

# Mechanisms for long-term persistence of large, multi-drug resistant plasmids that confer resistance to 3<sup>rd</sup> generation cephalosporins

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A family of multi-drug resistant (MDR), bla<sub>CMY-2</sub> positive, Inc A/C plasmids, has recently disseminated worldwide in multiple bacterial species and can be found in variety of animal hosts. The success of these plasmids across diverse ecological niches suggests that factors other than antibiotic use contribute to successful dissemination. Plasmid “fitness” is a function of (1) the fitness burden conveyed to the host bacterium, (2) the ability to minimize segregation loss during binary fission, and (3) the ability to colonize new strains via conjugation. Large plasmids (e.g. > 100 Kb) are expected to incur high fitness costs on the host bacterium. Our initial studies have focused on plasmid peH4H (148,000 bp), which originated from a cattle *E. coli* isolate. We introduced this plasmid into a lab strain *E. coli* (Gene Hog) and passaged this strain and others for more than 1,000 generations in the absence of antibiotics. We found no segregation loss, indicating that either a plasmid conveys a distinct selective advantage or it has a very effective anti-segregation control system. Direct competition studies suggest that strains harboring peH4H have a fitness advantage. Furthermore peH4H appears to readily conjugate to recipient strains when in the presence of a “helper” plasmid. Based on our preliminary findings we propose to identify the segregation control system employed by these plasmids, analyze transcriptomic changes to the host bacterium upon introduction of peH4H, and determine how plasmid genes are regulated. From these studies we will determine exactly why these large MDR plasmids are so successful in the absence of continuous selection pressure.