

# In Vitro Approach for Identification of the Most Effective Agents for Antimicrobial Lock Therapy in the Treatment of Intravascular Catheter-Related Infections Caused by *Staphylococcus aureus*

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Infection of intravascular catheters by *Staphylococcus aureus* is a significant risk factor within the health care setting. To treat these infections and attempt salvage of an intravascular catheter, antimicrobial lock solutions (ALSs) are being increasingly used. However, the most effective ALSs against these biofilm-mediated infections have yet to be determined, and clinical practice varies greatly. The purpose of this study was to evaluate and compare the efficacies of antibiotics and antiseptics in current clinical use against biofilms produced by reference and clinical isolates of *S. aureus*. Static and flow biofilm assays were developed using newly described *in vivo*-relevant conditions to examine the effect of each agent on *S. aureus* within the biofilm matrix. The antibiotics daptomycin, tigecycline, and rifampin and the antiseptics ethanol and Taurolock inactivated established *S. aureus* biofilms, while other commonly used antistaphylococcal antibiotics and antiseptic agents were less effective. These findings were confirmed by live/dead staining of *S. aureus* biofilms formed and treated within a flow cell model. The results from this study demonstrate the most effective clinically used agents and their concentrations which should be used within an ALS to treat *S. aureus*-mediated intravascular catheter-related infections.

The use of intravascular catheters (IVCs) in modern health care has increased over the last decades. Infection of these devices by surface-adhering bacteria, resulting in catheter-related blood stream infections (CRBSIs), is associated with significant patient morbidity and mortality, prolonged hospitalizations, and excess hospital-related costs. The Centers for Disease Control and Prevention (CDC) attributes 1 to 2% mortality among critically ill patients due to CRBSI (1).

Biofilms formed by *Staphylococcus*, in particular *Staphylococcus epidermidis* and *Staphylococcus aureus*, have for many years been recognized as the most frequent cause of CRBSI (2, 3). These biofilms are highly resistant both to the action of the immune and adaptive immune defense systems and to the action of antimicrobial agents, resulting in persistent infections and treatment failure.

The majority of guidelines recommend catheter removal and systemic antimicrobial treatment as a first-line or combination of CRBSI (4). However, clinical circumstances, for example, lack of alternative access sites, bleeding disorders, or extended coagulase time, often preclude device removal. Alternative strategies, such as the use of antimicrobial lock therapy (ALT), to treat these biofilm-related IVC infections, have generated considerable interest in recent years. Antimicrobial lock solutions (ALSs) have been used with variable success to kill the bacteria of the IVC in order to eradicate biofilms (5). This technique provides very high concentrations of antimicrobial agents at the site of infection. However, concerns around selection of resistant organisms, toxicity, and treatment failure have thus far limited their widespread application to the treatment of CRBSIs. The Infectious Diseases Society of America (IDSA) has issued guidelines on the management of CRBSIs, recommending the use of ALT for the salvage of an IVC associated with CRBSI (6). However, the choice of which antibiotic (or antibiotics) to be used in the ALT is often based on the *in vitro* susceptibility determined using conventional susceptibility testing, which may not accurately indicate that the antibiotic is

active against the same organisms at higher density and embedded within the biofilm matrix (7).

A range of antibiotics, including vancomycin, daptomycin, gentamicin, rifampin, and linezolid, have been used to eradicate biofilms in patients with CRBSI (8, 9). Recently, various antiseptic agents, including ethanol, HPA, and sodium citrate derivatives, have been studied, with significant variations in their effectiveness reported (10–12). There is also studies investigating the effectiveness of ALT have been limited to that they do not mimic features such as physiologically relevant flow (e.g., flow blood flow), the degradation of access proteins on IVC surfaces, and the nutrient limited environment found in the host. Furthermore, studies often utilize only a small number of reference strains using a static well performance plate biofilm assay. Therefore, there remains insufficient insight and knowledge about which agents, or combination of agents, is likely to be most effective in ALT against biofilm-forming *Staphylococcus* under *in vivo* conditions (13). The aim of this study was to use a biofilm model of IVC infections, more representative of the clinical environment, to investigate the

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