

Complicated Catheter-Associated Urinary Tract Infections Due to *Escherichia coli* and *Proteus mirabilis*

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INTRODUCTION

Indwelling urinary catheters are standard medical devices utilized in both hospital and nursing home settings to relieve urinary retention and urinary incontinence. Of the almost 100 million catheters that are sold annually worldwide, one-quarter of them are sold in the United States (50). The most common urinary catheter in use is the Foley indwelling urethral catheter, a closed sterile system that is comprised of a tube inserted through the urethra and held in place by an inflatable balloon to allow urinary drainage of the bladder. Although these devices were originally designed for short-term use in patients, indwelling catheter use is now commonplace in the long-term setting.

Due to the frequent and sometimes unnecessary use of indwelling catheters during hospitalization (21 to 50% of

patients) (153), many patients are placed at risk for complications associated with the use of these devices. A study of 1,540 nursing home residents determined that the risk of hospitalization, length of hospitalization, and length of antibiotic therapy were three times higher in catheterized residents than in noncatheterized residents (205). The most notable complication associated with indwelling urinary catheters is the development of nosocomial urinary tract infections (UTIs), known as catheter-associated UTIs (CAUTIs). Infections of the urinary tract associated with catheter use are significant not only due their high incidence and subsequent economic cost but also because of the severe sequelae that can result.

CAUTIs, the most common type of nosocomial infection, account for over 1 million cases annually (401) or over 40% of all nosocomial infections in hospitals and nursing homes (382, 383, 438) and constitute 80% of all nosocomial UTIs (132). Due to this high incidence, the overall cost for medical intervention of nosocomial UTIs is staggering, with an estimated \$424 million to \$451 million spent annually in the United States to manage these infections (157). Furthermore, catheter-

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ter-associated bacteremia is estimated to cost approximately \$2,900 per episode (339). Costs for treatment of nosocomial UTIs include antimicrobial therapy, increases in length of stay during hospitalization, physician visits, and morbidity (98). These costs will inevitably rise due to advances in preventive medicine that extend life expectancy, increasing the elderly population. This population today (those ≥ 65 years old) accounts for approximately 12.6% (37,849,672) of the total population of the United States (301,139,947) (422); their care accounts for about one-third (6) of the estimated \$1 trillion in U.S. health expenditures (279).

Individuals requiring an indwelling catheter are predisposed to the development of CAUTIs due to the presence of an indwelling catheter device and potentially pathogenic multi-drug-resistant organisms in the hospital setting. Despite the imminent threat of infection from these potent opportunistic nosocomial multiresistant strains, most cases of catheter-associated bacteriuria or the presence of bacteria in the urine are asymptomatic.

However, when an episode of CAUTI becomes symptomatic, the resulting sequelae can range from mild (fever, urethritis, and cystitis) to severe (acute pyelonephritis, renal scarring, calculus formation, and bacteremia). Left untreated, these infections can lead to urosepsis and death (284, 438). These complicated infections commonly recur and result in long-term morbidity due to the presence of encrustation and blockage of the catheter by crystalline biofilms that increase resistance to the host immune response and to antibiotics (394). Since the incidence of symptomatic CAUTIs is a major health concern due to the complications and recurrence associated with this type of infection, research directed at understanding the pathogenesis of CAUTIs is warranted and should lead to new and improved diagnosis, prevention, and treatment options.

PATHOGENESIS OF CAUTIs

Despite innate mechanical safeguards against microbial infection of the intact human urinary tract, specific organisms are capable of colonizing and persisting in this environmental niche. Similar to other mucosal pathogens, uropathogens employ specific strategies to infect the urinary tract, including colonization of a urinary catheter and/or mucosal site (uroepithelial cells), evasion of host defenses, replication, and damage to host cells. The insertion of a foreign body such as an indwelling catheter into the bladder increases the susceptibility of a patient to UTIs, as these devices serve as the initiation site of infection by introducing opportunistic organisms into the urinary tract. The majority of these uropathogens are fecal contaminants or skin residents from the patient's own native or transitory microflora that colonize the periurethral area (56, 66, 217, 288, 462). Transitory microflora that originate from hospital personnel or from contact with other patients may represent antibiotic-resistant nosocomial strains, complicating treatment for these infections. Bacterial entry into the bladder can occur at the time of catheter insertion, through the catheter lumen, or along the catheter-urethral interface (439). The preferred mechanism of bladder entry during CAUTIs is extraluminal (66%), where organisms ascend from the urethral meatus along the catheter urethral interface. Organisms can

also enter the bladder intraluminally (34%), where the bacteria migrate into the bladder as a result of manipulation of the catheter system (400, 440).

Indwelling urinary catheters further favor the colonization of uropathogens by providing a surface for the attachment of host cell binding receptors that are recognized by bacterial adhesins, thus enhancing microbial adhesion. Upon insertion, urinary catheters may damage the protective uroepithelial mucosa, which leads to the exposure of new binding sites for bacterial adhesins (108). Lastly, the presence of the indwelling catheter in the urinary tract disrupts normal host mechanical defenses, resulting in an overdistension of the bladder and incomplete voiding that leaves residual urine for microbial growth (133).

Organisms capable of infecting the urinary tract during catheterization use approaches to establish infection that are similar to those used by organisms that cause uncomplicated UTIs. However, due to the introduction of a foreign body, organisms causing CAUTIs require fewer recognized virulence factors to colonize and establish infection than those required by pathogens to infect a fully functional urinary tract.

Bacterial adhesins initiate attachment by recognizing host cell receptors located on surfaces of the host cell or catheter. Adhesins initiate adherence by overcoming the electrostatic repulsion observed between bacterial cell membranes and surfaces to allow intimate interactions to occur (61). These factors are differentially expressed during the course of infection not only for the recognition of different surfaces and cell types that the uropathogen encounters (e.g., in bladder versus the kidney) but also to evade the host immune response. These bacterial cell surface structures recognize specific host cell surface and extracellular matrix components such as proteins, glycoproteins, glycolipids, and carbohydrates. Gram-negative uropathogens produce an assortment of adhesins including those attached to the tip of hair-like projections, known as fimbriae or pili, as well as adhesins anchored directly within bacterial cell membranes, known as nonfimbrial adhesins.

Once firmly attached on the catheter surface or the uroepithelium, bacteria begin to phenotypically change, producing exopolysaccharides that entrap and protect bacteria. These attached bacteria replicate and form microcolonies that eventually mature into biofilms (Fig. 1). During biofilm development, intracellular communication by quorum sensing regulates formation and detachment from biofilms through the collective expression of genes after cellular populations reach a threshold concentration. The rate of genetic material exchange occurring within the biofilm is greater than that between planktonic cells (134, 326), thereby allowing the potential spread of antibiotic resistance genes and other traits. Once established, biofilms inherently protect uropathogens from antibiotics and the host immune response (63). The shedding of daughter cells from actively growing cells and the shearing of biofilm aggregates from the mature biofilm seed other sections of the catheter and bladder.

Many uropathogens use flagellum-mediated motility and type IV pilus-mediated (twitching) motility to facilitate the spread of infection from the initial colonization site to the urinary tract. Twitching motility via type IV pili cycles through periods of extension, attachment, and retraction in gram-neg-

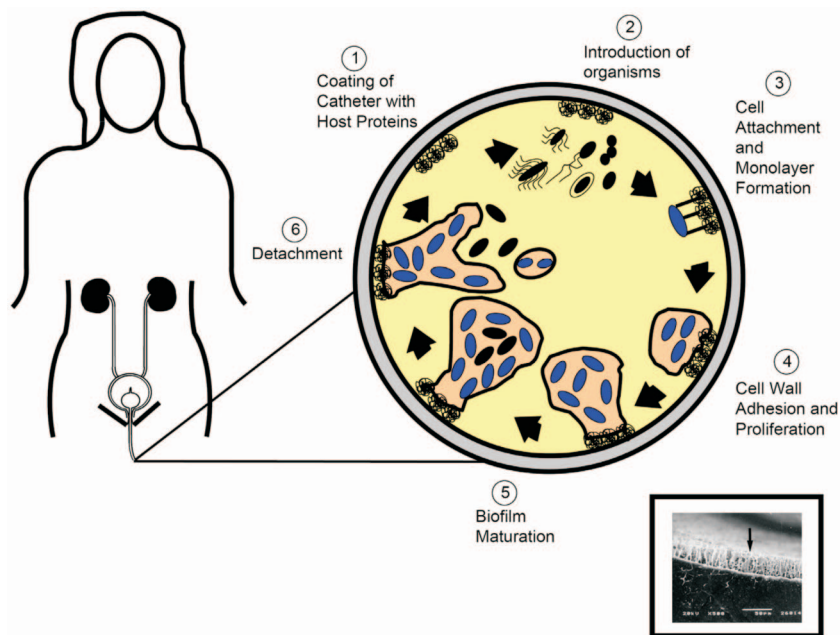


FIG. 1. Pathogenesis of biofilm formation on urinary catheters during CAUTIs. The inset (reprinted from reference 393) shows a scanning electron micrograph of a urinary catheter encrusted with *P. aeruginosa*.

ative bacteria (33, 256) and is thought to play a significant role in virulence (128).

Once colonized on the catheter and uroepithelium, uropathogens must adapt to the urinary tract environment and acquire nutrients. The production and secretion of degradative enzymes and toxins into the local environment may lead to a breakdown of tissue, releasing nutrients. As iron is a limiting nutrient in the human host (447), uropathogens have developed complex iron acquisition systems such as heme transporters, ferric and ferrous iron transport systems, and siderophore iron uptake systems to circumvent host iron-sequestering mechanisms. Certain uropathogens are capable of using urea, found in high concentrations in human urine (up to 500 mM) (35, 170), as a nitrogen source due to the expression of urease. As a consequence of urease-mediated hydrolysis of urea to ammonia and carbon dioxide, the local environment becomes alkalized, which leads to the precipitation of polyvalent ions that become enmeshed in the biofilms on catheters and urinary epithelial surfaces (118). These crystalline biofilms must be removed from the host to completely resolve the infection, since antimicrobial agents may be ineffective at eliminating biofilm-associated bacterial populations.

To maintain an infection in the human urinary tract, pathogens must be capable of evading the host immune response. Gram-negative uropathogens enact a number of mechanisms of host immune evasion, including the production of capsules, immunoglobulin A (IgA) proteases, and lipopolysaccharides (LPSs). Capsules, comprised of repeating units of polysaccharides, play a role in evading the immune system by resisting phagocytosis, antimicrobial peptides, and the bactericidal effects of human serum (46, 311, 454). Capsular structures elicit a poor immunogenic response due to their structural similarities to polysialic acid residues found on human cells (415). Additionally, this barrier plays a role in late biofilm develop-

ment (346) and protects against desiccation and phage attack. It also assists in accelerating the urinary stone crystallization process via electrostatic interactions that accumulate urinary ions at the bacterial surface (55, 87, 408). During the course of UTIs, antibodies that recognize antigenic components of uropathogens are produced. However, proteases targeting immunoglobulins and other host defense components such as complement (C1q and C3) and antimicrobial peptides (human beta-defensin 1 [hBD1] and human cathelicidin LL-37) protect uropathogens from the host response (24). LPS, a requisite constituent of gram-negative bacterial outer membranes, is composed of three components: a lipid A molecule that anchors LPS to the membrane, a core consisting of polysaccharides, and a variable O antigen. This macromolecule elicits a potent inflammatory response that initiates the development of septic shock in systemic infections. Various components of LPS have been demonstrated to be important for resistance to antimicrobial peptides (95) and complement-mediated lysis. A summary of virulence factors expressed by gram-negative bacteria is shown in Fig. 2.

During the course of researching literature for this review, it was surprising how little research has been directed specifically towards virulence associated with CAUTIs given the staggering number of patients that develop this type of infection annually. As a reflection of this finding, the majority of this review will discuss the virulence factors that are involved in the pathogenesis of UTIs caused by two gram-negative bacterial etiologic agents associated with CAUTIs, *Escherichia coli* and *Proteus mirabilis*, and how these factors may contribute to infections associated with indwelling catheters. When applicable, known virulence factors that are associated with the pathogenesis of CAUTIs will be described. Lastly, the review will conclude with methods used for the prevention and treatment of patients who develop these infections.

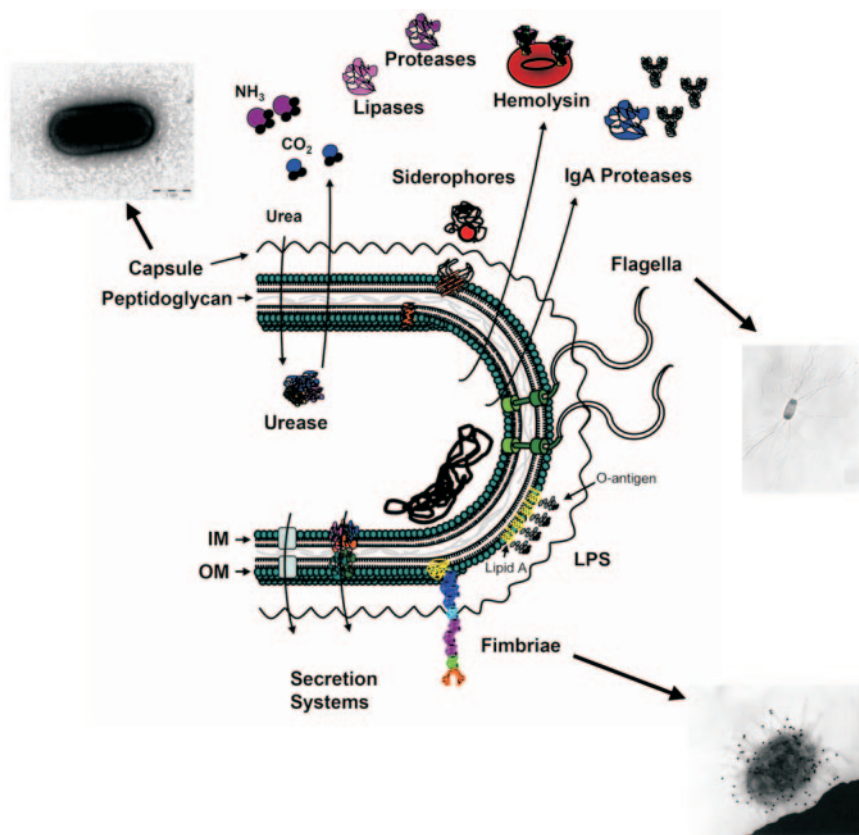


FIG. 2. Virulence factors of the gram-negative uropathogens *E. coli* and *P. mirabilis*. IM, inner membrane; OM, outer membrane. (The micrographs are reprinted from references 172, 219, and 346 with permission.)

CAUTIs DUE TO *E. COLI*

E. coli, undoubtedly the most researched microorganism, is a facultative anaerobe that is a member of the family *Enterobacteriaceae*. While both commensal and uropathogenic *E. coli* (UPEC) strains colonize the large intestines of humans, only UPEC strains are primarily selected for growth in the urinary tract. Virulence factors that differentiate these avirulent commensals from virulent strains of *E. coli* were acquired on mobile genetic elements by horizontal gene transfer; examples of such transfer can be found on the *E. coli* chromosome in the form of pathogenicity islands (125). These virulence factors enable *E. coli* strains to colonize and persist in the human host despite highly effective host defenses (278). *E. coli* strains have evolved to cause a variety of human diseases including sepsis, meningitis, diarrhea, and UTIs (276). These organisms are serotypically diverse, spanning over 250 serotypes based on O, H, and K antigens (292). Strains of *E. coli* associated with infections of the urinary tract are referred to as UPEC strains and are a subset of strains called extraintestinal pathogenic *E. coli* strains, which cause UTI, sepsis, and meningitis.

UPEC strains are the most commonly isolated organisms in community-acquired UTIs (70 to 90%) and among the most commonly isolated in nosocomially acquired UTIs (50%) including CAUTIs (202). *E. coli* has been identified as the causative agent in 90% of all case of UTI in ambulatory patients (167). UPEC strains can be classified into four phylogenetic

groups, designated A, B1, B2, and D, with strains classified as B2 and D usually causing the most extraintestinal infections including UTIs (287). Since these organisms are capable of colonizing the intestinal and vaginal tracts as well, these sites can serve as potential reservoirs for UTIs and CAUTIs (83, 160).

As with other organisms, UPEC strains possess an arsenal of virulence factors that specifically contribute to their ability to cause disease in the human urinary tract. Genes encoding hemolysin, P fimbriae, S fimbriae, and cytotoxic necrotizing factor 1 (CNF1), for example, have been identified on various pathogenicity islands in different UPEC strains (125). This genetically heterogenous group of organisms varies in its capacity to colonize and persist in the urinary tract (99, 158). DNA microarray analysis of *E. coli* CFT073, a pyelonephritis strain, compared transcriptional profiles of this strain grown in LB, in human urine, and in the murine bladder cystitis model of infection (126, 376) and verified the in vivo expression of type 1 pili, iron acquisition proteins, and capsule (15, 335, 336, 345). In a prevalence study conducted by Kanamaru et al. (180), who compared 427 *E. coli* strains (377 UTI isolates and 50 fecal isolates) using PCR assays, the putative virulence factors *iroN*, *iha*, *kpsMT*, *ompT*, and *usp* were found 2.0 to 4.3 times more frequently in UTI isolates than in fecal isolates and were strongly associated with a specific anatomical site of infection (i.e., kidney or bladder).

Since UPEC strains are more commonly associated with infections of the intact urinary tract, it is thought that less-virulent organisms are capable of causing complicated UTIs such as CAUTIs. These bacteria may express less and perhaps different virulence factors during this process compared to organisms that are able to infect structurally and functionally normal urinary tracts (261). It has been implied that UPEC strains that infect the catheterized urinary tract have a reduction in the expression of P fimbriae and possibly other factors such as hemolysin, serum resistance, colicin production, and certain H, O, and K serotypes (261). An analysis of 70 clinical urinary strains of *E. coli* isolated from patients with spinal injuries undergoing long-term bladder catheterization identified that these strains rarely possess a complete arsenal of virulence factors possessed by strains isolated from cases of uncomplicated UTI (26). Among 70 urinary isolates, the prevalences of virulence factors were as follows: mannose-resistant hemagglutinins, 30%; P fimbriae, 17%; hemolysin, 27%; K antigens, 28%; and aerobactin, 33% by bioassay and 39% by gene probe (26). These findings indicate that the presence of a urinary catheter and a neuropathic bladder increases susceptibility to colonization of the urinary tract (26). Despite its prominent role in CAUTIs, limited research specifically addressing UPEC and its ability to cause these types of infections has been performed. Because of this, we will focus on the most recent developments in the research on UPEC and its role in UTIs and, when applicable, any research that is devoted to the field of bacterial virulence during CAUTIs.

Adhesins

UPEC strains and other uropathogens must attach to uroepithelial cells and the catheter surface to colonize and initiate CAUTI and may express a variety of adhesins to assist in this initial attachment. These adhesins also contribute to the direct triggering of host and bacterial signaling pathways, assisting in the delivery of bacterial products to host tissues, and promoting bacterial invasion into host cells (271). A study by Reid et al. (321) suggested that nonspecific adhesins, not specific fimbriae, expressed by UPEC are responsible for attachment to urinary catheter material. It is unknown which specific adhesin molecules are involved in the colonization of UPEC on catheter surfaces. However, potential adhesins associated with UTIs, including type 1, P, S, FC1, and F9 fimbriae and Iha and Dr adhesins, could possibly play a role during CAUTIs. The most extensively studied adherence factors of UPEC are type 1 and P fimbriae (271); an in-depth description of these structures has been reported previously (96, 271).

Type 1 fimbriae, the most frequently expressed virulence factor of UPEC (80 to 100% of strains), are composite helical cell surface structures consisting of repeating major pilin FimA subunits, tip fibrillum (FimF and FimG), and tip adhesin FimH assembled via the chaperone (FimC)-usher (FimD) pathway (344). These pili undergo phase variation and are regulated by the recombinases FimB and FimE.

In a study by Mobley et al. (261) examining urine cultures of 51 long-term catheterization patients over a year, type 1 fimbriae were expressed by a significantly higher number of UPEC isolates causing the most persistent infections than by strains causing transient infections. These pili are thought to be crit-

ical for the interaction of UPEC with uroepithelial cells during colonization of the bladder (59, 211, 272, 403). FimH of type 1 pili is thought to be involved in the adherence of these organisms to the bladder epithelium through the recognition and binding of the mannosylated integral membrane glycoproteins uroplakin Ia (467) and uroplakin Ib located on superficial epithelial cells. This tip adhesin also recognizes extracellular matrix proteins including collagen (types I and IV), fibronectin, and laminin as well as Tamm-Horsfall protein. Therefore, these bacterial adhesive structures are able to recognize epithelia (bladder and kidney), immune cells (macrophages, neutrophils, and mast cells), erythrocytes, and extracellular matrix proteins. This tip adhesin may also mediate bacterial autoaggregation and biofilm formation (313, 347, 348).

In addition, type 1 pili are believed to induce an inflammatory response associated with UPEC attachment and invasion (202) through the binding of FimH to specific mast cell receptors that initiate this response by the secretion of inflammatory mediators (1). Lastly, Snyder et al. (377) demonstrated that the expression of type 1 fimbriae coordinately affects the expression of P fimbriae in an inverse manner that may coordinate sequential events during colonization during UTIs *in vitro* and *in vivo* as examined by CFT073-specific DNA microarray analysis and mutagenesis studies using a mouse model. This adhesin has been associated with the invasion process as will be discussed later in the review. As these pili have been suggested to be expressed for the initial interactions between UPEC and various surfaces, it is speculated that type 1 pili could be involved in the initial interactions with the catheter surface or in interactions with uroepithelial cells during CAUTIs associated with UPEC.

P fimbriae or pyelonephritis-associated pili (*pap*) are the second most common virulence factors associated with UPEC uropathogenesis. The genetic determinants responsible for the production of these fimbriae are encoded on the UPEC chromosome by the *papABCDEFGHIJK* operon. P fimbriae are composed of heteropolymeric fibers consisting of different protein subunits (148), including proteins involved in the structure of the pilus (major pilin PapA, pilus anchor PapH, tip fibrillum PapKEF, and tip adhesin PapG), pilus assembly (periplasmic chaperone PapD and outer membrane usher PapC), and pilus regulation (PapB and PapI). Some studies have shown that P pili are needed by UPEC strains during UTIs; others have failed to show this requirement. UPEC strains expressing P fimbriae attach to globoside residues present on human kidney epithelial cells, which is suggested to play a role in the virulence associated with pyelonephritis (present in 80% of pyelonephritic *E. coli* strains) as well as ascending UTI (79, 310). Attachment to uroepithelial cell digalactoside receptors mediated by these fimbriae has been shown to induce cytokine secretion (interleukin-6 [IL-6] and IL-8) by this cell type *in vitro* (137). Studies have proposed that P pili may be important in establishing a bacterial reservoir in the intestinal mucosa (113, 233). However, experimental evidence suggests that these adhesins appear to have a less important role in colonizing abnormal or obstructed urinary tracts (156, 416). Based on these findings, it is thought that P pili may have either no role or a limited role during CAUTIs caused by UPEC.

UPEC is capable of expressing other surface adhesins including S pili (271), F1C pili, F9 fimbriae, IrgA adhesin, and Dr

adhesins (271). S pili, consisting of the major subunit SfaA and the minor subunits SfaG, SfaH, and SfaS, recognize and bind sialyl galactosides on human kidney epithelial cells (191) and have been shown to play a role in UTIs caused by UPEC in rats (238). F1C pili, encoded by 14% of UPEC isolates, recognize and attach to kidney epithelial (distal tubules and collecting ducts) and endothelial (bladder and kidney) cells (183). Recently, Ulett et al. described a novel fimbria for UPEC strain CFT073 known as F9 fimbriae (420). These fimbriae were suggested to play a role during biofilm formation and are found in other UPEC and other pathogenic *E. coli* strains. The precise role of these surface structures during infection is currently unknown. UPEC expresses an iron-regulated gene homologue adhesin IrgA, designated Iha, during UTIs. This outer membrane protein is prevalent among clinical UPEC strains (38 to 74%) (16, 166) compared to fecal *E. coli* isolates (14 to 22%). Recombinant Iha from the pyelonephritogenic *E. coli* isolate CFT073 conferred adherence to cultured T-24 human uroepithelial cells to nonadherent *E. coli* strain ORN172 (163). In addition, a mutant in *iha* was more attenuated in a mouse model of ascending UTI than wild-type strain CFT073 and UPEC76 (CFT073 *pap* mutant) (163).

The Dr adhesin family of UPEC includes the uropathogen-associated fimbrial adhesin Dr and nonfimbrial adhesins (AFA-I, AFA-II, AFA-IV, Nfa-I, and Dr-II) (271) and has been associated with cystitis (30 to 50%) in children (114). The Dr operon consists of six genes encoding the main structural subunit DraA, the chaperone DraB (308), the usher DraC (308), the potential invasins DraD (106), DraP, and the adhesin DraE (465). The structural adhesin DraE determines the receptor-binding specificity of Dr adhesins (285). This adhesin is believed to be important in bacterial persistence in the urinary tract through the invasion of bladder and kidney epithelia via the interaction of these fimbriae with decay-accelerating factor (CD55) (286), a regulatory protein that protects tissues from autologous complement-mediated damage. The Dr adhesin also binds type IV collagen (286) and integrins, thus promoting recognition and the adherence of these organisms to interstitial compartments of the kidney, neutrophils, and erythrocytes. Strains of UPEC expressing Dr adhesin were capable of causing chronic experimental pyelonephritis in C3H/HeJ mice (114). They were also capable of long-term survival in human epithelial cells and persisting in the kidneys of experimental animals for months (215). It is unknown if this family of adhesins is expressed during CAUTIs caused by UPEC.

In summary, UPEC is known to express a number of adherence factors that assist in its ability to persist in the urinary tract. However, there is limited research on how this organism adheres to catheter surfaces. It can be speculated that some of the known adhesins that UPEC uses during UTIs could be expressed during CAUTIs caused by this organism as host cell components attach to the catheter surface to provide binding sites. However, more extensive research on the adherence of UPEC during CAUTIs is warranted and necessary to better understand the pathogenesis of this infection.

Motility

It is postulated that once UPEC is established on the catheter surface, flagellum-mediated motility is important for the

ascent of this uropathogen from the catheter to the bladder and subsequently to the upper urinary tract (ureter and kidney). The synthesis of the flagellar structure is coordinated in a complex regulon consisting of several operons arranged in a hierarchical system (discussed in detail in a review by Fernández and Berenguer [96]). The filament of flagella consists of flagellin, the major filament subunit encoded by the *fliC* gene, that extends into the extracellular milieu from the outer membrane. The filament is connected to the flagellar hook FlgE through its attachment to the junction proteins FlgK and FlgL and the filament scaffolding protein FlgD (96). Two recent mutagenesis studies by Lane et al. (208) and Wright et al. (459) demonstrated that flagella, while not absolutely required for virulence during UTIs, greatly enhanced the persistence and fitness of UPEC during this type of infection. Therefore, flagellum-mediated motility should likely be considered to be important for the movement of UPEC on the catheter surface and from the catheter surface to the uroepithelium. This, however, has not been directly demonstrated.

Invasion and Biofilm Formation

Once initial attachment and permanent adherence commence on either the surface of catheters or uroepithelial cells, the establishment of UPEC infection occurs through the colonization of the bladder by the invasion of host cells and the subsequent formation of biofilms. As demonstrated in murine models, UPEC has developed mechanisms to invade host cells, and several reviews discussed this phenomenon in detail (32, 271). UPEC strains have been observed in vitro and in vivo to be internalized by bladder epithelial cells (103, 241, 251) and renal epithelial cells (82, 294, 380, 442). Several adhesins and toxins have been implicated to be involved in the process of invasion, including type 1 fimbriae, the Afa/Dr adhesin family (Dr, Dr-II, F1845, Afa-1, and Afa-3), S pili, P pili, and CNF1. Type 1 fimbria-mediated invasion is dependent upon FimH expression (241). *E. coli* strains that express Dr adhesins have been observed to invade epithelial cells including Caco-2 intestinal cells (115, 286). Dr adhesin-mediated invasion of uroepithelial cells is dependent upon the presence of the decay-accelerating factor receptor on host cells (117) and may contribute to persistence within the upper urinary tract (271). Research into the roles of S and P pili (241) during bacterial invasion of epithelial cells has not been studied in depth. However, it has been proposed that these pili, in conjunction with toxins, may facilitate the invasion of host tissues (115, 116).

CNF1, discussed later in the review, has been implicated in the invasion of UPEC into uroepithelial cells. This secreted toxin enters the host via the low-pH-mediated endocytotic pathway (54) and then constitutively activates key Rho GTPases that signal the reorganization of the actin cytoskeleton in the host cell. CNF1 has been shown to induce apoptosis in bladder epithelial cells via terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling. Thus, this toxin may play a role in bladder cell shedding in vivo and exposing the underlying tissue for bacterial invasion (257).

The ability of UPEC strains to persist in the urinary tract has been demonstrated by Justice et al. to reside within the superficial umbrella cells of C3H and BALB/c murine bladders due to the formation of biofilm structures known as intracellular

bacterial communities (IBCs) (175). These IBCs are formed in a sequential manner. First, during murine cystitis, UPEC cells are attached to the cell surface by type 1 fimbriae and then invade the uroepithelium (240, 241) 1 to 3 h after the initial inoculation. Localized actin rearrangements occur and engulf the bound organism via zipper-like phagocytosis (240). After being internalized in the murine superficial bladder cell, UPEC replicates rapidly, forming clusters known as early IBCs (175). Recently, type 1 fimbriae have been shown to have an additional intracellular role during this stage of IBC formation (460). As IBCs mature, around 6 to 8 h after inoculation of the murine bladder (175), they more closely resemble classical biofilm structures (88), where the bacterial doubling time is increased (from approximately 30 to 60 min) and the bacterial cell length is shortened (0.7 μm versus 3 μm). At this stage, pod-like protrusions are observed on the surface of murine bladder epithelial cells (8). Around 12 h postinoculation, bacterial detachment is observed (175) as either a whole community or individual highly motile cells that burst out of the murine bladder lumen in a process referred to as fluxing (7). It is postulated that IBCs and biofilms contribute to the persistence of these organisms due to the increase in resistance to antibiotics and the host immune response. IBC formation has not yet been substantiated in humans.

There are several factors that are known to contribute to the formation of biofilms by *E. coli*. These include fimbriae, curli, flagella, antigen 43, and extracellular matrix molecules including cellulose, colanic acid, and poly- β -1,6-*N*-acetyl-D-glucosamine (67, 68, 74, 81, 437, 469). Specifically, biofilm formation mediated by type 1 fimbriae may assist in the colonization of urinary catheter surfaces (271).

There have been recent studies examining biofilm formation on catheters and UPEC. Ferrières et al. (97) showed that certain catheter materials such as silicone and silicone-latex actually select for and promote biofilm formation for the most virulent UPEC strains, whereas asymptomatic bacteriuria strains form better biofilms on polystyrene and glass (Fig. 3). Koseoglu et al. (194) revealed that UPEC type O4 had formed mature biofilms after 12 to 24 h and developed biofilms completely in almost all latex/silicone balloon catheter samples after 4 to 7 days as examined by scanning electron microscopy.

Biofilm formation is critical for initiating and maintaining CAUTIs. Therefore, any factors involved in this process are likely important virulence factors during CAUTIs caused by UPEC. However, there are currently very few studies that have examined biofilm formation during these infections.

Avoidance of Host Immune Response

Once established, bacteria must express factors to avoid the host immune response in order to persist in the urinary tract; these factors include fimbriae that are subject to phase variation, capsules, and LPS. As mentioned above, type 1 fimbriae are subject to phase variation to evade the host immune response. UPEC strains, as with other uropathogens, have been shown to produce an exopolysaccharide capsule as a means of avoiding the immune response and thereby contributing to serum resistance. There are over 80 types of these capsular polysaccharides (CPSs) (K antigens), with K1 capsules most frequently observed among urinary and clinical isolates (160, 291). These organisms produce

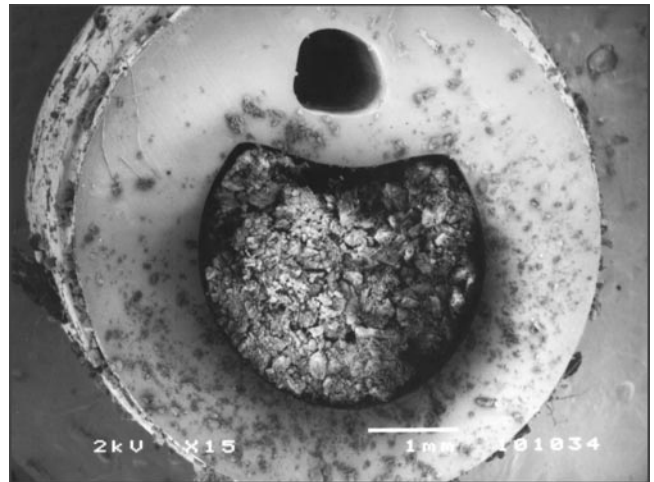


FIG. 3. Cross section of a silicone catheter removed from a patient after blockage. Crystalline material can be seen completely occluding the catheter lumen. (Reprinted from reference 387a) with permission of the publisher.)

group II or group III capsules, compared to commensals that express group I polysaccharide capsules (161). These capsules are thin, highly anionic structures that tend to aggregate spontaneously (174). The gene locus encoding the group II capsule has been described for UPEC strain NU149 and is organized into three regions. The genes involved in the assembly and transport of capsules are located at conserved regions designated regions 1 (*kpsFEDUCS*) and 3 (*kpsMT*). In contrast, region 2 (*kfiABCD*) is unique to each serotype and is involved in capsular biosynthesis (451). Schwan et al. (355) described the down-regulation of the *kpsFEDUCS* operon, possessing the genes of capsule assembly region 1 responsible for K-antigen expression (451), in UPEC upon type 1 fimbria attachment to mannose-coated Sepharose beads as a possible initiating event of UTIs. These acidic polysaccharide capsules assist in the avoidance of phagocytosis and complement activation (161). There are no studies that have investigated the role of these structures during CAUTIs caused by UPEC.

Upon entry into the bladder, UPEC contacts uroepithelial cells and initiates a robust innate immune response through the activation of various signaling pathways. Cell activation is accomplished by interactions between bacterial surface molecules, such as fimbriae and LPS, and uroepithelial cell receptors. LPS, a critical constituent of the gram-negative cell wall, was shown to be important for resistance to antimicrobial peptides (95) and complement-mediated lysis. However, the compound activates cells of innate immunity via its interaction with Toll-like receptor 4 (TLR4) and CD14 located on the host cell membrane (349, 350). These TLR molecules (TLR1 to TLR10) are pattern recognition receptors expressed on the cell surfaces of leukocytes and epithelial cells that recognize preserved molecular motifs located on various pathogens (202). TLR4 is important in these events since mice deficient in this surface receptor are incapable of efficiently clearing UPEC from the bladder (127). These initial interactions activate signaling pathways that subsequently lead to the release of cytokines (tumor necrosis factors, gamma interferon, IL-8, and IL-1) that

guide neutrophil infiltration, stimulate complement and coagulation (130, 351), and trigger the shedding of superficial bladder facet cells (273). Prior to expulsion from the host, intracellular UPEC emerges from these exfoliated bladder epithelial cells to invade the exposed underlying tissues to persist in the host. Type 1 fimbriae, as described by Blomgran et al. (30), interact with neutrophils in a mannose- and LPS-dependent manner, which leads to subsequent neutrophil apoptosis. It is suggested that LPS plays an important role in the persistence of UPEC during UTIs and CAUTIs. However, there is currently no experimental evidence that substantiates these claims.

Damage to the Host and Acquisition of Nutrients

Once established on catheter and uroepithelial surfaces, uropathogens such as UPEC strains must adapt to the urinary tract environment and acquire nutrients. This is accomplished in part by the production of degradative enzymes, toxins, and nutrient acquisition systems. These factors include toxins such as hemolysin, CNF1, and autotransporters such as secreted autotransporter toxin (Sat), Pic (139, 295), and Tsh (139) to break down host peptides and iron acquisition proteins to acquire iron sequestered by the host.

UPEC strains expressing toxins such as hemolysins, CNF, and Sat have been isolated (121, 144, 202). Of the 91 UTI isolates examined by Caprioli et al. (47), 37% produced both the hemolysin HlyA and CNF1, whereas only 1 of 114 fecal isolates produced CNF1. The two common forms of hemolysin expressed by UPEC are the alpha-hemolysin HlyA (384), and cell-associated beta-hemolysin. The presence of *hlyA* was found to be 31 to 48% for UTI isolates, compared to only 15% for fecal isolates (239). The genes encoding these heat-labile secreted pore-forming proteins are found on either plasmids or the chromosome (252). Hemolysin has been shown to lyse human kidney proximal tubule cells (263). The effect of these toxins is the induction of cell lysis due to an increase in intracellular osmotic pressure caused by the dissipation of calcium ion gradients. A proposed function of these toxins is to cause the release of host cell iron into the extracellular environment that can then be captured by the bacterium.

The toxins classified as CNF (CNF1 or CNF2) are known to constitutively activate the Rho GTPases RhoA, Rac1, and Cdc42, which regulate the actin cytoskeleton (75). Cells exposed to this toxin form enlarged multinucleated cells, as exhibited by membrane ruffling, the formation of focal adhesions and actin stress fibers, and DNA replication in the absence of cell division. The existence of the CNF1 gene was found in 27 to 41% of UTI isolates but in only 9% of fecal isolates (239). CNF1 has been shown to modulate polymorphonuclear leukocyte function through the down-regulation of phagocytosis and altering the distribution of the complement receptor CR3 (CD11b/CD18). A study by Falzano et al. (94) supplied evidence that CNF1 of UPEC is capable of blocking the cell cycle G₂/M transition in the T24 uroepithelial cell line.

UPEC has been shown to secrete a number of autotransporters during UTIs. The autotransporter toxin known as Sat (121) is a vacuolating cytotoxin that damages kidney epithelial cells during acute pyelonephritis in the mouse model (122). UPEC strains have also been found to express two other au-

totransporters, Pic and Tsh, that possess serine protease activity (139). These proteins are expressed *in vivo* during pyelonephritis in the mouse model (139). Since these proteins have been shown to cause host damage during UTIs in an animal model, it is plausible that these autotransporters could be expressed by UPEC during CAUTIs. However, research to address this hypothesis has not been undertaken.

Although nutrient acquisition systems are important for the survival of UPEC during growth in the urinary tract, this review focuses on the nutrient acquisition systems for iron, as these are the best-studied systems. Iron acquisition systems are important virulence factors produced during UTIs and CAUTIs, as this nutrient is limiting in urine (363). Since iron acquisition is important for the viability of UPEC during infection, these organisms produce multiple systems including siderophore-siderophore receptor and heme uptake systems (334). As stated above, *E. coli* strains likely use alpha-hemolysin as another means of retrieving iron from the host (290). The known siderophore systems of UPEC include the catechol enterobactin and the hydroxamate aerobactin along with their corresponding receptors, such as the IreA, IronN, and Iha receptors (161, 378). Most *E. coli* strains produce the siderophore enterobactin. However, the genes encoding aerobactin are found significantly more often in strains isolated from the urinary tract (69.4% of 124 isolates [$P = 0.001$]) than in fecal *E. coli* samples (41.2% of 51 isolates) (77). UPEC strains have been shown by hydrolysis fluorescence detection to produce novel siderophores including salmochelins, C-glycosylated enterobactins that are dependent upon the biosynthesis of enterobactin and the *iroBCDEN* operon (468), and yersiniabactin (423).

Iron acquisition systems have been shown to be important during UTIs caused by UPEC. Snyder et al. demonstrated that five iron acquisitions were upregulated in UPEC strain CFT073 during infection in the CBA mouse model of ascending UTI compared to static *in vitro* growth in LB (376). Recently, Reigstad et al. (322) revealed that heme- and siderophore-associated iron acquisition systems play key roles in IBC development in female C3H/HeJ mice. Further research is needed to determine if these acquisition systems are important during CAUTIs caused by these organisms.

CAUTIs DUE TO *PROTEUS MIRABILIS*

Proteus species, members of the family *Enterobacteriaceae* (301), are distinguishable from most other genera by their ability to swarm across an agar surface. These organisms are widely distributed in the environment, including polluted water, soil, and manure, where *Proteus* plays a role in the decomposition of organic matter from animals, and in the intestinal tract of mammals. *Proteus* species are the causative agent of a variety of opportunistic nosocomial infections including those of the respiratory tract, eye, ear, nose, skin, burns, throat, and wounds; it also may cause gastroenteritis (302, 331). Antibodies specific for *Proteus* have been isolated from patients with active rheumatoid arthritis (76, 90), suggesting some association with this malady. *Proteus* bacilli are more commonly associated with UTIs in those individuals with structural or functional abnormalities, especially ascending infections in patients undergoing urinary catheterization (441, 444). Colonization of the intestinal tract allows *Proteus* to establish reservoirs for

transmission into the urinary tract by intermittent colonization of the periurethral region. This intermittent colonization can lead to the subsequent contamination of the catheter, thus allowing nosocomial infections to develop (53).

Proteus-associated UTIs may be difficult to treat, and the bacterium persists due to complications associated with this type of infection, including bladder and kidney stone formation (urolithiasis) that can lead to the obstruction of catheters and the urinary tract (200, 356, 444). The three species of *Proteus* associated with UTIs are *Proteus mirabilis*, *Proteus vulgaris*, and *Proteus penneri*. While UTIs caused by *P. vulgaris* (361) and *P. penneri* (199, 200) have been identified, *P. mirabilis* is the third most common cause of complicated UTI (12%) and the second most common cause of catheter-associated bacteriuria in patients catheterized long term (15%) (439).

P. mirabilis is a common cause of CAUTIs. It was observed by Roberts et al. (327) that *P. mirabilis* has the greatest ability to attach to catheters out of all gram-negative organisms. As monitored by a low-light optical imaging system, catheter sections colonized with bioluminescent *P. mirabilis* were inserted into murine bladder lumen. These animals developed severe cystitis that persisted significantly longer than that in mice challenged with bacterial suspensions alone and required prolonged antibiotic treatment to reduce the infection (178). To establish and maintain infections of the urinary tract and colonization of catheters, *Proteus* species must adapt to the catheterized urinary tract and produce an arsenal of strictly regulated virulence factors.

Adhesins

Indwelling urinary catheters serve as the initiation site of CAUTIs by introducing uropathogens such as *Proteus* spp. into the urinary tract and providing a surface for coating by host cell debris and protein that may be recognized by bacterial adhesins. *P. mirabilis* strains tend to attach to catheters with a greater propensity than other gram-negative bacteria (327). Studies have demonstrated that *P. mirabilis* strains are capable of attaching to a number of catheter polymers including ethylene, propylene, polystyrene, sulfonated polystyrene, silicone, and red rubber (135, 327).

To facilitate binding to these different surfaces, *P. mirabilis* must be capable of producing a variety of adherence factors, such as fimbriae and hemagglutinins, that are thought to play an important role in the establishment of CAUTIs. *Proteus* species have been shown to produce various fimbriae and hemagglutinins involved in the colonization of the urinary tract and possibly catheter surfaces, including MR/P fimbriae (289), MR/K hemagglutinin (289), uroepithelial cell adhesin (UCA)/nonagglutinating fimbriae (NAF) (456), *P. mirabilis* fimbriae (PMF) (10), and ambient-temperature fimbriae (ATF) (243). Others have also been predicted for the genome sequence of strain HI4320 (M. Pearson, J. Parkhill, and H. L. Mobley, unpublished data).

MR/P fimbriae are perhaps the best-understood fimbriae expressed by *P. mirabilis* strains during UTIs. These fimbriae are thick channeled (7 to 8 nm) and are classified as mannose-resistant fimbriae (36, 371). These fimbriae assemble through the chaperone-usher pathway (379). The genes required for the expression of MR/P fimbriae on the cell surface are en-

coded on the *Proteus* chromosome on two divergent transcripts, *mrpABCDEFGHJ* (designated the *mrp* operon) and *mrpI* (14). Some of the proteins encoded by the *mrp* operon include the fimbrial structural subunit MrpA (13); the terminator for fimbrial assembly, MrpB (222); the minor fimbrial subunit MrpG (224); the tip adhesin MrpH (219); and the repressor of flagellin synthesis, MrpJ (223).

Expression of MR/P fimbriae is subject to phase variation (13, 14). The *mrpI* gene encodes a site-specific recombinase that reverses the orientation of the 251-bp invertible element that precedes the *mrp* operon. Expression of MR/P fimbriae correlates with the orientation of this invertible element (466). MrpI orients the invertible element in either an "on" position, allowing the expression of the MR/P fimbria, or an "off" position, in which the promoter is in the opposite orientation and is thus unable to drive transcription.

Many studies have suggested that MR/P fimbriae play a role in the virulence observed during UTIs caused by uropathogenic *P. mirabilis* strains. In the CBA model of ascending UTI, infection with *P. mirabilis* elicited a strong immune response to MrpA, the major structural subunit of MR/P fimbria, indicating that MR/P fimbriae were expressed in vivo (11). Isogenic mutants incapable of expressing MR/P fimbriae were attenuated when examined in this mouse model (12, 219, 221, 224). A mutant constitutively expressing MR/P fimbriae outcompeted the wild-type strain in the murine bladder but not the kidneys in a cochallenge experiment, thereby establishing MR/P fimbriae as being an important bladder colonization factor for *P. mirabilis* (221). Tissue binding studies by Sareneva et al. (343) revealed the propensity of this fimbrial type to adhere specifically to the human renal tubular epithelial cells and to the exfoliated uroepithelial cells of urinary sediment.

Experiments conducted by Jansen et al. (155) suggest that MR/P fimbriae dictate the localization of bacteria in the bladder and contribute to biofilm formation, a process essential for the establishment of CAUTIs. A *P. mirabilis* HI4320 construct with an invertible element locked in the "on" position colonized the luminal surfaces of murine bladder umbrella cells and formed significantly more biofilms after 2 days of growth in urine ($P = 0.05$) compared to a construct with the invertible element in the "off" position. The off-position mutant colonized the lamina propria underlying exfoliated uroepithelium. Although studies have associated the expression of MR/P fimbriae with virulence during UTIs caused by *P. mirabilis*, there is no direct evidence substantiating a role of these fimbriae in CAUTIs.

On the other hand, MR/K fimbriae have been linked with the attachment of organisms to catheter surfaces and with the persistence of catheter-associated bacteriuria (262, 331, 461). These fimbriae have also been detected during *Providencia stuartii* infections in catheterized elderly patients (262). Expression of these thin (4- to 5-nm) nonchanneled type 1 mannose-resistant fimbriae (36, 371) enables *P. mirabilis* to attach tightly to the Bowman's capsule of the host kidney glomeruli and to the tubular basement membranes (343). Although more associated with *P. penneri* strains (461), it is speculated that MR/K fimbriae play a possible role in the initial adherence to catheter biomaterials during *P. mirabilis* CAUTIs.

Besides MR/P and MR/K fimbriae, the other fimbriae produced by *P. mirabilis* during UTIs may contribute to attach-

ment to the catheter surface. Surface adhesins determined not to be involved in the hemagglutination caused by MR/P and MR/K fimbriae have been identified in *P. mirabilis*, including UCA/NAF (10, 29, 60, 406, 456), PMF (244), and ATF (242). Wray et al. (456) characterized UCA, a nonagglutinating fimbria from *P. mirabilis* HU1069 that was demonstrated to weakly attach to exfoliated human desquamated uroepithelial cells. The 540-bp *ucaA* gene, which encodes the major fimbrial subunit of UCA, has nucleic acid homology to the F17A gene of *E. coli* F17 pilin (58%) (60) and was identified in all 26 *P. mirabilis* strains tested (29). Due to its homology to the F17 pilin of *E. coli* (60), it has been suggested that these fimbriae might be involved in the colonization of the intestines by these organisms (58). Based on studies conducted by Bahrani et al. (10, 11, 13), there was some ambiguity as to which fimbrial types were identified as UCA (456) since thin (4-nm) and thick (6-nm) fimbrial filaments were observed by electron microscopy, and multiple bands were isolated on sodium dodecyl sulfate-polyacrylamide gels (406). These fimbrial subunits were isolated and characterized from *P. mirabilis* strain 7570 from a patient with struvite urolithiasis and renamed NAF by Tolson et al. (406). The N-terminal sequence of this fimbrial subunit was confirmed to be identical to the N-terminal sequence from *P. mirabilis* strain HU1069 of the study reported by Wray et al. and not homologous to the N termini of MR/P, ATF, or PMF fimbrial subunits (406). Bacteria expressing NAF adhered strongly to a number of cell lines in vitro, including uroepithelial cells (407) and MDCK (Madin-Darby canine kidney) (5, 216) and EJ/28 urinary tract tumor (214) cell lines. Purified NAF from *P. mirabilis* binds to a number of glycolipids such as asialo-GM1, asialo-GM2, and lactosyl ceramide, as demonstrated by thin-layer chromatography overlay assays and solid-phase binding assays (216). Because of its homology to fimbriae that assist in intestinal tract colonization, it is possible that these fimbriae may play a role in the initiation of CAUTIs by allowing *P. mirabilis* to attach and establish in the intestines and thus form a reservoir of organisms that can potentially cause CAUTIs. However, there have been no definitive studies examining this possibility.

PMF are encoded by genes located in the *pmf* gene cluster and consist of five polypeptides: PmfA, the 18.9-kDa major subunit of PMF (10); PmfC (93.1 kDa); PmfD (28.2 kDa); PmfE (38.9 kDa); and PmfF (19.7 kDa). The *pmf* gene cluster has >25% amino acid sequence identity with the *pap*, *mvp*, and *sfa* fimbrial gene clusters. However, *pmfE* has been identified as being unique to this gene cluster. Thus far, no regulatory elements for the production of these fimbriae have been identified (245). There are conflicting results as to the function these fimbriae during UTI. In a study by Massad et al. (244), PMF were demonstrated to play a role during the colonization of the bladder since an isogenic mutant in the *pmfA* gene of *P. mirabilis* HI4320 was 83-fold more attenuated than the wild-type strain during independent challenge in the CBA mouse model of ascending UTI (244). However, in this same study, PMF could not be shown to be involved with attachment to human uroepithelial cells since attachment to this cell type is similar in both the wild type and the *pmfA* mutant. Its role in the colonization of the kidney is also questionable since no significant difference between numbers of the wild type and *pmfA* was observed in kidney tissue (244). Contrary to this, a

study by Zunino et al. (472) showed that there was significant attenuation observed in the kidney and bladder by the isogenic *pmfA* mutant compared to the parent strain Pr2921 during cochallenge in a model of ascending UTI in female CD-1 mice. Furthermore, attachment of this isogenic *pmfA* mutant to T24/83 human-derived bladder carcinoma cells and human uroepithelial cells was significantly less than that of the wild type. These conflicting results require resolution.

ATF were classified as a new fimbrial type, as examined by electron microscopy and immunogold labeling (243), and were identified in all eight *P. mirabilis* strains analyzed. The genes responsible for the production of ATF are organized in the *atf* gene cluster and encode a 19-kDa major-subunit AtfA (243), the chaperonin-like protein AtfB, and the outer membrane usher AtfC (243). AtfA has significant amino acid sequence identity to type 1 major fimbrial subunits of several enteric species (38% to 41%) (243). An allelic-replacement *atf* mutant colonized the murine urinary tract at a gene comparable to that of the wild type in independent challenge and outcompeted the wild type in cochallenge experiments in the murine model of ascending UTI (243). As nonclinical strains of *Proteus* were shown to express AtfA, as observed by Western blot analysis (470), it is suggested that these fimbriae are involved in the colonization of *P. mirabilis* in the environment and are most likely not involved in CAUTIs.

Currently, only MR/K fimbriae are known to be associated with the process of attachment during CAUTIs. Clearly, additional studies must ascertain whether known factors or currently uncharacterized factors are involved in adherence, as this process is essential for these types of infections. The identification of novel adherence factors as well as other virulence factors will be facilitated by the recent annotation of the *P. mirabilis* HI4320 genome by the Sanger Centre in conjunction with the Mobley Laboratory.

Motility

In general, flagella on the surface of bacterial pathogens are thought to assist in host colonization and dissemination, initial attachment, and sensing of the extracellular environment (25). For *Proteus* species, these surface structures are important in the process known as swarming, a distinct characteristic of these organisms. Therefore, it is speculated that flagellar motility and, potentially, swarming are important during CAUTIs, as the ability of *P. mirabilis* to disseminate from the initial site of colonization on the catheter surface to the uroepithelial cells of the urinary tract is critical for the establishment of these types of infections.

Swarming is a surface-induced multicellular differentiation process that allows organisms to move in a coordinated manner and expand the population to new locations over solid surfaces (258, 318, 453). During growth in liquid medium, *Proteus* species assume the form of an infectious single-cell, motile, 1.0- to 2.0- μm -long bacillus that displays a distinct phenotype including the presence of peritrichous flagella on its cell surface and swimming behavior. However, when transferred onto solid medium, these swimmer cells differentiate into hyperflagellated, multinucleated, nonseptated elongated swarmer forms measuring 20 to 80 μm in length. These differentiated swarmer cells migrate out from the original inocula-

tion site in a rapid and highly coordinated manner that is dependent upon multicellular interactions and cell-to-cell signaling (18).

Swarmer cells align themselves in multicellular rafts and are enveloped in the extracellular slime material of the colony migration factor Cmf that is required for and facilitates translocation through a reduction in surface friction (124, 172, 381). The swarming process continues until the cell number is reduced by cell loss or when the bacterial mass changes the direction of motion (18). The cessation of movement, known as consolidation, is accompanied by the dedifferentiation and replication of swarmer cells into vegetative swimmer cells. This periodicity distinguishes *P. mirabilis* swarming from other swarming processes. For a more in-depth description on the process of *Proteus* swarming, refer to reviews by Rather (318) and Rozalski et al. (331).

Since swarming is such a dominant characteristic of this genus, any factors that affect or regulate this phenomenon would likely affect the fitness of the organism. The swarming phenomenon is a metabolically complicated and demanding process that must genetically coordinate the expression of over 50 genes (21), including those involved in the production, assembly, and operation of flagella and virulence factors such as flagellin, urease, hemolysin, and the ZapA metalloprotease (4, 24).

To identify potential genes that may be involved in the process of swarming, transposon mutagenesis studies of *P. mirabilis* using Tn5 transposons were performed (20, 21, 23, 41). Those studies identified over 50 genes that were involved in the swarming process and included genes that encoded proteins involved in flagellar biosynthesis (20), flagellar rotation (20), surface elongation (20), control and coordination of multicellular motility (20), production of LPS and peptidoglycan (23), and cell division (23). Burall et al. (41) identified a mini-Tn5 transposon mutant in *dsbA* of *P. mirabilis* HI4320 that was defective in the colonization of the murine urinary tract and in swarming. This gene encodes an oxidoreductase that forms disulfide bonds in periplasm proteins. However, no definitive role of this gene during swarming has been defined. The utilization of signature-tagged mutagenesis has greatly assisted in the identification of genes not previously associated with the swarming process, and any of these genes could play an essential part in the pathogenesis of CAUTIs caused by *P. mirabilis*.

The definitive roles of flagella and swarmer cell differentiation in the virulence of *P. mirabilis* during UTIs remain controversial, but some of these suggested roles include dissemination of *P. mirabilis* from the initial site of infection to other sections of the catheter or to the urinary tract and avoidance of the host immune response. Flagella are believed to contribute to the virulence of swimmer cells by allowing motility from the catheter to the bladder epithelium and onward, ascending into the ureters and kidneys. An isogenic, nonpolar, nonmotile, flagellum-negative mutant in *flaD* of *P. mirabilis* WPM111 was attenuated 100-fold compared to the wild type in the CBA model of ascending UTIs, indicating the importance of flagella in murine UTIs (259).

The biosynthesis of flagella is a key process in both motility and swarming and involves numerous genes on the *Proteus* chromosome (22). Flagellin is encoded by *flaC*, and the *flaD* gene encodes the flagellar filament capping protein (19). Stud-

ies suggest that the major flagellin protein for *P. mirabilis* is subject to antigenic variation through homologous recombination as three copies of flagellin-determinant gene (*flaA*, *flaB*, and *flaC*) that reside on the *P. mirabilis* genome with only one copy that is actively expressed (19, 275). It was proposed that flagellin gene rearrangement is a mechanism for host immune system evasion by *P. mirabilis* and is extremely relevant for *Proteus* infections since flagella are highly immunogenic. As a result, any antigenic change could increase the survival of *Proteus* species in the urinary tract through the evasion of secretory IgA directed toward flagella during colonization in the bladder (19). Due to its relevance during UTIs, it is probable that antigenic variation via flagellin gene rearrangement is a method of host immune response evasion by *P. mirabilis* during CAUTIs.

Swarming cell differentiation is thought by some to be important for the virulence of *P. mirabilis* during UTIs since several virulence factors, including flagellin, urease, the hemolysin HmpA, and the IgA metalloprotease ZapA, are upregulated in the differentiated swarmer cell compared to swimmer cells (4, 101). Mutants in FlhA synthesis, proteins required for flagellar synthesis, are nonmotile due to the loss of *fliC* transcription but also have reduced transcription of *hpmA* hemolysin (123). Therefore, it has been suggested, based in part on evidence of the coordinate expression of virulence factors during swarming cell differentiation, that factors involved in the swarming process are critical for pathogenesis and that a similar signal must be regulating both swarming and virulence (3, 4, 52, 258). Interestingly, however, swarmer cells are rarely observed in the murine model of ascending UTI, bringing into question the relevance of this morphotype in the absence of a catheter (154).

Swarming may play a role in the migration of *Proteus* strains on catheter materials; however, swarmer cells of *Proteus* species are capable of migrating across 1-cm-long sections of Foley catheters consisting of either hydrogel-coated latex, hydrogel/silver-coated latex, silicone-coated latex, and all silicone in vitro (337). Swarmer cells of *P. mirabilis* have been observed to migrate through populations of *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis* and then continue to migrate with little or no reduction over hydrogel-coated latex catheter sections (337). Nonswarming mutants were shown to have lost the ability to migrate over these catheter sections (172). However, upon their introduction into models of the catheterized bladder, these mutants were just as capable of encrusting and blocking catheters as the wild type (171). It seems that while swarming might have a role in the initiation of infection, facilitating the passage of the cells from the urethral meatus to the bladder, it is not required for the rapid formation of crystalline biofilm once bacteria have colonized the residual urine in the bladder.

There is conflicting experimental evidence about the significance of swarming during the ascension of *P. mirabilis* into the urinary tract and during pyelonephritis. Swarming-defective mutants and motile, nonswarming mutants of *P. mirabilis* were significantly attenuated in the colonization of the kidney compared to the wild type upon intravesical (bladder) inoculation of mice (2). Furthermore, the swarming-defective mutant was still able to colonize the murine bladder albeit at a lower rate than that of the wild-type strain. In contrast, the nonswarming

mutant was incapable of colonization of the murine kidney. These findings suggest that swarming is important for *P. mirabilis*-associated ascending UTIs and pyelonephritis. Histological analysis of murine renal tissue has supported these results, as swarmer cells were found to be the predominant cell type (2). Other studies suggested that flagella and/or swarming is not involved in the process of ascension into the upper urinary tract by *P. mirabilis*. A clinical strain of *P. mirabilis* that lacks flagella has been isolated (471). In addition, confocal microscopy studies by Jansen et al. (154) demonstrated that the predominant *P. mirabilis* morphotype was the short swimmer cell, not the swarmer cell, in the mouse model of ascending UTI. However, the differences observed between these experimental results are most likely due to differences in experimental parameters for each study (318). More conclusive studies are required to resolve these conflicts.

Biofilm Formation

After the initial colonization of the catheter surface, *Proteus* species, as with other uropathogens, form distinctive crystalline biofilm structures during CAUTIs. These structures assist in the persistence of *P. mirabilis* in the urinary tract by protecting these organisms from antibiotics and the host immune response and obviously contribute to adhesion to surfaces (146). Urinary stone formation during *Proteus*-mediated UTI is characteristic of this type of infection and is critical for the development of crystalline biofilms. Bacterially derived stones account for up to 30% of all urinary tract stones worldwide and account for approximately 75% of the urinary stones classified as staghorn calculi (141). Upper urinary tract stones are classified as staghorn calculi if stone formation occurs in the renal pelvis and extends out into at least two calyces (253). Crystalline biofilms are especially problematic during CAUTIs since catheters become blocked due to encrustation caused by the formation of these structures.

It should be recognized that there are powerful physical and chemical factors involved in the initiation and development of the crystalline biofilms that block catheters. Experiments in parallel-plate flow cells showed that when urine cultures flow over polymer surfaces, the pH of the urine can be a major factor in determining bacterial adhesion. For example, some polymers with strongly-electron-donating surfaces will resist colonization by cells until the pH of the urine rises above the pH at which calcium and magnesium phosphates precipitate out of solution. In alkaline urine, macroscopic aggregates of cells and crystals form in the urine, settle on the polymer surface, and initiate crystalline biofilm formation (391). These observations indicate that to stop biofilm formation on devices in the urinary tracts of patients infected with *P. mirabilis*, it is essential to prevent the rise in urinary pH and the crystallization of apatite and struvite (Fig. 4).

The luminal surfaces of catheters, particularly those of latex-based catheters, are extremely irregular in nature (64). The engineering techniques used in catheter manufacture produce especially rough surfaces on the rims of the catheter eyeholes. Experiments in physical models of the catheterized bladder in which catheters were removed at various time intervals after infection with *P. mirabilis* to monitor the initial stages of encrustation demonstrated the vulnerability of the eyeholes to

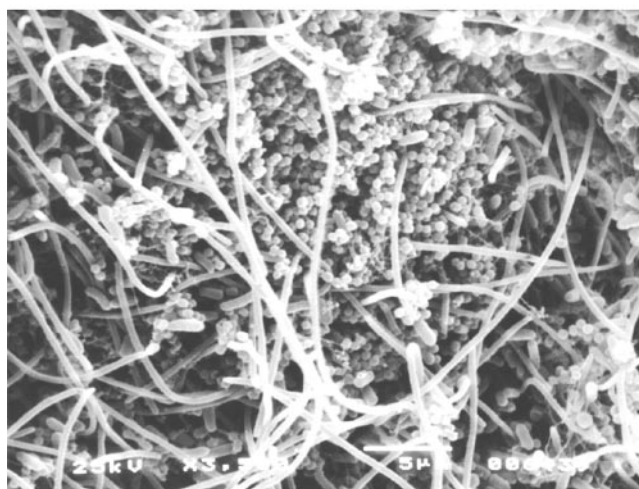
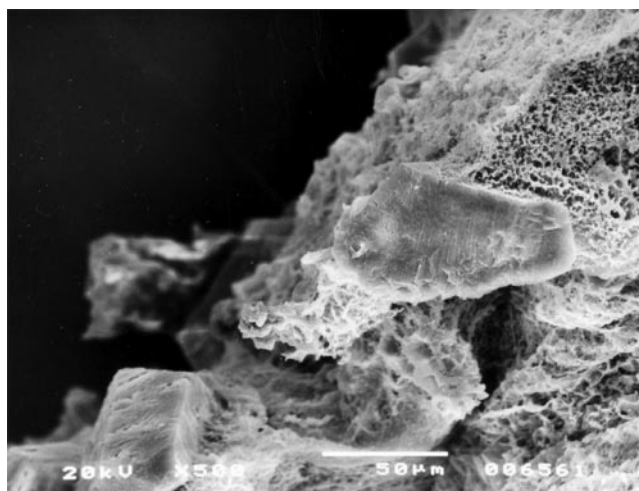


FIG. 4. Crystalline material that blocked a patient's catheter after just 4 days. The large coffin-shaped crystals were shown by X-ray microanalysis to be a form of magnesium ammonium phosphate (struvite), and the microcrystalline aggregates were shown to be calcium phosphate (apatite). A four-membered bacterial community was isolated from this crystalline biofilm composed of *E. coli*, *P. aeruginosa*, *E. faecalis*, and *P. mirabilis*. (Modified from reference 390a with permission from Elsevier.)

colonization (386). Scanning electron microscopy revealed that within 2 h, bacterial cells were trapped in the crevices in the uneven surfaces of the eyelets. At 4 h, microcolonies of cells had developed in the surface depressions, and by 6 h, with the rise in urinary pH, crystals had started to form in the biofilm. Extensive crystalline biofilm was obvious at 20 h and was spreading down the catheter lumen. Blockage generally occurred at the eyehole or in the balloon region of the lumen.

Bacterial urease and capsule polysaccharides are two major factors known to be involved in urinary crystal formation and, hence, crystalline biofilm formation in *P. mirabilis* (277). Since urea is present in concentrations of up to 500 mM in human urine (35, 170), it is not surprising that bacterial ureases play a pivotal role in *Proteus*-associated UTI. Urease contributes to the development of urinary stones due to urease-mediated hydrolysis of urea to ammonia and carbon dioxide that alkali-

linizes the local environment. This increase in urinary pH causes the local supersaturation and precipitation of calcium phosphate and magnesium-ammonium phosphate from urine to form crystals of carbonate apatite [$\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$] and struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), respectively (118). These crystals accumulate in the biofilms of catheters and urinary epithelial surfaces and eventually obstruct the flow of urine through the catheter and from the bladder or kidney. Incontinence can develop due to urine leakage around the catheter or retention of urine in the bladder that can seriously complicate the care of patients undergoing long-term bladder catheterization (392). Urease is produced by *Proteus* species known to cause clinical infections (*P. mirabilis*, *P. vulgaris*, and *P. penneri*) (266), and the urease produced by *P. mirabilis* is the best characterized one.

The *P. mirabilis* urease is a 250-kDa multimeric nickel metalloenzyme that is produced in the cytoplasm (266). As this enzyme is inducible in urea, it is assumed to be constitutively expressed during growth in urine (58). This urease is homologous to the urease of *Klebsiella aerogenes* (266), and the urease operon of *P. mirabilis* has homology to the urease operon of *Providencia stuartii* (169, 265, 435) and likely all urease genes. This operon possesses seven genes, *ureDABCEFG*, that encode proteins involved in the production of urease. The urease apoenzyme is composed of a trimer of trimers consisting of the polypeptides UreA, UreB, and UreC [(UreABC)₃] (264) and is activated upon the insertion of the divalent nickel metallocenter into each of the UreC subunits. The insertion and assembly of the nickel ions into the metallocenter are accomplished by the urease accessory proteins UreD chaperone (258), UreE (nickel ion donor), UreF, and UreG (264). The exact mechanism for the assembly of urease is not fully understood (331).

The urease gene cluster is regulated by UreR and the histone-like nucleoid structuring protein (H-NS). The 33-kDa polypeptide UreR, a member of the AraC/XylS family of transcriptional activators (281), initiates transcription of the genes encoding the urease subunits and accessory proteins and of its own gene in a urea-inducible manner through binding to the intergenic region between *ureR* and *ureD* (84, 150, 405). UreR binds the promoters of the *ureR* and *ureD* genes in the absence of urea, albeit with less affinity than in the presence of urea, suggesting that the organism is prepared for the rapid induction of urease (84, 150, 405). The transcriptional repressor H-NS recognizes and binds to the poly(A) tracts of the intergenic region between the *ure* genes and bends the DNA to repress the transcription of *ureR* in the absence of urea induction (57), as shown in an *E. coli* model system (312). Both UreR and H-NS regulators were able to displace each other from the *ureR-ureD* intergenic region in a gel shift assay (312).

The importance of urease as a virulence factor of *Proteus*-associated UTIs as well as CAUTIs has been demonstrated in the CBA mouse model. An insertion mutation in the *ureC* gene abolished urease activity and was attenuated in the murine model (168). This urease-negative construct colonized the bladder and kidneys in 100-fold-fewer bacteria than the urease-positive strain after 2 days postinoculation and caused no urolithiasis during infection (159). A study by Li et al. (225) revealed that catheterized CBA mice were more susceptible to infection by the wild-type *P. mirabilis* strain after 7 days than

uncatheterized CBA mice. However, although catheterized CBA mice were more susceptible to bladder colonization by the *ureC* insertion mutant than uncatheterized mice, the mutant was unable to colonize the kidneys under any circumstances (225). These results suggest that even though urease is important during colonization of the urinary tract during uncatheterized infection, it is not necessary for the initial colonization of the bladder during CAUTIs in the mouse model.

Stone formation is the primary role of urease during UTI caused by urease-producing organisms. A secondary role of urease during *Proteus* UTI is the accumulation of toxic levels of ammonia from urease-mediated hydrolysis of urea that damages tissue including renal epithelia (277). Ammonia has been demonstrated to be toxic to the protective uroepithelial glycosaminoglycan layer (119) present at the bladder surface to effectively block the adherence of bacteria to the uroepithelium. However, the cytotoxic effects observed for urease are not as severe as those observed for HpmA hemolysin (260).

Besides bacterial urease, capsular structures assist in crystalline stone formation observed during UTIs and CAUTIs associated with *P. mirabilis*. These structures are believed to accelerate struvite crystal growth (55, 249) by aggregating precipitated components of urine into stones (250). *Proteus* CPSs tend to be acidic due to the presence of uronic acid, pyruvate, or phosphate groups, thus enabling this structure to bind to metal cations such as Ca^{2+} and Mg^{2+} (331). Purified partially anionic CPS of *P. mirabilis* ATCC 49565 added to artificial urine at a pH of 7.5 to 8.0 induced more struvite formation than other CPS types, as examined by particle counting (Coulter counter) and by phase-contrast microscopy (87). With the exception of one polymer (curdlan) that did not bind Mg^{2+} ions, the enhancement of struvite crystallization by CPS polymers was inversely proportional to their Mg^{2+} binding ability. Therefore, it is suggested that the weak binding of the Mg^{2+} ion by the partial anionic structure of *P. mirabilis* CPS enhances struvite crystallization by enabling the weakly concentrated Mg^{2+} ions to be readily released from LPS for crystal formation (87, 331).

Once a mature biofilm develops on the surface of uroepithelial cells or catheters, organisms within the mushroom-shaped structure communicate with each other, utilizing diffusible chemical signals that regulate a variety of cellular functions including glutamine (3), autoinducer-2 (AI-2) (354), cyclic dipeptides, and putrescine (396). However, the role of these signaling molecules in the process of biofilm formation or swarming during *P. mirabilis* UTIs or CAUTIs is unclear.

In short, crystalline biofilms are known to form during CAUTIs associated with *P. mirabilis* and are responsible for some of the more severe sequelae experienced. Only a few of the proteins involved with this process in *P. mirabilis*, including bacterial urease, have been identified and studied. Therefore, more extensive studies need to be completed, including the identification of these potential proteins.

Avoidance of Host Immune Response

Besides the formation of crystalline biofilms, *P. mirabilis* uses several immunoavoidance factors to persist in the urinary tract, including antigenic variation, capsules, IgA proteases, and LPS. As mentioned above, the flagellin protein of *P. mira-*

bilis may undergo antigenic variation as a means of avoiding an antibody response. CPS, also referred to as slime material or glycocalyx, is a highly hydrated polymer present on the bacterial cell surface. These structures have several known functions during UTIs and CAUTIs caused by *P. mirabilis*, such as a role in crystalline stone formation (250), which is discussed above. Other known functions of CPS include protection against the host immune response and antibiotics, attachment to surfaces (146), and, potentially, swarming. Little is known about these structures in *Proteus* species. Studies of certain O antigens of *P. mirabilis* (O6 and O57) and *P. vulgaris* (O19) demonstrated that capsular antigen structures that are identical to the O-specific chains of their LPS are produced (27, 307, 419). One capsular structure of *P. mirabilis* (ATCC 49565) has been identified as being an acidic CPS consisting of a high-molecular-weight polymer of branched trisaccharide units composed of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine), 2-acetamido-2,6-dideoxy-L-galactose (*N*-acetyl-L-fucosamine), and D-glucuronic acid (27).

The colony migration factor *Cmf*, an extracellular slime material, from wild-type *P. mirabilis* WT19 is an acidic CPS composed of a tetrasaccharide repeating unit. This substance is rich in galacturonic acid and *N*-acetylgalactosamine, as determined by glycosyl composition and linkage analyses and by one- and two-dimensional nuclear magnetic resonance spectroscopy (316). A mutant in *cmfA* was attenuated in a model of experimental uropathogenicity compared to the wild type, thereby demonstrating a reduced ability to colonize the urinary tract (2). As described above, this factor facilitates surface colony expansion of the swarm cell population through a reduction in surface friction (124). Therefore, it is proposed that this factor contributes to the uropathogenicity of *P. mirabilis* during CAUTIs by facilitating the translocation of differentiated cell populations on catheter surfaces.

To combat invading microbes, during the course of UTIs, secretory IgA, the dominant immunoglobulin form in mucus secretion, is produced against antigenic components of uropathogens by the host immune system. This immunoglobulin is important for the protection of the mucous membrane and underlying tissue of the urinary tract during infection and is fairly resistant to proteolytic cleavage. However, proteases targeting immunoglobins and other host defense components such as complement (C1q and C3) and antimicrobial peptides (hBD1 and human cathelicidin LL-37) protect uropathogens from the host response (24). Only a few bacterial species produce proteases against IgA (e.g., *Neisseria* spp., *Haemophilus* spp., and *Streptococcus* spp.) (270, 309), including an IgA protease activity that has been identified for *Proteus* species.

While not produced by *Providencia* spp. and *Morganella* spp., all clinical strains of *P. mirabilis* and *P. penneri* and many strains of *P. vulgaris* examined produced an EDTA-sensitive protease (229, 358, 359). The purified metalloprotease appeared to be a composite of a single band and a double band (53 and 50 kDa, respectively), as visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and all three bands were proteolytically active (229). The optimal pH for these enzymes was pH 8, which would coincide with the action of urease that alkalizes urine (229). The production and activity of the *P. mirabilis* metalloprotease appear to occur during UTIs since breakdown fragments detected in urine specimens

from patients with clinical evidence of upper UTIs are not detected in urine specimens from patients infected with a non-proteolytic strain. Also, fragments were identical to the breakdown fragments observed when purified IgA was degraded by purified metalloprotease, as examined by immunoblotting (362).

The EDTA-sensitive IgA extracellular metalloprotease produced by a chronic urinary tract isolate of *P. mirabilis* cleaves the heavy chain of the serum immunoglobulin IgA into two fragments (359). The cleavage sites for this protease differed from those of other microbial IgA1 proteases in that it cleaves outside the hinge region (228, 358). The substrate specificity of the protease isolated from a chronic UTI isolate, *P. mirabilis* 64676, was expanded to include the cleavage of subclasses of IgA (secretory IgA1 and IgA2) and IgG, both present in urine (331), and the nonimmunoglobulin substrates such as gelatin, secretory component, and casein (228). Cleavage of IgG occurs at the hinge region as a two-stage process that involves a pepsin-like activity and then a papain-like activity to yield Fab and Fc fragments (228).

Wassif et al. (445) isolated and characterized this protease from *P. mirabilis* BB2000 and designated it ZapA. ZapA is a 55-kDa recombinant extracellular metalloprotein that is a member of the serralyisin family of zinc metalloproteases and a member of the ABC transporter family (433). It is produced during swarmer cell differentiation and is stimulated by divalent cations ($\text{Ca}^{2+} > \text{Mg}^{2+}$) (433). This protease was found to be important during *Proteus*-mediated UTIs, as mutants in the *zapA* gene fail to degrade IgA and are attenuated compared to the wild type during infection in a CBA mouse model of ascending UTI (433). The genes for ZapA production and secretion are organized into the *zapEABCD* operon, and based on homologies to other known systems, the system consists of the ZapA-specific ABC transporter and the polypeptides ZapB, which possesses an ATP-binding cassette; ZapC, the membrane-spanning fusion protein; ZapD, which is involved in outer membrane transport; and ZapE, a second metalloprotease (433). ZapE was homologous to other extracellular proteases, including ZapA (42% identity) from *P. mirabilis* (445), PrtC (45% identity) and PrtA (38%) from *Erwinia chrysanthemi* (112), serralyisin (44% identity) from *Serratia marcescens*, and AprA (36% identity) from *Pseudomonas aeruginosa* (89). The substrate specificity of ZapA includes both human and mouse serum and secretory forms of IgA (IgA1 and IgA2) and IgG (445). Additional substrates include many urinary tract proteins such as complement (C1q and C3), cell matrix (collagen, fibronectin, and laminin), cytoskeletal proteins (actin and tubulin), and the innate immune system antimicrobial peptides hBD1, a component of the human renal tubule innate immune response, and human cathelicidin LL-37, as determined by *in vitro* assays (24).

Because of its broad specificity, there are several probable roles for IgA protease production during *Proteus*-associated UTI. Its primary role may be to evade the host immune response, including antibody-mediated opsonization and degradation of immune system components. The secretion of the protease by *P. mirabilis* during UTI or during CAUTIs could serve to cleave IgG and IgA into fragments with defective immune function, leading to decreased respiratory bursts in neutrophils (227). These proteases appear to inactivate effec-

tors of the innate immune system, such as the antibacterial peptides hBD1 and LL-37, that have critical roles in preventing infection at the uroepithelial surface (24, 353). As this protease has such a broad specificity, one of its main functions during UTIs and CAUTIs could be to degrade host cell proteins for amino acid and peptide acquisition. Since the production of this enzyme and the ability to invade human uroepithelial cells coincide with swarming cell differentiation, IgA protease also plays a role in invasion during infection.

Lastly, to evade the host immune response, *P. mirabilis*, as with other gram-negative organisms, possesses LPS. It is well reported that *Proteus* is an antigenically heterogeneous genus due to structural differences among the O-specific polysaccharide chain of LPS. Currently, there are 60 O serogroups (212, 303), including 49 numbered serogroups (O1 to O49) (212) of *P. mirabilis* and *P. vulgaris* and 19 specific H-antigen (flagellum) serotypes as classified previously (195, 304). Immunological studies of LPS have also established a number of additional serogroups for *P. penneri* strains (364, 473, 474). The common O serotypes isolated from clinical strains of *Proteus* species are O3, O27, O10, and O28 serotypes (212).

LPS is the main structural component of the gram-negative outer membrane and is one of the major virulence factors associated with these organisms. LPS is composed of three components: a lipid A molecule that anchors LPS to the membrane, a core consisting of polysaccharides, and a variable O antigen. Chemically, the LPS of *Proteus* species has been classified into 16 total chemotypes for *P. vulgaris* and *P. mirabilis* (366) and 7 total chemotypes for *P. penneri* (474). The composition and structure of several O-specific polysaccharides of *Proteus* species have been described (27, 48, 49, 102, 176, 185, 186, 188–190, 195, 304–307, 365, 367–370, 419, 425–432). In most *Proteus* strains, the general structure of the O-specific polysaccharides has been found to be acidic due to the presence of uronic acids and various noncarbohydrate acidic components, including phosphate groups (187, 331). For a more detailed description of these structures and LPS, refer to the review by Rozalski et al. (331).

LPS is known to induce a number of effects on its host, including fever, hypotension, disseminated intravascular coagulation, and subsequent shock (324). Various components of LPS have been demonstrated to be important for resistance to antimicrobial peptides (95) and to complement-mediated lysis. However, their role in the pathogenesis of UTIs is unclear.

Certain studies suggested that despite the presence of incomplete LPS in certain rough mutants, *P. mirabilis* strains are still pathogenic in the urinary tract (201, 213, 331). Other studies have indicated, however, that LPS is a virulence factor of *Proteus*-associated UTIs. In particular, nonpolar mutants of the *waaE* gene, a gene involved in the biosynthesis of the inner core of LPS through the substitution of alpha-L-glycero-D-manno-heptopyranose I at the O-4 position by a beta-D-glucopyranose residue (152), of *P. mirabilis* showed reductions in adhesion to uroepithelial cells. In addition, pathogenicity in female Wistar rats, swarming motility, and the ability to form biofilms in vitro were also reduced in these mutants (152).

Due to the acidic, negatively charged residues on its surface, a possible function of LPS during UTIs and CAUTIs is its ability to attach and bind cations such as Ca^{2+} and Mg^{2+} , leading to the accumulation of these ions around bacterial cells

and the acceleration of the crystallization process (408). Studies by Torzewska et al. (408) indicated that the level of Ca^{2+} and Mg^{2+} cation binding on the surface of *Proteus* organisms is dependent upon the sugar composition of the *Proteus* LPS as determined by in vitro crystallization in artificial urine.

Certain *P. mirabilis* LPS structures have played a role in this organism's resistance to antimicrobial peptides (120, 248, 395) and defensin molecules. Defensins are a major component of innate host defense against bacteria, including the potent molecule polycationic hBD3 found on epithelia (31).

In summary, *P. mirabilis* strains can persist in the urinary tract during UTIs or CAUTIs by evading the host immune response and are known to express several immunoavoidance factors. Currently, there is no direct evidence to confirm that many of these factors are produced during CAUTIs and are important during these infections. Biofilms have been shown to protect organisms against the host immune response (146) and are assumed to protect *P. mirabilis* against this response during CAUTIs. As *P. mirabilis* is known to produce a capsule and capsules are one mechanism of evading the host immune response, it has been suggested that these structures protect these uropathogens against the immune system during UTIs and CAUTIs. The colony migration factor Cmf, an acidic CPS, was also proposed to assist in swarming across catheter surfaces. As the IgA protease ZapA has been demonstrated to be expressed during swarming and as swarming has been shown to occur during the migration of *P. mirabilis* across catheter sections, it can be suggested that this protease is expressed during CAUTIs caused by these organisms. The LPS of *P. mirabilis* is thought to function in the avoidance of the immune response and the accumulation and acceleration of the crystallization process.

Damage to the Host and Acquisition of Nutrients

Once colonized on the catheter and the uroepithelium, uropathogens must adapt to the urinary tract environment and acquire nutrients. This is accomplished by the production of degradative enzymes, toxins, and nutrient acquisition systems. Uropathogens such as *P. mirabilis* can express and secrete degradative enzymes and toxins into the local environment to break down tissue, releasing nutrients and/or assisting in the spreading of the infection. Degradative enzymes target substrates located in the host for use as energy sources, including nutrients located in host fluids (e.g., urea from urine) and host cell surface and extracellular matrix components such as collagen, fibronectin, elastin, and hyaluronan. *Proteus* species produce several of these factors, and these factors include proteases to break down host polypeptides and peptides (e.g., ZapA) and ureases to obtain carbon and nitrogen sources, hemolysin to permeabilize host cell membranes, and iron acquisition systems to acquire iron sequestered by the host.

Hemolysis of human and sheep erythrocytes has been demonstrated to be mediated by 126 different urinary and soil isolates of *Proteus* species (*P. mirabilis* and *P. vulgaris*) (196, 197, 201), and the level of hemolytic activity is significantly higher in *P. mirabilis* strains than in *P. vulgaris* strains (296, 297). Hemolysins identified in *Proteus* species include HlyA and HpmA. HlyA, an extracellular calcium-dependent pore-forming cytotoxin (448, 450), is produced by strains of *P. pen-*

neri (330, 360) and by 40% of *P. vulgaris* strains tested but not by *P. mirabilis* strains (192). The *hly* genes from *P. vulgaris* are homologous to the *hly* genes of *E. coli* (192) and represent the *Morganella*, *E. coli*, *Proteus*, and *Pasteurella* family of hemolysins (192, 193, 399, 449). In addition to this hemolysin, *P. vulgaris*, *P. penneri*, and *P. mirabilis* strains produce an unrelated hemolysin known as HpmA, as identified by immunoblot and DNA-DNA hybridization. These 166-kDa hemolysins, or cell-associated hemolysins (177, 192, 196, 197, 360, 449), are calcium-independent (177, 196, 360) pore-forming toxins that insert into target eukaryotic membranes, causing the efflux of Na⁺ ions and subsequent cell lysis (34). The production of HpmA is observed only in actively growing and replicating organisms in the presence of erythrocytes (297, 299), with maximum production occurring during the late logarithmic phase in *P. mirabilis* strains (398). This hemolysin and its secretory protein HlyB have significant nucleotide identity (52.1%) to the *shlA* and *shlB* hemolysin genes of *S. marcescens* (230). Cleavage of the 29-amino-acid N-terminal leader sequence is required for the activation and secretion of the HpmA hemolysin of *P. mirabilis* 477-12 (421) into the periplasm. In addition, the amino-terminal region of HpmA is required for hemolytic activity (398). The secretion and activation of HpmA are assisted by the 63-kDa potential outer membrane protein HpmB (230).

The HpmA hemolysin is thought to play a role in the virulence of *Proteus* UTIs and CAUTIs since the production of the HpmA hemolysin is upregulated coordinately with other virulence factors during swarming differentiation (4, 101). It was suggested that hemolysin assists in the spread of *Proteus* into the kidneys via tissue damage (260) and in tissue invasiveness (298, 299, 329, 330). HpmA cytotoxic activity against a number of cell culture lines in vitro including Vero, human renal proximal tubular epithelial (52, 260), Daudi, Raji, and U937 cells by a chromium release assay (260, 398) has been observed. The cytotoxicity of the HpmA hemolysin observed is dependent upon the in vivo condition examined. For example, the lethal dose of a hemolysin-negative mutant was six times higher than that of the wild-type strain upon intravenous challenge (398). However, a mutant in the *hpmA* gene of *P. mirabilis* WPM111 showed no significant difference in colonization compared to the wild type in the murine model of ascending UTI (398). While hemolytic activity observed in *P. mirabilis* strains has been correlated to cell invasiveness (298, 329), the nonhemolytic *P. mirabilis* strain WPM111 was shown to be 10- to 100-fold more invasive in human renal proximal tubular epithelial cells than hemolytic wild-type strain BA6163 based on a gentamicin protection assay. This result could be misleading, however, since hemolysin-mediated pores could allow the entry of gentamicin into cells (52). Therefore, the definitive role of *Proteus* hemolysin during UTIs has not been firmly established.

Extracting essential nutrients from host tissues and the surrounding extracellular milieu is critical for any microbe to establish a UTI or CAUTI. The composition of the host environment directly affects which acquisition systems are expressed during infection. Although the acquisition of various metals and carbon and energy sources as well as the presence or absence of oxygen are important in determining what metabolic pathways are enacted by pathogens and are critical for the fitness of any organism, we will focus primarily on the

acquisition of iron, as its role in virulence in different organisms is well established.

Iron is an essential element for the production of cytochromes and as an enzyme cofactor. Proteins involved in iron acquisition are critical virulence factors produced during a UTI (9, 42, 45, 457) and, potentially, CAUTIs, since this nutrient is limiting in urine (363). The addition of exogenous iron reduces the susceptibility of animals to the development of *P. mirabilis* pyelonephritis (131). While most enterobacteria produce phenolate (enterobactin)- and/or hydroxamine (aerobactin)-type siderophores during iron-limiting conditions (42, 45, 296), none of these traditional siderophores have been demonstrated to be produced in *Proteus*, *Providencia*, or *Morganella* species (92, 296, 323). It has been proposed that these bacterial genera utilize α -keto acids derived from the deamination of amino acid by amino acid deaminase to bind iron and act as potential siderophores (85). The 51-kDa amino acid deaminase of *P. mirabilis* is encoded by the *aad* gene (246), and attempts to generate a mutant deficient in this gene have been unsuccessful, suggesting that mutations in this gene are lethal (246). Traditionally, the expression of most siderophores is repressed by the binding of the Fur protein on the promoters of iron-regulating genes. However, no Fur binding site has been identified upstream of the *aad* gene (246). Since deaminase activity was not affected by low-iron conditions, Aad may have other functions aside from the acquisition of iron (58). A signature-tagged mutagenesis study of *P. mirabilis* HI4320 conducted by Burrall et al. (41) identified potential iron acquisition protein homologues such as the *Pasteurella multocida* membrane-bound heme receptor HasR, the phosphopantetheinyl transferase NrpG, the *Yersinia pestis* putative outer membrane protein IrgA, and a *Y. pestis* putative iron transport permease. However, the mechanism of iron acquisition for *P. mirabilis* is currently unknown. With the accessibility of the genomic sequence of *P. mirabilis* HI4320 made available by the Sanger Centre, the identification of more potential iron acquisition genes will be facilitated, and perhaps a better understanding of this process will emerge.

In summary, *P. mirabilis*, as with other uropathogens, must be capable of adapting to the urinary tract environment and acquiring nutrients. This is thought to be accomplished by the production of degradative enzymes such as urease and proteases, toxins such as the hemolysin HpmA, and iron nutrient acquisition proteins.

PREVENTION OF CAUTIs

Over 5 million patients per year receive urinary catheterizations (236), and all are at risk for developing CAUTIs. Current effective infection control measures against CAUTIs are ones that only delay the onset of bacteriuria, since no methods that prevent these types of infections have been developed (410). This section will describe the different practices that have been proposed for reducing the risk of developing CAUTIs. These include avoiding unnecessary catheterization, selecting alternative catheterization procedures, maintaining a closed drainage system, and eliminating bacterial colonization of the meatus. The development of new biomaterials that reduce biofilm formation on the surface of catheters, the addition or impregnation of antimicrobial agents on catheter materials,

and the use of probiotics will be described as potential prevention measures. Lastly, future promising technologies for the prevention of CAUTIs will be discussed.

Limiting Catheter Usage

Selective and limited catheter use is critical to reduce the numbers of patients at risk for developing CAUTIs. Similar to the phenomenon observed for antibiotic use, urinary catheters have been overly used in the nosocomial and institutional settings because of their relative ease of application. Studies suggest that this is the case, as 21 to 38% of initial catheterizations had no justifiable indication (153, 274, 342). One of the causes of overuse has been due to “forgotten” catheters, where the physicians or students have not removed catheters after their use is no longer needed. In fact, one survey determined that 28% of inpatient physicians and students did not know which of their patients had received catheterization (341). A reduction in CAUTI due to the presence of forgotten catheters could be achieved through the utilization of a computerized reminder system (340). However, the restriction of urinary catheters to only those patients that truly require catheterization, such as to relieve urinary tract blockage, to allow drainage of neurogenic bladders and urinary retention, to facilitate healing of the genitourinary tract after surgery, or to measure the urine output in postoperative or critically ill patients with accuracy (455), is a key preventive measure for CAUTIs. These devices should be promptly removed after their required use as to reduce the risk of infection (236).

Once it is determined that a patient requires urinary catheterization, the duration of the catheterization affects the risk of developing infections associated with this device. With the risk of bacteriuria associated with catheter insertion being 3 to 10% per day (282), it is estimated that 10 to 50% of patients undergoing short-term catheterization (up to 7 days) and nearly 100% of patients that require long-term catheterization (>28 days) will develop a CAUTI (129, 269, 387). During the course of catheterization, it has been recommended that urinary catheters should be changed every 8 to 10 days (332) to reduce the risk of infection. Drainage bags should be emptied at a minimum of every 4 to 6 h to avoid bacteria entering the catheter lumen (280).

Condom and Suprapubic Catheters and Intermittent Catheterization

Besides their selective and limited use, alternative methods of catheterization that potentially reduce the risk of CAUTIs, including condom and suprapubic catheters and intermittent catheterization, are available. Condom catheters, while useful for male patients that lack bladder outlet obstruction, require meticulous care to avoid complications such as skin maceration and have been associated with an increase risk of UTIs (140, 455). The use of suprapubic catheters, indwelling catheters inserted above the pubic bone directly into the bladder, shows promise in terms of risk of infection (142, 237). However, no controlled clinical studies have been conducted to confirm its benefits (455). Those individuals with spinal cord injuries or individuals with dysfunctional emptying of the bladder commonly utilize intermittent catheterization or temporary inser-

tion of a catheter. As with suprapubic catheters, there is a lack of studies examining the effectiveness of intermittent catheterization in the reduction of the number of CAUTIs compared to indwelling catheters.

Closed-System Foley Catheters and Proper Use by Health Care Professionals

Since its inception, there have been relatively few changes in the original design of the standard Foley indwelling catheter. However, minor modifications to this system, the size of catheter used, and proper catheter care play crucial roles in reducing the risk of CAUTIs. The introduction of the closed catheter system, one in which the collection tube is fused to the drainage bag, was critical for the reduction of CAUTIs, and variations to this drainage system have been attempted to further decrease the rate of infection. Some of these changes include the addition of a urine-sampling port in the drainage tubing and preconnected catheter/collecting tube systems (109). Infections associated with indwelling urinary catheters have dramatically reduced from 100% to less than 25% for up to 2 week of catheterization due to the usage of closed drainage systems versus ones that were open (203).

Choosing the correct catheter size assists in decreasing the risk of developing a CAUTI. It has been suggested that smaller catheters (14 French or 16 French) and 5-ml balloons should be utilized (65), as larger catheters have been shown to be a risk factor for the development of UTI (455). These larger catheters tend to increase the amount of residual urine (91) that can lead to the reinoculation of the bladder (93) and increase the risk of blockage of the periurethral glands that leads to UTI, urethral irritation, and erosion (91). Long-term catheterization using a 30-ml balloon has been associated with urinary leakage, bladder wall irritation, and bladder spasms. However, larger catheters have been shown to be of benefit for short-term use after genitourinary surgery to decrease bleeding (409).

Proper aseptic instillation of the catheter by hospital and institutional personnel is critical for the reduction of CAUTIs as well as the avoidance of collection bag contamination upon emptying (17). Cross-contamination is responsible for many outbreaks of nosocomial UTI, as patients with asymptomatic UTI and hospital personnel can become reservoirs of infections (455). The separation of catheterized patients (234) and increased handwashing have been recommended to reduce cross-contamination. Routine bacterial monitoring has been suggested as a control measure to reduce the number of carriers and subsequently cross-contamination (203).

Prevention of Bacterial Colonization of the Urinary Meatus and Urinary Tract

Studies have been performed to determine if the prevention of bacteria colonized on the meatus from entering the bladder is a plausible method to reduce instances of CAUTIs. Although studies have shown that catheterized patients are at a higher risk for infection if their meatus is colonized with various microorganisms (107, 108), two prospective studies concluded that the current method of meatus care (either twice-a-day cleansing with povidone-iodine solution followed by

povidone-iodine ointment or daily cleansing with soap and water) was ineffective in reducing CAUTIs in patients with closed drainage systems (37, 43). Once organisms have entered the urinary tract, methods to eradicate these organisms prior to replication have been implemented. These methods include irrigation of the bladder and the use of prophylactic systemic antibiotics. Continuous irrigation of the bladder with nonabsorbable antibiotics such as neomycin-polymyxin was shown to be ineffective in reducing the rate of CAUTIs and was in fact associated with frequent disruptions of the closed drainage system (443). Although treatment with prophylactic antibiotics does delay infection (38, 109), this method is not recommended since the effect is only temporary, with a tendency to be selective for those strains that are antibiotic resistant.

Novel Surfaces

Once a uropathogen gains access to the urinary tract, adhesion to the catheter material and the uroepithelium is imperative to the initial establishment of biofilms in CAUTIs. Bacterial attachment to catheter surfaces is dependent upon the hydrophobicity of the organisms and the biocompatible surface of the catheter. Following urine application in the catheter lumen, a conditioning layer is formed from the deposition of proteins, minerals, polysaccharides, and other host-derived factors in the urine (80). This also occurs on the outside surface of the catheter due to the interaction with the host urogenital surfaces. The resulting attachment of host factors provides binding sites for uropathogens. Therefore, the development of surfaces that are resistant to organism colonization is important for the prevention of CAUTI and is ongoing. Although several control measures for the prevention of biofilm formation on catheters have been examined, no single biosurface, including silicone, polyurethane, composite biomaterials, or hydrogel-coated materials has been shown to be effective in preventing colonization due to the development of the conditioning layer (80, 236, 417) (Fig. 5).

Catheters Containing Antimicrobial Agents

Preventive measures that have been examined but that have not yielded any proven benefit in the prevention of bacteriuria in catheterized patients include the application of antimicrobial solutions and lubricants on the catheter surface prior to catheter insertion and the addition of antimicrobial agents in the collection bag (17). Conversely, in separate prospective, randomized trials, antimicrobial-impregnated catheters containing either nitrofurazone (235) or the combination of the broad-spectrum antibiotics minocycline and rifampin (72) demonstrated significant reductions in bacterial CAUTIs. However, these trials were limited, and the emergence of resistant strains was not resolved (236).

Besides antibiotics, impregnation of catheter material with antiseptics, such as silver compounds, has been studied as a possible preventive measure, with conflicting results on the efficacy of these silver-coated urinary catheters in the prevention of bacteriuria. Clinical trials have shown conflicting results as to the efficacy of silver oxide-coated catheters compared with uncoated catheters. In a prospective clinical trial involving 482 acutely hospitalized patients, silver oxide-coated catheters

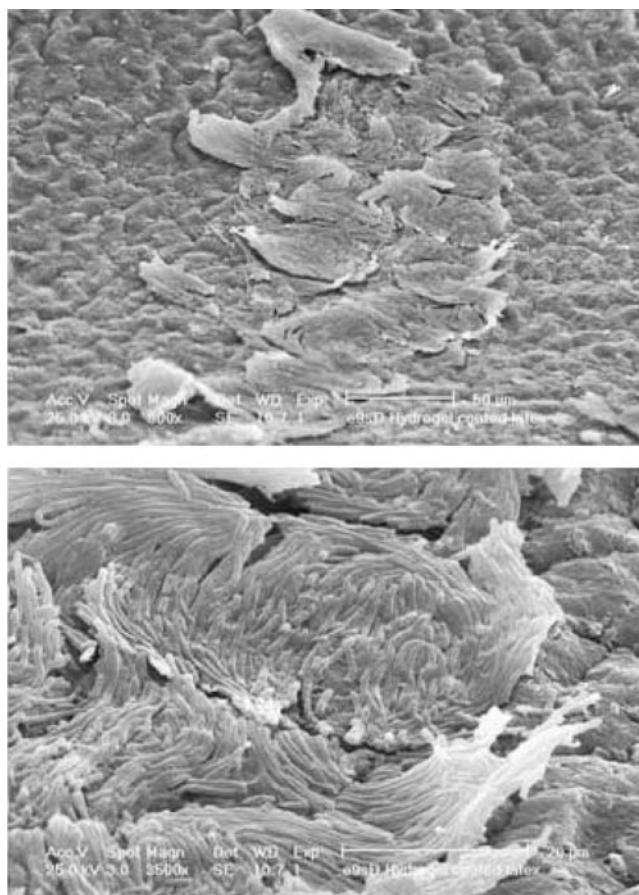


FIG. 5. Scanning electron micrographs of a section of a hydrogel-coated latex catheter over which swarmer cells of *P. mirabilis* are migrating. The sections have been removed from the laboratory model described by Sabbuba et al. (337). Multicellular rafts of typical swarmer cells are visible on the irregular surface of the catheter, migrating from left to right. The micrographs were kindly provided by Rob Broomfield of Cardiff School of Biosciences, Cardiff University.

reduced the incidence of UTI only among women not receiving antimicrobial agents (19% for control catheter versus 0 for silver catheter) ($P = 0.04$) compared with a control silicone catheter (165). A randomized study of 1,309 patients catheterized longer than 24 h failed to demonstrate the effectiveness of a silicone catheter coated externally with 5% silver oxide compared to a standard silicone elastomer-coated latex catheter. However, these silver oxide catheters did show a significantly increased incidence of bacteriuria in male patients and a significantly increased occurrence of staphylococcal bacteriuria (325).

Similarly, there are conflicting studies regarding the efficacy of silver hydrogel urinary catheters in the prevention of nosocomial UTIs (206, 404). Adherence of several strains that cause CAUTIs (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *E. faecalis*, and *K. pneumoniae*) was reduced on silver hydrogel-treated silicone or latex catheters compared with latex or silicone catheters, as shown by radiolabeled-cell assays (104) (Fig. 6).

The silver catheter that is being increasingly used is a hydrogel silver alloy latex-based device. This catheter has metallic silver in a gold and platinum coating linked to a latex base

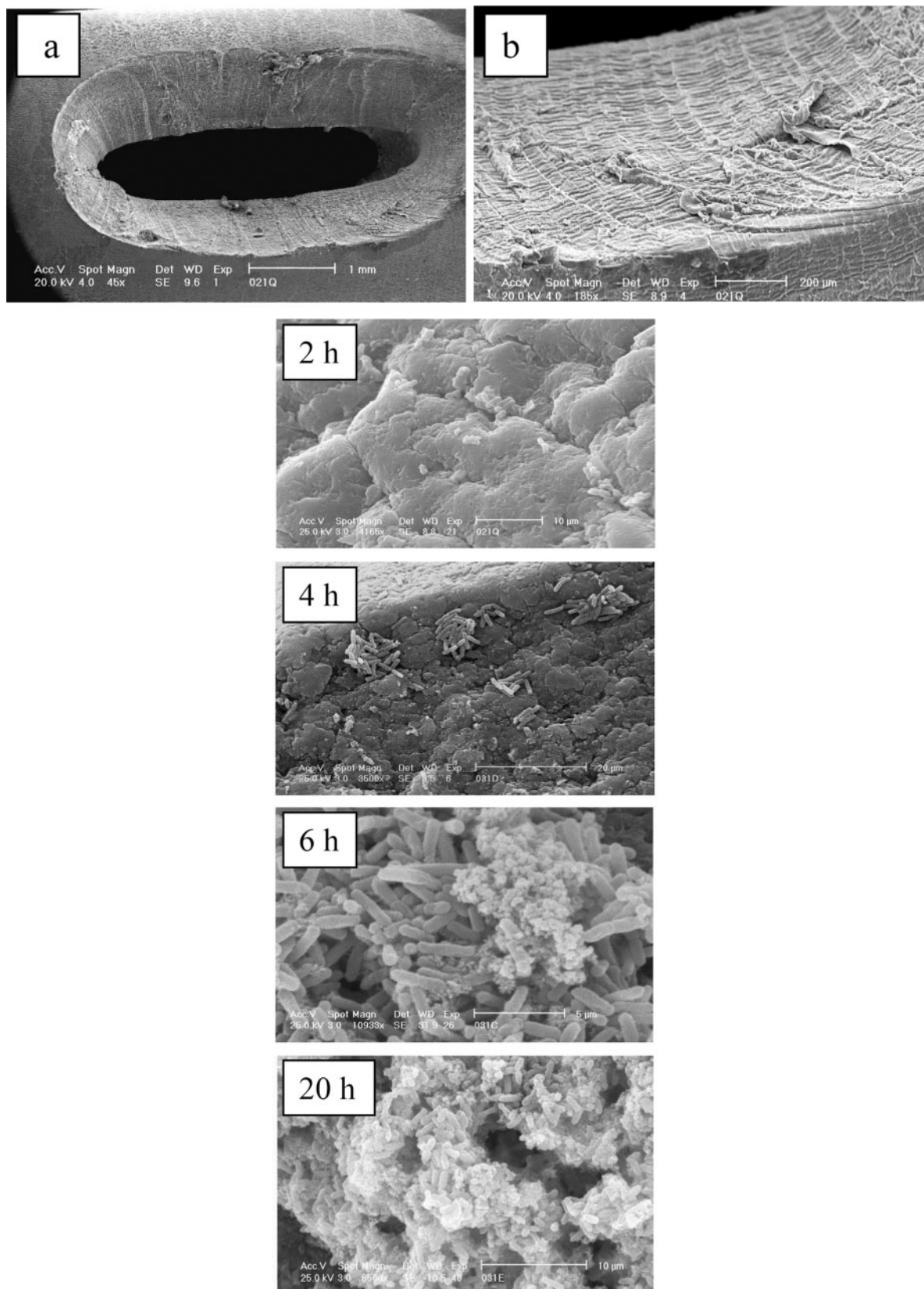


FIG. 6. Early stages in the formation of a crystalline *P. mirabilis* biofilm on a hydrogel-coated latex catheter. Catheters were removed for examination by scanning electron microscopy after incubation for various times in a laboratory model of the catheterized bladder. The irregular nature of the surface of the eyelet is shown in a and b. After 2 h, in the model, cells can be seen trapped in crevices. At 4 h, microcolonies have formed in surface depressions. At 6 h, microcrystalline material accumulated in the developing biofilm as the pH of the urine rose. At 20 h, extensive crystalline biofilm formed at the eyehole. (Reproduced from reference 386 with kind permission from Springer Science and Business Media.)

on both the external and internal surfaces. Silver ions are apparently released into the surrounding fluids to exert their antibacterial activity. The outer hydrogel layer gives the catheter its lubricity (255). Despite the fact that this catheter has undergone extensive clinical trials, it is still the subject of considerable controversy. Perhaps the most impressive evidence of its efficacy comes from a study reported by Karchmer et al. (182), who compared the infection rates in hospitalized patients given the hydrogel/silver alloy-coated or silicone-coated latex catheters. In this trial, wards rather than patients were randomized into two groups. During the first 6 months of the study, group 1 wards were supplied with the silver-coated catheters, and group 2 wards were supplied with the silicone-coated catheters. After 1 month, "washout-period" group 1 wards received the silicone-coated catheters, and group 2 received the silver catheters. Surveillance for nosocomial infections was performed by infection control practitioners using CDC criteria (110). These definitions register both symptomatic UTI and asymptomatic bacteriuria. Analysis of the data showed that 154 infections were recorded among the 13,945 (1.1%) patients fitted with the silver catheter, compared to 189 of the 13,933 (1.36%) patients who had the silicone-coated catheter ($P = 0.07$). When the data were expressed as infection rates per patient days, infections on the wards using silver catheters were calculated to be 2.66 infections per 1,000 patients days, compared to 3.35 per 1,000 days in the control wards ($P = 0.04$). Secondary bloodstream infections complicated the care of 14 of the 343 patients with UTI. The rate of these infections (0.04%) in patients on units supplied with the silver catheters was not significantly different ($P = 0.42$) from that (0.07%) when the patients were receiving the silicone-coated catheters. Several other studies reported no significant reductions in infection rates with this catheter. A meta-analysis of trials involving a total of 2,355 patients determined that silver alloy catheters (odds ratio, 0.24; 95% confidence interval, 0.11 to 0.52) were significantly more protective against bacteriuria than silver oxide catheters (odds ratio, 0.79; 95% confidence interval, 0.56 to 1.10) (363). This conclusion was reached even though those authors referred to the difficulties in comparing efficacies from studies that used different criteria of infection (usually bacteriuria at levels ranging from 10^2 to 10^5 CFU/ml). They also recognized that methodologically poor studies showed a benefit and that the more rigorous studies did not. Niel-Weise et al. (283) were critical of that review, expressing the opinion that meta-analysis should be based on clinical studies of high methodological quality. They concluded from their own review of the literature that because of the fundamental problems with many of the clinical trials, there was insufficient evidence to recommend the use of the silver alloy catheters.

The subsequent literature on the silver alloy latex catheter has failed to resolve the controversy over its efficacy. For example, Rupp et al. (333) based their conclusion that the silver catheter reduces catheter-associated UTI on a study that used historical control data to assess its effect. They reported that over a 2-year period during which the silver catheter was used, the incidence of catheter-associated UTI was 2.6/1,000 catheter days, compared to the rate in the previous 2 years of 6.13/1,000 catheter days ($P = 0.002$). Their definition of infection included both symptomatic UTI and asymptomatic bacte-

riuria, with 43% of the infections recorded as being asymptomatic. Although the trial was not properly controlled, they calculated that the annual savings that might ensue from the use of the silver catheter would be from \$5,811 to \$535,452. Gentry and Cope (111) also used a definition of infection that included bacteriuria and symptomatic infection. They reported a CAUTI rate of 5.1% when the silver catheters were used, compared to a rate of 7.7% in a baseline period. However, analysis of their raw data suggests that the rate in the baseline period was in fact 7.3%. There are, of course, fundamental problems with this type of uncontrolled study that make it difficult to accept the conclusions that the use of the silver catheter produced a 33.5% (or even 30%) drop in infections. In both periods, there were only four cases of CAUTI recorded. No statistical analysis of the data was performed to test whether these rates were in fact significantly different.

The data available from the clinical trials on antimicrobial catheters have convinced some authors that coating with silver or impregnating with nitrofurazone has "engineered out" the risk of infection in catheterized patients (73, 223). Others are more skeptical, as a recent comprehensive review by Brosnahan et al. (39), for example, concluded that while data from clinical trials on hospitalized adults undergoing short-term catheterization suggest that the use of silver alloy catheters might reduce the risk of infection, the evidence is not strong, and the trials are generally of poor quality. Trautner et al. (412) pointed out that there is no evidence that antimicrobial catheters prevent bacteriuria in patients undergoing long-term catheterization (>28 days). They also expressed the view that clinical trials using bacteriuria (at whatever level) as the criterion for infection are fundamentally flawed. They argued that it is important to appreciate the distinction between UTI and asymptomatic bacteriuria in order to attempt a rational assessment of the evidence in the literature concerning the prevention of catheter-associated UTI. Most cases of catheter-associated bacteriuria did not progress to symptomatic UTI. It would clearly be more impressive if clinical trials showed that the various antimicrobial catheters could significantly reduce the incidence of pyelonephritis or bloodstream infections. Johnson et al. (164) concluded that while both nitrofurazone and silver alloy-coated catheters seem to reduce the development of asymptomatic bacteriuria in comparison to latex or silicone control catheters, during short-term use, there is little or no data on the effect of these devices on symptomatic UTI, morbidity, secondary bloodstream infection, or mortality rates. They expressed the view that the lack of data on these clinically more meaningful end points means that it is difficult to make definitive recommendations to decision makers. The clinical benefit and cost savings have yet to be demonstrated directly in a randomized, properly controlled trial with any of these devices in any patient population.

It is surprising, given the clinical significance of catheter encrustation and blockage, that clinical trials to test the ability of antimicrobial catheters to prevent this complication have not been reported in the literature. Laboratory studies in models of the catheterized bladder have shown that both the silver alloy-coated catheter and the nitrofurazone catheters are rapidly blocked by crystalline *P. mirabilis* biofilm (267, 268). It is not difficult to explain these observations, as the amounts of silver that diffuse into the urine from the catheter are not

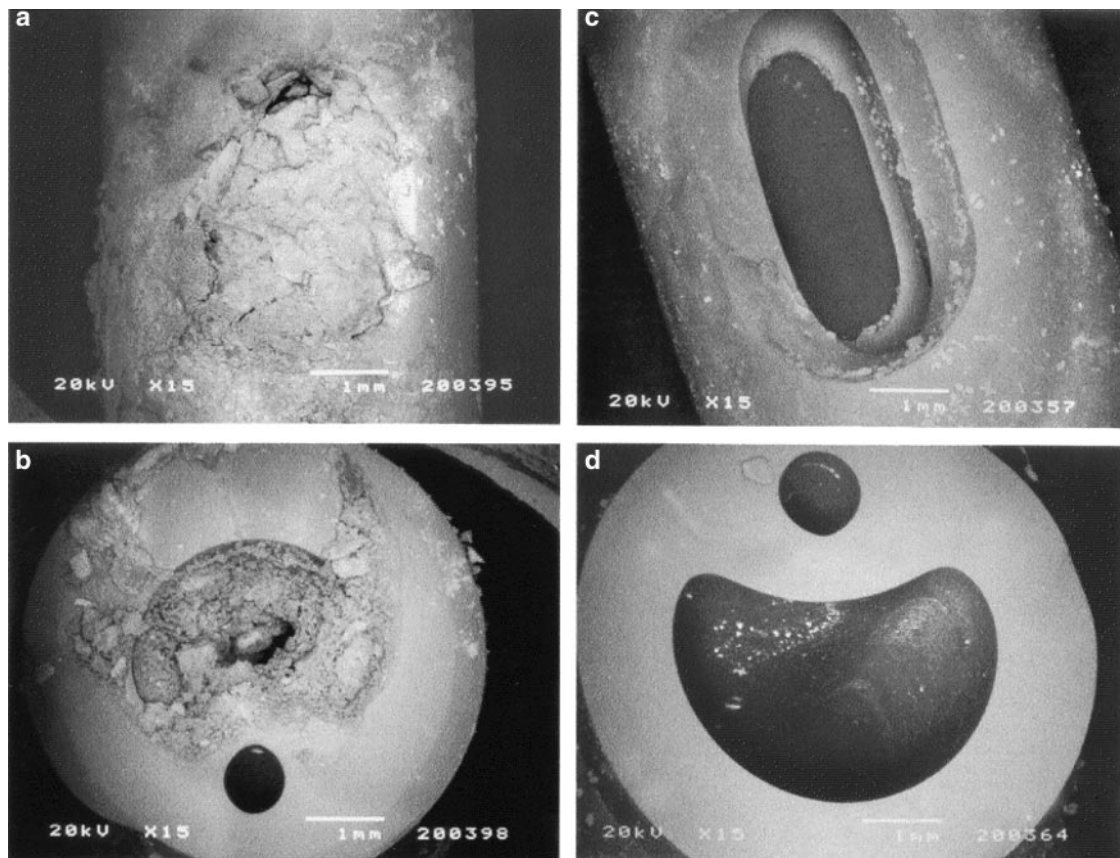


FIG. 7. Scanning electron micrographs showing the extent of encrustation at the eyeholes and in the central channels of control (a and b) and triclosan-treated (c and d) silicone catheters. The control catheter was removed from the *P. mirabilis*-infected bladder model when it was blocked at 30 h. The triclosan-treated catheter drained freely for the experimental period and was removed from the model at 7 days. (Reprinted from reference 388 with permission from Elsevier.)

sufficient to control the rise in pH, and nitrofurazone has limited activity against *P. mirabilis* (162).

The application of electric current to catheters fitted with silver electrodes releases ions into urine that inhibit bacterial growth and was shown to significantly reduce the rate of *P. mirabilis* catheter encrustation (51). This principle may have applications to prevent encrustation during long-term catheterization (51).

Besides silver, other antiseptics have been examined for their efficacy against bacterial colonization. Catheters impregnated with synergistic combinations of chlorhexidine, silver sulfadiazine, and triclosan prevented the adherence of a broad spectrum of extraluminal bacteria on their outer surfaces compared to silver hydrogel latex- and nitrofurazone-treated silicone catheters in an in vitro urinary tract model (105).

Catheter encrustation is a problem of long-term bladder management, and to prevent its development, it is necessary that any antibacterial agent coated onto or incorporated into catheters should diffuse into the urine and prevent the rise in urinary pH and the crystallization of the calcium and magnesium phosphates. Maintaining the release of effective concentrations of antibacterials for the lifetime of long-term catheters is a challenge. Bibby et al. (28) suggested that the catheter balloon could be used as a reservoir for substantial quantities of antibacterial chemicals and that the membrane of the bal-

loon might ensure its controlled release into the residual urine over extended periods. They found that mandelic acid diffused through the catheter balloon, achieving concentrations of around 0.1 mg/ml in urine. Unfortunately, mandelic acid is not very active against *P. mirabilis* or other urinary pathogens, being bactericidal in urine at concentrations of around 5 mg/ml (328). The biocide triclosan, however, is extremely active against *P. mirabilis*. The MIC of this agent for *P. mirabilis* isolates from encrusted catheters was found to be 0.2 μ g/ml (173). In experiments in laboratory models supplied with artificial urine and infected with *P. mirabilis*, triclosan was shown to diffuse through the balloons of all-silicone catheters into the residual urine. The rise in urinary pH and crystalline biofilm formation on the catheters was inhibited. Catheters with their retention balloons inflated with water were blocked within 24 h, while catheters inflated with triclosan (10 mg/ml in 5% [wt/vol] polyethylene glycol) drained freely and showed minimal encrustation at the end of a 7-day experimental period (Fig. 7) (388). The strategy was also effective when artificial urine was replaced with pooled human urine and when latex-based catheters were tested. Triclosan was found to have impregnated the all-silicone catheters but not the latex-based devices (173). These experiments were all performed under conditions in which a catheter is introduced into residual urine that is heavily colonized by *P. mirabilis* (10^8 CFU/ml) and has

a pH of above 8.0. A low flow rate (0.5 ml/min) of concentrated urine was used to simulate the low fluid intake characteristic of many elderly patients undergoing long-term catheterization. As the triclosan strategy inhibits encrustation under these severe experimental conditions, it may well extend the lifespan of catheters in patients infected with *P. mirabilis*.

Probiotics

Bacterial interference or the instillation of nonpathogenic strains into the bladder has the potential for the prevention of symptomatic infection through the hindrance of uropathogen colonization (319). In vitro (411, 414) and in vivo (70) studies have shown that nonpathogenic strains of *E. coli* 83972 reduce the colonization of catheters by a variety of uropathogens. In one study, 21 patients inoculated with *E. coli* 83972 experienced no symptoms of bacteriuria, while this same patient group experienced a mean of 3.1 symptomatic UTIs per year prior to colonization (147). On this same line of investigation, Trautner et al. (413) colonized a urinary catheter surface with a colicin-producing avirulent strain of *E. coli* that subsequently prevented the colonization of a uropathogenic clinical isolate of *E. coli* in vitro.

Future Promising Technologies

Some approaches that offer promise in the reduction of bacterial adherence to and biofilm formation in the catheter and the urinary tract include the sensing of bacterial encrustation, utilization of biofilm inhibitors on catheter surfaces, development of hydrophilic and nutrient-scavenging biomaterials, and use of low-energy surface acoustic waves. A simple sensor has been developed to detect early stages of urinary catheter encrustation and to avoid the clinical crises induced by catheter blockage. In laboratory models of colonization by *P. mirabilis*, the sensor signaled encrustation at an average time of 43 h before catheters were blocked with crystalline biofilm (389). A subsequent clinical study confirmed that sensors located in urine collection bags could detect infection by *P. mirabilis* and the early stages of catheter encrustation in patients. The mean time interval between the sensor giving the signal and the catheter being blocked was 12 days, thus allowing catheter replacement in ample time to avoid emergency referrals caused by catheter blockage (390).

Identifying the bacterial factors involved in biofilm formation on catheters and developing and/or determining inhibitors of these factors will be critical in preventing bacteriuria. One such factor is the protein GlmU (*N*-acetyl-D-glucosamine-1-phosphate acetyltransferase). This protein is involved in the biosynthesis of the activated nucleotide sugar UDP-GlcNAc, which is an essential precursor of peptidoglycans and LPSs in gram-positive and gram-negative bacteria, respectively (315). It also has a role in the synthesis of the β -1,6-*N*-acetyl-D-glucosamine polysaccharide adhesin required for biofilm formation in *E. coli* and *Staphylococcus epidermidis* (151). Therefore, inhibitors of GlmU in conjunction with protamine sulfate show promise for use in anti-infective coatings for urinary catheters (44). These compounds have antibiofilm activity against clinical isolates such as *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. epidermidis*, and *E. faecalis*, and *N,N'*-(1,2-

phenylene)dimalleimide-plus-protamine sulfate-coated silicone catheters have a reduced bacterial colonization rate for *P. aeruginosa* and *S. epidermidis* than catheters coated with silver hydrogel in vitro (44).

Signal molecules of quorum sensing that are critical for biofilm formation for their role in coordinating population behavior during host invasion and colonization are produced by uropathogens (393). Therefore, determining factors that inhibit quorum sensing may be important for the inhibition of biofilm formation and subsequent bacteriuria. Furanones, compounds isolated from a marine red macroalga, *Delisea pulchra*, that interfere with quorum sensing (184), are of limited use due to toxicity, and studies examining the efficacy of these compounds for clinical use were found to be variable (410). Therefore, other less toxic alternatives need to be discovered.

The development of new biomaterials for the manufacture of catheters that are not suitable for bacterial colonization and are nontoxic to patients is a logical progression in the prevention of bacteriuria during catheterization. The use of LoFric catheters (catheters with a hydrophilic coating such as polyvinyl pyrrolidone and salt) was associated with less hematuria and a significant decrease in the incidence of UTIs (424). This is believed to be due to the difference in water binding ability to reduce friction and urethral trauma (231), and, as such, these catheters were preferred by patients for intermittent self-catheterization due to higher comfort levels (78).

A promising method of bacteriuria prevention is the development of biomaterials that scavenge essential nutrients such as iron. Preliminary studies showed that catecholamine inotropes encourage biofilm formation by *S. epidermidis* by transferring iron to the bacteria from the host iron binding protein transferrin (232). The addition of exogenous lactoferrin to *P. aeruginosa* prevents biofilm formation through the stimulation of twitching motility (373). However, catheters composed of biomaterials that scavenge iron have yet to be developed for use in clinical trials (385).

Recently, Hazan et al. (136) examined the effect of low-energy surface acoustic waves on biofilm formation. It was revealed that Foley urinary catheters attached with elastic wave-generating actuators inserted into the urinary tracts of male rabbits maintained urine sterility for up to 9 days, compared to 2 days in control catheterized animals (136). Those findings were substantiated by the presence of diminished biofilm development on these catheters, as determined by scanning electron microscopy and bioburden analyses (136).

DIAGNOSIS AND TREATMENT OF CAUTIs

Even with appropriate preventive measures in place, catheterized patients, and in particular those with long-term catheterization, will experience bacteriuria due to cross-contamination. As mentioned above, most cases (90%) of bacteriuria during catheterization are asymptomatic (401). However, treatment of asymptomatic bacteriuria remains controversial among physicians, with striking a balance between the prevention of UTI-related morbidity and the emergence of antimicrobial resistance. Treatment of catheter-associated bacteriuria is not recommended, since asymptomatic bacteriuria is inevitable in catheterized patients, and in healthy individuals,

these infections clear up spontaneously after catheter removal. Also, antibiotic therapy has little benefit for long-term catheterization (434). Because bacteriuria is a common occurrence, the treatment of catheterized patients may lead to the emergence and selection of antibiotic-resistant strains and increase the risk for the development of multiresistant nosocomial strains. Most physicians believe that asymptomatic bacteriuria should be not treated unless the patient is either immunosuppressed (e.g., after organ transplantation), at risk for bacterial endocarditis, about to undergo urinary tract instrumentation, or pregnant (438). However, the presence of bacteriuria serves as a bacterial reservoir for the potential progression of these organisms into symptomatic infection such as cystitis. They may also subsequently lead to significant sequelae such as pyelonephritis, bacteremia, and, possibly, mortality. Therefore, this condition is a major health risk factor for those individuals who are utilizing indwelling urinary catheters.

The CDC definition of a nosocomial UTI (110) requires a symptomatic infection with the presence of fever, urgency, increased frequency of urination, dysuria, and a urine culture of $>10^5$ CFU/ml (40) with no more than two species of organisms (133). However, CAUTIs may represent a more unique diagnostic situation since the typical symptoms and signs of infection (bacteriuria [401], pyuria [69], and suprapubic pain) are unreliable due to the presence of the catheter. The presence of bacteriuria cannot be used as the sole reliable diagnostic indicator of symptomatic infection, and as mentioned above, most cases of bacteriuria are asymptomatic (401) and spontaneously resolve after catheter removal.

As with bacteriuria, pyuria (the presence of white blood cells [WBCs] in urine) is utilized as an indicator of infection. However, Tambyah and Maki determined that there is a poor correlation of pyuria with CAUTIs (402). WBC counts for catheterized patients are apparently skewed since pyuria can occur in the absence of infection due to irritation of the host uroepithelium caused by the catheter (402). Also, urease-producing uropathogens such as *Proteus* species often convert normally acidic urine into an alkaline state that can lyse WBCs, thereby reducing the WBC count during an infection (402). Suprapubic pain cannot be used as an accurate indicator of infection, as catheters themselves can cause this type of pain. Therefore, catheterized patients must exhibit other symptoms and signs prior to being diagnosed with and treated for a CAUTI.

We suggest that for preliminary diagnosis, once the patient exhibits the symptoms described by the CDC mentioned above, urinalysis and urine culture should be performed using a urine sample collected from a newly inserted sterile catheter (71) or suprapubic catheter. These steps reduce the incorrect identification of the causative agents responsible for the infection so that an appropriate treatment can be administered.

Urinalysis examines the physical, chemical, and microscopic properties of urine. In a standard urinalysis, the odor, color, and clarity of urine are first evaluated for the possible presence of urine with a strong odor, hematuria (red urine), pyuria (cloudy urine), or phosphate crystal deposits (cloudy urine) (372). After a quick visual examination, chemical analysis of the urine is conducted by dipstick urinalysis. These dry reagent sticks measure a variety of characteristics of urine such as specific gravity, urinary pH, and the presence of nitrites, leu-

kocyte esterase, protein, and peroxidase. The detection of the protein leukocyte esterase in urine is indicative of the presence of neutrophils and thus pyuria (372). The detection of nitrites in urine suggests the presence of bacterial infection since many gram-negative and some gram-positive bacteria are capable of reducing nitrates to nitrite (372). However, non-nitrate-reducing organisms such as enterococci, *Staphylococcus saprophyticus*, and *Acinetobacter* spp. will appear as false negatives. Normally, urine is slightly acidic but can range from pH 4.5 to 8 (372). The presence of alkaline urine is suggestive of an infection with a urease-producing organism such as *Proteus* spp. The detection of peroxidase in urine can indicate hematuria, myoglobinuria, and hemoglobinuria (372).

If the dipstick analysis results suggest that an infection is present, the presence of bacteriuria can be established using several methods that determine bacterial cell count. In one method, the patient's wet-mount spun urine sample is examined microscopically for the presence of crystals, urinary casts, epithelial cells, blood cells, and bacteria. The quantitative loop method determines bacterial number by inoculating plates using calibrated platinum loops and counting colonies directly (293). Several automated methods of ascertaining bacterial counts are currently available, including staining bacterial cells with safranin (Bac-T-Screen) or acridine orange (Autotrak) and measuring the amount of bacterial ATP by bioluminescence (Monolight and LUMAC systems) (86, 375, 458).

Once these initial tests are completed, the presence of a CAUTI is confirmed by the determination of bacterial cell and blood cell counts. Two urine cultures with repeated isolation of the same uropathogen with >100 colonies (100 CFU per ml of urine) or the isolation of a single uropathogen with $<10^5$ colonies per ml for a patient being treated with antimicrobial agents is indicative of these types of infections (110). In conjunction with bacterial cell counts, a urine culture containing an abnormal number of red blood cells (hematuria, >5 red blood cells per high-power field) and leukocytes (pyuria, >10 WBCs per ml^3) signifies the presence of CAUTIs (452).

Once a catheterized patient is believed to have a symptomatic UTI, the catheter is removed if possible due to the high rate of relapse (383), and empirical therapy is administered. Specific effective treatment of symptomatic UTI is a challenge due to the often polymicrobial nature of CAUTIs in patients catheterized long term (438), antibiotic resistance, and biofilm formation (62). Therefore, empirical therapy consists of broad-spectrum antibiotics and is dependent upon the condition of the patient and the site of infection as well as previous culture and drug sensitivity determinations at individual institutions (179). After the administration of empirical therapy, drug sensitivities of suspected uropathogens are determined to tailor the antimicrobial regimen to a narrow-spectrum, more potent drug (446). Most patients are administered antimicrobial therapy for at least 10 to 14 days. However, parenteral therapy is usually initiated in severely ill patients and then switched to an oral treatment after improvement (383). Parenteral antibiotic therapy is utilized during febrile infections or for those patients unable to tolerate oral medications during the course of treatment and lasts for 14 to 21 days (438). Parenteral antibiotics that are used to treat CAUTIs include certain broad-spectrum and recently developed cephalosporins (ceftriaxone), ticarcillin-clavulanate, and piperacillin-tazobactam (434). If a patient

either has been on short-term catheterization, has not been critically ill, or is believed not to have a polymicrobial infection, treatment consists of either trimethoprim-sulfamethoxazole (TMP-SMX) (Bactrim, Cotrim, Septra), fluoroquinolones, or nitrofurantoin (Furadantin or Macrobid) (65). TMP-SMX, taken orally or parenterally, disrupts folate metabolism and is effective against most uropathogens other than *E. coli* except for *Pseudomonas* and *Enterococcus* spp. (374). Fluoroquinolones inhibit DNA gyrase (145), reduce biofilm formation (320, 463), and can reach high urinary concentrations. These drugs are effective in treating *Pseudomonas*, *Proteus*, and other resistant gram-negative organisms (143, 374). The UTI-specific antibiotic nitrofurantoin is effective against most uropathogens except *Pseudomonas* and *Proteus* spp. (149).

Unlike the drug therapies administered for patients catheterized short term, treatment for those individuals who are undergoing long-term catheterization differs, as these infections are more likely to be polymicrobial in nature. Those individuals undergoing long-term catheterization or suffering from polymicrobial infections and who are noncritical are treated with TMP-SMX or an expanded-spectrum cephalosporin such as cefuroxime (Ceftin) (65, 383). Catheterized patients that are seriously ill require a two-drug regimen that includes ampicillin with either the monobactam aztreonam (Azactam), the expanded-spectrum cephalosporin cefprozil (Cefzil), the broad-spectrum cephalosporin ceftriaxone (Rocephin), gentamicin or another aminoglycoside or ciprofloxacin (Cipro), or other fluoroquinolones (65, 464). Once antibiotic therapy has been administered, the resolution of symptoms and not the absence of bacteriuria indicates that the infection has been cleared.

If a patient does not respond to treatment and/or is suspected of having complicated or recurrent pyelonephritis or a blockage due to urinary stone formation, the patient will undergo diagnostic imaging. During an emergency, an ultrasound can be used for patients with loin pain and fever (100) for a risk-free assessment of the presence of abscesses, stones, and urine flow blockage. Nuclear scans, employing injections of small amounts of radioactive tracers, are used to detect kidney scarring, including dimerceptosuccinic acid scintigraphy. To rule out kidney stones or obstructions, magnetic resonance imaging and computed tomography are performed. X rays, in conjunction with contrast agents, can be used to examine different parts of the urinary tract to determine the existence of structural abnormalities, urethral narrowing, and incomplete emptying. Two examples are an intravenous pyelogram (an X ray for kidney obstructions or abnormalities) and a voiding cystourethrogram (an X ray of the bladder and urethra).

Lastly, cystoscopy can be used to detect structural abnormalities and interstitial cystitis in those individuals with recurrent UTI. A study using cystoscopy has indicated that 38 of 61 (62%) patients suffering from recurrent encrustation and blockage of their catheters had bladder stones (338). It was revealed that pairs of *P. mirabilis* isolates from the stones and catheter biofilms of six of these patients were identical, as determined by restriction enzyme digest profiles of bacterial DNA (338). This finding indicates that bladder stones harbor strains of *P. mirabilis* that can rapidly recolonize replacement catheters with crystalline biofilm. It is suggested that the detection and removal of these stones for those patients diag-

nosed with CAUTIs could be accomplished using flexible cystoscopy to assist in resolving the problem of chronic catheter encrustation and blockage.

The formation of crystalline urinary stones is a serious complication associated with CAUTIs, and as such, the removal of these stones is critical for full recovery from catheter-associated infection, since any remaining stone fragments can serve as a nidus for infections and the formation of new stones (253). Multimodality therapy is the key to a more successful removal of struvite stones, and the combination of open surgery and medical therapy is synergistic (436). Open surgery, while recommended for the complete removal of urinary stones, does not ensure complete removal, as the incidence of residual stone fragments is 12 to 36% (226). However, surgical removal of residual stones has been demonstrated to be important, as 41% of patients with untreated unilateral struvite stones have a 5-year survival rate (314).

The minimally invasive percutaneous nephrolithotomy, with or without extracorporeal shockwave lithotripsy (ESWL), is the preferred nonsurgical alternative (254, 436), with stone-free rates that range from 23 to 86% (207). Percutaneous nephrolithotomy, performed under general anesthesia, involves an incision into the back and the insertion of an endoscope to remove stones from the kidney. Monotherapy with ESWL should be used for those patients with smaller staghorn stones (<500-mm² surface area) (357). ESWL effectively fragments urinary crystals through the utilization of sound waves to allow passage of these fragments out of the urinary tract. After either procedure, the insertion of an indwelling ureteral stent may be required to allow the proper drainage of urine. Medical therapy, such as culture-specific antibiotics, urease inhibitors, and urine acidification, complements these above-described treatments to reduce the morbidity associated with persistent UTIs and stone recurrence (436). Acetohydroxamic acid (Lithostat) is the most commonly used urease inhibitor. Chemolysis by urinary acidification is possible by the administration of oral ammonium chloride, glucono-delta-lactone, and magnesium salt carbonate or a citric acid-based solution such as hemiacidrin or Suby solution G (397). However, there are conflicting reports about the efficacy of citric acid for use in chemolysis (138, 247). Once administration of the appropriate treatment is accomplished, therapeutic success for urinary stones is measured by the alleviation of patient symptoms and stone-associated morbidity, such as infection.

PROGNOSIS OF CAUTIs

The prognosis of catheter-associated infection is complicated due to the occurrence of chronic or recurrent UTIs, complicated UTI, and pyelonephritis. If left untreated, these infections can lead to abscess formation, renal obstruction, and scarring and eventually will lead to bacteremia, sepsis, and, possibly, death. These infections are difficult to treat due to the presence of biofilms and crystals that protect uropathogens from proper treatment. Only after the complete eradication of biofilm and crystals in the urinary tract can a catheter-associated infection be eliminated.

To decrease morbidity and mortality associated with infection of urinary catheters, new methods of prevention and treatment need to be continually developed. The development of

affordable catheters constructed with anti-infective surfaces may be key to the success in preventing infection (236). The identification and development of agents that prevent attachment and subsequent biofilm formation are also feasible as preventative measures. Inhibitors of other known bacterial virulence factors such as siderophores and the continued identification of new virulence factors associated with CAUTIs will aid in understanding the pathogenicity as well as identifying new targets for the development of potential preventive and/or treatment options for these infections. The development of catheters coated with antiseptics and new catheter surface technologies that can release greater quantities of ionic silver or other anti-infective agents into the aqueous environment may assist in the prevention of CAUTIs caused by intraluminal organisms (236). Alternative catheterization methods such as conformable catheters with a collapsible intraurethral segment can reduce the amount of urethral tissue trauma. Such catheters have been developed but have not been clinically evaluated in randomized controlled trials and are not commercially available (236). Other preventable measures include the development of vaccines against uropathogens (236), including potential vaccines (198, 418) or vaccine candidates for *E. coli* (181, 209, 210, 336, 352) and *P. mirabilis* (218, 220, 300). The continuing development of technological advancements of minimally invasive instruments, novel urinary acidification agents, less toxic urease inhibitors, and chemicals to enhance the protective glycosaminoglycan layer can lessen the morbidity associated with catheter-associated stone formation. Advancements might also provide potential alternative treatment options for the clearance of urinary stones (317). As the number of individuals at risk for catheter-associated infections continues to rise due to advances in medicine, continued research efforts for better prevention and treatment options to reduce morbidity and mortality associated with these infections will be critical to keep CAUTIs at a minimum.

Some 20 years ago, in an important editorial, Calvin Kunin (204) posed the question, "Can we build a better catheter?" He commented that at a time when impressive technological advances have taken place in many areas of medical care, it is difficult to understand why we are still not able to solve the apparently simple problem of draining urine from a disabled bladder without causing infection and all the associated complications. Building better catheters should certainly be possible, as there is plenty of scope for improving their design, and it is disappointing that little has changed in the meantime. There have been some advances in our understanding of the pathogenesis of CAUTI, including a better appreciation of the central role of bacterial biofilms in these infections. The current level of morbidity associated with the use of catheters is unacceptable, as it undermines the quality of life for so many elderly and disabled people. It is clear that if we are to design effective strategies to avoid these problems, there is a pressing need for more information about the factors that initiate and control the development of catheter biofilms by urinary tract pathogens such as *E. coli* and *P. mirabilis*.

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