

Microbial Inactivation Properties of an Antimicrobial / Antithrombotic Catheter Solution (AAT-023)

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Introduction

- Microbial infections are the most serious complications associated with indwelling central venous catheters. Four hundred thousand catheter related bloodstream infections (CRBSI) occur annually in the USA. Systemic antibiotics alone eradicate only one-third of CRBSI, because intraluminal colonization and biofilm serve as a nidus for infection.
- Filling a catheter lumen with high concentration of antibiotics (ciprofloxacin, minocycline, rifampin, vancomycin, or gentamicin) can decrease bacterial growth, and improve catheter salvage rates. However using antibiotics in catheter locks prophylactically increases bacterial resistance.
- Heparin is commonly used as an anticoagulant catheter lock. However, sodium heparin stimulates biofilm formation of some bacterial strains (e.g. *S. aureus*) on plastic surfaces and has no antibacterial effect.
- Mixtures of antibiotics and chemical compounds e.g. minocycline-edetate calcium disodium (MEDTA), or chemical composites e.g. tauridine (2%)-polyvinylpyrrolidone (5%) (T/PVP) have been used for catheter lock.

Published Clinical Uses of Citrate

- Sodium citrate is used in blood products and plasmapheresis.
- Numerous studies of catheter locks of 1% to 46.7% report no significant adverse events.
- At lower concentrations 1% to 4% citrate was as effective as heparin as an anticoagulant.

Published Clinical Uses of Methylene Blue

- | Disease Treated | MB Dose |
|---|------------------------|
| Hepatopulmonary syndrome | 3 mg/kg for 15 min |
| Post-cardiac bypass hypotension | 2 mg/kg |
| Septicemia with hypotension | 2 mg/kg |
| Hemodialysis patients w/ hypotension | 1 mg/kg bolus |
| Parathyroid gland identification | 5-7.5 mg/kg |
| Methemoglobinemia acute and chronic | 1-2 mg/kg up to 7mg/kg |
| Pelvic surgical procedures (identify ureters) | 1 mg/kg |
| Dose range for 70 kg patient | 70 mg-490 mg |
| (AAT-023 - 0.05% MB in 5 ml) | 2.5 mg |

Materials & Methods

- A novel solution (AAT-023) consisting of 0.05% methylene blue (MB) in 7% sodium citrate at pH 6.2 with preservatives was tested in saline solution and in presence of media and 5% bovine albumin against gram positive and gram negative bacteria and fungi from hospital isolates and ATCC collection. Some of the tested organisms were: *E. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus* (also MARS), *S. epidermidis*, *C. albicans*, *A. niger* and *V. vulnificus*. The challenge suspensions were $>10^8$ CFU/mL for bacteria, or $>10^4$ for fungi.
- A clinical isolate of *S. aureus* was used to evaluate anti-biofilm properties. The microorganism was perfused in human serum for 3 hours followed by AAT-023 solution for 2 days with 3 hour bacterial challenges in-between.

Results

Comparison of Antimicrobial Effectiveness between Heparin and AAT-023

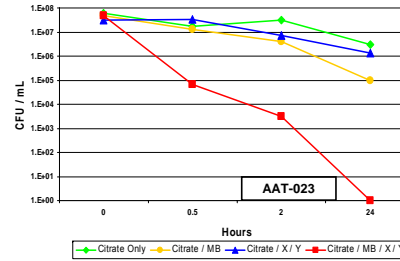
	Heparin (Baseline)				AAT-023			
	1 hr	24 hr	48 hr	72 hr	1 hr	24 hr	48 hr	72 hr
<i>S. aureus</i> 33591	Green	Green	Green	Green	Red	Red	Red	Red
<i>S. epidermidis</i> 12228	Green	Green	Green	Green	Red	Red	Red	Red
<i>E. faecalis</i> 376	Green	Green	Green	Green	Red	Red	Red	Red
<i>E. coli</i> 25922	Green	Green	Green	Green	Red	Red	Red	Red
<i>E. coli</i> 35218	Green	Green	Green	Green	Red	Red	Red	Red
<i>P. aerug</i> 27853	Green	Green	Green	Green	Red	Red	Red	Red
<i>C. albicans</i> 10231	Green	Green	Green	Green	Red	Red	Red	Red

Legend: Fully Alive (Green), ~3-4 Log Kill (Yellow), Complete Kill (Red)

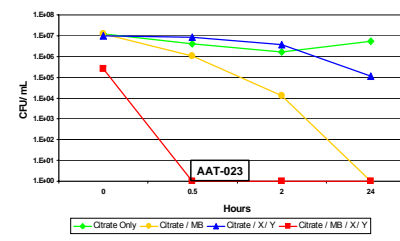
- Studies of the AAT-023 components demonstrated synergistic rather than additive effects.

Synergistic Antimicrobial Effectiveness:

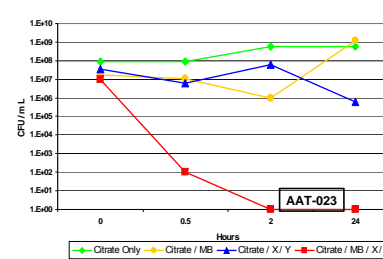
S. aureus in presence of media and albumin.



E. coli in presence of media and albumin.



P. aeruginosa in presence of media and albumin.



- All organisms have an MIC of 25% or less of the original concentration of AAT-023. There was no change in MIC over time.

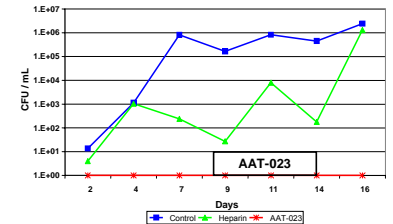
MICs of several organisms vs. % AAT-023

	50%	25%	12.50%	6.25%	3.12%	1.56%	Controls**
<i>S. aureus</i> 33591	+	+	+	+	+	+	-/+
<i>S. aureus</i> 29213	+	+	+	+	+	+	-/+
MRSA*	+	+	+	+	+	+	-/+
<i>S. epidermidis</i> *	+	+	+	+	+	+	-/+
<i>E. coli</i> 35218	+	+	+	+	+	+	-/+
<i>E. coli</i> 25922	+	+	+	+	+	+	-/+
<i>P. aeruginosa</i> 27853	+	+	+	+	+	+	-/+
<i>E. faecalis</i> *	+	+	+	+	+	+	-/+
<i>P. mirabilis</i> *	+	+	+	+	+	+	-/+
<i>C. albicans</i> 10231	+	+	+	+	+	+	-/+
<i>C. albicans</i> *	+	+	+	+	+	+	-/+

*Hospital isolates
**NeaPos controls

- AAT-023 eradicates CFU in biofilm, from 10^5 CFU/mL to zero, even after repeated exposure to *S. aureus* in plasma.

Biofilm Antimicrobial Effectiveness: *S. aureus* Colonization Inhibition and Elimination.

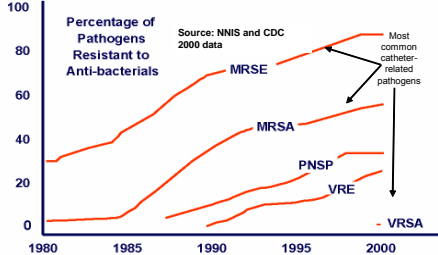


- For most tested strains, microbial reduction above 99% was obtained in the first few minutes of incubation with AAT-023, and in others this reduction was achieved in less than 1 hour (as seen to the left). In control (saline solution), the concentration of microorganisms remained unchanged for several days.

Discussion

- AAT-023 has broad and rapid antimicrobial properties against planktonic bacteria. Antimicrobial properties are not diminished in the presence of media and 5% albumin, so the product should be effective even if diluted by blood at the tip of the catheter. AAT-023 eliminates staphylococcus within established biofilms. Other studies have demonstrated safety, lack of toxicity, and a lack of microbial resistance to AAT-023.
- A randomized prospective multi-center trial is now beginning to compare the effects of AAT-023 and heparin on the incidence of CRBSI in central venous catheters for dialysis.

Growing Resistance to Antibiotics



MRSE=Methicillin-Resistant Staphylococcus epidermidis
MRSA=Methicillin-Resistant Staphylococcus aureus
PNSP=Penicillin Non-susceptible Streptococcus Pneumoniae
VRE=Vancomycin-Resistant Enterococci
VRSA=Vancomycin-Resistant Staphylococcus aureus

Antibacterial-Antithrombotic Catheter Lock-Design Objectives
We developed a new antimicrobial/antithrombotic lock solution with the following properties:

- Anticoagulant properties comparable to heparin.
- Components previously approved for IV administration- citrate and methylene blue (MB).
- Safety for use prophylactically.
- Ability to kill planktonic bacteria and fungal strains within 60 minutes.
- Ability to kill sessile bacteria in biofilm.
- No known bacterial resistance to components.
- Not an antibiotic.
- Relative density of 1.040 which is \approx to blood of a typical dialysis patient.