

L. LONG¹, H. G. KIM¹, B. FERREK¹, A. CIRINO¹, M. A. GHANNOUM^{1,2,3};¹Case Western Reserve Univ., Cleveland, OH, ²Univ. Hosp. Case Med. Ctr., Cleveland, OH, ³Great Lakes Pharmaceuticals, Cleveland, OH.

Introduction

Nosocomial infections due to *Staphylococcus aureus* have increased recently and their treatment is complicated by the emergence of multi-drug-resistance.

Staphylococcal infections are associated with the formation of biofilms on indwelling medical devices, such as indwelling vascular catheters and damaged tissues.

Antimicrobial Lock therapy provides a means for delivering high concentrations of drug to resistant biofilms coating catheters. B-Lock™, a triple combination of trimethoprim, EDTA and 25% ethanol is a new product entering clinical development for prevention and treatment of catheter infections. We examined the efficacy of B-Lock™ as a catheter lock solution against biofilms formed by methicillin-resistant *Staphylococcus aureus* (MRSA) in a rabbit model of catheter-related biofilm infection.

Materials and Methods

The experiments were performed following Institutional Animal Care and Use Committee guidelines. Female New Zealand White rabbits weighing between 2.5 to 3.5 kg were purchased from Covance, Inc., Princeton, NJ and housed at the Case Western Reserve University Animal Facility under standard laboratory conditions.

Inoculum: MRSA ATCC 43300 was obtained from the CMM Culture Collection and used as the infecting organism. Several Petri dishes were plated with MRSA on Brain Heart Infusion (BHI) agar and incubated at 37°C for 24 hours. Using a sterile loop colonies were harvested and tubes with 25 ml each of sterile BHI broth were inoculated. The tubes were then placed in a shaking water bath at 37°C overnight. After centrifugation and washing two times with sterile normal saline (NaCl 0.85%), the cells were re-suspended in the same solution. Ten-fold dilutions of the cell suspension were prepared and measured using a spectrophotometer. A standard 300 µl inoculum consisting of 10⁷ CFU of MRSA, 100 U of heparin (Abbott Laboratories, North Chicago, Ill.), and sterile normal saline was prepared.

Materials and Methods (Cont.)

Procedure: Rabbits were allowed to acclimatize for 7 days prior to surgery. Intramuscular anesthesia using a cocktail of ketamine and xylazine was given prior to surgery. Silicone catheters were placed in the right external jugular vein and tunneled subcutaneously as described previously (Figure 1) [1]. In three experiments, catheters were infected with MRSA 10⁷, which was 'locked' in the lumen of the catheter for 24 hours. The organisms were then removed and daily heparin flushes were performed. Mature biofilms are formed by day three at which time blood samples were obtained from the catheter and submitted for culture to confirm the presence of *S. aureus* (SA). Animals were randomized into groups: 1) B-Lock™ lock therapy for 2 hours a day for 7 days (n = 4), and 2) untreated control (UC, n = 5). After seven days of treatment, the animals were anesthetized and blood cultures were obtained through the catheter and peripherally via cardiac puncture. The animals were then euthanized with a pentobarbital cardiac injection. The catheter was removed for quantitative culture and scanning electron microscopy (SEM).

SEM: Catheter segments were fixed in 2% glutaraldehyde and prepared for SEM observation according to previously published protocol [2]. The fixed and dehydrated catheter segments were sputter coated with gold-palladium (60/40) and viewed under a Phillips XL30 scanning electron microscope.

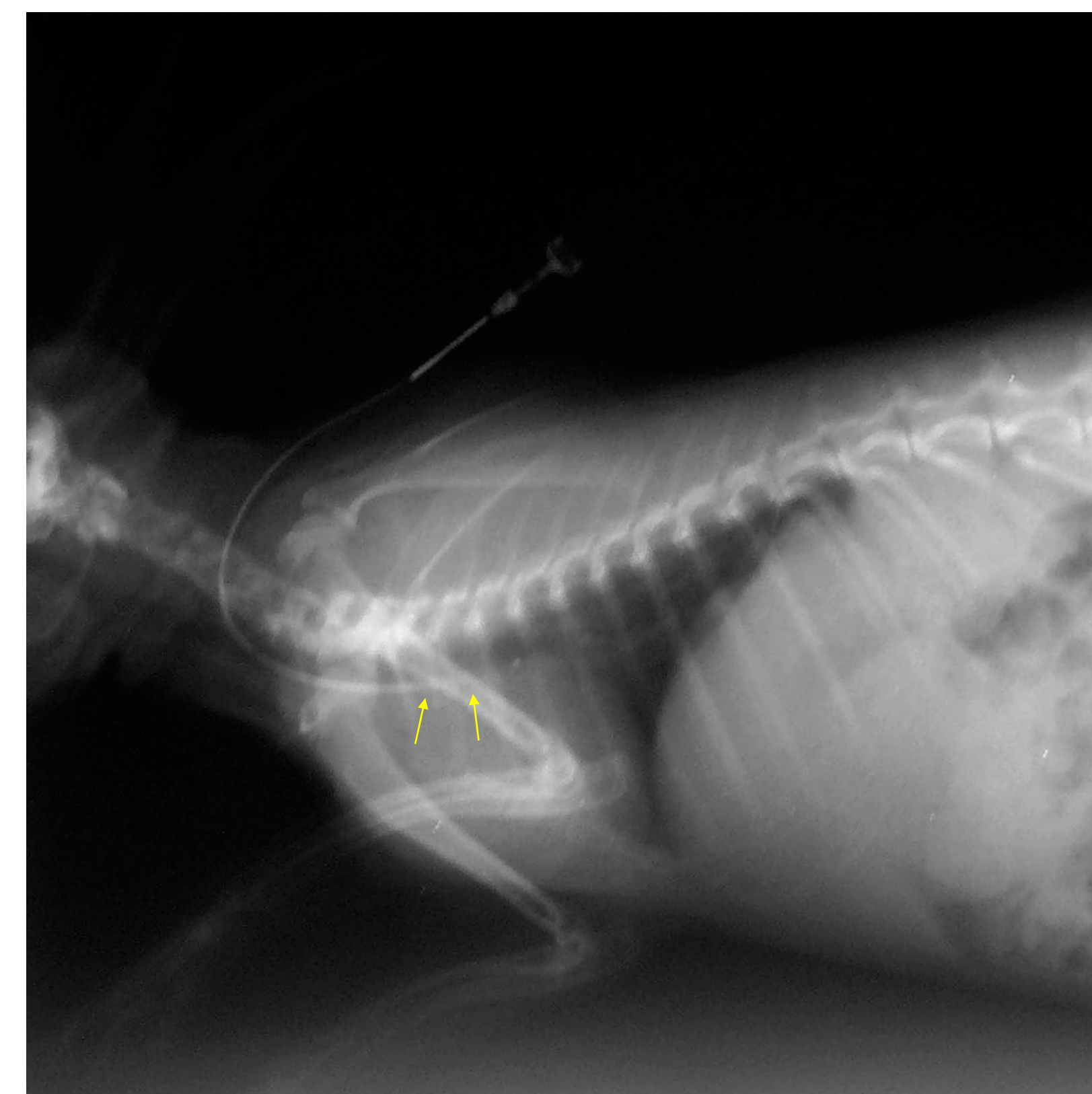


Figure 1. Post-operative venogram demonstrating placement of the central venous catheter within the right external jugular vein and the right anterior vena cava (arrows).

Treatment	Average Log CFU ± SD
B-Lock™	0
B-Lock™	0
B-Lock™	0
B-Lock™	0.82 ± 0.99
Untreated Control	4.26
Untreated Control	3.80 ± 0.08
Untreated Control	3.30
Untreated Control	2.53
Untreated Control	2.24 ± 0.10

Table 1. Bacterial colony forming units (CFU) per catheter

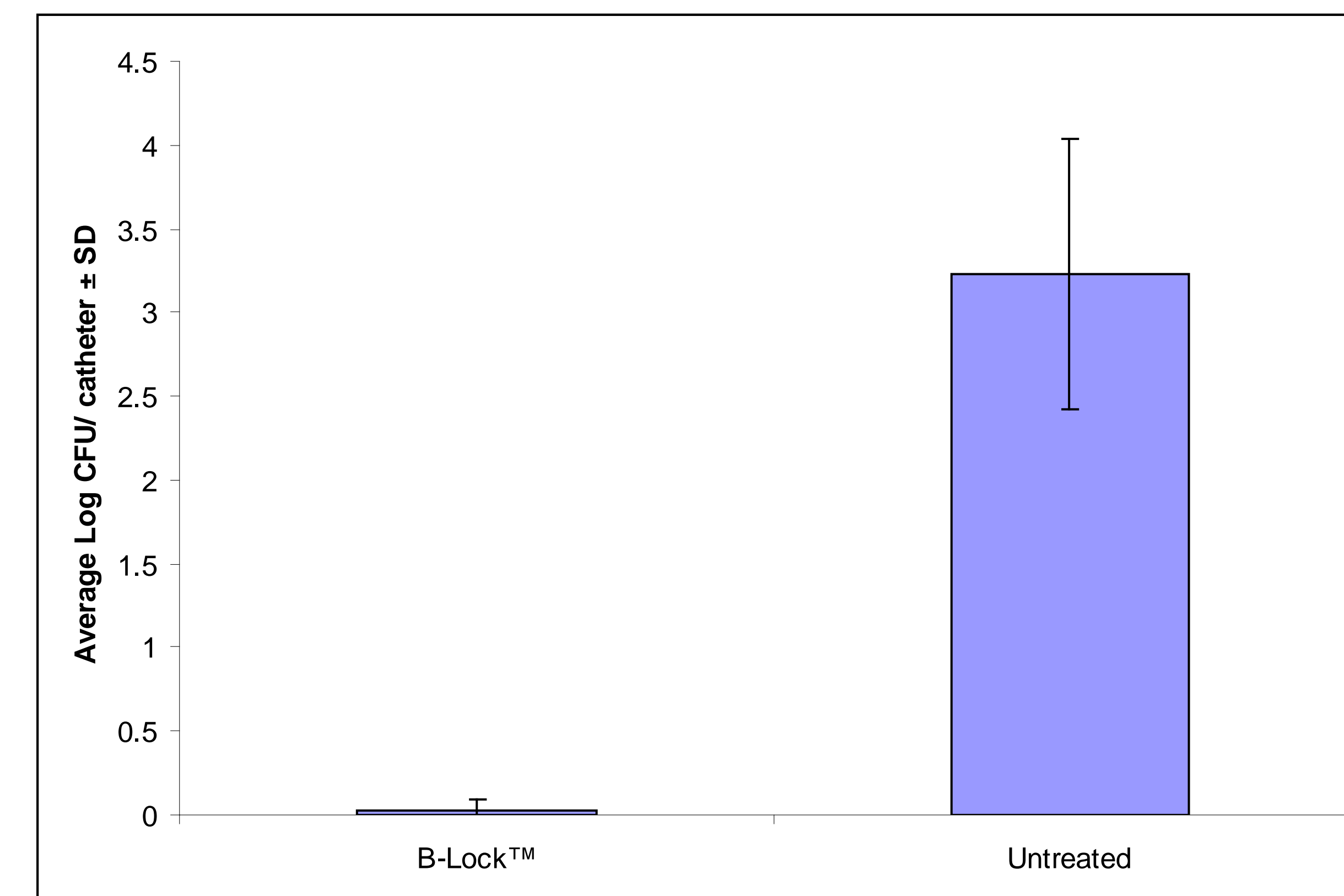


Figure 2. Bacterial burden of catheter segments obtained from treated and untreated animals with catheter-associated biofilms.

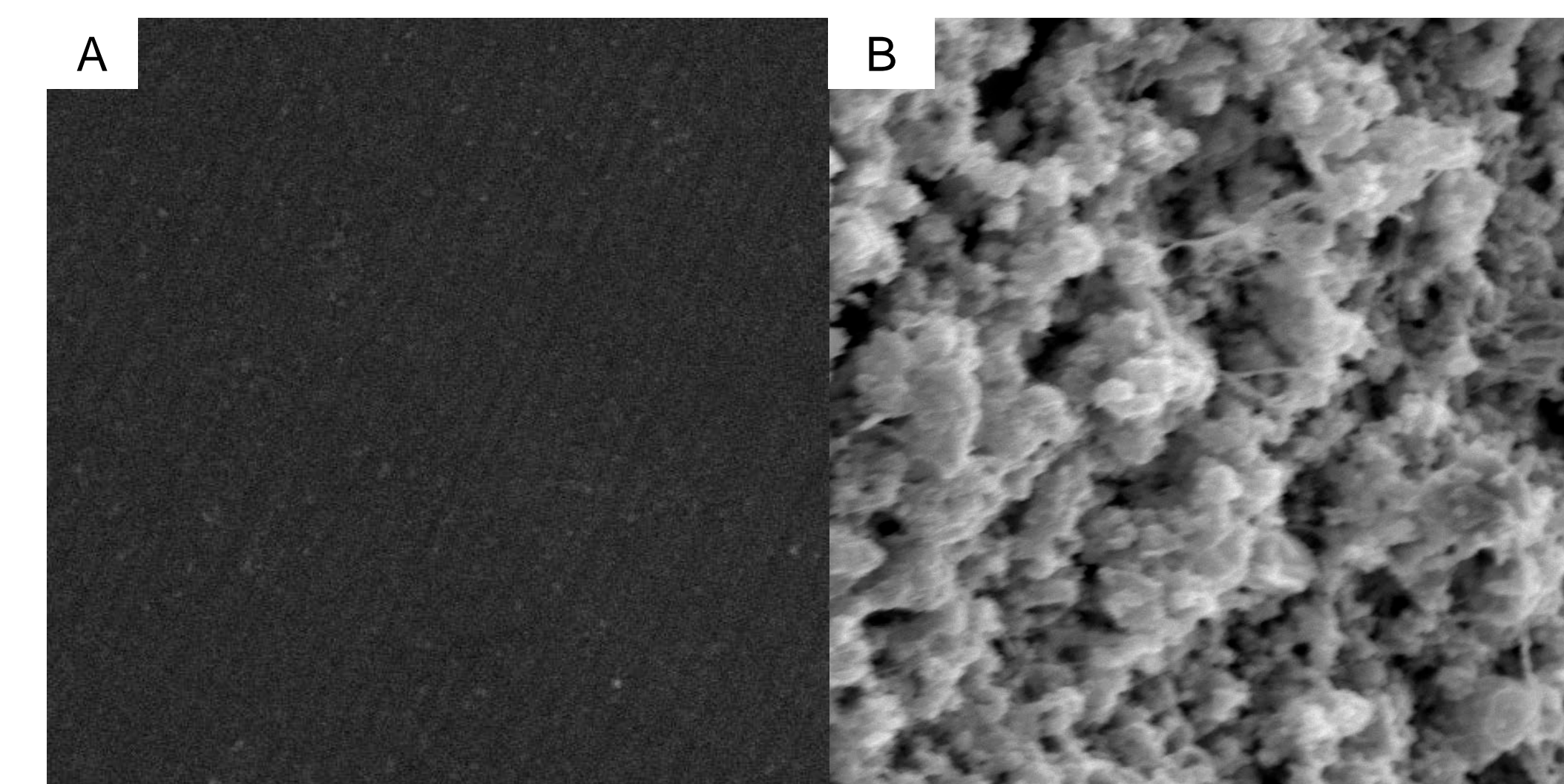


Figure 3. Representative photographs of scanning electron micrograph of intraluminal catheter surfaces; A. B-Lock™-treated, B. Untreated control

Results

Blood Cultures: All blood culture samples obtained from the catheters on day 3 post-infection (prior to the initiation of lock therapy) were positive for SA, confirming the presence of catheter associated biofilm infections.

Quantitative Analysis: As can be seen by Figure 2, the untreated controls behaved as expected yielding an average fungal burden of log₁₀ 3.23 ± 0.81 CFU/ catheter. B-Lock™-treated catheters yielded an average fungal burden of 0.03 ± 0.06 CFU/ catheter (*P*-value 0.0032 compared to the untreated control). Lock therapy with B-Lock™ cleared the MRSA infections in three catheters, yielding zero CFUs in each, and the fourth yielded extremely low CFUs (Table 1).

SEM: As can be seen by Figure 3, SEM results showed eradication of biofilms from catheters exposed to B-Lock™, while robust biofilm formation was observed on UC catheters.

Conclusions

Antibiotic lock therapy with B-Lock™ was highly efficacious in treating methicillin-resistant *Staphylococcus aureus* catheter-related biofilm infections. B-Lock™ may represent a useful treatment strategy for catheter salvage in bacterial blood stream infections. Randomized clinical trials evaluating the use of the tested catheter lock solution for the treatment of MRSA infection are warranted.

References

- Schinabeck MK, Long LA, Hossain MA, Chandra J, Mukherjee PK, Mohamed S, Ghannoum MA. Rabbit model of *Candida albicans* biofilm infection: liposomal amphotericin B antifungal lock therapy. *Antimicrob Agents Chemother.* 2004 May;48(5):1727-32.
- Chandra, J., P. K. Mukherjee, S. D. Leidich, F. F. Faddoul, L. L. Hoyer, L. J. Douglas, and M. A. Ghannoum. 2001. Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. *J. Dent. Res.* 80:903–908.

Acknowledgment This work is supported by a grant from Great Lakes Pharmaceuticals.