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REVIEW

Proteus spp. as Putative Gastrointestinal Pathogens

Amy L. Hamilton,[a,b] Michael A. Kamm,[a,b] Siew C. Ng,[c] Mark Morrison[d]

[a]Department of Gastroenterology, St Vincent's Hospital, Melbourne, Australia

[b]Department of Medicine, The University of Melbourne, Melbourne, Australia

[c]Department of Medicine and Therapeutics, Institute of Digestive Disease, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Science, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

[d]The University of Queensland Diamantina Institute, Faculty of Medicine, Translational Research Institute, Brisbane, Australia

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SUMMARY *Proteus* species, members of the *Enterobacteriaceae* family, are usually considered commensals in the gut and are most commonly recognized clinically as a cause of urinary tract infections. However, the recent identification of *Proteus* spp. as potential pathogens in Crohn's disease recurrence after intestinal resection serves as a stimulus to examine their potential role as gut pathogens. *Proteus* species possess many virulence factors potentially relevant to gastrointestinal pathogenicity, including motility; adherence; the production of urease, hemolysins, and IgA proteases; and the ability to acquire antibiotic resistance. Gastrointestinal conditions that have been linked to *Proteus* include gastroenteritis (spontaneous and foodborne), nosocomial infections, appendicitis, colonization of devices such as nasogastric tubes, and Crohn's disease. The association of *Proteus* species with Crohn's disease was particularly strong. *Proteus* species are low-abundance commensals of the human gut that harbor significant pathogenic potential; further investigation is needed.

KEYWORDS bacteriology, Crohn's disease, *Enterobacteriaceae*, gastrointestinal disease, infections, inflammatory bowel disease, *Proteus*

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Address correspondence to Michael A. Kamm, mkamm@unimelb.edu.au.

INTRODUCTION

Proteus species are members of the *Enterobacteriaceae* family of bacteria. Most commonly, they are recognized clinically as a cause of urinary tract infections. Although *Proteus* spp. are typically considered commensals in the gastrointestinal (GI) tract, their abundance as a proportion of the microbial community is very low (<0.05%) (1). As a result, their detection in disease states using 16S profiling, and possibly metagenomics, may have rendered *Proteus* spp. undetectable due to bioinformatic abundance thresholds.

The recent identification of *Proteus* spp. as potential pathogens in Crohn's disease recurrence after intestinal resection (2, 3) serves as a stimulus to examine their potential role as gut pathogens. This review aims to provide an overview of the genus *Proteus* in terms of its known virulence factors as well as to collate the evidence surrounding the role of *Proteus* spp. in the pathophysiology of gastrointestinal diseases.

CHARACTERISTICS OF THE *PROTEUS* GENUS

Proteus spp. are Gram-negative bacteria belonging the *Enterobacteriaceae* family and are common commensals of the gastrointestinal microbiota (4). The first isolates were reported and characterized by Hauser in the late 19th century (5). The genus is currently comprised of *Proteus mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri*, *P. terrae*, and *P. cibarius*, along with the unnamed genomospecies 4, 5, and 6 (6–10). In humans, all current members of the genus, except for *P. cibarius* and *P. terrae*, have been isolated from clinical specimens (4, 5, 7, 9, 11). Typically, the human gut is colonized by various combinations of *P. vulgaris*, *P. mirabilis*, and *P. penneri*, but they comprise less than 0.05% of the gut microbiota of healthy subjects (Fig. 1) (1, 12). While *Proteus* spp. are widely recognized as pathobionts and the gut is the reservoir of these bacteria, the research focus on this genus has been on their role in urinary tract infections rather than intestinal manifestations (13–15).

Many recent studies of the gut "microbiome" in health and disease have revealed that there are gross alterations in the relative proportions of key bacterial taxa associated with active disease, which is generically referred to as "dysbiosis." One of the hallmarks of dysbiosis in the inflammatory bowel diseases (IBDs) is the population expansion of the phylum *Proteobacteria*, specifically the *Enterobacteriaceae* (16). Other genera within the *Enterobacteriaceae* family, such as *Escherichia*, *Shigella*, *Salmonella*, and *Klebsiella*, have received due attention in this regard, while *Proteus* has not been comprehensively investigated.

Pathogenic Features

Proteus species are short (1.5- to 2-μm) straight rods that demonstrate dimorphism as "swimming" and "swarming" forms, as do some other members of the *Enterobacteriaceae* family (17). Swimmer cells predominate in liquid environments as single cells with 4 to 10 peritrichous flagella (Fig. 2, bottom right) (5, 14). The swarming behavior of *Proteus* species results in a characteristic bull's-eye pattern on a plate culture, as a result of a cyclic process of swarming and consolidation phases (18). When *Proteus* cells are placed in a viscous environment or on a solid surface, they undergo differentiation to filamentous, multinucleated, highly flagellated swarmer cells (Fig. 2, top and bottom left). Following this differentiation, a consolidation phase occurs, where the cells revert to a shorter morphotype, and metabolic preparation occurs prior to the next swarming cycle (18).

Proteus mirabilis undergoes swarming differentiation at much higher concentrations of agar (1.5 to 2%) than other swarming bacteria (19). When *Proteus* spp. swarm, there is a dramatic increase in the production of secreted proteins, including virulence factors such as the protease ZapA (17, 20, 21). *In vivo*, swarmer cells have been demonstrated in mouse models of ascending urinary tract infection only infrequently, with the occasional swarmer cell being isolated from the kidneys and bladder stones of infected mice (22, 23). The swarming phenotype can occur under both aerobic and anaerobic conditions (24) and can be induced by the concentration of amino acids, in particular

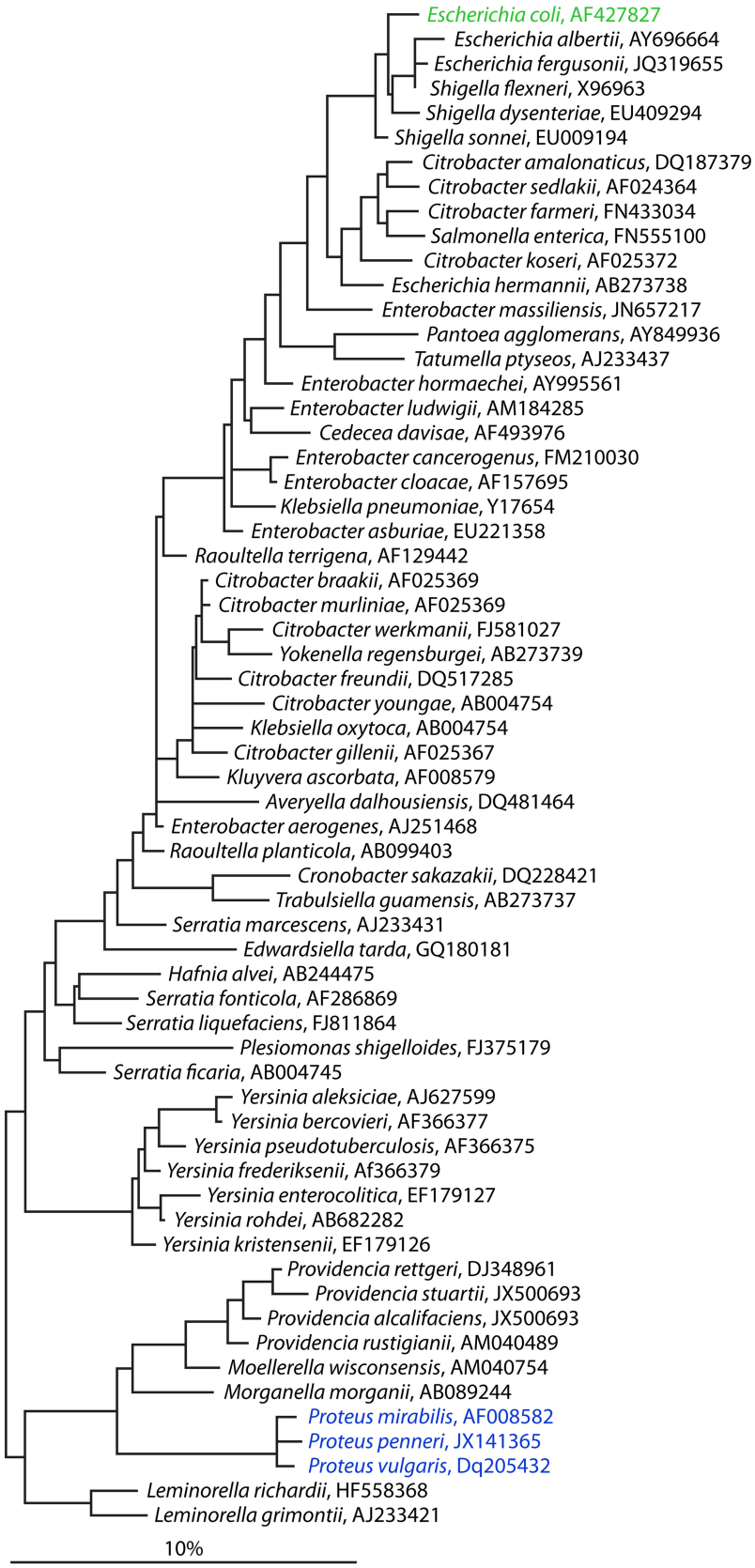

FIG 1 Phylogenetic tree showing the species from the *Enterobacteriaceae* family that colonize the human gastrointestinal tract. GenBank accession numbers of the16S rRNA gene sequences are provided for each species, and the family names are indicated. *E. coli* is highlighted in green, and the *Proteus* genus is shown in blue. (Reproduced from reference 133.)

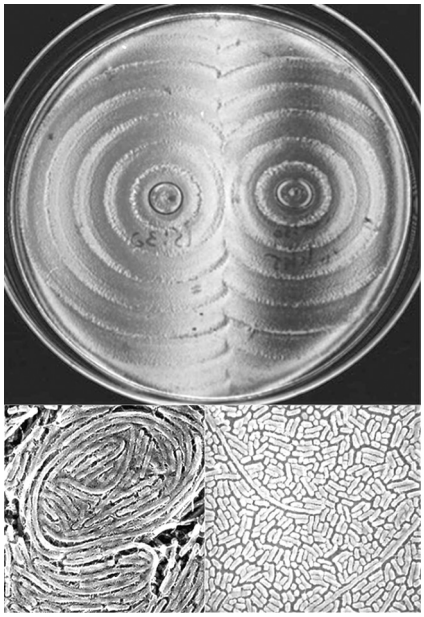

FIG 2 (Top) Strain of *P. mirabilis* inoculated twice, 1 h apart, demonstrating the macroscopic characteristic bull's-eye pattern produced by periodic swarming. (Reproduced with permission from reference 134.) (Bottom left) Interacting *P. mirabilis* swarmer cells; (bottom right) combination of swimmer and swarmer cells within a biofilm. (Both panels reproduced from reference 135 with permission from Elsevier.)

glutamine (25, 26). It has also been demonstrated that a more acidic pH, as might be expected to occur in the proximal small bowel and cecum, dramatically increased swarming behavior (27–29). Swarming has been shown to be an important factor in intracellular invasion and persistence, with 15- to 20-fold more swarmer cells than swimmer cells being capable of the intracellular invasion of uroepithelial cells (30). There is some evidence that swarming *Proteus* strains are more invasive in urinary tract mouse models than are swarm-defective mutant strains (22). Furthermore, a number of metabolites present in the intestinal tract have been shown to promote swarming, including choline, glutamine, and the most abundant polyamine in the gut, putrescine (25, 31–33). While we cannot yet conclude definitively that swarming behavior occurs in the gut *in vivo*, the combination of a viscous surface (such as the gut mucosa), the high availability of glutamine and polyamines such as putrescine (26, 31, 33), and electron acceptors for anaerobic respiration such as choline (32) makes it likely that the gut environment may be permissive for swarming.

Adhesion and mucosal attachment. Adhesion to epithelial surfaces is essential for the pathogenesis of *Proteus* infections in both the urinary and gastrointestinal tracts. Sequencing of *Proteus mirabilis* strain HI4320 revealed 17 fimbrial gene sets (operons), more than any other bacterial genome currently characterized (34–36). Six of the fimbrial types that *Proteus mirabilis* can produce have been characterized (Table 1), including mannose-resistant *Proteus*-like fimbriae (MR/P fimbriae), mannose-resistant *Klebsiella*-like fimbriae (MR/K fimbriae), nonagglutinating fimbriae (NAF) (also known as uroepithelial cell adhesin [UCA]), ambient-temperature fimbriae (ATF), *P. mirabilis* P-like pili (PMP), and *P. mirabilis* fimbriae (PMF) (5, 36). These fimbriae and adhesins play a major role in the formation of bacterial biofilms, a common complication of both urinary and gastrointestinal instrumentation (37). It is likely that the MR/P and NAF/UCA fimbrial types are most important in gastrointestinal pathogenesis, due to their role in epithelial adhesion (38–41).

A comparison of the 17 individual chaperone-usher fimbrial operons across the 7 sequenced *P. mirabilis* strains as well as 58 clinical isolates showed 99% conservation in 13 of 17 fimbrial operons, demonstrating that these genes are highly conserved across

TABLE 1 Fimbriae and pili expressed by *Proteus* species[a]

Fimbrial type	Structure	Characteristic(s)	Contribution(s) to gastrointestinal pathogenicity	Reference(s)
MR/P	7-nm–8-nm, "thick" channeled fimbriae	Important for epithelial cell adhesion	Expression may allow mucosal cell adhesion in the gastrointestinal tract and contribute to intestinal persistence	38, 39, 69, 136
		MR/P expression undergoes phase variation, allowing for a molecular "switch" that turns on/off the expression of the *mrp* operon depending on the environment and oxygen availability, e.g., on in bladder colonization and off in kidney colonization	The repeating structures of flagellin proteins contribute to immunogenicity in the gut	
		MR/P fimbriae are potent immunogens and potential vaccine candidates		
		MR/P$^+$ *Proteus* strains are more genotoxic and cytotoxic to bladder/kidney-derived epithelial cells than MR/P$^-$ mutants	MR/P$^+$ *Proteus* strains are also likely more cytotoxic to intestinal epithelial cells	
MR/K	4-nm–5-nm, "thin" nonchanneled fimbriae	Expression is more common in *P. penneri* strains than in *P. mirabilis* strains	*P. penneri* has not been linked to gastrointestinal pathogenicity	42, 137
NAF/UCA	4-nm, "thin" nonchanneled fimbriae	Recognizes glycolipids, including asialo-GM1, asialo-GM2, lactosyl ceramide, and galectin-3		40, 41, 138
		Important for epithelial cell adhesion and colonization		
		Not present in *P. vulgaris*		
ATF		Highly expressed at 23°C, moderately expressed at 37°C	The structural subunit AtfA has significant homology with the major subunit of type 1 fimbriae from other enteric pathogens, such as *S. enterica* serovar Typhi	37, 40, 139–141
		Not considered to contribute to UTI or biofilm formation; may be more important for enteric colonization and environmental survival		
		Believed to be important for survival and persistence outside a mammalian host, at room temp		
PMP		Identified in uropathogenic *P. mirabilis* strains isolated from dogs		142
		Not present in *P. vulgaris*		
		PmpA fimbrial subunits show high homology to P-fimbriae from uropathogenic *Escherichia coli*		
		Cell surface assembly has not been confirmed		
PMF		Important in biofilms and responsible for the formation of higher-volume biofilms		37

[a]UTI, urinary tract infection.

strains isolated from various clinical sites (36). Of these, it is likely that at least two, and up to six, of the characterized fimbriae can be assembled on the cell surface at any one time (36, 42).

The regulation of motility and the expression of adhesion factors are tightly coupled; of the 17 fimbrial operons, at least 10 gene clusters possess a homolog of the *mrpJ* gene, a repressor of motility (43). MrpJ downregulates the flagellar master regulator *flhDC* via binding to the promoter sequence (43). When these cells revert back to the swimming morphology, the expression of MR/P and NAF fimbriae returns (37, 44).

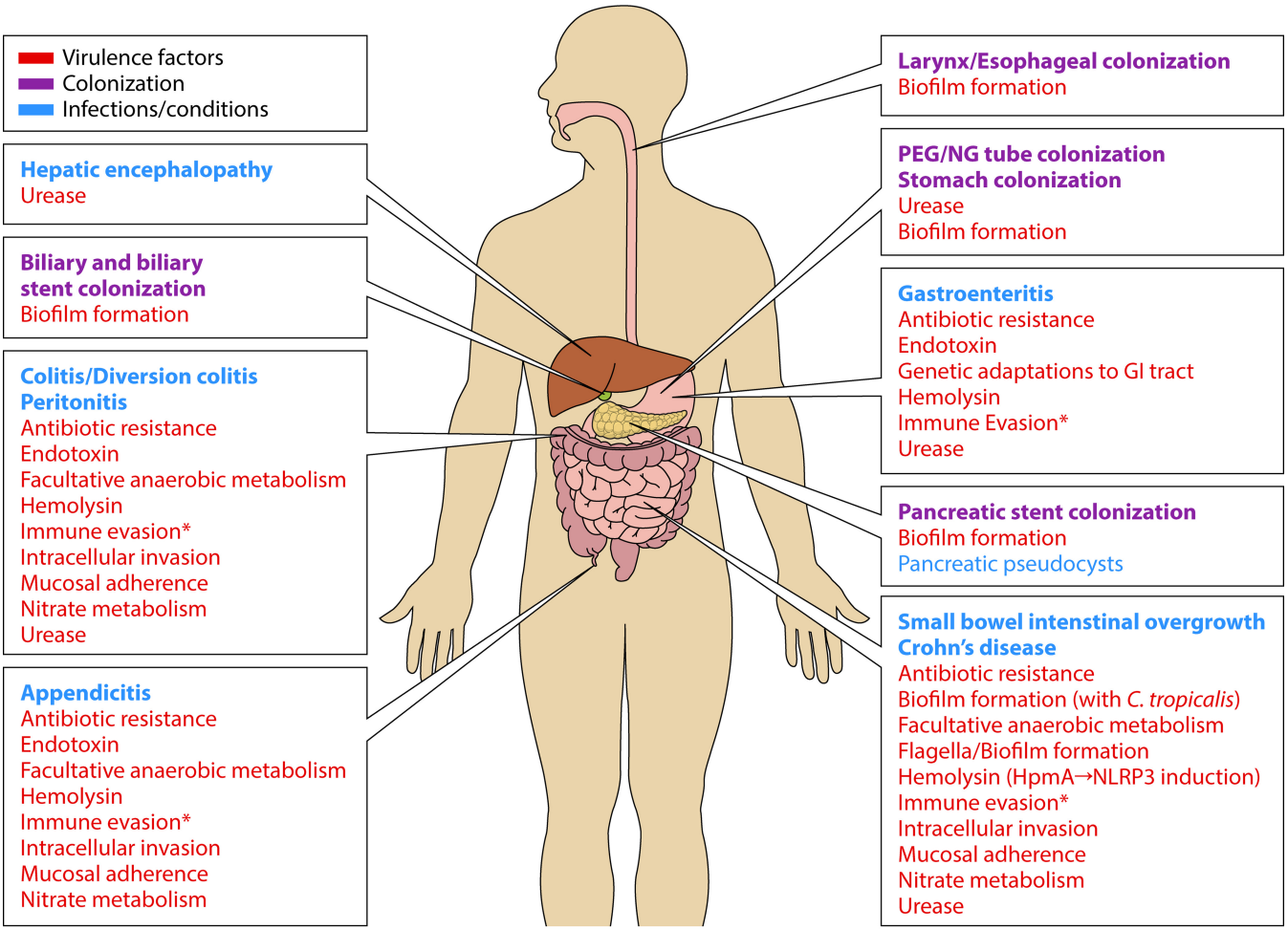

FIG 3 Potentially important *Proteus*-related virulence factors in relation to anatomical disease location and disease. *, immune evasion includes the production of the ZapA metalloprotease, O-antigens, and flagellin variation.

Furthermore, the induction of MR/P fimbriae appears coupled to the availability of oxygen (45). MR/P fimbriae are phase variant and can be "switched" on or off by a site-specific DNA recombinase (MrpI) that inverts a promoter region flanked by inverted repeats. This "invertible element" switches on or off the MR/P fimbrial operon, depending on the environmental conditions. There appears to be a growth advantage to MR/P fimbrial expression under low-oxygen conditions, such as those that would be present in the intestinal tract, likely contributing to the adhesiveness and persistence of *Proteus* species in the gut (45). The adaptation of *Proteus* species to mucosal surfaces by way of both fimbrial expression (for adherence) and swarming motility could increase the invasiveness, persistence, and pathogenicity of these species in the gut (Table 1 and Fig. 3).

Urease. The urease enzyme is a microbiological adaptation to metabolize urea, the most abundant nitrogenous waste product of human metabolism (46). Urease generates ammonia and carbonic acid as end products, with this ammonia providing a rich source of nitrogen for microbial metabolism in the gut (47). Urease confers a survival advantage to *Proteus* by providing nitrate for nonfermentative anaerobic respiration. This in turn promotes the population expansion of the *Enterobacteriaceae* (including *Proteus*) (48, 49). Additionally, as with *Helicobacter pylori*, the presence of this enzyme likely confers a survival advantage through increasing the local pH of the environment, allowing urease-positive organisms to survive in more-acidic environments such as the

TABLE 2 Cell culture lines capable of intracellular uptake of *Proteus mirabilis*

Cell line	Cell type	Reference(s)
CaCo-2[a]/CaCo-2BBe[a]	Colorectal adenocarcinoma/C2BBe1 (brush border-expressing cells) Models for apical microvilli and tight junctions	60, 62, 132, 143, 144
HT29[a]	Enterocytes, undifferentiated or differentiated (polarized, in the absence of glucose)	60, 62, 132, 143, 145
HT29-18N2[a]	Mucus-secreting differentiated cells	60, 132
HT29-FU[a]	Mucus-secreting differentiated cells	
HT29-MTX[a]	Mucus-secreting differentiated cells	
INT407[a]	Embryonic intestinal epithelial cells (possibly HeLa-contaminated line)	61
HCT-8[a]	Ileocecal colorectal adenocarcinoma	
T24	Transitional cell bladder carcinoma	
HeLa	Cervical carcinoma	146
Vero	African green monkey kidney cells	
L-929	Mouse fibroblasts	
Human blood lymphocytes		

[a]Gastrointestinal cell line.

upper digestive tract. *Proteus* spp. have a wide pH range at which they are able to grow, from pH 5 to 10, with an optimal pH of 7 to 8 (9). Urease activity has been confirmed for *P. mirabilis*, *P. vulgaris*, and *P. penneri*, and production is regulated by both the environmental concentration of the substrate (urea) and increased chromosomal transcription in swarming cells (50–52).

Colonization of the upper gastrointestinal tract by urease-producing *Proteus* species may cause occasional false-positive results for the [^{13}C]urea breath test (UBT), especially if the patient has already been treated with proton pump inhibitors and antibiotics for *H. pylori* eradication (53). Given that urease is a known contributor to the pathogenesis of *H. pylori*-mediated damage in the upper gastrointestinal tract, it may also contribute to the pathogenicity of *Proteus* spp. in the gut in the setting of environmental perturbations, such as changes in pH.

Hemolysins. The *Proteus* genus produces two distinct cytotoxic hemolysins, HpmA and HlyA (51, 54, 55). *P. mirabilis* and most *P. vulgaris* strains produce only HpmA, most *P. penneri* strains produce HlyA, and a few isolated *P. vulgaris* strains produce both HpmA and HlyA (54, 56, 57). HpmA has been shown to lyse erythrocytes, bladder epithelial cells, B-cell lymphoma cells, and monocytes, while HlyA can lyse erythrocytes, fibroblasts, and neutrophils (55, 56). HpmA is a cell-associated hemolysin, encoded on the *hpm* locus along with HpmB (an activator and chaperone of HpmA). The expression of these hemolysin proteins is tightly coupled to the swimming-swarming cycle, with swarming cells being 18-fold more cytotoxic than swimmer cells (30). HpmA has also been shown to lyse erythrocytes under anaerobic conditions and at multiple temperatures (58). The contribution of hemolysins to gastrointestinal pathogenesis may occur through the lysis of innate immune cells, the induction of the NOD-like receptor protein 3 (NLRP3) inflammasome, and downstream interleukin-1β (IL-1β) release (59).

Intracellular invasion and persistence. Intracellular invasion by *Proteus mirabilis* has been assessed mainly by using cellular invasion assays in cell lines ranging from uroepithelial cells to colonic cell types.

In a cell-based urinary tract model, swarming cells were 15-fold (0.18% intracellular entry) more invasive to uroepithelial cells than swimmer cells (0.012% intracellular entry) (30). After the invasion of uroepithelial cells, swarmer cells start to divide, develop septums, and differentiate back to an average of 50 to 300 swimmer cells within the cytoplasm (30). *P. mirabilis* has been identified as being intracellularly invasive in a number of cell lines (summarized in Table 2). There are differences in the intracellular invasion and uptake pathways depending on the cell type, with intracel-

TABLE 3 Substrates degraded by *Proteus mirabilis* ZapA metalloprotease

Substrate	Locations	Reference(s)
Structural cell components		
Actin, β-tubulin, fibronectin, collagen, laminin	All cells	21
Innate immune components		
Immunoglobulins (IgA1, IgA2, IgG)		21, 147
Complement proteins (C1q, C3)		21
Defensins (human beta defensin 1)	Epithelial surfaces, small bowel, and large bowel	21, 148, 149
Cathelicidin peptide LL-37	Epithelial surfaces, wounds	21, 150

lular vacuoles containing *P. mirabilis* having a single membrane within urothelial cells and a double membrane within intestinal cells (60, 61). These mechanisms may contribute to effective intracellular colonization (cytoplasmic colonies), evasion of the host immune system, and resistance to antibiotics (60).

Translocation of Gram-negative *Enterobacteriaceae* (including *P. mirabilis*) has been demonstrated *in vitro*, in terms of both uptake and intracellular persistence. By using Caco-2 and HT-29 cells (enterocyte cell lines), it was demonstrated that *P. mirabilis* not only is invasive but also can survive intracellularly for $\geq$20 h without affecting the viability of enterocytes (62). The origin of the *P. mirabilis* isolate influences the invasiveness of *Proteus* species in laboratory studies, with fecal isolates showing higher invasion efficiencies (61).

Proteus mirabilis is a common cause of pathogenic infection of bladders augmented with bowel segments (enterocystoplasty). A study investigated the colonization of colonic cell lines by *P. mirabilis* as a model for stone formation in enterocystoplasty. The HT29-18N2 cell line (a mucus-secreting intestinal goblet cell subclone of HT-29 [63]) underwent widespread cellular destruction, with extensive intracellular bacterial colonies. Furthermore, upon confocal microscopy, there was colocalization of *P. mirabilis* and both human colonic mucin MUC2 and human gastric mucin (MUC5AC) (63). There is experimental evidence that *Proteus* spp. can invade at least the outer mucous layer of the gut and the inner layer to the epithelial surface if there are certain immune defects. These defects include the absence of the Lypd8 protein or defects in innate immunity (the T-bet$^{-/-}$ $\times$ Rag2$^{-/-}$ colitis [TRUC] mouse model) (64–66). *Proteus mirabilis* has many invasive characteristics; however, they remain to be directly characterized in the context of gastrointestinal disease.

Immune evasion. In addition to intracellular invasion, *P. mirabilis* also secretes an extracellular metalloprotease (ZapA) that has been shown to degrade a wide range of substrates (Table 3). This enzyme has a key role in the evasion of innate immune destruction by *Proteus* species by the proteolytic digestion of secretory IgA, IgG, and other cellular components in the urinary and gastrointestinal tracts (20, 21). Kerr et al. showed ZapA cleavage products (IgA and IgG) in the urine of patients with *P. mirabilis*, reflecting protease activity *in vivo* (67). The expression of this protein has been shown to be correlated tightly with cellular differentiation from swimmer to swarming cells by *P. mirabilis*, with increased transcription of the ZapA locus (20). ZapA hydrolyzes human β-defensin 1, a constitutively expressed innate immune antimicrobial peptide that is expressed in the colonic epithelium, as well as secretory IgA (20, 21). The expression of ZapA in the gut may provide a survival advantage to *Proteus* spp. by perturbing host mucosal immune responses, increasing the likelihood of persistent colonization. The genomic rearrangements encoding the flagellin proteins of *Proteus mirabilis* and the antigenic variability of *Proteus* species lipopolysaccharide (LPS) (both reviewed below) also contribute to evasion of the host immune system (Fig. 3).

Endotoxin and flagellins. As a Gram-negative pathogen, *Proteus* species possess intrinsic characteristics similar to those of other *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, including the production of flagellin

and the proinflammatory cell wall component LPS (68, 69). A constituent of LPS, endotoxin (lipid A), is highly immunostimulatory (70). Endotoxin is sensed by the innate immune system (specifically Toll-like receptor 4) and activates the downstream signaling of NF-κB (71). This, in turn, triggers a proinflammatory cascade (mediated by tumor necrosis factor alpha [TNF-α]) that can lead to acute sepsis (72, 73).

In addition to lipid A, *Proteus* species LPS includes O-antigens (polysaccharides that are repeating oligosaccharide units, each made up of 2 to 8 sugar residues) that are highly structurally diverse. Approximately 80 O-antigen serogroups have been reported, derived from a total of 60 O-antigen gene clusters (11, 73). Antibodies to O-antigens are not uncommon in human sera, for example, 25% of blood donors have anti-*P. mirabilis* antibodies to the O36 serogroup, which is just one of the many serogroups (74). The expression levels of virulence factors, such as urease, proteases (ZapA), and hemolysin, can vary significantly between the O-antigen serogroups, with negatively charged O-polysaccharide serogroups having higher ureolytic, proteolytic, and swarming activities (75). This heterogeneity of surface structures occurs across all *Proteus* species.

In addition, bacterial flagellins, the repeating protein subunits from which flagella are built, are highly immunogenic due to their three-dimensional structure (76). Bacterial flagellin is sensed by Toll-like receptor 5, which activates a number of downstream inflammatory pathways, including MyD88 (72, 76, 77). Recombination of the flagellin genes *flaA* and *flaB*, leading to hybrid flagellin proteins with significant antigenic variation, also contributes to innate immune evasion by *Proteus* spp. (78, 79) These features contribute to the overall pathogenicity of *Proteus* spp. via stimulation of the host innate immune system by bacterial products (Fig. 3).

Conjugation, plasmid acquisition, and antibiotic resistance. *Proteus* species are inherently antibiotic resistant. Resistance to polymyxins is mediated via covalent modifications of lipid A (80). The substitution of L-arabinoso-4-amine for either the Kdo residue or the ester-linked lipid A phosphate moiety increases the overall charge of the usually negative LPS to zero, reducing the binding of cationic polymyxins (69, 80, 81). They also possess intrinsic resistance to colistin, tigecycline, and tetracycline (82). Sequencing of *P. mirabilis* strain HI4320 demonstrated the presence of genes for a conjugal transfer pilus, which allows the horizontal genetic transfer of plasmids encoding antibiotic resistance (35).

While most species of *Proteus* remain sensitive to a range of antibiotics, increasing rates of acquired antibiotic resistance in the *Enterobacteriaceae* are a growing problem (83). In 2007 to 2008, observations of *P. mirabilis* strains acquiring *Salmonella* genomic island 1 (SGI1) were reported by groups in China and Palestine (84). SGI1 is a mobile genomic element first identified in *Salmonella* Typhimurium that integrates into the recipient chromosome and carries multiple genes encoding resistance to streptomycin, trimethoprim, tetracycline, sulfonamides, chloramphenicol, fluoroquinolones, and a broad spectrum of β-lactam antibiotics (84). A further SGI1-positive *P. mirabilis* strain (NKU) has also been identified in Europe (84). Recently, an SGI1-positive *P. mirabilis* strain acquired a plasmid containing the New Delhi metallo-β-lactamase 1 gene, leading to the identification of extensively drug-resistant (XDR) *P. mirabilis* strain PM58, which is resistant to all antibiotics used for *Enterobacteriaceae* except aztreonam (82). A recently sequenced *P. mirabilis* isolate (NO-051/03) from a patient with a soft tissue infection in Europe had acquired the resistance genes for trimethoprim, β-lactams, phenicols, sulfonamides, and aminoglycosides (85).

In gastrointestinal disease, the antibiotic sensitivity profile of *Proteus* species is relevant to pathogenicity under conditions that may be exacerbated by antibiotic perturbation of the gut microbiome. Given the potential for a "bloom" of *Enterobacteriaceae* (including *Proteus* species) both in the presence of an inflammatory process (86) and with a perturbation of the enteric environment via surgery (87), the use of antibiotics should be considered a possible potentiating factor.

Vaccine Candidates

A full review of the treatment of *Proteus* infection is outside the scope of this review.

Purified MR/P fimbrial proteins have been tested for their antigenic potential as vaccine candidates (88, 89). There has been success by using the intranasal delivery of MrpH, the fimbrial tip adhesin of *P. mirabilis* (88). A fusion protein comprised of MrpH and mannose-binding protein delivered intranasally provided 75% protection from *P. mirabilis* ascending urinary tract infection in a mouse model (88). A fusion protein comprised of MrpH from *P. mirabilis* and FimH from uropathogenic *E. coli* delivered intranasally with monophosphoryl lipid A (as an adjuvant) induced robust IgG and IgA responses in mice (89). A clinical study of an inactivated bacterial cell suspension of four bacterial species, including a strain of *P. vulgaris*, was trialed in 159 patients with a history of recurrent urinary tract infections, compared with 160 patients maintained on prophylactic sulfamethoxazole-trimethoprim (90). The group of patients who received the vaccine had a mean number of 0.36 urinary tract infections in 3 months, versus 1.60 for those receiving sulfamethoxazole-trimethoprim ($P < 0.0001$) (90).

PROTEUS SPECIES AS GASTROINTESTINAL PATHOGENS

Colonization by *Proteus* Species

Proteus species are known human digestive tract commensal organisms with abundances varying according to location (Fig. 3). Colonization occurs early. Infants from Sweden and Pakistan were assessed for *Enterobacteriaceae* based on mode of delivery (vaginal versus cesarean) and breastfeeding behavior (91). Cesarean births in Pakistan were associated with *Proteus* species colonization within 3 days, with 11 of 21 cesarean-delivered and 1 of 9 vaginally delivered infants being positive for *Proteus* spp. ($P = 0.049$) (91).

Zilberstein et al. cultured mucosal samples from the upper ($n = 20$) and lower ($n = 24$) digestive tracts of healthy controls. *Proteus* species were present in 8% of gastric samples, 46% of duodenal and jejunal samples, 19% of ileal samples, 13% of cecal samples, and 38% of samples from the transverse colon (12).

Müller compared the recovery of *Proteus* species from the stool specimens of 1,422 healthy subjects. *P. mirabilis* was identified in 2.7% of healthy subjects, which is a probable underestimate as the epithelial preference of *P. mirabilis* means that it is likely undersampled in stool specimens (92). *Proteus penneri* and *P. vulgaris* were isolated from 0.9% and 4.2%, respectively, of the same population (92). A smaller culture-based study of 60 patients with gastrointestinal symptoms who tested negative for parasites demonstrated a colonization rate of 33% (20/60 patients) for *P. vulgaris* (93).

Proteus species, especially *P. mirabilis*, are often antibiotic resistant, conferring a survival advantage when colonizing the gastrointestinal tract. In a study of multiple-drug-resistant Gram-negative bacteria in the rectum of long-term-care patients, 52 drug-resistant strains were identified, 15 of which were *P. mirabilis* strains (94). A total of 87% of patients colonized with *P. mirabilis* were also cocolonized with at least one other resistant Gram-negative bacterium (range, 1 to 4 species; median, 2 species). Of the 15 patients with a resistant *P. mirabilis* strain, only 1 patient spontaneously cleared the organism, compared to 30 to 75% clearance for other bacterial species ($P = 0.007$ for clearance of other species versus *P. mirabilis*, as determined by a log rank test), demonstrating the ability of *P. mirabilis* to cause more-persistent colonization than other Gram-negative species (94). Of the 15 *P. mirabilis* strains recovered, 13 were genetically distinct, demonstrating the heterogeneity of *P. mirabilis* populations between patients within the same health care facility (94).

Proteus spp. in Gastroenteritis

Proteus species have been associated with infectious gastroenteritis. In a comparison of 1,271 patients with diarrhea, *P. mirabilis* was more prevalent in patients with diarrhea (10.8% in affected cases versus 2.7% in healthy subjects; $P < 0.001$) (92). However, that study did not take into account the possible administration of antibiotics to affected patients or the potential bystander or overgrowth effect (92). *P. mirabilis* has also been

associated with foodborne gastroenteritis in an outbreak in Beijing, China, associated with the consumption of stewed pork (95). Shi et al. investigated genetic adaptation to the digestive tract of *P. mirabilis* species isolated from the vomit and feces of patients, obtained at the time of a foodborne outbreak (96). When three clinical isolates were compared to four local and reference strains of *P. mirabilis*, obtained from food, a healthy subject, and two patients with urinary tract infections (including reference strains HI4320 and BB2000), there was evidence of strain-level genetic adaptation to the digestive tract (96). All seven isolates harbored drug resistance genes, but only the three isolates (one from vomit, one from stool, and one from infected food) contained digestive tract toxicity genes, including one with a complete type 4 secretion system (T4SS) not identified previously in *P. mirabilis*. Active horizontal gene acquisition has been demonstrated for *Proteus mirabilis*, including a protein from *Yersinia enterocolitica*, a known GI pathogen and member of the *Enterobacteriaceae* family (96). In summary, *Proteus* species can be linked to diarrheal states, but their primary pathogenic role has not been confirmed.

Proteus spp. in the Upper Gastrointestinal Tract

Colonization of the upper gastrointestinal tract, including the esophagus and stomach, by *Proteus* species in infants and older adults has been reported, often associated with instrumentation of the oropharynx (97–101). In 13 infants with feeding tubes without gastrointestinal symptoms, *Proteus* was isolated from the throat in 8% of patients, from the gastric juice in 15% of patients, and from the duodenal fluid in 8% of patients (98). In elderly patients with nasogastric feeding tubes (NGTs), *Proteus* species were isolated from the oropharynx in 24% of patients and from gastric fluids in 26% of patients (100). In another study, colonization of the oropharynx with *Proteus* species was present in 13% of patients with percutaneous endoscopic gastrostomy (PEG) and 21% of patients with NGTs, and colonization of the stomach was present in 4% and 23% of the same patients, respectively (99).

Association with Hepatobiliary Disease

Early culture-based surveys of patients undergoing biliary surgery showed the occasional isolation of *Proteus* species from the biliary tract (13% of bile samples) (102). *P. mirabilis* has also been recovered from bile obtained during endoscopic retrograde cholangiopancreatography (ERCP) in 6% of cultured samples (103).

In a metagenomic analysis using multitag pyrosequencing, the rectosigmoid mucosal community of healthy individuals was compared with that from patients with cirrhosis. Patients with cirrhosis had an elevated proportion of *Proteus* species compared with controls; the relative abundance in healthy controls was 0.0%, versus 0.1% in patients with cirrhosis ($P < 0.00001$) (104). Urease-producing microbes, such as *Proteus* spp., in the gut are known to contribute to the pathogenesis of hepatic encephalopathy through the breakdown of urea to ammonia and carbonic acid (104, 105).

In a series of patients undergoing liver resection, *Proteus vulgaris* bacteremia was identified in two patients, and polymicrobial infections were identified in eight patients (106). In the hepatobiliary tract, *Proteus* spp. are an uncommon cause of infection and are usually related to surgical interventions, such as ERCP or abdominal surgery. Implantable devices, such as stents, are also at risk of colonization and biofilm formation (107).

Pancreatic Disease

There are isolated reports of *Proteus* species infections of the pancreas, including a patient with a large infected pancreatic pseudocyst compressing the common bile duct. The cystic contents were polymicrobial, including *Proteus vulgaris*, *Morganella morganii*, *Stenotrophomonas maltophilia*, and *Pseudomonas aeruginosa* (108).

Proteus species were found in biofilms that form on biliary and pancreatic stents placed via ERCP in 14 of 100 stents (107).

Intestinal Disease

There are a number of reported links between *Proteus* spp. and various intestinal conditions, including small bowel intestinal overgrowth, Crohn's disease, and ulcerative colitis.

A small study of the downstream effects of small bowel ulceration caused by nonsteroidal anti-inflammatory drugs in rats identified a mixed population of *E. coli* and *P. mirabilis*. When treated with metronidazole, rats were protected from ulcer development (109). *Proteus* species were recovered from jejunal fluid in 11% of patients with small intestinal bacterial overgrowth syndrome (SIBOS), which often follows the expansion of facultative anaerobic bacterial communities (110). Viable bacterial translocation of *Proteus mirabilis* across the intact intestinal barrier has been demonstrated from the cecal and colonic mucosa of a monoassociated mouse model, with bacteria being additionally isolated from the mesenteric lymph nodes and the liver (111).

Crohn's disease. Recent research has implicated *Proteus* spp. in inflammatory bowel diseases, with evidence being derived both from patient-based microbiome surveys and mechanistic research. Ambrose et al. used culture-based techniques to compare the recovery of pathogenic gut bacteria from the ileal serosa and mesenteric lymph nodes in 45 Crohn's disease patients and 43 patients having surgery for other indications (112). Overall, Crohn's disease cases were more likely to have pathogenic bacteria recovered from the small bowel serosa (12/45 [27%] patients versus 6/41 [15%] controls). Of the 12 patients with positive serosal cultures, 4/12 patients were positive for *Proteus* spp. (33%). Additionally, involved and uninvolved mesenteric lymph nodes were assessed: 15/45 patients (33%) with involved nodes had a positive culture, and of these samples, 1/15 (7%) grew *Proteus* spp. Eleven of 45 samples of uninvolved nodes harbored bacteria, with 1/11 (9%) being positive for *Proteus* species (112). Although these data are not significant due to small numbers, they demonstrate the recovery of *Proteus* species from patients with Crohn's disease, while the recovery of *Proteus* species from lymph nodes establishes bacterial translocation, in line with the other members of the *Enterobacteriaceae* family (113).

Two microbiome studies have linked an overabundance of *Enterobacteriaceae* and *Proteus* spp. to Crohn's disease. In a pediatric study, *Proteus* species comprised 3/18 (16.7%) Gram-negative bacterial strains recovered from 12 Crohn's disease patients, compared to the total absence of *Proteus* species recovered from patients with ulcerative colitis, indeterminate colitis, and lymphonodular hyperplasia and from controls (114). A microarray-based study comparing patients with active Crohn's disease with matched healthy controls identified an overrepresentation of *Proteus* species overall, and *Proteus vulgaris* in particular, and other members of the *Proteobacteria* phylum in patients with active ileal disease requiring surgery (115).

A number of studies have addressed the mechanisms by which *Proteus* and other *Enterobacteriaceae* may contribute to the development of inflammatory bowel diseases. The TRUC mouse model of ulcerative colitis has been used to demonstrate that *Proteus mirabilis* and *Klebsiella pneumoniae* can elicit colitis and that this propensity for the development of colitis can be transmitted to wild-type mice via microbiome transfer (65).

Crohn's disease has been associated with changes in nitrogen metabolism. Ammonia produced from the breakdown of urea by bacterial urease provides a source of nitrogen for respiration and amino acid synthesis by pathogenic facultative anaerobes from the *Proteobacteria* phylum (49).

Interactions between the *Enterobacteriaceae*, which include *Proteus* species, and fungi (*Candida tropicalis*) have recently been implicated in the dysbiosis that characterizes Crohn's disease (116). When Crohn's disease patients were compared with first-degree relatives of Crohn's disease patients and healthy controls, bacterial dysbiosis was identified in affected patients and unaffected first-degree relatives. Crohn's disease patients had an increased presence of *Candida tropicalis*. Complex interactions between *Enterobacteriaceae* species (*Serratia marcescens* and *E. coli*) and *Candida*

tropicalis were confirmed in laboratory studies, showing that flagellated and/or fimbriated bacteria combined with fungal hyphae to form a robust biofilm, with these three species combined forming the thickest biofilm ($P < 0.0001$). It was postulated that biofilms enriched for immunomodulatory microbial components (lipopolysaccharides and oligomannans, etc.) may perpetuate inflammation in dysbiotic patients through the induction of proinflammatory cytokine responses and of apoptosis. *Proteus* spp. were also strongly positively correlated with the abundance of *Candida* in patients with familial Crohn's disease ($r = 0.709$; $P < 0.005$), raising the possibility that *Proteus* spp. are capable of the same interactions, although this was not demonstrated, possibly due to their low abundance (116).

Seo et al. demonstrated that in the presence of intestinal injury and colonization with *P. mirabilis*, a marked proinflammatory IL-1β response occurs, via the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome (59). This occurs only in the presence of the *P. mirabilis* hemolysin HpmA, which appears to induce host macrophage induction of NLRP3. Preexisting injury or inflammation was necessary for the induction of NLRP3 activity and IL-1β by *P. mirabilis* (dextran sodium sulfate [DSS]-induced colitis), as inflammatory monocytes were required, suggesting that *P. mirabilis* may act to perpetuate and accelerate preexisting inflammation rather than induce it. *Proteus mirabilis* was as efficient at inducing IL-1β as pathogenic *Salmonella* spp., but the presence of the HpmA hemolysin was essential for the induction of IL-1β. IL-1β has been shown to be associated with disease activity in IBD patients (117, 118); however, in that study, most other anaerobic or facultative anaerobic commensals induced TNF-α expression but not IL-1β (59). When hemolysin expression levels were compared across *P. mirabilis* strains from multiple clinical sources (pyelonephritis, catheter-associated, and fecal isolates), fecal isolates had the highest hemolytic activity and had significantly higher hemolytic titers than those of the catheter-associated strain ($P = 0.001$) but not those of the pyelonephritis strain ($P = 0.065$) (119).

Proteus species have been associated with the postoperative recurrence of Crohn's disease by two independent groups (2, 3). Metagenomic surveys of patients at the time of surgery and 6 and 18 months postoperatively demonstrated that patients were more likely to have disease recurrence in the presence of detectable *Proteus* genera ($P = 0.008$) and the absence of detectable *Faecalibacterium* ($P < 0.001$) (3). The combination of detectable *Proteus* species and absent *Faecalibacterium* ($<$0.1%) in postoperative ileal biopsy specimens was associated with an increased risk of recurrence, with an odds ratio (OR) of 14 (95% confidence interval [CI], 1.7 to 110; $P = 0.013$). Smoking, an independent risk factor for postoperative disease recurrence, was also associated with an increased presence of *Proteus* spp. ($P = 0.0130$) (3). In another study by Mondot et al. of 20 Crohn's disease patients undergoing ileocolonic resection, the presence of a *Proteus mirabilis* operational taxonomic unit (OTU) was predictive of recurrence at 6 months postoperatively (2).

A recent review of consecutive Crohn's disease patients with intra-abdominal abscesses as a result of active disease demonstrated infection with *Proteus* spp. in 4.8% of cases, which was associated with high rates of quinolone resistance (120). Whether the presence of *Proteus* spp. in association with postoperative recurrence is a primary pathogenic event or secondary to disease recurrence remains to be elucidated. However, the association in both studies was established prospectively and longitudinally, with predictive association, making a pathogenic role more likely.

Other large intestinal diseases. Kanareykina et al. obtained samples (mouth, stomach, small intestine, and feces) from 65 patients with ulcerative colitis; performed culture-based enumeration; and identified *Proteus mirabilis*, *Proteus vulgaris*, or the closely related species *Morganella morganii* or *Providencia rettgeri* (Fig. 1) in nearly all cases (121). In 40/65 patients, these species were recovered from more than one anatomic site. A *Proteus* species protein "vaccine" was then administered and resulted in clinical improvement in moderate to severe cases of ulcerative colitis as well as a decrease in bacterial counts. However, no details of the vaccine composition or any

objective disease activity metrics were described, and the study overall was of low quality (121).

A recent study of children with and without appendicitis showed increases in the relative abundances of 12 genera, of which *Proteus* species were the only representatives of the *Enterobacteriaceae* family (0.015% versus 0%; $P = 0.028$) (122).

Proteus bacteria have been implicated in the perpetuation of colonic inflammation in diversion colitis (123). Inflammatory conditions of the bowel increase the local concentrations of inducible nitric oxide synthase, leading to high levels of nitrate that cannot be metabolized, except by the microbiota (124). This favors the expansion of bacteria that are able to metabolize nitrate under anaerobic conditions, leading to a survival advantage and population expansion of *Proteus* spp. and other nitrate-reducing *Enterobacteriaceae* (48, 123).

Nosocomial Infections and *Proteus* Species Complicating Gastrointestinal Disease

Proteus species, especially *P. mirabilis* and *P. vulgaris*, are common causes of nosocomial opportunistic infections. Many patients with preexisting gastrointestinal diseases are liable to secondary *Proteus* infections, often in the context of polymicrobial infections. *Proteus* species can also cause peritonitis following perforations of the gastrointestinal tract; in one report of 383 patients with peritonitis, *Proteus* species were identified in 87 (23%) patients (125).

Proteus species can colonize medical devices placed in the gastrointestinal tract, including ventriculoperitoneal shunts (126), nasogastric tubes (99, 100), biliary and pancreatic stents (107), and tracheoesophageal voice prostheses (127). *Proteus* bacteria have been shown to be contaminants of gastroscopes and colonoscopes after insufficient disinfection (128). Infections can also be acquired in the hospital setting due to environmental contamination, with *P. vulgaris* persisting on dry, hard surfaces for up to 2 days (129). There are reports of hospital-based and community epidemics of infection with person-to-person spread, with most patients acquiring gastrointestinal carriage prior to infection (13, 130).

CONCLUSIONS

Proteus species are hardy, adaptable, and potentially pathogenic residents of the human gastrointestinal tract and have been underappreciated as a cause of gastrointestinal disease. Host-microbe and microbe-microbe interactions by *Proteus* spp., and the pathogenicity of this genus that may result from population expansion in response to environmental changes, are emerging as important aspects of disease associated with this genus. The possible contribution of *Proteus* spp. to intestinal diseases and infections has been somewhat neglected. Research into the virulence of *Proteus* spp. in the urinary tract using the bacteriology of ileal conduits (131) and intestinal segments (60, 132) for bladder augmentation suggests that *Proteus* spp. should be examined more closely for their potential as gastrointestinal pathogens.

There is increasing evidence that *Proteus* species may play a role in inflammatory bowel disease through the direct action of the bacteria, compounded by host immune evasion and perturbation. As Gram-negative organisms, *Proteus* species are intrinsically proinflammatory as a result of the production of lipopolysaccharide (LPS) and immunostimulatory flagellin proteins. There may be an association between *Proteus* species and inflammatory bowel disease, especially Crohn's disease, mainly through population expansion and immune activation. Their low population abundance does not preclude a potential large pathogenic effect.

Genetic characterization of enteric isolates compared to urinary tract isolates will be important for determining the effect of virulence factors on gastrointestinal pathogenesis.

Research on the gut microbiome as an ecosystem is informing our understanding of *Proteus* species, yet there are still unanswered questions. These include obtaining confirmation that *Proteus* species can swarm within the human gut and addressing the

effect of individual environmental changes (e.g., surgery, pH, or oxygen concentrations) on the mucosa-associated *Proteus* population.

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M.A.K. and A.L.H. devised the concept. A.L.H. acquired data, screened papers, interpreted the data, and wrote the manuscript. M.A.K., S.C.N., and M.M. provided critical revision of the manuscript for important intellectual content.

REFERENCES

1. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. 2012. Human gut microbiome viewed across age and geography. Nature 486:222–227. https://doi.org/10.1038/nature11053.
2. Mondot S, Lepage P, Seksik P, Allez M, Treton X, Bouhnik Y, Colombel JF, Leclerc M, Pochart P, Dore J, Marteau P, GETAID. 2016. Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. Gut 65:954–962. https://doi.org/10.1136/gutjnl-2015-309184.
3. Wright EK, Kamm MA, Wagner J, Teo SM, Cruz P, Hamilton AL, Ritchie KJ, Inouye M, Kirkwood CD. 2017. Microbial factors associated with postoperative Crohn's disease recurrence. J Crohns Colitis 11:191–203. https://doi.org/10.1093/ecco-jcc/jjw136.
4. Penner JL. 2005. Genus XXIX. *Proteus*, p 745–753. *In* Brenner DJ, Krieg NR, Staley JT, Garrity GM (ed), Bergey's manual of systematic bacteriology, 2nd ed, vol 2. The *Proteobacteria*: part B, the *Gammaproteobacteria*. Lippincott Williams & Wilkins, Philadelphia, PA.
5. Manos J, Belas R. 2006. The genera *Proteus*, *Providencia*, and *Morganella*, p 245–269. *In* Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), The prokaryotes. Springer, New York, NY.
6. Drzewiecka D. 9 January 2016. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol https://doi.org/10.1007/s00248-015-0720-6.
7. O'Hara CM, Brenner FW, Steigerwalt AG, Hill BC, Holmes B, Grimont PA, Hawkey PM, Penner JL, Miller JM, Brenner DJ. 2000. Classification of *Proteus vulgaris* biogroup 3 with recognition of *Proteus hauseri* sp. nov., nom. rev. and unnamed *Proteus* genomospecies 4, 5 and 6. Int J Syst Evol Microbiol 50(Part 5):1869–1875. https://doi.org/10.1099/00207713-50-5-1869.
8. O'Hara CM, Brenner FW, Miller JM. 2000. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. Clin Microbiol Rev 13:534–546. https://doi.org/10.1128/CMR.13.4.534-546.2000.
9. Hyun DW, Jung MJ, Kim MS, Shin NR, Kim PS, Whon TW, Bae JW. 2016. *Proteus cibarius* sp. nov., a swarming bacterium from Jeotgal, a traditional Korean fermented seafood, and emended description of the genus *Proteus*. Int J Syst Evol Microbiol 66:2158–2164. https://doi.org/10.1099/ijsem.0.001002.
10. Behrendt U, Augustin J, Sproer C, Gelbrecht J, Schumann P, Ulrich A. 2015. Taxonomic characterisation of *Proteus terrae* sp. nov., a N2O-producing, nitrate-ammonifying soil bacterium. Antonie Van Leeuwenhoek 108:1457–1468. https://doi.org/10.1007/s10482-015-0601-5.
11. Yu X, Torzewska A, Zhang X, Yin Z, Drzewiecka D, Cao H, Liu B, Knirel YA, Rozalski A, Wang L. 2017. Genetic diversity of the O antigens of *Proteus* species and the development of a suspension array for molecular serotyping. PLoS One 12:e0183267. https://doi.org/10.1371/journal.pone.0183267.
12. Zilberstein B, Quintanilha AG, Santos MA, Pajecki D, Moura EG, Alves PR, Maluf Filho F, de Souza JA, Gama-Rodrigues J. 2007. Digestive tract microbiota in healthy volunteers. Clinics (Sao Paulo) 62:47–54. https://doi.org/10.1590/S1807-59322007000100008.
13. Chow AW, Taylor PR, Yoshikawa TT, Guze LB. 1979. A nosocomial outbreak of infections due to multiply resistant *Proteus mirabilis*: role of intestinal colonization as a major reservoir. J Infect Dis 139:621–627. https://doi.org/10.1093/infdis/139.6.621.
14. Mobley HL, Belas R. 1995. Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. Trends Microbiol 3:280–284. https://doi.org/10.1016/S0966-842X(00)88945-3.
15. Coker C, Poore CA, Li X, Mobley HLT. 2000. Pathogenesis of *Proteus mirabilis* urinary tract infection. Microbes Infect 2:1497–1505. https://doi.org/10.1016/S1286-4579(00)01304-6.
16. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. 2012. IBD—what role do proteobacteria play? Nat Rev Gastroenterol Hepatol 9:219–230. https://doi.org/10.1038/nrgastro.2012.14.
17. Armbruster CE, Mobley HLT. 2012. Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. Nat Rev Microbiol 10:743–754. https://doi.org/10.1038/nrmicro2890.
18. Pearson MM, Rasko DA, Smith SN, Mobley HLT. 2010. Transcriptome of swarming *Proteus mirabilis*. Infect Immun 78:2834–2845. https://doi.org/10.1128/IAI.01222-09.
19. Rather PN. 2005. Swarmer cell differentiation in *Proteus mirabilis*. Environ Microbiol 7:1065–1073. https://doi.org/10.1111/j.1462-2920.2005.00806.x.
20. Walker KE, Moghaddame-Jafari S, Lockatell CV, Johnson D, Belas R. 1999. ZapA, the IgA-degrading metalloprotease of *Proteus mirabilis*, is a virulence factor expressed specifically in swarmer cells. Mol Microbiol 32:825–836. https://doi.org/10.1046/j.1365-2958.1999.01401.x.
21. Belas R, Manos J, Suvanasuthi R. 2004. *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. Infect Immun 72:5159–5167. https://doi.org/10.1128/IAI.72.9.5159-5167.2004.
22. Allison C, Emody L, Coleman N, Hughes C. 1994. The role of swarm cell differentiation and multicellular migration in the uropathogenicity of *Proteus mirabilis*. J Infect Dis 169:1155–1158. https://doi.org/10.1093/infdis/169.5.1155.
23. Li X, Zhao H, Lockatell CV, Drachenberg CB, Johnson DE, Mobley HLT. 2002. Visualization of *Proteus mirabilis* within the matrix of urease-induced bladder stones during experimental urinary tract infection. Infect Immun 70:389–394. https://doi.org/10.1128/IAI.70.1.389-394.2002.
24. Alteri CJ, Himpsl SD, Engstrom MD, Mobley HLT. 2012. Anaerobic respiration using a complete oxidative TCA cycle drives multicellular swarming in *Proteus mirabilis*. mBio 3:e00365-12. https://doi.org/10.1128/mBio.00365-12.
25. Armbruster CE, Hodges SA, Mobley HLT. 2013. Initiation of swarming motility by *Proteus mirabilis* occurs in response to specific cues present in urine and requires excess L-glutamine. J Bacteriol 195:1305–1319. https://doi.org/10.1128/JB.02136-12.
26. Allison C, Lai H-C, Gygi D, Hughes C. 1993. Cell differentiation of *Proteus mirabilis* is initiated by glutamine, a specific chemoattractant for

swarming cells. Mol Microbiol 8:53–60. https://doi.org/10.1111/j.1365-2958.1993.tb01202.x.
27. Nugent SG, Kumar D, Rampton DS, Evans DF. 2001. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. Gut 48:571–577. https://doi.org/10.1136/gut.48.4.571.
28. Fujihara M, Obara H, Watanabe Y, Ono HK, Sasaki J, Goryo M, Harasawa R. 2011. Acidic environments induce differentiation of *Proteus mirabilis* into swarmer morphotypes. Microbiol Immunol 55:489–493. https://doi.org/10.1111/j.1348-0421.2011.00345.x.
29. Pickard JM, Zeng MY, Caruso R, Nunez G. 2017. Gut microbiota: role in pathogen colonization, immune responses, and inflammatory disease. Immunol Rev 279:70–89. https://doi.org/10.1111/imr.12567.
30. Allison C, Coleman N, Jones PL, Hughes C. 1992. Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. Infect Immun 60:4740–4746.
31. Milovic V. 2001. Polyamines in the gut lumen: bioavailability and biodistribution. Eur J Gastroenterol Hepatol 13:1021–1025. https://doi.org/10.1097/00042737-200109000-00004.
32. Jameson E, Fu T, Brown IR, Paszkiewicz K, Purdy KJ, Frank S, Chen Y. 2016. Anaerobic choline metabolism in microcompartments promotes growth and swarming of *Proteus mirabilis*. Environ Microbiol 18:2886–2898. https://doi.org/10.1111/1462-2920.13059.
33. Sturgill G, Rather PN. 2004. Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. Mol Microbiol 51:437–446. https://doi.org/10.1046/j.1365-2958.2003.03835.x.
34. Schaffer JN, Pearson MM. 2015. *Proteus mirabilis* and urinary tract infections. Microbiol Spectr 3:UTI-0017-2013. https://doi.org/10.1128/microbiolspec.UTI-0017-2013.
35. Pearson MM, Sebaihia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, Abdellah Z, Arrosmith C, Atkin B, Chillingworth T, Hauser H, Jagels K, Moule S, Mungall K, Norbertczak H, Rabbinowitsch E, Walker D, Whithead S, Thomson NR, Rather PN, Parkhill J, Mobley HLT. 2008. Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. J Bacteriol 190:4027–4037. https://doi.org/10.1128/JB.01981-07.
36. Kuan L, Schaffer JN, Zouzias CD, Pearson MM. 2014. Characterization of 17 chaperone-usher fimbriae encoded by *Proteus mirabilis* reveals strong conservation. J Med Microbiol 63:911–922. https://doi.org/10.1099/jmm.0.069971-0.
37. Scavone P, Iribarnegaray V, Caetano AL, Schlapp G, Härtel S, Zunino P. 2016. Fimbriae have distinguishable roles in *Proteus mirabilis* biofilm formation. Pathog Dis 74:ftw033. https://doi.org/10.1093/femspd/ftw033.
38. Scavone P, Villar S, Umpierrez A, Zunino P. 2015. Role of *Proteus mirabilis* MR/P fimbriae and flagella in adhesion, cytotoxicity and genotoxicity induction in T24 and Vero cells. Pathog Dis 73:ftv017. https://doi.org/10.1093/femspd/ftv017.
39. Jansen AM, Lockatell V, Johnson DE, Mobley HLT. 2004. Mannose-resistant Proteus-like fimbriae are produced by most Proteus mirabilis strains infecting the urinary tract, dictate the in vivo localization of bacteria, and contribute to biofilm formation. Infect Immun 72:7294–7305. https://doi.org/10.1128/IAI.72.12.7294-7305.2004.
40. Rocha SP, Pelayo JS, Elias WP. 2007. Fimbriae of uropathogenic *Proteus mirabilis*. FEMS Immunol Med Microbiol 51:1–7. https://doi.org/10.1111/j.1574-695X.2007.00284.x.
41. Lee KK, Harrison BA, Latta R, Altman E. 2000. The binding of *Proteus mirabilis* nonagglutinating fimbriae to ganglio-series asialoglycolipids and lactosyl ceramide. Can J Microbiol 46:961–966. https://doi.org/10.1139/w00-083.
42. Adegbola RA, Old DC, Senior BW. 1983. The adhesins and fimbriae of *Proteus mirabilis* strains associated with high and low affinity for the urinary tract. J Med Microbiol 16:427–431. https://doi.org/10.1099/00222615-16-4-427.
43. Pearson MM, Mobley HLT. 2008. Repression of motility during fimbrial expression: identification of fourteen *mrpJ* gene paralogs in *Proteus mirabilis*. Mol Microbiol 69:548–558. https://doi.org/10.1111/j.1365-2958.2008.06307.x.
44. Latta RK, Grondin A, Jarrell HC, Nicholls GR, Berube LR. 1999. Differential expression of nonagglutinating fimbriae and MR/P pili in swarming colonies of *Proteus mirabilis*. J Bacteriol 181:3220–3225.
45. Lane MC, Li X, Pearson MM, Simms AN, Mobley HL. 2009. Oxygen-limiting conditions enrich for fimbriate cells of uropathogenic *Proteus mirabilis* and *Escherichia coli*. J Bacteriol 191:1382–1392. https://doi.org/10.1128/JB.01550-08.
46. Mora D, Arioli S. 2014. Microbial urease in health and disease. PLoS Pathog 10:e1004472. https://doi.org/10.1371/journal.ppat.1004472.
47. Rutherford JC. 2014. The emerging role of urease as a general microbial virulence factor. PLoS Pathog 10:e1004062. https://doi.org/10.1371/journal.ppat.1004062.
48. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tsolis RM, Stewart VJ, Bäumler AJ. 2013. Host-derived nitrate boosts growth of *E. coli* in the inflamed gut. Science 339:708–711. https://doi.org/10.1126/science.1232467.
49. Ni J, Shen T-CD, Chen EZ, Bittinger K, Bailey A, Roggiani M, Sirota-Madi A, Friedman ES, Chau L, Lin A, Nissim I, Scott J, Lauder A, Hoffmann C, Rivas G, Albenberg L, Baldassano RN, Braun J, Xavier RJ, Clish CB, Yudkoff M, Li H, Goulian M, Bushman FD, Lewis JD, Wu GD. 2017. A role for bacterial urease in gut dysbiosis and Crohn's disease. Sci Transl Med 9:eaah6888. https://doi.org/10.1126/scitranslmed.aah6888.
50. Mobley HL, Jones BD, Penner JL. 1987. Urease activity of *Proteus penneri*. J Clin Microbiol 25:2302–2305.
51. Mobley HL, Chippendale GR, Swihart KG, Welch RA. 1991. Cytotoxicity of the HpmA hemolysin and urease of Proteus mirabilis and Proteus vulgaris against cultured human renal proximal tubular epithelial cells. Infect Immun 59:2036–2042.
52. Mobley HL, Island MD, Hausinger RP. 1995. Molecular biology of microbial ureases. Microbiol Rev 59:451–480.
53. Osaki T, Mabe K, Hanawa T, Kamiya S. 2008. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. J Med Microbiol 57:814–819. https://doi.org/10.1099/jmm.0.47768-0.
54. Swihart KG, Welch RA. 1990. The HpmA hemolysin is more common than HlyA among *Proteus* isolates. Infect Immun 58:1853–1860.
55. Swihart KG, Welch RA. 1990. Cytotoxic activity of the *Proteus* hemolysin HpmA. Infect Immun 58:1861–1869.
56. Senior BW. 1993. The production of HlyA toxin by *Proteus penneri* strains. J Med Microbiol 39:282–289. https://doi.org/10.1099/00222615-39-4-282.
57. Cestari SE, Ludovico MS, Martins FH, da Rocha SP, Elias WP, Pelayo JS. 2013. Molecular detection of HpmA and HlyA hemolysin of uropathogenic *Proteus mirabilis*. Curr Microbiol 67:703–707. https://doi.org/10.1007/s00284-013-0423-5.
58. Kaca W, Rozalski A. 1991. Characterization of cell-bound and cell-free hemolytic activity of *Proteus* strains. Eur J Epidemiol 7:159–165. https://doi.org/10.1007/BF00237360.
59. Seo S-U, Kamada N, Muñoz-Planillo R, Kim Y-G, Kim D, Koizumi Y, Hasegawa M, Himpsl SD, Browne HP, Lawley TD, Mobley HLT, Inohara N, Núñez G. 2015. Distinct commensals induce interleukin-1β via NLRP3 inflammasome in inflammatory monocytes to promote intestinal inflammation in response to injury. Immunity 42:744–755. https://doi.org/10.1016/j.immuni.2015.03.004.
60. Mathoera RB, Kok DJ, Verduin CM, Nijman RJM. 2002. Pathological and therapeutic significance of cellular invasion by *Proteus mirabilis* in an enterocystoplasty infection stone model. Infect Immun 70:7022–7032. https://doi.org/10.1128/IAI.70.12.7022-7032.2002.
61. Oelschlaeger TA, Tall BD. 1996. Uptake pathways of clinical isolates of *Proteus mirabilis* into human epithelial cell lines. Microb Pathog 21:1–16. https://doi.org/10.1006/mpat.1996.0037.
62. Wells CL, van de Westerlo EMA, Jechorek RP, Erlandsen SL. 1996. Intracellular survival of enteric bacteria in cultured human enterocytes. Shock 6:27–34. https://doi.org/10.1097/00024382-199607000-00007.
63. Phillips TE, Huet C, Bilbo PR, Podolsky DK, Louvard D, Neutra MR. 1988. Human intestinal goblet cells in monolayer culture: characterization of a mucus-secreting subclone derived from the HT29 colon adenocarcinoma cell line. Gastroenterology 94:1390–1403. https://doi.org/10.1016/0016-5085(88)90678-6.
64. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, Glickman JN, Glimcher LH. 2007. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell 131:33–45. https://doi.org/10.1016/j.cell.2007.08.017.
65. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. 2010. *Enterobacteriaceae* act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe 8:292–300. https://doi.org/10.1016/j.chom.2010.08.004.
66. Okumura R, Kurakawa T, Nakano T, Kayama H, Kinoshita M, Motooka D,

Gotoh K, Kimura T, Kamiyama N, Kusu T, Ueda Y, Wu H, Iijima H, Barman S, Osawa H, Matsuno H, Nishimura J, Ohba Y, Nakamura S, Iida T, Yamamoto M, Umemoto E, Sano K, Takeda K. 2016. Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. Nature 532:117–121. https://doi.org/10.1038/nature17406.
67. Kerr MA, Loomes LM, Senior BW. 1995. Cleavage of IgG and IgA in vitro and in vivo by the urinary tract pathogen *Proteus mirabilis*. Adv Exp Med Biol 371A:609–611. https://doi.org/10.1007/978-1-4615-1941-6_128.
68. Eaves-Pyles T, Murthy K, Liaudet L, Virag L, Ross G, Soriano FG, Szabo C, Salzman AL. 2001. Flagellin, a novel mediator of *Salmonella*-induced epithelial activation and systemic inflammation: I kappa B alpha degradation, induction of nitric oxide synthase, induction of proinflammatory mediators, and cardiovascular dysfunction. J Immunol 166: 1248–1260. https://doi.org/10.4049/jimmunol.166.2.1248.
69. Rózalski A, Sidorczyk Z, Kotełko K. 1997. Potential virulence factors of *Proteus* bacilli. Microbiol Mol Biol Rev 61:65–89.
70. Raetz CR, Whitfield C. 2002. Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635–700. https://doi.org/10.1146/annurev.biochem.71.110601.135414.
71. Akira S, Uematsu S, Takeuchi O. 2006. Pathogen recognition and innate immunity. Cell 124:783–801. https://doi.org/10.1016/j.cell.2006.02.015.
72. Takeuchi O, Akira S. 2010. Pattern recognition receptors and inflammation. Cell 140:805–820. https://doi.org/10.1016/j.cell.2010.01.022.
73. Knirel YA, Perepelov AV, Kondakova AN, Senchenkova SN, Sidorczyk Z, Rozalski A, Kaca W. 2011. Structure and serology of O-antigens as the basis for classification of *Proteus* strains. Innate Immun 17:70–96. https://doi.org/10.1177/1753425909360668.
74. Arabski M, Grabowski S, Konieczna I, Kaca W, Kondakova AN, Perepelov AV, Senchenkova SN, Shashkov AS, Knirel YA. 2008. Serotyping of clinical isolates belonging to *Proteus mirabilis* serogroup O36 and structural elucidation of the O36-antigen polysaccharide. FEMS Immunol Med Microbiol 53:395–403. https://doi.org/10.1111/j.1574-695X.2008.00440.x.
75. Stankowska D, Kwinkowski M, Kaca W. 2008. Quantification of *Proteus mirabilis* virulence factors and modulation by acylated homoserine lactones. J Microbiol Immunol Infect 41:243–253.
76. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410: 1099–1103. https://doi.org/10.1038/35074106.
77. López-Yglesias AH, Zhao X, Quarles EK, Lai MA, VandenBos T, Strong RK, Smith KD. 2014. Flagellin induces antibody responses through a TLR5- and inflammasome-independent pathway. J Immunol 192:1587–1596. https://doi.org/10.4049/jimmunol.1301893.
78. van der Woude MW, Bäumler AJ. 2004. Phase and antigenic variation in bacteria. Clin Microbiol Rev 17:581–611. https://doi.org/10.1128/CMR.17.3.581-611.2004.
79. Murphy CA, Belas R. 1999. Genomic rearrangements in the flagellin genes of *Proteus mirabilis*. Mol Microbiol 31:679–690. https://doi.org/10.1046/j.1365-2958.1999.01209.x.
80. Olaitan AO, Morand S, Rolain J-M. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 5:643. https://doi.org/10.3389/fmicb.2014.00643.
81. Vaara M, Vaara T, Jensen M, Helander I, Nurminen M, Rietschel ET, Mäkelä PH. 1981. Characterization of the lipopolysaccharide from the polymyxin-resistant *pmrA* mutants of *Salmonella typhimurium*. FEBS Lett 129:145–149. https://doi.org/10.1016/0014-5793(81)80777-6.
82. Qin S, Qi H, Zhang Q, Zhao D, Liu Z-Z, Tian H, Xu L, Xu H, Zhou M, Feng X, Liu H-M. 2015. Emergence of extensively drug-resistant *Proteus mirabilis* harboring a conjugative NDM-1 plasmid and a novel *Salmonella* genomic island 1 variant, SGI1-Z. Antimicrob Agents Chemother 59:6601–6604. https://doi.org/10.1128/AAC.00292-15.
83. Iredell J, Brown J, Tagg K. 2016. Antibiotic resistance in *Enterobacteriaceae*: mechanisms and clinical implications. BMJ 352:h6420. https://doi.org/10.1136/bmj.h6420.
84. Doublet B, Poirel L, Praud K, Nordmann P, Cloeckaert A. 2010. European clinical isolate of *Proteus mirabilis* harbouring the *Salmonella* genomic island 1 variant SGI1-O. J Antimicrob Chemother 65:2260–2262. https://doi.org/10.1093/jac/dkq283.
85. D'Andrea MM, Giani T, Henrici De Angelis L, Ciacci N, Gniadkowski M, Miriagou V, Torricelli F, Rossolini GM. 2016. Draft genome sequence of *Proteus mirabilis* NO-051/03, representative of a multidrug-resistant clone spreading in Europe and expressing the CMY-16 AmpC-type beta-lactamase. Genome Announc 4:e01702-15. https://doi.org/10.1128/genomeA.01702-15.
86. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB. 2007. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*. Cell Host Microbe 2:119–129. https://doi.org/10.1016/j.chom.2007.06.010.
87. Alverdy JC, Hyoju SK, Weigerinck M, Gilbert JA. 2017. The gut microbiome and the mechanism of surgical infection. Br J Surg 104:e14–e23. https://doi.org/10.1002/bjs.10405.
88. Li X, Lockatell CV, Johnson DE, Lane MC, Warren JW, Mobley HLT. 2004. Development of an intranasal vaccine to prevent urinary tract infection by *Proteus mirabilis*. Infect Immun 72:66–75. https://doi.org/10.1128/IAI.72.1.66-75.2004.
89. Habibi M, Asadi Karam MR, Shokrgozar MA, Oloomi M, Jafari A, Bouzari S. 2015. Intranasal immunization with fusion protein MrpH·FimH and MPL adjuvant confers protection against urinary tract infections caused by uropathogenic *Escherichia coli* and *Proteus mirabilis*. Mol Immunol 64:285–294. https://doi.org/10.1016/j.molimm.2014.12.008.
90. Lorenzo-Gómez MF, Padilla-Fernández B, García-Criado FJ, Mirón-Canelo JA, Gil-Vicente A, Nieto-Huertos A, Silva-Abuin JM. 2013. Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus prophylactic treatment with antibiotics. Int Urogynecol J 24:127–134. https://doi.org/10.1007/s00192-012-1853-5.
91. Adlerberth I, Carlsson B, de Man P, Jalil F, Khan SR, Larsson P, Mellander L, Svanborg C, Wold AE, Hanson LA. 1991. Intestinal colonization with *Enterobacteriaceae* in Pakistani and Swedish hospital-delivered infants. Acta Paediatr Scand 80:602–610. https://doi.org/10.1111/j.1651-2227.1991.tb11917.x.
92. Müller HE. 1986. Occurrence and pathogenic role of *Morganella-Proteus-Providencia* group bacteria in human feces. J Clin Microbiol 23:404–405.
93. Amin OM. 2011. The contribution of pathogenic bacteria to GI symptoms in parasite-free patients. J Bacteriol Parasitol 2:109. https://doi.org/10.4172/2155-9597.1000109.
94. O'Fallon E, Gautam S, D'Agata EM. 2009. Colonization with multidrug-resistant gram-negative bacteria: prolonged duration and frequent cocolonization. Clin Infect Dis 48:1375–1381. https://doi.org/10.1086/598194.
95. Wang Y, Zhang S, Yu J, Zhang H, Yuan Z, Sun Y, Zhang L, Zhu Y, Song H. 2010. An outbreak of *Proteus mirabilis* food poisoning associated with eating stewed pork balls in brown sauce, Beijing. Food Control 21:302–305. https://doi.org/10.1016/j.foodcont.2009.06.009.
96. Shi X, Lin Y, Qiu Y, Li Y, Jiang M, Chen Q, Jiang Y, Yuan J, Cao H, Hu Q, Huang S. 2016. Comparative screening of digestion tract toxic genes in *Proteus mirabilis*. PLoS One 11:e0151873. https://doi.org/10.1371/journal.pone.0151873.
97. Thomas S, Raman R, Idikula J, Brahmadathan N. 1992. Alterations in oropharyngeal flora in patients with a nasogastric tube: a cohort study. Crit Care Med 20:1677–1680. https://doi.org/10.1097/00003246-199212000-00013.
98. Challacombe DN, Richardson JM, Anderson CM. 1974. Bacterial microflora of the upper gastrointestinal tract in infants without diarrhoea. Arch Dis Child 49:264–269. https://doi.org/10.1136/adc.49.4.264.
99. Segal R, Dan M, Pogoreliuk I, Leibovitz A. 2006. Pathogenic colonization of the stomach in enterally fed elderly patients: comparing percutaneous endoscopic gastrostomy with nasogastric tube. J Am Geriatr Soc 54:1905–1908. https://doi.org/10.1111/j.1532-5415.2006.00964.x.
100. Segal R, Pogoreliuk I, Dan M, Baumoehl Y, Leibovitz A. 2006. Gastric microbiota in elderly patients fed via nasogastric tubes for prolonged periods. J Hosp Infect 63:79–83. https://doi.org/10.1016/j.jhin.2005.11.005.
101. Ehrenkranz NJ. 1970. Bacterial colonization of newborn infants and subsequent acquisition of hospital bacteria. J Pediatr 76:839–847. https://doi.org/10.1016/S0022-3476(70)80363-8.
102. Lou MA, Mandal AK, Alexander JL, Thadepalli H. 1977. Bacteriology of the human biliary tract and the duodenum. Arch Surg 112:965–967. https://doi.org/10.1001/archsurg.1977.01370080063010.
103. Misra V, Misra SP, Singh PA, Dwivedi M, Verma K, Narayan U. 2009. Significance of cytomorphological and microbiological examination of bile collected by endoscopic cannulation of the papilla of vater. Indian J Pathol Microbiol 52:328–331. https://doi.org/10.4103/0377-4929.54986.
104. Bajaj JS, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, Monteith P, Noble NA, Sikaroodi M, Gillevet PM. 2012. Colonic mucosal

microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. Am J Physiol Gastrointest Liver Physiol 303:G675–G685. https://doi.org/10.1152/ajpgi.00152.2012.
105. Lai D, Gorbach SL, Levitan R. 1972. Intestinal microflora in patients with alcoholic cirrhosis: urea-splitting bacteria and neomycin resistance. Gastroenterology 62:275–279.
106. Wang X, Andersson R, Soltesz V, Bengmark S. 1992. Bacterial translocation after major hepatectomy in patients and rats. Arch Surg 127:1101–1106. https://doi.org/10.1001/archsurg.1992.01420090109016.
107. Vaishnavi C, Kapoor P, Kochhar R. 2014. Su1148. Bacterial biofilms produced in stents retrieved from patients with biliary and pancreatic diseases. Gastroenterology 146:S-389. https://doi.org/10.1016/S0016-5085(14)61396-2.
108. Yeh CL, Lai KH, Lo GH, Lin CK, Hsu PI, Chan HH, Tsai WL, Lin CP. 2003. Endoscopic treatment in a patient with obstructive jaundice caused by pancreatic pseudocyst. J Chin Med Assoc 66:555–559.
109. Collins AJ, Reid J, Soper CJ, Notarianni LJ. 1995. Characteristics of ulcers of the small bowel induced by non-steroidal anti-inflammatory drugs in the rat: implications for clinical practice. Br J Rheumatol 34:727–731. https://doi.org/10.1093/rheumatology/34.8.727.
110. Bouhnik Y, Alain S, Attar A, Flourie B, Raskine L, Sanson-Le Pors MJ, Rambaud JC. 1999. Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. Am J Gastroenterol 94:1327–1331. https://doi.org/10.1111/j.1572-0241.1999.01016.x.
111. Wells CL, Erlandsen SL. 1991. Localization of translocating *Escherichia coli*, *Proteus mirabilis*, and *Enterococcus faecalis* within cecal and colonic tissues of monoassociated mice. Infect Immun 59:4693–4697.
112. Ambrose NS, Johnson M, Burdon DW, Keighley MRB. 1984. Incidence of pathogenic bacteria from mesenteric lymph nodes and ileal serosa during Crohn's disease surgery. Br J Surg 71:623–625. https://doi.org/10.1002/bjs.1800710821.
113. O'Brien CL, Pavli P, Gordon DM, Allison GE. 2014. Detection of bacterial DNA in lymph nodes of Crohn's disease patients using high throughput sequencing. Gut 63:1596–1606. https://doi.org/10.1136/gutjnl-2013-305320.
114. Conte MP, Schippa S, Zamboni I, Penta M, Chiarini F, Seganti L, Osborn J, Falconieri P, Borrelli O, Cucchiara S. 2006. Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. Gut 55:1760–1767. https://doi.org/10.1136/gut.2005.078824.
115. Mondot S, Kang S, Furet JP, Aguirre de Carcer D, McSweeney C, Morrison M, Marteau P, Doré J, Leclerc M. 2011. Highlighting new phylogenetic specificities of Crohn's disease microbiota. Inflamm Bowel Dis 17:185–192. https://doi.org/10.1002/ibd.21436.
116. Hoarau G, Mukherjee PK, Gower-Rousseau C, Hager C, Chandra J, Retuerto MA, Neut C, Vermeire S, Clemente J, Colombel JF, Fujioka H, Poulain D, Sendid B, Ghannoum MA. 2016. Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial Crohn's disease. mBio 7:e01250-16. https://doi.org/10.1128/mBio.01250-16.
117. Leal RF, Planell N, Kajekar R, Lozano JJ, Ordás I, Dotti I, Esteller M, Masamunt MC, Parmar H, Ricart E, Panés J, Salas A. 2015. Identification of inflammatory mediators in patients with Crohn's disease unresponsive to anti-TNFα therapy. Gut 64:233–242. https://doi.org/10.1136/gutjnl-2013-306518.
118. Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D. 1990. Role of interleukin 1 in inflammatory bowel disease—enhanced production during active disease. Gut 31:686–689. https://doi.org/10.1136/gut.31.6.686.
119. Mobley HL, Chippendale GR. 1990. Hemagglutinin, urease, and hemolysin production by *Proteus mirabilis* from clinical sources. J Infect Dis 161:525–530. https://doi.org/10.1093/infdis/161.3.525.
120. Reuken PA, Kruis W, Maaser C, Teich N, Büning J, Preiß JC, Schmelz R, Bruns T, Fichtner-Feigl S, Stallmach A. 5 February 2018. Microbial spectrum of intra-abdominal abscesses in perforating Crohn's disease: results from a prospective German registry. J Crohns Colitis https://doi.org/10.1093/ecco-jcc/jjy017.
121. Kanareykina SK, Misautova AA, Zlatkina AR, Levina EN. 1987. *Proteus* dysbioses in patients with ulcerative colitis. Nahrung 31:557–561. https://doi.org/10.1002/food.19870310570.
122. Jackson HT, Mongodin EF, Davenport KP, Fraser CM, Sandler AD, Zeichner SL. 2014. Culture-independent evaluation of the appendix and rectum microbiomes in children with and without appendicitis. PLoS One 9:e95414. https://doi.org/10.1371/journal.pone.0095414.
123. Neut C, Guillemot F, Colombel JF. 1997. Nitrate-reducing bacteria in diversion colitis: a clue to inflammation? Dig Dis Sci 42:2577–2580. https://doi.org/10.1023/A:1018885217154.
124. Neut C, Bulois P, Desreumaux P, Membree J-M, Lederman E, Gambiez L, Cortot A, Quandalle P, van Kruiningen H, Colombel J-F. 2002. Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn's disease. Am J Gastroenterol 97:939–946. https://doi.org/10.1111/j.1572-0241.2002.05613.x.
125. Hau T. 1990. Bacteria, toxins, and the peritoneum. World J Surg 14:167–175. https://doi.org/10.1007/BF01664869.
126. Liu KL, Lee TC, Lin MT, Chen SJ. 2007. Education and imaging. Gastrointestinal: abdominal abscess associated with a ventriculoperitoneal shunt. J Gastroenterol Hepatol 22:757. https://doi.org/10.1111/j.1440-1746.2007.04964.x.
127. Ticac B, Ticac R, Rukavina T, Kesovija PG, Pedisic D, Maljevac B, Starcevic R. 2010. Microbial colonization of tracheoesophageal voice prostheses (Provox2) following total laryngectomy. Eur Arch Otorhinolaryngol 267:1579–1586. https://doi.org/10.1007/s00405-010-1253-8.
128. Machado AP, Pimenta AT, Contijo PP, Geocze S, Fischman O. 2006. Microbiologic profile of flexible endoscope disinfection in two Brazilian hospitals. Arq Gastroenterol 43:255–258. https://doi.org/10.1590/S0004-28032006000400002.
129. Kramer A, Schwebke I, Kampf G. 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 6:130. https://doi.org/10.1186/1471-2334-6-130.
130. Cherry WB, Lentz PL, Barnes LA. 1946. Implication of *Proteus mirabilis* in an outbreak of gastroenteritis. Am J Public Health Nations Health 36:484–488. https://doi.org/10.2105/AJPH.36.5.484.
131. Chan RC, Reid G, Bruce AW, Costerton JW. 1984. Microbial colonization of human ileal conduits. Appl Environ Microbiol 48:1159–1165.
132. Mathoera RB, Kok DJ, Visser WJ, Verduin CM, Nijman RJ. 2001. Cellular membrane associated mucins in artificial urine as mediators of crystal adhesion: an in vitro enterocystoplasty model. J Urol 166:2329–2336. https://doi.org/10.1016/S0022-5347(05)65581-4.
133. Rajilic-Stojanovic M, de Vos WM. 2014. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev 38:996–1047. https://doi.org/10.1111/1574-6976.12075.
134. Rauprich O, Matsushita M, Weijer CJ, Siegert F, Esipov SE, Shapiro JA. 1996. Periodic phenomena in *Proteus mirabilis* swarm colony development. J Bacteriol 178:6525–6538. https://doi.org/10.1128/jb.178.22.6525-6538.1996.
135. Wilkerson ML, Niederhoffer EC. 1995. Swarming characteristics of *Proteus mirabilis* under anaerobic and aerobic conditions. Anaerobe 1:345–350. https://doi.org/10.1006/anae.1995.1037.
136. Li X, Lockatell CV, Johnson DE, Mobley HLT. 2002. Identification of MrpI as the sole recombinase that regulates the phase variation of MR/P fimbria, a bladder colonization factor of uropathogenic *Proteus mirabilis*. Mol Microbiol 45:865–874. https://doi.org/10.1046/j.1365-2958.2002.03067.x.
137. Yakubu DE, Old DC, Senior BW. 1989. The haemagglutinins and fimbriae of *Proteus penneri*. J Med Microbiol 30:279–284. https://doi.org/10.1099/00222615-30-4-279.
138. Wray SK, Hull SI, Cook RG, Barrish J, Hull RA. 1986. Identification and characterization of a uroepithelial cell adhesin from a uropathogenic isolate of *Proteus mirabilis*. Infect Immun 54:43–49.
139. Massad G, Bahrani FK, Mobley HL. 1994. *Proteus mirabilis* fimbriae: identification, isolation, and characterization of a new ambient-temperature fimbria. Infect Immun 62:1989–1994.
140. Zunino P. 2000. Virulence of a *Proteus mirabilis* ATF isogenic mutant is not impaired in a mouse model of ascending urinary tract infection. FEMS Immunol Med Microbiol 29:137–143. https://doi.org/10.1111/j.1574-695X.2000.tb01516.x.
141. Massad G, Fulkerson JF, Jr, Watson DC, Mobley HL. 1996. *Proteus mirabilis* ambient-temperature fimbriae: cloning and nucleotide sequence of the *aft* gene cluster. Infect Immun 64:4390–4395.
142. Bijlsma IG, van Dijk L, Kusters JG, Gaastra W. 1995. Nucleotide sequences of two fimbrial major subunit genes, *pmpA* and *ucaA*, from canine-uropathogenic *Proteus mirabilis* strains. Microbiology 141(Part 6):1349–1357. https://doi.org/10.1099/13500872-141-6-1349.
143. Hess DJ, Henry-Stanley MJ, Erickson EA, Wells CL. 2002. Effect of tumor necrosis factor alpha, interferon gamma, and interleukin-4 on bacteria-enterocyte interactions. J Surg Res 104:88–94. https://doi.org/10.1006/jsre.2002.6417.
144. Wells CL, Jechorek RP, Olmsted SB, Erlandsen SL. 1993. Effect of LPS on

epithelial integrity and bacterial uptake in the polarized human enterocyte-like cell line Caco-2. Circ Shock 40:276–288.
145. Wells CL, VandeWesterlo EM, Jechorek RP, Erlandsen SL. 1996. Effect of hypoxia on enterocyte endocytosis of enteric bacteria. Crit Care Med 24:985–991. https://doi.org/10.1097/00003246-199606000-00019.
146. Rozalski A, Dlugonska H, Kotelko K. 1986. Cell invasiveness of *Proteus mirabilis* and *Proteus vulgaris* strains. Arch Immunol Ther Exp (Warsz) 34:505–512.
147. Senior BW, Loomes LM, Kerr MA. 1991. The production and activity in vivo of *Proteus mirabilis* IgA protease in infections of the urinary tract. J Med Microbiol 35:203–207. https://doi.org/10.1099/00222615-35-4-203.
148. O'Neil DA, Porter EM, Elewaut D, Anderson GM, Eckmann L, Ganz T, Kagnoff MF. 1999. Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. J Immunol 163: 6718–6724.
149. Ramasundara M, Leach ST, Lemberg DA, Day AS. 2009. Defensins and inflammation: the role of defensins in inflammatory bowel disease. J Gastroenterol Hepatol 24:202–208. https://doi.org/10.1111/j.1440-1746.2008.05772.x.
150. Vandamme D, Landuyt B, Luyten W, Schoofs L. 2012. A comprehensive summary of LL-37, the factotum human cathelicidin peptide. Cell Immunol 280:22–35. https://doi.org/10.1016/j.cellimm.2012.11.009.

Amy L. Hamilton is a Clinical Scientist from St Vincent's Hospital and the University of Melbourne. She graduated from Monash University, Melbourne, Australia, with a bachelor of science, with Honors in Microbiology and Immunology. She has worked on a number of investigator-initiated clinical trials in gastroenterology and is passionate about translational research in inflammatory bowel disease as a way of improving long-term outcomes for patients. She has a special interest in the gut microbiome and its role in the etiology of inflammatory bowel diseases, with this research focus allowing her to utilize both basic science and translational skills. She has completed her Ph.D. in the Department of Medicine at the University of Melbourne, addressing the microbiology of Crohn's disease after surgical resection. She has been a member of Professor Michael A. Kamm's research group since 2010.

Michael A. Kamm is Gastroenterologist at St Vincent's Hospital Melbourne and Professorial Fellow at the University of Melbourne, Melbourne, Australia, since 2008. He was previously Chairman of Medicine and Director of the Physiology and Inflammatory Bowel Disease Units at St Mark's Hospital in London, United Kingdom, from 1989 to 2008 and Professor of Gastroenterology at Imperial College London. He treats and researches in the fields of inflammatory bowel disease (IBD) and functional bowel disorders. Professor Kamm has conducted multidisciplinary research, spanning basic science, translational, and clinical aspects of gut disorders. In the field of the gut microbiota and IBD, he has studied mechanisms of inflammation, including recognition of bacterial antigens by dendritic cells and the immunological and clinical therapeutic effects of putative prebiotics and probiotics. Most recently, he conducted investigator-initiated studies into the perioperative management of Crohn's disease ("POCER" study) and fecal microbiota transplantation in ulcerative colitis ("FOCUS" study), both changing outcomes for patients and identifying specific bacteria associated with clinical outcomes.

Siew C. Ng is Professor at the Department of Medicine and Therapeutics, The Chinese University of Hong Kong. She received her medical degree from St Bartholomew's and Royal London School of Medicine and obtained her Ph.D. from Imperial College London. Dr. Ng's main research interests include inflammatory bowel disease, gut microbiota, and colorectal cancer, aiming to further our understanding of the pathogenesis of inflammatory intestinal disorders via clinical trials and translational research. She is President of the Hong Kong IBD Society, Scientific Secretary of the International Organization of IBD, and member of the Management Committee of the International IBD Genetics Consortium. She has published over 150 peer-reviewed papers in international journals, including *Nature Genetics*, *Nature Communications*, *Lancet*, *Gastroenterology*, and *Gut*. She is a pioneer of IBD Epidemiologic and Microbiome research in Asia-Pacific. Her work has received over 20 Prestigious National and International Awards.

Mark Morrison is the Chair in Microbial Biology and Metagenomics at The University of Queensland Diamantina Institute (UQDI), since October 2013. An authority in metagenomic and molecular microbiology, he is currently Australia's science representative to the International Human Microbiome Consortium. Professor Morrison obtained his Ph.D. from the University of Illinois, Urbana, IL. After 20 years in the United States, he returned to Australia in 2006 as CSIRO's first appointment via the Science Leader Scheme (leading the Gut Health Flagship Research Program). Since 2007, he has held a Professorship within the School of Chemistry and Molecular Biosciences, The University of Queensland. Professor Morrison aims to translate genomic and metagenomic data sets into sound biological frameworks, helping to produce novel diagnostic, organismal, and enzyme-based technologies. While Professor Morrison's focus was initially on microbial physiology and metabolism, he has since attained international acclaim for his efforts to successfully develop and use genomics and related methods to study anaerobic "commensal" gut bacteria.