Antimicrobial peripherally inserted central catheters (PICCs) might reduce the incidence of central line-associated bloodstream infections (CLABSI). We tested the biocompatibility of a novel gendine-coated (combination of chlorhexidine [CHX] and gentian violet [GV]) PICC in a rabbit intravascular model and tested antimicrobial efficacy in comparison with commercially available minocycline/rifampin (M/R)- and CHX-treated PICCs in an in vitro biofilm colonization model. Gendine-coated and uncoated control PICCs were inserted in the jugular veins of rabbits for 4 days. Histopathological analysis was performed at the end of the 4-day period, and circulating levels of CHX and GV in the blood were measured at different time points using liquid chromatography–mass spectrometry. The antimicrobial efficacy of the PICCs was tested following simulated intravascular dwells of 24 h and 1 week against clinical isolates of methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumanii, Enterobacter cloacae, Candida albicans, and Candida glabrata. Rabbits implanted with gendine-coated PICCs exhibited reduced levels of thrombosis and inflammation compared to those of the rabbits with uncoated controls. No GV was detected in blood samples over the entire study period, and trace concentrations of CHX were detected. The gendine-coated PICCs completely prevented the adherence of all pathogens from 24 h to 1 week (P ≤ 0.001), while M/R-treated, CHX-treated, and control PICCs did not. Gendine-coated PICCs were highly effective in preventing biofilm formation of multidrug-resistant pathogenic bacteria and fungi. Gendine-coated PICCs were biocompatible in an intravascular setting. Further, the pharmacokinetic testing established that acute systemic exposures of CHX and GV from the gendine-coated catheters were well within safe levels.
on the surfaces of indwelling catheters leads to biofilm formation in a complex development process that involves distinct stages of primary adherence, cell-to-cell interaction, microcolony formation, and development of a mature biofilm (17). Biofilms are extremely complex communities embedded in extracellular polymeric substances composed primarily of polysaccharides, proteins, and nucleic acids (18), which retain nutrients for the constituent cells and defend them from the host immune response and antimicrobial agents (19). As many as 60% of all microbial infections are related to biofilm formation (20); biofilms are clinically important, accounting for >80% of microbial infections in the body (21). We also tested the gendine-coated catheter in vivo for acute biocompatibility in a rabbit intravascular model utilizing histopathological examination and pharmacokinetics of circulating antiseptic levels in the blood by liquid chromatography-mass spectrometry.

**MATERIALS AND METHODS**

**Sequential coating of catheter with gendine.** Gendine-coated PICCs were produced by applying a proprietary sequential treatment process to polyurethane PICCs following extrusion. This incorporated gendine into the catheter walls and the luminal and external surfaces. Gendine consisted of a mixture of gentian violet (Sigma-Aldrich, St. Louis, MO) and chlorhexidine (Sigma-Aldrich). Commercially available polyurethane PICCs, which were uncoated (nonantimicrobial), M/R treated (Spectrum; Cook Medical, Bloomington, IN), and CHX treated (Chlorogard; Arrow International, Inc., Reading, PA) were included in the study as controls and comparators. Uncoated and gendine-coated PICCs were gamma sterilized at the MD Anderson Cancer Center Radiation facility, and the M/R- and CHX-treated PICCs were packaged sterile.

**Testing the baseline antimicrobial efficacy of gendine-coated catheters by biofilm colonization.** Uncoated control, M/R-treated, CHX-treated, and gendine-coated catheters were tested for the inhibition of biofilm formation using a biofilm colonization model (16, 22). Segments (1-cm length) of uncoated control, M/R-treated, CHX-treated, and gendine-coated PICCs were tested for the inhibition of biofilm formation by methicillin-resistant *Staphylococcus aureus* (MRSA) strain MDACC4798, vancomycin-resistant enterococci (VRE) strain MDACC3238, *P. aeruginosa* strain MDACC4689, *Escherichia coli* strain MDACC2131, *Acinetobacter baumannii* strain MDACC2012, Enterobacter cloacae strain MDACC2265, *Candida albicans* strain MDACC009-3072, and *Candida glabrata* strain MDACC0786. These isolates were collected from infected patients at our hospital (MD Anderson Cancer Center [MDACC]). Triplicate PICC segments were first placed into sterile 24-well tissue culture plates containing 1 ml of human donor plasma to enhance the formation and binding of blood proteins and were incubated for 24 h at 37°C. Following incubation, the plasma was then replaced with Mueller-Hinton broth containing 5.0 × 10⁷ cells of various organisms and incubated for an additional 24 h. After incubation, the microbial inoculum was discarded, and segments were washed by shaking for 30 min in 1 ml of 0.9% sterile saline. The segments were then removed with sterile sticks, placed in 5 ml of 0.9% saline, and sonicated for 15 min. We also tested the catheter used in each rabbit, and blinding was also employed for the individual collecting the tissue and blood samples. The severity of the inflammatory reaction was based on the quantification of inflammatory cells (neutrophils, lymphocytes, and plasma cells).

**Liquid chromatography-mass spectrometry analysis of chlorhexidine and gentian violet.** Chlorhexidine (CHX) and gentian violet (GV) were detected and quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled to a Waters Acquity ultra-performance liquid chromatography (UPLC) apparatus (Milford, MA) with temperature-controlled autosampler. The analytical column used to chromatographically resolve the compounds was a Phenomenex Kinetex phenyl-hexyl, 2.6-μm particle size, 2.1 by 100-mm column (Torrance, CA). The chromatographic method used a linear gradient of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile starting at 5% B to 95% B in 4.75 min. The flow rate was maintained at 0.300 ml/min with a column temperature at 60°C. The total run time for the analysis was 10 min, with CHX having a retention time of 3.29 ± 0.1 min and GV having a retention time of 0.9± 0.1 min.
Biocompatibility and Efficacy of Gendine-Coated Catheters

RESULTS

Baseline efficacy of inhibition by gendine-coated catheters. After 24 h of microbial challenge, gendine-coated catheter segments completely prevented the formation of biofilms of MRSA, VRE, P. aeruginosa, E. coli, A. baumannii, E. cloacae, C. albicans, and C. glabrata and were significantly superior to uncoated control catheters, showing 5- to 7-log reductions in the number of viable biofilm colonies \((P < 0.005)\). (Fig. 1, 2, and 3). In addition, the gendine-coated catheters showed better antimicrobial efficacy than M/R-treated catheters in the most species, except \(A. baumannii\) \((P \leq 0.03)\), reducing by 2 to 4 logs the number of viable biofilm colonies. Compared with CHX-treated catheters, gendine-coated catheters also showed better efficacy in the most species except MRSA \((P < 0.005)\), reducing by 2 to 6 logs the number of viable biofilm colonies.

M/R-treated catheters showed a 5-log reduction in the number of viable biofilm colonies of MRSA (median [range], \(5.0 \times 10^2\) colonies [0 to 1.5 \(\times 10^3\) colonies]) (Fig. 1) and \(P. aeruginosa\) (median [range], \(7.0 \times 10^2\) colonies [0 to 4.3 \(\times 10^3\) colonies]) (Fig. 2), and a 3-log decrease in the number viable biofilm colonies of \(E. cloacae\) (median [range], \(8.6 \times 10^3\) colonies [1.8 \(\times 10^3\) to 1.4 \(\times 10^4\) colonies]), and a 2-log reduction in VRE (median [range], 7.8 \(\times 10^3\) colonies [3.5 \(\times 10^3\) to 1.1 \(\times 10^4\) colonies]) (Fig. 1) compared to the uncoated controls.

The CHX catheter showed a 5-log reduction in MRSA (median [range], 2.2 \(\times 10^2\) colonies [0 to 8.5 \(\times 10^2\) colonies]), a 3-log reduction in VRE (median [range], 1.2 \(\times 10^3\) colonies [4.5 \(\times 10^2\) to 1.8 \(\times 10^3\) colonies]), \(E. cloacae\) (median [range], 9.5 \(\times 10^3\) colonies [5.5 \(\times 10^3\) to 1.2 \(\times 10^4\) colonies]), and \(E. coli\) (median [range], 2.1 \(\times 10^3\) colonies [1.3 \(\times 10^3\) to 3.0 \(\times 10^3\) colonies]), a 2-log reduction in \(A. baumannii\) (median [range], 9.3 \(\times 10^3\) colonies [5.0 \(\times 10^4\) to 1.5 \(\times 10^5\) colonies]) and \(C. glabrata\) (median [range], 6.3 \(\times 10^3\) colonies [3.5 \(\times 10^5\) to 1.1 \(\times 10^6\) colonies]), and 1-log reduction in \(C. albicans\) (median [range], 9.6 \(\times 10^2\) colonies [1.4 \(\times 10^3\) to 1.2 \(\times 10^4\) colonies]) compared to the uncoated control (Fig. 1 to 3). We found complete inhibition of biofilm formation of pathogens tested for both neutralizing and nonneutralizing broth.

Durability of inhibition by gendine-coated catheters. When we tested the durability of the prolonged inhibition of MRSA, VRE (Fig. 1), \(P. aeruginosa\), \(E. coli\), \(A. baumannii\), \(E. cloacae\) (Fig. 2), \(C. albicans\), and \(C. glabrata\) (Fig. 3) biofilm formation, gendine-coated catheters completely inhibited biofilm growth of all pathogens tested after 1 week of incubation. The M/R-treated, CHX-treated, and uncoated catheters permitted the growth of all organisms to various densities (Fig. 1 to 3). Data analysis showed that from baseline through week 1, gendine-
Coated catheters had significantly better antimicrobial durability than that of the uncoated control catheters (P < 0.0001), M/R-treated catheters (P < 0.0001), and CHX-treated catheters (P ≤ 0.001) (Fig. 1 to 3).

Histopathology studies. A total of five rabbits were used for the histopathology study. Two rabbits received untreated control catheters, and three rabbits received gendine-treated catheters. Jugular veins and subcutaneous tissue through which a catheter...
had been tunneled were collected and analyzed from each rabbit. Three cross-sections of each submitted tissue were processed, sectioned, stained with hematoxylin and eosin, and examined histologically.

Pharmacokinetics of chlorhexidine and gentian violet assessed by LC-MS. Chlorhexidine and gentian violet were detected without interfering peaks from plasma spiked with gentian violet and chlorhexidine standards and from plasma collected from rabbits with implanted gendine-coated catheters. The correlation curve was linear for the concentrations ranging from 0.05 ng/ml to 2.5 ng/ml, with correlation coefficients of 0.9937 and 0.9995 for chlorhexidine and gentian violet, respectively. The limit of detection was found to be 0.05 ng/ml for both chlorhexidine and gentian violet. The mean levels (n = 3) of CHX were 0.91 ng/ml (at 2 h), 1.08 ng/ml (at 24 h), 0.41 ng/ml (at 72 h), and 0.29 ng/ml (at 96 h). No detectable levels of GV were found in any of the plasma samples (Table 1).

DISCUSSION

Our data show that gendine-coated catheters are highly effective in preventing biofilm colonization of multidrug-resistant Gram-positive and Gram-negative bacteria and fungi, including MRSA, VRE, P. aeruginosa, E. coli, A. baumannii, E. cloacae, C. albicans, and C. glabrata, compared to uncoated catheters (P < 0.0001). Furthermore, the gendine-coated catheters had greater antimicrobial activity and were significantly more effective than commercially available M/R- and CHX-treated catheters against most pathogens tested. The antimicrobial activity and durability of the gendine-coated catheters were also significantly superior to those of traditional M/R- and CHX-treated catheters in preventing biofilm colonization of various nosocomial pathogens tested after a week of immersion in serum.

Though M/R-treated CVCs have been associated with prolonged antimicrobial activity in vitro (26), have excellent activity against multidrug-resistant staphylococci (27), and performed well in completely preventing CLABSIs caused by staphylococci in large prospective randomized trials (26, 28, 29), they failed to completely prevent CLABSIs caused by Gram-negative bacteria, like K. pneumoniae, E. cloacae, and Pseudomonas species (28). The prevalence of Gram-negative bacterial CLABSIs increased from 14 to 19% of the CLABSI cases reported in 1986 to 1999 (30, 31) to 28% of the CLABSI cases reported in the last decade (32). Furthermore, Candida species contribute up to 15% of all CLABSIs, and the attributable mortality rate of health care-associated candidemia has been reported to be between 38% and 49% (33). Hence, our novel gendine-coated catheter promises to be useful in preventing Gram-negative bacterial CLABSIs and catheter-related infections caused by Gram-positive and fungi.

Gendine consists of gentian violet and chlorhexidine. Gentian violet is a triphenylmethane dye with antibacterial, antifungal, and antihelminthic activity. Gentian violet has been used topically...

FIG 4 Histological cross-section of jugular vein of rabbit implanted with uncoated catheter. Gamma-sterilized 5-Fr. uncoated PICC catheters (length, approximately 6 in.) were inserted through a small incision over the right jugular groove of each rabbit, and subcutaneous tissue was bluntly dissected to expose the jugular vein. The vein was incised, and an uncoated catheter was inserted through the jugular vein and directed toward the heart. The uncoated catheter was secured with surgical glue at the site to the venous incision and tacked to muscle to be secured. On day 4 (postmortem), all rabbits were manually restrained for mask induction of anesthesia with isoflurane. Three cross-sections of each submitted tissue were processed with paraffin, sectioned, stained with hematoxylin and eosin, and examined histologically.

FIG 5 Histological cross-section of jugular vein of rabbit implanted with gendine-coated catheter. Gamma-sterilized 5-Fr. gendine-coated PICC catheters (length, approximately 6 in.) were inserted through a small incision over the right jugular groove of each rabbit, and subcutaneous tissue was bluntly dissected to expose the jugular vein. The vein was incised, and a gendine-coated catheter was inserted through the jugular vein and directed toward the heart. The gendine catheter was secured with surgical glue at the site to the venous incision and tacked to muscle to be secured. On day 4 (postmortem), all rabbits were manually restrained for mask induction of anesthesia with isoflurane. Three cross-sections of each submitted tissue were processed with paraffin, sectioned, stained with hematoxylin and eosin, and examined histologically.
throughout the world for many years, primarily to treat skin lesions coat the oral cavities of neonates, and as an oral rinsing agent for patients with oropharyngeal candidiasis (34–36). Chlorhexidine is a cationic polybiguanide with antimicrobial activity and has also been used for a wide range of medical applications. A chlorhexidine-alcohol solution is used to disinfect skin surgical sites and as a hand scrub (37, 38). Chlorhexidine has also been used to treat the umbilical cord of newborn babies (39–41). Further, chlorhexidine has been used to impregnate latex gloves (42). Among these two antimicrobial components of gendine, chlorhexidine increases the outer membrane permeability of pathogens and leads to leakage of their cellular contents (43). Gentian violet induces cell penetration and DNA binding, which inhibits DNA replication through the production of hydroxyl/perhydroxy radicals (44, 45). As they have independent mechanisms of action, we believe the increase in permeability produced by chlorhexidine works synergistically with gentian violet.

As an antiseptic combination not used for the treatment of systemic infections, gendine promotes to be a more effective catheter treatment than those currently available and might reduce the risk of developing antibiotic-resistant pathogens. In addition, there are recent reports of pathogens that are increasingly resistant to chlorhexidine alone. Gram-negative bacteria, such as P. aeruginosa, A. baumannii, K. pneumoniae, and E. coli, are reported to be resistant to chlorhexidine (46–48), and MRSA isolates with resistance to chlorhexidine have been found (49). Hence, the CHX PICC, which contains chlorhexidine only, presents a risk of being ineffective against those organisms resistant to chlorhexidine and further contributes to the development of chlorhexidine-resistant pathogens. In fact, the results of this study (Fig. 2 and 3) highlight the limitations of the efficacy of the CHX PICC against multidrug-resistant Gram-negative and fungal organisms. In contrast, not only did the gendine-coated PICC have significantly greater antimicrobial efficacy, but as a combination antiseptic treatment, the gendine-coated catheter is likely to have a low risk of developing increasingly chlorhexidine-resistant bacteria and fungi.

Our pharmacokinetics studies showed very low levels of chlorhexidine ranging from 0.29 ng/ml to 1.1 ng/ml over the 4-day period in our rabbit model. These levels of chlorhexidine were well below the level that was safely tolerated in other studies. The detectable median level of chlorhexidine in the blood was 32 ng/ml in 23 infants after 9 days of receiving umbilical cord disinfection with 1% chlorhexidine (50). In a more recent study, among 27 blood samples collected from 12 chlorhexidine-exposed children, 23 (85%) had chlorhexidine levels up to 4.5 ng/ml, and 15% had up to 17 ng/ml (51). Detectable levels of chlorhexidine have been reported in blood samples of 34 out of 96 patients, ranging from 10 ng/ml to 83 ng/ml, following vaginal washing with a 0.2% chlorhexidine solution (52). In another study, 10 infants among 20 infants receiving 2% chlorhexidine antiseptics just prior to PICC placement (53) had detectable levels of chlorhexidine (1.6 to 206 ng/ml) in their serum. Since no detectable levels of gentian violet were found in the blood, we believe that chronic chlorhexidine exposure levels from the gendine-coated catheter might not be different from chronic chlorhexidine exposures with catheters.

Our current gendine-coated catheter study differs from a previous gendine-coated catheter study (22) in that antimicrobial testing was performed using a quantitative biofilm colonization model. Our team previously reported on the development of gendine-treated medical devices, such as CVVs, urinary catheters, endotracheal tubes, and gloves made of different types of polymers (22–24, 34). These gendine-coated devices also showed antimicrobial efficacy and durability against a wide range of bacteria and fungi (22–24, 34). Furthermore, gendine-impregnated urinary catheters were able to prevent urinary tract infections in a rabbit model (54) when challenged with E. coli.

Inflammatory changes vary slightly between uncoated control and gendine-coated catheters; however, no treatment-related effect can be discerned based upon the type and number of samples examined. With that said, the inflammatory and thrombotic changes observed in the control (untreated) catheters tended to exhibit increased severity compared to that of the gendine-coated catheters, although there were overlapping variations. Gentian violet has been reported in mammalian cells to inhibit NADPH oxidases, leading to NF-kB inhibition and anti-inflammatory activity (55), which might be a contributing factor.

No detectable levels of gentian violet were found in blood samples collected from rabbits, indicating that there is minimal to no accumulation or absorption of gentian violet in the blood. Gentian violet was reportedly used in hundreds of thousands of people

### Table 1: Chlorhexidine and gentian violet concentrations in plasma as assessed by liquid chromatography/mass spectrometry

<table>
<thead>
<tr>
<th>Rabbit groupa</th>
<th>Concn (ng/ml) at time (h):</th>
<th>Control rabbits</th>
<th>GEN-rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>CHX</td>
<td>GV</td>
<td>CHX</td>
</tr>
<tr>
<td>Rabbit groupa</td>
<td>#1</td>
<td>#2</td>
<td>#1</td>
</tr>
<tr>
<td>Control rabbits</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>#1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.92</td>
</tr>
<tr>
<td>#2</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.00</td>
<td>0.00</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>GEN-rabbits</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>#1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.92</td>
</tr>
<tr>
<td>#2</td>
<td>0.00</td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>0.00</td>
<td>0.00</td>
<td>0.91 ± 0.02</td>
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</table>

a For the control rabbits, uncoated catheters were inserted in the jugular vein of the rabbit. Two rabbits were used for the control group. For the GEN-rabbits, gendine-coated catheters were inserted in the jugular vein of the rabbits. Three rabbits were used for the experimental group. Before insertion of the catheters, blood samples were collected at 0 h. The detection limit for chlorhexidine (CHX) and gentian violet (GV) was 0.5 pg/ml.
in South America (55) as a direct additive to blood to prevent the transmission of Chagas’ disease at concentrations of 0.6 mM (>200,000 ng/ml) (45, 56) without major side effects and with reversible staining of patient tissues. A clinical trial studying the topical treatment of full- and partial-thickness wounds with 1% gentian violet in 70 geriatric patients found no adverse events related to gentian violet, and >90% of the wounds completely healed (57). A recent review reports the clinical use of gentian violet in the treatment of skin cancer (55), and this was well tolerated with successful outcomes.

This study has a number of limitations. First, the microbiologist in this study who conducted the biofilm colonization experiment on catheters was not blinded. Second, the focus of the study was to look at the acute toxicity to peak blood levels of chlorhexidine and gentian violet reached. We plan to study the effects of chronic toxicity on the prolonged implantation of catheters in the future.

Conclusions. Gendine-treated PICCs were very effective in preventing biofilm formation by a range of highly pathogenic Gram-positive and Gram-negative bacteria and fungi, and they were significantly superior to commercially available M/R- and CHX-treated PICCs. The pharmacokinetic testing established that acute systemic exposures of chlorhexidine and gentian violet from the gendine-coated catheters were either negligible, in the case of gentian violet, or well within safe levels, in the case of chlorhexidine. Furthermore, gendine-coated catheters were found to be biocompatible with a good safety profile in an intravascular setting and in tissues exhibited less severe implant responses than those with nonantimicrobial catheters, as assessed by histopathologic analysis in rabbits. In the future, larger animal studies would enable comparisons of statistical significance.

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REFERENCES


