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Dimethyl Sulfoxide Enhances Effectiveness of Skin Antiseptics and Reduces Contamination Rates of Blood Cultures

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Effective skin antisepsis is of central importance in the prevention of wound infections, colonization of medical devices, and nosocomial transmission of microorganisms. Current antiseptics have a suboptimal efficacy resulting in substantial infectious morbidity, mortality, and increased health care costs. Here, we introduce an *in vitro* method for antiseptic testing and a novel alcohol-based antiseptic containing 4 to 5% of the polar aprotic solvent dimethyl sulfoxide (DMSO). The DMSO-containing antiseptic resulted in a 1- to 2-log enhanced killing of *Staphylococcus epidermidis* and other microbes *in vitro* compared to the same antiseptic without DMSO. In a prospective clinical validation, blood culture contamination rates were reduced from 3.04% for 70% isopropanol–1% iodine (control antiseptic) to 1.04% for 70% isopropanol–1% iodine–5% DMSO ($P < 0.01$). Our results predict that improved skin antisepsis is possible using new formulations of antiseptics containing strongly polarized but nonionizing (polar aprotic) solvents.

Antiseptics are crucial for the prevention of postoperative and device-associated infections; such infections result in substantial additional morbidity and health care costs (1–6, 11, 14, 27). Recently, attention has focused on antiseptic hand washing to decolonize bacteria from the skin of health care workers (5). Bacterial skin flora typically becomes sequestered in layers of dead keratinized skin, sweat glands, and hair follicles, making effective skin decontamination difficult. In addition to wound infections and other true infections, antiseptic failure can cause blood specimen contamination and contribute to inappropriate treatment and increased costs (27). Specifically, in one report when blood cultures were analyzed alone, a single false-positive blood culture from a hospitalized inpatient cost the patient an additional $4,200 in unnecessary medication, additional follow-up testing, and increased length of stay (1).

Clinical studies of antiseptic efficacy usually employ blood culture contamination with skin flora as the indicator system due to the large number of samples screened and the standardization of antiseptic practices. Currently used antiseptics have a significant failure rate, resulting in inappropriate evaluations for sepsis, unnecessary antibiotics, and increased length of hospitalization (1, 2, 9, 27). This has been shown for iodine-alcohol as well as chlorhexidine-alcohol mixtures, where blood culture contamination rates ranging from 3% to 5% have been reported in a tertiary-care setting (2, 14, 18, 20). Thus, there remains a significant need for improved antiseptics. One element of discovery of new antiseptics is developing suitable models for screening new antiseptics. In addition to the choice of antiseptic used, it is important to remember that effective technique and dedicated phlebotomy teams have been shown to have major impacts on contamination rates as well (2, 14, 27). For the clinical study presented here, we attempt to study only the effect of antiseptic type in isolation from these other technique- and practice-related effects.

In this paper we describe a two-part study aimed at improving both the antiseptics used in clinical practice and the screening method used to determine candidate antiseptics. First, we describe a method that allows rapid *in vitro* screening of antiseptic agents. Second, we introduce a novel antiseptic containing the polar aprotic solvent dimethyl sulfoxide (DMSO), a biocompatible solvent with low toxicity (FDA directive 67/548/ec). Finally, we validate the performance of the DMSO-containing antiseptic in a clinical trial involving 1,590 antiseptic application events from patients receiving blood cultures at our institution.

MATERIALS AND METHODS

To determine the relative effectiveness of the new versus standard antiseptics, both *in vitro* and *in vivo* approaches were used. The *in vitro* method directly compares split samples using methods detailed below (Fig. 1). Figure 1 illustrates the simultaneous transfer, mixing, dilution, and plating of both antiseptics in a paired manner, providing the critical timing control necessary for this method. The *in vivo* studies achieve control of variability by using a dedicated phlebotomy team trained to perform skin antisepsis in an identical manner for both kit types, using kits that are identical in appearance except for coded labeling, and using phlebotomy and microbiology teams blinded to the study agents. All studies presented are acceptable under our institutional review processes and as per our Institutional Review Board-approved protocol for evaluation of skin antiseptics (LAB01-321, J. J. Tarrand, principal investigator).

Strains. We performed *in vitro* testing using the following strains: commercial strains *Staphylococcus epidermidis* ATCC 12228 and ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 (American Type Culture Collection, Manassas, VA) and laboratory strains of coagulase-negative staphylococci (CNS) A1, B2, C3, and D4 and *Acinetobacter baumannii* strains 1, 2, and 3 (Microbiology Laboratory, Department of Laboratory Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX).

Reagents. Isopropanol, atomic iodine, DMSO (product 472301; material safety data sheet, version 3.6), ethanol, and chlorhexidine gluconate, all as American Chemical Society (ACS) grade, were obtained from Sigma-Aldrich, Inc. (St. Louis, MO).

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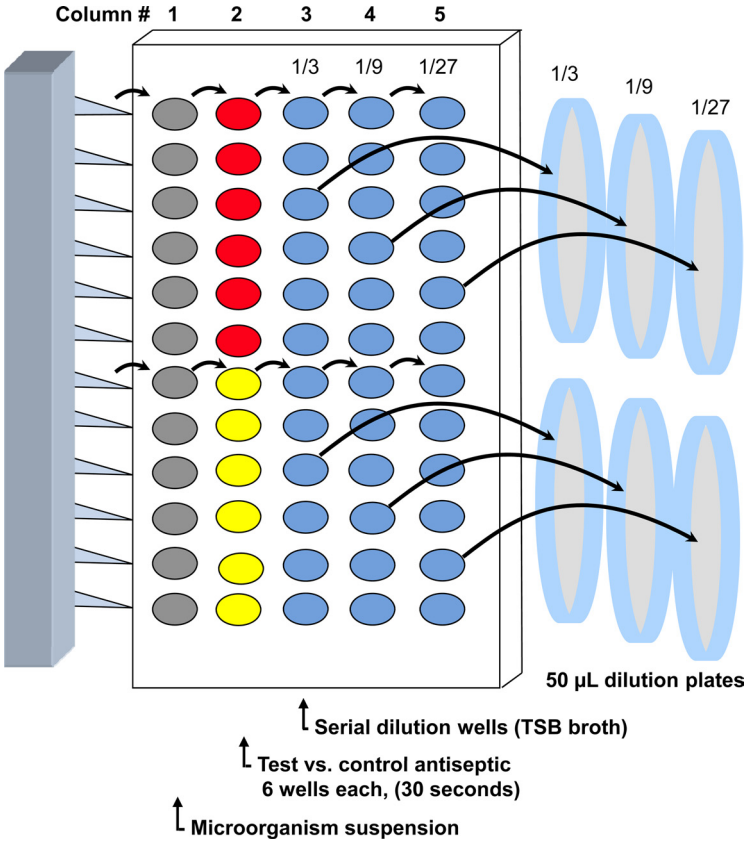

FIG 1 Method illustration. Samples of concentrated microorganism (12 wells) are simultaneously transferred using a multichannel pipette into test and control antiseptics. These wells are then further diluted in tryptic soy broth before being plated for colony counting.

Procedure for *in vitro* antiseptic evaluation. Microbial organisms were grown overnight at 35°C in three 35-ml blood culture bottles containing Columbia broth (Bactec Plus aerobic/F medium; Becton Dickinson, Sharpsburg, MD). The bottles were cooled to 4°C and centrifuged at 3,000 × *g* for 20 min, and the bacterial cells were resuspended in sterile 0.9% saline. The bacterial cells were allowed to incubate in saline at room temperature for 24 to 48 h to mimic the low temperature and low nutrition environment of human skin. These "aged" cells presumably also mimic the thick-walled sessile cells seen in stationary-phase bacterial cultures. The cells were centrifuged as above, resuspended in a minimal volume (~1.5 ml) of phosphate-buffered saline (PBS) or saline, placed in a pipetting trough, and distributed in 100-μl aliquots into the first 12-well column (column 1) of a 96-well U-bottom plate (Fig. 1). Thus, each well contained 100 μl of a concentrated suspension, estimated to contain approximately 1×10^{11} bacterial cells per ml based on the original turbidly of Bactec bottle growth (100 ml multiplied by approximately 10^9/ml). Fifty microliters of cells from this first column was then picked up using a 12-channel multichannel pipette and transferred to a second column (column 2) containing 150 μl of the test or control antiseptics (previously loaded into the plate) and mixed immediately (Fig. 1). Six wells contained the control antiseptic, and six wells contained the test antiseptic. The bacterial cells were allowed to interact with antiseptic for 30 s, and then a fresh 12-channel pipette was used to simultaneously transfer antiseptic-bacterial mixtures from column 2 to corresponding dilution wells containing tryptic soy broth (TSB; preloaded into the plate). Three serial dilutions were made rapidly by adding 50 μl of antiseptic-bacterial mixture to 100 μl of TSB broth medium to yield 1:3, 1:9, and 1:27 dilutions. This dilution step was done to rapidly stop antiseptic activity. Next, a fresh multichannel pipette was again used to simultaneously transfer 50-μl samples from each of these dilution wells onto sheep blood agar plates. The two plates were opened prior to the procedure and positioned closely together to simultaneously allow six channel tips to dispense to one plate for control wells and six channel tips to dispense to a second plate corresponding to the test antiseptic wells (Fig. 1). Plates were spread simultaneously using two sterile "hockey sticks." Colony count enumeration was performed after 18 to 24 h at 35°C. The starting alcohol concentration was usually 93.3%, and the starting DMSO concentration was 6.6%, to result in a final concentration of 70% alcohol and 5% DMSO following dilution of the 150-μl antiseptic well with the 50 μl of bacterial suspension containing approximately 10^{10} bacteria final per well. In some experiments, the isopropanol control antiseptic was supplemented with iodine or chlorhexidine gluconate; this mixture was then tested with or without DMSO (Table 1). For *in vitro* studies, the final antiseptic compositions and concentrations are as stated in Table 1.

Clinical evaluation of antiseptic effectiveness. To further demonstrate the effectiveness of the DMSO-containing antiseptic, we performed a clinical validation study using blood culture contamination rates as our measurable endpoint. All subjects were enrolled under LAB01-321, an approved protocol at M. D. Anderson Cancer Center. Patient demographics are shown in Table 2. We compared the rate of skin flora contamination of aseptically collected blood culture samples derived from patients exposed to either test or standard antiseptic. For this study only coagulase-negative staphylococci (CNS) and catalase-positive coryneform bacteria were classified as contaminants, and cultures were further required to show ≤1 CFU/ml of blood sample. All subjects had blood cultures ordered, and our phlebotomy team routinely disinfected ~50 cm^2 of antecubital skin using our standard application technique. Samples were randomized 1:1 between kits containing control antiseptic and those containing an experimental antiseptic. All kits were manufactured at the same time and stored at 4°C until use. Exposure to either antiseptic

TABLE 1 DMSO effect on antiseptic activity

Expt.	Organism	Alcohol	Iodine[b]	No. of CFU[a]	
				IP	IPD
A	*S. epidermidis* 12228	50% Ethanol		2,360	76
				1,730	58
B	*S epidermidis* 12228	50% Ethanol		400	5
				384	3
C	*S epidermidis* 12228	Isopropyl	Yes	399	5
				313	1
D	*S epidermidis* 12228	Isopropyl	Yes	146	1
E	*S epidermidis* 12228	Isopropyl	Yes	116	0
F	*S epidermidis* 12228	Isopropyl	Yes	51	0
G	CNS A1	Isopropyl		250	30
H	CNS B2	Isopropyl		15	0
I	CNS C3	Isopropyl		200	0
J	CNS D4	Isopropyl		147	10
K	*A. baumannii* strain 1	Isopropyl		1,500	8
L	*A. baumannii* strain 2	Isopropyl		47	2
M	*A. baumannii* strain 3	Isopropyl		~10,000	188
				~10,000	168
N	*E. coli*	Isopropyl		1,248	0
				1,174	0
O	*E. coli*	Isopropyl		192	0
				128	0
P	*E. coli*	Isopropyl		53	6
				43	3
Q	*P. aeruginosa*	Isopropyl		248	2
				188	0
R	*P. aeruginosa*	Isopropyl		190	8
				80	1

[a] Experiments A and B used a final concentration of 50% ethanol versus 50% ethanol plus 4% DMSO. The remainder of the experiments compared a final concentration of 70% isopropanol (IP) versus 70% isopropyl alcohol plus 4% DMSO (IPD). Plates were counted at 24 h. Some experiments show results for duplicate pairs of plates.
[b] In some cases, 2% iodine was added to 70% isopropanol with or without the addition of DMSO.

was a two-step process. Step 1 was to expose all antecubital sites to 1 ml of 60% isopropanol for 30 s. In step two, randomized subjects were exposed for 3 min to either the standard antiseptic, consisting of 1 ml 70% isopropanol–1% iodine–30% water (IPI), or the test antiseptic, consisting of 1 ml of 70% isopropanol–1% iodine–25% water–5% DMSO (IPID). Antiseptics were applied only by the Department of Laboratory Medicine's phlebotomy team, who were blinded to the type of antiseptic kit used. The DMSO-containing antiseptic had no difference in odor, color, or other observable difference compared to the standard antiseptic. The clinical microbiology technologist team, who were also blinded to the type of antiseptic kit, determined the level of sample contamination by using Isolator 10 (Wampole Laboratories, Cranbury, NJ) lysis-centrifugation blood culture tubes and by applying previously published criteria (21).

Statistical evaluation. Data from *in vitro* experiments were collected into random blocks (strata) and analyzed using a nonparametric, non-paired, two-tailed, signed rank test (Nebiyou Bekele, Department of Biostatistics, M. D. Anderson, Cancer Center). Comparison of continuous data was performed using a Student's *t* test. Clinical categorical data were compared using Fisher's exact test and two-tailed contingency statistics (Cytel Software Corp., Cambridge, MA.). *P* values of ≤ 0.05 were considered significant (7).

TABLE 2 Patient demographics

Parameter	Value for the antiseptic[a]	
	IPI	DIPI
No. of patients	331	326
No. of samples	822	768
Avg patient age (yrs [SD])	55.3 (14.1)	55.2 (14.7)
Male patient population (%)	56.5	59.8
Patients with leukemia (%)	38.6	42.6
Lymphoma/myeloma patients (%)	19.8	16.7
Patients with solid tumor (%)	41.6	40.7

[a] IPI, 70% isopropanol–1% iodine; DIPI, 5% DMSO–70% isopropanol–1% iodine.

RESULTS

Table 1 shows the effect of adding DMSO to isopropanol-based antiseptics versus isopropanol control mixtures on coagulase-negative staphylococci, including *S. epidermidis* ATCC strain 12228, and a variety of other bacteria. Inocula, dilutions, alcohol concentrations, and exposure times were all closely paired (Fig. 1). Plating of dilutions proceeded from the lowest dilution to the highest and, again, within a dilution isopropanol and isopropanol-DMSO plates were plated (spread) together. The plates derived following exposure to the DMSO-containing antiseptic showed reduced numbers of bacterial colonies by our *in vitro* method at 24 h compared to paired control antiseptic plates (Table 1).

Table 1 shows that the effect of antiseptics on *E. coli* plate counts was similar to that on CNS counts; 70% isopropanol–5% DMSO produced lower counts than 70% isopropanol ($P = 0.03$). Inocula, dilutions, alcohol concentrations, and exposure times were all closely paired. Plate counts at 24 h indicated that more bacteria were killed by the solution containing DMSO. Similar enhancement of killing was seen for *Pseudomonas aeruginosa*. *E.*

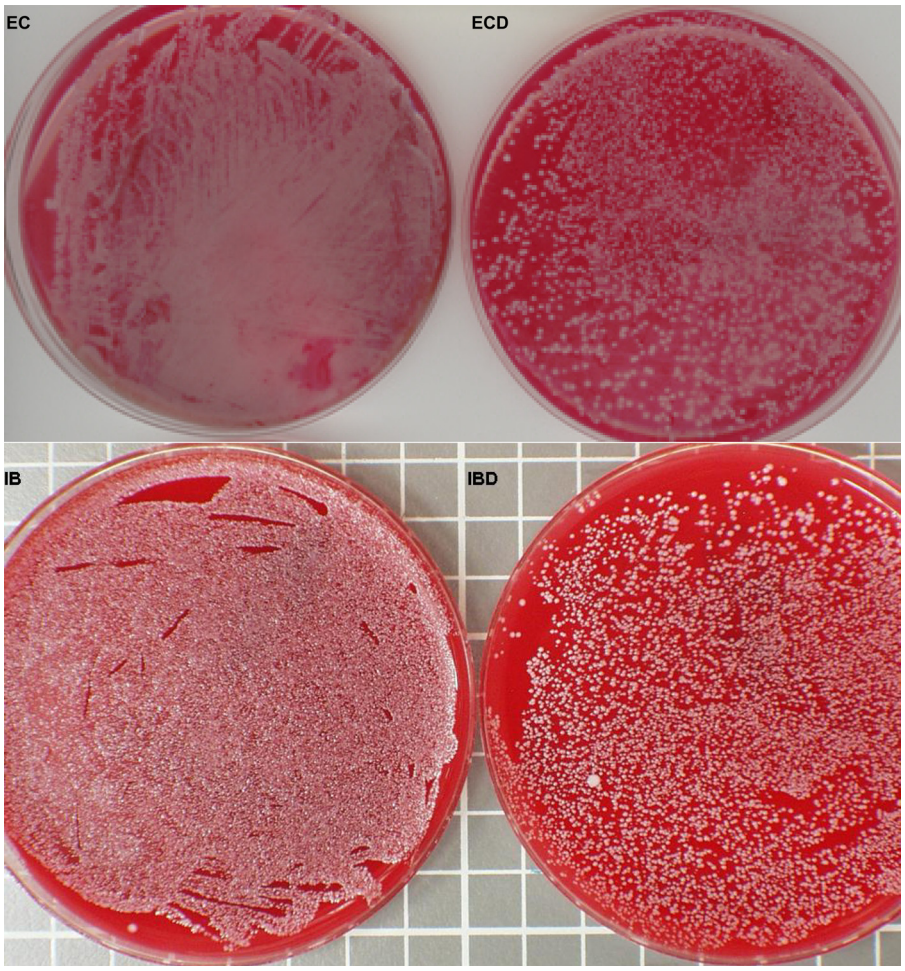

FIG 2 (Top) *S. epidermidis* killing in 50% ethanol–1% chlorhexidine gluconate (EC) compared to 50% ethanol–1% chlorhexidine gluconate–4% DMSO (ECD). (Bottom) *S. epidermidis* killing in 70% isopropanol–0.6% Brij 35 (IB) compared to 70% isopropanol–0.6% Brij 35–5% DMSO (IBD). Paired plates shown at a 1:3 dilution. Starting bacterial cell wells contain $\sim 10^{11}$ CFU/ml.

coli, *P. aeruginosa*, and *Acinetobacter baumannii* were tested since all are associated with nosocomial to skin contact transmission. Additional studies are presented in Fig. 2 showing a DMSO effect using matched plates of *S. epidermidis* exposed to different antiseptics (50% ethanol solutions containing 1% chlorhexidine gluconate with or without 4% DMSO or 50% ethanol containing 0.6% Brij 35 with or without 4% DMSO).

Although most of our data concern alcohol-based antiseptics, the effect of polar aprotic solvents is also seen in water-based solutions as well. When 18% DMSO versus 18% water was added to 10% povidone-iodine, enhanced killing activity was seen; 18% DMSO versus 18% water resulted in 2 versus 273 CFU, while 6% DMSO versus 6% water gave 26 versus 93 CFU.

Figure 3A shows the effectiveness of 70% isopropanol with the addition of various concentrations of DMSO. The addition of DMSO to isopropanol significantly increased antiseptic killing of *S. epidermidis* ATCC 12228, even at the lowest concentration tested (2.8% DMSO; *t* test, $P < 0.001$). However, DMSO in PBS without isopropanol had no antibacterial activity; 20% DMSO-PBS, pH 7.4, resulted in a mean CFU/plate of 250.5 (standard deviation [SD], 44.7; $n = 4$) versus 20% water-PBS, which resulted in a mean CFU/plate of 211.5 (SD, 13.3; $n = 4$; not significant). Figure 3B shows that when the DMSO concentration was held constant at 5%, increasing concentrations of isopropanol increased the activity of the alcohol-DMSO mixtures substantially ($P < 0.0001$). DMSO had no detectable effect at isopropanol concentrations of 40% or below in this series of experiments.

Finally, in the clinical validation portion of this study, we enrolled 1,590 antiseptic/blood culture events and evaluated the efficacy of the control versus DMSO-containing antiseptics. Enhanced antiseptic activity was seen when 5% DMSO–70% isopropanol–1% iodine (DIPI) was compared to 70% isopropanol–1% iodine (IPI). A 66% reduction in contamination was observed with DIPI versus IPI (1.04% versus 3.04%; Fisher exact, unpaired, two-tailed test, $P < 0.01$) (Table 3). The control antiseptic showed 23 CNS and 2 coryneform bacteria, and the test antiseptic showed three CNS and five coryneform bacteria. On average each patient had 2.5 samples collected during the study period, and no patient had more than one contaminant detected during the study time period. Toxicity was monitored using an incident report mechanism driven by the clinical team, phlebotomists, and patient reports. No time constraints were made on the incident reports. No reports of rash, irritation, redness, or other incident reports were made in association with the study patients.

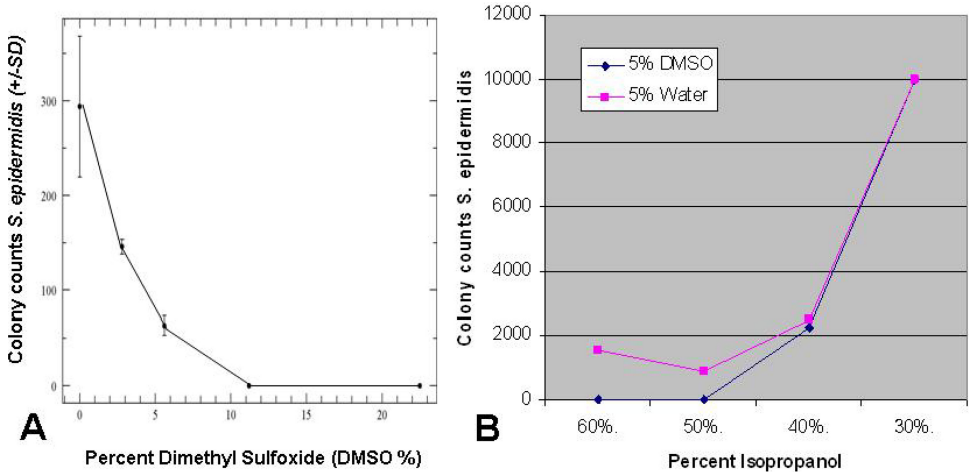

FIG 3 (A) Effect of DMSO concentrations on the activity of 70% isopropanol. (B) Effect of various isopropanol concentrations on antiseptic activity with 5% DMSO or water. *S. epidermidis* ATCC 12228 was used as the test organism. Note that 10,000 CFU is an estimate based on comparison to a dilution series of known standards.

DISCUSSION

The results of our *in vitro* and *in vivo* antiseptic studies showed that a new alcohol-based formulation containing DMSO resulted in increased antiseptic effectiveness. Further, this study demonstrated a useful dilutional screening method for antiseptic evaluation.

Preventing inappropriate treatment and decreasing the length of hospital stays have become national quality goals (6, 8, 9, 17), in part because of data showing that the cost of potentially preventable wound infections exceeds $3 billion annually in the United States alone (10, 12, 13, 16, 24, 26, 29).

Dimethyl sulfoxide has been previously used for drug delivery in topical therapeutic compositions. High concentrations (50% or greater) of DMSO are necessary in this setting, where DMSO facilitates the gradual absorption of drugs, such as nitroglycerin, directly through the dermis (28). We have used DMSO previously as an enhancement to standard oxidase testing (22). However, in the current paper we show surprising improvements in *in vitro* and *in vivo* antisepsis using only low concentrations of DMSO. The mechanism is not entirely clear. Aqueous channels (pores) have been shown to result from the swelling and increased mobility of phospholipid head groups in lipid bilayers using 27% DMSO (19). We hypothesize that this action of DMSO enhances the access of active agents (alcohol, iodine, and chlorhexidine) to critical bacterial cell structures such as bacterial pores.

Skin bacteria appear to exist in a sessile state, with low energy charge, possibly due to low temperature, low water activity, and low nutrition availability to bacterial cells (15, 25). A period of 24 h of bacterial cell aging *in vitro* seemed to mimic the behavior of bacteria from directly scraped skin cells exposed to test or control antiseptic in our studies. The 24-h aging time period is entirely arbitrary; nonetheless, the *in vitro* screening model using aged bacteria had the best agreement with skin scraping as well as clinical validation findings. Finally, it should be emphasized that the killing rates with modern alcohol-based antiseptics are very rapid indeed, and careful timing and pairwise sampling are critical to control variability in this model. We are not aware of other *in vitro* models suitable for antiseptic screening.

In general, iodine or other antiseptic-adjuvant-containing combinations are superior to alcohol antiseptics alone (18). All of the antiseptics that we tested (alcohol, alcohol-iodine, alcohol-chlorhexidine, alcohol-Brij 35, and water-povidone-iodine) have shown a proportional enhancement of killing with the addition of small amounts of DMSO. The addition of DMSO to water-based povidone-iodine was also superior to povidone alone. This is important since water-based antiseptics have a lower fire risk and are used extensively in the surgical settings. Other polar aprotic solvents such as dimethylacetamide showed a moderate to weak enhancing effect; however, this agent has additional toxicity concerns.

In our clinical validation involving 1,590 antiseptic/blood culture procedures, we found a 1.04% contamination rate for DMSO-containing tincture of iodine. This is below typical blood culture contamination rates (20, 23, 24, 29) and one-third of the rate seen with the standard iodine tincture in our validation trial. Interestingly, this rate, although low, does not approach the 100-fold reduction seen in the *in vitro* model. Perhaps the model is failing is some way, or perhaps this may relate to bacteria hidden in sebaceous glands, sweat glands, hair follicles, or sequestered in lacunae of the stratum corneum, suggesting an irreducible level of contamination for living skin. We performed earlier studies that showed similar trends but had to be stopped due to slow accrual (iodine-DMSO studies were stopped with 52 samples at 3 years, with isopropyl-iodine (IPI) contaminants in 5 of 30 patients and DMSO-isopropyl-iodine (DIPI) contaminants in 0 in 22 patients; chlorhexidine-DMSO studies were stopped with 365 samples at 2

TABLE 3 Control versus DMSO-containing antiseptic applied prior to blood culture

Antiseptic[a]	No. of contaminated blood cultures detected	No. of samples tested
IPI	25	822
DIPI*	8	768

[a] IPI, 70% isopropanol–1% iodine; DIPI, 5% DMSO–70% isopropanol–1% iodine. *, $P < 0.01$ for the reduction of contamination with DIPI versus IPI (Fisher's exact test).

years with isopropyl-chlorhexidine (IPC) contaminants in 4 of 175 patients and DMSO-isopropyl-chlorhexidine (DIPC) contaminants in 1 of 190 patients).

Rapid *in vitro* screening may facilitate further antiseptic development. Here, we demonstrate that the inclusion of small amounts of the polar aprotic solvent DMSO can improve the effectiveness of several currently used skin antiseptics. A new class of antiseptics based on inclusion of polar aprotic solvents may offer general improvements in skin antisepsis, including lower rates of wound infection, catheter infection, blood culture contamination, and nosocomial infection derived from health care worker hands.

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