Antibiotic activity in an *ex vivo* model of canine pyometra

K.A. Von Dollen, M.G. Papich, M.J. Jones, T.M. Beachler, T.L. Harris, S.K. Lyle, K.E. Ellis, C.S. Bailey
Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Pyometra is a serious medical condition of bitches, affecting 1 in 4 older intact bitches. Treatment entails either ovariohysterectomy, or antimicrobial therapy in combination with ecbolics and supportive care. Since uterine cultures are difficult to obtain and vaginal cultures have a low diagnostic value, antimicrobial therapy is largely selected empirically based on the most common bacterial etiology, *Escherichia coli*. Antibiotic failure is not uncommon and can result in treatment failure and clinical decomposition. In this work, we utilized an *ex vivo* model to quantitatively evaluate the activity of three frequently used antibiotics in either standard growth medium (Mueller Hinton Broth; MHB) or pyometra fluid against two strains of *E. coli.* We hypothesized that pyometra fluid would suppress antibiotic activity of all three antibiotics compared to MHB. Known CFU of either ATCC 25922 strain (ATCC, 7.5 x 10^4 CFU/mL) or a clinical strain of *E. coli* bacteria (CLINISO, 1.27 x 10^5 CFU/mL) were added to 2 mL of each fluid type. Based on MIC values, both strains were classified as wild-type according to EUCAST.[1] The ATCC strain was susceptible based on clinical breakpoints to cefazolin and enrofloxacin and resistant to amoxicillin/clavulanic acid.[2] The clinical strain was susceptible to enrofloxacin, intermediate to cefazolin, and resistant to amoxicillin/clavulanic acid.[2] Ciprofloxacin (0.015 μg/mL), amoxicillin/clavulanic acid (4 μg/mL), or cefazolin (1 μg/mL) were selected for testing because cefazolin and amoxicillin/clavulanic acid are used clinically, and ciprofloxacin is an active metabolite of enrofloxacin with similar activity against *E. coli*. Concentrations selected for testing were in the range for wild-type strains of *E. coli.*[1] Each combination of antibiotic, bacterial strain and fluid type was incubated in triplicate (ATCC) or quintuplicate (CLINISO). Bacterial growth was quantified at 8 and 24 hours in a modified time-kill trial as described by the Clinical and Laboratory Standards Institute.[3] Logarithmic growth was compared across fluid environments and antibiotic treatments using an ANOVA with split-plot design and Tukey all-pairwise comparison test (Statistix 10, Tallahassee FL). At 8 and 24 hours, both strains of *E. coli* grew in both fluid types (3-4 log increase in bacterial numbers from inoculation). In MHB at 8 hours, ciprofloxacin resulted in a 0.4 log decrease of CLINISO and a 3.17 log decrease of ATCC ($p<0.05$), whereas neither cefazolin nor amoxicillin/clavulanic acid inhibited bacterial growth compared to controls ($p>0.05$). In MHB at 24 hours, ciprofloxacin resulted in a 0.15 log decrease in CLINISO and a 2.98 log decrease in ATCC, whereas neither cefazolin nor amoxicillin/clavulanic acid inhibited bacterial growth compared to controls ($p>0.05$). In pyometra fluid, no antibiotic inhibited bacterial growth at either 8 or 24-hour time points. These data show that while ciprofloxacin is bactericidal in laboratory tests, it may only have bacteriostatic effects against canine clinical isolates. Further, at the concentrations tested, no antibiotic inhibited bacterial growth in canine purulent uterine fluid. These findings suggest that antibacterial activity against *E. coli* may be diminished in canine pyometra fluid and underscore the vital importance of fluid evacuation in cases of pyometra.

Keywords: Pyometra, antibiotic, canine, bitch

References

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