

Prevalence and antimicrobial resistance of high concern Gram-negative pathogens isolated from distinct broiler production systems in the Southeastern United States

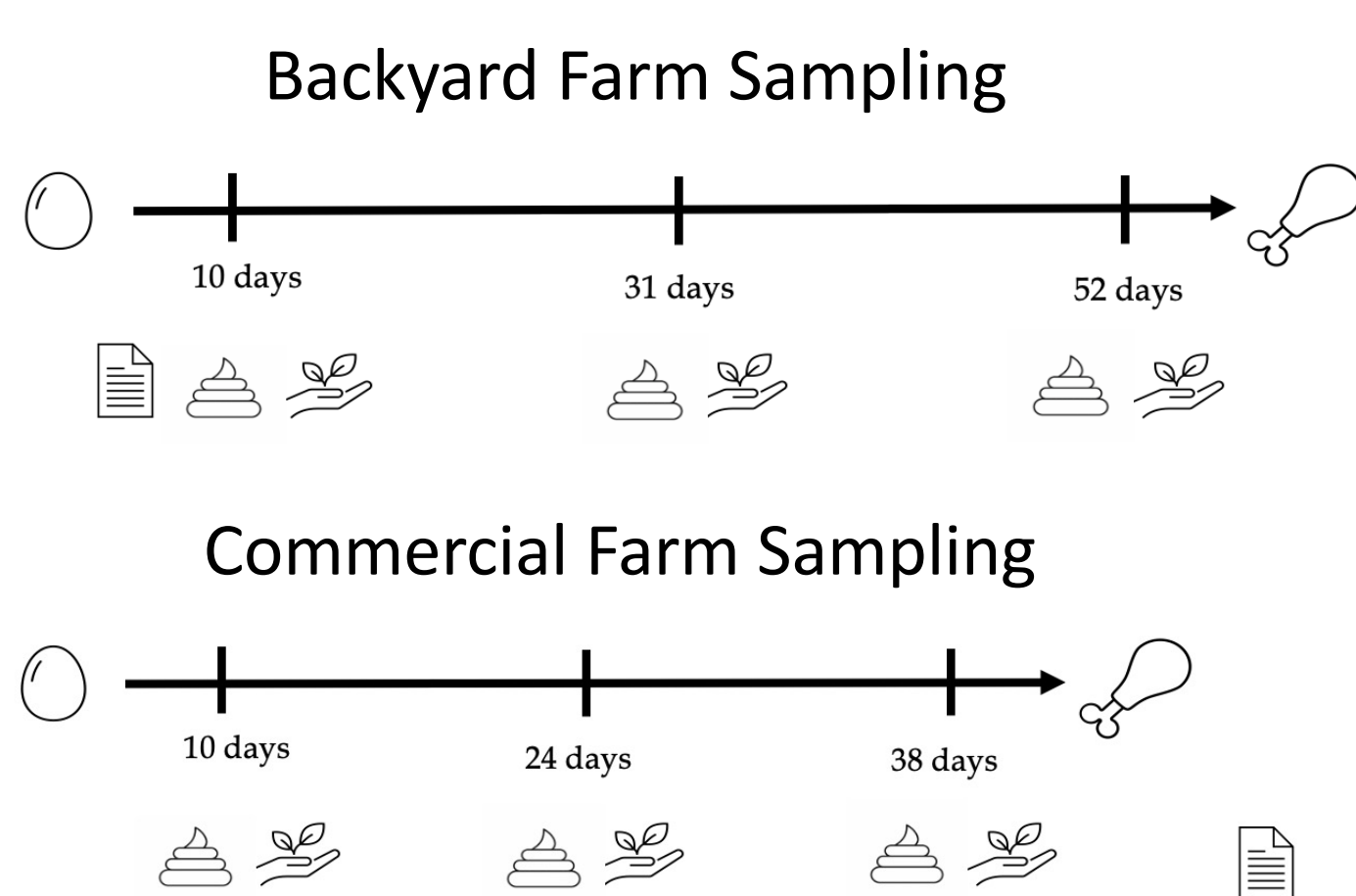
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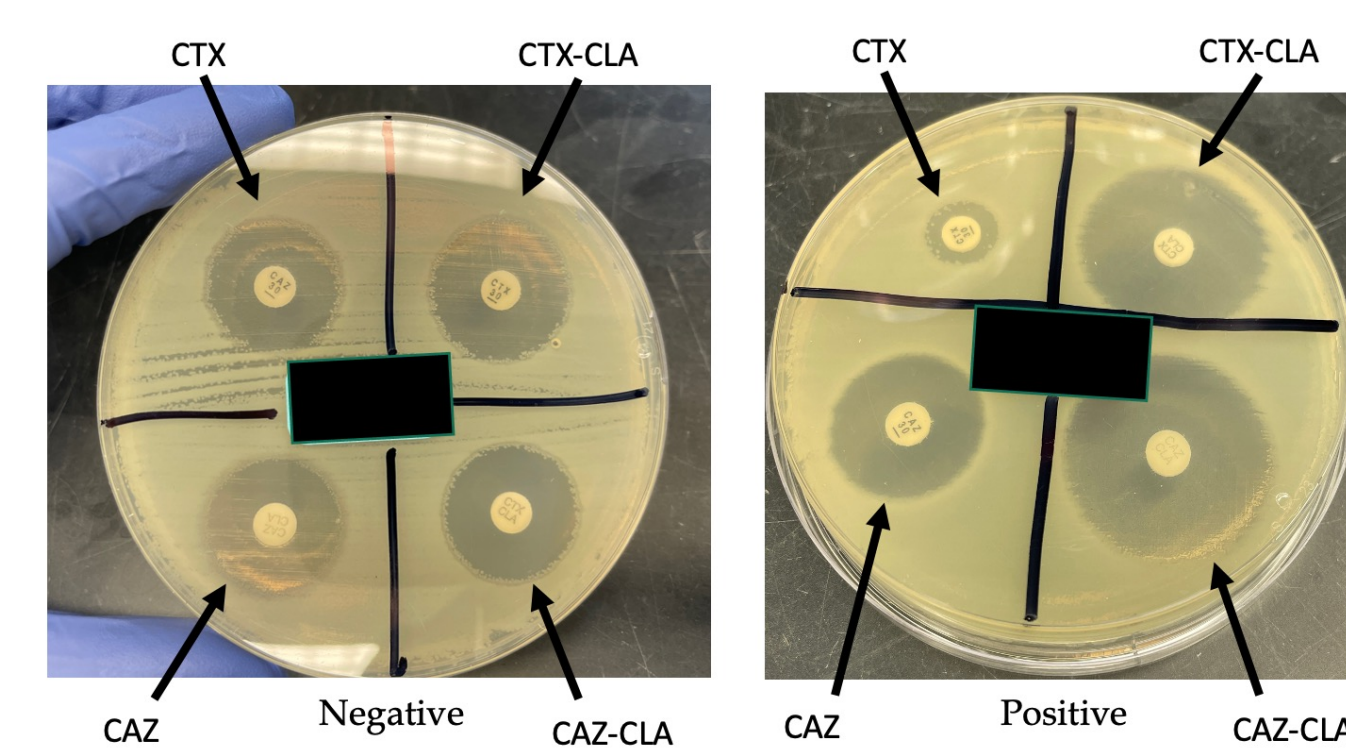
Background

- ❖ Broiler chicken is the number one consumed meat in the United States.¹
- ❖ Popularity of owning backyard poultry has greatly increased given consumer demand for local foods.²
- ❖ Chickens are known to be a reservoir for antimicrobial resistant pathogens as exhibited by the CDC reported *Salmonella* outbreaks yearly from 2019-2022.³
- ❖ Other high concern pathogens include *Campylobacter* and extended-spectrum beta-lactamase (ESBL) *E. coli*. ESBL pathogens are resistant to commonly used antimicrobials in medicine.⁴
- ❖ Due to lack of research focusing on pathogens in backyard poultry production, there is little comparative data between backyard and commercial farms.
- ❖ This study aims to compare prevalence and antimicrobial resistance of pathogens from various samples on backyard and commercial farms.
- ❖ A better understanding of antimicrobial resistant pathogen persistence in these environments is important to improve food and production safety.

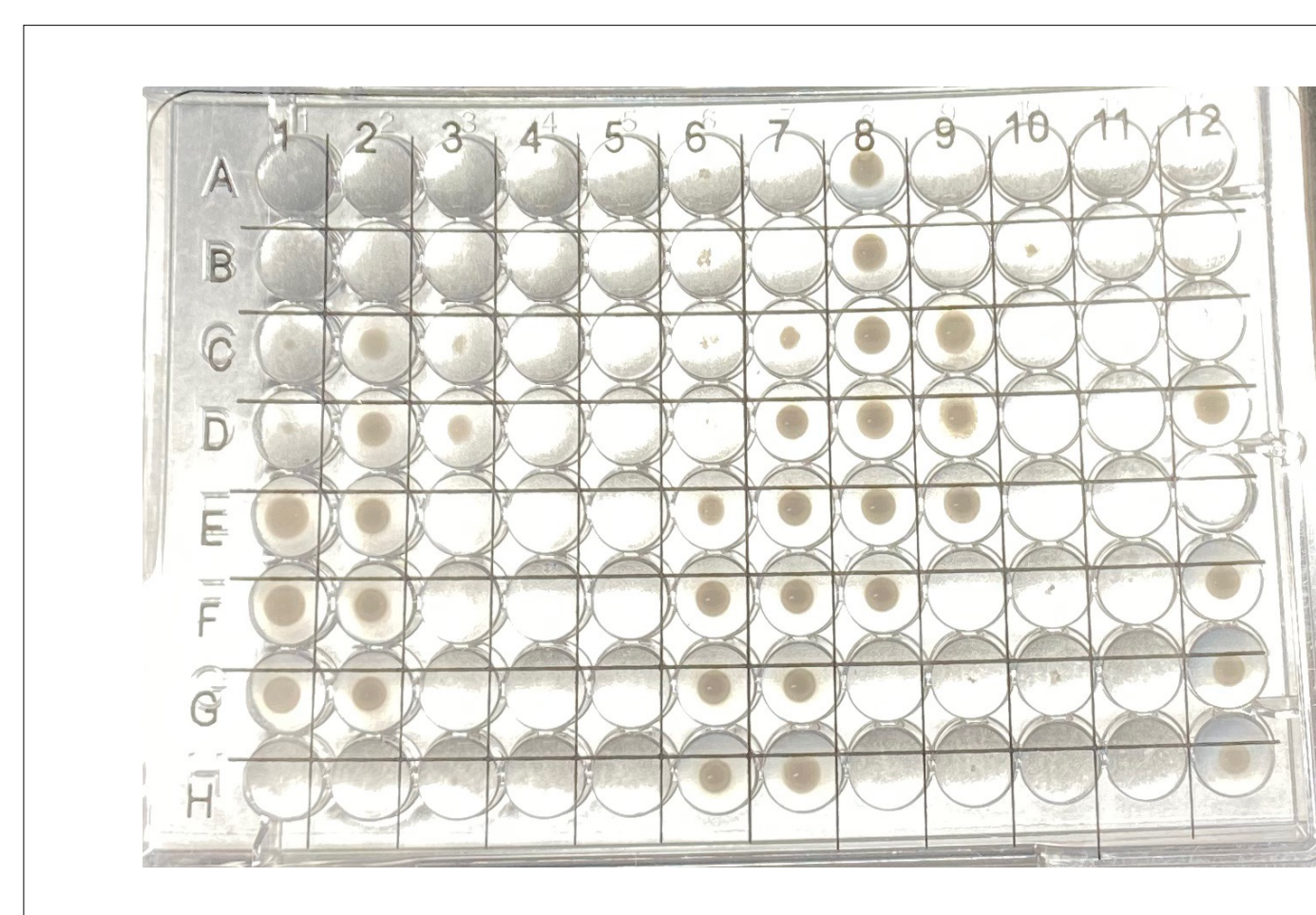
Methods



Sampling Scheme. This shows what days of production we sample each type of farm. Birds on commercial farms move through production faster so the timeline is shortened. Both fecal and environmental samples are collected at each of the 3 visits. Processing procedure for pathogens was adopted from the National Antimicrobial Resistance Monitoring System (NARMS) protocol. A questionnaire was also used to record farm management practices.



Disc Diffusion. This assay tests *E. coli* isolates for the ESBL phenotype. The discs used are 3rd generation cephalosporins alone (cefotaxime (CTX) and ceftazidime (CAZ)) as well as those in combination with a beta-lactamase inhibitor (clavulanic acid (CLA)). Positive ESBL phenotypes are identified by a zone of inhibition around the antimicrobial with clavulanic acid of 5mm or greater than that of the antimicrobial alone.



| Plate Code: | CMV3AGNF | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-------------|----------|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|----|----|
| F0X | A21 | ORL | AKO | AVO | CIP | GEN | NAL | XNL | FIS | AMP | STR | | |
| F0X | A21 | ORL | AKO | AVO | CIP | GEN | NAL | XNL | FIS | AMP | STR | | |
| F0X | A21 | CHL | AKO | AUS2 | CIP | GEN | NAL | XNL | FIS | AMP | STR | | |
| F0X | A21 | CHL | AKO | AUS2 | CIP | GEN | NAL | XNL | FIS | AMP | STR | | |
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| F0X | A21 | TET | AKO | AUS2 | CIP | GEN | NAL | XNL | FIS | AMP | STR | | |
| F0X | A21 | TET | AKO | AUS2 | CIP | GEN | NAL | XNL | FIS | AMP | STR | | |
| A21 | CHL | TET | AKO | CIP | CP | NAL | XNL | FIS | EXT | STR | POS | | |
| A21 | CHL | TET | AKO | CIP | CP | NAL | XNL | FIS | EXT | STR | POS | | |

Antimicrobial Susceptibility Testing (AST). A broth microdilution method was used to determine the minimum inhibitory concentration for each isolate and antimicrobial concentrations. NARMS and CLSI breakpoints were applied to determine if isolates were resistant, susceptible, intermediate, or susceptible dose-dependent.

| | | | |
|--------------|--|----------------------------|---|
| Resistant | → Therapeutic failure likely. ⁵ | Susceptible | → Therapeutic success likely. ⁵ |
| Intermediate | → Therapeutic effect uncertain. ⁵ | Susceptible Dose-Dependent | → Effects change with dose or frequency (multiple dosing options exist). ⁵ |

Results

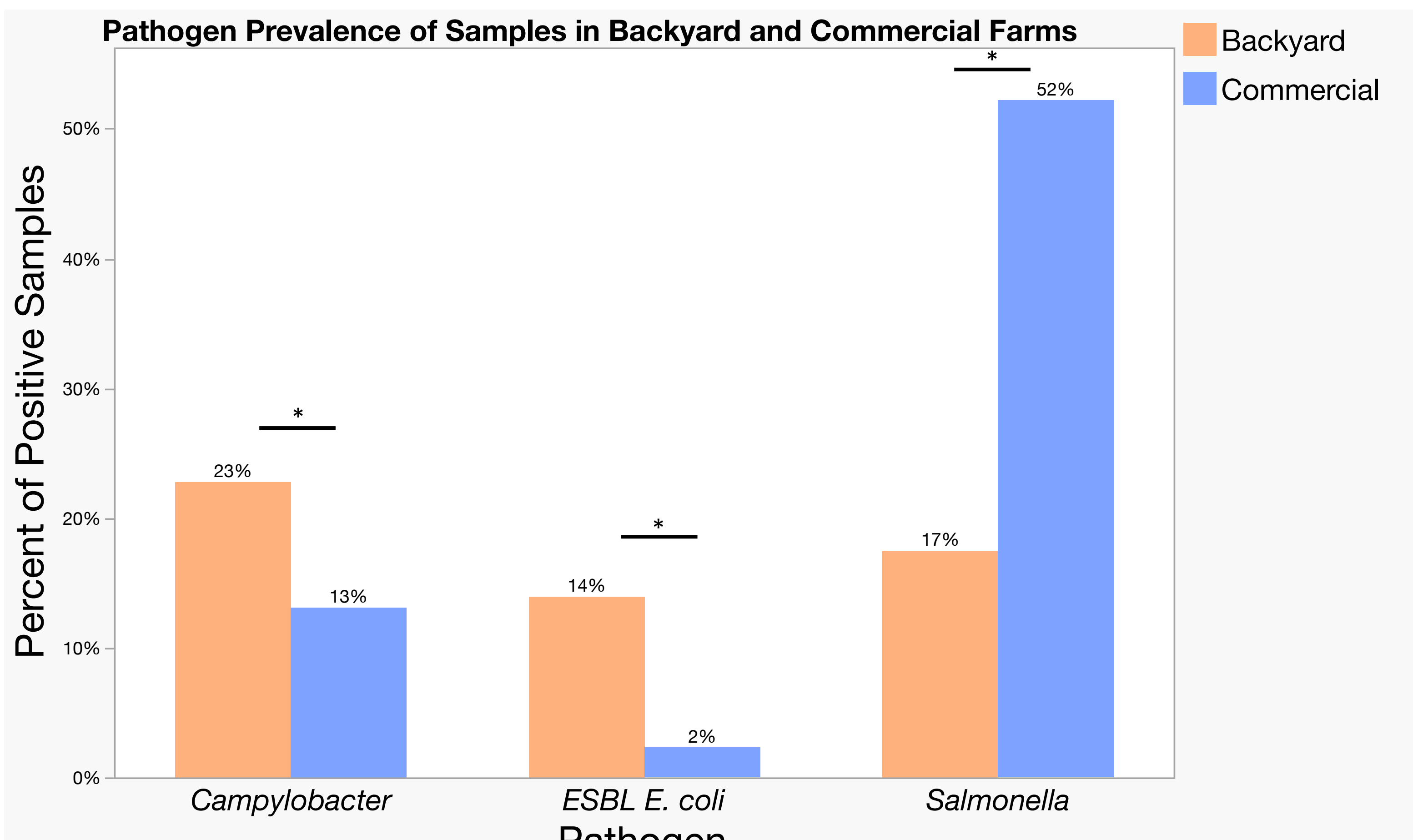
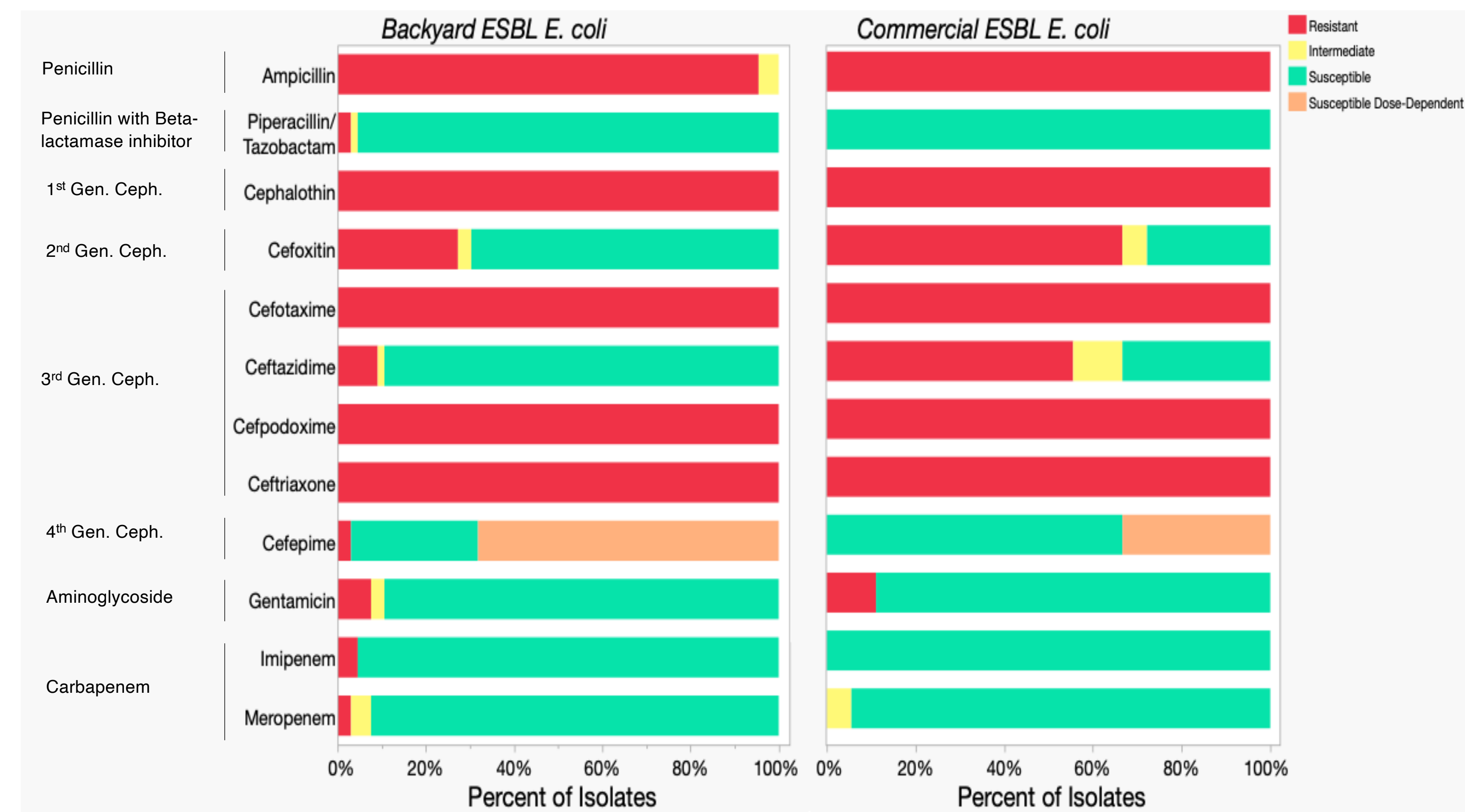


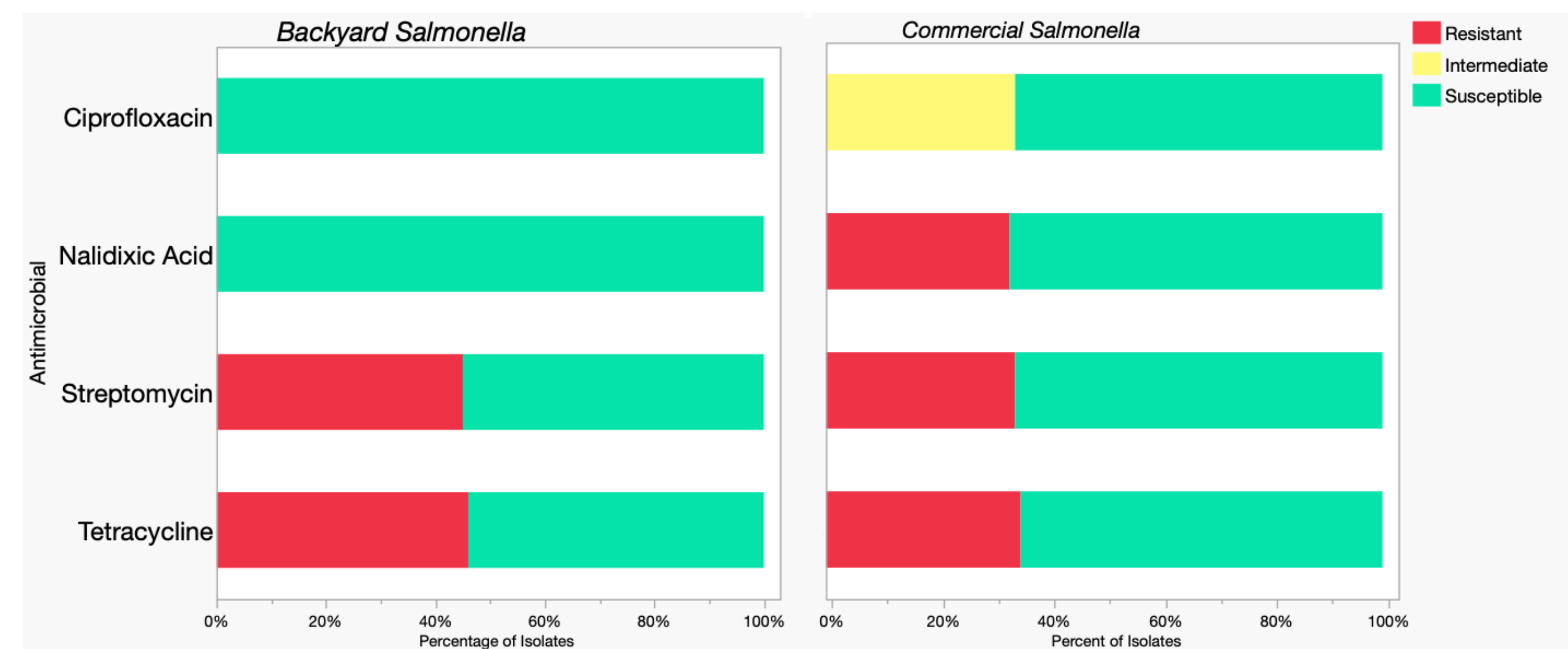
Figure 1. Pathogen Prevalence. Bar chart shows the differences between commercial (N=10) and backyard (N=8) farm pathogen prevalence. For all pathogens, percentages of pathogen prevalence were significant in relation to farm type (Chi-squared test, p-value= .05). Percentages were also found to be significantly different between each other (Two proportion Z-test, * = p-value less than .05).



Key Points:

- * Cephalosporins are grouped into 5 generations. Each generation aims to increase its coverage, particularly with Gram-negative infections in the 3rd and 4th generations (considered "extended-spectrum").⁷
- * Resistance was found to Penicillin, 1st, 2nd, 3rd generation Cephalosporins and potentially growing resistance to 4th generation Cephalosporin, Cefepime. This is expected with ESBL pathogens.⁸
- * Resistant and intermediate isolates were associated with last resort antimicrobials, Carbapenems. This is a major concern with ESBL pathogens that is being witnessed here in backyard farm and commercial broiler environments.⁸

Figure 2. ESBL E. coli Antimicrobial Susceptibility Testing. Stacked bar chart shows the proportion of ESBL *E. coli* isolates that are resistant, intermediate, susceptible, or susceptible dose-dependent to the presented antimicrobials on the ThermoFisher ESBL Plate (ESB1F). N= 66 backyard and 18 commercial isolates.



*All backyard and commercial farm isolates were susceptible to Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin, Cefiturox, Ceftriaxone.

*Additional resistance found in commercial isolates (percent of isolates): 0.25% Cefoxitin, 0.49% Gentamicin, 8.85% Trimethoprim/Sulfamethoxazole, 33.66% Sulfisoxazole, 9.09% Chloramphenicol

Key Points:

- *Ciprofloxacin and Nalidixic acid are considered first-line antimicrobials for treating *Salmonella* infection in humans.^{9,10} All *Salmonella* isolates show susceptibility to these antimicrobials in backyard farms but not in commercial.
- *Streptomycin and Tetracycline resistance is evident in both backyard and commercial farms. This is consistent with findings from 2022 CDC reported backyard *Salmonella* outbreak.³
- *101 Commercial *Salmonella* isolates are deemed multi-drug resistant (MDR), meaning they are resistant to three or more classes of antimicrobials. Resistance to the quinolone, aminoglycoside, sulfonamide, and tetracycline class were common among MDR isolates.

Figure 3. Salmonella Antimicrobial Susceptibility Testing. Stacked bar chart showing the percentage of *Salmonella* isolates that are resistant, intermediate, or susceptible to the presented antimicrobials on the ThermoFisher Gram Negative NARMS Plate (CMV3AGNF). N=128 backyard and 407 commercial isolates.

Summary

- ❖ To our knowledge, this is the first study to investigate prevalence and antimicrobial susceptibility testing of *Salmonella*, *Campylobacter*, and ESBL *E. coli* isolated from backyard and commercial broiler farms in the Southeastern United States.
- ❖ High concern pathogens are prevalent in both bird fecal and environmental samples of both farm types.
- ❖ Multi-drug resistant *Salmonella* were found in commercial farms.
- ❖ Resistance to last resort antimicrobials, carbapenems, was found in ESBL *E. coli* isolates.
- ❖ The results from this study are important from a One Health perspective, as it has implications for human, animal, and environmental health alike.
- ❖ Future Directions: *Campylobacter* AST, finish sampling and AST for all 10 backyard farms, risk factor assessment based on questionnaire responses

Acknowledgements

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