



The potential of intramammary cephapirin and cefalonium treatment to select for ESBL producing Enterobacteriaceae in the bovine gut

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Introduction

The selection and spread of Extended Spectrum β -lactamase (ESBL)-producing Enterobacteriaceae within animal production systems and potential spill over to humans is a major health concern. Cephapirin and cefalonium are widely used antibiotics to treat intramammary infections in dairy cows and could possibly select for ESBL producing bacteria in their intestinal tract even when administered locally due to distribution of the antibiotic throughout the body albeit at lower than MIC concentrations. However, the minimal concentration to select for resistance, the Minimum Selective Concentration (MSC), in a complex environment like the gastrointestinal tract, is unknown. Therefore, we studied the potential effects of low doses of cephapirin and cefalonium to select for ESBL producing *E. coli* in rich media and fresh faecal fermentations of dairy cows as a model for intramammary application of cephapirin and cefalonium and compared it to the maximum expected concentrations of cephapirin and cefalonium in the intestinal tract.

Materials & methods

ESBL and non-ESBL *E. coli* isolates from dairy farms have been collected in 4 different European countries. A 1:3 and 1:1 mixture of these isolates have been made and mixed with respectively lysogeny broth (LB) media (rich media) and fresh fecal samples from 10 dairy cows from each of the 4 countries (baseline mixture). For the rich media competition assay, the 1:3 mixture with LB has been incubated for 6 hours at 37°C under different low concentrations of cephapirin and cefalonium for their potential to select for Cefotaxime (CTX) resistance as an indicator for ESBL selection. In the fecal fermentation competition assay, the 1:2 mixture with fecal samples was mixed with anaerobic standard ileal efflux medium and anaerobically incubated overnight under different concentrations of cephapirin and cefalonium. In both experiments, 0.25 $\mu\text{g/ml}$ CTX was used as positive control. 96 individual *E. coli* isolates were picked after incubation on MacConkey agar plates for further susceptibility testing. The percentage of resistant colonies to CTX in each of the conditions have been assessed to determine the MSC for ESBL producing *E. coli*. To determine the maximum expected concentrations of cephapirin and cefalonium in the intestinal content of dairy cows after intramammary treatment a literature search has been conducted.

Results

In both experiments, an increase in CTX resistant colonies was seen between overnight incubation at 8 $\mu\text{g/ml}$ cephapirin and 4 $\mu\text{g/ml}$ cefalonium compared to the blanc samples. In rich media, there was a significant increase in CTX resistance at a concentration of 8 $\mu\text{g/ml}$ cephapirin and 4 $\mu\text{g/ml}$ cefalonium compared to the blanc samples (respectively $p=0.007$ and $p=0.023$) (Figure 1). This increase was not seen at lower concentrations.

In the fecal fermentations, a 37% increase in CTX resistance was observed for cephapirin ($p=0.079$), and 13% for cefalonium ($p=0.190$) at the highest concentrations compared to the blanc samples (Figure 2). For lower concentrations hardly any increase was visible. These findings indicate that the MSC for ESBL *E. coli* is above 0.8 $\mu\text{g/ml}$ and 0.4 $\mu\text{g/ml}$ for cephapirin and cefalonium respectively.

Based on available literature the maximum expected concentrations of cephapirin and cefalonium in the intestinal content of adult dairy cows after intramammary administrations with doses according to the label will be close to or below 0.1 $\mu\text{g/g}$.

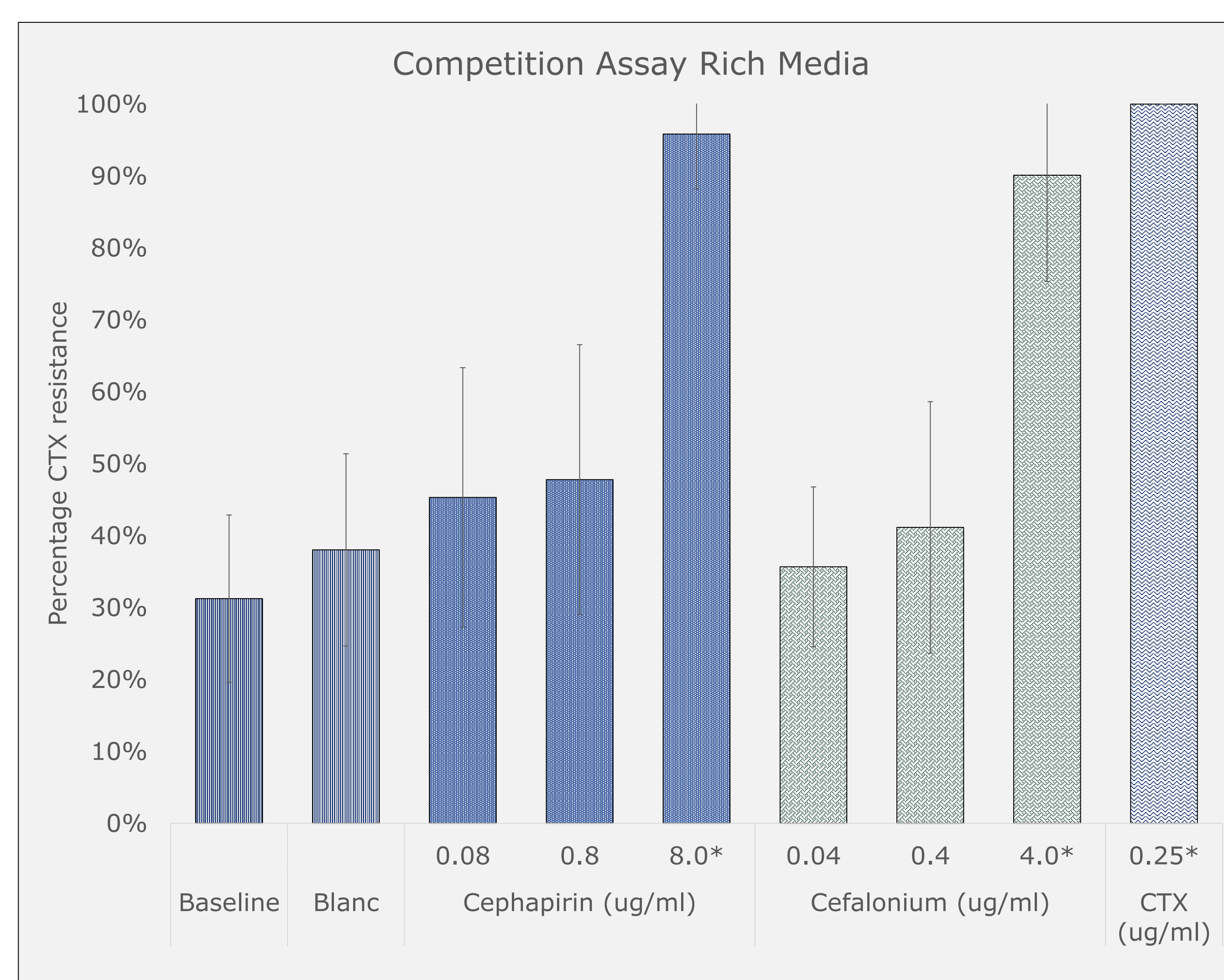


Figure 1. Competition assay rich media. The bars show the percentage of CTX resistant *E. coli* colonies at different concentrations of antibiotics after incubation. * $p<0.05$

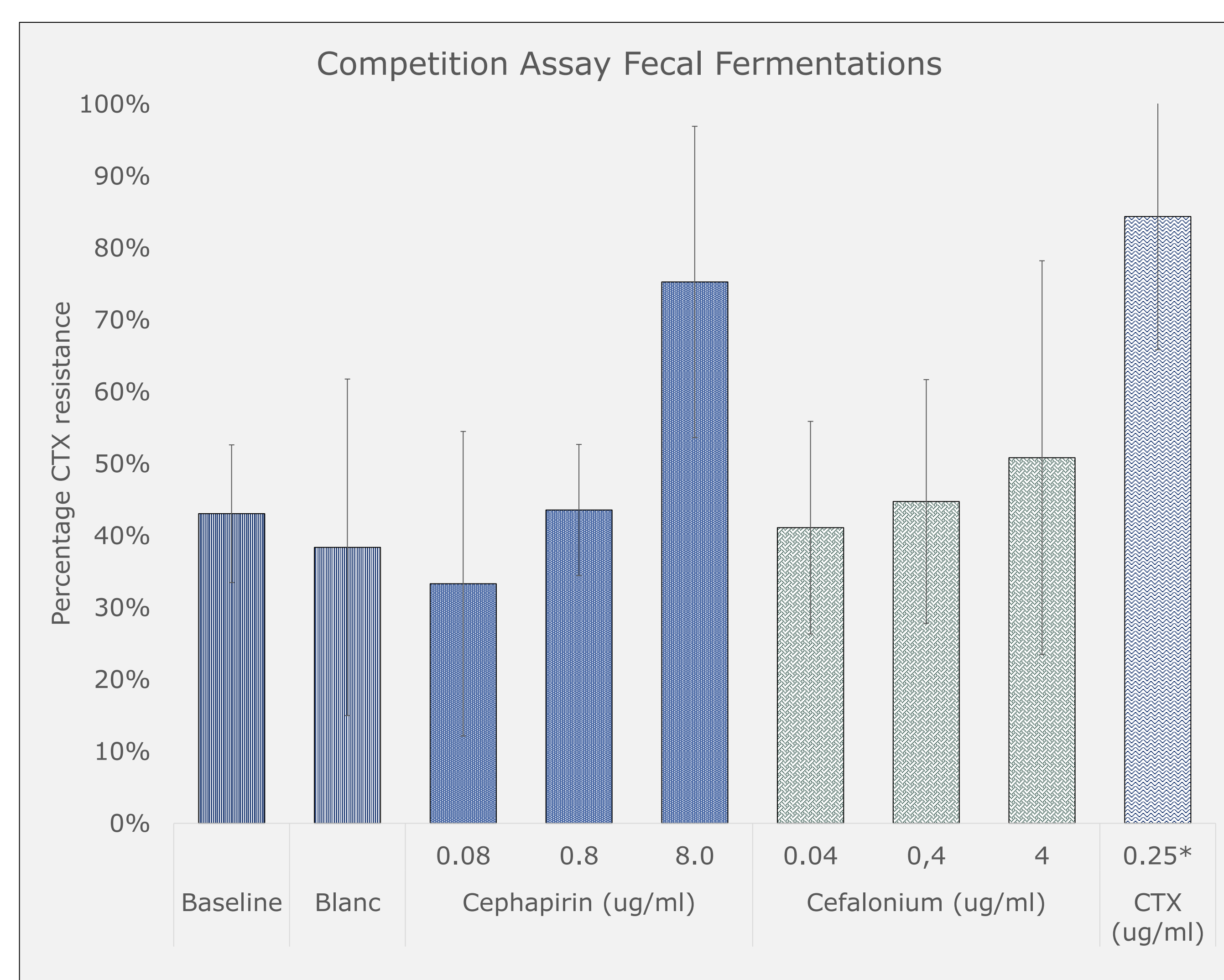


Figure 2. Competition assay fecal fermentations. The bars show the percentage of CTX resistant *E. coli* colonies at different concentrations of antibiotics after incubation. * $p<0.05$

Conclusion

Based on our findings, the Minimum Selective Concentration for Cefotaxime resistance of cephapirin and cefalonium is expected to be substantially higher than the calculated maximum concentration of cephapirin and cefalonium in the intestinal content of dairy cows after intramammary treatment. This would imply a limited potential of intramammary administered cephapirin and cefalonium to select for ESBL-producing *E. coli* in the bovine gut.

References

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