

Antibiotic and Non-Antibiotic Combinations For The Treatment Of Biofilm Associated Bacterial Endometritis In Mares

Treatment of chronic bacterial endometritis in mares with antimicrobials is sometimes unsuccessful. Chronic infections may be associated with a biofilm which protects bacteria from the host immune system and provides antibiotic tolerance. Equine uterine Gram-negative bacterial isolates can form a biofilm *in vitro* and are likely to form a biofilm *in vivo*.

A series of *in vitro* studies were conducted to assess biofilm dispersal and/or bacterial killing for antibiotics and non-antibiotic agents alone or in combination against Gram-negative bacteria. Our data indicates that antibiotics and non-antibiotic agents are more effective against biofilm if administered concurrently (i.e. in the same syringe). The attached table (Table 1) explains how to make up clinical treatments for local infusion into the uterus based on the *in vitro* data. The amount of either antibiotic or non-antibiotic agent for each infusion are the minimum effective concentrations against *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The treatment period should be at least 72 hours in duration, with repeated treatments every 24 hours (i.e. a uterine infusion of the selected combination once every 24 hours for 3 consecutive days). This treatment protocol resulted in complete biofilm dispersal and bacterial killing *in vitro*.

It is important to note that some non-antibiotic agents and antibiotics should not be combined in the same syringe. For example, the *in vitro* data indicated that mixing acetylcysteine with antibiotics in the same syringe resulted in reduced activity of the antibiotics.

We recommend antibiotic sensitivity testing for all Gram-negative organisms. Bacteria inherently resistant to an antibiotic will still be resistant when that antibiotic is used in combination with a non-antibiotic agent.

Source of antibiotics:

- Amikacin sulfate (250 mg/ml), Fort Dodge® Animal Health (available through veterinary distributors)
- Ceftiofur sodium sterile powder (1 or 4 gram vials reconstituted to 50 mg/ml), Zoetis Inc. (available through veterinary distributors)
- Ciprofloxacin (10 mg/ml), human IV infusion preparation, Claris Lifesciences Inc. (available through human pharmacies)

Source of non-antibiotic agents:

- TRIZEDTA, 16 oz Crystals, Dechra Veterinary Products (available veterinary distributors)
- DMSO 99% purity, FWI Inc. (available through veterinary distributors)
- Ceragyn™ Uterine Infusion, Ceragyn LLC. (available through veterinary distributors)
- Hydrogen Peroxide Topical Solution U.S.P. 3%, (available through drug stores and veterinary distributors)

For questions regarding biofilm or endometritis therapy, please contact Dr. Ryan Ferris, rferris@colostate.edu



Table 1. Antibiotic and Non-Antibiotic Combinations For The Treatment Of Biofilm Associated Bacterial Endometritis In Mares

Tris EDTA- final concentration in the syringe should be 50 mM Tris and 3.5 mM EDTA

Note: Tris-EDTA and Tricide are similar; however Tricide is not equivalent to Tris-EDTA in regards to bacterial killing
To make Tris-EDTA: 16oz bottle of Dechra Triz-EDTA crystals; add 8 oz of sterile water (this is different than the bottle instructions). The 2x concentration of Tris-EDTA solution will be further diluted by the antibiotics below to the proper final concentration.

Antibiotic	Drug Amount	Tris EDTA	QS	Final volume	Notes:
Amikacin (250 mg/ml)	4 mls (1 gram)	30 mls	16 mls sterile fluid (Saline, LRS, Sterile H ₂ O)	60 mls	10 mls of 8.4% sodium bicarbonate should be added to the amikacin
Ceftiofur (1 gram reconstituted in 20 mls)	20 mls (1 gram)	30 mls	10 mls sterile fluid (Sterile H ₂ O)	60 mls	
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	40 mls	0	80 mls	Split between two syringes

H₂O₂- 1% final concentration in the syringe

A 3% stock solution is available at many drug stores and veterinary distributors

Antibiotic	Drug Amount	H ₂ O ₂	QS	Final volume	Notes:
Amikacin (250 mg/ml)	4 mls (1 gram)	20 mls	26 mls sterile fluid (Saline, LRS, Sterile H ₂ O)	60 mls	10 mls of 8.4% sodium bicarbonate should be added to the amikacin
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	20 mls	0	60 mls	

Ceragyn Infusion Product- >30% final volume in the syringe

Antibiotic	Drug Amount	Ceragyn	QS	Final volume	Notes:
Amikacin (250 mg/ml)	4 mls (1 gram)	20 mls	26 mls sterile fluid (Saline, LRS, Sterile H ₂ O)	60 mls	10 mls of 8.4% sodium bicarbonate should be added to the amikacin
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	20 mls	0	60 mls	

DMSO- 30% final concentration in the syringe

99% stock solution is used for calculations below

Antibiotic	Drug Amount	DMSO	QS	Final volume	Notes:
Ceftiofur (1 gram reconstituted in 20 mls)	20 mls (1 gram)	20 mls	20 mls sterile fluid (Sterile H ₂ O)	60 mls	
Ciprofloxacin (10 mg/ml)	40 mls (400 mg)	20 mls	0	60 mls	

