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Exploring Fluoroquinolone-Resistance Dynamics Using a Distinct in Vitro Fermentation Chicken Caeca Model

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Abstract : Resistance to fluoroquinolones (FQ) has evolved increasingly over the years, posing a significant challenge for the treatment of human infections, particularly gastrointestinal tract infections caused by zoonotic bacteria transmitted through the food chain and environment. In broiler chickens, a relatively high proportion of FQ resistance has been observed in Escherichia coli indicator, Salmonella and Campylobacter isolates. We hypothesize that flumequine (Flu), used as a secondary choice for the treatment of poultry infections, could potentially be associated with a high proportion of FQ resistance. To evaluate this hypothesis, we used an in vitro fermentation chicken caeca model. Two continuous single-stage fermenters were used to simulate in real time the physiological conditions of the chicken caeca microbial content (temperature, pH, caecal content mixing, and anoxic environment). A pool of chicken caecal content containing FQ-resistant E. coli obtained from chickens at slaughter age was used as inoculum along with a spiked FQ-susceptible Campylobacter jejuni strain isolated from broilers. Flu was added to one of the fermenters (Flu-fermenter) every 24 hours for two days to evaluate the selection and maintenance of FQ resistance over time, while the other served as a control (C-Fermenter). The experiment duration was 5 days. Samples were collected at three different time points: before, during and after Flu administration. Serial dilutions were plated on Butzler culture media with and without Flu (8mg/L) and enrofloxacin (4mg/L) and on MacConkey culture media with and without Flu (4mg/L) and enrofloxacin (1mg/L) to determine the proportion of resistant strains over time. Positive cultures were identified by mass spectrometry and matrix-assisted laser desorption/ionization (MALDI). A subset of the obtained isolates were used for Whole Genome Sequencing analysis. Over time, E. coli exhibited positive growth in both fermenters, while C. jejuni growth was detected up to day 3. The proportion of Flu-resistant E. coli strains recovered remained consistent over time after antibiotic selective pressure, while in the C-fermenter, a decrease was observed at day 5; a similar pattern was observed in the enrofloxacin-resistant E. coli strains. This suggests that Flu might play a role in the selection and persistence of enrofloxacin resistance, compared to C-fermenter, where enrofloxacin-resistant E. coli strains appear at a later time. Furthermore, positive growth was detected from both fermenters only on Butzler plates without antibiotics. A subset of C. jejuni strains from the Flu-fermenter revealed that those strains were susceptible to ciprofloxacin (MIC < 0.12 µg/mL). A selection of E. coli strains from both fermenters revealed the presence of plasmid-mediated quinolone resistance (PMQR) (qnr-B19) in only one strain from the C-fermenter belonging to sequence type (ST) 48, and in all from Flu-fermenter belonged to ST189. Our results showed that Flu selective impact on PMQR-positive E. coli strains, while no effect was observed in C. jejuni. Maintenance of Flu-resistance was correlated with antibiotic selective pressure. Further studies into antibiotic resistance gene transfer among commensal and zoonotic bacteria in the chicken caeca content may help to elucidate the resistance spread mechanisms.

Keywords : fluoroquinolone-resistance, escherichia coli, campylobacter jejuni, in vitro model
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