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Impact of Chlorhexidine–Silver Sulfadiazine-Impregnated Central Venous Catheters on In Vitro Quantitation of Catheter-Associated Bacteria

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To assess the impact of the antiseptic effects of silver sulfadiazine–chlorhexidine-impregnated central venous catheters on catheter culture systems, a series of in vitro experiments was performed. Segments of antiseptic- and non-antiseptic-impregnated catheters were sonicated in thioglycolate broth and removed. After the addition of 10^3 CFU of *Staphylococcus epidermidis* per ml, aliquots of catheter-exposed broth were subcultured onto blood agar at 15-min intervals. Decreased mean colony counts were noted at 45 min for broth exposed to antiseptic-impregnated catheters compared with the colony counts for broth exposed to non-antiseptic-impregnated catheters (170 versus 540 CFU/ml). These effects, which were also demonstrated by the roll-plate method, were abrogated by the use of medium containing inhibitors of silver sulfadiazine and chlorhexidine. To assess the duration of the antiseptic effects, catheter segments were suspended for up to 14 days in phosphate-buffered saline, incubated with 10^6 CFU of *S. epidermidis* per ml, and cultured. Inhibition of bacterial growth by antiseptic-impregnated catheters disappeared after 14 days. These studies suggest that antiseptic compounds elute from catheters during broth- and solid medium-based culturing processes, making necessary the addition of inhibitors of these compounds in culture media. They further suggest that the antimicrobial effects of antiseptic-impregnated catheters wane within several days of placement.

Infections of intravascular catheters have emerged as major sources of morbidity and mortality in hospitalized patients. The National Nosocomial Infection Surveillance System estimates the incidence of nosocomial bacteremias associated with central venous catheters to be 5.8/1,000 catheter days in surgical intensive care units and 6.9/1,000 catheter days in medical intensive care units. Catheter-related bacteremias (CRBs) account for the vast majority of nosocomial bloodstream infections (3). The case fatality rate for CRBs has been estimated to be 10 to 20% (2).

Attempts to reduce the incidence of CRBs have focused on prevention of the entry of bacteria into the catheter system from the catheter hub and the catheter insertion site. Appropriate tubing changes (4), insertion site antisepsis (5), and the use of sterile barrier precautions during insertion (10) have all been shown to diminish, but not eliminate, the risk of CRB.

Recently, clinical data supporting the use of antiseptic central venous catheters impregnated on the external surface with silver sulfadiazine and chlorhexidine for the prevention of CRB have appeared in abstract form (7). Although in vitro experiments have suggested a gradual release of antiseptic compounds from these devices over several days (8), the effects of eluted antiseptics on standard catheter culturing techniques are unknown. We report here on a series of in vitro studies designed to assess the magnitude and duration of the effects of antiseptic impregnation of intravascular catheters on semiquantitative catheter cultures.

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MATERIALS AND METHODS

Central venous catheters. Non-antiseptic-impregnated and silver sulfadiazine–chlorhexidine-impregnated, three-lumen central venous catheters (Arrow International, Reading, Pa.) were divided into 5-cm segments by aseptic technique for all experiments.

Organisms. In all experiments a biofilm-producing strain of *Staphylococcus epidermidis*, strain ATCC 35984, was used.

Neutralizing medium. The neutralizing medium (formulation graciously provided by Shanta Modak, Columbia University, New York, N.Y.) consisted of 30 g of powdered Trypticase soy broth, 6 g of sodium oleate, 5 g of sodium thiosulfate, 1 g of tryptone, 50 ml of Tween 80, and 20 ml of lecithin, to which was added 200 ml of deionized water. The solution was mixed, diluted with 800 ml of deionized water, and autoclaved at 121°C (250°F) for 20 min.

Experiment 1. Experiment 1 was performed to screen for the elution of antiseptic compounds from catheters into culture media. A suspension of *S. epidermidis* adjusted to a density of 1.5×10^8 CFU/ml was inoculated onto blood agar plates as described previously for the standard disk diffusion test (9). Five-centimeter segments of antiseptic- and non-antiseptic-impregnated catheters were placed on separate plates. The plates were incubated at 35°C for 18 h and were visually inspected for inhibition of growth.

Experiment 2. Experiment 2 was performed to quantitate the effects of antiseptic-impregnated catheters on catheter culture techniques as performed in the clinical microbiology laboratory.

(i) Sonication. Five-centimeter segments of antiseptic- or non-antiseptic-impregnated catheters were placed in separate tubes containing 10 ml of thioglycolate broth and in tubes containing 10 ml of neutralizing medium (one segment per broth tube) (11). These experiments were performed in duplicate for antiseptic- and non-antiseptic-impregnated catheters. The tubes were sonicated in a water bath for 60 s and vortexed for 30 s, and catheter segments were immediately removed and discarded. An inoculum of 10^3 CFU of *S. epidermidis* per ml from a 2-h broth culture was added to each catheter-exposed broth tube and to tubes of unexposed thioglycolate broth. The tubes were then incubated at 35°C. Aliquots of 100 μl of broth were subcultured onto blood agar at 0, 15, 30, and 45 min and at 1, 3, 6, and 24 h after the addition of *S. epidermidis*. Colonies of staphylococci were counted after 24 h of incubation at 35°C.

(ii) Roll-plate method. Five-centimeter segments of antiseptic- or non-antiseptic-impregnated catheters were placed onto blood agar plates by using a sterile forceps and were rolled for approximately 15 revolutions (6). Inocula of 1.5×10^1 to 1.5×10^5 *S. epidermidis* organisms in 10-fold dilutions were added to separate plates and were spread evenly. The colonies were counted after 24 h of incubation at 35°C. These experiments were performed in duplicate for antiseptic- and non-antiseptic-impregnated catheters.

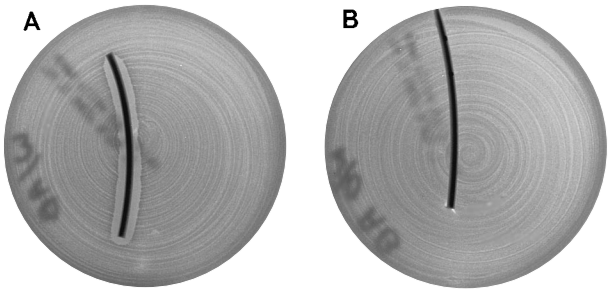

FIG. 1. Effect of antiseptic catheter on a lawn of *S. epidermidis*. A zone of inhibition is present around the antiseptic-impregnated catheter segment (A) but is absent around the non-antiseptic-impregnated catheter segment (B).

Experiment 3. Experiment 3 was performed to assess the duration of antimicrobial effects of antiseptic-impregnated catheters in a liquid system. Five-centimeter segments of antiseptic- or non-antiseptic-impregnated catheters were suspended in 500 ml of phosphate-buffered saline (PBS) in sterile beakers. Segments of regular or antiseptic-impregnated catheters were removed at 0, 3, 7, and 14 days; the PBS with the remaining catheters was replaced with fresh PBS on the same days as catheter segment removal. The removed catheters were exposed to 10^6 CFU of *S. epidermidis* per ml in 10 ml of Trypticase soy broth for 30 min and were then cultured as clinical specimens by the sonication and roll-plate methods as detailed above. These experiments were performed in duplicate for antiseptic- and non-antiseptic-impregnated catheters.

RESULTS

Effect of antiseptic-impregnated catheter on a lawn of *S. epidermidis*. There was a well-defined zone of inhibition surrounding the antiseptic catheter at 24 h of incubation (Fig. 1). In contrast, there was no visible inhibition of *S. epidermidis* by the non-antiseptic-impregnated catheter.

Effect of antiseptic-impregnated catheter on catheter culture techniques. Broth medium sonicated without catheters and with segments of non-antiseptic-impregnated catheters supported the rapid growth of *S. epidermidis*, with mean colony counts reaching quantities too numerous to count by 3 h (Fig. 2). However, broth sonicated with an antiseptic-impregnated catheter segment inhibited the growth of *S. epidermidis*, with a threefold decrease in colony counts at 1 h compared with the colony counts in broth exposed to a segment of a non-antiseptic-impregnated catheter. There was a 10-fold decrease in colony counts from 1 to 3 h and undetectable growth at 24 h in the antiseptic-impregnated catheter group. This effect was reversed with the use of media containing inhibitors of silver sulfadiazine and chlorhexidine, with growth equal to that in broth exposed to non-antiseptic-impregnated catheters.

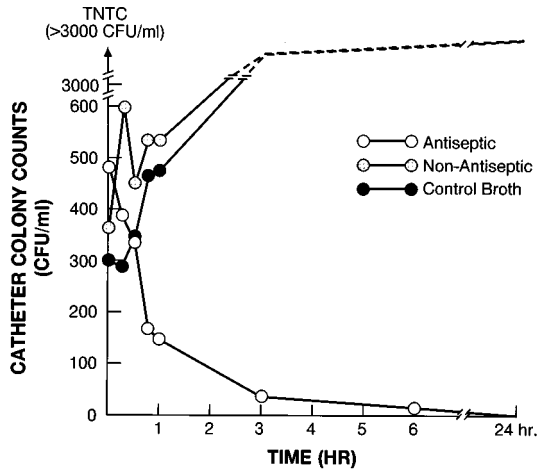

FIG. 2. Effect of antiseptic-impregnated catheter on catheter culture techniques. Mean colony counts of *S. epidermidis* from broth exposed to an antiseptic-impregnated catheter, a non-antiseptic-impregnated catheter, or no catheter (control) and then incubated for up to 24 h. TNTC, too numerous to count.

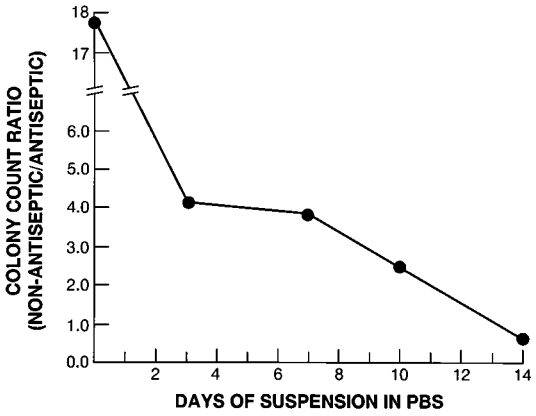

FIG. 3. Duration of antiseptic effects by the sonication method. Staphylococcal colony counts from antiseptic- and non-antiseptic-impregnated catheters suspended in PBS for up to 2 weeks and then incubated with *S. epidermidis* and cultured by the sonication method. The values for each time point are expressed as a ratio of colony counts from non-antiseptic-impregnated catheters to that from antiseptic-impregnated catheters.

A similar effect was observed by the roll-plate method. Blood agar plates on which antiseptic-impregnated catheters had been rolled exhibited a clear zone of inhibition of bacterial growth in the area where the catheter had been rolled, with uninhibited growth outside this area. With an inoculum of 1.5×10^2, 12-fold fewer colonies of *S. epidermidis* were seen on plates on which antiseptic-impregnated catheters had been rolled than on plates on which non-antiseptic-impregnated catheters had been rolled (mean, 15 versus 176 colonies).

Duration of antiseptic effects. Figure 3 shows the ratio of mean colony counts (sonication method) of *S. epidermidis* cultured from antiseptic- and non-antiseptic-impregnated catheters previously suspended in PBS for various periods of time. Because visual comparison with a density (McFarland) standard was used to estimate the inoculum for catheter exposure at each time point, colony counts for the non-antiseptic-impregnated (control) catheter varied at each time point. In order to standardize the data, a ratio of colony counts from non-antiseptic-impregnated catheters to that from antiseptic-impregnated catheters was used. At day 0, a high ratio (17.8) reflected the antimicrobial effects exerted by a fresh antiseptic-impregnated catheter. These effects gradually diminished and disappeared over time, with the colony count ratio approaching 1.0 at 14 days of suspension in PBS.

Similar results were obtained by the roll-plate method (Table 1). The wide disparity in colony counts between antiseptic- and non-antiseptic-impregnated catheters narrowed over the first week; the counts were similar by 10 days.

DISCUSSION

The disruption of normal cutaneous defenses against infection by intravascular catheters represents one of the best-recognized pathogenetic mechanisms for the development of nosocomial bacteremia. The most common pathogen associated with catheter-related infection is *S. epidermidis*, which possesses several virulence factors that facilitate its pathogenicity.

TABLE 1. Duration of antiseptic effects by the roll-plate method

Days of catheter suspension in PBS	Mean colony counts[a]			
	Non-antiseptic-impregnated catheter		Antiseptic-impregnated catheter	
	No. 1	No. 2	No. 1	No. 2
0	TNTC	TNTC	22	13
3	TNTC	TNTC	116	31
7	TNTC	284	287	65
10	TNTC	253	TNTC	290
14	TNTC	213	TNTC	232

[a] Staphylococcal colony counts from antiseptic-impregnated and non-antiseptic-impregnated catheters suspended in PBS for up to 2 weeks and then incubated with *S. epidermidis* and cultured by the roll-plate method. TNTC, too numerous to count (greater than 300 colonies per plate).

Entering at the catheter-cutaneous junction or the catheter hub, *S. epidermidis* interacts with the catheter itself and with various components of the surrounding glycoprotein sheath to quickly colonize the internal and/or external catheter surfaces. Growing colonies sequester themselves from host defenses by means of a polysaccharide biofilm, eventually escaping into the bloodstream to produce bacteremia and sometimes metastatic infection.

A catheter that could kill invading microorganisms represents a potentially important advance in the prevention of nosocomial device-related infections. The silver sulfadiazine–chlorhexidine-impregnated catheter used in the investigations described here has recently gained wide acceptance, despite a paucity of published clinical data supporting its efficacy. The results of Maki et al. (7) suggest that, compared with non-antiseptic-impregnated catheters, antiseptic-impregnated catheters are associated with a fourfold reduction in catheter-related infections. Similarly, Clemence et al. (1) were able to demonstrate a twofold reduction in the rate of CRB in an intensive care unit setting after the introduction of antiseptic-impregnated catheters. Because this reduction persisted for 7 months after the withdrawal of antiseptic-impregnated catheters from the study hospital, the portion of the reduction of CRBs attributable to antiseptic-impregnated catheters is in question. Few other studies have assessed the clinical benefit of these devices.

The accurate diagnosis of intravascular catheter-related infections continues to challenge both the clinician and the microbiologist. Because the signs and symptoms of such infections are frequently nonspecific or absent, the clinician relies on culture data to support the diagnosis. Unfortunately, no single laboratory method has emerged as a "gold standard" for the diagnosis of catheter-related infection. The reasons for this deficiency are probably multifactorial and include the need for catheter removal by most methods, an artifactual lowering of colony counts by prior antibiotic therapy, and contamination during the collection and processing of catheter specimens.

In addition, many catheter culture methods have technical aspects that limit their usefulness. Cumbersome rolling, flushing, or diluting steps frequently result in poor reproducibility and culture contamination. The investigations described in this report suggest an additional source of variability: elution of antiseptic compounds from the catheters themselves into the broth medium used to culture the catheter. The potential impact of antiseptic-impregnated catheters on quantitative catheter culture data is supported by the finding of antimicrobial activity in the broth medium following sonication with an antiseptic-impregnated catheter, despite immediate removal of the catheter following sonication. This activity was evident after only 15 min of exposure of *S. epidermidis* to antiseptic-impregnated catheter-exposed broth, but it was abrogated by the use of a broth culture medium containing inhibitors of chlorhexidine and silver sulfadiazine. Our clinical microbiology laboratory now uses this medium in its sonication catheter culture method. A similar antiseptic effect was observed by the roll-plate method.

In their original description of the antiseptic-impregnated catheter, Modak and Sampath (8) documented the release of radiolabelled silver and chlorhexidine into saline solution over a period of 12 days. Forty-five percent of the total chlorhexidine content of the catheter was released over the first 48 h, with 2 to 4% released daily thereafter. The rate of silver release was also highest in the first 48 h (22%), tapering to 1 to 4% on subsequent days. Our third experiment was designed to measure the elution of antimicrobial activity from an antiseptic-impregnated catheter over time. In the system described here, the antiseptic-impregnated catheter inhibited the growth of microorganisms for 1 week, with antimicrobial activity disappearing after 10 to 14 days. The fact that antistaphylococcal activity was absent after 10 days when it was measured by the roll-plate method and after 14 days when it was measured by the sonication method may reflect technical differences between the two culture methods.

These findings have important implications for the diagnosis of infections involving antiseptic-impregnated catheters by culture. It is critical that the media used in both broth and solid medium-based systems for culturing catheters include inhibitors of the antiseptic compounds contained in the catheters. Failure to use such inhibitory agents could lead to an underestimation of the quantity of catheter-associated bacteria. In the case of the silver sulfadiazine–chlorhexidine-impregnated catheter currently in use, this appears to be most important in the culture of catheters that have been in place for less than 2 weeks. The effect of the release of antiseptic compounds on qualitative or quantitative blood cultures drawn through the catheter is unknown and is the subject of ongoing investigation.

Our experiments provide strong supporting evidence of the powerful antimicrobial effects of a fresh silver sulfadiazine–chlorhexidine-impregnated catheter. However, it is notable that the antiseptic activity of this catheter is released in a flow system and reaches undetectable levels by 10 to 14 days. In addition, although bacterial adherence was reduced in the first week of catheter life, staphylococci were detectable by culture even on new catheter segments. If these in vitro findings can be extrapolated to the in vivo setting of high-volume blood flow past a catheter in a large central vein, then even freshly placed antiseptic-impregnated catheters may not be invulnerable to infection. Moreover, catheters in place for longer than 10 to 14 days may offer little advantage over non-antiseptic-impregnated catheters in the prevention of infection. The applicability of these data to patient care awaits the results of large-scale clinical trials of these devices.

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