Prevalence of Cefotaxime Resistant *Enterobacteriaceae* in Beef Cattle in Florida

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Synopsis
The animal factors that affect antibiotic resistance, specifically cefotaxime resistance, have yet to be studied in detail. The prevalence of cefotaxime (a third-generation cephalosporin) resistance in beef claves and adult cattle in Florida was tracked over a period of one year. The findings of this study indicate that environmental factors may influence the prevalence of cefotaxime resistance in beef cattle.

Summary
Third-generation cephalosporins are used extensively in human medicine and, to some extent, as a therapeutic agent in veterinary medicine. The animal factors that affect antibiotic resistance in general and cefotaxime resistance specifically have not been studied in detail. We tracked cefotaxime resistance in adult cattle from eleven different farms and followed a cohort of beef calves for one year at one particular farm. Calves and adult cattle had never been exposed to any prophylactic antibiotic. The prevalence of cefotaxime resistant bacteria in calves was 60%, 50%, 68% and 6% for March, June, August, and December sampling, respectively. Animal factors of age, breed group, and husbandry management practices were not significantly associated with cefotaxime resistance in calves. The prevalence of cefotaxime resistant Enterobacteriaceae bacteria in adult cattle varied among farms, ranging from 5.2% to 100%. The bacteria isolated from adult cattle were resistant to high concentrations of cefotaxime and demonstrated multi-drug resistance against ten different antibiotics. The findings of this study suggest that antibiotic resistance develops in nature and may be transmitted to food animals from the environment. The basic mechanism in the development of antibiotic resistance is not yet understood.

Introduction
Antibiotic resistance causes more than 23,000 deaths and $55 billion in the US (overall societal costs). Natural bacterial resistance plays a vital role in the evolution and spread of antibiotic resistance (Walsh and Duffy, 2013). It appears the acquisition of microbial resistance is independent of antibiotic usage in human and veterinary medicine (Call et al., 2008). Previous studies have reported the presence of antibiotic resistance in food animals. However, the origin of resistance remains unknown (Johnson et al., 2009; Hiroi et al., 2012; Mollenkopf et al., 2012). The US is an intensive user of antibiotics and cephalosporins account for 14% of total dispensed antibiotics in the US (Braykov et al., 2013; Laxminarayan et al., 2013). Cefotaxime is a third generation cephalosporin widely used to treat infections and surgical care (Page et al., 1993). Resistance to antibiotics including cephalosporins has been reported from hospitals in more than 100 countries around the world (WHO, 2014). The resistance is usually mediated by production of extended spectrum β- lactamase (ESBL) enzymes by bacteria (Bush and Fisher, 2011). Knowledge involving the factors that influence the dynamics of resistance can be useful in controlling antibiotic resistance (Alekshun and Levy, 2007). This study provides insight to understanding the prevalence of cefotaxime resistance in food animals. Further environmental assessment is needed to better understand the dynamics of antibiotic resistance in pre-harvest animal production.

Animal management and sample collection
The fecal samples were collected in four different seasons of the growing period, March (n=259), June (n=263), August (n=261), and December (n=193). Due to culling of calves for reasons unrelated to this study, only 193 calves were available for the December sample. The sampling scheme resulted in 188 animals that had all four sampling time points. Fecal samples were collected directly from the rectal-anal junction (RAJ) of animals.

Identification and characterization of Cefotaxime resistant bacteria
This study utilized a combination of culture-based and nucleic acid-based methods for the detection and enumeration of cefotaxime resistant bacteria from the fecal samples. We screened fecal samples on Tryptic Soy Agar and MacConkey agar both supplemented with Cefotaxime (4 mg/L). For the genetic characterization, the DNA of cefotaxime resistant isolates was used as a template for the multiplex PCR to amplify nine ESBL genes.

Antibiotic resistance profiling and identification of resistance genes
The isolates were tested against 10 antimicrobials by the standard Kirby Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2011). Resistant bacterial colonies on MacConkey plus cefotaxime agar were purified and then *bla* TEM *and bla* CTX-M genes were amplified using specific primers. The PCR products were eluted and sent for sequencing to the Cancer and Genetics Research Center (CGRC) at UF.

Statistical analyses
All statistical analysis was conducted using the STATA software package (STATA® MP 11.2, StataCorp, College Station, Texas, USA) with a significance threshold of $\alpha<0.05$.

Results
Prevalence of Cefotaxime resistant bacteria in beef calves over one year
We found high prevalence of cefotaxime resistance in young 1-3 month old beef calves even if they had no history of antibiotic usage (Figure 1). Our results indicate that cefotaxime resistance levels are higher in warmer climates (June and August) than in December (Figure 1), indicating there might be influence of climate on cefotaxime resistance. No significant association between animal breed or sex and the occurrence of cefotaxime resistance could be established.

Cefotaxime resistance in beef heifers and cows
All 1,365 samples showed growth on the Tryptic Soy Agar (TSA) containing cefotaxime. Cefotaxime resistance ranged from 6% (farm # 5) to 100% (farm # 7) on MacConkey agar (Figure 2A and 2B). MacConkey agar is selective for *Enterobacteriaceae* members which have the highest capability to transmit the resistance to other bacteria. MacConkey positive isolates will be utilized for our future experiments. Results here indicated that, although cephalosporins were not used for prophylactic treatment in these farms, cefotaxime resistance was widely prevalent and resistance had developed irrespective of anthropogenic selection pressure (Figure 2B). As shown in Figure 3, most of the bacterial isolates tested in this study had a minimum inhibitory concentration (MIC) of more than 20 μg/ml, confirming that they were intrinsically resistant to the therapeutic treatment of cefotaxime at a clinical level. Nucleotide Blast search of our CTX-M positive isolates showed that the isolates in this study were carrying genes which resembled previously reported *bla* CTXM-15 and *bla* CTXM-1 genes. These results indicate that cefotaxime resistance in these isolates is genetically related to clinical isolates and thereby pose severe public health risks.

Acknowledgements
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Figures

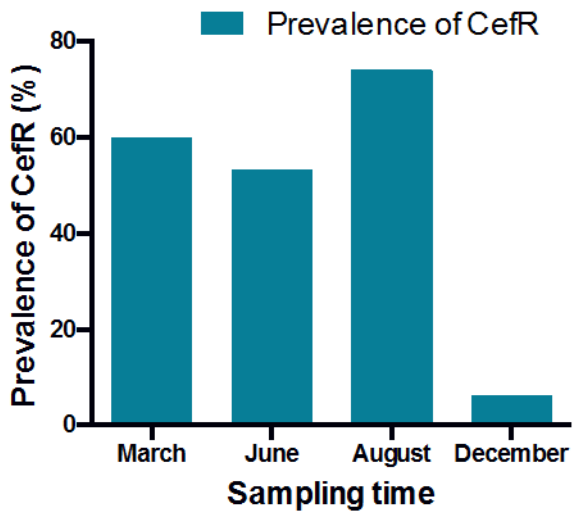

Figure 1. Prevalence of Cefotaxime resistance in beef calves.

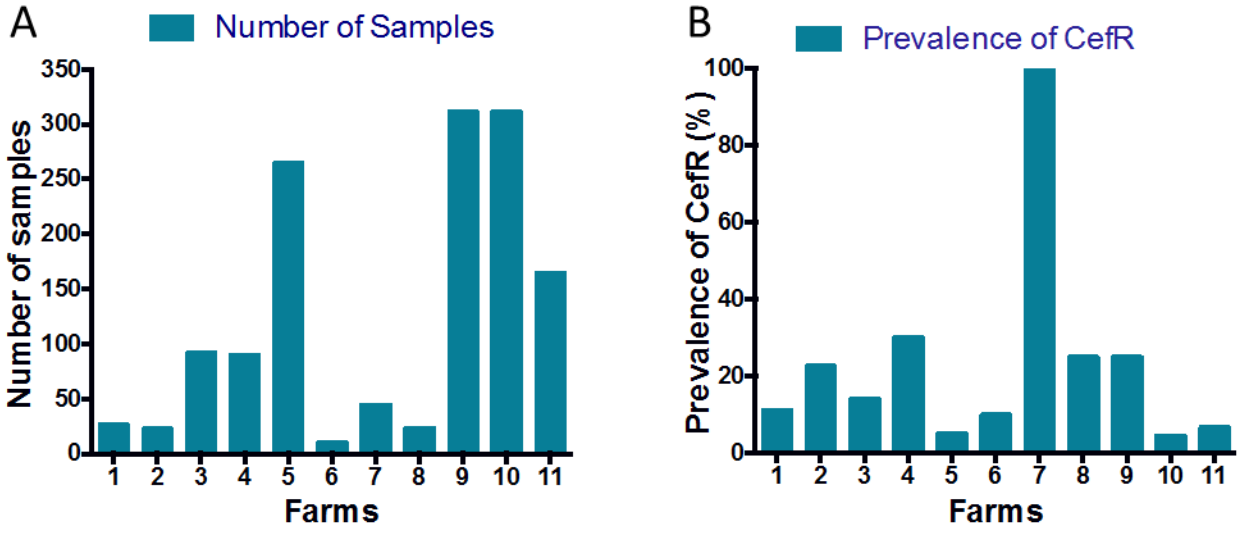

Figure 2. Number of fecal samples (A) and prevalence of cefotaxime resistance in 11 cattle farms (B).

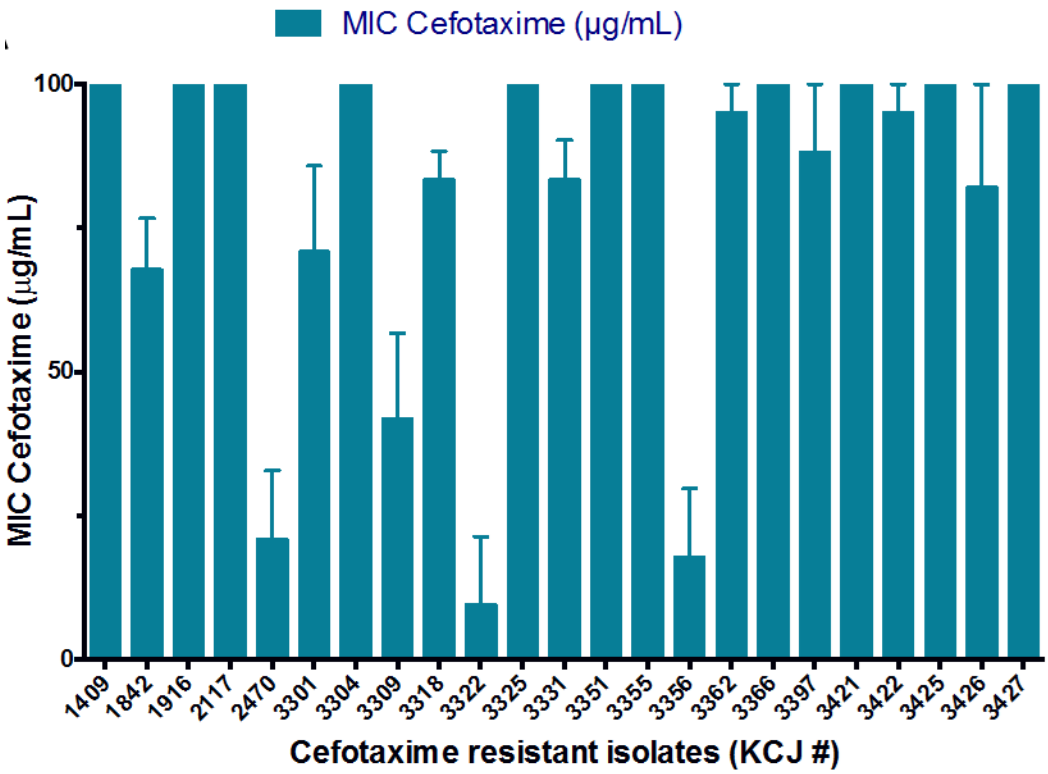

Figure 3. Minimum inhibitory concentration (MIC) test for the 23 cefotaxime resistant isolates.