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Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*

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Abstract

Proteus mirabilis, named for the Greek god who changed shape to avoid capture, has fascinated microbiologists for more than a century with its unique swarming differentiation, Dienes line formation and potent urease activity. Transcriptome profiling during both host infection and swarming motility, coupled with the availability of the complete genome sequence for *P. mirabilis*, has revealed the occurrence of interbacterial competition and killing through a type VI secretion system, and the reciprocal regulation of adhesion and motility, as well as the intimate connections between metabolism, swarming and virulence. This Review addresses some of the unique and recently described aspects of *P. mirabilis* biology and pathogenesis, and emphasizes the potential role of this bacterium in single- species and polymicrobial urinary tract infections.

In Homer's *Odyssey*, Proteus was pursued by many for his ability to foretell the future to anyone capable of capturing him, but he changed shape to evade his pursuers. The name *Proteus* was first used in bacterial nomenclature by Hauser in 1885 to describe a shape-shifting bacterium isolated from putrefied meat[1]. *Proteus mirabilis*, a Gram-negative, dimorphic, motile member of the family Enterobacteriaceae, has fascinated scientists for more than 125 years owing to its ability to differentiate from short rods into elongated, multinucleate swarm cells that express thousands of flagella[2]. Members of the genus *Proteus* are widely distributed in nature and can be isolated from soil, stagnant water, sewage and the intestinal tract[3]. *P. mirabilis* is a leading agent of pyelonephritis, urolithiasis, prostatitis and catheter-associated urinary tract infections (CAUTIs) and causes approximately 3% of all nosocomial infections and up to 44% of CAUTIs in the United States[4–6].

CAUTIs are the most common health care-associated infections worldwide, accounting for up to 40% of hospital- acquired infections[7]. The duration of catheterization is the most important risk factor associated with CAUTI development, as roughly 10–50% of patients undergoing short-term catheterization (1–7 days) develop only bacteriuria, whereas essentially all patients catheterized for 28 days or longer develop a CAUTI[8]. CAUTIs are thought to be caused by self-inoculation of the catheter; indeed, for *P. mirabilis*, the strains causing bacteriuria in a given patient match the faecal isolates from that patient[9].

P. mirabilis is generally not the first organism found on the catheter surface, but it is common in long-term catheterization[10,11]. When *P. mirabilis* colonizes a catheter, the bacterial cells develop in protected communities known as biofilms. The biofilms that are initially formed on catheters tend to be monomicrobial, but rapidly become polymicrobial

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during long-term catheterization, with up to 72% of catheters being colonized by two or more species[12]. Catheter bio-films and urine samples taken from patients undergoing long-term catheterization frequently contain combinations of *P. mirabilis*, *Morganella morganii*, *Providencia stuartii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*[5,7,13–16]. Data compiled over the past 30 years reveal that, depending on the study parameters and sample population, up to 77% of CAUTIs are polymicrobial, and *P. mirabilis* is generally the most common organism isolated from these infections[13–18].

P. mirabilis has many virulence factors that contribute to the establishment of a UTI in a mouse model of infection[19]. The bacterium expresses fimbriae, or bacterial appendages tipped with adhesive proteins, that mediate attachment to cells in the urinary tract and probably also to the catheter. In addition, *P. mirabilis* produces urease, which hydrolyses urea to carbon dioxide and ammonia. This reaction provides an abundant source of nitrogen for the bacterium, but also causes the formation of crystalline biofilms that block the catheter. Furthermore, *P. mirabilis* can differentiate into swarm cells that contribute to the establishment of infection by migrating along the catheter. Inside the urinary tract, the bacterium has mechanisms to scavenge nutrients and evade the host immune response, and is capable of reciprocal expression of fimbriae (for adherence) and flagella (for motility when it needs to ascend the urinary tract).

There have been many recent advances in our understanding of *P. mirabilis* pathogenicity, including the publication of the complete 4.06 Mb genome sequence of a CAUTI isolate (*P. mirabilis* str. HI4320)[20], the identification of novel pathogenicity factors by signature-tagged mutagenesis[21–23], and the elucidation of the intricate connections between metabolism, swarming motility, urease activity and nitrogen availability in the establishment and persistence of *P. mirabilis* UTIs. This Review aims to address the most recent findings concerning *P. mirabilis* pathogenicity and to place these findings in the context of CAUTI progression, identifying the stage of infection (for example, initial entry to the urinary tract, ascending the tract and immune evasion) to which they contribute.

Furthermore, given the growing appreciation in the field for the polymicrobial nature of many infections, it is imperative to consider how knowledge gained from single-species investigations can be applied to the study and treatment of polymicrobial infection. Although few studies have addressed polymicrobial CAUTIs to date, *P. mirabilis* is frequently isolated from these infections. It is therefore important to consider how *P. mirabilis* and other common causes of CAUTIs interact on catheters and during infections, and how these interactions influence the course of an infection. The final section of this Review discusses *P. mirabilis* pathogenicity factors in the context of polymicrobial infection and considers ways in which this organism interacts with other uropathogens.

Entry to the urinary tract

To establish a CAUTI, *P. mirabilis* must first gain access to the urinary tract. Although the catheter provides a direct route to the bladder, *P. mirabilis* needs to adhere tightly to the catheter to resist the flow of urine and must traverse this surface or disrupt the flow of urine to gain entry to the bladder.

Adherence to catheters

P. mirabilis readily colonizes the lumen and external surface of all current catheter types, and adherence is enhanced in the presence of urine[24–28]. *P. mirabilis* encodes 17 putative fimbrial operons, the most encoded by any sequenced bacterial species[20]. Five fimbrial types have been characterized and reviewed[29] (TABLE 1), but little is known about the contribution of fimbriae to catheter adherence and colonization. *P. mirabilis* expresses

mannose-resistant *Klebsiella*-like (MRK) fimbriae, a type that mediates catheter attachment by *P. stuartii*[30] and *Proteus penneri*[31] and might therefore contribute to colonization by *P. mirabilis*. The ambient-temperature fimbriae (ATFs) of *P. mirabilis* do not contribute to colonization of the mouse urinary tract but are optimally expressed at ambient temperature and could therefore influence catheter colonization[32,33].

Swarming motility

An overview of *P. mirabilis* swarming motility is presented in BOX 1. Swarming describes flagellum- dependent movement across a surface, in contrast to swimming through liquid or soft agar. This form of motility allows *P. mirabilis* to migrate across catheters, gaining entry to the urinary tract[4,34,35]. Swarming is required for migration across most catheter types, as non-swarming mutants traverse only hydrogel-coated latex catheters, by swimming through the water-filled channels in the matrix[36]. In addition, *P. mirabilis* catheter biofilms contain protruding swarm cells[37]. Although the role of these biofilm-associated swarm cells is unclear, they might seed dispersal from the catheter to the urinary tract[37].

Box 1

Proteus mirabilis swarming motility

Unlike species that swarm on 0.45% agar, such as *Escherichia coli*, *Proteus mirabilis* swarming motility occurs on 1.5% agar at both 30 °C and 37 °C, resulting in a characteristic bull's eye pattern (see the figure, part **a**). This pattern is caused by sequential rounds of swarm cell differentiation, swarming colony migration (designated S in part **a**), and consolidation with de-differentiation back to a swimmer-cell morphology (designated C in part **a**). On a solid surface, *P. mirabilis* differentiates into swarm cells that are multinucleate, 20–50-fold elongated and express thousands of flagella (see the figure, part **b** (arrow) and, at higher magnification, part **c**)2,73. In liquid culture, *P. mirabilis* cells are rod shaped, 1–2 μm in length and peritrichously flagellated (see the figure, part **d**). Recent work allowed visualization of the helical connections formed in swarm rafts during migration, revealing that these connections consist of interwoven flagellar filaments from adjacent swarm cells (see the figure, part **e** and, at higher magnification, part **f**))36. It is not yet clear how *P. mirabilis* coordinates the formation of these structures, but they seem to be required for normal swarming motility.

Although consolidation was once considered a resting stage, *P. mirabilis* is considerably more metabolically active during consolidation than it is during swarming, and overall gene expression is also higher[127,128]. The genes that are most highly upregulated during consolidation include those encoding proteins involved in amino acid import and synthesis, peptide uptake, central metabolic pathways, peptidoglycan remodelling and cell wall synthesis, as well as stress response proteins, proteases and many flagellar proteins. By contrast, few genes are upregulated in swarm cells compared to in consolidated cells, and swarming can occur in the absence of protein synthesis. This indicates that swarming is almost entirely devoted to flagellum- mediated motility, whereas consolidation seems to be the active stage during which *P. mirabilis* prepares for the next round of motility[128].

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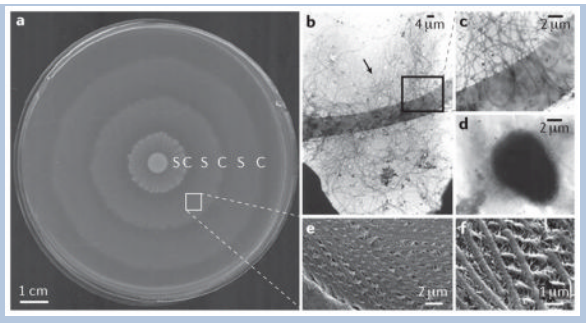


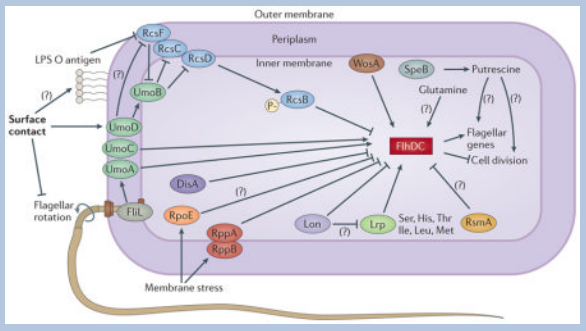
Most strains of *P. mirabilis* are unable to differentiate into swarm cells in liquid medium without the addition of a thickening agent, so it is thought that differentiation is triggered by surface contact or inhibition of flagellar rotation[38]. Several other factors have been implicated in the regulation of swarming motility (and are reviewed elsewhere[39,40]), including the upregulator of flagellar master operon (Umo) proteins, the regulator of colanic acid capsule synthesis (Rcs) phosphorelay, the RppAB two-component system, the carbon storage regulator (CsrA) homologue RsmA, Lon protease, the tyrosine decarboxylase DisA and leucine-responsive regulator (Lrp). An overview of the role of these factors in regulating swarm cell differentiation is presented in BOX 2. Interestingly, Lrp is part of a family of transcription factors that link gene regulation to metabolism, and its activity in *P. mirabilis* is regulated by leucine, isoleucine, serine, histidine, threonine and methionine[41,42]. Although glutamine does not regulate Lrp activity, this amino acid also appears to promote swarm cell differentiation[43], and additional metabolic intermediates that promote swarming under normally non-permissive conditions have been identified (C.E.A. and H.L.T.M., unpublished observations). Thus, the decision to swarm appears to be influenced by metabolic status and the presence of specific amino acids, including glutamine and histidine, which are two of the most concentrated amino acids in normal human urine[44].

Box 2

Swarm cell differentiation in *Proteus mirabilis*

Recent studies concerning *Proteus mirabilis* swarm cell differentiation have revealed a complex regulatory network, with most factors acting on the flagellar transcriptional regulator (FlhDC). Some of these factors alter gene expression, and others modify protein level; in the figure, all factors are shown as affecting FlhDC, for simplicity. The regulator of colanic acid capsule synthesis (Rcs) phosphorelay, composed of a response regulator (RcsB), a sensor kinase (RcsC), an outer-membrane activator (RcsF) and a phosphotransferase (RcsD; also known as RsbA), ultimately results in phosphorylated RcsB, which represses *flhDC*[113,129,130]. When *P. mirabilis* contacts a surface under conditions that are favourable for swarming, this signal is sensed and propagated by some combination of the inhibition of flagellar rotation, FliL, WosA and changes in the cell wall involving the O antigen of lipopolysaccharide (LPS)[38,131–133]. The signal is relayed by inhibition of RcsF and increased upregulator of flagellar master operon D (UmoD) activity, leading to activation of UmoB and, consequently, a reduction in phosphorylated RcsB, alleviating *flhDC* repression[132,134]. The importance of the Rcs phosphorelay in the regulation of swarm cell differentiation is further underscored by the finding that disruption of *rcsD* alleviates the requirement for surface contact and allows the formation of elongated cells in liquid culture[113]. In addition to the Rcs system, Lon protease negatively regulates swarming by degrading FlhD and possibly also leucine-responsive regulator (Lrp)[135]. RppA negatively regulates flagellin synthesis by decreasing expression of *flhDC* as well as modulating LPS synthesis[136,137], and RNA polymerase σ-

factor RpoE may respond to membrane stress sensed by the RppAB system by decreasing expression of *flhDC*[136]. The putative tyrosine decarboxylase DisA also negatively regulates swarming by decreasing expression of the class 2 and class 3 flagellar genes[138]. The carbon storage regulator (CsrA) homologue RsmA inhibits swarming when overexpressed, possibly by regulating *flhDC* expression[40,139]. The amino acid glutamine promotes swarm cell differentiation under normally non-permissive conditions, and the polyamine putrescine (generated by agmatinase (SpeB)) is required for swarming, although their mechanisms of action are not yet known[43,120]. Thus, initiation of swarming requires the integration of numerous signals and is intimately connected to the metabolic status of the bacterium, membrane integrity, and cell wall changes associated with surface contact.



Urease and catheter encrustation

The urease gene cluster of *P. mirabilis*, which comprises *ureRDABCEFG*, produces a cytoplasmic nickel metalloenzyme that is positively regulated by UreR and repressed by the nucleoid- associated protein H-NS[45–52]. UreR and H-NS compete for the same regulatory region upstream of *ureD*, resulting in tight control of urease expression: H-NS represses transcription of the *ure* operon at ambient temperature, but a shift to host body temperature and high concentrations of urea alleviate H-NS transcriptional repression, allowing UreR to activate urease expression[47]. Urease contributes to catheter colonization by hydrolysing urea to ammonia and carbon dioxide, thereby increasing the pH and facilitating the precipitation of polyvalent ions in urine, leading to the formation of struvite (magnesium ammonium phosphate) and apatite (calcium phosphate) crystals[19,53,54]. Bacterial adherence typically occurs when the urine pH increases to ~8.2 and crystals deposit on the catheter[27].

The crystals accumulate within biofilms on the catheter and eventually block the catheter lumen, obstructing urine flow and leading to complications such as incontinence and painful distension of the bladder (caused by urinary retention)[55]. This in turn leads to vesicoureteral reflux, bacteriuria, ascending infection, pyelonephritis and possibly septicaemia[56]. *P. mirabilis* str. HI4320 generally causes urinary stones and severe pyelonephritis in experimental UTIs, and urease is expressed by this strain within the bladder and kidneys[57]. Furthermore, mutation of the gene encoding the major structural subunit of urease, UreC, severely reduces *P. mirabilis* colonization of the bladder and kidneys, prevents stone formation and limits kidney damage, indicating that urease is a major contributing factor to both the severity and persistence of *P. mirabilis*-mediated UTIs[58–60].

Ascending UTIs and pathogenesis

The urinary tract consists of two kidneys, two ureters, the bladder and the urethra (FIG. 1). Catheterization provides a direct route to the bladder, and the combined actions of fimbriae, adhesins, swarming motility and urease promote entry of *P. mirabilis* to the urinary tract, but

the bacterium then faces new challenges when it is inside the host. To establish a UTI, *P. mirabilis* must adhere to the uroepithelial cells lining the bladder. The bacterium also generally does not stay confined to the bladder, but ascends the ureters to infect the kidneys. Therefore, *P. mirabilis* must modulate the expression of the adherence and motility factors that are important for ascension and must produce specialized virulence factors for survival within the urinary tract. A mouse model of an ascending UTI, in which *P. mirabilis* is introduced into the bladder via a catheter that is inserted into the urethra and carefully removed immediately following infection, is commonly used to study the contribution of these virulence factors to infection. This model allows for direct infection of the bladder, but the initial inoculum does not reach the kidneys unless the bacteria actively ascend.

Fimbriae and adhesins

Although *P. mirabilis* fimbriae and adhesins are important determinants of pathogenicity, as they promote adherence to the uroepithelium, most studies suggest that individual *P. mirabilis* fimbriae and adhesins are necessary, but not sufficient, to establish a UTI. For instance, the autotransporter AipA mediates adhesion to human kidney and bladder epithelial cell lines and contributes to colonization of the kidneys and spleen in a mouse model, but an *aipA* mutant still colonizes the bladder and kidneys at relatively high levels[61]. Even with the loss of two distinct fimbrial types, *P. mirabilis* retains the ability to adhere to bladder cells and establish a UTI, albeit at a reduced level[62]. Therefore, determining the role of individual fimbrial types is complex.

Despite these challenges, recent work has elucidated the contributions of several fimbria types to UTIs (TABLE 1). Non-agglutinating fimbriae (NAFs; also known as uroepithelial cell adhesin (UCA) fimbriae) facilitate adherence to uroepithelial cell lines[63,64], MRK fimbriae mediate adherence to Bowman's capsules of the kidney glomeruli[63], and mannose-resistant *Proteus*-like (MRP) fimbriae mediate adherence to the lumen and cytoplasm of tubular cells in the kidney and to epithelial cells present in urine[63]. *P. mirabilis* fimbriae (PMFs) are also thought to contribute to colonization of the bladder, but are not necessary for kidney colonization[65,66]. However, the exact contribution of PMFs to infection remains unclear, as expression of the major structural subunit (PmfA) is lower during infection than in non-infecting bacteria[67].

MRP fimbriae are the best studied fimbrial type of *P. mirabilis*, and they contribute to colonization of the urinary tract, as disruption of the *mrp* operon decreases bacterial load in the urine, bladder and kidneys[68,69]. MRP fimbriae are subjected to phase variation (FIG. 2), and phase variation *in vivo* favours the expression of these fimbriae[70]. Furthermore, inhibiting *mrp* expression results in a slight defect in colonization, whereas constitutive *mrp* expression allows the bacteria to out-compete the wild-type strain in the bladder, indicating that MRP fimbriae provide an advantage during bladder colonization[71]. Along with the finding that MRP fimbriae are present in 94% of *P. mirabilis* isolates obtained from the mouse bladder during infection, these results strongly indicate that MRP fimbriae contribute to colonization of the bladder and probably the entire urinary tract[72].

Reciprocal regulation of fimbriae and flagella

Bacteria generally do not express adherence factors at the same time as flagella, suggesting that there is an underlying mechanism of reciprocal regulation[2,73]. For instance, *P. mirabilis* swarm cells express thousands of flagella but few fimbriae, and expression of the *mrp* operon is highest when the expression of flagellar genes is reduced[67,74]. Recent work determined that the last gene in the *mrp* operon, *mrpJ*, encodes a transcriptional regulator that directly represses motility by binding to the promoter region of the flagellar transcriptional regulator (*flhDC*) operon[75,76]. Furthermore, locking the invertible element

(IE) of the *mrp* operon in the OFF position, and thus inhibiting *mrpJ* expression, favours faster swarming motility[72]. Interestingly, *P. mirabilis* str. HI4320 encodes 14 additional *mrpJ* paralogues, ten of which are associated with other fimbrial operons[76]. Overexpression of most of these paralogues similarly represses motility and, for some paralogues, inhibits swarm cell differentiation[76], indicating that *P. mirabilis* is exquisitely committed to regulating the expression of fimbriae and flagella. The proteins encoded by all 15 *mrpJ*-type genes contain a conserved DNA-binding domain, although it is not known whether all paralogues bind the *flhDC* promoter[76]. MrpJ might also contribute to phase variation of the fimbrial *mrp* operon[77], providing another layer of complexity.

Further insight into the reciprocal expression of MRP fimbriae and flagella comes from analysis of gene expression in a mouse UTI model. Transcriptome profiling revealed that, during infection, the most highly upregulated *P. mirabilis* genes are components of the *mrp* operon, whereas genes involved in motility are highly down-regulated[67]. An analysis of temporal expression, however, showed that the *mrp* operon is highly expressed at days 1 and 3 post-infection but is not as highly expressed by day 7, whereas the expression of genes encoding flagellar components is decreased for only 1 day following infection[67]. This finding suggests that the expression of MRP fimbriae is crucial for early stages of infection but decreases over time, possibly to allow for flagellum- mediated motility. This is a noteworthy possibility, as swarm cells seem to be rare during the early stages of a UTI, but might have a crucial role in later stages of infection[78–80].

Metabolic requirements for infection

On the basis of *in vivo* gene expression and other study data, *P. mirabilis* nitrogen metabolism appears to be crucial for the establishment of a UTI. During experimental infection, *P. mirabilis* increases glutamate dehydrogenase (*gdhA*) expression and decreases expression of glutamine synthetase (*glnA*)[67]. GdhA mediates the conversion of ammonia and α-ketoglutarate to glutamate when ammonia is abundant, whereas GlnA is part of the GS–GOGAT system (glutamine synthase–glutamine oxoglutarate amino-transferase system) for the high-affinity conversion of limited amounts of ammonia to glutamine to generate glutamate under nitrogen-limited conditions. Therefore, the data suggest that *P. mirabilis* uses GdhA and the ammonia produced by urease activity to generate glutamate during infection[67]. This hypothesis has yet to be tested, but the portion of the tricarboxylic acid cycle that is required for α-ketoglutarate synthesis (involving the enzymes encoded by *gltA, acnB* and *icd*) is upregulated during UTIs[67]. Furthermore, *P. mirabilis gdhA* mutants exhibit less colonization of the bladder, kidneys and spleen than wild-type *P. mirabilis*, underscoring the importance of this system during UTIs[67].

Metal acquisition

As the urinary tract is an iron-limited environment and iron is essential to the function of many proteins and enzymes, scavenging iron from the host is imperative for pathogenic bacteria. *P. mirabilis* was originally thought to lack siderophores[81], leaving a large gap in our understanding of how this species persists in the urinary tract. However, iron is clearly necessary for *P. mirabilis* pathogenicity, as five genes found to be associated with iron acquisition result in attenuation of virulence when they are mutated[21,23]. One of these genes, *hmuR2*, encodes a haem receptor that contributes to bladder and kidney colonization[82]. Two other putative iron receptors encoded by PMI0842 and PMI2596 similarly contribute to *P. mirabilis* pathogenicity[83].

Following annotation of the *P. mirabilis* str. HI4320 genome and microarray analysis of gene expression under iron-limited conditions, it is now known that *P. mirabilis* encodes at least 21 putative iron acquisition systems, two of which have been well characterized[20,84].

In keeping with its unique nature, *P. mirabilis* str. HI4320 encodes a previously undescribed non-ribosomal-peptide synthetase (NRPS)-independent siderophore (NIS) system, now known as proteobactin (encoded by the operon *pbtABCDEFGHI*)[84]. *P. mirabilis* also contains the *nrp* operon, encoded on the mosaic pathogenicity island ICE*Pm1* (integrative and conjugative element of *P. mirabilis* 1) and upregulated during iron limitation[85,86]. The *nrp* genes have homology to the yersiniabactin synthesis genes, but the *nrp* system of *P. mirabilis* is distinct from the yersiniabactin synthesis systems of other species[84]: although *P. mirabilis* can utilize enterobactins produced by other species, it cannot produce or utilize yersiniabactin, despite the high similarity between the yersiniabactin synthesis genes and the *nrp* locus[84].

Proteobactin and the siderophore encoded by the *nrp* operon seem to be the primary mechanisms for iron chelation in *P. mirabilis*[84]. One study found these loci to be more prevalent in UTI isolates (present in all ten isolates tested) than in non-UTI isolates (present in only five of the ten tested isolates), suggesting that these loci contribute to fitness within the urinary tract[84]. Both siderophores are important for colonization of the bladder, although the yersiniabactin-related siderophore provides a greater contribution to overall fitness[84]. Furthermore, transcriptome profiling has verified that these iron uptake systems are upregulated *in vivo*[67].

Zinc is another metal that is crucial for the function of numerous proteins and enzymes. *P. mirabilis* has a functional zinc uptake system (ZnuABC) that is required for growth under zinc limitation[87]. Mutation of *znuC* limits swimming and swarming motility, possibly owing to an impact on the flagellar master regulator FlhC, as in some species FlhC contains a zinc-binding site[88]. ZnuB is expressed during mouse UTIs and is recognized by the humoral response, and ZnuC is upregulated during mouse UTIs[67,83]. However, the contribution of the ZnuABC system to virulence remains unclear, as a *znuC* mutant colonizes the urinary tract to a similar level as the wild-type bacterium but is outcompeted during a challenge with both bacteria simultaneously[87].

Toxins

Haemolysin was proposed as a virulence factor in *P. mirabilis* because strains with high haemolysin production are more lethal than strains with low haemolysin production, and the cytotoxicity of *P. mirabilis* to human renal epithelial cells is largely due to haemolysin[89–91]. Haemolysin is also thought to facilitate bacterial spread within the kidney and development of pyelo-nephritis during ascending UTIs[19]. However, mutation of *hpmA*, the gene encoding this toxin, does not appear to affect kidney colonization or tissue damage during infection[74,92,93], indicating that either haemolysin is not as active during infection as the *in vitro* data suggest or the activity of other virulence factors masks its contribution.

Proteus toxic agglutinin (Pta), encoded by ICE*Pm1*, is a bifunctional outer-membrane autotransporter that mediates cell–cell aggregation and also contains a catalytically active α-domain (a subtilisin-like alkaline protease domain) capable of lysing kidney and bladder cells[86,94]. This unusual adhesin–toxin was first identified as an outer-membrane surface-expressed protein that is recognized by the mouse immune system[83], and loss of *pta* results in a significant colonization defect in the bladder, kidneys and spleen, as well as reduced pathology[92,94]. Notably, simultaneous inactivation of *pta* and *hpmA* results in a greater reduction in cytotoxicity than is caused by either mutation alone[92], indicating that Pta and HpmA have an additive effect. Pta production by the *hpmA* mutant might therefore explain why no difference in pathogenicity is observed between the haemolysin mutant and the parent strain.

The genome of *P. mirabilis str.* HI4320 encodes three uncharacterized potential autotransporters that possess serine protease motifs and may also act as toxins (PMI0844, PMI2126 and PMI2341)[20,67]. This strain also encodes several other proteins that, on the basis of their similarity to known toxins, are themselves potential toxins, including PMI0004 and PMI2043 (which have homology to the cytotoxin RtxA), PMI1747 and PMI1748 (which together form a putative binary toxin, XaxAB), PMI0023 (a putative intimin–invasin), and PMI2028, PMI2029 and PMI2030 (which together form a putative type I secretion system)[20]. However, none of the genes encoding these proteins is significantly upregulated during an experimental UTI[67].

P. mirabilis also encodes a type III secretion system (T3SS) for possible injection of effector proteins into target eukaryotic cells[20]. Although the *P. mirabilis* T3SS genes are expressed in liquid culture, mutation of *P. mirabilis* homologues of genes that are essential for T3SS function in other systems (*spa47* and *exsD*) has no impact on the secreted protein profile of this bacterium or on its pathogenesis in a mouse model, indicating that the T3SS might not be a virulence determinant for *P. mirabilis*[95]. However, another putative T3SS gene, *ipaD*, is among the most highly upregulated genes during an experimental UTI, leaving it unclear exactly what this system contributes to virulence[67].

Persistence and immune evasion

Once *P. mirabilis* gains access to the urinary tract, it has a remarkable ability to persist despite antibiotic treatment and catheter changes[55]. To persist within the host, bacteria must successfully evade innate and adaptive immune responses. One mechanism of immune evasion for many species is to vary the expression or composition of antigenic structures, such as outer-membrane proteins or fimbriae. In *P. mirabilis*, the MRP fimbriae are phase variable[96]. It is not yet known whether the 16 other types of fimbriae undergo phase variation, but any modulation of expression in this wide range of fimbriae would contribute to fitness. In addition, *P. mirabilis* str. HI4320 contains 13 putative orphan fimbrial genes that are not part of a complete operon and might contribute to further fimbrial diversity[20,97]. Flagellin is also thought to contribute to immune evasion via antigenic variation[4,98,99].

P. mirabilis encodes a metalloproteinase, serralysin (ZapA), that cleaves serum and secretory immuno-globulin A1 (IgA1), IgA2 and IgG, thereby providing protection from the mucosal immune response[100]. ZapA might also cleave complement components C1q and C3, cell matrix components such as collagen, fibronectin and laminin, cytoskeletal proteins such as actin and tubulin, and certain antimicrobial peptides[101]. The importance of ZapA is underscored by the finding that mutation of *zapA* results in a dramatic decrease in the recovery of bacteria from the urine, bladder and kidneys[102].

The formation of urinary stones by *P. mirabilis* generates a protective and nutrient-rich environment for the bacterium[19]. Stone formation and catheter encrustation together physically contribute to persistence by causing retention of urine, generating a reservoir of bacteria and preventing wash-out[55,56]. In addition, *P. mirabilis* bacteria have been visualized within urinary stones during a mouse UTI[80]. Urinary stones are thought to limit bacterial exposure to antibiotics and antibodies, and also, as a result of nutrient limitation, to possibly limit replication of the bacteria sequestered inside the stone, which would make these bacteria less sensitive to antimicrobials that target replication[19,80].

P. mirabilis is also capable of invading urothelial cells to survive intracellularly[89,90,103], probably representing another mechanism for immune evasion and persistence. Interestingly, one study reported that cell invasion provides protection against antibiotic treatment, although determining the exact contribution of invasion was complicated by the

fact that *P. mirabilis* also caused the formation of crystals within the invaded cells, so these crystals might have been responsible for the protection[103].

Multicellular interactions

In light of the finding that up to 77% of CAUTIs are polymicrobial and that *P. mirabilis* is generally the most common organism isolated from these infections[13–18], it is important to identify ways in which *P. mirabilis* influences the pathogenicity of other organisms and vice versa. Although to date little work has focused on the interactions between uropathogens, several *P. mirabilis* virulence factors have the potential to enhance the pathogenicity of other species.

Swarming and interbacterial competition

P. mirabilis swarming represents a multicellular behaviour, and this unique type of motility facilitates the migration of non-motile species, such as *K. pneumoniae* and *Staphylococcus aureus*, on a catheter surface[5,34]. Therefore, swarming has the potential to influence polymicrobial UTIs by facilitating entry of other species into the urinary tract, although it is yet to be determined whether polymicrobial infection provides a direct benefit for *P. mirabilis*.

Another fascinating aspect of the interbacterial interactions during swarming is the Dienes phenomenon, in which two *P. mirabilis* swarming colonies of a single strain merge with each other, whereas swarms of different strains form a distinct boundary where they meet, known as a Dienes line[104]. Formation of the Dienes line requires direct cell–cell contact between living bacteria and is thought to involve killing of one strain at the boundary[105]. Interestingly, competitive killing is observed only during swarming, as strains that are sensitive to killing on swarm agar are not outcompeted in broth culture or on agar that is not swarming permissive[105].

One explanation for the formation of the Dienes line involves the production of the bacteriocin proticine, which is capable of killing sensitive strains. Indeed, boundaries form between *P. mirabilis* strains that differ in proticine production and sensitivity[106]. However, some strains that are deficient in proticine production still form boundaries with other strains lacking proticine production, indicating that another mechanism mediates Dienes line formation[107]. In the search for this mechanism, a transposon mutant was identified that forms a boundary rather than merging with its parent strain, and the disrupted locus was named identification of self (Ids)[107]. Further work determined that *idsABCDEF* constitutes an operon, that *idsD* and *idsE* seem to encode strain-specific factors which are essential for self recognition, and that *idsB, idsC* and *idsF* encode factors which are essential for self recognition but can be complemented by *ids* genes from other strains[107,108]. As two swarm fronts merge, only a subset of cells in the advancing swarm expresses the *ids* genes and traverses the boundary of the other swarm, and this subset is sufficient to propagate the signal of self versus non-self[108]. Furthermore, *ids* expression decreases as an advancing swarm approaches another swarm of the same strain.

The *ids* system alone, however, does not fully explain the interstrain interactions that occur within the Dienes line, as certain mutations within this locus can result in boundary formation without any apparent competitive killing[105]. The *ids* locus encodes putative type VI secretion system (T6SS) effector proteins, so it was hypothesized that T6SS is involved in Dienes line formation[107]. In agreement with this hypothesis, our laboratory has identified additional loci encoding T6SS effector and structural proteins involved in Dienes line formation (C. J. Alteri and H.L.T.M., unpublished observations). The T6SS is thought to be involved in maintenance of a balanced relationship between the bacterium and the host as

well as in mediating competitive interbacterial interactions, and the T6SS of *Vibrio cholerae* was recently shown to be involved in interspecies competition and killing[109]. The T6SS of *P. mirabilis*, along with proticine and the *ids* genes, may mediate a combination of inter-bacterial killing during swarming and Dienes line formation, competition on the catheter surface, and even possibly some form of interaction with the host during a UTI.

Urease, pH and nitrogen metabolism

Despite the fact that urease is produced by other common agents of CAUTIs, such as *P. stuartii* and *M. morganii*, *P. mirabilis* is the main species responsible for the formation of crystalline biofilms on catheters and the resulting blockage that causes urine retention, urine reflux and bacteriuria[11,12]. Therefore, *P. mirabilis* alone can substantially affect whether or not other species persist on the catheter and reach the urinary tract to cause infection. As the crystalline biofilms seem to provide protection for *P. mirabilis*[19,80], these structures may provide similar protection to other species present during a polymicrobial infection, if these species can coexist within the biofilms.

The potent urease of *P. mirabilis* results in the urinary tract containing an abundance of ammonia that would not be present during a single-species infection by a urease-negative organism such as uropathogenic *E. coli* (UPEC), so other species might benefit from the generation of this preferred nitrogen source during a polymicrobial infection. For UPEC, this hypothesis is supported by the finding that expression of *glnA*, which is induced by nitrogen limitation, is upregulated fourfold in bacteria taken from the urinary tract[110]. Co-infection with *P. mirabilis* may alleviate the nitrogen-limited conditions for UPEC, thereby enhancing the growth or persistence of this urease-negative organism. Our laboratory has found that simultaneous co-infection with *P. mirabilis* and UPEC enhances colonization for both species, suggesting that these uropathogens readily coexist in the urinary tract (C. J. Alteri and H.L.T.M., unpublished observations). The reason for this enhanced colonization and the overall impact of this effect on pathology are active areas of research.

Conversely, the dramatic increase in pH as a result of urease activity might have a negative impact on other species. For instance, *P. mirabilis* outcompetes some urease-negative organisms (such as *Enterobacter cloacae*) and some less-potent urease-positive organisms (such as *M. morganii*, *P. aeruginosa* and *K. pneumoniae*) when co-cultured in a bladder model, even when *P. mirabilis* is introduced 72 hours after catheter colonization by the other organism[12]. This appears to be due, in part, to the urease activity of *P. mirabilis*, as the decrease in viability for these other species correlates with the rise in pH that occurs shortly after the introduction of *P. mirabilis*.

Horizontal gene transfer

The 94 kb ICE*Pm1* mobile pathogenicity island of *P. mirabilis* is present in several *P. stuartii* and *M. morganii* isolates, with up to 100% sequence identity for some genes, suggestive of DNA transfer between these species[86]. ICE*Pm1* is more prevalent in *P. mirabilis* clinical isolates taken from the urine of catheterized individuals than in clinical isolates taken from other body sites, suggesting that this mobile genetic element contributes to colonization and pathogenicity within the urinary tract. As CAUTIs are generally caused by self-inoculation with gut micro-biota[9], the acquisition of ICE*Pm1* might be the reason why normally commensal microorganisms become able to colonize the urinary tract, or might explain why these commensals become pathogenic when they reach this site. Furthermore, it has been demonstrated that ICE*Pm1* can excise from the *P. mirabilis* chromosome and integrate into other ICE*Pm1*-deficient *P. mirabilis* strains and at least one *E. coli* strain, as long as the integrase, chromosome-partitioning protein A (ParA) and type IV secretion system are intact[111]. These studies support the notion of horizontal gene

transfer between potential uropathogens, a phenomenon that may also allow for transfer of antimicrobial resistance.

Cell–cell communication

N-acyl homoserine lactone (AHL) signalling molecules are utilized by several Gram-negative species to sense population density and coordinate gene expression[112]. *P. mirabilis* lacks a clear AHL synthase (LuxI) homologue and does not seem to produce this type of signalling molecule[20,113]. However, *P. mirabilis* encodes a LuxR family transcriptional regulator and seems to produce compounds with AHL-like activity; one study found that the addition of exogenous AHL to a *P. mirabilis* population has a strain-specific impact on virulence factor expression, swarming and biofilm formation[20,114–117]. AHLs secreted by other species on the catheter or during a polymicrobial UTI may therefore modulate *P. mirabilis* swarming or virulence. Fatty acids have also been proposed to influence swarming in a manner similar to AHLs, probably influencing swarm cell differentiation and *flhDC* expression, as well as biofilm formation, via the Rcs phosphorelay[118].

The quorum sensing molecule autoinducer 2 (AI-2), encoded by *luxS*, can mediate both intra- and interspecies interactions. *P. mirabilis* possesses a *luxS* homologue and produces AI-2 (REF. 119). However, mutation of *luxS* in *P. mirabilis* str. BB2000 does not significantly affect swarming, virulence factor production, or survival in a mouse model, suggesting that AI-2 does not contribute to pathogenicity[119]. This lack of phenotype might indicate that *P. mirabilis* uses LuxS strictly as part of the activated methyl cycle, particularly as *P. mirabilis* str. HI4320 contains no clear homologue of the Lsr system for sensing and responding to AI-2. However, AI-2 produced by *P. mirabilis* might influence gene expression in other species that use this signalling molecule.

Putrescine has also been proposed as an extracellular signal that is capable of mediating cell–cell communication[120]. As putrescine is a component of the outer membrane for some *P. mirabilis* strains[121], the signalling capabilities of this molecule remain unclear. If *P. mirabilis* utilizes putrescine for signalling, the bacteria may respond to putrescine produced by other species or scavenged from the host. Indeed, *P. mirabilis* upregulates a putrescine transporter during experimental infection[67], although the importance of putrescine during UTIs is unknown.

Summary

Advances in our understanding of *P. mirabilis* pathogenicity have provided new insights into the regulation of swarm cell differentiation, the possible contribution of swarm cells during a UTI, unique aspects of *P. mirabilis* metabolism and iron acquisition, and previously unrecognized pathogenicity factors (summarized in FIG. 1 and TABLE 2). Despite these advances, many questions remain. There are many different fimbrial types encoded by *P. mirabilis*, and the binding specificity, role during infection and contribution to catheter colonization are unclear for many of these fimbriae. Investigations into *P. mirabilis* toxins will also be of interest, as only haemolysin and Pta have been well characterized to date, and there is ambiguity concerning the role of the T3SS. With respect to swarming, our knowledge of the regulatory network for sensing when conditions are favourable for swarming is incomplete, and the ability of *P. mirabilis* to coordinate swarming behaviour through cell–cell signalling remains an area of debate. What drives the rare event of swarm cell differentiation during a UTI and whether these isolated swarm cells contribute to infection are also unknown. Similarly, why *P. mirabilis* requires proticine, Ids factors and a T6SS to distinguish between strains and whether any of these systems are used for interspecies interactions are unclear.

The development of a vaccine for *P. mirabilis* UTIs is warranted owing to the increasing drug resistance among *P. mirabilis* isolates and the severe complications of infection. Vaccine development thus far has focused on using fimbrial subunits or the Pta autotransporter to reduce colonization and elicit a protective antibody response, but no vaccine strategy has yet provided complete protection against *P. mirabilis* UTIs in a mouse model[92,122–126]. The identification of novel virulence determinants and a better understanding of *P. mirabilis* pathogenicity will aid the design of effective vaccines. Furthermore, an understanding of the factors involved in catheter colonization, biofilm development and swarming will contribute to the development of therapeutics aimed at limiting the persistence of this troublesome species on catheters.

In light of the knowledge that many CAUTIs are poly-microbial, it is also important to consider how knowledge gained from single-species studies can be extended to polymicrobial infection. *P. mirabilis* clearly has the potential to facilitate entry of other species to the urinary tract and possibly to enhance their persistence, and it is therefore intriguing to speculate that therapeutics aimed at limiting *P. mirabilis* colonization may also impact the CAUTI burden caused by other species. Further investigation is necessary to determine how urinary tract pathogens interact during polymicrobial CAUTIs and to fully elucidate the impact of *P. mirabilis* on other uropathogens, as well as its contribution to the persistence and severity of polymicrobial CAUTIs.

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Glossary

Pyelonephritis	An infection of the kidney parenchyma
Urolithiasis	The formation of calculi (stones) in the kidney, bladder or urethra
Prostatitis	Inflammation of the prostate gland
Bacteriuria	The presence of bacteria in the urine
Signature-tagged mutagenesis	A high-throughput method for generating pools of bacterial strains that contain transposon insertions to disrupt genes: in this method each transposon has a unique tag
Nucleoid-associated protein	A protein that is associated with the region of a bacterial cell containing genetic material (the nucleoid) and that contributes to maintaining the supercoiled structure of the nucleic acid
Autotransporter	A bacterial outer-membrane protein that is produced as a single polypeptide, consisting of a passenger domain which is transported through the outer membrane and a β-barrel domain that anchors the protein to the outer membrane and facilitates transport of the passenger domain
Bowman's capsule	A cup-shaped double membrane in the kidney that filters blood to remove organic waste, excess inorganic salts and water, which are then concentrated into urine

O antigen	The bacterial cell wall antigen of lipopolysaccharide. O antigen is composed of repeating oligosaccharide subunits made up of 3–5 sugars
Phase variation	Reversible alteration in the expression of antigenic proteins on the cell surface (generally ON versus OFF) to produce a clonal population of phenotypically heterogeneous bacteria
GS–GOGAT system	A metabolic pathway that uses glutamine synthetase (GS), glutamine oxoglutarate aminotransferase (GOGAT) and ATP to assimilate ammonia into glutamate under nitrogen limitation. By contrast, when ammonia is abundant, this process is carried out by glutamate dehydrogenase in a one-step reaction that does not require ATP. The GS–GOGAT system is also known as the glutamate synthase cycle
Siderophores	Low-molecular-mass organic compounds with a high affinity for chelating or binding iron. These compounds are produced by microorganisms to scavenge ferric iron
Integrative and conjugative element	A self-transmissible mobile genetic element that resides within the chromosome of a host cell and can excise and transfer to a new host via conjugation
Yersiniabactin	A siderophore produced by *Yersinia* spp. and many other bacterial species

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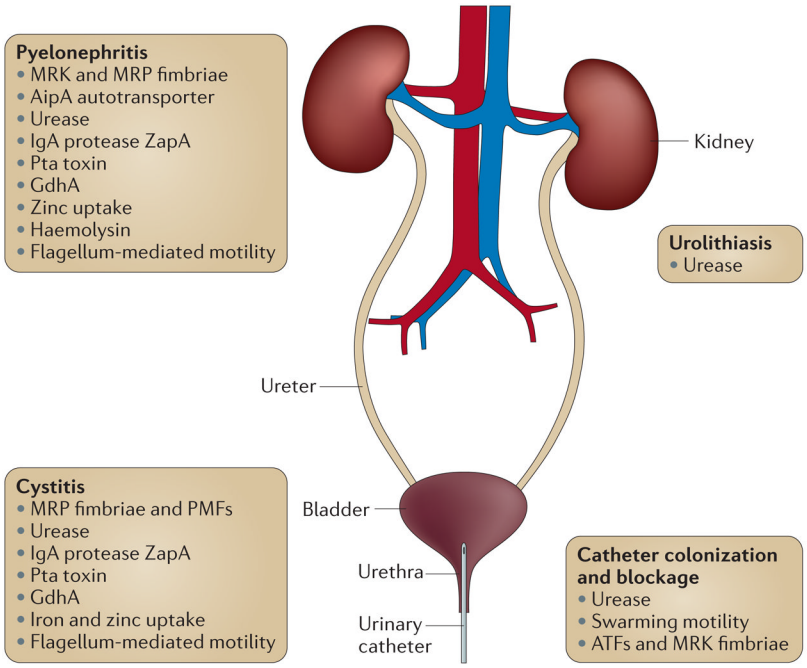

Figure 1. Ascending urinary tract infection and *Proteus mirabilis* virulence factors

An overview of key *Proteus mirabilis* virulence factors that contribute to catheter colonization and blockage, infection of the bladder (cystitis) and kidneys (pyelonephritis), and to the formation of urinary stones (urolithiasis). A full list of *P. mirabilis* pathogenicity factors and their contribution to infection is provided in TABLE 2. ATFs, ambient-temperature fimbriae; GdhA, glutamate dehydrogenase; IgA, immunoglobulin A; MRK, mannose-resistant *Klebsiella*-like; MRP, mannose-resistant *Proteus*-like; PMFs, *P. mirabilis* fimbriae; Pta, *Proteus* toxin agglutinin; ZapA, serralysin.

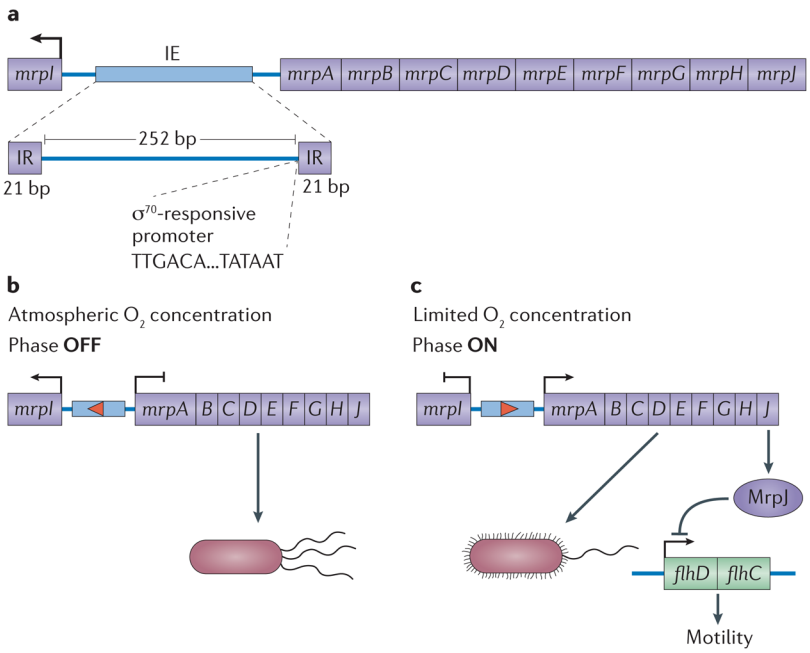

Figure 2. The mannose-resistant *Proteus*-like fimbrial operon: organization and phase variation

a. The mannose-resistant *Proteus*-like (*mrp* **Nature**) fimbrial operon consists of *mrpI*, an intergenic region containing an invertible element (IE), and *mrpABCDEFGHJ*. The 252 bp IE is flanked by 21 bp inverted repeats (IRs) and contains an RNA polymerase σ^{70}-responsive promoter that is predicted to drive transcription of *mrpABCDEFGHJ* when the IE is in the ON position, resulting in the formation of MRP fimbriae. A recombinase encoded by *mrpI* is divergently transcribed from the rest of the operon (using a separate promoter). When expressed, MrpI reverses the orientation of the IE and the promoter contained within it, driving phase variation. As MrpI is the sole recombinase in *Proteus mirabilis*[71], the IE can be phase locked by mutating *mrpI*. **b.** Aerobic conditions favour *mrpI* expression and the active conversion of fimbriate bacteria to the non-fimbriate form[77]. **c.** Reduced oxygen levels favour expression of the *mrp* operon, including *mrpJ*, which represses motility by inhibiting expression of the flagellar transcriptional regulator (*flhDC*) operon[77].

Table 1

Fimbriae of *Proteus mirabilis*

Type of fimbriae	Contribution to catheter colonization	Adherence capabilities *in vitro*	Contribution to UTI pathogenesis
ATFs	Possible	Unknown	Not associated
MRK fimbriae	Likely	Bowman's capsules (kidney)	Kidney colonization
MRP fimbriae	Unknown	Kidney tubular cells, and epithelial cells from urine	Bladder and kidney colonization, and reciprocal regulation of motility
NAFs	Unknown	Uroepithelial cells	Unknown
PMFs	Unknown	Uroepithelial cells	Bladder colonization

ATFs, ambient-temperature fimbriae; MRK, mannose-resistant *Klebsiella*-like; MRP, mannose-resistant *Proteus*-like; NAFs, non-agglutinating fimbriae; PMFs, *Proteus mirabilis* fimbriae; UTI, urinary tract infection.

Table 2

Contribution of *Proteus mirabilis* virulence determinants to urinary tract infections

Virulence determinants	Entry to the urinary tract		Ascending urinary tract infections		
	Catheter colonization	Swarming	Urine	Bladder	Kidneys
Fimbriae	ATFs and/or MRK fimbriae?	MrpJ represses expression of fimbriae	MRP fimbriae, PMI3001	MRP fimbriae and PMFs	MRK and MRP fimbriae
Adhesins	ND	ND	ND	PMI2575	AipA
Motility	Not relevant	FlgE *, FliF *, FliL	CheW *, FlaD	CheW *, FliF *, FlaD	CheW *, FlgE *, FliF *, FlaD
Regulators of gene expression	ND	DisA, HexA, Lrp, Rcs proteins, RppAB, RsmA, Umo proteins, WosA	AsnC *, HdfR *, HexA, NhaR *, UreR	AsnC *, HexA, HdfR *, UreR	AsnC *, HexA, NhaR *, UreR
Urease	Promotes colonization	Coordinately expressed	UreF *	UreC, UreF *	UreC, UreF *
Proteases and toxins	ND	Lon, HpmA, ZapA (coordinately expressed)	ZapA	Pta, ZapA	Pta, ZapA, U32 peptidase family protease *, HpmA?
Metabolic pathways	ND	AceE *, CyaA *, SdhC * (upregulated)	CarA *,CbbC *, CyaA *, Edd *, GuaB *, SdaA *, SdhC *	AceE *, CarA *, CyaA *, GdhA, GuaB *, SdhC *	AceE *, CarA *, CbbC *, CyaA *, Edd *, GdhA, GuaB *, SdhC *
Metal acquisition	ND	ZnuC	ZnuC	HmuR2, Nrp, Pbt, ZnuC, PMI0842	HmuR2, ZnuC,PMI0842, PMI2596
Other	ND	CpsF *, CysJ, DppA, WaaL	DppA, DsbA *, ExbD *, HemY *, MetN *, MrcA *, NrpG *, ParE *, PpiA *, PstC *, PstS *, SerC *, SurA *, YidA *, PMI1000 *, PMI1193 *, PMI1448 *, PMI3359 *, PMI3705 *	CpsF *, DppA, DsbA *, ExbD *, MrcA *, ParE *, PstC *, PstS *, SurA *, TaaP, PMI1193 *, PMI2014 *	CpsF *, CysJ, DppA, DsbA *, ExbD *, MetN *, MrcA *, NrpG *, ParE *, PpiA *, PstC *, PstS *, SufI *, SurA *, PMI0283 *, PMI1184 *, PMI1193 *, PMI3705 *

ATFs, ambient-temperature fimbriae; GdhA, glutamate dehydrogenase; HpmA, haemolysin; HmuR2, haemin receptor; Lrp, leucine-responsive regulator; MRK, mannose-resistant *Klebsiella*-like; MRP, mannose-resistant *Proteus*-like; ND, no data; Pbt, proteobactin; PMFs, *Proteus mirabilis* fimbriae; Pta, *Proteus* toxic agglutinin; Rcs, regulator of colanic acid capsule synthesis; Umo, upregulator of flagellar master operon; Ure, urease operon; ZapA, serralysin; ZnuC, zinc uptake protein C.

* Identified by signature-tagged mutagenesis.