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Prevention of Biofilm Colonization by Gram-Negative Bacteria on Minocycline-Rifampin-Impregnated Catheters Sequentially Coated with Chlorhexidine

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Resistant Gram-negative bacteria are increasing central-line-associated bloodstream infection threats. To better combat this, chlorhexidine (CHX) was added to minocycline-rifampin (M/R) catheters. The *in vitro* antimicrobial activity of CHX-M/R catheters against multidrug resistant, Gram-negative *Acinetobacter baumannii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* was tested. M/R and CHX-silver sulfadiazine (CHX/SS) catheters were used as comparators. The novel CHX-M/R catheters were significantly more effective ($P < 0.0001$) than CHX/SS or M/R catheters in preventing biofilm colonization and showed better antimicrobial durability.

Central venous catheters (CVCs) are essential medical devices in the care of critically ill and cancer patients. Despite their significant usefulness, CVCs are also the source of 87% of the bloodstream infections that occur in intensive care units (1), with attributable mortality rates ranging from 13 to 25% and increases in hospital stays ranging from 7 to 12 days (2, 3). In the United States, more than 5 million CVCs are inserted annually (4, 5) and are responsible for 250,000 to 400,000 annual cases of health care-associated bloodstream infections (6, 7). Hence, central-line-associated bloodstream infections (CLABSIs) are the most common and serious complications associated with indwelling CVCs (5).

CVCs coated with antimicrobial agents have been proven to considerably reduce the risk of CLABSIs, and the use of antimicrobial CVCs has become a standard of care (8, 9). Two antimicrobial CVCs, minocycline-rifampin (M/R) and chlorhexidine-silver sulfadiazine (CHX/SS), were clinically proven to reduce CLABSIs at a time when skin-derived, Gram-positive bacteria were the most significant pathogens and were given CDC category 1A recommendations in the most recent guidelines for the prevention of intravascular-catheter-related infections (8–10). However, significant advances in skin antisepsis and sterile barrier precautions have shifted the epidemiologic threat increasingly to Gram-negative bacteria (11). The *in vitro* efficacy of M/R and CHX/SS against emerging Gram-negative pathogens is limited. In this report, we describe the development of a chlorhexidine-minocycline-rifampin (CHX-M/R) CVC. We compared the antimicrobial adherence activity and durability of M/R, CHX/SS, and CHX-M/R CVCs in a well-established *in vitro* model for the preventing biofilm colonization by multidrug-resistant clinical isolates of *A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. maltophilia*. These organisms were found to contribute to the majority of Gram-negative CLABSIs in the United States from 2009 to 2010 (12). Developing an antimicrobial catheter with broad-spectrum antifungal and antibacterial activity that includes resistant Gram-negative bacteria is of paramount importance because of the mortality rates associated with Gram-negative bacteremia, which may exceed 40% (13, 14).

CHX-M/R was prepared by a proprietary sequential coating method. Briefly, polyurethane catheter material was first impregnated with a mixture of minocycline and rifampin. After drying, catheters were treated on the lumen and exterior surfaces with a CHX-polymer coating. A previous study with CHX-M/R (15) employed the reverse sequence. The updated method provided a smoother surface finish. Commercially available triple lumen 7Fr. M/R (Cook Medical, Bloomington, IN) and CHX/SS (Arrowgard BluePlus; Arrow International, Inc., Reading, PA) catheters were tested as comparators. An uncoated polyurethane CVC was used as a positive control.

The antimicrobial activity of catheters was tested against *A. baumannii* strain 2021, *E. cloacae* strain 2265, *E. coli* strain 2131, *K. pneumoniae* strain 2856, *P. aeruginosa* strain 4698, and *S. maltophilia* strain 5572 in a well-established biofilm colonization model (11, 15, 16). Briefly, uncoated and coated catheter segments were incubated at 37°C for 24 h in donor plasma in triplicate. The donor plasma was then replaced with 5.0×10^5 cells in Muller-Hinton broth containing Gram-negative bacteria and incubated for an additional 24 h. After washing, the segments were sonicated in 5 ml of 0.9% sterile saline for 15 min and 100 μl of liquid from each segment was serially diluted and spread onto Trypticase soy agar–5% sheep blood agar plates for quantitative culture. Plates were then incubated at 37°C inverted for 24 h and then examined for colony growth.

To test the durability of inhibition of biofilm colonization on the CVCs, uncoated and coated catheters were incubated for up to 3 weeks in serum at 37°C and then challenged with bacteria for 24 h to assess retained antimicrobial activity in the biofilm colonization model (11, 15, 16). The shelf stability of the CHX-M/R cath-

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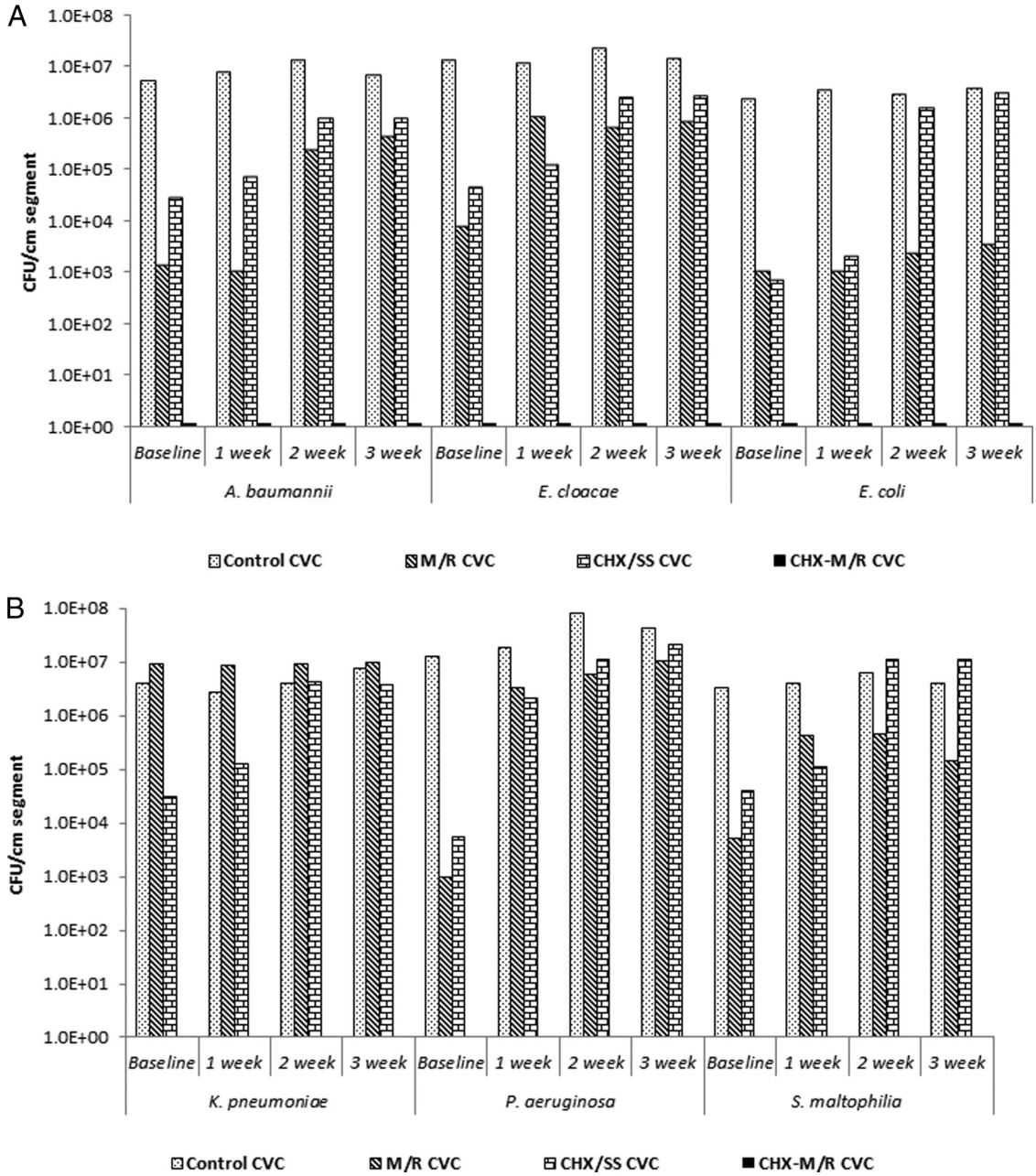

FIG 1 *In vitro* antimicrobial activity for 24 h (baseline) and durability for up to 3 weeks of different antimicrobial-coated catheters against *A. baumannii*, *E. cloacae*, and *E. coli* (A) and *K. pneumoniae*, *P. aeruginosa*, and *S. maltophilia* (B).

eters was tested by storing them at 25°C for 1 year and then retesting their baseline antimicrobial activity in the biofilm colonization model (11, 15, 16). Statistical analyses were performed by using SAS version 9.1 (SAS Institute Inc., Cary, NC) for the Kruskal-Wallis test, the Wilcoxon rank sum test, and two-way nonparametric analysis of variance.

Biofilm colonization on coated CVCs. CHX-M/R CVC segments completely prevented biofilm colonization of all pathogens tested at 24 h (baseline). The inhibition of CHX-M/R CVCs was significantly greater than that obtained for M/R, CHX/SS, and uncoated control CVCs ($P < 0.0001$) (Fig. 1A&B).

M/R-coated CVC segments exhibited a 5-log reduction of *P. aeruginosa* (median [range], 9.5×10^2 [2.6×10^1 to 5.1×10^3] versus 1.3×10^7 [1.2×10^7 to 2.3×10^8]) (Fig. 1B), a 4-log reduction of *E. cloacae* (median [range], 8.0×10^3 [3.2×10^3 to 3.1×10^4] versus 1.3×10^7 [1.2×10^7 to 2.8×10^7]) (Fig. 1A), and a 3-log reduction relative to uncoated controls in viable biofilm colony counts of *A. baumannii* (median [range], 1.4×10^3 [0 to 2.8×10^3] versus 5.6×10^6 [3.9×10^6 to 7.5×10^6]), *E. coli* (median [range], 1.1×10^3 [0 to 2.4×10^3] versus 2.4×10^6 [8.5×10^5 to 5.1×10^6]) (Fig. 1A), and *S. maltophilia* (median [range], 5.2×10^3 [2.9×10^3 to 8.2×10^3] versus 3.2×10^6 [1.5×10^5 to 7.4×10^6]) (Fig. 1B).

CHX/SS catheter segments exhibited a 4-log reduction in via-

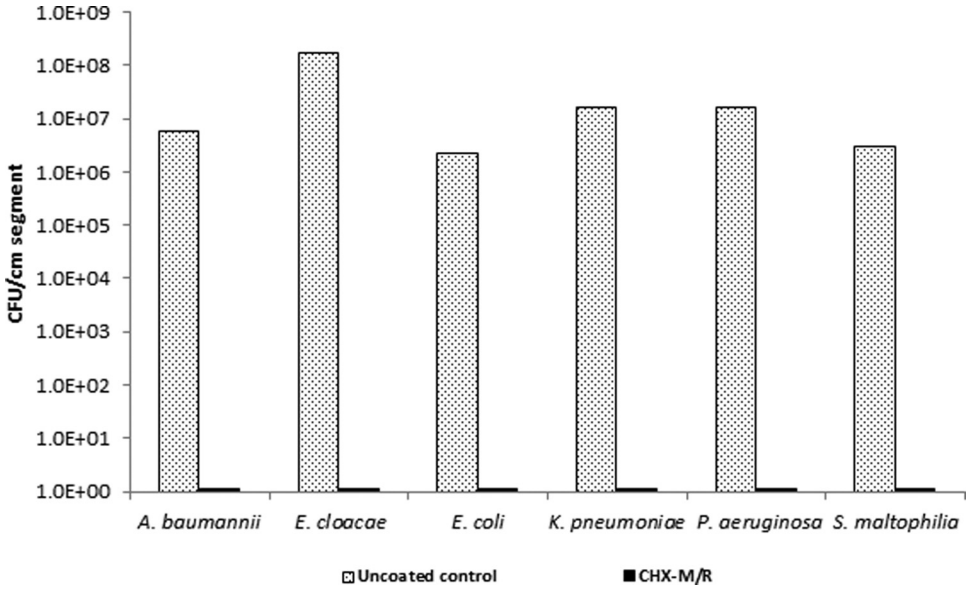

FIG 2 Antimicrobial activities of CHX-M/R catheters against *A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. maltophilia* after 1 year of storage at 25°C.

ble biofilm colony counts of *E. coli* (median [range], 7.3×10^2 [0 to 1.3×10^3] versus 2.4×10^6 [8.5×10^5 to 5.1×10^6]) (Fig. 1A), *P. aeruginosa* (median [range], 5.4×10^3 [2.8×10^3 to 8.8×10^3] versus 1.3×10^7 [1.2×10^7 to 2.3×10^8]) (Fig. 1B) and a 3-log reduction in viable biofilm colony counts of *E. cloacae* (median [range], 4.5×10^4 [1.8×10^4 to 5.8×10^4] versus 1.3×10^7 [1.2×10^7 to 2.8×10^7]) (Fig. 1B) compared to uncoated control CVCs. The difference between the antimicrobial activities of M/R and CHX/SS CVCs against *A. baumannii*, *E. cloacae* (Fig. 1A), *P. aeruginosa*, and *S. maltophilia* (Fig. 1B) was significant ($P = 0.01$).

Durability of antimicrobial-coated CVCs. CHX-M/R catheter segments retained antimicrobial durability against *A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. maltophilia* compared to M/R, CHX/SS, and uncoated control CVCs over 3 weeks. Weekly results were significant ($P < 0.0001$) (Fig. 1A and B) throughout the 3-week incubation of catheters in serum.

Shelf life of CHX-M/R CVCs. After 1 year of real-time storage of CHX-M/R catheters at 25°C, antimicrobial activity was fully retained. Biofilm colonization of Gram-negative bacteria was fully inhibited compared to uncoated control catheters ($P < 0.0001$); indicating that the baseline antimicrobial activity of CHX-M/R was stable (Fig. 2).

CHX has been reported to increase outer membrane permeability in Gram-negative organisms by binding to anionic moieties on the cell membrane, thus disrupting the transmembrane transport system (17). We demonstrated here a synergistic augmentation of the activity of M/R against Gram-negative bacterial biofilm colonization through complete prevention of the formation of biofilms of Gram-negative pathogens. Our recent study showed that CHX-M/R CVCs had prolonged antimicrobial durability against Gram-positive bacteria, fungi, and *P. aeruginosa* (15). Thus, the broad-spectrum activity of the CHX-M/R catheter has the potential to bring the rate of CLABSIs close to zero.

A large multicenter prospective randomized trial showed that CHX/SS-coated CVCs have a short antimicrobial durability and are not as effective in preventing infection as M/R-coated CVCs (18). The CVC impregnated with M/R has been associated with prolonged antimicrobial durability *in situ* for around 50 days (4) and has excellent activity against resistant staphylococci (19). However, in large prospective randomized trials the antibiotic M/R catheter, which performed well in completely preventing CLABSI caused by staphylococci (4, 20, 21), failed to completely prevent CLABSI caused by Gram-negative pathogens such as *K. pneumoniae*, *E. cloacae*, and *Pseudomonas* species (20).

Among 217 bacteremias caused by *S. maltophilia*, 73% were diagnosed as catheter-related infections in cancer patients (22). Furthermore, the prevalence of Gram-negative bacillus CLABSIs has increased from 14 to 19% of the CLABSI cases reported from 1986 to 1999 (23, 24) to 28.2% of the CLABSI cases reported in the last decade (25). Therefore, the novel antimicrobial CVC impregnated and coated with CHX-M/R should be highly useful in preventing these rapidly emerging Gram-negative CLABSIs, as well as catheter infections caused by Gram-positive bacteria and fungi (15).

Conclusions. A novel CHX-M/R CVC was superior to M/R and CHX/SS CVCs in inhibiting biofilm colonization by various resistant Gram-negative bacteria. The CHX-M/R CVC also displayed superior antimicrobial durability against the same Gram-negative bacteria. Clinical testing is warranted and necessary to prove that this treatment significantly reduces bloodstream infections caused by resistant Gram-negative bacteria.

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