Molecular variations in Klebsiella pneumoniae and Escherichia coli FimH affect function and pathogenesis in the urinary tract

David A. Rosen  
*Washington University School of Medicine in St. Louis*

Jerome S. Pinkner  
*Washington University School of Medicine in St. Louis*

Jennifer N. Walker  
*Washington University School of Medicine in St. Louis*

Jennifer Stine Elam  
*Washington University School of Medicine in St. Louis*

Jennifer M. Jones  
*Washington University School of Medicine in St. Louis*

*See next page for additional authors*

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David A. Rosen, Jerome S. Pinkner, Jennifer N. Walker, Jennifer Stine Elam, Jennifer M. Jones, and Scott J. Hultgren

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

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Type 1 pili mediate binding, invasion, and biofilm formation of uropathogenic *Escherichia coli* (UPEC) in the host urothelium during urinary tract infection (UTI) via the adhesin FimH. In this study, we characterized the molecular basis of functional differences between FimH of the UPEC isolate UT89 and the *Klebsiella pneumoniae* cystitis isolate TOP52. Type 1 pili characteristically mediate mannose-sensitive hemagglutination of guinea pig erythrocytes. Although the adhesin domain of *K. pneumoniae* TOP52 FimH (FimH$_{52}$) is highly homologous to that of *E. coli*, with an identical mannose binding pocket and surrounding hydrophobic ridge, it lacks the ability to agglutinate guinea pig erythrocytes. In addition, FimH-dependent biofilm formation in *K. pneumoniae* is inhibited by heptyl mannose, but not methyl mannose, suggesting the need for contacts outside of the mannose binding pocket. The binding specificity differences observed for FimH$_{52}$ resulted in significant functional differences seen in the pathogenesis of *K. pneumoniae* UTI compared to *E. coli* UTI. Infections in a murine model of UTI demonstrated that although the *K. pneumoniae* strain TOP52 required FimH$_{52}$ for invasion and IBC formation in the bladder, FimH$_{52}$ was not essential for early colonization. This work reveals that a limited amount of sequence variation between the FimH of *E. coli* and *K. pneumoniae* results in significant differences in function and ability to colonize the urinary tract.

Bacterial adherence to host mucosal surfaces is often an important first step in the infection process. This is especially true in the case of urinary tract infections (UTIs) (59). It is estimated that half of all women will experience at least one UTI in their lifetime (49), the vast majority of which are caused by uropathogenic *Escherichia coli* (UPEC) and other *Enterobacteriaceae* (48). An essential step in UPEC infection of the bladder is adherence to the host urothelial surface via type 1 pili (2, 27, 29). Type 1 pili are assembled via the chaperone/usher pathway (3, 30, 53). They are adhesive hair-like fibers consisting of cylindrical pilus rods composed of FimA pilin subunits and small-tip fibrillae composed of FimF, FimG, and the adhesin FimH (6, 31). The FimH adhesin recognizes mannosalated uraplinkins and $\beta$-1, $\alpha$-3 integrin receptors on the luminal surface of bladder urothelial cells (17, 29, 63). Binding of UPEC to host cells induces a cascade of signaling events that ultimately leads to bacterial internalization and the formation of biofilm-like intracellular bacterial communities (IBCs) (1, 17, 22, 32, 39, 43, 51). IBC formation is also dependent on type 1 pili (62). Ultimately bacteria disperse from this intracellular niche and progress to infect other urothelial cells.

Type 1 piliated bacteria have historically been characterized by their ability to agglutinate guinea pig red blood cells (RBCs) in a mannose-sensitive manner (14, 15, 52). This mannose-sensitive hemagglutination (MSHA) phenotype of *E. coli* expressing type 1 pili requires the FimH adhesin. FimH consists of two domains: an amino-terminal adhesin domain (AD; receptor binding domain) and a carboxy-terminal pilin domain (PD) (8, 29, 31). FimH recognizes mannosylated glycoproteins, including those present on the host urothelium through its AD. FimH-mediated adhesion can be inhibited by $\alpha$- or $\rho$-mannose or oligosaccharides containing terminal mannose residues (5, 19–21). Additionally, it has been demonstrated that the FimH AD binds more tightly to $\alpha$-D-mannosides with longer alkyl chains. Heptyl mannose was found to have the highest affinity for FimH (5). In animal models, neutralization of the adhesin by FimH-specific antibodies protects from UPEC cystitis (35, 36). X-ray crystal structures of FimH reveal a highly conserved mannose binding pocket at the tip of the FimH AD surrounded by a distal hydrophobic ridge (8, 29). Minor sequence differences in *E. coli* FimH, many of which are not located in close proximity to the mannose binding pocket, have been found to correlate with differential binding phenotypes (54–56).

*Klebsiella pneumoniae* is the second leading cause of gram-negative UTI but is a much less prevalent etiologic agent than UPEC. *K. pneumoniae* genes encode numerous chaperone/usher pilin, including type 1 pili and type 3 pili (23). While type 1 pili have historically been defined by their MSHA phenotype, type 3 pili display a mannose-resistant hemagglutination (MRHA) with tannin-treated RBCs (47). Type 1 pili of *K. pneumoniae* are highly homologous to those of UPEC (23) and have been previously implicated in UTI pathogenesis (18, 40). The fim operon of *K. pneumoniae*, encoding type 1 pili, contains a terminal fimK gene, not present in UPEC, which plays a role in suppressing the expression of type 1 pili (50). *K. pneumoniae* binds, invades, and forms IBCs within host urothelial cells, albeit less efficiently than UPEC in the murine cystitis model. Similar to UPEC, *K. pneumoniae* also expresses type 1 pili within these IBCs (50). In this study, we discovered that type 1-piliated *K. pneumoniae* cells are unable to mediate hem-
agglutination of guinea pig erythrocytes despite the presence of wild-type FimH containing an identical mannose binding pocket to E. coli FimH. We analyzed functional and structural differences in FimH of the K. pneumoniae strain TOP52 and the effects of these differences on UTI pathogenesis.

MATERIALS AND METHODS

Bacterial strains and culture conditions. A complete list of bacterial strains and plasmids used in this study can be found in Table 1. The clinical strains used include UTI89, a UPEC cystitis isolate (43), and TOP52 1721 (abbreviated E. coli fimH).

A targeted deletion of fimH in K. pneumoniae TOP52 was constructed using the red recombinase method as previously described (10, 44), with pKD4 as a template and the primers indicated (Table 2). Subscripts 89 and 52 were used to indicate a given domain was from UTI89 or TOP52, respectively. Single ADs and PDs were added together as templates in a PCR to create a full-length fimH gene that was subsequently cloned into the arabinose-inducible pBAD33 vector (abbreviated pBAD). The four permutations of the ADs and PDs yielded fimH complementation vectors pADpADpADpAD (pBAD1). All constructs were verified and sequenced using pBAD plasmid primers.

HAs. Hemagglutination assays (HAs) were performed with guinea pig RBCs (optical density at 660 nm [OD660] of 2.0; Colorado Serum Company) as previously described using serial dilutions in microtiter plates with and without the addition of 100 mM methyl α-D-mannopyranoside (20).

Biofilm assays. Bacteria were grown in LB broth in wells of microtiter plates in the presence of 0.01% arabinose and either no mannose, 1 mM methyl arabinose, 100 mM methyl mannose, or 1 mM heptanol mannose. After 48 h of growth at room temperature, wells were rinsed and then stained with crystal violet, and biofilms were quantified as previously described (46).

Modeling of K. pneumoniae FimH. K. pneumoniae TOP52 FimH was modeled onto the X-ray crystal structure of E. coli FimH from the 396 isolate FimH complex structure (PDB identification 1KLF) (29) using the protein structure threading program Threuc (4). The resulting model was compared to the 396

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Plasmids

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structure and UTI89 amino acid sequence. (A structure for UTI89 FimH has not been solved to date, and J96 FimH differs by only 4 amino acids [aa] from UTI89 FimH.) Figures were rendered in the molecular modeling program Pymol (11).

**Mouse infections.** Bacterial strains were used to inoculate 8-week-old female C3H/HeN mice (National Cancer Institute) by transurethral catheterization as previously described (42). Twenty-five-mliliter static cultures were inoculated from freezer stocks and grown at 37°C for 18 h and then subcultured 1:250 into 25 ml fresh medium. These cultures were then grown statically at 37°C for 18 h and centrifuged for 5 min at 5,800 rpm, and the resultant pellet was resuspended in phosphate-buffered saline (PBS) and diluted to approximately 2 × 10^9 CFU/ml. Fifty microliters of this suspension was used to infect each mouse with an inoculum of 1 × 10^5 to 2 × 10^5 CFU. All studies were approved by the Animal Studies Committee at Washington University School of Medicine.

**Organ titers, gentamicin protection assays, and IBC enumeration.** To quantitatively bacteria present in mouse organs, bladders and kidneys were aseptically harvested at the indicated times postinfection, homogenized in PBS, serially diluted, and plated onto LB agar plates. Luminal and intracellular bacteria were quantified using an ex vivo gentamicin protection assay as previously described (33). For ex vivo enumeration of IBCs, infected bladders were harvested at 1 h postinfection, bisected, splayed, washed with PBS, fixed with 3% paraformaldehyde for 10 min, and absorbed onto mouse infection, fixed with 1% paraformaldehyde for 1 h at room temperature and lacZ stained as previously described (33). IBCs were visualized and counted using an Olympus SZX12 dissecting microscope (Olympus America).

**fim operon phase assay.** To determine the orientation of the type 1 pilus phase-variable promoter switch (fimS) in UTI89 ΔfimH, a phase assay was performed as previously described (58). Briefly, PCR primers were used to amplify fimH promoter region including fimS. The PCR product was then digested with the restriction endonuclease HinfI (New England Biolabs) and was sequenced. The TOP52 produced no detectable hemagglutination of guinea pig erythrocytes. Deletion of fimH was also negative for hemagglutination. Thus, type 1-piliated K. pneumoniae TOP52 is unable to mediate MSHA.

**FimH52 and FimH89 are highly similar in amino acid sequence and predicted structure.** To further investigate the functional differences of K. pneumoniae TOP52 type 1 pili, we sequenced fimH52 (GenBank accession no. EU327536) and compared it to other known K. pneumoniae FimH sequences and the sequence of E. coli UTI89 FimH (FimH89). The FimH52 amino acid sequence shares 100% identity with the FimH adhesin domain of K. pneumoniae strain IA565 (23), 99.6% amino acid identity with the FimH of K. pneumoniae ATCC 700721 strain (41), and 85.3% amino acid identity with K. pneumoniae strain IA551 (16). FimH52 has 86.4% amino acid identity to FimH of E. coli UTI89 (FimH89) (Fig. 1A) and maintains the general bidomain composition of E. coli FimH with an amino-terminal adhesin domain (amino acids [aa] 1 to 157) and a carboxy-terminal pilin domain (aa 161 to 279) separated by a short linker region.

We threaded K. pneumoniae FimH onto the X-ray crystal structure of FimH from the complex structure of FimC-H from the J96 E. coli isolate (29). We then overlaid J96 FimH and TOP52 FimH and compared the positions and identities of amino acid differences in FimH52 and FimH89 (Fig. 1B). This comparison assumes that residues conserved between J96 FimH and UTI89 FimH have the same conformation as shown in the three-dimensional J96 FimH structure. There are only four amino acid differences between FimH of these two strains. Seventeen AD amino acid differences and 21 PD amino acid differences exist between FimH52 and FimH89. Interestingly, FimH52 displays full conservation of the residues known to interact with mannose in the mannose binding pocket (orange) and those that form the surrounding hydrophobic ridge (green in Fig. 1). Residue differences exist in areas adjacent to the receptor binding site and in other more distal parts of the molecule, which may together alter the molecular details of the interaction with mannose. Two differences in FimH52 primary sequence exist in residues adjacent to known mannose-binding residues (His312 and Ser141, changed from Arg and Asp, respectively, in FimH89). The threaded model FimH52 suggests that these residues would lie ~8.5 Å away from the bound mannose moiety. Arg312 and Asp141 form a salt bridge in E. coli FimH that helps stabilize the structure of the FG

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*a* HA titer data are representative of three independent experiments.

**RESULTS**

Type 1-piliated K. pneumoniae TOP52 is hemagglutination negative. In contrast to UPEC, statically passaged K. pneumoniae TOP52 produced no detectable hemagglutination of guinea pig RBCs despite expression of type 1 pili (Table 3). The MSHA titer of the UPEC strain UTI89 was 1:512. Deletion of fimH abolished the ability of UTI89 ΔfimH to produce MSHA. UTI89 ΔfimH produced a low MRHA titere of 1:4, unlike UTI89. Wild-type K. pneumoniae TOP52 did not agglutinate guinea pig erythrocytes. Deletion of fimH to create TOP52 ΔfimH was also negative for hemagglutination. Recently, we discovered that deletion of fimK, a gene unique to Klebsiella fim gene clusters, resulted in a hyper-type 1-piliated phenotype (50). The hyperpiliated TOP52 ΔfimK was also hemagglutination negative. The fimX recombinase has been shown to have fimB-like properties (7, 25), and its overexpression results in increased expression of type 1 pili in both E. coli (25) and K. pneumoniae (50). The hyperpiliated TOP52/pifmX was also hemagglutination negative. Thus, type 1-piliated K. pneumoniae TOP52 is unable to mediate MSHA.

**TABLE 3. FimH52-specific inability of K. pneumoniae TOP52 to agglutinate guinea pig RBCs**
loop that contains mannose binding residues Gln133, Asn135, and Asp140 and forms part of the hydrophobic ridge. Arg132 NH1 also makes two hydrogen bonds to Gln59 OE1 and Glu89 OE1. In FimH52, His132 is only able to make a single hydrogen bond with Glu89 OE2. Differences in these and other residues may help explain the inability of \textit{K. pneumoniae} TOP52 to agglutinate guinea pig RBCs.

The inability of \textit{K. pneumoniae} TOP52 to agglutinate guinea pig RBCs is specific to the adhesin domain of FimH52. Although all residues involved in direct interactions with the mannose moiety and all those in the surrounding hydrophobic ridge are identical between FimH52 and FimH89, nearly 14% of amino acids differ between the two proteins. We hypothesized that if this variation in FimH sequence accounts for the inability to agglutinate guinea pig RBCs.

To test the expression of exogenous \textit{fimH} in the UTI89 \textit{ΔfimH} background, phase assays were conducted analyzing the phase-variable promoter switch of type 1 pili (Fig. 2). The \textit{fim} operon of wild-type \textit{E. coli} UTI89 was primarily phase on after static growth; however, loss of \textit{fimH} in UTI89 \textit{ΔfimH} and the UTI89 \textit{ΔfimH/pBAD} vector control resulted in bacterial populations that were primarily in the phase-off orientation. Complementation with either \textit{p}fimH89 or \textit{p}fimH52 did not result in a robust off-to-on switch as the populations remained primarily phase off with similarly low levels of piliated bacteria. However, enough phase-on bacteria were present to detect an MSHA titer with \textit{p}fimH89 complementation (Table 3).

FimH52 and FimH89 function was investigated further by constructing FimH chimeras. We used the chimeras to complement \textit{fimH}-knockout strains and then examined the final pilus assembly on each strain by immunoelectron microscopy.

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phase switch being primarily off in these complementations, the majority of bacteria were bald without noticeable pili. However, similar subpopulations of bacteria existed in each sample that were moderately piliated and immunolabeling at the tips of pili was observed for UTI89, UTI89/pBAD, UTI89/fimH, and UTI89/fimH/pBAD. UTI89 was largely phase on, while the UTI89,fimH strains were all primarily phase off despite complementation.

TOP52, UTI89, and UTI89/pBAD both produced an MSHA titer of 1:32, while UTI89/fimH produced an MSHA titer of 1:512, while UTI89/fimH/pBAD produced an MSHA titer of 1:8. While the majority of bacteria were bald without noticeable pili, piliated bacteria could be found in all four samples. Wild-type UTI89 produced an MSHA titer of 1:512, while UTI89/fimH/pBAD produced an MSHA titer of 1:8. The low level of type 1 pilus expression explains the inability to fully complement UTI89/fimH with each construct (Fig. 3). The low level of type 1 pilus expression explains the inability to fully complement UTI89/fimH with each construct (Fig. 3).

Wild-type UTI89 produced an MSHA titer of 1:512, while wild-type TOP52 was hemagglutination negative. UTI89/fimH, UTI89/fimH/pBAD, TOP52/fimH, and TOP52/fimH/pBAD all lacked the ability to agglutinate guinea pig RBCs. UTI89/fimH/pBAD is capable of agglutinating guinea pig RBCs with the native UTI89 PD or with the PD of K. pneumoniae TOP52. These results demonstrate that the K. pneumoniae TOP52 FimH inability to agglutinate guinea pig RBCs is specific to its AD. The AD of E. coli UTI89 FimH is capable of agglutinating guinea pig RBCs with the native UTI89 PD or with the PD of K. pneumoniae TOP52. Thus, variations between the FimH ADs of E. coli UTI89 and K. pneumoniae TOP52 are likely responsible for their differences in function.

**TABLE 4.** Adhesin domain-specific hemagglutination deficiency of *K. pneumoniae* TOP52 FimH

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*HA titer data are representative of three independent experiments.

**FIG. 2.** The fim operon of UTI89 ΔfimH is primarily in the phase-off orientation. Phase assays of the fimS invertible promoter region of the fim operon were done for E. coli UTI89, UTI89 ΔfimH, UTI89 ΔfimH/pBAD, UTI89 ΔfimH/pimH, and UTI89 ΔfimH/pimH52. UTI89 was largely phase on, while the UTI89 ΔfimH strains were all primarily phase off despite complementation.

**FIG. 3.** fimH constructs in UTI89 ΔfimH are expressed in some bacteria and localized at the tips of pili. Immunoelectron microscopy using an anti-FimH antibody was performed against UTI89 ΔfimH/pimH (A), UTI89 ΔfimH/pAD52PD90 (B), UTI89 ΔfimH/pAD52PD90 (C), and UTI89 ΔfimH/pimH52 (D). While the majority of bacteria did not appear to be expressing type 1 pili, piliated bacteria could be found in all four samples. Piliated bacteria displayed FimH immunostaining at the distal tips of pili.
FimH-dependent. Thus, although FimH\(_{52}\) is unable to mediate hemagglutination, it is capable of mediating biofilm formation. *E. coli* UTI89 formed a robust biofilm, while UTI89 \(\Delta\)fimH did not. The formation of *E. coli* UTI89 biofilm was fully inhibited by 100 mM methyl mannose or 1 mM heptyl mannose (Fig. 4). In contrast, TOP52/p\(\Delta\)fimX biofilm formation was not affected by the presence of 100 mM methyl mannose, but was fully inhibited by 1 mM heptyl mannose.

Therefore, TOP52/p\(\Delta\)fimX forms a FimH-dependent biofilm that is inhibited by heptyl mannose but not methyl mannose. This phenotype is distinct from those of *E. coli* UTI89 FimH-dependent biofilms, which are fully inhibited by the presence of 100 mM methyl mannose.

**E. coli** UTI89 and *K. pneumoniae* TOP52 both require fimH for effective persistence in the urinary tract. To analyze the respective roles of FimH\(_{52}\) and FimH\(_{89}\) in urinary tract infection, \(10^7\) CFU of *E. coli* UTI89, UTI89 \(\Delta\)fimH, *K. pneumoniae* TOP52 or TOP52 \(\Delta\)fimH were inoculated into the bladders of C3H/HeN mice by transurethral catheterization. Bladders and kidneys were harvested at various time points postinoculation, and bacterial titers were determined. In the bladder (Fig. 5A), *E. coli* UTI89 had significantly higher titers than UTI89 \(\Delta\)fimH at 6 h (\(P < 0.0001\)), 24 h (\(P < 0.0001\)), and 336 h (\(P = 0.0007\)) postinfection. UTI89 \(\Delta\)fimH was cleared from the bladder as time progressed. *K. pneumoniae* TOP52 had slightly but significantly higher titers (\(P = 0.0244\)) than TOP52 \(\Delta\)fimH in the bladders of mice at 6 h postinoculation. By 24 h, there was no significant difference between TOP52 and TOP52 \(\Delta\)fimH bladder titers. However, by 336 h postinfection, TOP52 \(\Delta\)fimH had significantly lower titers than wild-type TOP52 (\(P = 0.0012\)). In the kidneys (Fig. 5B), UTI89 had significantly higher titers than UTI89 \(\Delta\)fimH at 6 h postinfection (\(P < 0.0001\)), however the two strains had similar titers at both 24 and 336 h postinfection. TOP52 and TOP52 \(\Delta\)fimH had similar levels of bacterial burden in the kidneys at all time points tested. Thus, FimH in *K. pneumoniae* TOP52 does not play a critical role early in bladder infection as is the case with *E. coli* UTI89; however, FimH is required for effective persistence in the bladder in both strains.

**FimH\(_{52}\) is required for *K. pneumoniae* TOP52 bladder invasion and IBC formation.** In order to further assess the role of FimH\(_{52}\) in acute *K. pneumoniae* TOP52 cystitis, bladder invasion assays were performed at 1 h postinfection with UTI89, UTI89 \(\Delta\)fimH, TOP52, or TOP52 \(\Delta\)fimH. In these assays, luminal bacteria were collected by successive bladder washes (Fig. 5C), prior to gentamicin treatment of the bladder to kill extracellular bacteria, as previously described (33). After 1.5 h of incubation in gentamicin, bladders were washed and homogenized and cell titers were determined to reveal the intracellular bacterial burden (Fig. 5D). UTI89 had 100-fold-higher luminal bacterial counts compared to UTI89 \(\Delta\)fimH at 1 h postinfection (\(P = 0.0043\)). However, TOP52 and TOP52 \(\Delta\)fimH had similar levels of luminal colonization. At this 1-h time point, UTI89 had significantly higher levels of intracellular bacteria than UTI89 \(\Delta\)fimH (\(P = 0.0055\)), which did not have any intracellular titers above the limit of detection (5 CFU). TOP52 also invaded into the bladder tissue and had intracellular bacterial titers that were significantly higher than TOP52 \(\Delta\)fimH (\(P = 0.0095\)), which did not have titers above the limit of detection.

To determine if the presence of FimH\(_{52}\) affects the ability of TOP52 to form IBCs, we visualized and quantified IBCs by lacZ staining of whole, mounted, fixed bladders as described previously (33) at 6 h postinoculation of UTI89, UTI89 \(\Delta\)fimH, TOP52, or TOP52 \(\Delta\)fimH (Fig. 5E). UTI89 formed a wide range of IBCs with a median of 25.5 per bladder, while UTI89 \(\Delta\)fimH formed no detectable IBCs. TOP52 had a median of 2.0 IBCs per bladder, while TOP52 \(\Delta\)fimH was unable to produce detectable IBCs (\(P = 0.0009\)). These data suggest that *K. pneumoniae* TOP52 FimH\(_{52}\), in contrast to *E. coli* UTI89 FimH\(_{89}\), does not play a significant role in early bladder colonization. However, FimH\(_{52}\) is required for TOP52 invasion and IBC formation in the murine bladder, as is the case for UTI89.

**fimH\(_{52}\) does not restore the ability of UTI89 \(\Delta\)fimH to effectively infect the bladder.** *E. coli* UTI89 relies on FimH to successfully cause UTI in the murine model. The ability of fimH\(_{52}\) to restore the ability of UTI89 \(\Delta\)fimH to bind, invade, and infect murine bladders was investigated by using UTI89 \(\Delta\)fimH complemented with pBAD vector control, p\(\Delta\)fimH\(_{89}\), and p\(\Delta\)fimH\(_{52}\). In 1-h gentamicin protection assays (Fig. 6A and B), UTI89 \(\Delta\)fimH complemented with p\(\Delta\)fimH\(_{89}\) had significantly higher luminal titers than the same strain complemented with vector control (Fig. 6A, \(P = 0.0001\)) despite the known expression deficiencies observed above. Additionally, UTI89 \(\Delta\)fimH/p\(\Delta\)fimH\(_{52}\) had significantly higher 1-h luminal titers than UTI89 \(\Delta\)fimH/p\(\Delta\)fimH\(_{89}\), which had colonization levels similar to those of the vector control. Examination of the intracellular population at 1 h (Fig. 6B) revealed that the UTI89 \(\Delta\)fimH/pBAD vector control did not have titers above the limit of detection, whereas UTI89 \(\Delta\)fimH/p\(\Delta\)fimH\(_{89}\) did produce significantly higher burdens of intracellular bacteria (\(P = 0.0028\)). UTI89 \(\Delta\)fimH/p\(\Delta\)fimH\(_{52}\) was able to invade the bladder tissue, but at significantly lower levels compared to UTI89 \(\Delta\)fimH/p\(\Delta\)fimH\(_{89}\).
At 6 h postinoculation, UTI89/fimH had significantly higher burdens of bacteria in the bladder than both UTI89/fimH/pBAD (P = 0.0043) and UTI89 ΔfimH (P = 0.0032). Complementation of UTI89 ΔfimH with fimH from either UTI89 or TOP52 did not significantly affect 6-h kidney titers compared to those of the vector control. The fimH52 gene was not able to restore UTI89 ΔfimH to levels above that of the vector control, while complementation with fimH89 yielded higher bacterial burdens at 1 and 6 h. This suggests a potential defect in the function of FimH52 in the bladder compared to FimH89.

**DISCUSSION**

FimH of the *K. pneumoniae* strain TOP52 (FimH52) has an amino acid sequence highly homologous to the sequence en-
coded by dozens of fimH genes that have been sequenced from *E. coli* (34, 54, 56). The residues that form the mannose binding pocket (Asn46, Asp47, Asp54, Gln133, Asn135, and Asp140) and hydrophobic ridge (Phe1, Ile13, Tyr48, Ile52, Tyr137, and Phe142) are completely identical between FimH52 and all known FimH adhesins of *E. coli*. Despite this identity, FimH52 has a receptor specificity unique from that of UPEC FimH. FimH52 is unable to mediate agglutination of guinea pig erythrocytes, whereas all known UPEC FimH adhesins are defined by their ability to mediate MSHA. Different *E. coli* FimH variants have been classified as high-affinity monomannose binders or lower-affinity trimannose binders (45, 54). However, both trimannose and monomannose variants display MSHA of guinea pig erythrocytes.

*E. coli* FimH recognizes mannose and has been shown to be able to interact with Manα1, 3Manβ1, 4GlcNAcβ1, 4GlcNAC in an extended binding site (61). These additional interactions between FimH and extended oligomannose moieties are mimicked by butyl α-D-mannose (61). Extended alkyl-α-mannosides have higher affinities for *E. coli* FimH compared to methyl-α-D-mannopyranoside (methyl mannose), with heptyl α-D-mannopyranoside (heptyl mannose) having the lowest dissociation constant ($K_d$) of 5 nM (5). *K. pneumoniae* FimH-dependent biofilms could only be inhibited by heptyl mannose and not methyl mannose, arguing that *K. pneumoniae* FimH requires additional contacts of the alkyl chain outside of the mannose binding pocket.

FimH52 differs at 17 positions from *E. coli* FimH and was threaded onto the three-dimensional structure of *E. coli* FimH. In the DE loop, adjacent to the hydrophobic ridge, Val94 and Asn96 of *E. coli* UTI89 FimH (FimH89) are changed to Ile and Asp, respectively, in FimH52. In the G strand, immediately C terminal to key residues in the hydrophobic ridge, Val145 in FimH89 is changed to Ile in FimH52. Combined, these differences may alter the structural stability of the hydrophobic ridge of FimH52 through changes in hydrophobic and hydrogen bond contacts. Thus, although FimH52 is unable to bind methyl mannose, these amino acid changes may facilitate interactions with longer oligomannose substrates (61).

The inability of FimH52 to mediate hemagglutination may be due to amino acid changes in proximity to the mannose binding pocket. Gln133 and Asp140 *E. coli* FimH residues are required for HA titers and mannose binding (29). Two differences in *K. pneumoniae* TOP52 primary sequence exist in residues adjacent to these mannose-binding residues at positions 132 and 141. Data from the threaded model suggest that at least two hydrogen bonds are lost in FimH52 with the combined differences in residues 132 and 141, which may have a destabilizing effect on interactions at the mannose site around the Asp140 and Gln133 mannose-binding resi-
dues. Sequence variation in regions of FimH not in close proximity to the mannose binding pocket may also significantly affect FimH function (56, 57).

Studies have suggested that fimbrial shafts can influence binding specificities of type 1 pili (16, 38). These effects do not account for the binding specificity differences observed for FimH52. FimH52 assembled into E. coli UTI89 type 1 pili was also hemagglutination negative, and FimH90 assembled into K. pneumoniae TOP52 type 1 pili produced an MSHA titer. Thus, the major functional disparities between E. coli and K. pneumoniae type 1 pili were specific to the AD of FimH, not the strain background or fimbrial shaft. However, fimbrial shafts may influence FimH binding in more subtle ways that could have been missed in this study due to the lower expression of type 1 pili in fimh-knockout backgrounds.

The binding specificity differences observed for FimH52 result in dramatic functional differences seen in K. pneumoniae UTI pathogenesis compared to E. coli UTI pathogenesis. Although K. pneumoniae TOP52 requires FimH for invasion and IBC formation in the murine bladder, FimH is not essential for early colonization. TOP52 and TOP52 ΔfimH have similar 1-h luminal bladder titers, 24-h whole-bladder titers, and only modest titer differences at 6 h postinfection. The small but significant differences at 6 h likely represent the intracellular population of bacteria in IBCs within TOP52-infected bladders that are absent in TOP52 ΔfimH-infected bladders. K. pneumoniae may use a different, non-type 1 pilus adhesin for initial binding to the bladder surface that E. coli lacks. This would explain why TOP52 ΔfimH had higher 1-h luminal titers and 6-h whole-bladder titers compared to UTI89 ΔfimH. K. pneumoniae contains a gene that encodes type 3 pili; however, these pili have not been implicated in binding to the bladder surface and are thought to mediate attachment to the basolateral surface of tracheal epithelial cells and basement membrane components (60). In addition to type 1 and type 3 pilus, K. pneumoniae genes encode at least two other non-pilus adhesins. The CF29K and KPF-28 adhesins may play important roles in mediating attachment within the mammalian intestine, but their role in UTI has not been investigated (9, 12).

For many years, the glycoprotein uroplakin Ia has been considered the main receptor mediating FimH-dependent adhesion in the bladder (42, 63). Recently, it has been shown that host cell integrins also can mediate type 1 pilus-dependent invasion of urothelial cells (17). We currently do not know if K. pneumoniae FimH52 is capable of binding these receptors. It is possible that FimH52 may only be capable of binding integrin receptors (and not uroplakin Ia) for invasion of urothelial cells but not necessarily mediating significant adhesion to the uroplakin-coated bladder surface. Alternatively, K. pneumoniae FimH may have evolved for binding to a receptor in a different environment from the bladder.

This work focused on a single uropathogenic isolate of K. pneumoniae, and it is important to extend this work to other strains. The sequence of TOP52 FimH was almost identical to those of other sequenced K. pneumoniae FimH proteins and thus may be representative. The inability of K. pneumoniae TOP52 to agglutinate guinea pig RBCs is not an isolated finding. The ATCC 700271 strain also lacks a MSHA titer. The first studies of fimbiae and adhesive properties of 154 K. pneumoniae isolates found that 57.6% of strains produced little or no MSHA titer (13). Many researchers considered this to be due to poor type 1 expression in K. pneumoniae. However, K. pneumoniae TOP52 remained hemagglutination negative when expression of type 1 pili was increased by deletion of fimK or overexpression of fimX. Additionally, expression of E. coli type 1 pili at similar levels to TOP52 type 1 pili resulted in a positive MSHA.

This study suggests that limited sequence variation between the FimH of E. coli and K. pneumoniae results in differences in function and ability to colonize the urinary tract. Despite its poor adhesive properties in the urinary tract, FimH of K. pneumoniae remains an important virulence factor. It enables K. pneumoniae to progress through an IBC pathway during UTI and ultimately persist in the host. K. pneumoniae FimH likely requires ligand-receptor contacts outside of the mannose binding pocket for efficient binding. Further insight into these structural determinants will aid in our understanding of the altered host-pathogen interactions of K. pneumoniae UTI.

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Differences in E. coli and K. pneumoniae FimH


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