
Ampicillin	AM10	Beta-lactam	10 µg	≥17	14-16	≤13
Amoxicillin/ Clavulanic Acid	AMC30	Beta-lactam	20/10 µg	≥18	14-17	≤13
Azithromycin	AZM15	Macrolide	15 µg	≥13	N/A	≤12
Chloramphenicol	C30	Phenicol	30 µg	≥18	13-17	≤12
Ciprofloxacin	CIP5	Quinolone	5 µg	≥31	21-30	≤20
Cefotaxime	CTX	Cephem	30 µg	≥26	23-25	≤22
Doxycycline	D30	Tetracycline	30 µg	≥14	11-13	≤10
Gentamycin	GM10	Aminoglycoside	10 µg	≥15	13-14	≤12
Meropenem	MEM10	Carbapenem	10 µg	≥23	20-22	≤19
Streptomycin	S10	Aminoglycoside	10 µg	≥15	12-14	≤11
Sulfamethoxazole /Trimethoprim	SXT	Sulfonamide	23.75/1.25 µg	≥16	11-15	≤10
Tetracycline	TE30	Tetracycline	30 µg	≥15	12-14	≤11

References

Clinical and Laboratory Standards Institute. M100 Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Clinical and Laboratory Standards Institute. Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2018.

Results

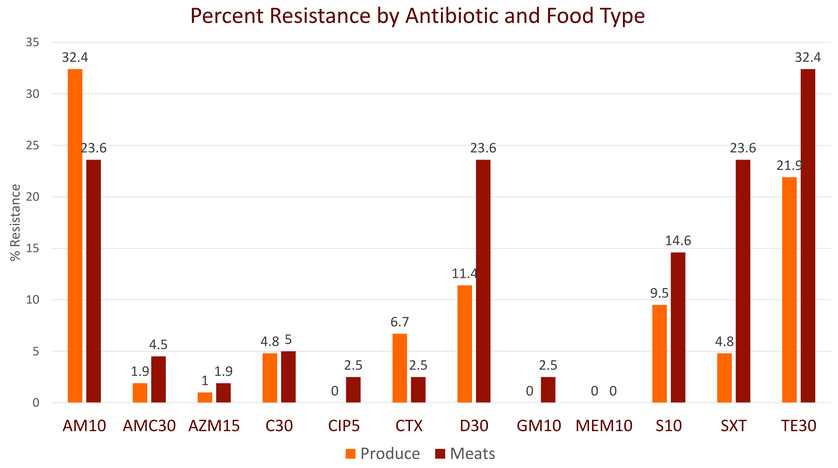


Figure 1: Results of antibiotic resistance testing for both produce (105) and meat (157) samples. Number of isolates characterized by their zones of inhibition as resistant in the form of percent resistance from the total.

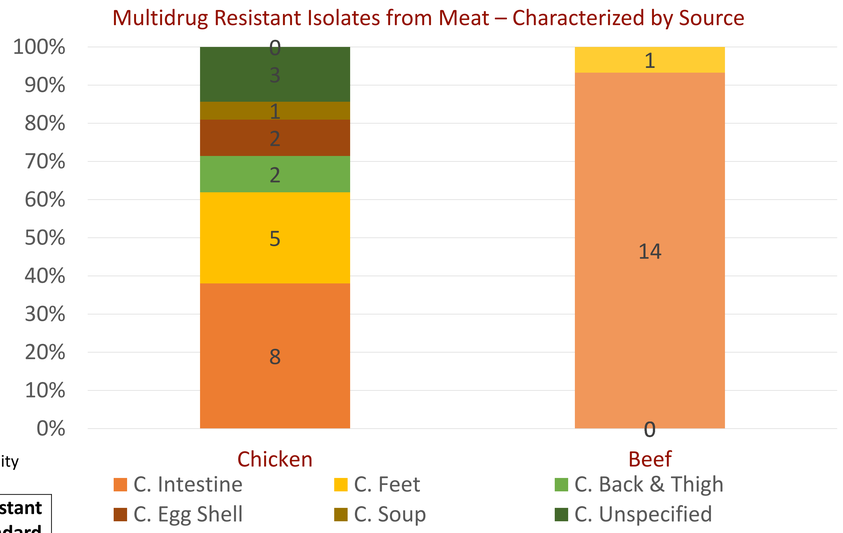


Figure 3: Multidrug-resistant isolates from meat characterized by source, either chicken or beef. 15 of 36 isolates were found in beef samples, 21 of 36 isolates were found in chicken samples.

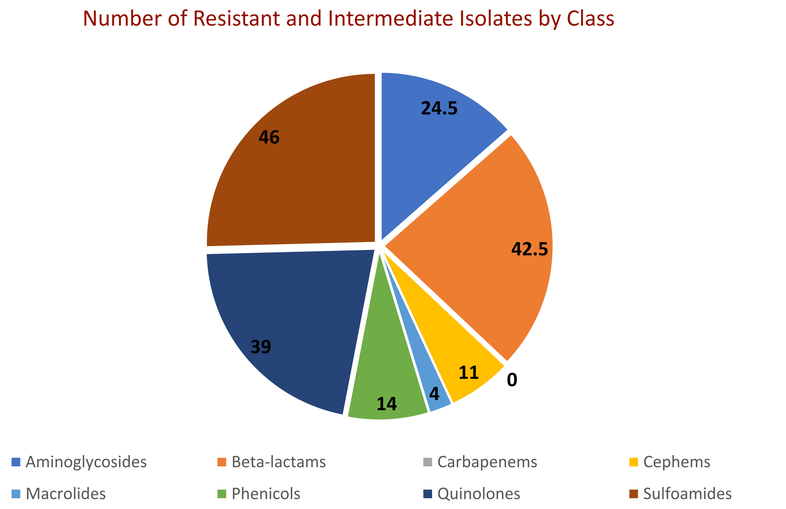


Figure 4: Pie chart of resistant and intermediate isolates, from produce and meat combined (262), organized by class. Tetracycline and Aminoglycoside classes were averages

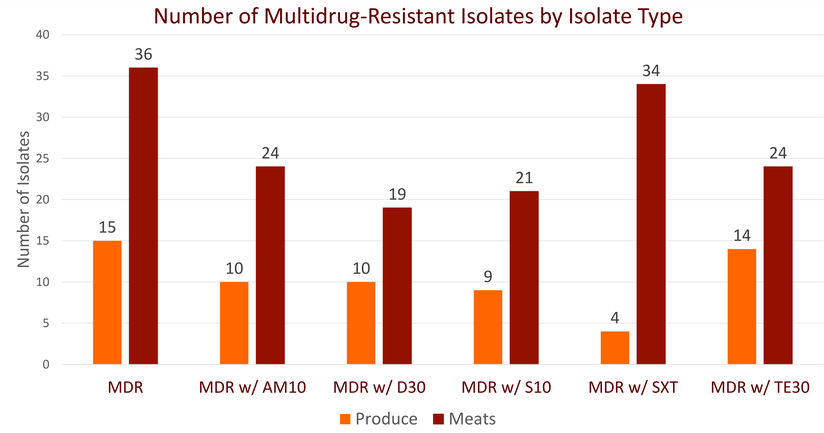


Figure 2: Multidrug-resistant isolates characterized by the antibiotic included in the resistance. Multidrug-resistant isolate is defined as resistance shown against at least 3 different antibiotics.

Main Takeaways

Resistance Patterns

- The data indicated multiple different resistance patterns across *E. coli* collected from food samples
- Multidrug resistant E. coli was more prevalent in meat samples over produce samples, with more sources coming from chicken over beef
- Produce showed less of an overall presence of antibiotic-resistant bacteria compared to samples collected from meat
- The *E. coli* that were multidrug-resistant were not resistant to the same classes of antibiotic in every case. This indicates they are different strains.
- It was interesting to find the high presence of sulfamethoxazole/trimethoprim resistant bacteria in meat samples while there was a low amount present in produce samples

Importance of the data

- The results of this study show the importance of proper cooking methods of meat products, especially chicken and beef, to 165°F in order to kill possible antibiotic resistant bacteria lingering in raw food
- To the same point, the presence of antibiotic resistant bacteria in produce is just as shocking, indicating that caution should be taken into account by individuals that may be most harmed by illnesses due to antibiotic resistant bacteria.

Antibiotic Resistance

- Resistance against ampicillin was most commonly found among produce
  - One in three isolates were resistant to AM10
- Tetracycline resistance was high in meat samples at 32.4%
  - One in three isolates were resistant to TE30
  - One in four isolates were resistant to ampicillin, doxycycline, or sulfamethoxazole/trimethoprim

Resistance by class

- Aminoglycosides
  - Out of all the food isolates tested, if the *E. coli* was resistant to gentamycin, it was also resistant to streptomycin 100% of the time
- Beta-lactams
  - Out of all the food isolates tested, if the *E. coli* was resistant to amoxicillin/clavulanic acid, it was also resistant to ampicillin 89% of the time
- Tetracyclines
  - Out of all the food isolates tested, if the *E. coli* was resistant to doxycycline, it was also resistant to tetracycline 100% of the time

Acknowledgements

Funding for this work was provided in part, by the National Science Foundation under award number 2009717, and by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture