Comparative Efficacy of Commercially Available and Emerging Antimicrobial Urinary Catheters Against Bacteriuria Caused by *E. coli* In Vitro

Gilly Regev-Shoshani, Mary Ko, Adam Crowe, and Yossef Av-Gay

OBJECTIVES To compare the efficacy of both commercially available and emerging urinary catheter technologies in relation to their effects on bacteriuria caused by *Escherichia coli* in vitro. Antiseptic urinary catheters have recently become commercially available and others are in the developmental stage.

METHODS Silver alloy-coated catheters, antibiotic Nitrofurazone (NF)–coated catheters, and nitric oxide (NO)–coated catheters were tested against a noncoated control for their antiseptic ability. Inhibition of bacterial growth, biofilm formation, and the number of live bacteria within the biofilm, using up to 10^3 bacterial load were evaluated. Experiments were performed either in *E. coli* containing Luria broth media or in urine infected with *E. coli*.

RESULTS NF- and NO-coated catheters had equivalent antimicrobial activity and eradicated all bacteria in planktonic and biofilm states. Silver-coated catheters had no effect on *E. coli* growth or biofilm formation compared with the control, although silver-coated catheters did inhibit bacterial levels within the biofilm by 50%.

CONCLUSIONS NF- and NO-coated catheters are highly effective in preventing planktonic growth and biofilm formation. Silver-coated catheters were not found to be effective in this study. UROLOGY 78: 334–340, 2011. © 2011 Elsevier Inc.

Catheter-associated bacteriuria (CAB) is the most common nosocomial infection worldwide. It accounts for up to 40% of hospital-acquired infections in the US each year, adding a burden of up to $500 million to the cost of health care in the US.[1] CAB carries with it a 2.8-fold increased risk of death and results in bacteremia in approximately 3% of patients, constituting a serious complication.[2] Bacteriuria is typically caused by a single organism,[3] with *Escherichia coli* being the most frequently isolated species IDSA (2009)[4]. Together, *E. coli* and *Pseudomonas aeruginosa* account for more than 39% of cases.[5] Because of the widespread use of urinary catheterization, CA-bacteriuria results in considerable antimicrobial use. According to some studies, the daily rate of bacteriuria in catheterized patients ranges from 3–8%, and the incidence of bacteriuria is directly related to the duration of catheterization.[6-8] By one month, nearly all patients with an indwelling catheter will have bacteria in their urine.[4]

CAB is difficult to treat with current antimicrobial strategies because of the antibiotic resistance biofilm that forms from free-floating bacteria that adhere to the surface of catheters and colonize them. These biofilm bacteria are highly differentiated and extremely resistant to antibiotics.[9] Biofilms are especially relevant in catheterization, because indwelling urinary catheters provide a surface for the attachment of microbial host cell binding receptors that are recognized by bacterial adhesins. This in turn facilitates microbial adhesion and subsequent colonization with uropathogens. Catheterization also disrupts the uroepithelial mucosa, exposing new binding sites for bacterial adhesins.[10] Once biofilms are established, they shed cells that seed other sections of the catheter and bladder. They also protect pathogenic bacteria from antimicrobials and the host immune response.

Various approaches have been developed to prevent biofilm formation, including the application of antiseptic lubricating gels at the catheter insertion point, the use of antireflux valves, and the application of a taped seal to the catheter drainage tubing junction.[11] In addition to these measures, catheter coatings are also being investigated to determine whether they can inhibit the forma-

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From the Department of Medicine, Division of Infectious Diseases, Universality of British Columbia, 2733 Heather St. Vancouver, British Columbia, V5Z 3J5, Canada

Reprint requests: Dr. Yossef Av-Gay, Department of Medicine, Division of Infectious Diseases, University of British Columbia, 2733 Heather St. Vancouver, BC, Canada. V5Z 3J5. E-mail: yossi@interchange.ubc.ca

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tion of biofilms. Some studies with anti-adherence agents (eg, heparin) have shown promise.[12,13] Other studies have antibiotic coating to eradicate bacteria. In addition, gendine-coated, silver alloy–coated, and nitric oxide–coated catheters have demonstrated inhibition of biofilm formation in some recent studies.[5,14]

Silver is a very effective antibacterial substance and silver alloy–coated catheters have been used in recent years in an effort to reduce infection rates. A recent Cochrane review of 23 trials comparing types of indwelling urinary catheters for short-term catheterization in hospitalized adults found that silver alloy–coated catheters are protective against CAB.[15] However, silver oxide catheters were not associated with a statistically significant reduction in CAB, and other meta-analyses have similarly concluded that silver oxide–coated catheters are ineffective. Recent IDSA guidelines (2009) described the treatment effect observed with silver alloy–coated catheters as being significantly smaller in more recent studies than in earlier research.

Johnson et al[16] noted that some of the prepost studies that demonstrated benefits of silver alloy–coated catheters may have demonstrated enhancement of the catheters' purported benefits because of already improving background bacteriuria rates, replacement of pre-existing catheters, co-interventions, such as nursing education, or sample differences between study periods. Bjarnsholt et al[17] demonstrated that up to 100 times more silver would be needed to achieve efficacy against biofilm organisms compared with planktonic organisms. Recent research has thus examined the use of silver-containing nanoparticles as a way to apply more silver to the catheter surface as well as the surrounding urinary environment.[18] Some other catheter products in development combine silver with other substances to enhance their efficacy.[19]

Antibiotic-coated catheters may reduce the risk of CAB by preventing or delaying onset in hospitalized patients.[11,20] They have demonstrated antimicrobial effects against bacteriuria pathogens in several in vitro studies,[20,21] and some in vivo studies also report positive effects.[8] Ciprofloxacin, gentamicin, norflaxin, nitrofurazone, and combinations of compounds, such as chlorhexidine and protamine sulfate have been successfully incorporated into catheter coatings.[10] Nitrofurans have proven to be effective against a wide spectrum of gram-positive and gram-negative bacteria, including a variety of strains of the common urinary pathogens. Furthermore, bacterial resistance to nitrofurans has not appreciably evolved over time, despite decades of clinical use.[22] In vitro studies indicate that Nitrofurazone catheters (NFCs) might have a stronger antibacterial effect than silver hydrogel catheters.[17] However, nitrofurans have been demonstrated to be ineffective against most strains of *P. aeruginosa*, and they do not inhibit viruses or fungi. NFCs are also very expensive; on average, they cost 130% more than noncoated silicone catheters.[16]

Nitric oxide (NO) has been shown to be bacteriostatic and bactericidal.[23] Miller et al[24] demonstrated that multiple 30-minute treatments of 160 ppm NO resulted in more than a 5 $\log_{10}$ colony-forming unit per milliliter (cfu/mL) decrease in the bacterial load of *Staphylococcus aureus*, *E. coli*, and *P. aeruginosa*. NO has also been investigated for its potential to prevent biofilm formation. Catheters impregnated with gaseous nitric oxide (gNO) demonstrated slow release of NO over a 14-day period, were rendered antiseptic and were able to prevent bacterial colonization and biofilm formation on their luminal and exterior surfaces. NO-impregnated catheters inhibited the growth of *E. coli* within the surrounding media and eradicated bacterial concentrations of up to 10^4 cfu/mL.[14]

This study compared the efficacy of commercially available and emerging antimicrobial urinary catheters with respect to their effects on *E. coli* infection in vitro.

MATERIAL AND METHODS

An untreated control and 3 different coated catheters were used in this study.

Control: 6-mm diameter Folysil silicon Foley catheter Ch/Fr 18 (catalog no. AA6118; Coloplast, Corp, Minneapolis, MN)

NOX: the same catheter as control after impregnation with NO as described later

AG: BARDEX I.C. silver-coated anti-infective Foley catheter Ch/Fr 18 (catalog no. 0165SI18; Bard, Inc. Covington, GA)

NFC: Release NF—Nitrofurazone-coated silicone Foley catheter Ch/Fr 16 (catalog no. 95216; Rochester Medical, Stewartville, MN)

The NOX catheter was an NO-impregnated control catheter. A Coloplast, Foley catheter was cut aseptically into 2-cm sections and impregnated with NO (Airgas Speciality Gases, Chicago, IL) in a previously described exposure chamber under propriety conditions and using a proprietary technique.[25,26] Catheter pieces not treated with NO, used as controls, were stored in a sterile sealed vial until use.

E. coli bacterial culture was obtained from American Type Culture Collection (ATCC #25922). Bacteria were grown to 0.5 McFarland standard. One-milliliter aliquots of these preparations containing approximately 2.5×10^8 cfu/mL were stored at −70°C. On the day of the experiments, the fresh stock was removed from the freezer and thawed, and 2 mL of Luria broth (LB) was added. Cultures were further diluted with LB to 10^3 cfu/mL.

E. coli culture (2 mL) at a concentration of 10^3 cfu/mL was added to a vial containing a 2-cm piece of catheter and incubated for 24 hours at 37°C. After 24 hours, samples were vortexed and plated (using a 10^3 time dilution) on LB agar plates and then incubated at 37°C for 24 hours. The colony-forming unit (CFU) was counted and calculated to represent the CFU per mL.

E. coli culture (200 μL) at a concentration of 10^3 cfu/mL was added to 1.8 mL of urine (reaching a final concentration of 10^2 cfu/mL) containing a 2-cm section of catheter and incubated at 37°C for 72 hours. Urine was collected in a sterile vial from a male volunteer on the day of the experiment. After 72 hours,

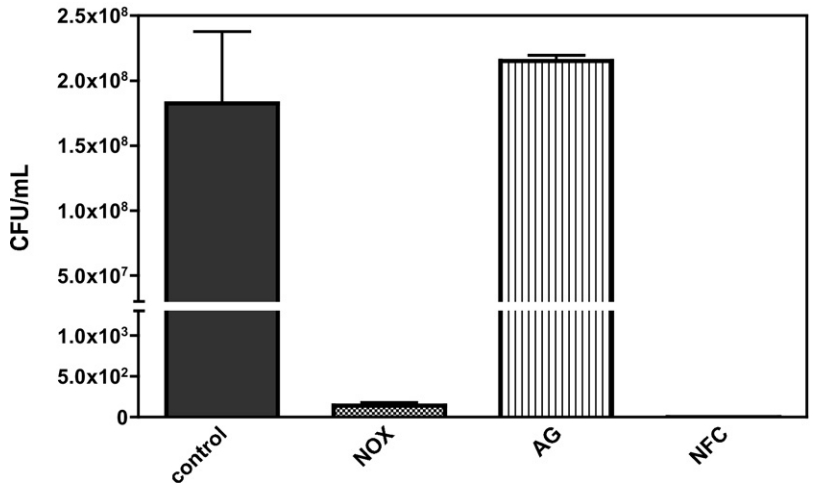

Figure 1. Comparison of *E. coli* growth in media containing pieces of the NOX, AG, and NFC catheters vs media from control catheter, after immersion of the catheters for 24 hours in suspension composed of 10^3 cfu/mL and incubated for 24 hours at 37°C.

samples were vortexed and plated onto LB agar plates and incubated at 37°C overnight. The CFU were counted and calculated to represent the CFU/mL. To qualitatively evaluate the colonization potential, 1, 10, and 100 μL of each sample were plated of a 3-compartment LB agar Petri plate. Plates were incubated overnight at 37°C.

Catheter sections (2 cm) were immersed in tubes containing 3 mL of *E. coli* culture at 10^3 cfu/mL and incubated at 37°C for 24 hours. After 24 hours, catheter sections were washed twice using 3 mL of sterile saline (0.9% wt/vol NaCl) and transferred aseptically to an LB agar plate. Each catheter section was rolled once on the plate then incubated at 37°C for 24 hours.

Catheter pieces (2 cm each) were added, aseptically, to 1.8 mL of urine (collected as stated previously) plus 200 μL of *E. coli* culture at 10^3 cfu/mL and incubated at 37°C for 72 hours. After 72 hours, catheter pieces were washed twice in 3 mL sterile saline then cut into 2 pieces of equal size, aseptically.

One half of each catheter section was added to 1.5 mL of crystal violet dye in water (1% wt/vol) for 15 minutes then washed twice in 4 mL distilled water (dH_2O). Washed catheter pieces were then added to 2 mL of 95% ethanol to relinquish crystal violet bound to the surface of the catheter. The absorbance at 595 nm of each ethanol sample was measured using a spectrophotometer and used as an indicator of biofilm formation.

The other half of each catheter section was added to 2 mL of sterile distilled water (dH_2O) and sonicated at a level of 5 for 30 seconds. The extent of colonial *E. coli* released from each catheter was determined by plating each sample on LB agar plates and incubating at 37°C overnight. The CFU were counted and calculated to represent the CFU per mL.

RESULTS

After being immersed in bacterial culture for 24 hours, the antibacterial activity of the NOX and NFC catheters vastly exceeded that of both the untreated (control) and AG catheters (Fig. 1). Control and AG catheters reached concentrations of 1.8×10^8 and 2.1×10^8 cfu/mL, respectively. NOX catheter pieces contained an average bacterial concentration of 2.5×10^2 cfu/mL after the 24-hour incubation, revealing an effective reduction in the concentration of planktonic *E. coli*. No bacteria were observed in the sample containing NFC catheters.

After being immersed in urine plus bacteria for 72 hours, the solution containing the AG catheter had a similar bacterial concentration as the control: 2.0×10^8 and 5.0×10^7, respectively; whereas the solutions containing the NOX and NFC catheters completely eradicated bacteria in urine. The results of the plated solutions are shown in Figure 2.

The rolling of catheter pieces on LB agar plates provides a qualitative measure of the presence of bacterial colonization on the surface of each catheter. As shown in Figure 3, both the control and AG catheters contained extensive colonization on the surface of the catheter, whereas for catheters NOX and NFC, no bacterial colonization was observed.

Crystal violet studies indicate the extent of biofilm formation on each catheter type. Each experiment was repeated 3 times with 3 replicates in each of the experiments. The AG catheter showed the highest average absorbance, indicating extensive biofilm formation (Fig. 4A). The AG catheter rendered an average absorbance 8–10 times greater than all other catheter types, even the control. The NOX catheter had half the absorption of the control and the NFC catheter had 40% less. This indicates that both the NOX and NF catheters had less biofilm formation on their surface when compared with the control. Comparative analysis of variance test ($P < .005$) shows that all 3 tested catheters were significantly different from the control, and NF and NOX were found to be significantly different from AG. Figure 4B shows the amount of colonized bacteria released from the catheter surface. The NOX and NFC catheters proved effective at eradicating bacteria embedded within the biofilm, whereas the control and AG catheters produced bacteria concentration of 7.5×10^4 and 9.8×10^2 cfu/mL, respectively.

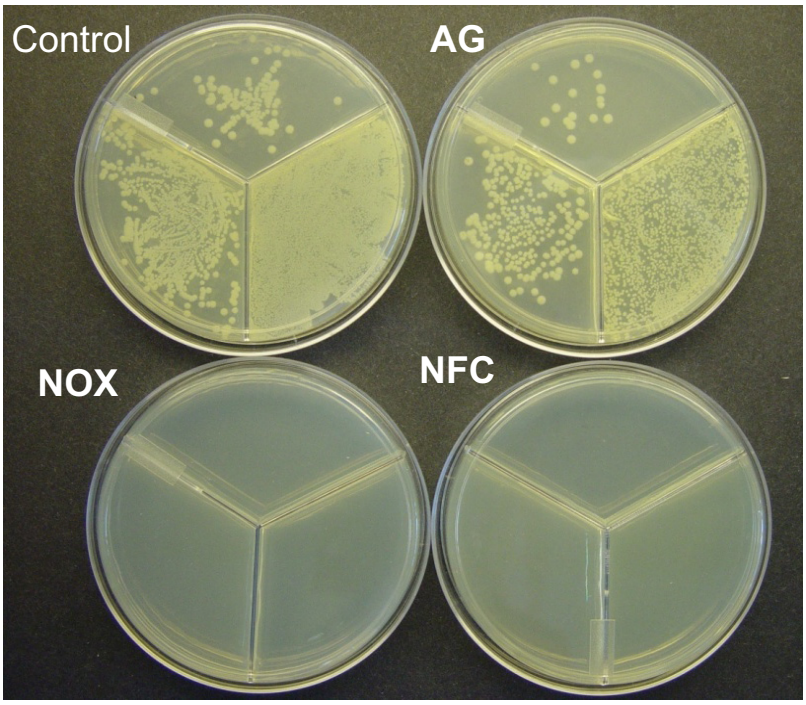

Figure 2. Comparison of *E. coli* growth in urine after 72 hours' exposure to pieces of NOX, AG, NFC, and control catheters. Within each 3-compartment LB agar Petri plate, 1, 10, and 100 μL of each sample were plated and incubated overnight at 37°C.

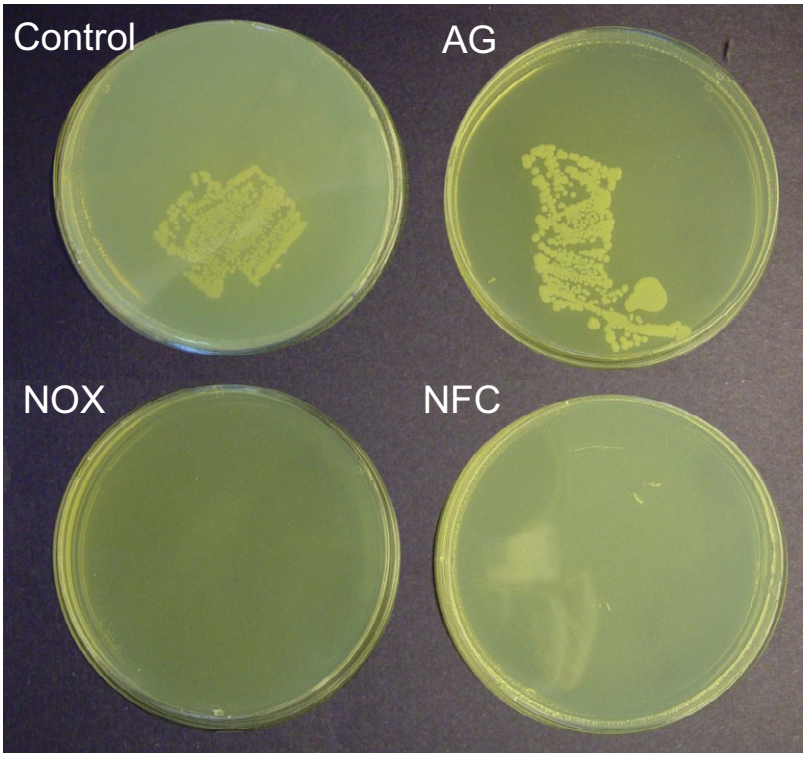

Figure 3. Comparison of *E. coli* colonization on NOX, AG, and NFC catheters vs control after immersion of catheters for 24 hours in suspension containing 10^3 cfu/mL of *E. coli*. In each LB Petri dish, a catheter was rolled over the surface and then incubated at 37°C overnight.

COMMENT

Results from this study demonstrate that NF- and NO-impregnated urinary catheters possess similar antimicrobial properties. This study did not find the silver-coated catheter to be effective. Our results agree with the recent IDSA guidelines describing the treatment effect observed with silver alloy–coated catheters as being significantly smaller in more recent studies than in earlier research.[4]

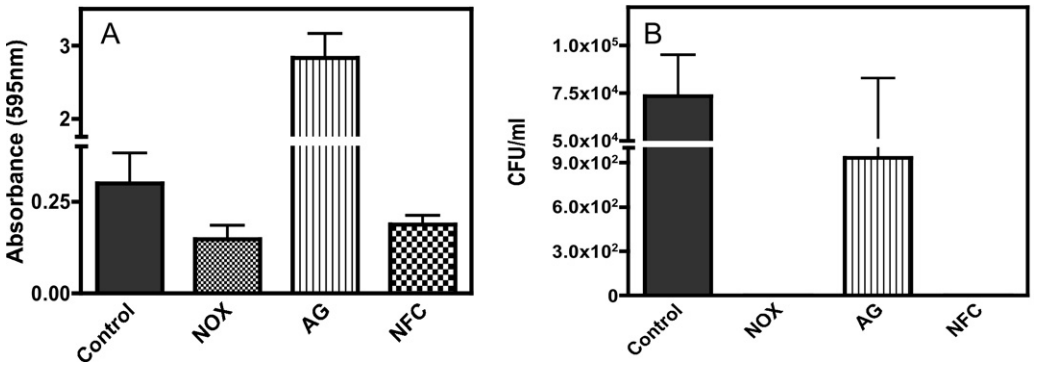

Figure 4. Comparison after 72 hours of incubation in urine of **(A)** colonized biofilm formation on NOX, AG and NFC catheters vs control demonstrated by absorbance at 595 nm of the Crystal Violet that was attached to the catheter pieces after extraction of color with ethanol, and **(B)** bacterial growth from the biofilms from the different catheters.

This study shows that NO-impregnated catheters are comparable with antimicrobial-coated urinary catheters in their level of antimicrobial activity. NO-coated catheters prevented *E. coli* growth in urine for 72 hours, which is the same as the NFCs. Other studies have shown NFCs to be useful for inhibition of CAB in patients who have an indwelling urinary catheter for 5–7 days[27] and to be efficient in reducing CAB.[16] Other data collected in our laboratory (not published) show that NO-coated catheters can inhibit CAB for at least 7 days. This may have some implications in the future translation to the clinical realm.

Antibiotics and NO kill bacteria in different ways, thus, their specificity is not the same. Antibiotics are specific to an organism or a group of organisms, whereas NO is not. NF was found to be effective against *E. coli* in this study, but most gram-negative isolates are nonfermenters,[27] which imparts resistance to NF. The mechanism by which NO prevents bacterial adhesion is still unknown. It has a broad range of antimicrobial, antifungal, and antiviral activity.[24] One hypothesis is that NO, through reactive intermediate species such as N_2O_3 and N_2O_4, destroys the function of bacterial adhesion proteins that mediate surface adhesion.[14] Another hypothesis is that NO creates a physical barrier on the surface of the catheter. Darling and Evans have suggested that NO produces a chemical modification of surface thiols or metal centers involved in critical enzymatic or regulatory function. Inactivation of cysteine proteases is another suggested general mechanism of NO-related antimicrobial activity.[28]

An ever-increasing clinical problem of significant concern is the ability of disease-causing organisms to adapt metabolically and genetically to the drugs used to treat them. Nowhere is this more evident than in the development of antimicrobial resistance by bacteria. Bacteria that are not intrinsically resistant to an antimicrobial drug may develop resistance through de novo mutation or through the acquisition of resistance genes from another organism by horizontal transfer.[29] Biofilms, formed on the surface of catheters, represent an ideal niche for plasmid exchange among bacteria and thus lead to higher antibiotic resistance of bacterial cells growing as biofilm as compared with planktonic cells. As for NO, it has been suggested that bacteria, such as *Staphylococcus aureus*, *P. aeruginosa*, and *E. coli* have the ability to metabolically adapt to nitrosative stress and to decrease their sensitivity to NO antimicrobial action.[30] However, because of NO's broad nonspecificity, rendered by its multiple intracellular biochemical targets, the risk of developing resistance to NO is likely to be ameliorated.

CONCLUSIONS

These in vitro results show that NF- and NO-impregnated catheters act better to prevent bacteriuria than silver alloy–coated catheter and have a better antiseptic effect. Both NO- and NF-impregnated catheters were highly effective in preventing planktonic growth and biofilm formation. This is consistent and supportive of the IDSA guidelines. This is an in vitro study that may have further important clinical implications for postoperative surgical short-term catheterization. More in vivo data are required here to extend this to clinical scenario.

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EDITORIAL COMMENT

Catheter-associated urinary tract infection (CAUTI) is the most common hospital-acquired infection. Recently there has been increased attention to this because hospitals will no longer be reimbursed by Medicare for costs associated with CAUTI. The mainstays of prevention of CAUTI include avoiding catheters when possible, limiting their use to the minimal time span necessary, and practicing sterile placement techniques and appropriate catheter care. Currently, a number of companies produce catheters with specific coatings—typically some type of silver alloy—to inhibit bacterial growth.

In this study, the authors compared several different catheters coated with various substances to assess their ability to decrease bacteriuria. They noted that although those coated with antibiotic Nitrofurazone as well as those coated with nitric oxide significantly reduced bacterial growth, those coated with a silver alloy as well as the control catheters did not appear to decrease bacterial growth. The logical extension of this is that this phenomenon may translate into fewer CAUTIs in patients in whom Nitrofurazone or nitric oxide catheters are used. In fact, although a number of studies have demonstrated that catheters impregnated with various substances reduce the rate of asymptomatic bacteriuria, there is currently no evidence that this results in fewer CAUTIs.

Although the results of the current study are intriguing and deserve further study, it would be premature to recommend specially coated catheters to prevent CAUTI. Until there are studies to definitively demonstrate that the decreased rates of bacteriuria translate to fewer CAUTIs, there is no justification for the use of these catheters. In fact, most of the "antibacterial" catheters currently available depend on a silver alloy for their effect. Based on the results of this study, which found no significant antimicrobial activity in the silver alloy group, one could question whether there is even any potential for a decrease in CAUTI with such catheters. In addition, given the significantly higher costs of these "antibacterial" catheters, one should be very cautious and demand clinical outcomes data before starting to use them. It is hoped that translational studies that look for real improvements in CAUTI rates in patients with Nitrofurazone- and nitric oxide–impregnated catheters will be performed.

Howard B. Goldman, M.D., Center for Quality and Patient Safety, Glickman Urologic and Kidney Institute, The Cleveland Clinic; and Lerner School of Medicine, Case Western Reserve University, Cleveland, Ohio

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REPLY

We agree with the remarks outlined in the editorial and would like to stress the following: for an antimicrobial catheter to be considered effective, it must be able to completely inhibit