Satu Olkkola

Antimicrobial Resistance and Its Mechanisms among *Campylobacter coli* and *Campylobacter upsaliensis* with a Special Focus on Streptomycin

Campylobacteriosis is the most common cause of human bacterial gastroenteritis in the developed world. The most often isolated causative agent from diseased humans is *C. jejuni*, but also *C. coli* and *C. upsaliensis*, common colonizers of pigs and dogs, respectively, are known to cause disease. Campylobacteriosis is usually self-limiting but antimicrobial treatment is warranted in severe cases, with macrolides and fluoroquinolones being the first and second options, respectively. Intravenous aminoglycosides are indicated in Campylobacter bacteraemia. However, high rates of fluoroquinolone-resistant *Campylobacter spp.* have emerged in many parts of the world. Also, in several studies, high proportions of streptomycin-resistant *C. coli* or *C. upsaliensis*, have been found. Yet, the mechanisms of STR resistance have been only partially characterized in *C. jejuni* and *C. coli* and completely ignored in C. upsaliensis.

The primary aim of this thesis was to investigate the molecular mechanisms of STR resistance in porcine *C. coli* and canine *C. upsaliensis* isolates. We were able to associate high level of STR resistance in porcine C. *coli* to mutations in the rpsL gene. In *C. upsaliensis*, a mutation in rpsL was also noted in all the low- and high-level STR-resistant isolates. All highly STR-resistant *C. upsaliensis* isolates had, in addition to the rpsL mutation, significant truncation of rsmG, encoding a conserved methyltransferase responsible for methylation of the ribosomal STR binding site. Even though STR resistance conferring mutations in rpsL and rsmG have been well documented in other bacterial species, they were first time described in *Campylobacter spp.* in the present study. Further, using genomics and insertional mutagenesis, a novel STR resistance-conferring gene was identified in the intermediately STR-resistant *C. coli* isolates. This gene is homologous, albeit at a low level, to other previously described aminoglycoside 6-adenylyltransferase encoding genes, and does not appear to originate from Gram-positive bacterial species. Based on our findings, we hypothesize that this gene could have evolved from a proto-resistance element in *Campylobacter spp.* Altogether these results provide a significant advance in understanding the mechanisms of STR resistance in *Campylobacter spp.* and will aid in predicting the phenotypic resistance from genome data.

Fluoroquinolone resistance-associated mutations in the DNA gyrase-encoding gene gyrA were characterized in porcine *C. coli* treated with danofloxacin as well as among canine *C. upsaliensis.* The commonly described C257T mutation was found in both species. In *C. coli* this caused the amino acid change T86I in DNA gyrase and high levels of ciprofloxacin resistance, while in *C. upsaliensis* the predicted amino acid change was T86M causing only minor increase in CIP MIC but a high level of nalidixic acid resistance. Therefore, danofloxacin does not seem to induce novel mutations in *C. coli* in vivo but the same mutation appears not to be sufficient to cause a high level of fluoroquinolone resistance in *C. upsaliensis*.