Antimicrobial Resistant E. coli from European Starlings in Concentrated Animal Feeding Operations

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Introduction
The use of antibiotics in animal agriculture for disease prevention, therapy, and growth promotion is generally recognized to be linked to increased antimicrobial resistance (AMR) in associated bacterial communities. A complex microbial ecology exists at the interface of livestock, with numerous inputs and outputs that contribute to AMR in these systems. Of particular interest, wildlife found in association with animal production facilities have been shown to harbor gut bacteria that have similar types of AMR to bacteria present in livestock. As a result, these wildlife species are potential reservoirs and reintroduce AMR into livestock production, and through their normal movement and migratory patterns they can spread AMR bacteria on local-to-global spatial scales.

Bird species that are in frequent contact with livestock production may play the most important role in the dissemination and propagation of AMR of any other type of wildlife given their large host ranges. European starlings (Sturnus vulgaris) are of particular concern, as they can congregate in large flocks in and around animal production facilities—with roosts reaching as many as 50,000 to 100,000 birds. They also travel long distances, coming in frequent contact with livestock in pastures and feedlots, especially in the absence of natural food sources.

Objectives
The objectives of this study are to (1) determine the prevalence and antimicrobial susceptibility profiles of AMR in the indicator bacteria Escherichia coli (E. coli) isolated from European starlings associated with concentrated animal feeding operations (CAFOs); and (2) establish prevalence of important genetic determinants linked to resistance of β-lactam antibiotics, which are considered priority drugs for both animal and human health.

Materials and Methods
Feedlots with severe European starling infestations (e.g., experiencing more than 10,000 starlings per day) were identified in previous studies. All feedlots were located in major beef cattle production areas in the U.S., including Colorado, Iowa, Kansas, Missouri, and Texas. Up to 30 European starlings were collected at each livestock facility. Contents of starling gastrointestinal tracts were plated onto microbiological media containing the antibiotics cefotaxime (CTX) or ciprofloxacin (CIP), which allowed our team to isolate E. coli resistant to these drugs (Fig 1).

Confirmation of bacteria as E. coli was accomplished using matrix-assisted laser desorption/ionization biotyping (MALDI-TOF MS), a method that allows for rapid identification of large numbers of bacterial isolates. Rapid identification of bacteria via MALDI-TOF MS is performed by direct comparisons of mass spectra obtained from pure isolates on agar plates to spectra from known bacterial standards contained in a mass spectral reference library. Next, susceptibility of the E. coli to important antimicrobials was determined using disk diffusion (a classic phenotypic method for characterizing AMR). This method involves utilization of disks embedded with antibiotics placed directly on an agar surface containing a bacterial isolate. The antibiotics subsequently diffuse into the agar. Bacteria that can grow in close proximity to the disk are generally considered resistant—because they are still able to grow when exposed to high concentrations of the antibiotic. In contrast, where there is a large zone of no bacterial growth around the disk, the bacteria are susceptible to the antibiotic—because they are not able to grow when exposed to high concentrations of the antibiotic.

In this study, determination of similarity between bacterial isolates was an important task because it allows for tracking of routes of transmission and spread of bacteria between different environments. To accomplish this, polymerase chain reaction (PCR) was used to detect genes conserved in similar isolates (phylogenetic
typing). PCR was also used to determine the presence of genes that encode β-lactamases, which often mediate resistance to β-lactam antibiotics in these bacteria. The β-lactam category of antibiotics includes antibiotics important in both human and veterinary medicine (e.g., penicillin).

**Results and Discussion**

Of the 238 total *E. coli* isolates available in this study, most were resistant to eight or more different types of antibiotics. More than 97% of the isolates were classified as multi-drug resistant (MDR), which is defined as resistance to three or more antibiotic classes. Grouped by drug classes (14 total drug classes tested), a majority of the isolates were resistant to six or more drug classes.

The overwhelming majority of isolates were found to have AMR to penicillins (ampicillin and piperacillin), and a first-generation cephalosporin (cefazolin). Additionally, resistance to second-generation cephalosporins, third-generation cephalosporins, and monobactams (aztreonam) was observed. Cephalosporins and monobactams are β-lactam antibiotics, but due to structural differences they commonly are more resistant to most β-lactamases compared to penicillins. Genes responsible for resistance to the β-lactam antibiotics were also found in the characterized bacterial isolates, with three different types of genes that are known to easily transfer between bacteria, thereby spreading AMR in microbial communities.

This study showed that *E. coli* isolated from starlings associated with livestock production/CAFOs contain AMR phenotypes that are important in animal and human health. Among them were resistances to fluoroquinolones (ciprofloxacin and nalidixic acid) and β-lactam antimicrobials, including important classes of antibiotics such as third-generation cephalosporins. Additionally, we determined the presence of three important genes conferring resistance to β-lactams in these isolates.

European starlings are already considered invasive species, and starling control is actively being carried out on many agricultural operations; however, this study provides additional rationale for livestock producers, scientists, local, state, and federal officials, and other stakeholders to actively continue collaborating to mitigate the starling problem in CAFOs.

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