American Journal of Infection Control 41 (2013) e77-e79

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Brief report

Disinfection of needleless connectors with chlorhexidine-alcohol provides long-lasting residual disinfectant activity

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Key Words:
Central line-associated bloodstream infection
Line maintenance bundle

The optimal disinfection method for needleless connectors (NCs) is unclear. We used an experimental model of microbial NC contamination to test different scrub times (swipe, 5, 15, 30 seconds) of chlorhexidine-alcohol versus alcohol and for residual disinfectant activity. Swipe with alcohol did not adequately disinfect NCs, particularly when contaminated with *Staphylococcus aureus* or *Pseudomonas aeruginosa*. With ≥5-second scrub, chlorhexidine-alcohol and alcohol performed similarly, but chlorhexidine-alcohol showed residual disinfectant activity for up to 24 hours.

Central line-associated bloodstream infections (CLABSIs) are associated with substantial morbidity and cost. In pediatrics, most CLABSIs are related to line maintenance rather than insertion.[1] Disinfection of needleless connectors (NCs) prior to line entry is likely a critical factor in preventing late CLABSIs.[2]

There is variability in policies and clinical practice around disinfection of NCs, including scrub duration and the disinfectant itself.[1] Short (≤5 second) scrub times with alcohol may be inadequate,[3] but this is controversial.[4,5] Use of a 30-second chlorhexidine-alcohol scrub for NC disinfection, as tested in a recent pediatric intensive care unit collaborative study,[1] has important implications for practice and cost. Scrubbing for 30 seconds imposes a significant time burden for clinicians and may lead to delays in medication administration. Furthermore, chlorhexidine-alcohol pads are more expensive than alcohol pads.

We undertook this laboratory study to compare NC disinfection with 3.15% chlorhexidine gluconate-70% isopropyl alcohol versus 70% isopropyl alcohol using different scrub durations. We also assessed whether a chlorhexidine-containing product would have residual antimicrobial activity on NCs.

MATERIALS AND METHODS

Bacterial and yeast strains

The bacterial and yeast strains used were *Enterococcus faecalis* (strain 12030), *Pseudomonas aeruginosa* (strain PAO1), *Staphylococcus aureus* (strain Newman), *Staphylococcus epidermidis* (strain M187), and *Candida albicans* (strain SC5314). A *P aeruginosa* PAO1 biofilm mutant lacking the polysaccharide Psl (strain WFPA60)[6] was also tested to validate the model.

Inoculation

Our model of NC contamination and sample sizes were based on prior work by Menyhay and Maki.[3] The top surfaces of 1 brand of positive-pressure NC (MaxPlus; MP1000; Carefusion, San Diego, CA, 9-20 NCs/group/experiment) were inoculated by 15-second exposure to a suspension of bacteria (optical density [OD] at 650 nm [OD_{650}] 0.5) or yeast (OD_{650} 1.6) in sterile water. After drying for 24 hours at room temperature, contaminated NCs were attached to a sterile syringe and flushed with 5 mL broth media (tryptic soy broth for bacteria, yeast peptone dextrose for *Candida albicans*). The contamination level in flushed media was assessed by the OD_{650}

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Supported by a grant from the Program for Patient Safety and Quality at Boston Children's Hospital.
Conflicts of interest: None to report.

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http://dx.doi.org/10.1016/j.ajic.2012.10.018

Table 1
Contamination of NCs after disinfection by scrubbing for the indicated time with chlorhexidine-alcohol CHGA or alcohol alone

		Swipe		5 Seconds		15 Seconds		30 Seconds	
	None	CHGA	Alcohol	CHGA	Alcohol	CHGA	Alcohol	CHGA	Alcohol
Staphylococcus aureus	10/10	0/10	3/9*	0/10	2/9	0/10	1/10	0/10	0/10
Staphylococcus epidermidis	10/10	0/10	2/10	1/10	0/10	0/9	0/10	0/10	1/10
Enterococcus faecalis	10/10	0/10	2/10	0/10	0/10	0/10	2/10	0/10	0/10
Pseudomonas aeruginosa	10/10	0/10	5/10*	0/10	0/10	0/10	1/10	0/10	0/10
Candida albicans	10/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

CHGA, Chlorhexidine-alcohol.
NOTE. Values are presented as number positive/number tested.
*$P < .05$ compared with CHGA swipe group.

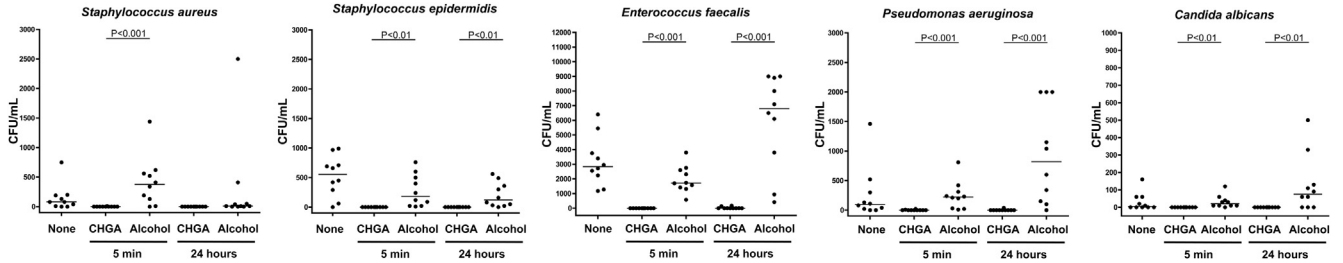

Fig 1. Residual disinfectant activity after scrub of NCs with chlorhexidine-alcohol or alcohol alone. After pretreatment with chlorhexidine-alcohol (CHGA) or alcohol alone, needleless connectors (NCs) were inoculated 5 minutes or 24 hours later. Contamination was measured by colony-forming units in 100 μL of the collected medium plated on agar. Each point represents the result from 1 NC, and bars represent medians. Each experiment was repeated 2 or 3 times, and results of a representative experiment are shown. *P* values were determined using Kruskal-Wallis test with Dunn's multiple comparison tests.

after overnight growth (37°C for bacteria, 30°C for yeast, 250 rpm), with contamination defined as $OD_{650} > 0.1$.

Disinfection

Contaminated NCs were scrubbed with 70% isopropyl alcohol pad (Webcol; Tyco Healthcare Group, Mansfield, MA) or 3.15% chlorhexidine gluconate-70% isopropyl alcohol pad (Chlorascrub; American Solumed, Newport Beach, CA). Scrubbing consisted of 180° back-and-forth twisting motion for <1 second ("swipe"), 5, 15, or 30 seconds (1 duration per NC). After disinfection, NCs were allowed to dry for at least 30 seconds and then flushed with media and cultured as above. The contamination level was quantified both by counting colony-forming units (CFU) in the flushed media on agar (plated immediately, a less sensitive measure of contamination) and by measuring the OD_{650} after overnight growth, with contamination defined as $OD_{650} > 0.1$. Pure culture was confirmed by subculturing a diluted aliquot on tryptic soy agar and MacConkey agar. Each experiment was repeated 2 or 3 times.

Residual disinfectant activity

Uncontaminated NCs were scrubbed for 15 seconds with either alcohol alone or chlorhexidine-alcohol and allowed to dry at room temperature for either 5 minutes or 24 hours. The pretreated NCs were then inoculated with bacteria or yeast as described above. After drying for 24 hours at room temperature, the NCs were flushed and cultured as described above. Each experiment was repeated 2 or 3 times.

Statistical analysis

The percent contamination for different disinfectants at the same scrub time was compared using Fisher exact test. CFU/milliliter in flushed media were compared using Kruskal-Wallis analysis of variance with Dunn's multiple comparison test. All analyses were performed with Prism software (GraphPad Software, San Diego, CA).

RESULTS

Needleless connector contamination model

We first validated the model by testing the ability of a biofilm mutant of *P aeruginosa* to contaminate the abiotic surface of the NC. As predicted from the known role of the Psl polysaccharide in *P aeruginosa* biofilm formation,[6] the Psl mutant contaminated only 35% of NCs (7 of 20) compared with 75% of NCs (15 of 20) by wild-type *P aeruginosa* strain PAO1 ($P < .05$).

Duration and type of disinfection

For disinfection experiments, the CFU in flushed media were nearly all below the limit of detection, so we instead used the more sensitive method of measuring the OD_{650} after overnight growth. For 15- or 30-second scrub with chlorhexidine-alcohol, none of the NCs showed contamination (all $OD_{650} < 0.1$) for all 5 microbes (Table 1). For NCs contaminated by *S aureus* and *P aeruginosa*, a swipe (<1 second scrub) with alcohol was significantly less effective than with chlorhexidine-alcohol (Table 1).

Residual disinfectant activity

As expected, there was no residual activity of alcohol alone. NCs pretreated with chlorhexidine-alcohol showed significant residual activity for all microbes tested (Fig 1). Similar differences were seen when contamination was assessed by overnight growth (not shown).

DISCUSSION

Proper disinfection of NCs before central line access is critical, but the optimal disinfectant and scrub duration are unknown.

In an in vitro model, we found that disinfection of NCs with chlorhexidine-alcohol was superior to alcohol alone for brief scrub times and that chlorhexidine-alcohol has residual disinfection activity on NCs up to 24 hours after application.

Although this study was limited to 1 type of NC, prior in vitro studies have shown that NCs from different manufacturers behave similarly.[5,7,8] Furthermore, positive displacement mechanical valve NCs have been associated with increased rates of CLABSI compared with split-septum NCs,[9] making study of disinfection methods important.

Our results align with previous clinical studies suggesting that disinfection of NCs with chlorhexidine-alcohol may be superior to alcohol alone.[7,8] In an in vitro study of *Enterococcus faecalis* contamination, Menyhay and Maki showed that a novel NC cover composed of a chlorhexidine-alcohol-impregnated sponge was superior to scrub with alcohol.[3] Others, however, reported that 15-second scrubs with chlorhexidine-alcohol or alcohol were equally effective at disinfecting NCs inoculated with a pool of microbes (*S aureus*, *S epidermidis*, *P aeruginosa*, and *C albicans*).[10] Investigations testing alcohol alone have reported no benefit of longer scrub times.[4,5]

Our finding of residual disinfectant activity of chlorhexidine-alcohol on NCs has potentially important clinical implications. More research is needed to assess whether the residual effect exists in clinical settings.

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