

Characterization of antimicrobial resistance in atypical *E. coli* and *Klebsiella pneumoniae*

Sara F. Dobry¹, Joshua B. Daniels^{1,2}, Lisa Orfe¹, Thomas Besser^{1,2}, and Douglas Call¹

¹Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; ²Washington Animal Disease Diagnostic Laboratory, Pullman, WA

Bacterial resistance to third generation cephalosporins poses a significant therapeutic challenge in both human and veterinary medicine. One gene that conveys such resistance, the cephamycinase, *bla*_{CMY-2}, has disseminated to a large number of *Salmonella* serovars and to both pathogenic and commensal *E. coli* found in cattle. The plasmids that harbor *bla*_{CMY-2} are widely disseminated, but many of these plasmids cannot be mobilized in the laboratory and at least one plasmid backbone under study has apparently lost both the origin of transfer and mobilization genes. This apparent lack of mobility is at odds with the widespread distribution and clearly successful dissemination of these plasmids in a genetically diverse population of enteric bacteria. Two hypotheses might explain this pattern: i) conjugation is far more efficient in anaerobic and microaerophilic conditions present in the gastrointestinal niche compared with conventional conjugation studies; or ii) plasmid transfer originates from a large, but unrecognized reservoir of enteric flora and the fate of these plasmids in *E. coli* and *Salmonella* is not important to long-term persistence in the gastrointestinal niche.

The purpose of this study was to examine the mobility of *bla*_{CMY-2} bearing plasmids from non-*E. coli* fecal coliform bacteria isolated from cattle. Eight ceftazidime resistant isolates were originally collected from cattle and presumptively identified as non-*E. coli* coliforms by culture and conventional biochemical identification methods. PCR amplification and sequencing of a fragment of the 16S rRNA locus revealed that six of the eight isolates were atypical *E. coli* and two were *Klebsiella pneumoniae*. PCR, Southern blots, and electroporation experiments confirmed that seven isolates harbored a plasmid-borne *bla*_{CMY-2} gene. Conjugation experiments were conducted using filter mating both with and without helper plasmids. Only one *E. coli* isolate harbored a transmissible plasmid. This preliminary study confirmed earlier observations that *bla*_{CMY-2} bearing plasmids are difficult to mobilize under laboratory conditions. While we were unable to identify additional *bla*_{CMY-2} plasmid bearing coliforms, our observations indicate that poor plasmid mobility is not limited to *E. coli* and *Salmonella* hosts.