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Next-Science: A Novel Antimicrobial Agent that Inhibits Biofilm Development by *Escherichia coli* Clinical Isolates on Urinary Tract Catheters

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Abstract

Catheter-associated urinary tract infections (CAUTI) constitute about 40% of health-care associated infections in the United States. It is estimated that about 15% to 25% of hospitalized patients receive a urethral catheter at some point during hospitalization predisposing them to the development of CAUTI. Pathogenic bacteria colonize the surface of the catheter and develop highly resistant structures termed biofilms which protect them from the effects of diverse antibiotics. *Escherichia coli* are among the main causative agents of CAUTI. Due to the emergence of antibiotic resistant strains, it is critical to develop new novel antimicrobial agents. We recently showed that a novel anti-biofilm agent, Next Science, inhibited biofilm development by wound pathogens. In this study, we tried to determine if treating urinary tract catheters (UTC) with NS prevents the development if *E. coli* biofilms.

Three types of UTCs were cut into small pieces that were treated with NS. Biofilm development by an *E. coli* laboratory strain and several *E. coli* clinical isolates on treated and untreated pieces was accomplished using the microtiter plate assay. Biofilms developed on inner and outer surfaces of the catheters were quantified by determining the number of microorganisms (colony forming units) on each piece and visualized using confocal laser scanning microscopy (CLSM).

In comparison with untreated catheters, all three types of NS-treated catheters prevented biofilm development by tested *E. coli* strains. In addition, CLSM demonstrated the presence of *E. coli* biofilms on the inner and outer surfaces of the untreated but not treated catheters. Our results suggest that NS is a novel antimicrobial treatment to prevent biofilm development by pathogenic *E. coli* strains on UTCs.

Keywords: Urinary tract infections; Biofilm; Antimicrobial agents; *E. coli*

Introduction

Catheter-associated urinary tract infection (CAUTI) constitutes about 40% of healthcare associated infections in the USA [1]. Placing more than 30 million bladder catheters annually results thousands of cases of CAUTI [2]. Another contributing factor for CAUTI is the increased use of indwelling catheters; most hospitalized patients are catheterized for 2-4 days [3]. During catheterization, bacteria enter the urinary tract either through the extraluminal or the intraluminal route [4]. The bacteria utilize the extraluminal route early during the insertion of the catheter [4]. Alternatively, they may utilize this route later as the perineal bacteria ascend along the outer surface of the catheter [5]. The intraluminal route is utilized either when contaminated urine refluxes into the bladder from the collection bag or when a break in the closed drainage system occurs [5]. Most of CAUTI are caused by a single bacterial species (monomicrobial) including *Staphylococcus epidermidis, Enterococcus faecalis, E. coli*, and *Proteus mirabilis* [4-6]. These bacteria initially colonize the urinary tract catheter.

After colonizing the surface of the urinary catheter, contaminating bacteria form biofilms. The formation of the biofilm is essential for the development of CAUTI [7]. Factors within the bladder accumulate on the surface of the catheter and facilitate biofilm formation. These factors, which include host proteins, organic molecules, and conditioning film of electrolytes are important in the initial bacterial attachment [8]. Under certain conditions, bacteria form biofilms on both biotic and abiotic surfaces [6,8]. During biofilm formation, proliferating bacteria secrete extracellular polymeric substance (EPS) materials which protect them from the effects of antibiotics as well as different host responses [9]. Due to the significant role of these biofilms in the development of CAUTI, numerous approaches were utilized to prevent their formation on either the inner or outer surface of the urinary catheter. These approaches included modification of the catheter surface with antimicrobials included coating, matrix loading, and immersion in an antimicrobial agent. Among the antimicrobials that produced variable results are: hydrogels, silver, triclosan, nitric oxide, antibiotics, and quorum-sensing inhibitors [4,10]. The development of additional antimicrobial agents to prevent biofilm development on urinary tract catheters is essential. We recently described Next Science (NS), a novel antimicrobial agent that destroys the biofilm and eliminates individual the bacteria within it. NS gel prevented wound infection by different wound pathogens [11]. In addition, NS-treated tympanostomy tubes inhibited biofilm development by middle ear pathogens [12]. In this study, we examined the effectiveness of NS in preventing biofilm development by *E. coli* strains on urinary catheters.

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Materials and Methods

Bacterial strains and growth media

Bacterial strains utilized in this study included the *E. coli* strain MM294 [13] and four *E. coli* clinical isolates (CF77, CF266, CF347, and CF358) obtained from cystic fibrosis (CF) patients presented at the pediatric clinic at Texas Tech University Medical Centre. The isolates were obtained through a protocol approved by the Institutional Review Board at Texas Tech University Health Sciences Centre, Lubbock, Texas. For general growth, strains were grown overnight in Luria-Bertani (LB) broth. Biofilms were developed using tryptic soy broth (TSB). To examine the biofilms using confocal laser scanning microscopy, we utilized the *E. coli* strain MM294/ pMRP9-1. Plasmid pMRP9-1 carries the gene that codes for green fluorescence protein (GFP) [14]. To maintain plasmid pMRP9-1 in MM294, we grew the strain in the presence of carbenicillin at a concertation of 50 µg/mL.

Next-Science

Next-Science is a proprietary agent (PCT/US2012/059263) (Next Science, Jacksonville, FL) that was designed to eliminate biofilms by destabilizing the EPS matrix through chelation of calcium and to kill bacterial pathogens by removing proteins from bacterial membranes leading to cell lysis [11]. Next Science (NS) was obtained in an aqueous solution at a stock concentration of 1.3 mg/mL and was further diluted in phosphate buffered saline (PBS).

Biofilm development

The microtiter plate culture assay was utilized to develop the biofilms as previously described [15,16]. Briefly, aliquots of overnight cultures of the tested strain in LB broth were harvested, washed in 1 mL PBS, and diluted in PBS to an OD_{600} of 0.02. Biofilms were developed on three types of urinary catheters; polyvinyl chloride (PVC) intermittent catheters, silicone catheters, and silicone-coated catheters. Each catheter was cut into small pieces (0.25 cm) and the pieces were washed in PBS and used in the biofilm assay. Catheter pieces were placed into the wells of a sterile 24-well polystyrene microtiter plate (Costar; Corning, Tewksbury, MA) containing TSB. Each well, which contained 1 mL of TSB, was inoculated with about 300 colony forming units (CFU) of the tested strain.

To investigate the effectiveness of NS in inhibiting biofilm development on the catheter pieces, 1 mL of either sterile PBS (negative control) or NS solution (50 µg/mL) was added to each well. After 24 h of additional incubation at 37°C, the biofilms were quantified and visualized. Each experiment was repeated at least three times to confirm the reproducibility of the results.

Quantification of the biofilms

Each catheter piece was placed into a 1.5 mL microcentrifuge tube containing 1 mL of TSB and vigorously vortexed to disrupt the biofilm. The bacterial suspension was then serially diluted 10- fold in TSB and 10 µL aliquots of each dilution were spotted onto LB agar plates. The plates were incubated at 37°C for 24 h and the numbers of microorganisms (CFU) in each catheter piece was determined using the following formula: (CFU counted X dilution factor X100). Each experiment was repeated at least 3 times for reproducibility.

Visualization of the biofilms

At the end of the biofilm development, each catheter piece was gently rinsed in 1 mL of PBS and the biofilm was visualized by confocal laser scanning microscopy (CLSM) using an Olympus IX71 Fluoview 300 confocal laser scanning microscope (Olympus America). The catheter piece was longitudinally split to visualize the biofilm on the inner and outer surfaces. Three-dimensional biofilm image reconstructions were performed using NIS Elements 2.2 software (Nikon Instruments).

Statistical analyses

Analyses were done using GraphPad Prism 6.05 (GraphPad Software, San Diego, CA). Differences between pairs were assessed by unpaired two-tailed t tests.

Results and Discussion

To prove the concept, we utilized the *E. coli* strain MM294, which is a laboratory strain. However, unlike other *E. coli* laboratory strains, MM294 maintained its wild type phenotype and none of its genes was mutated to facilitate cloning or expression studies. Preliminary experiments confirmed that NS completely inhibited the planktonic growth of MM294 (data not shown). We utilized three types of urinary catheters; PVC, silicone, and silicone coated (Materials and Methods). Each catheter was cut into 0.25 cm pieces and biofilm development on each piece was conducted using the microtiter plate assay as described in Materials and Methods. As shown in Figure 1, MM294 formed a considerable biofilm on all three types of catheters. In addition, at a concentration of 50 µg/mL, NS completely inhibited the development of MM294 biofilms (Figure 1). We recovered no CFU from any of the catheter pieces (Figure 1). To confirm the effectiveness of any antimicrobial agent on a catheter, analysis of the inner and outer surfaces of the catheter is essential. Therefore, we visualized the MM294 biofilm on both sides on the catheter using CLSM. We selected the silicone catheter for such analysis. We utilized MM294 strain containing plasmid pMRP9-1 which carries the gene that codes for green fluorescence protein (GFP). As shown in Figure 2, MM294/ pMRP9-1, formed a well-developed biofilm on both sides of the silicone catheter. However, treatment with NS inhibited the development of the biofilm on both sides of the catheter (Figure 2). These results suggest that NS is effective in preventing *E. coli* biofilms on the exterior and interior surfaces of the silicone catheter.

We next examined, using the microtiter plate assay, the effect of NS on biofilms formed by *E. coli* clinical isolates obtained from patients on the silicone catheter. While all five tested isolates formed considerable biofilms on both sides of the catheter, they failed to do that in the presence of NS (Figure 3).

Antibiotic resistant mutants among different bacterial pathogens are developing at an alarming rate and the pathogens that cause CAUTI are no exception. One of the pathogens that contributes significantly

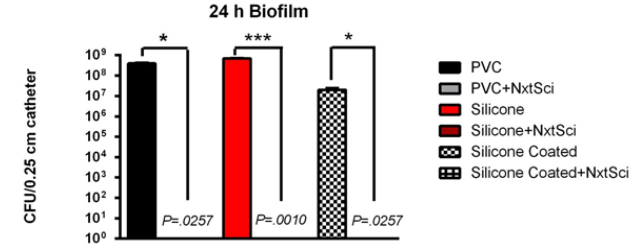

Figure 1: Next Science inhibits biofilm development by the *E. coli* strain MM294 on PVC, silicone, or silicone-coated urinary catheters. Biofilms were developed as described in the text and the number of microorganisms (CFU/0.25 cm piece of catheter) was determined. Values represent the average of three independent experiments, ± standard error of the mean.

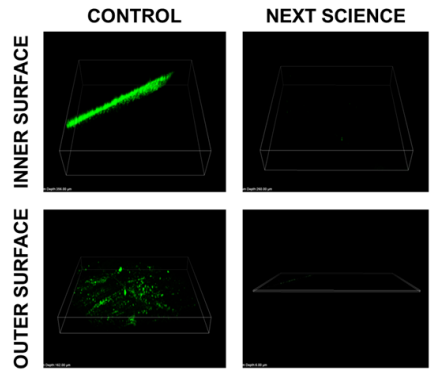

Figure 2: Next Science inhibits biofilm development by the *E. coli* strain MM 294 carrying the green fluorescent plasmid pMR9-1 on the outer and inner surface of the silicone coated catheter. Biofilms were developed as described in the text and visualized using confocal laser scanning microscopy.

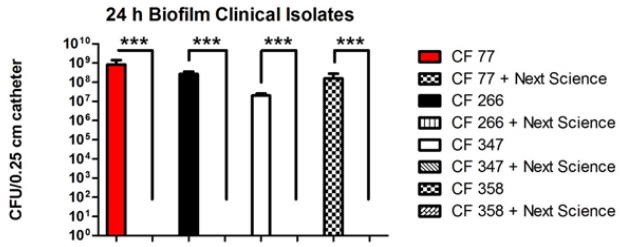

Figure 3: Next Science inhibits biofilm development by three *E. coli* clinical isolates on the surface of a silicone catheter. Biofilms were developed as described in the text and the number of microorganisms (CFU/0.25 cm piece of catheter) was determined. Values represent the average of three independent experiments, ± standard error of the mean.

to CAUTI is *E. coli* [4,5]. In this study and using *E. coli* strains, we demonstrated the effectiveness of NS in: 1) preventing biofilm development on three different types of urinary catheters (Figure 1); 2) preventing biofilm development on both sides of a silicone catheter (Figure 2); and 3) preventing biofilm development by several clinical isolates on a silicone catheter (Figure 3). Thus, in clinical applications and within the urinary bladder of a catheterized patient, NS would potentially kill *E. coli* planktonic cells around the catheter and prevent those that survived from establishing a biofilm on either side of the catheter. We would also extend this analysis to include other pathogens (other than *E. coli*) obtained from patients with urinary tract infections. Another application of NS in to eliminate biofilms formed on urinary catheters.

Conclusion

An ideal approach in this regard is to utilize two agents; one that penetrates/destroys the EPS matrix (such as lysozyme) and another to kill the individual bacteria within the biofilm (such as antibiotics) [17,18]. However, as we previously demonstrated through *in vitro* and *in vivo* biofilm analyses NS is unique in that it contains both activities [11]. Therefore, in future experiments we would develop partial biofilms on both sides of the catheter and then treat the infected catheter with NS.

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