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Antimicrobial Activity of B-Lock against Bacterial and *Candida* spp. Causing Catheter-Related Bloodstream Infections▽

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The triple combination trimethoprim, EDTA, and ethanol (B-Lock), is an antimicrobial lock solution for use in indwelling intravascular catheters to prevent and treat catheter-associated infections. B-Lock demonstrated MICs of ≤0.05% (percentage of solution) against *Candida* spp. ($n = 125$) and 0.003% to 25% against bacterial strains ($n = 175$). B-Lock was also fungicidal against the majority of the *Candida* strains at 6% to 25%. B-Lock demonstrates potential value for the prevention and treatment of catheter-associated infections.

Central venous access is an important tool for the appropriate treatment and support of patients in the intensive care unit (ICU), hospitals, and outpatient settings for many life-threatening diseases, such as cancer and end-stage renal disease. However, central line-associated bloodstream infections contribute to patient morbidity and mortality, extended length of hospital stay, and increased cost of care (4–6, 8, 9, 11). Therefore, prompt intervention to prevent and salvage the functionality of the indwelling catheter is essential.

The Infectious Diseases Society of America (IDSA) guidelines for intravascular catheter-related infection recommend that antibiotic lock therapy should be used for catheter salvage (7). B-Lock catheter lock solution (B-Lock), a sterile, clear liquid solution consisting of (by wt/vol) 0.5% trimethoprim (5 mg/ml), 19% ethanol (25% by volume), and 3% calcium disodium EDTA (Ca EDTA) in phosphate-buffered saline (PBS) buffer, is an antimicrobial lock solution developed for prevention of microbial infections in indwelling intravascular devices (catheters) (1, 2).

The MIC of B-Lock was determined against 25 clinical strains each of the *Candida* spp. *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* (including fluconazole-susceptible and fluconazole-resistant isolates) and 25 clinical strains each of *Acinetobacter baumanii*, methicillin-resistant and -susceptible coagulase-negative S*taphylococcus*, methicillin-resistant and -susceptible *Staphylococcus aureus*, *Enterobacter* sp., *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* according to Clinical and Laboratory Standards Institute (CLSI) methodology (1, 2).

As can be seen in Table 1, B-Lock solution inhibited all isolates, both bacterial and fungal, at concentrations of 25% or lower, with 100% of *Candida* isolates ($n = 125$) inhibited at ≤0.05% and 90% of bacterial stains ($n = 200$) inhibited at 12.5%. Importantly, B-Lock had the lowest MIC_{50}, defined as the lowest concentration to inhibit 50% of the strains tested, against *C. albicans* (MIC_{50} of 0.0015%), one of the most prevalent species infecting immunocompromised patients, and against *C. glabrata* (MIC_{50} of 0.0015%), which is increasingly reported from systemic infections. In addition, B-Lock inhibited half of the *C. krusei* strains tested, which are innately resistant to multiple antifungals, at a concentration of 0.003%.

Activity against bacteria was strain specific, with the greatest inhibitory activity against *E. faecalis*, commonly found in bloodstream infections (MIC_{50} of 0.0125%). Importantly, B-Lock was also able to inhibit the growth of methicillin-resistant *S. aureus* (MRSA), methicillin-resistant coagulase-negative *Staphylococcus*, and *P. aeruginosa*, with MIC_{50}s of 0.05%, 1.6%, and 6.25%, respectively.

B-Lock was fungicidal against the majority of *Candida* strains tested, as defined by a ≥99.9% reduction in colony count from the initial inoculum. Similarly, B-Lock was cidal against 50% of the MRSA isolates at a concentration of 0.4%, and against 50% of the *E. faecalis* and methicillin-susceptible *S. aureus* strains at a concentration of 0.8%. Overall, B-Lock demonstrated greater cidal activity against Gram-positive bacteria than against Gram-negative organisms.

The fact that B-Lock demonstrates antimicrobial activity against such a broad range of systemic microbial pathogens suggests a distinct advantage in that this lock solution targets both bacteria and fungi and alleviates the need to use specific antifungal/antibacterial solutions. The underlying reason for the broad-spectrum activity for B-Lock could be explained on the basis of its active ingredients (ethanol and trimethoprim). Exposure to ethanol has long been suspected to have deleterious effects on bacterial cell membranes. Fried and Novick (3) postulated that ethanol may directly disrupt the interactions between phospholipid hydrocarbon chains or alter the aqueous-phospholipid interface. Since the majority of the enzymes for the synthesis of phospholipids, cell wall, and outer membrane components are associated with the cytoplasmic membrane, ethanol disruption of the membrane structure could lead to a defect in the cell division process itself. The membrane damage from ethanol may facilitate the entry of trimethoprim into the cell, thereby facilitating the inhibition of the target enzyme dihydrofolate reductase, which plays a central role in the synthesis of nucleic acid precursors (10).

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TABLE 1. MIC and MBC or MFC values of B-Lock against microorganisms associated with catheter infections

Isolate[a]	B-Lock MIC (% solution)		B-Lock MBC or MFC (% solution)[b]	
	Range	50%/90%[c]	Range	50%/90%[c]
Bacterial				
Acinetobacter baumanii	0.8–12.5	6.25/12.5	12.5–>25	>25/>25
Coagulase-negative staphylococcus, methicillin resistant	0.003125–25	1.6/25	0.25–>25	>25/>25
Coagulase-negative staphylococcus, methicillin susceptible	0.00625–25	0.2/6.25	0.025–>25	>25/>25
Enterobacter sp.	0.0125–25	0.4/25	0.1–>25	>25/>25
Enterococcus faecalis	0.003125–25	0.0125/12.5	0.05–>25	0.8/>25
Escherichia coli	0.00625–25	0.4/25	0.0125–>25	12.5/>25
Klebsiella pneumoniae	0.0125–25	3.125/25	0.05–>25	>25/>25
Pseudomonas aeruginosa	0.1–25	6.25	1.6–>25	>25/>25
Serratia marcescens	0.1–12.5	0.2	1.6–>25	>25/>25
Staphylococcus aureus, methicillin resistant	0.025–25	0.05	0.1–>25	0.4/>25
Staphylococcus aureus, methicillin susceptible	0.0125–12.5	0.025	0.05–>25	0.8/>25
Fungal				
Candida albicans	0.0002–0.003	0.0015/0.003	12.5–>50	50/50
Candida glabrata	0.0004–0.006	0.0015/0.003	25–>50	50/50
Candida krusei	0.0008–0.006	0.003/0.006	25–>50	>50/>50
Candida parapsilosis	0.0012–0.025	0.006/0.012	50–>50	50/>50
Candida tropicalis	0.0004–0.05	0.006/0.025	25–>50	50/50

[a] Throughout, $n = 25$, except for *S. marcescens* ($n = 18$).
[b] Minimal bactericidal concentrations (MBC) are shown for bacterial isolates, and minimal fungicidal concentrations (MFC) are shown for fungal isolates.
[c] 50%/90%, MIC_{50}/MIC_{90}, MBC_{50}/MBC_{90}, or MFC_{50}/MFC_{90}.

In conclusion, our study shows that B-Lock possesses a broad-spectrum antimicrobial activity against microorganisms known to cause central line-associated bloodstream infections. Evaluation of this solution for its efficacy to prevent such infections is warranted.

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