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From Catheter to Kidney Stone: The Uropathogenic Lifestyle of *Proteus mirabilis*

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Abstract

Proteus mirabilis is a model organism for urease-producing uropathogens. These diverse bacteria cause infection stones in the urinary tract and form crystalline biofilms on indwelling urinary catheters, frequently leading to poly-microbial infection. Recent work has elucidated how *P. mirabilis* causes all of these disease states. Particularly exciting is the discovery that this bacterium forms large clusters in the bladder lumen that are sites for stone formation. These clusters, and other steps of infection, require two virulence factors in particular: urease and MR/P fimbriae. Highlighting the importance of MR/P fimbriae is the cotranscribed regulator, MrpJ, which globally controls virulence. Overall, *P. mirabilis* exhibits an extraordinary lifestyle, and further probing will answer exciting basic microbiological and clinically relevant questions.

Introduction to *Proteus mirabilis*

P. mirabilis is a gastrointestinal (GI) organism notorious for causing catheter-associated urinary tract infections (CAUTIs, see Glossary) and urinary stones. It is a model organism for urease⁺ bacteria, a phylogenetically diverse group of bacteria encoding the virulence factor urease, that often form resilient crystalline biofilms on catheters and potentially deadly stones in the urinary system. Recent studies have elucidated key steps in *P. mirabilis*'s virulence lifestyle and the intracellular regulatory mechanisms that control these stages of infection. The combination of this new information with previous foundational studies has shed light on how this organism, and perhaps other urease⁺ bacteria, successfully initiates and establishes infection of the urinary system.

This review focuses on the recent advances in the *P. mirabilis* field in the context of CAUTIs and urinary stones. We first briefly describe these two disease states. Next, we summarize the *P. mirabilis* lifecycle from catheter colonization to the development of urinary stones. Then, we describe the role of urease and fimbriae during infection, as recent developments have further characterized the function and/or regulation of these two classes of virulence

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Appendix B Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tim.2016.11.015.

factors [1]. Lastly, we discuss polymicrobial infections as they, until recently, have been the subject of relatively few studies, yet may be important for understanding *P. mirabilis*-associated diseases. We propose that *P. mirabilis* and other urease⁺ bacteria are an understudied group of urinary pathogens, and that continued research into these organisms will provide insight into the treatment of potentially serious or deadly urinary tract diseases.

CAUTIs and Urinary Stones

CAUTIs are a predominant nosocomial infection within the US and are particularly prevalent in patients with long-term indwelling catheters [2,3]. CAUTIs associated with urease⁺ organisms are especially problematic as they can cause urinary stones and crystalline biofilms on catheters, which are notoriously difficult to treat. In recognition of their significant health and economic burden, CAUTIs are one of the top four healthcare-associated infections (HAIs) that the US is actively trying to eliminate, per the Center for Disease Control's (CDC's) National State and Healthcare-Associated Infections progress report.¹ Unfortunately, the incidence of CAUTI has not changed from years 2009 to 2014, unlike the other HAIs on this list, which saw decreases anywhere from 8 to 50%.¹

Patients with an indwelling catheter are more likely to develop urinary stones [4,5]. Most are classified as infection stones, meaning that they have a bacterial etiology and often consist of **struvite** and/or **apatite** minerals [6]. Although the prevalence of infection stones has decreased over the past 30 years, the overall prognosis is still poor: the 20-year mortality rate for infection stones is 28% with nonsurgical management, and 7% with surgical intervention [4]. Obstructive pyelonephritis, or a kidney stone that has blocked the kidney ducts, is one of the more severe complications of urinary stones, requiring immediate treatment to avoid serious complications such as **urosepsis** and death [4,7]. Even with stone removal, residual stone fragments may remain in the urinary system, putting these patients at a 40–85% risk for recurrent stones, versus a 0–10% risk for patients who had complete stone removal [8].

Infection stones, particularly those composed of struvite, and crystalline biofilms are almost always caused by bacteria that produce the urea-hydrolyzing enzyme, urease [3,9]. Uropathogenic genera that encode urease include *Proteus*, *Staphylococcus*, *Providencia*, *Ureaplasma*, and less frequently, *Pseudomonas* and *Klebsiella* [9,10]. Infection stones and CAUTIs are often polymicrobial and may include these species as well as urease-negative bacteria, such as *Escherichia coli*, which potentially further complicates treatment of CAUTI-associated stones [11–15]. *P. mirabilis* in particular is a model organism used to study CAUTIs, infection stones, and now polymicrobial urinary tract infections (UTIs).

The Uropathogenic Lifestyle of *P. mirabilis*: From GI to UTI

Although *P. mirabilis* is an intestinal organism known to externally cross over to the urethra, it does not usually cause UTIs in patients with normal, unobstructed urinary tracts [16]. However, the presence of a chronic, indwelling catheter within the urethra and bladder

¹Appendix A Resources

www.cdc.gov/HAI/pdfs/progress-report/hai-progress-report.pdf.

permits less inhibited ascension of the bacteria up the catheter and into the urinary system (Figure 1) [17–19]. To do this, *P. mirabilis* may employ swarming motility, where the bacteria lengthen, become hyperflagellated, and move as a coordinated community across surfaces [18]. Swarming has been correlated with production of virulence factors, including urease and crystalline biofilm formation (discussed below) [20–22]. However, the contribution of swarming to UTI remains under debate, as swarming-defective mutants can still form crystalline biofilms, and differentiated swarmer cells are rarely observed during UTI [20,23]. Swarming motility will not be further discussed in this review, as it has been extensively covered elsewhere [21,22].

P. mirabilis CAUTIs are exacerbated by the formation of catheter-associated biofilms, or multicellular communities that are surrounded by a matrix of macromolecules such as polysaccharide, protein, and extracellular DNA [3,24]. Although biofilm formation on catheters by bacterial pathogens is a common feature of CAUTI, the potent urease activity of *P. mirabilis* in particular leads to crystalline biofilms, which can block catheter flow and are hypothesized to protect cells within crystalline armor, making them particularly difficult to eradicate [25–27].

Once *P. mirabilis* gains access to the bladder in a mouse model of UTI, it may invade the bladder **urothelium** as early as a half hour after infection (Figure 1) [1]. Some research has begun to characterize this process through the use of various cell lines [28–34]. However, invasion of the bladder epithelium, or urothelium, does not seem to be the dominant lifestyle for *P. mirabilis* in the bladder [1,35]. Instead, *P. mirabilis* forms large intraluminal clusters that can extend the entire length of the bladder. Importantly, these clusters serve as the site of stone formation (Figure 1) [1]. After entering the bladder, *P. mirabilis* can ascend to the kidney, cause kidney stones, and cross into the bloodstream [36–39].

The lifecycle of *P. mirabilis* in the bladder is a complicated, multistep process (Figure 1). In the next section, we focus on two virulence factors, urease and mannose-resistant *Proteus*-like (MR/P) fimbriae, as they exert multiple, key functions during infection, including having a role in the aforementioned bladder clusters. Other known virulence factors required in experimental UTI in mice are listed in Figure 2 and described in detail elsewhere [21,22], but they will not be discussed further in this review.

Urease

Urease is a nickel metalloenzyme that catalyzes the hydrolysis of urea, a highly water-soluble molecule concentrated into the urine of mammals as a mechanism to eliminate excess nitrogen [40]. Specifically in *P. mirabilis*, and less potently in *Providencia* spp., urease is induced by the regulator UreR in the presence of urea (approx. 400 mM in human urine) [41]. Urea hydrolysis via urease results in the production of carbonic acid and two molecules of ammonia, and among uropathogens, the hydrolysis rate of *P. mirabilis* urease is especially rapid. In aqueous solutions at equilibrium, the two ammonia molecules can dramatically increase the net pH (Figure 1). During CAUTIs, the ammonia and carbon dioxide bind with Mg^{2+} and Ca^{2+} found in the urine, respectively, resulting in struvite and carbonate apatite [41]. These minerals precipitate from the urine and form crystalline

deposits on catheters and/or aggregate into stones in an organ within the urinary system [6,42]. This biochemical process is important for *P. mirabilis* to form both crystalline biofilms and urinary stones (Figure 2).

Bacterial catheter colonization can occur *in vitro* as early as 15 min post-inoculation. As *P. mirabilis* propagates, and adherent bacteria grow and divide into microcolonies, the pH rises, leading to the formation of crystals, which can further facilitate bacterial attachment to the catheter [43–45]. In fact, the higher the urease activity, the faster a catheter encrusts *in vitro* [46]. As the layer of crystalline biofilm continues to grow, bacteria and crystals become tightly associated [44]. In a **flow system**, bacteria can subsequently disseminate from microcolonies across the surface of the catheter, where the crystalline biofilm process can begin again [45]. *P. mirabilis* strains with a mutation within the *ure* operon (*ureDABCEFG*) are unable to form crystalline biofilms, thus solidifying the role of this enzyme in this process (Figure 2) [25,44,45].

In addition to catheter encrustation, *P. mirabilis* uses urease to establish both bladder and kidney infection in a mouse model of ascending UTI [37]. Indeed, *ure* gene expression can be seen in both the bladders and kidneys at 7 days post-infection (dpi), and *ure* genes are consistently expressed in voided urine from 1 to 7 days post-infection [47,48]. A *ureC* mutant exhibits bladder colonization defects as early as 24 h post-infection (hpi) and fails to form clusters [1]. Urease mutants have difficulty overcoming this early defect, as they exhibit a lower CFU in both the bladder and kidney, have a less severe pathology in these organs, and fail to form urinary stones anywhere from 2 to 14 dpi [36,37,49].

The requirement for urease at early time points (24 hpi) of bladder colonization suggests that urease may be doing more than just supplying mineral deposits within a cluster. This is in accordance with an *in vitro* analysis of catheter encrustation, which found urease to be important for initiating biofilm formation on catheters seemingly independently of crystal formation [43]. One explanation is that the methods used for these studies were not able to observe microscopic mineral deposits. In contrast, since urease activity produces ammonia, it is possible that *P. mirabilis* uses urea as a nitrogen source for growth; however, various *in vitro* conditions do not expose a growth defect for a urease mutant [1,36,49], urease-encoding genes are not induced during nitrogen starvation [50], and *in vivo* microarray analysis suggests that urease activity is not important for nitrogen uptake and assimilation [48]. Ammonia liberated by urease may also contribute to urothelial damage, potentially exposing niches for adherence or making cellular nutrients available to the bacteria [37]. It is possible that the contribution of urease to CAUTI is multifactorial, and additional functions remain unknown.

Fimbriae

Fimbriae, or pili, are hair-like appendages that coat the exterior of both Gram-negative and Gram-positive bacteria [51]. They can be involved in a number of processes such as biofilm formation, surface attachment, and evasion of the host immune response [52]. One of the five major classes of fimbriae, chaperone-usher, represents the most abundant group of surface appendages on Gram-negative bacteria [53]. These fimbriae are typically composed

of many copies of one major protein subunit that polymerizes into the characteristic linear structure. This assembly is coordinated by a periplasmic chaperone and a membrane-bound usher [54]. Many fimbriae possess an adhesin protein at the tip, which mediates direct contact with a specific surface [54].

The *P. mirabilis* HI4320 type strain genome contains at least 17 different operons that encode chaperone-usher fimbriae, the most within a single strain of any bacterium known to date [55]. In contrast with other fimbria-producing species, most of these 17 operons are conserved among *P. mirabilis* strains isolated from varied human and animal anatomical sites, as well as distinct parts of the world over a 30-year time frame [56]. Presumably, maintaining a large repertoire of attachment mechanisms is advantageous for *P. mirabilis*, perhaps by permitting the bacteria to colonize a variety of environmental niches.

Out of these 17 fimbrial operons, five have been characterized *in vivo*. At least four fimbrial operons are implicated in *P. mirabilis* virulence in mouse models of UTI: MR/P [57–59], uroepithelial cell adhesin (UCA, also known as nonagglutinating fimbria, or NAF) [60], *P. mirabilis* fimbriae (PMF) [61,62], and Fimbria 14 [63]. Analysis of a fifth fimbria, the ambient-temperature fimbria (ATF), suggested that it was not required for infection [64]. Although ATF production increases in an MR/P mutant strain *in vivo*, the significance of this is unknown [35]. Here, we focus on MR/P fimbriae, the best-characterized fimbriae that have a newly elucidated importance for bladder infection.

MR/P Fimbriae

MR/P fimbriae are critical for bladder and kidney infection in mice. Bacteria in the bladder, ureters, and kidneys produce MR/P fimbriae at 24–48 hpi, with the highest percentage found in the bladder [35]. Additionally, *mrp* genes within the *mrp* operon (*mrpABCDEFGHJ*) are the most highly-induced *in vivo* [48], are required at early steps of infection, including cluster formation [1], and are critical for wild-type levels of bladder colonization at later time points [57–59,65]. Together, these data solidify MR/P fimbriae as a key virulence factor for *P. mirabilis* to establish and maintain its hold on a host's urinary system.

The bladder and/or kidney receptors for MR/P are unknown. Some evidence suggests that MR/P may be important for urothelial cell adherence and/or proper localization of *P. mirabilis* in the bladder [35,65,66]. Interestingly, cells constitutively producing MR/P (MR/P 'on') and cells that do not express the *mrp* operon (MR/P 'off') associate with distinct locations in the bladder, although 'on' and 'off' bacteria are recovered in similar numbers [35]. The *P. mirabilis* MR/P 'on' strain and an *E. coli* strain engineered to express *mrp* exhibit enhanced **hemagglutination**, aggregation during growth in culture, and/or biofilm formation [35,59]. These *in vitro* phenotypes are reminiscent of the *in vivo* cluster, and suggest that MR/P may be important for initiating or maintaining cluster formation within the bladder. Therefore, MR/P could be responsible for both direct host binding as well as cluster formation.

Without clusters, mineral deposition does not occur, and therefore MR/P is likely important for stone formation. Indeed, scanning electron microscopy (SEM) studies of MR/P 'on' and 'off' strains in the bladder suggested that the MR/P 'on' strain formed more biofilm-like

clusters [35]. Conversely, mixed populations of MR/P-OFF and MR/P-ON are more likely to occur in mice with bladder stones whereas the majority of the population is MR/P-ON in mice without bladder stones [67]. This may suggest that MR/P is not important for maintaining the cluster or stone, or that only subpopulations of bacteria expressing *mrp* are needed during this step in infection. The microenvironment in stones may drive this variation, as pH, leukocyte attack, and nutrient accessibility vary greatly. In particular, MR/P fimbriae are induced during oxygen restriction and they are also immunogenic [68]. Additionally, if clusters represent a form of biofilm, then perhaps MR/P ‘off’ cells are disseminating from the cluster/stone using flagella-mediated motility, which is repressed by an MR/P regulatory protein, MrpJ (described below).

MR/P is critical for biofilms in microtiter plate models [35,69]. However, it remains untested whether MR/P is important for mediating crystalline biofilm formation on catheters. Identifying the role of MR/P and other fimbriae for catheter encrustation is important, as it will create a better overall picture of the UTI lifestyle of *P. mirabilis* from catheter ascension to urinary dissemination.

To successfully colonize a host, *P. mirabilis* must appropriately regulate MR/P production during the infection process. Indeed, at the end of the *mrp* operon is *mrpJ*, a gene that autoregulates *mrp* [70]. Additionally, MrpJ controls non-*mrp* genes, many of which are involved in virulence [70,71]. This suggests that MrpJ may be a more global regulator of virulence in *P. mirabilis*.

MrpJ Regulation

MrpJ is a predicted helix-turn-helix (HTH) protein that is required for a mouse model of ascending UTI [71,72]. It positively autoregulates the *mrp* operon by binding to a region within the promoter, which presumably leads to recruitment of the σ^{70} -dependent RNA polymerase holoenzyme [71]. Interestingly, chromatin immunoprecipitation (ChIP) evidence suggests that MrpJ interacts with an additional element approximately 800 bp upstream of the *mrp* operon [71]. This element is located within the divergently transcribed *mrpI* gene, which encodes a recombinase that alters the orientation of an invertible element found within the *mrpA* promoter; the orientation of this invertible element determines whether *mrp* is expressed [66]. This additional site of DNA-MrpJ interaction suggests that MrpJ may exert further, uncharacterized control of the transcription of *mrp*, *mrpI*, or both [71,72]. The possibility that MrpJ controls transcription through looping of DNA between these two binding sites seems particularly compelling, although the mechanism of regulation remains unknown.

In addition to the *mrpA* promoter, MrpJ directly interacts with the promoter of *flhDC*, an operon that encodes the master flagellar regulator [70–72]. As opposed to *mrp* regulation, MrpJ represses the expression of *flhDC*, thus inhibiting motility [70–72]. This is an intuitive solution that permits *P. mirabilis* to simultaneously control two opposing actions: adherence and motility. Further ChIP analysis revealed that MrpJ interacts with at least two regions in *flhDC* [71]. These two regions are located in the promoter and the coding region [71]. Similarly to *mrp*, MrpJ may regulate *flhDC* by binding to two different sites and/or through DNA looping.

MrpJ also controls the expression of a variety of other genes, many of which are known or putative virulence factors (Figure 2, bold) [71]. These include cell-surface molecules such as other fimbriae, lipoproteins, and lipopolysaccharide (LPS), the Pta toxin, and the ZapA metalloprotease; the latter two (and PMF fimbriae) have been demonstrated to be important for infection in mice (Figure 2) [28,71,73–75]. *P. mirabilis* can provoke extensive urinary tissue injury, particularly when urolithiasis occurs [1]. Proteases such as ZapA and Pta may contribute to this damage and, as a result, (i) reveal alternative binding targets for MrpJ-regulated fimbriae, and (ii) release cellular nutrients. Notably, *P. mirabilis*, unlike uropathogenic *Escherichia coli* (UPEC), utilizes glycolysis during UTI, implying that this pathogen has access to and relies on different carbon sources in the host [48,76]. ZapA may also aid bacterial survival by cleaving antimicrobial peptides found in urine [73]. LPS modifications, lipoproteins, and other fimbriae can affect bacterial adherence, biofilm formation, or immune evasion in bacterial pathogens, although all of these topics have not been examined for *P. mirabilis* [77–79].

Interestingly, one of the most highly-upregulated classes of genes encodes type VI secretion system (T6SS) proteins. In *P. mirabilis*, this system permits bacteria to identify other cells of the same strain *in vitro*, as strains with different sets of T6SS genes leads to killing of the non-identical strain [80,81]. However, these studies identified T6SS activity during swarming, when the *mrp* genes are repressed; this suggests that the T6SS is also active under other, MrpJ-activated conditions, such as during UTI, and that T6SS effectors may also target the host. Indeed, T6SS contribute to infection caused by other pathogens [82]. The importance of the T6SS for UTI, or whether MrpJ directly binds to targets identified in the microarray, remain an active area of research.

Overall, the MrpJ regulon is more extensive and complex than originally anticipated. There exist many unknowns, such as whether there is a different mechanism of control for targets of activation versus repression, and the identification of a consensus binding site. These outstanding questions merit further studies on MrpJ to elucidate differential control over its regulon, a process that is key for infection.

Fimbrial Cross-Regulation

Ten of the 17 fimbrial operons encoded by *P. mirabilis* contain an *mrpJ* paralog [55]. Similarly to MrpJ, at least two of these factors (AtfJ and Fim8J) autoregulate their own operon, whereas one does not (UcaJ); the remainder are uncharacterized [83]. Most of these paralogs repress motility to varying degrees [70]. Interestingly, mechanistic studies of one paralog, AtfJ, found that this factor requires a different combination of residues for autoregulation than for motility repression [83]. Some of this specificity is mediated by the unique C-terminal domain in AtfJ [83]. Together, these data suggest that MrpJ paralogs may use different protein/DNA interactions to differentially regulate target genes.

Given the degree of similarity among these regulators, it is reasonable to hypothesize that *P. mirabilis* has mechanisms in place to prevent ineffective regulatory cross-talk in environments that require one or more fimbriae. MrpJ paralogs may control which fimbriae are produced in a hierarchical, temporal fashion. Indeed, fimbrial cross-regulation exists at the transcriptional level [71,83], although the exact mechanisms remain uncharacterized.

Another intriguing concept is whether MrpJ paralogs form heterodimers to either prevent or promote transcription at particular promoters. These questions and others may not only lead to a better understanding of CAUTIs and stone formation, but also have a broader relevance for regulatory mechanisms used in other bacterial species.

Polymicrobial Infections

Patients with polymicrobial UTIs (pUTIs), or mixed UTIs, generally have long-term, indwelling catheters; in fact, anywhere from 75 to 85% of cultures isolated from indwelling catheters are polymicrobial (Table 1) [11,84]. Until recently, pUTIs have been overlooked clinically [85], perhaps because it was not clear whether multiple strains represented a true etiology of the UTI, or whether the additional strains were simply contamination [86]. Likewise, because modeling polymicrobial infections is considerably more difficult than single-species infections, there have been relatively few studies in pUTI. There is conflict as to how to address pUTIs associated with CAUTI in the clinic: whereas the IDSA (Infectious Diseases Society of America) emphasizes the total number of bacteria in a urine sample regardless of the number of isolated strains [87], the NHSN (National Healthcare Safety Network) does not analyze samples with more than two bacterial isolates.ⁱⁱ So far, there are no established clinical guidelines specifically discussing polymicrobial urinary stones.

P. mirabilis pUTI has been observed with other species such as *P. aeruginosa*, *Providencia stuartii*, *Klebsiella pneumoniae*, and *Enterococcus faecalis* [12,84,88]. Multispecies interactions may result in synergistic benefits for urinary pathogens. For example, the presence of two urease⁺ organisms, *P. mirabilis* and *P. stuartii*, enhances urease activity *in vitro*, and coinfection *in vivo* increased the number of mice with observable urinary stones [85]. Likewise, cocolonization of UPEC and *P. mirabilis* in mice increased the CFU of *E. coli* in the bladder and of both of these organisms in the kidneys, particularly at 7 dpi [76]. The authors of this study proposed that these bacteria do not directly compete within the bladder because they utilize different carbon metabolism pathways in the host; this is supported by the observation that the same metabolic gene mutations in each of these organisms resulted in differential fitness defects *in vivo*, but not *in vitro* [76]. These results pose a compelling hypothesis, as *E. coli* often lives within intracellular bacterial communities (IBCs) in host urothelial cells, whereas the majority of *P. mirabilis* cells exist in luminal clusters [1,89]. It is possible that *E. coli* can integrate into urinary stones, which could explain its enhanced CFU during mixed UTIs, particularly if the cluster is protecting *E. coli* from attack by the host's innate immune cells. Antagonistic interactions are also possible during pUTI. In one study, *P. mirabilis* was isolated less often from catheters colonized by *Morganella morganii* or *Enterobacter cloacae*; however, investigation of laboratory-grown biofilms showed that *P. mirabilis* readily blocked catheters when mixed with these species [12]. Further clinical CAUTI studies, particularly surveys that do not discard samples containing three or more species, will help focus laboratory studies on the most important interactions.

ⁱⁱwww.cdc.gov/nhsn/pdfs/psemanual/7psecauticurrent.pdf.

Concluding Remarks

Although research on *P. mirabilis* has elucidated how this organism may cause CAUTI and urinary stones, there remain many more questions that would provide critical insights into how this organism causes disease (see Outstanding Questions). To paint a better picture of *P. mirabilis*'s lifestyle, further research needs to connect the dots between catheters, bladder, and kidney infections, perhaps by using an indwelling catheter mouse model. This includes better understanding of MR/P fimbriae and urease as their exact functions during all steps of infection remains enigmatic. Additionally, multispecies interactions during pUTIs remain an exciting field with many areas of clinical importance that are ripe for investigating. This is particularly relevant given the recent observations that the healthy bladder is not necessarily sterile [90].

P. mirabilis uses an amazing assembly of mechanisms to cause urinary tract disease, and further research will certainly lead to a continued appreciation of this bacterium's remarkable approach to causing infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary

Apatite	a common urinary stone mineral with the chemical formula: $[Ca_{10}(PO_4)_6CO_3]$ [6].
CAUTI	catheter-associated urinary tract infection. A UTI that occurs when a urinary catheter is indwelling for >2 days and symptoms appear during catheterization or a day before removal ⁱⁱ .
Flow system	a method for growing bacterial biofilms utilizing a flow chamber with continuously cycling fresh or spent media [91].
Hemagglutination	an <i>in vitro</i> assay that assesses whether factors such as bacteria, viruses, or antibodies can agglutinate red blood cells.
Struvite	a common infection stone mineral with the composition: $[(NH_4)(MgPO_4 \cdot 6H_2O)]$ [6].
Urosepsis	a possibly life-threatening systemic inflammatory response (sepsis) to a urogenital infection [92].
Urothelium	epithelial cells that line the inner surface of the renal pelvis, ureters, bladder, and urethra [93].

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Trends

P. mirabilis is a model organism for urease⁺ organisms that are associated with CAUTIs and infectious stones.

P. mirabilis forms extensive crystalline biofilms on catheters that are infamously difficult to treat.

Once in the bladder, *P. mirabilis* can form luminal clusters that are the site of bladder stone formation.

At least two virulence factors, urease and MR/P fimbriae, are required for both cluster formation and crystalline biofilm formation on catheters.

The MR/P regulator, MrpJ, controls a variety of other known and putative virulence factors.

Polymicrobial infections can lead to synergistic enhancement of UTI and may have clinically relevant consequences.

Outstanding Questions

What is the current incidence of urease⁺ bacteria in CAUTI and urinary stones? What are the healthcare and economic burdens of these organisms?

How does *P. mirabilis* form/disseminate from clusters?

What is the lifestyle of *P. mirabilis* in the kidney?

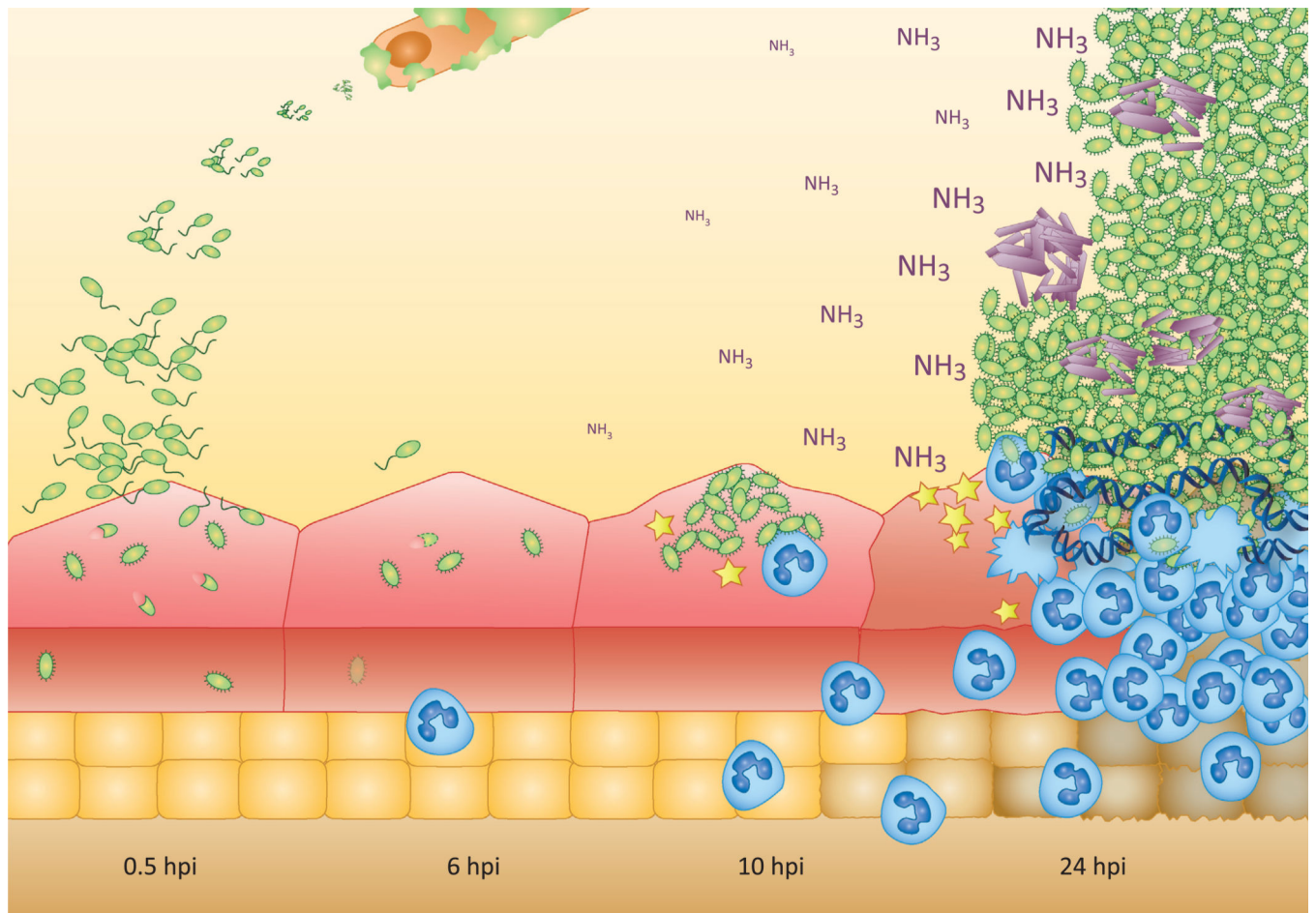
Does urease have a function outside of crystal/stone formation?

What is the function of MR/P fimbriae during UTI?

How do MrpJ paralogs achieve specificity, and is there crosstalk between these regulators?

What are the lifestyles of other urease⁺ bacteria in the urine? How might polymicrobial infections alter these lifestyles?

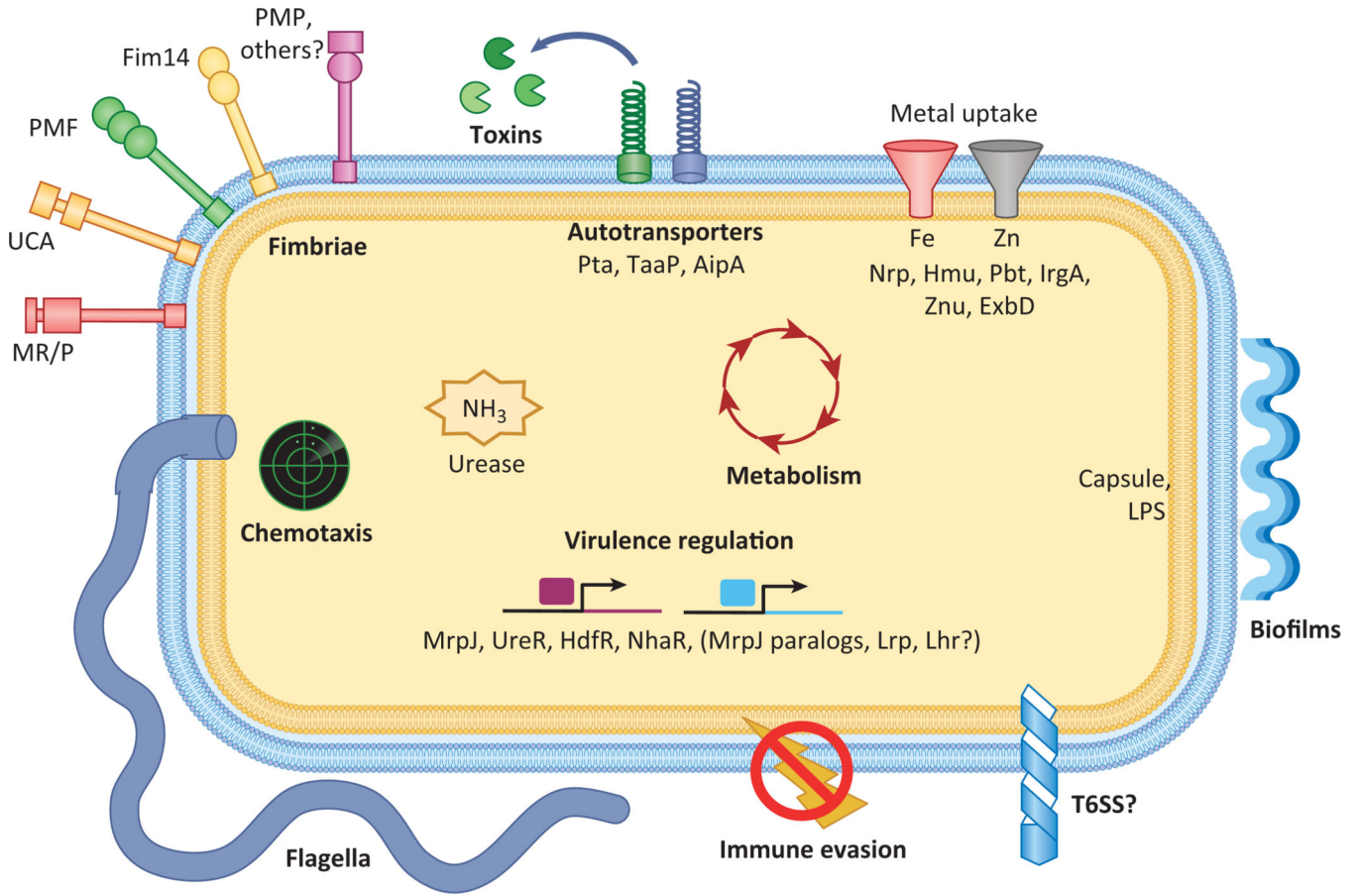
How might the urinary microbiome affect uropathogens' ability to cause disease?



Trends in Microbiology

Figure 1. Lifestyle of *Proteus mirabilis*

P. mirabilis cells form crystalline biofilms on the surface of catheters. Once inside the bladder (0.5–6 hours post-infection[hpi]), this organism can invade into urothelial cells of the bladder. As early as 10–24 hpi, *P. mirabilis* forms intraluminal clusters that can extend the length of the bladder and are associated with urothelial cell destruction. Host innate immune cells such as neutrophils (blue) are recruited to the site of infection and can form NETs (neutrophil extracellular traps). Figure used with permission from Schaffer *et al.* [1].



Trends in Microbiology

Figure 2. Concepts of *Proteus mirabilis* Pathogenesis during Urinary Tract Infection (UTI)
 Bolded classes of virulence factors have genes that are regulated by MrpJ. For more extensive information on these virulence factors, see references [21,22,99,100]. **Adherence:** binding catheters, host tissues, and neighboring bacteria may all contribute to disease. Adherence is mediated by chaperone-usher fimbriae and autotransporter adhesins. **Urease:** involved in stones, crystalline biofilms, and possibly nutrition or host sensing. **Motility:** *P. mirabilis* swarms across catheters and may ascend to the kidneys using swimming motility. Both forms of motion are mediated by flagella. Chemotaxis proteins allow the bacteria to follow chemical gradients. **Metabolism:** likely permits establishment of a nutritional niche, competition with other species, and response to host cues. **Metal scavenging:** iron and zinc uptake are essential for growth, but are sequestered by the host; therefore, specialized proteins are required for bacteria to scavenge these metals. **Toxins:** proteins such as HpmA and Pta may aid in nutrient accessibility, immune evasion, or provision of surfaces to colonize. **Biofilm formation:** Crystalline biofilms readily form on catheters, and bacterial clusters in the bladder may be a biofilm-mediated process. **Immune evasion:** this can include antibody and antimicrobial peptide degradation, polymyxin resistance, lipopolysaccharide (LPS) variation, and physical obstruction of phagocytosis. **Virulence regulation:** required to coordinate all steps of infection. MrpJ-controlled systems in this

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figure are bolded. **Type 6 secretion system (T6SS)**: involved in self-recognition; unknown role during UTI.

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Table 1
Studies Assessing the Prevalence of Polymicrobial Infections of Indwelling Catheters

Refs	# of samples	Population	Catheter duration	Method	% No detectable bacteria	% Single species	% Polymicrobial
Armbruster, 2016 [88]	182 ^a	Nursing home patients	3 to 360 days	Urine culture	0 ^b	69	31
Breitenbacher, 1984 [11]	15 ^a	Nursing home patients	1 year	Urine culture	0 ^c	14	86
Dedeic-Ljubovic and Hukic, 2009 [94]	1809 ^a	Hospitalized patients with spinal cord injuries	unknown	Urine culture	6	48	46
Frank <i>et al.</i> , 2009 [95]	8	Men catheterized after total prostatectomy	2 weeks	rRNA sequencing of catheter surface	12	0	88
Ganderton <i>et al.</i> , 1992 [84]	50	Nursing home patients	3 to 83 days (median 35 days)	Culture of catheter surface	0 ^c	25	75
Holá <i>et al.</i> , 2010 [96]	535	Hospital patients	NS ^d	Sonication and culture of catheters	0 ^b	13	87
Macleod and Stickler, 2007 [12]	106	Patients in hospitals or community care	NS ^d	Sonication and culture of catheters	0 ^b	28	72
Matsukawa <i>et al.</i> , 2005 [97]	86	Elderly men	1–35 days (mean of 3)	Culture of catheter swabs	46.5	39.5	14
Matsukawa <i>et al.</i> , 2005 [97]	86	Elderly men	1–35 days (mean of 3)	Urine culture	70	21	9
Tenney and Warren, 1987 [98]	7	Afebrile patients	>30 days	Urine culture	14	0	86
Warren <i>et al.</i> , 1982 [15]	619 ^a	Patients in a chronic care facility	1 month	Urine culture	2	21	77

^aMultiple samples from the same patients were included in the analysis.

^bOnly culture-positive samples were assessed.

^cStudy does not state whether there were samples with no detectable bacteria.

^dNS, not specified.