Identifying source and dissemination pathways of antimicrobial resistance on dairies

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Introduction

The goal of the research was to assess an approach to determine source and dissemination pathways for antimicrobial resistant (AMR) *Escherichia coli* in dairy environments. If the source of AMR bacteria on dairies and the path of transmission can be identified, then steps can be taken to limit its generation and spread into niches that would not otherwise be populated by AMR *E. coli*, including into the human population. The objectives of this project were to determine the intensity of sampling needed to measure diversity of isolate resistance and assess transmission dynamics within a dairy farm.

Materials and Methods

Sampling

This study is field-based; working with commercial dairy herds that maintain both a milking herd and rear their replacement animals on the same physical site and house at least 200 preweaned calves. For this pilot study, all isolates were from fecal samples taken from a single commercial dairy herd in central Washington. We defined 8 production niches based on housing and function: preweaned calves, weaned calves, breeding age heifers , early lactation (fresh) cows, lactating cows, non-lactating (dry) cows, lactating cows to be sold ("do not breed"=DNB), and cows in the hospital pen. Our on-farm sampling target was 9 animals per niche and 4 isolates per animal. A minimum of 3 samples were taken from each pen that housed animals.

E. coli isolation

We used *E. coli* as our model bacterium for resistance phenotypes.

1. ~ 0.10 grams of fecal sample diluted to 10^{-5} in sterile saline, plated to MAC
2. Incubated 18-24 hours at 37°C, randomly selected 8 lactose positive colonies from each plate to Columbia blood agar
3. From the blood agar, 4 oxidase test negative and indole test positive isolates were tested for susceptibility to 15 antibiotics
4. Resistance phenotypes were generated by concatenating minimum inhibitory concentration (MIC) results

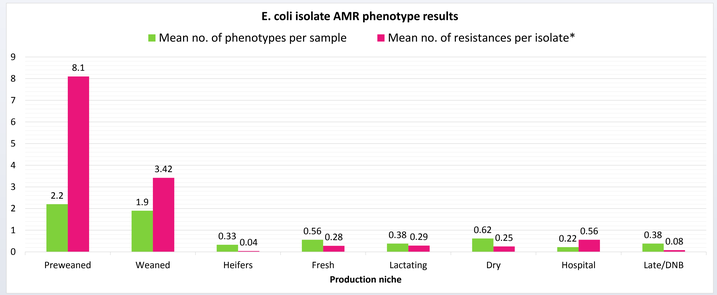
Data analysis

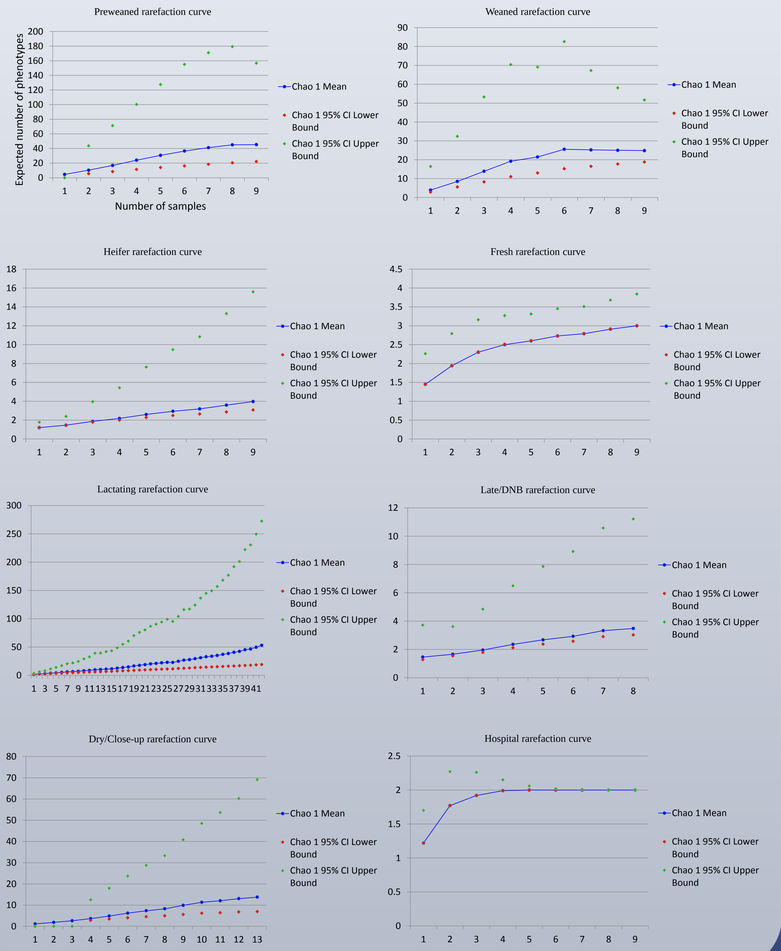
For each niche, we assessed phenotypic AMR diversity based on the distribution of profiles within that niche. Biological diversity was quantified by phenotype richness (the number of AMR profiles in each niche). The relationship between sample size and diversity within each niche was modeled with rarefaction curves based on Chao1 estimates, reported with a 95% confidence interval.

Table 1: Antibiotics tested

Antibiotic	Resistance concentration
Ampicillin	8 µg/ml
Chloramphenicol	8 µg/ml
Sulfisoxazole	256 µg/ml
Kanamycin	16 µg/ml
Amikacin	16 µg/ml
Trimethoprim/Sulfamethoxazole	2/38 µg/ml
Streptomycin	32 µg/ml
Tetracycline	4 µg/ml
Amoxicillin/Clavulanic Acid	8/4 µg/ml
Naladixic Acid	16 µg/ml
Gentamicin	4 µg/ml
Ceftiofur	2 µg/ml
Cefotaxime	1 µg/ml
Cefoxitin	8 µg/ml
Chloramphenicol	8 µg/ml
Ciprofloxacin	0.12 µg/ml

Results





Discussion

In this preliminary study, we examined the resistance phenotypes of *E. coli* from fecal samples collected from a single commercial dairy herd. Isolates from preweaned calves had the greatest phenotypic diversity and the greatest degree of resistance. All isolates from adult animals had comparable low levels of both diversity and resistance. These results suggest that preweaned calf *E.coli* isolates are phenotypically distinct from the rest of the dairy, and may be a source for generation of AMR bacteria. Since this is just one sampling from one herd, these findings are far from conclusive. The rarefaction curves for every niche besides the lactating cows are approaching an asymptote, which indicates that the sample sizes were sufficiently large to capture the phenotypic diversity of the population. The sampling size may need to be increased for lactating cows to be representative of diversity in the niche. This sampling method can be used for a larger project that is currently under development to analyze source and dissemination pathways for resistance that will collect more samples across multiple dairies and time points.

Fig. 1: A pen of dairy cows feeding

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