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REVIEW

Rapid Growth and Metabolism of Uropathogenic *Escherichia coli* in Relation to Urine Composition

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SUMMARY Uropathogenic *Escherichia coli* (UPEC) strains cause a majority of urinary tract infections (UTIs). Since UPEC strains can become antibiotic resistant, adjunct or alternate therapies are urgently needed. UPEC strains grow extremely rapidly in patients with UTIs. Thus, this review focuses on the relation between urine composition and UPEC growth and metabolism. Compilation of urinary components from two major data sources suggests the presence of sufficient amino acids and carbohydrates as energy sources and abundant phosphorus, sulfur, and nitrogen sources. In a mouse UTI model, mutants lacking enzymes of the tricarboxylic acid cycle, gluconeogenesis, and the nonoxidative branch of the pentose cycle are less competitive than the corresponding parental strains, which is consistent with amino acids as major energy sources. Other evidence suggests that carbohydrates are required energy sources. UPEC strains in urine *ex vivo* and *in vivo* express transporters for peptides, amino acids, carbohydrates, and iron and genes associated with nitrogen limitation, amino acid synthesis, nucleotide synthesis, and nucleotide salvage. Mouse models confirm the requirement for many, but not all, of these genes. Laboratory evolution studies suggest that rapid nutrient uptake without metabolic rewiring is sufficient to account for rapid growth. Proteins and pathways required for rapid growth should be considered potential targets for alternate or adjunct therapies.

KEYWORDS *Escherichia coli*, carbon metabolism, iron metabolism, nitrogen metabolism, nucleotide metabolism, urinary tract infection

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INTRODUCTION

For clinicians, urinary tract infections (UTIs), and particularly recurrent urinary tract infections (RUTIs), constitute a major and growing health challenge (1). In women with RUTIs, the classic dogma regarding their pathogenesis has held that these infections occur in an antegrade fashion, with bacteria moving in from the vagina or perineum and ascending the urethra to finally enter the bladder. Thus, each episode of infection was viewed as a recurrent reinfection with the same or a different strain, depending on the urethral flora. This dogma led to RUTI prevention approaches such as vaginal hormone treatments to modify the vaginal pH, improve vaginal trophicity, and diminish bacterial adherence (1). Another explanation for RUTI was provided through the work of Mulvey et al., which suggested that bacteria can attach to the bladder surface, become internalized, and thus persist in the bladder wall (2). The mainstay of RUTI treatment has been dominated by antibiotics, but the persistence mechanism just described attenuates their effectiveness. Furthermore, antibiotic effectiveness is decreasing because of widespread allergy and resistance issues, which are particularly relevant to the older population of postmenopausal women who are often affected by RUTIs; new therapies to treat RUTIs are urgently needed (3). Not surprisingly, nonantibiotic therapies have received recent attention (4–6).

Uropathogenic *Escherichia coli* (UPEC) causes 80 to 90% of community-acquired UTIs, 30 to 50% of nosocomially acquired UTIs, and most RUTIs (7, 8). Mouse models provide much of our current molecular information on UTIs by UPEC strains (9). Bacteria in the bladder bind to the urothelium using a type 1 pilus, and the protein at its tip, FimH, is a mannose-binding lectin that binds proteins, such as uroplakin 1a, on the urothelium (10). Once bound, UPEC invades the bladder epithelial layer and forms intracellular bacterial communities (IBCs) in the acute phase of infection (9). Only 0.1 to 0.01% of the bacteria are internalized, and 1% of these bacteria form IBCs (11). Bacteria from the IBCs are released and begin the infectious cycle again (9). In addition, after the superficial layers slough off, bacteria attach to deeper layers, forming quiescent intracellular reservoirs (QIRs) inside the bladder wall (9). QIRs potentially provide inocula for reinfection after antibiotic treatment depletes extracellular bacteria in the bladder lumen. Consistent with the findings from this animal model, recent data from De Nisco and colleagues demonstrated the presence of bacteria in the bladder walls of women with RUTIs (12). From the bladder, the infection can spread to the kidneys (pyelonephritis) and the bloodstream (sepsis).

Numerous factors contribute to a UTI. Important components of UTI susceptibility are the properties of uropathogenic bacteria, which include the FimH adhesin, a variable group of virulence factors, and the ability to grow extremely rapidly in urine and the bladder (13–15). Another critical factor is the variations in glycosylation of the urothelium, to which UPEC strains bind, and the Tamm-Horsfall protein, which binds to bacteria and prevents binding to the urothelium. Evidence suggests that the carbohydrate expression profile of the urothelium is more important than variations in adhesin binding (16, 17). Still another crucial factor is the effectiveness of several host defense mechanisms (18). In addition, there is a resident genitourinary microbiome that might protect against bacterial invasion (19, 20). Consistent with this possibility is the observation that the microbiome changes with disease (21, 22).

Rapid growth in urine and the resulting elevation in bacterial numbers increase the chance of adhesion, internalization, formation of IBCs and QIRs, and spreading past the urinary tract. UPEC strains have been shown to grow better in urine than nonpathogenic strains (23); in fact, UPEC strains grow extraordinarily fast in urine, and rapid growth has been proposed as a virulence factor (14, 24). Urine composition is likely to be crucial for maintaining a healthy genitourinary microbiome or encouraging UPEC growth. One wonders what urine components and metabolic pathways allow UPEC to grow readily in urine. Age-related changes or fluctuations in urine composition may account for increased susceptibility to infection. This review addresses the relation between urine composition and bacterial growth, as well as bacterial adaptations that

promote rapid growth of UPEC strains in urine during UTIs. A thorough understanding of UPEC metabolism and bacterial adaptations to urine may suggest targets for alternate or adjunct therapies to reduce the frequency of UTIs.

RAPID BACTERIAL GROWTH IN URINE IS A VIRULENCE FACTOR

Several studies demonstrated extremely rapid growth of uropathogens and UPEC strains in urine. The first quantitative study of bacterial growth in urine stated that "even without added glucose, urine generally supports multiplication of the usual pathogens of the urinary tract about as well as does nutrient broth" (25). A subsequent study showed that "normal urine is noninhibitory to small inocula of Enterobacteriaceae and enterococci but that it is not as conducive to growth for some of the indigent, resident flora" (26). When 12 human UPEC strains and 10 human intestinal *E. coli* strains were compared, both groups grew equally well in broth (average of 1.9 generations per hour), but the UPEC strains grew faster in pooled urine (averages of 1.92 and 1.20 generations per hour, respectively) and had a shorter lag before exponential growth (averages of 1.6 and 3.2 h, respectively) (23). Calculations indicated that the rapid growth was sufficient to maintain a bacterial presence without adhesion-mediated surface growth. A more recent study found an average doubling time of 22.4 min (2.7 generations per hour) for *E. coli* in the bladders of 38 women with cystitis symptoms (14). In addition, analysis of RNA from UPEC strains isolated from urine samples from women with UTIs, compared to UPEC strains grown in the laboratory, indicated elevated expression of genes associated with rapid growth, such as genes for ribosomal proteins, components of the transcriptional and translational machinery, tRNA processing, and cell division proteins (27, 28).

Recent studies are increasingly recognizing the importance of rapid growth and metabolism for virulence. A study of 43 human UPEC strains that cause RUTIs did not find a pattern of virulence factors, and urovirulence was proposed to involve conserved functions, such as metabolism (15). Another study found that one particular group of human UPEC strains, the ST131 lineage, had a low virulence factor score (number of virulence factors), but the greatest metabolic potential (more metabolic activities), compared to a collection of 300 human UPEC isolates. For this lineage, metabolism was proposed to be an important component of fitness (29). Growth and metabolism have been proposed to be virulence factors (24).

The rapid growth of UPEC strains in urine and the resulting high cell density may be a mechanism to overcome host defenses; if so, then rapid growth in urine may often be the first step in a UPEC-mediated UTI. The resulting high cell density in the bladder may facilitate pilus-dependent attachment. The relation between pilus synthesis and growth in urine is complex. Pilus synthesis was low for three strains grown in pooled human urine (30), and human urine blocked pilus synthesis and function (31). In contrast, pilus gene expression from UPEC strains isolated directly from urine of UTI patients was variable (27, 32). This evidence supports the conclusion that only a minority of bacteria attach to and enter urothelial cells (9). A high bacterial density may protect the few piliated cells from host defense mechanisms. If rapid growth is a virulence factor, then interventions that slow the growth rate are therapeutically relevant, perhaps by reducing UPEC adhesion to and entry into urothelial cells.

UPEC GENETIC DIVERSITY AND ADAPTABILITY

UPEC strains show great diversity in gene content, virulence factors, genomic islands, and pathogenicity islands (28, 33–37). For example, one study of 44 nonduplicate human UPEC isolates found 27 different sequence types (38). One reason for this diversity is the large pan-genome, that is, the total number of genes present from all *E. coli* strains. Analysis of 2,085 sequenced *E. coli* genomes indicated a core genome of 3,188 gene families and a pan-genome size between 60,000 and 89,000 gene families. The pan-genome size is actually an underestimate, since it continues to be a linear function of the number of genomes sequenced (39). Several classification schemes have defined *E. coli* subgroups, including the Clermont scheme, which divides *E. coli*

into phylogenetic groups A, B1, B2, C, D, E, and F (40); most UPEC strains are in the B2 group (7, 15). Extensive horizontal gene transfer is suggested not only by the large pan-genome size but also by the observation that the CRISPR (clustered regularly interspaced short palindromic repeat) systems, which block horizontal gene transfer, were less active in UPEC strains than in commensals (41). When the extent of UPEC diversity began to be appreciated, the possibility of different UPEC pathotypes was suggested (42). A pathotype is defined as a group of strains that has a particular set of virulence factors and therefore a distinct pathogenesis. Recent studies showed the absence of distinct genetic signatures for urovirulence, resulting from a great diversity of virulence factor combinations, which argued against distinct genetic pathotypes (15, 38).

In addition to a large pan-genome, rapid evolution also increases UPEC diversity. A well-studied *E. coli* strain that causes asymptomatic bacteriuria was introduced by intravesical inoculation into six human volunteers and allowed to colonize the bladder. At monthly intervals, the infecting bacterium was reisolated and resequenced; the results indicated rapid and divergent evolution (43). Human UPEC isolates are often mutator strains, which further contributes to genetic diversity (44, 45). The high frequency of mutator strains may result from high levels of reactive oxygen species in urine, which in turn could result from rapid growth, bacterial interactions with epithelial cells, or the host inflammatory response (46–48). In summary, a variety of mechanisms have the potential to contribute to UPEC diversity.

UPEC METABOLIC DIVERSITY AND FLEXIBILITY

An analysis of 20 human commensal and pathogenic *E. coli* strains showed that much of their genetic diversity was due to different cohorts of metabolic genes, which could account for rapid niche adaptation (49). Consistent with this diversity of metabolic genes is the observation that the pathways of amino acid and carbohydrate degradation differed substantially in three model *E. coli* strains that grew well in the urinary tract (30). These diverse metabolic genes were found in both extraintestinal and commensal *E. coli* strains, which suggested that extraintestinal virulence was a coincidental consequence of commensalism (49, 50). For example, growth of both commensal and pathogenic *E. coli* strains in the mouse intestine involved cometabolism of up to 9 sugars and several microaerobic and anaerobic respiratory systems (51–53).

Rapid growth must occur after passage from the intestinal to urinary tract, and *E. coli* quickly adapts to different environments. One major factor in dealing with changing environments is a simple and flexible program for metabolic regulation. A massive analysis of expression of central metabolic genes in nonpathogenic *E. coli* K-12, combined with a metabolomic analysis, showed that 90% of the changes in transcription of central metabolic genes were the result of three metabolites, i.e., cyclic AMP, fructose-1,6-bisphosphate, and fructose-1-phosphate, acting through two transcription factors, Crp and Cra (54). The importance of Crp for UPEC growth and metabolism has been confirmed (55, 56). This simple regulatory program could potentially explain how *E. coli* can handle the transition between the intestinal and urinary tracts.

The rapid growth of UPEC strains in urine may suggest an alteration of the metabolic program just described, and, consistent with this possibility, UPEC strains have acquired novel regulators that control or modulate metabolism. The QseBC two-component regulatory system, which is commonly found in UPEC strains, controls the expression of 36 regulators (57). The KguSR two-component regulatory system, which was also found in UPEC strains, stimulates α-ketoglutarate and energy metabolism during anaerobic growth (58), which is significant because anaerobic growth normally represses α-ketoglutarate dehydrogenase activity (59). Finally, several virulence genes in a high-pathogenicity island found in the well-studied UPEC strain CFT073 were shown to control energy metabolism (60).

Genetic diversity, adaptability, metabolic diversity, and metabolic flexibility have important implications. In its natural environment, the intestinal tract, *E. coli*'s ability to consume a variety of different nutrients has been proposed to impair or block growth of invading pathogens (61, 62). This same metabolic flexibility becomes problematic

TABLE 1 Solutes in urine at above 1 mM and some trace metals

Component	Concn[a]		
	Set 1[b]	Set 2[c]	Set 3[d]
Urea	280	300[e]	280
Cl^-	140	88[e]	
Na^+	130	125	113
K^+	43	36	58
NH_3/NH_4^+	23	23[e]	37
SO_4^{2-}	17	24[e]	17
Creatinine		10	
PO_4^{2-}	6.2	14[e]	
Ca^{2+}	7.0	2.0	5.3
Mg^{2+}	2.7	2.6	3.2
Citrate		2.5	
Hippuric acid		2.3	
Uric acid	0.58	1.9	2.3
Glucuronic acid		1.7	
Glycine		1.2	
Cysteine		1.0	
Trimethylamine *N*-oxide		0.9	
Taurine		0.8	
Fe^{2+}/Fe^{3+}	[2.5]	[0.89]	[5]
Cu^{2+}	[0.4]	[0.17]	
Ni^{2+}	[3.4]	[0.08]	

[a]Values are in millimolar, except for those in brackets, which are in micromolar.
[b]Values are a summary of those obtained using technology prior to 1974 (63). When an average was provided in the reference, that value is provided in the table. When a range without an average was given in the original reference, the midpoint of the range is provided here. The results were originally reported as daily excretion per body weight. For the purpose of presentation, the reported values were normalized to the midpoint of the creatinine range, which was assumed to be 10 mM.
[c]Results are from the human urine metabolome, which is considered the most reliable source of information (64). This reference combines a literature review with experimental validation using current technologies. The reported values were normalized to creatinine, which was assumed to be 10 mM.
[d]Results are from Ipe et al. (68) and were reported as concentrations.
[e]Results are from the human metabolome database (65). The urine metabolome is contained within the human metabolome, but the numbers from the two databases do not always agree. The human metabolome database provides individual entries and a range for each metabolite from each study, and it does not provide an overall average. The numbers provided in this table are the average of averages for each study entry and from range midpoints when an average was not provided. The use of midpoints adds some error, since outlier values can distort the midpoint.

when *E. coli* enters a different environment, such as the urinary tract, and outcompetes beneficial bacteria. Metabolic flexibility undoubtedly is a major factor in the success of *E. coli* as a pathogen in numerous niches.

URINE COMPOSITION

To understand rapid bacterial growth in urine, it is necessary to know the components of human urine. The most abundant components in urine are summarized in Tables 1 to 3, which are generally derived from two data repositories. The first data set summarizes 400 components measured prior to 1974 (63). The second set is the human urine metabolome database (64), which has become a subset of information from the human metabolome database (65). The metabolome data set summarizes 2,651 metabolites that have been detected in urine (64). In the tables, the values are the averages of multiple measurements from adults and are expressed as concentrations, even though the original results are often normalized to the creatinine level, which was assumed to be 10 mM for the calculations. In Tables 1 and 2, a third data set in which the values were reported as concentrations is presented; the calculated values from the first two data sets agree reasonably well with those from the third data set.

A major source of uncertainty for all values is the large range for some of these values: the average variation for a metabolite in the human urine metabolome is ±50%, with variations as high as ±350% (64). The variations may exist not only between individuals but also for one individual over time. These ranges are not shown in the tables, but a large range could be important for understanding UTIs, since an individual

TABLE 2 Amino acids in urine in mM

Amino acid	Concn (mM)		
	Set 1[a]	Set 2[b]	Set 3[c]
Alanine			
Total	0.55		
Free		0.22	0.28
Arginine			
Total	0.13		
Free	0.045	0.08	0.20
Asparagine			
Free		0.094	
Aspartate			
Total	0.63		
Free	0.015	0.11	
Cystine			
Total	0.35		
Free	0.27	0.12	
Cysteine			
Free		0.73	
Glutamate			
Total	1.46		
Free	0.27	0.075	
Glutamine			
Free	0.10–2.1	0.38	0.53
Glycine			
Total	4.27		
Free	1.44	0.94	1.1
Histidine			
Total	0.86		
Free	0.64	0.44	0.66
Isoleucine			
Total	0.075		
Free	0.030	0.013	0.020
Leucine			
Total	0.113		
Free	0.049	0.029	
Lysine			
Total	0.27		
Free	0.13	0.18	
Methionine			
Total	0.046		
Free	0.016	0.011	
Phenylalanine			
Total	0.090		
Free	0.051	0.067	
Proline			
Total	0.26		
Free	0.051	0.037	
Serine			
Total	0.28		
Free	0.14	0.23	0.53

(Continued on following page)

TABLE 2 Amino acids in urine in mM

Amino acid	Concn (mM)		
	Set 1[a]	Set 2[b]	Set 3[c]
D-Serine			
Free		0.115[d]	
Threonine			
Total	0.21		
Free	0.10	0.14	
Tryptophan			
Total	0.096		
Free	0.048	0.060	0.064
Tyrosine			
Total	0.19		
Free	0.054	0.091	0.093
Valine			
Total	0.13		
Free	0.038	0.049	0.040

[a]See footnote *b* of Table 1.
[b]See footnote *c* of Table 2.
[c]Values from a third data set are presented because concentrations were directly measured, which allows comparison to calculated results from the first two data sets (120).
[d]From Huang et al. (70).

may become susceptible to infection only when nutrient concentrations become favorable. Other sources of variability include differences in sample collection, daily fluctuation within individuals, and nonuniformity in sample populations (gender, age, time of collection, etc.).

Major Components: Electrolytes, Osmolytes, and Components over 1 mM

The major electrolytes and osmolytes and their approximate concentrations are urea (300 mM), Na^+ (120 mM), Cl^- (115 mM), K^+ (45 mM), NH_4^+ (30 mM), SO_4^- (20 mM), PO_4^- (10 mM), Ca^{2+} (5 mM), and Mg^{2+} (3 mM) (Table 1). A few additional compounds, whether organic or inorganic, are present near 1 mM: creatinine (assumed to be 10 mM for purpose of comparison), citrate (2.5 mM), hippuric acid (2.3 mM), glucuronic acid (1.7 mM), uric acid (1.6 mM), glycine (1.2 mM), cysteine (1 mM), trimethylamine *N*-oxide (0.9 mM), and taurine (0.8 mM) (calculated from Table 3 in reference 64). Figure 1 shows the distribution of major solutes, excluding urea, Na^+, Cl^-, and K^+, which constitute ~85% of the measured solutes.

The average pH of urine is about 6.0, with a typical range from 5 to 8 (66–69). Potential buffers are citrate (highest pK_a of 5.4), phosphate (pK_a of 7.2), and ammonia (pK_a of 9.2). Considering the low concentrations of citrate and phosphate, urine has little buffering capacity.

Growth Components: Carbon, Nitrogen, and Sulfur Sources

Since UPEC strains grow rapidly in urine, sources of carbon, energy, nitrogen, phosphate, and sulfur must be readily available. Possible carbon sources are carbohydrates, amino acids (both free and in peptides), nucleobases, and fatty acids. The nucleobases are not present in sufficient quantity to be a major source of carbon or energy. The fatty acid content is difficult to assess, especially since cell debris, which contains membrane and other insoluble material, is removed before assay of components in urine.

The free amino acid concentration is estimated to be 3 to 4 mM, and the combined total of amino acids, free and in peptides, is estimated to be 5 to 7 mM (Table 2). These values are based on the sum of measured free amino acids (from several sources) and the observation that 20 to 40 mg total (peptides plus free) amino acids is excreted per day, whereas 13 to 20 mg free amino acids is excreted (63), which suggests that 60% of the amino acids are free and 40% are in peptides. The five most abundant amino

TABLE 3 Carbohydrates, tricarboxylic acid cycle intermediates, nucleobases, and nucleotides in urine

Component	Concn[a]		
	Set 1[b]	Set 2[c]	Set 3[d]
Carbohydrates			
Arabinose		0.13	
Arabitol		0.32	0.21
Erythritol		0.33	0.48
Fucose		0.11	
Galactose		0.12	
Gluconate		0.17	
Glucuronate		1.7	
Glucose	0.19	0.38	
Glycerol	0.05	0.13	
Lactose		0.12	
Mannitol		0.32	0.21
Sorbitol		0.10	0.058
Xylose		0.20	
Xylulose		0.20	
TCA cycle intermediates			
2-Ketoglutarate	0.28	0.048	0.091
Acetate	0.08	0.13	0.20
cis-Aconitate		0.21	
Citrate	2.9	2.4	2.0
Fumarate	0.015	0.007	0.007
Isocitrate		0.57	
Malate	0.062		0.006
Succinate	0.053	0.062	0.056
Nucleosides and nucleobases			
Adenine	[7.4]	[29]	[16]
Adenosine		[14]	[14]
Hypoxanthine	[51]	[72]	[51]
Guanine	[2.0]		[2.8]
Guanosine		[0.8]	
Cytosine		[41]	
Cytidine			[2.6]
Uracil		[95]	[73]
Uridine			[4.1]
Pseudouridine	0.21	0.29	
Uric acid	0.58	1.9	2.3

[a]See footnote *a* of Table 1.
[b]See footnote *b* of Table 1.
[c]See footnote *c* of Table 1.
[d]See footnote *e* of Table 1.

acids (from the average from several databases) are glycine (1.6 mM), cysteine (0.97 mM), histidine (0.56 mM), glutamine (0.46 mM), and serine (0.30 mM). A few papers state that D-serine is the most abundant amino acid, but this is a misreading of the original paper; D-serine is the most abundant D-amino acid (70) and the 11th most abundant amino acid overall. *E. coli* has pathways to degrade only about half of the amino acids (71), and the concentration of utilizable amino acids is about 2 mM. The vast majority (~90%) of utilizable amino acids contain three carbons, which means that urine contains about 6 mM carbon atoms available for energy generation. For comparison, LB contains about 100 mM amino acids (72), and *E. coli* grew to a much higher density in LB than in urine (73). The five most abundant amino acids in LB are glycine (4 to 18 mM), glutamate (11 mM), valine (9.5 mM), leucine (7.9 mM), and serine (7.5) (72). In addition to a major difference in the total concentrations of amino acids, LB and urine clearly differ in their amino acid composition.

Carbohydrates are plentiful, even though no carbohydrate except glucuronic acid is present at above 1 mM (Table 3). The free carbohydrate concentration is at least 4.3 mM, and the sum of carbohydrate carbon atoms is about 25 mM. The carbohydrate

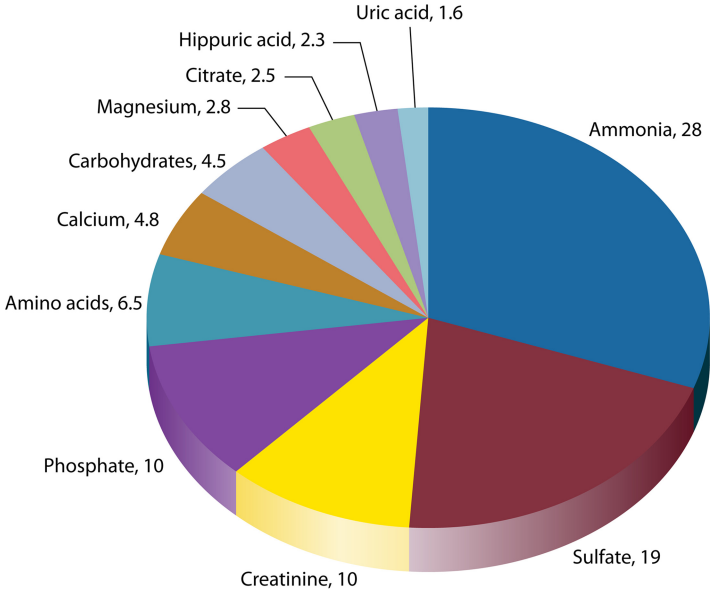

FIG 1 Major components of urine above 1 mM. For the purpose of presentation, this chart excludes urea (290 mM), Na^+ (123 mM), Cl^- (115 mM), and K^+ (46 mM). The values for all components are in millimolar and are from the tables. The amino acids and carbohydrates are the sums of individual components. The total carbohydrate content is the sum of 14 carbohydrates in Table 2, and undoubtedly more are present.

content is an underestimate because not all measured carbohydrates were included in the table. The point is not to provide an exhaustive list but to note that there are low concentrations of numerous carbohydrates and that their sum is substantially higher than the amino acids.

Urine also contains some tricarboxylic acid (TCA) cycle intermediates, nucleosides, and nucleobases (Table 3). Citrate (2 to 3 mM) accounts for over 80% of the TCA cycle intermediates in urine (Table 3), but a nonpathogenic strain of *E. coli* degrades citrate only under anaerobic conditions (74). The sum of the other TCA cycle intermediates is about 0.4 mM. The common nucleosides are in a range from 1 to 14 μM. The common nucleobases are more abundant and are present in a range from 17 to 85 μM, with the curious exception of guanine (~2 μM).

Urine contains a variety of other components. The most abundant nitrogenous compounds are urea, ammonia, and then the amino acids. Ammonia is the preferred nitrogen source of *E. coli*, and the high ammonia concentration suggests that urine is not nitrogen limited; however, gene expression studies actually suggest nitrogen limitation (discussed below). The three most abundant sulfur sources are sulfate (~20 mM), cysteine (~1 mM), and taurine (~0.8 mM), which indicates that urine is not obviously sulfur limited. Urine also contains measurable amounts of vitamins/cofactors, hormones, modified versions of amino acids and nucleobases, and numerous other compounds (63).

URINE COMPONENTS AFFECT GROWTH AND UTI SUSCEPTIBILITY

Several observations suggest that urinary composition is an important factor for UTI susceptibility. First, trauma patients are susceptible to UTIs, and urine from patients at 1 and 5 days after severe trauma supported a substantially higher *E. coli* growth yield, i.e., final cell density, than urine from healthy volunteers (75). Urine from the trauma patients had 55% less urea, 34-fold more urinary protein, substantially higher levels of all free amino acids, and higher levels of glucose and iron, and, as described below, all of these factors affect bacterial growth. Second, elderly women are more susceptible to UTIs, and although a systematic analysis of their urine has not been performed, older women had urine with a lower specific gravity (i.e., urea concentration) and probably diminished kidney function (76); the diminished urea concentration could promote

TABLE 4 Metabolic defects that affect growth in urine or competitive fitness in a mouse model

Category and defective gene(s)	Function	Growth in urine[a]	Defect(s) in UTI[b]	Strain(s) tested	Reference(s)
Central metabolic and catabolic enzymes					
dppA	Dipeptide transport		B, not K	CFT073	78
oppA	Oligopeptide transport		B and K	CFT073	78
talA talB[c]	Nonoxidative branch of pentose cycle		B and K	CFT073	121
sdhB	Tricarboxylic acid cycle	+	B and K	CFT073	78
fumC	Tricarboxylic acid cycle		B, not K	CFT073	121
pckA	Gluconeogenesis	+	B and K	CFT073	78
pta	Acetogenesis		K, not B	CFT073	82
ackA	Acetogenesis		K, not B	CFT073	82
pta ackA[c]	Acetogenesis		B and K	CFT073	82
dsdA	D-Serine catabolism	-/+	None	CFT073	87
sdaA sdaB	Serine degradation		B, not K	CFT073	85
lacZ	β-Galactoside degradation		B and K	UTI89	94
galK	Galactose degradation		B and K	UTI89	94
vpeBC	Carbohydrate transport		K, not B	AL511	91
Amino acid synthesis					
argBCH	Arginine synthesis	−/+		CFT073	97
argC	Arginine synthesis	− or −/+	K, not B	ABU83972, CP9	73, 96
argE	Arginine synthesis	−		ABU83972	96
argG	Arginine synthesis	−/+	None	CFT073	78
carAB	Arginine and pyrimidine synthesis	− or −/+	B and K	CFT073, ABU83972	96, 97
glnA	Glutamine synthesis	−		P28	95
ilvA	Isoleucine synthesis	−		ABU83972	96
ilvC	Branched-chain amino acid synthesis	−	None	ABU83972	96
metE	Methionine synthesis	−	None	ABU83972	96
Nucleotide metabolism					
apt hpt	Purine salvage	−/+	B, not K	CFT073	97
guaA	Purine synthesis and salvage	− or −/+	B and K	CFT073, CP9	73, 97
purA	Purine synthesis and salvage	− or −/+	B and K	CFT073, ABU83972	96, 97
purE apt hpt[c]	Purine synthesis and salvage	−/+	B and K	CFT073	97
pyrF upp	Pyrimidine synthesis and salvage	−/+	B and K	CFT073	97

[a]No entry indicates that growth was not examined in urine or that a mouse coinfection experiment was not performed.
[b]Mutants were coinfected with an isogenic parental strain without a mutation, and the effects of the infection were observed. B, infection in the bladder; K, infection in the kidneys.
[c]The defects were cumulative; defects were less pronounced with fewer mutations.

growth. Third, patients with culture-confirmed UTIs often had a variety of alterations of urine composition, including high proteinuria, which could provide a potential source of carbon and energy (reviewed in reference 69). Finally, diabetics are more susceptible to UTIs than are nondiabetics (77). In summary, changes in a variety of urinary components can enhance bacterial growth.

ENERGY METABOLISM DURING GROWTH IN URINE AND DURING UTI

The methods to assess the importance of a particular metabolic pathway for a UTI are (i) growth of wild-type and mutant strains in urine, (ii) proteomic and transcriptomic analysis of cells either grown in urine or isolated from urine of mice or humans with UTIs, and (iii) competitive fitness experiments in a mouse model in which wild-type and mutant strains compete in the same animal. Table 4 describes all metabolic mutants that either had impaired growth in urine or were less competitive in a mouse coinfection experiment. Table 5 describes metabolic mutants that were shown not to affect growth in urine or competitive fitness. The results of the experiments summarized are largely from analysis of one strain, CFT073: of the 60 metabolic mutants analyzed, 46 were CFT073 derivatives. Given the genetic diversity of UPEC strains, CFT073 metabolism may or may not be representative of that of other UPEC strains. CFT073 is a member of phylogenetic group B2, but not all UPEC strains are in group B2. An additional complication is that most of our knowledge of metabolism during UTIs is

TABLE 5 Metabolic defects that do not affect competitive fitness in a mouse model

Category and defective gene	Function	Comment	Strain tested	Reference(s)
Central metabolic and catabolic enzymes				
pfkA	Glycolysis	Major isozyme	CFT073	121
pgi	Glycolysis	Grew well in urine	CFT073	78, 121
pykA	Glycolysis	One of two isozymes	CFT073	121
tpiA	Glycolysis	Grew well in urine	CFT073	78, 121
gnd	Oxidative pentose cycle	Grew well in urine	CFT073	78, 121
talA[a]	Nonoxidative pentose cycle	One of two isozymes; grew well in urine	CFT073	78, 121
talB[a]	Nonoxidative pentose cycle	One of two isozymes	CFT073	121
edd	Entner-Doudoroff pathway	Grew well in urine	CFT073	78, 121
frdA	Reductive tricarboxylic acid cycle	Anaerobic metabolism	CFT073	121
acs	Acetate assimilation		CFT073	82
Amino acid and polyamine metabolism				
argG	Arginine synthesis	Less growth in urine	CFT073	78
dsdA	D-Serine catabolism	Slower growth in urine	CFT073	87
ilvC	Branched-chain amino acid synthesis	Less growth in urine	ABU83972	96
metE	Methionine synthesis	Less growth in urine	ABU83972	96
serA	Serine synthesis	Grew well in urine	CFT073	78
speB	Putrescine synthesis	Minor pathway	CFT073	78
Nucleotide metabolism				
purE[b]	Purine synthesis	Grew well in urine	CFT073	97
pyrF[c]	Pyrimidine synthesis	Grew well in urine	CFT073	97
upp[c]	Pyrimidine salvage	Grew well in urine	CFT073	97
Carbohydrate catabolism				
araF	Arabinose catabolism		CFT073	78
nanA	Sialic acid catabolism		CFT073	78
uxuA	Hexuronate catabolism		CFT073	78
xylA	Xylose catabolism		CFT073	78

[a]Unlike each single mutant, the *talA talB* double mutant is less competitively fit (121).
[b]The *apt hpt* double mutant is less competitively fit in a mouse UTI model, and the *purE apt hpt* triple mutant is even less fit (97).
[c]Unlike each single mutant, the *pyrF upp* double mutant is less competitively fit (97).

from mouse models, and such infections may or may not be representative of human infections, especially RUTIs.

Central Metabolic Pathways

In a mouse model, mutants lacking enzymes of the tricarboxylic acid (TCA) cycle, gluconeogenesis, the nonoxidative branch of the pentose cycle, and peptide transport were less competitive than parental strains. In contrast, mutants lacking key enzymes of glycolysis, the Entner-Doudoroff pathway, and the oxidative branch of the pentose cycle were as competitive as their parental strains (78). Figure 2 shows a schematic of these pathways and gene products whose loss was tested in a mouse UTI model. Such a pattern is most consistent with amino acids, not carbohydrates, as the energy source. Transcriptomic analysis of *E. coli* RNA isolated directly from the urine of women with UTIs showed expression of electron transport components that use oxygen or nitrate as a terminal electron acceptor (27). The expressed terminal reductases suggested conditions that varied from aerobic (high oxygen) to microaerobic (low oxygen) to anaerobic (nitrate consumption) (27). The fumarase-nitrate reductase regulator (Fnr), which controls the transitions between aerobic and anaerobic growth, has been shown to be a major regulator of UPEC metabolism (79).

A metabolomic analysis of urine from patients with UTIs found elevated levels of acetate and trimethylamine (80). Trimethylamine generation suggests anaerobic respiration with trimethylamine *N*-oxide, which is abundant in urine, as the terminal electron acceptor (81). Transcriptome analysis confirmed the production of acetate, since the enzymes of acetate generation were elevated and enzymes of acetate assimilation were reduced in bacteria from the urine of women with UTIs (27). Loss of enzymes necessary for acetogenesis attenuated virulence (82). Acetate formation from either carbohy-

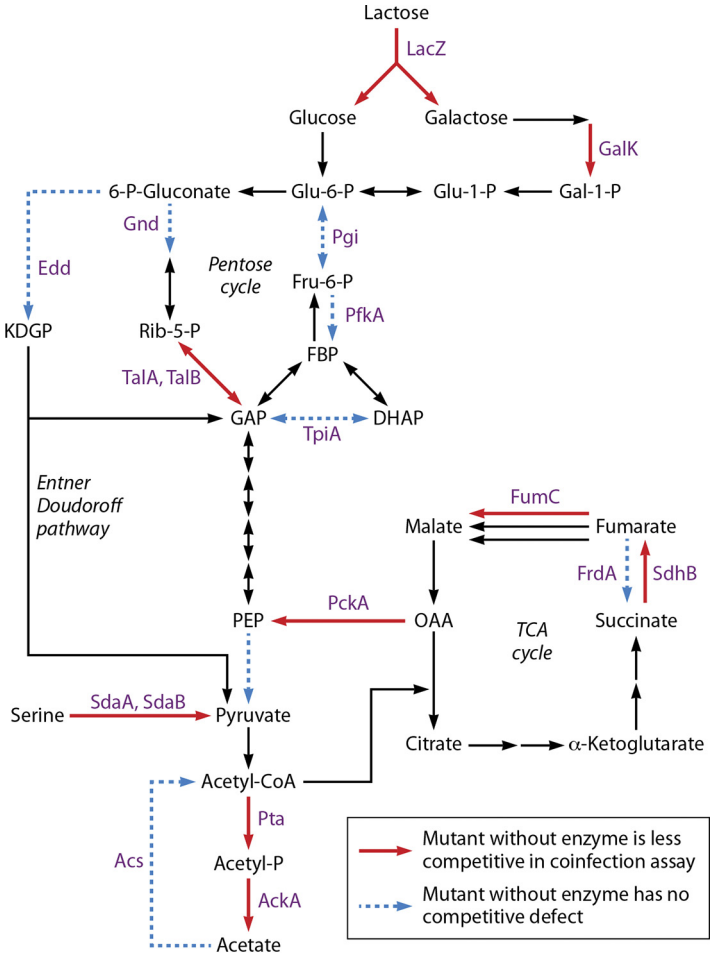

FIG 2 Central metabolic pathway requirement for UTIs. Protein designations are provided only for mutants that were tested in a mouse UTI coinfection model. See Tables 4 and 5 for more-detailed information. The thick arrows indicate reactions required for a mouse UTI, and the dashed arrows indicate reactions that are not required. Double-headed arrows represent reversible reactions. The pentose cycle is simplified to show a linear sequence, when it is actually a sequence of eight reactions that interconnect with glycolysis. Metabolite abbreviations: DHAP, dihydroxyacetone phosphate; Fru-6-P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; Gal-1-P, galactose-1-phosphate; GAP, glyceraldehyde-3-phosphate; Glu-1-P, glucose-1-phosphate; Glu-6-P, glucose-6-phosphate; KDGP, 2-keto-3-deoxygluconate-6-phosphate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; Rib-5-P, ribose-5-phosphate. Enzyme abbreviations: AckA, acetate kinase; Acs, acetyl coenzyme A synthetase; Edd, phosphogluconate dehydratase; FrdA, fumarate reductase; FumC, fumarase C; GalK, galactokinase; Gnd, 6-phosphogluconate dehydrogenase; LacZ, β-galactosidase; PckA, phosphoenolpyruvate carboxykinase; PfkA, phosphofructokinase I (the major isozyme); Pgi, glucose-6-phosphate isomerase; Pta, phosphotransacetylase; PykA, pyruvate kinase (isozyme II); SdaA, L-serine deaminase isozyme I; SdaB, L-serine deaminase, isozyme II; SdhB, succinate dehydrogenase subunit; TalA, transaldolase isozyme A; TalB, transaldolase isozyme B; TpiA, triose-phosphate isomerase.

drates or possibly acetogenic amino acids (serine, glycine, alanine, and cysteine) suggests substrate-level phosphorylation. The diversity and flexibility of energy-generating pathways—substrate-level phosphorylations along with oxidative phosphorylation driven by multiple electron transport pathways—will allow UPEC strains to handle diverse and changing environments.

Amino Acid Utilization

Amino acids have been proposed to be the major energy source for UPEC strains in mouse models of UTIs, based on the requirement for gluconeogenesis but not glycolysis. Furthermore, amino acid utilization is also consistent with expression of transporters for amino acids and peptides from bacteria either grown in urine or from patients with a UTI (27, 28, 30, 78, 83, 84). The peptide transporters OppA and DppA were

reported to be highly expressed in most of these studies, and loss of either resulted in a competitive disadvantage in a mouse infection model (78). Transporters for alanine, arginine, aspartate, glutamate, glutamine, glycine, histidine, and serine were also reported to be highly expressed in some of these studies, but high expression in urine did not necessarily correlate with a requirement for some of these transporters during an infection (78). The contribution of most of the amino acid transporters to UTIs has not been evaluated in an animal model.

E. coli can utilize only about half of the amino acids as energy sources (71), but which amino acids in urine are degraded for energy are not known. Only one study examined the effect of loss of a specific amino acid catabolic pathway on a UTI: loss of L-serine degradation resulted in a competitive growth defect in the murine bladder (85). Another gene involved in amino acid catabolism, the *tkt1* gene, is prevalent among extraintestinal *E. coli* of the B2 phylogenetic group (which includes many UPEC strains) and codes for a dipeptidase that cleaves at least two alanine-containing dipeptides (86). Several studies suggested the importance of D-serine degradation, but a more recent study noted problems with the previous studies and concluded that D-serine degradation did not contribute to colonization in the murine urinary tract (87).

Carbohydrate Utilization

Carbohydrates are not thought to be a major energy source in urine because mutants lacking gluconeogenic enzymes are less fit in a competitive mouse infection model, but mutants lacking some glycolytic enzymes are as fit as the corresponding parental strains. However, other considerations suggest not only that carbohydrate metabolism contributes to virulence but also that carbohydrates may be major energy sources. First, with the exception of glucuronate, at 1.7 mM, all urinary carbohydrates appear to be under 0.4 mM. However, as noted above, utilizable carbohydrates are more abundant than utilizable amino acids in urine. Given *E. coli*'s ability to simultaneously utilize multiple carbohydrates (51), the contribution of carbohydrate catabolism to energy metabolism and virulence during UTIs should not be dismissed. Second, the observation that glycolytic mutants of CFT073 are not less competitive in a mouse UTI model would seem to suggest that carbohydrates are not energy sources. However, the glycolytic mutants that were tested in the mouse model lacked Pgi (phosphoglucoisomerase), PfkA (one isozyme of phosphofructokinase), or PykA (one isozyme of pyruvate kinase), and these enzymes are not needed for the degradation of most urinary carbohydrates, such as glucuronic acid, listed in Table 3. Third, the observation that gluconeogenic mutants of CFT073 are at a competitive disadvantage in a mouse model suggests amino acids over carbohydrates as the energy source. However, since degradation of many carbohydrates in urine does not generate hexose phosphates, gluconeogenesis would be required for synthesis of hexose phosphates from these carbohydrates for polysaccharide synthesis. Fourth, genes that degrade hexuronates, sorbitol, fructose, arabinose, xylose, and other carbohydrates were highly expressed during growth in urine (30, 78, 83, 88, 89). Fifth, carbohydrate catabolic genes appear in pathogenicity-associated islands (90–93). Sixth, carbohydrate utilization genes were more prevalent in the B2 phylogenetic group than in the A and B1 phylogenetic groups, and the B2 group is more often associated with human UTIs: the B2 group has been specifically proposed to utilize carbohydrates as their energy source (28). Seventh, two studies showed that loss of carbohydrate catabolic genes impaired virulence. In the first study, the expression of genes for lactose and sorbitol catabolism was induced in intracellular bacterial communities during UTIs, and their loss reduced virulence (94). In the second study, loss of the Vpe carbohydrate permease, which transports an unknown carbohydrate, impaired virulence and reduced exopolysaccharide formation (91). Finally, as mentioned above, UPEC strains grew better in urine than nonpathogenic strains, but both grew equally well in broth. A major difference between urine and broth is that urine contains carbohydrates (Table 3), but the broth used does not contain utilizable carbohydrates (23, 72). The difference in growth in urine versus broth might be carbohydrate utilization. The impor-

tance of carbohydrate metabolism for UPEC strains during UTIs should be considered an open question and may be strain specific.

IMPORTANT BIOSYNTHETIC PATHWAYS

Amino Acid Synthesis and the Paradoxical Induction of the Nitrogen-Regulated Response

Urine contains many amino acids and nucleobases for macromolecular synthesis, but some building blocks are not sufficiently abundant and must be synthesized. The first studies to examine precursors that must be synthesized during growth in urine examined which amino acid or vitamin auxotrophs (mutants with specific nutritional deficiencies) grew to a lower cell density in urine. Guanine, arginine, and glutamine auxotrophs grew very poorly (<5%); leucine, methionine, serine, phenylalanine, and proline auxotrophs grew less well than an isogenic parental strain (5 to 50%); and nicotinamide, histidine, tryptophan, cysteine, and adenine auxotrophs grew as well as an isogenic parental strain, which suggested that compounds of the last group were present in excess (95). A similar study showed that genes involved in arginine and guanine synthesis were required for growth in human urine. Although a guanine auxotroph completely failed to grow in urine, it was only about three times less virulent than a wild-type strain in a mouse model (73). An arginine auxotroph grew as fast as the parental strain in urine but reached a much lower final cell density. Compared to a prototropic strain, an arginine auxotroph colonized mice as well in the bladder but less well in the kidneys (73). An arginine auxotroph does not have a growth defect *in vivo*, perhaps because arginine is continually replenished in the bladder. Two more thorough and recent studies both found reduced growth in urine for mutants with defects in synthesis of arginine, methionine, the branched-chain amino acids, purines, and pyrimidines (96, 97). A microarray analysis compared gene expression of UPEC strains grown in human urine and in a minimal medium and found expression in urine for genes of arginine, aspartate, methionine, lysine, serine, alanine, and glutamate synthesis (96). In summary, growth in urine requires synthesis of several amino acids.

UPEC growth in urine of mice or humans induces glutamine synthetase (GlnA) (27, 28, 83). GlnA expression implies nitrogen limitation, which is unexpected, since urine is rich in nitrogen sources, especially ammonia (~30 mM), which is the preferred nitrogen source of *E. coli* (98). GlnA assimilates ammonia into glutamine, which is required for synthesis of several amino acids and all the nucleotides. In addition, the *glnA* gene is part of a complex *glnA-ntrBC* operon that codes for the major regulators of the nitrogen-regulated (Ntr) response, NtrB and NtrC. Elevated GlnA implies not only ammonia restriction but also induction of 100 genes of the Ntr response. Ntr genes include several coding for amino acid and peptide transporters (98), including DppA-BCDF, which was important for growth in urine and during UTI in a mouse model. Despite the presence of ammonia, a urine component(s) must limit glutamine synthesis, which in turn will limit the synthesis of several amino acids and induce several genes of amino acid biosynthesis. In addition, low intracellular glutamine will induce the Ntr response, which could account for elevated expression of amino acid and peptide transporters. Despite the induction of the Ntr response, the inhibitory component that limits glutamine synthesis does not prevent a high growth rate, perhaps because of expression of amino acid and peptide transporters.

Purine and Pyrimidine Synthesis

The nucleobases in urine are in the range of 17 to 85 μM, except for guanine, which is present at about 2 μM, and the nucleobases are more abundant than the nucleosides. When adenine is more abundant than guanine, as in urine, adenine is toxic (99). The generation of the high-adenine/low-guanine urinary environment could conceivably be a host defense mechanism. Nucleotide synthesis can occur via *de novo* or salvage pathways, and evidence suggested that both contribute to UTIs. Mutants defective in GMP synthesis grew poorly in urine (73, 95, 96), and purine and pyrimidine biosynthetic genes were highly expressed in bacteria from women with UTIs (27, 28).

Furthermore, mutants defective in GMP or pyrimidine synthesis had attenuated virulence in a mouse model (73, 100). The effects on virulence (i.e., coinfection studies) were greatest with defects in both the *de novo* and salvage pathways, that is, in strains with separate lesions in both pathways, or in a strain with a defective enzyme that participates in both pathways, such as GuaA (97).

UREA, OSMOLALITY, AND GROWTH

The major components of urine osmolality are urea and inorganic ions (see Table 1). Urea is membrane permeative and can denature proteins and nucleic acids, whereas inorganic ions tend to stabilize macromolecules (101). RNA profiling of pathogenic CFT073 showed that the effects of high urea and inorganic ions were different (102). High salt increased expression of genes of osmotic stress (synthesis or transport of osmoprotectants) and anaerobic metabolism (e.g., nitrate reduction) and reduced expression of genes of motility (flagella and chemotaxis) and purine and pyrimidine metabolism. High urea increased expression of genes for some chaperones and virulence factors, such as the capsule and pili, and lowered expression of genes of acid stress and sulfur metabolism.

A combination of urea and low pH is bacteriostatic (103), perhaps because low pH exacerbates the denaturing effects of urea. Higher pH and lower urea should stimulate bacterial growth, and clinical evidence supports this conclusion. Urine specific gravity (a function of urea) decreased with age (76), which may partially account for an increased frequency of UTIs in elderly patients. Furthermore, urea concentration decreased in trauma patients, and such patients are more susceptible to UTIs (75).

IRON, METABOLISM, AND VIRULENCE

Iron is required for the activity of several important metabolic enzymes, especially those of energy metabolism, and iron availability affects metabolism (104). Genes of iron uptake are highly expressed in *E. coli* grown in urine or isolated from urine of mice or humans with UTIs, which implies iron limitation (27, 28, 30, 32, 83, 88, 105). However, iron limitation is difficult to reconcile with rapid growth and metabolism, which implies adequate intracellular iron. Three forms of iron are transported into cells: ferric ions (Fe^{3+}), ferrous ions (Fe^{2+}), and iron-containing heme (106). Ferric iron binds citrate or a variety of secreted ferric-binding siderophores. Each *E. coli* strain contains several iron uptake systems; for example, CFT073 has at least ten ferric uptake systems, and other UPEC strains often have at least seven uptake systems (28, 105, 107). All of these systems are expressed under conditions with low iron, since the Fur protein complexed to Fe^{2+} represses all of these uptake systems (106).

Multiple studies show that iron uptake systems contribute to virulence. Genes for synthesis of the siderophore aerobactin are part of a genomic island, which contributed to colonization of the human bladder (108). The gene for one siderophore receptor was found in pathogenic, but not commensal, isolates (109). Loss of iron uptake genes reduced virulence in mouse coinfection models (reviewed in reference 105). Loss of specific systems reduced both the growth rate and growth yield in urine, and the effects of loss of multiple systems were cumulative (110). As a countermeasure, host mechanisms inactivate siderophore activity (reviewed in reference 79).

The iron uptake systems are redundant to some extent, since loss of one or two systems often, but not always, had little effect *in vivo* or *in vitro* (110, 111). Loss of some systems impaired kidney colonization, which suggested a tissue specificity for these systems (111). On the other hand, loss of more than one system reduced the growth rate and growth yield in human urine and reduced competitive fitness in a mouse model (110). The effect on growth yield implies that multiple systems extract more environmental iron, and the effect on growth rate implies that multiple systems are required to maintain sufficient intracellular iron for rapid growth and metabolism. An additional function of multiple iron uptake systems may be to bind iron to a siderophore that can be used only by the organism that synthesizes the siderophore

and has the appropriate receptor. In other words, multiple uptake systems confer a competitive advantage to those bacteria with them (110).

RAPID GROWTH AND LABORATORY EVOLUTION

UPEC strains grow faster than commensal strains in urine, which implies acquisition of adaptations that allow faster growth. Laboratory evolution studies suggest two strategies for faster growth: faster nutrient uptake and metabolic fluxes in standard pathways (112) or metabolic rewiring, which can be described as the repurposing of pathways for new functions. Metabolic rewiring has been observed only when the starting strain for an adaptive evolution experiment was lacking or defective in a major metabolic process, and metabolic rewiring bypassed the defect (113–115). As described above, UPEC strains grown in urine or from urine of patients with UTIs had elevated levels of RNA from genes for uptake systems compared to when these strains were grown in broth. Such gene expression suggests that rapid nutrient uptake promotes rapid growth.

CONCLUSION: THE CONTROL OF UROPATHOGENS

Rapid growth in urine and the urinary tract is a prominent property of UPEC strains, and this review has summarized the relation between urine composition and UPEC growth. Numerous unresolved issues remain. First, how much do urine composition and bacterial growth contribute to UTI susceptibility? The fact that UPEC strains grow more rapidly in urine than nonpathogenic strains suggests that rapid growth and metabolism are virulence factors. Second, why do UPEC strains, compared to nonpathogenic strains, grow better in urine but grow equally well in broth? Two non-mutually exclusive explanations are possible: (i) urine, unlike broth, contains carbohydrates, and UPEC strains utilize carbohydrates more efficiently, or (ii) amino acids are the primary carbon and energy source for both types of strains, and UPEC strains utilize urinary amino acids more efficiently than the nonpathogenic strains. The second explanation would appear to be less likely, since both types of strains have been reported to grow equally well in amino acid-rich broth which lacks carbohydrates (23). Third, most of our knowledge about UPEC metabolism is based on CFT073, and considering the genetic diversity, metabolic flexibility, and adaptability of *E. coli*, CFT073 may or may not be representative of other UPEC strains. Fourth, although we have a general sense of carbon and energy sources during UTIs (amino acids and carbohydrates), we have virtually no information about which specific amino acids and carbohydrates are important; multiple carbon sources are likely to be used simultaneously, and particular combinations of energy sources may have synergistic effects. Are the same amino acids and carbohydrates used for each UTI, or do variations in urine composition from patient to patient determine which compounds provide energy? Finally, how are iron and nitrogen limitation compatible with rapid growth?

Antibiotics are the main treatment for UTIs, but problems with antibiotics include resistance, allergy, and effects on intestinal flora; development of alternative treatments is ongoing. Promising alternative treatments include vaccines against surface proteins that transport iron, siderophore-protein conjugates, or the pili that bind to the urothelium or agents that block adhesion, such as mannosides or other small molecules that bind to pili (9, 116, 117). It is beyond the scope of this review to evaluate dietary effects on urine composition, but there is a literature on this topic. One popular strategy for UTI prevention is oral mannose supplementation, which, like the mannosides described above, potentially blocks bacterial adhesion; several small clinical studies suggest mannose effectiveness, but virtually nothing is known about urinary mannose and UTI susceptibility and frequency (118).

Rapid growth and metabolism are nontraditional virulence factors, and antimetabolite agents could be effective as an adjunct therapy. *E. coli* possesses impressive metabolic flexibility, and metabolic vulnerabilities may be specific to a particular UPEC strain or to the urine composition of an individual. Rapid growth may require simultaneous utilization of several energy sources. A potential antimetabolite-based therapy should target enzymes that are required regardless of the variations in available energy

sources, such as enzymes of essential biosynthetic pathways. For example, we summarized evidence that glutamine synthetase or enzymes that contribute to both *de novo* and salvage pathways of nucleotide metabolism are potential targets. Identification of metabolic vulnerabilities will require additional information on the requirements for rapid growth and may need to take into account urine composition and the metabolism of the infecting uropathogen.

Although the primary focus of this review was to investigate the scientific relationship between urine composition and rapid UPEC growth in urine, clinicians dealing with recurrent urinary tract infections are also keenly interested in many relevant aspects of this research which could pave the way to several translational projects in treating or preventing RUTIs in women. For example, increased water intake reduced the frequency of RUTIs for women with low fluid intake, perhaps because of nutrient dilution that impairs bacterial growth (119). Once the compounds that promote growth of uropathogens are more thoroughly understood, a personalized analysis of urine composition may become part of a personalized combination therapy that includes antibiotics, antimetabolites, and dietary interventions that affect urine composition.

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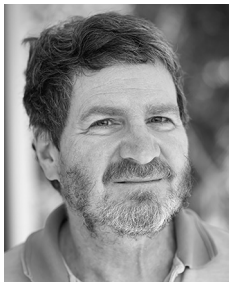
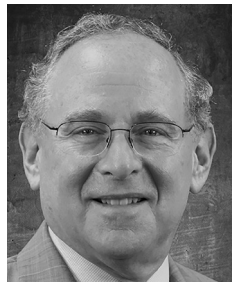


Larry Reitzer is currently Professor of Biology at the University of Texas at Dallas. His Ph.D. thesis at Washington University (St. Louis, MO) was one of the first demonstrations that rapidly growing cells (tissue culture or tumors) used glutamine as the major energy source. His postdoctoral work analyzed nitrogen limitation and the nitrogen-regulated (Ntr) response in *Escherichia coli*, including the demonstration of "activation-from-a-distance" regulation in bacteria. As an independent investigator, he has studied metabolism and gene expression in bacteria. One focus was continued study of the genes and regulation of the Ntr response. Another focus has been the discovery and analysis of aspects of amino acid metabolism, including the pathways of arginine, cysteine, and putrescine catabolism. Current research is focused on the metabolism of uropathogenic *E. coli* and its relation to motility and virulence.



Philippe Zimmern, M.D., is a Professor of Urology at University of Texas (UT) Southwestern Medical Center and the recipient of the Felecia and John Cain Chair for Women's Health in Urology in honor of Dr. Philippe Zimmern. His expertise covers urinary incontinence, prolapse, and urinary tract infections. After his urology training in France, a fellowship at UCLA, and a second urology training at UCLA, Dr. Zimmern joined the faculty at UT Southwestern in 1995. He is a past president of the Society for Urodynamics and Female Urology (SUFU). Dr. Zimmern codirects a two-year endowed fellowship program in female pelvic medicine and reconstructive surgery (FPMRS). He has been a visiting professor in many countries, has coauthored nearly 400 publications, 29 surgical movies, and 67 book chapters, and has coedited four textbooks on FPMRS topics. He is FPMRS certified and a member of FACS. He received the inaugural Leaders in Excellence Award at UT Southwestern (2019).