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The natural history and composition of urinary catheter biofilms: early uropathogen colonization with intraluminal and distal predominance

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Abstract

Purpose: To determine the composition and site of initiation of bacterial biofilm location on indwelling urinary catheters, and to track biofilm progression over time.

Materials & Methods: Indwelling urinary catheters were collected from two tertiary care centers following removal from patients. Indwelling time was noted, and catheters were de-identified. Catheters were sectioned, stained for biofilms, and analyzed using spectrophotometry and visualization. Biofilm colonization patterns were analyzed using descriptive statistical analyses and bacterial composition was determined using next-generation sequencing.

Results: 33 catheters from 26 males and 7 females were collected with indwelling times ranging from 15 minutes to 43 days and analyzed. Biofilm colonization was consistently high on the region of the balloon throughout indwelling times. After week 1, the distal third of the catheter had higher biofilm colonization than the proximal third (week 2: $p=0.034$). At all indwelling times, the intraluminal surface of the catheter had greater biofilm colonization than the outer surface. Next-generation sequencing detected potential uropathogenic bacteria in 10 of 10 analyzed samples.

Conclusions: The catheter balloon, its distal aspect, and its lumen were the predominant locations of biofilms, comprised of uropathogenic bacteria. Strategies to prevent or treat biofilms should be targeted to these areas.

Keywords

biofilm; urinary tract infection; catheter; antibiotic resistance; cystitis; infection; *Escherichia coli*

Introduction

Urinary tract infections (UTIs) are among the most common and costly infections worldwide.^{1,2} In the United States, approximately 80% of UTIs are associated with indwelling urethral catheters, and are termed catheter-associated urinary tract infection (CAUTIs).³ Despite multiple reduction efforts, CAUTIs account for about 1 million cases per year in the U.S.⁴ and are associated with increased morbidity and mortality.¹

CAUTIs are initiated via peri-meatal uropathogen introduction into the urethra and ascension toward the bladder.¹ Biofilms protect bacteria from urine turbulence as well as antibiotic penetration and host immune responses, and thus biofilm formation leads to a survival advantage, and persistent and antibiotic resistant infections.⁵ Biofilms are initiated when free-floating bacteria are deposited onto surfaces submerged in fluid such as a urinary catheter.⁶ Biofilms comprise multicellular bacterial communities with scaffolding of extracellular DNA, exopolysaccharides, and pili. Bacterial biofilm eradication is important in the prevention and treatment of CAUTI as well as curbing antibiotic resistance⁷, and gaining an understanding of biofilm formation and progression on catheters is critical.

We sought to understand the location and timing of initial biofilm initiation and progression over indwelling time. Using next-generation sequencing, which allows for improved characterization of multispecies biofilms relative to conventional culture methods^{8–11}, we aimed to determine the identity of bacterial species that composed the biofilms. We hypothesized that catheters would be colonized rapidly, starting with the extraluminal and distal aspect of the catheter. We reasoned that the proximity of this area to the perirectal flora would result in colonization distally with subsequent proximal spread. In our study, we detected biofilms within hours following catheter insertion. The biofilms predominated distally and intralumenally as well as on the balloon portion. The biofilms were commonly comprised of uropathogenic bacteria containing antibiotic resistance genes.

Methods

Medical staff at two tertiary care sites were instructed to place removed urethral urinary catheters in a repository, and note the indwelling time and patient sex, for investigator retrieval. Specimens were stored at 4°C until the time of analysis (within 24 h of collection). Silver-coated catheters were excluded from collection. Other patient information was not collected, and thus our institutions' IRBs approved the study without the need for patient consent.

Nonadherent bacteria and debris from catheters were removed through rinsing in two liters of water, and then 10 mL of water irrigated through the catheter lumen via a sterile syringe. Thus, remaining bacteria were adherent in the form of biofilms. Catheters were bisected translumenally and stained for adherent biofilms with 0.1% crystal violet solution for 15

minutes. Catheters were rinsed twice (1 liter each) using 1x PBS and dried by capillary action on absorbent paper, and further sectioned into 1 cm pieces. Each piece was placed in a 24-well plate and 1 ml 80% ethanol, 20% acetone solution was added to each well to solubilize the crystal violet. Aliquots from the plate were read spectrophotometrically at 595 nm (OD_{595}) for all segments of each catheter. 30 segments of each catheter (starting proximally, in the bladder lumen) were thus analyzed. Catheters were grouped by indwelling time (Group 1: 0-7 days; Group 2: 8-14 days; Group 3: 15-28 days; Group 4: 29+ days). Mean OD_{595} for each segment by group was graphed and standard error was indicated with error bars. Photographic documentation was obtained for the determination of biofilm predominance on the catheter lumen versus extraluminal surface, and representative catheters from each indwelling time group are displayed in Figure 2. For quantitative analysis, mean OD_{595} values for each catheter third were calculated and compared using ANOVA and post-hoc t-tests wherein $p < 0.05$ was considered significant.

Intraluminal catheter swabs were obtained, incubated overnight in liquid LB at 37°C, and examined for turbidity to determine the presence of microbial growth. Samples were then mixed with glycerol and preserved in cryovials at -80°C. Aliquots (500 µl) from the glycerol stocks were shipped to the MicroGen Diagnostics (Lubbock, TX) laboratory for speciation by next-generation sequencing analysis. Briefly, the MicroGenDX assay uses both qPCR and 16s ribosomal RNA to test against the database of 25,000 species of bacteria and fungi as well as relative proportion of species within the sample. The assay also detects resistance factors for antibiotics. Bacterial species and resistance genes detected were recorded in Table 1 with corresponding indwelling times.

Results

33 catheters were collected following various indwelling times (mean 15 days, range: 15 min to 43 d) from patients (26 male, 7 female) in two tertiary care centers. Viability of biofilm samples was confirmed based on turbidity of overnight liquid media culture from catheter swabs. The group of catheters that ranged from 0-7 days indwelling time (mean 3 days), “Group 1”, contained 10 catheters (Figure 1A, Figure 2A). Interestingly, biofilms were detected as early as several hours indwelling time. Biofilms then progressed as a function of indwelling time (Figure 3A, 3B). Overall biofilm progression trends did not differ between catheters indwelling in male versus female patients (Figure 4). Biofilms predominated at the balloon portion of the catheter and within the catheter lumen. Although visually (Figure 2A) the proximal portion of the catheters showed predominance, there was no significant difference quantitatively between the proximal and distal catheter thirds (Figure 1A, $p=0.53$).

Group 2 comprised 6 catheters (Figure 1B, Figure 2B), which ranged from 8-14 days (mean 11.5 d) indwelling time. The mean biofilm absorbance of group 2 segments (0.959) was greater than that of group 1 (0.197) ($p<0.05$) (Figure 3B). Group 2 also exhibited biofilm predominance near the balloon site and intraluminally (Figure 2B). In Group 2, the distal third of the catheter showed biofilm predominance both visually and quantitatively relative to the proximal third ($p=0.034$) (Figure 1B and 2B).

Group 3 consisted of 11 indwelling catheters with indwelling times from 15-28 days (mean 26 d). The mean biofilm absorbance of group 3 (1.18) was significantly greater than that of Group 1 (0.197) ($p<0.05$), but not Group 2 ($p=0.959$) (Figure 3B). Similar to Groups 1 and 2, Group 3 exhibited biofilm predominance near the balloon site and intraluminally (Figure 2C). Although visually, Group 3 had biofilm predominance on the distal portion of the catheters, there was no statistically significant difference between the proximal and distal thirds of the catheters (Figure 2C, $p=0.62$).

Group 4, consisting of 6 indwelling catheters, had indwelling times of 29 days or more (range: 30-43 d, mean 36 d). Biofilms predominated throughout all portions of catheters in this group – distally and proximally, luminal and extraluminal (Figures 1D and 2D). There was no visual or quantitative difference in the proximal versus distal thirds, nor did the balloon-containing portions exhibit relative predominance visually (Figures 1D and 2D). The mean biofilm absorbance of group 4 (2.28) was greater than that of Group 1 (0.197) ($p<0.05$), Group 2 (0.959) ($p<0.05$), and Group 3 (1.18) (Figure 3B, $p<0.05$).

To determine the bacterial species that composed the biofilms on the urinary catheters, swab samples were obtained during biofilm staining and 10 samples were sent for next-generation sequencing analysis using the MicroGenDX platform. Bacteria were detected on all analyzed samples (Table 1). The most commonly detected bacteria were *Enterococcus faecalis* and *Escherichia coli*. Other bacteria detected included *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Bacterial resistance genes were detected in 4 of the 10 samples. Detected genes conferred resistance to beta-lactam, quinolone, aminoglycoside, and tetracycline drugs. There were no fungi detected in the samples.

Discussion

Catheter-associated urinary tract infections are one of the most common infections worldwide.^{12, 13} Despite the United States' prioritization of combating CAUTIs over the past decade, the incidence of CAUTI increased by 6% from 2009 to 2013.^{14–16} Biofilms, comprising bacteria adherent to the urinary catheter and one another, result in persistent infections that are resistant to antibiotics.⁵ The present investigation sought to determine the catheter location of bacterial biofilm initiation and progression, with the goal to inform the optimal positioning of antibacterial coatings and other strategies on the next generation of urinary catheters, allowing for maximization of efficacy and minimization of cost.

Nickel *et al.* reported the proliferative behavior of biofilms on urinary catheters in an *in vitro* urinary drainage system and an animal model.^{17, 18} They demonstrated early biofilm predominance on the luminal aspect and distal end of their catheter system, with progression proximally. Further, in a strict closed-drainage system, which has been shown to decrease CAUTI incidence¹⁹, the investigators found that the extraluminal aspect of the catheter exhibited relatively higher biofilm predominance, albeit more slowly progressing. Thus, authors hypothesized that the etiology of biofilm formation on catheters is due to colonization near the catheter drainage bag spigot and areas of potential breakage of the closed drainage system, as well as periurethral colonization. Remarkably, in spite of the

importance of understanding biofilm formation to inform novel catheter development, there has been limited investigation into the natural history of biofilm formation on urinary catheters since these early studies.

The results of the present study confirm and extend the *in vitro* and animal model results of Nickel *et al.* Bacterial biofilms formed as early as the first fifteen minutes of indwelling time in patients in our study, similar to what was demonstrated *in vitro*.²⁰ The intraluminal and distal aspects of our catheters exhibited the greatest predominance of biofilms. These findings have important implications for preventative CAUTI strategies. First, closed-drainage catheter systems may reduce CAUTI due to their slowing of the biofilm progression particularly on the intraluminal aspect of the catheters, the location of most predominant biofilm formation. Second, although catheter irrigation with antiseptic agents has had mixed results in CAUTI reduction,^{21,22} additional work is needed to explore such aseptic strategies, as they are poised to target the intraluminal aspect of the catheter specifically. In spite of closed-drainage protocols in the collecting facilities, every catheter analyzed in the present study exhibited intraluminal biofilm formation, and next-generation sequencing detected potential uropathogenic bacteria in 10 of 10 analyzed samples. Thus, routine antiseptic irrigation may be an important step in the management of catheterized patients.

Biofilms predominated on the distal aspect of the catheter. This finding suggests that, although sterile insertion technique is widely accepted and recommended as a preventative strategy against CAUTIs²³, it is unlikely to be sufficient for prevention, as the majority of the biofilms propagated on the distal portion of the urinary catheter where aseptic insertion technique would not be directly preventive. The balloon portion of the catheter, also exhibited intense biofilm formation relative to the neighboring segments. A novel catheter on the market makes use of two balloons in an effort to prevent bladder collapse and thus maximize drainage.²⁴ Our results suggest that a second balloon may serve as an additional strong nidus for biofilm formation, which may offset any benefit achieved through improved drainage. Further studies are warranted to evaluate the risks and benefits of additional urinary catheter balloons. Further, the findings of our study may help to save in the cost of silver and other catheter coatings, as coating could be concentrated on the intraluminal and distal aspects of catheters, with potentially minimal increase in biofilm formation.

Next-generation sequencing analysis detected bacterial species present in biofilms in 10 of 10 analyzed catheters (Table 1). Analyzed catheters ranged from 7–43 days indwelling time and the majority contained typical uropathogens in their biofilms, e.g. *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* (Table 1). *Staphylococcus aureus*, previously shown to be a uropathogen in the context of catheterization²⁵, was detected in 2 of the 10 catheters. *Staphylococcus epidermidis*, detected in one sample, while typically considered a contaminant in urine culture as it is resident to skin flora, has also been demonstrated as uropathogenic, particularly in catheterized patients.^{26–28} The ability to form biofilms is critical to the virulence of *S. epidermidis*.²⁸ The timing and progression of these organisms from catheter colonizers to causative agents of UTI are important phenomena that warrant additional investigation. The presence of uropathogens in biofilm samples in indwelling urinary catheters underscores the importance of biofilm prevention and

eradication as treatment for CAUTI and shows the utility of next generation sequencing platforms such as MicrogenDx in identifying bacteria that might be missed by conventional urine culture.

An exciting and promising strategy to prevent CAUTIs and other device-related infections, termed bacterial interference, is catheter coating with non-pathogenic bacteria or their adhesive molecules, to outcompete uropathogens and thus prevent colonization with undesirable bacteria. A small trial demonstrated a risk reduction when a nonpathogenic *E. coli* strain was instilled in bladders of spinal cord injury patients with chronic indwelling catheters.²⁹ Our results support new modifications of delivery systems for bacterial interference. For example, inoculating the drainage bag or drainage tubing may be sufficient to establish colonization, as we now understand biofilms predominate intraluminally and distally. As new evidence of bacterial colonization of healthy bladders is accumulating, the idea of purposely introducing a nonpathogenic bacterial population to reduce infection risk is becoming more palatable to patients and physicians.

The IDSA states that data are insufficient to recommend whether routine catheter change should be performed to prevent CAUTI.³⁰ In the present study, biofilms continued to progress over the course of the indwelling times investigated. There was an initial slow increase in biofilm formation, but by the third week, biofilms started to progress more rapidly (Figure 3). Such a rapid progression may have been triggered by quorum sensing or global epigenetic upregulation of growth factors. Our data suggest that a catheter change at approximately 3 weeks or sooner may be beneficial, as biofilm proliferation becomes rapid after this time (Figure 3).

Limitations include the inability to correlate biofilm formation with incidence of urinary tract infections or other pathologies due to the de-identification of the catheters. Future investigations are planned, which will include such demographic information as well as concomitant use of antibiotic therapy in the patient population. We also acknowledge that the sequencing data in the current study was limited by a modest sample size. In spite of the sample size limitation, we did generate strong evidence that biofilms were composed of uropathogenic bacteria and contained antibiotic resistance genes. Additionally, this study did not analyze the differences between males and females in catheter location of biofilm formation due to sample size limiting additional stratification. Such a future study will yield informative results as male and female urethral length and anatomy is markedly different. Future studies should make comparisons between the catheter portions internal to the urethra and external to the urethra, to delineate differences between the two aspects, and to further inform novel anti-septic strategies.

Conclusions

The catheter balloon, its distal aspect, and its lumen were the predominant locations of biofilms, and biofilms were detected within hours of indwelling time. All catheter samples analyzed for biofilm species were comprised of uropathogenic bacteria, and half of the analyzed samples contained antibiotic resistance genes. Antimicrobial strategies targeted to

our study's identified catheter regions of biofilm predominance may maximize efficacy against CAUTI and minimize cost in the next generation of urinary catheters.

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Abbreviations

UTI	urinary tract infection
CAUTI	catheter-associated urinary tract infection
IRB	internal review board
PBS	phosphate-buffered saline
LB	luria-bertani
OD	optical density
qPCR	quantitative polymerase chain reaction
IDSA	infectious disease society of America
NIH	National Institutes of Health

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Figure 1: Biofilms progress as a function of indwelling time and exhibit distal and balloon predominance.

Catheters were collected from patients with indwelling times of 0-7 days (A), 8-14 days (B), 15-28 days (C), or 29+ days (D). Absorbance OD₅₉₅, indicative of biofilm quantity, was plotted as a function of catheter segment with the most proximal (bladder end) segment on the left of each graph, and the most distal (peri-meatal end) segment on the right of each graph. Bar heights indicate the mean and error bars indicate the standard error. *p<0.05

Figure 2: Biofilm formation predominates on the intraluminal aspect relative to the extraluminal aspect of urinary catheters over indwelling times.

Catheters were collected from patients with indwelling times of 0-7 days (A), 8-14 days (B), 15-28 days (C), or 29+ days (D) as in Figure 1. Catheters were sectioned and stained for biofilms as described, and arranged with the most proximal segment on the top left and the most distal segment on the bottom right. Each panel indicates a representative catheter from the corresponding indwelling time group. Each row contains the intraluminal face of the catheter segment on the top and the extraluminal face of the segment on the bottom.

Figure 3: Biofilms progress on urinary catheters as a function of indwelling time, with an early lag phase, followed by rapid proliferation.

(A) Mean absorbance of all segments for each catheter, measured as OD₅₉₅, was plotted as a function of indwelling time. (B) Mean absorbance of all segments of each catheter group (Group 1: 0-7 days indwelling time; Group 2: 8-14 days indwelling time; Group 3: 15-28 days indwelling time; Group 4: 29+ days indwelling time). Error bars indicate standard error of the mean. *p<0.05

Figure 4: Catheters indwelling in male and female patients exhibit similar biofilm progression trends as a function of indwelling time

(A) Mean absorbance of all segments for each catheter, measured as OD₅₉₅, was plotted as a function of indwelling time and stratified by patient sex.

Table 1:

Bacterial biofilm composition and antibiotic resistances as determined by PCR and next-generation sequencing

Sample number	Indwelling time (days)	Organism(s) detected by next-generation sequencing	Proportion of sample	Resistance genes detected
1	30	Enterococcus faecalis	100%	none
2	9	Staphylococcus epidermidis	92%	none
		Pseudomonas fluorescens	7%	
3	10	Staphylococcus aureus	98%	none
4	41	Staphylococcus aureus	83%	none
		Acinetobacter calcoaceticus	16%	
5	7	Enterococcus faecalis	96%	none
		Klebsiella pneumoniae	3%	
6	43	Klebsiella pneumoniae	81%	Beta-lactam
		Enterococcus faecalis	17%	
7	40	Escherichia coli	79%	Beta-lactam
		Citrobacter freundii	10%	Macrolide
		Enterococcus faecalis	9%	Aminoglycoside
				Tetracycline
				Quinolone
8	21	Escherichia coli	93%	none
		Enterococcus faecalis	6%	
9	11	Escherichia coli	100%	Beta-lactam
				Quinolone
10	13	Escherichia coli	100%	Beta-lactam
				Quinolone