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Norinder, Birgit Stattin; Köves, Béla; Yadav, Manisha; Brauner, Annelie; Svanborg, Catharina

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Do *Escherichia coli* strains causing acute cystitis have a distinct virulence repertoire?

Birgit Stattin Norinder[1,2], Béla Köves[1], Manisha Yadav[1], Annelie Brauner[2], Catharina Svanborg[1*]

[1]Department of Microbiology, Immunology and Glycobiology, Institute of Laboratory Medicine, Lund University; SE-22362, Lund, Sweden

[2]Division of Clinical Microbiology, Department of Microbiology, Tumor and Cell Biology, Karolinska University Hospital and Karolinska Institutet; Nobels väg 16, Box 280, SE-17177 Stockholm, Sweden

*Correspondence to:

C Svanborg, [1]Department of Microbiology, Immunology and Glycobiology, Institute of Laboratory Medicine, Sölvegatan 23, 223 62 Lund, Sweden

E-mail: catharina.svanborg@med.lu.se

Abstract

Bacterial virulence factors influence the site and severity of urinary tract infections. While pyelonephritis-associated molecular traits have been defined, virulence factors specific for acute cystitis strains have not been identified. This study examined the virulence factor repertoire of 247 *Escherichia coli* strains, prospectively isolated from women with community-acquired acute cystitis. *Fim* sequences were present in 96% of the isolates, which also expressed Type 1 fimbriae. Curli were detected in 75%, 13% of which formed cellulose. *Pap* sequences were present in 47%, 27% were *papG+*, 23% were *prsG+* and 42% expressed P fimbriae. TcpC was expressed by 33% of the strains, 32% in a subgroup of patients who only had symptoms of cystitis and 42% in patients with signs of upper urinary tract involvement; most frequently by the *papG+/prsG+* subgroup. Strains with the full *fim, pap* and *TcpC* and curli virulence profile were more common in cystitis patients with than in patients without upper tract involvement ($p < 0.05$). The varied virulence profile of *E. coli* strains causing acute cystitis suggests that diverse bacterial strains, expressing Type 1 fimbriae trigger a convergent host response, involving pathways that give rise to the characteristic symptoms of acute cystitis.

Keywords: cystitis, *Escherichia coli,* P fimbriae, Type 1 fimbriae, TcpC, curli

1. Introduction

The severity of urinary tract infections (UTI) reflects the virulence and tissue specificity of the infecting strain. Acute pyelonephritis is caused by a restricted subset of uropathogenic *Escherichia coli* (UPEC) clones, distinguished for example by O:K:H serotypes or *E. coli* reference collection types combined with specific virulence factors with specific functions during the pathogenesis of infection [1]. Adhesins, including P and Type 1 fimbriae facilitate tissue attack and toxins perturb diverse cellular functions [1, 2]. TcpC, a homolog of the Toll/Interleukin-1 receptor domain is a new type of virulence factor, which acts by inhibiting Toll-like receptor (TLR) signaling [3]. These virulence factors increase the fitness of UPEC for the renal environment and aid them to resist elimination by the host defense. Through their interactions with host cells, the virulence factors trigger the innate immune response, leading to symptoms like fever, general malaise and flank pain.

Acute cystitis is a more common but less well-defined disease entity than acute pyelonephritis, characterized by inflammation of the lower urinary tract with symptoms like dysuria, frequency and suprapubic pain. Acute cystitis strains form an intermediary group with respect to O: K: H serotype diversity, ECOR types and certain virulence gene frequencies [1, 2, 4, 5]. Type 1 fimbrial expression alone has been discussed as major virulence factors in acute cystitis as these fimbriae enhance virulence in the murine urinary tract [6] [7-9], through attachment to the bladder mucosa. Receptor epitopes are provided by mannosylated host cell glycoconjugates in sIgA [10], uroplakins on bladder cells, CD48 on mucosal mast cells [11], integrins β1 and α3 [12] and Tamm-Horsfall Protein (THP) [13] and diverse signaling pathways trigger bacterial internalization and innate immunity. On the other hand, human inoculation studies have so far

not confirmed the role of Type 1 fimbriae for persistence and inflammation in the urinary tract [9, 14, 15]. Toxins such as hemolysin (hly) and cytotoxic necrotizing factor (CNF) enhance uroepithelial damage [16] and curli and cellulose support biofilm formation but there is no evidence that these properties are unique for acute cystitis strains or more abundant in this group [17]. Acute cystitis strains also express P fimbriae [4, 5, 18-20] and three PapG adhesin variants have been identified [21]. The reported frequencies of P fimbriated strains vary among acute cystitis isolates as shown by binding assays and PCR-based genotyping [5, 18-20, 22] and thus, the contribution of the PapG adhesin variants to bladder infection remain unclear.

In this study, we have used molecular epidemiology to address if strains causing acute cystitis have a distinct virulence factor repertoire. The results show that Type 1 fimbriae and curli are common in acute cystitis isolates but analysis of multiple virulence factors did not define a cystitis-specific virulence profile. These findings raise the question if the symptoms of acute cystitis actually result from the action of specific virulence factors, especially Type 1 fimbriae which are most abundant among these strains, or if the pathogenesis of acute cystitis is fundamentally different from that of acute pyelonephritis, in terms of the variety of organisms that can give rise to a similar symptom profile. Understanding the pathogenesis of acute cystitis thus remains a major challenge.

2. Materials and Methods

2.1. Patients

Women ≥18 years of age were enrolled in a controlled randomized treatment trial of symptomatic UTI in general practice [23]. They had significant bacteriuria (> 10^4 cfu/mL) and were assigned a diagnosis of acute cystitis based on frequency, dysuria and/or suprapubic pain, a temperature <38.0°C and no flank pain. Patients who also had flank pain and/or fever (>38.0°C) were diagnosed as having acute cystitis with upper urinary tract involvement. On admission, a history of previous UTI, concomitant disease and medical treatment were recorded. The UTI episode was classified as sporadic (< two episodes during the previous six months or < three during the previous 12 months) or recurrent and as uncomplicated or complicated if the patient had structural or functional abnormalities of the urinary tract.

2.2. Host response to infection

Blood samples were obtained at diagnosis and examined for C-reactive protein (CRP, cut off ≥10 mg/L), white blood cell counts (cut off ≥10x10^9/L) and erythrocyte sedimentation rate (ESR, cut off >25 mm/h).

2.3. Urine cultures

Midstream urine samples were obtained at diagnosis. Quantitative urine cultures identified 247 *E. coli* growing as monocultures, and the isolates were stored in deep agar stabs. For analysis, bacteria were grown overnight on tryptic soy agar plates at 37 °C. The urinary tract is normally sterile, and urinary tract infections are usually caused by a single bacterial strain, originating

from the fecal flora [24, 25]. Infections by multiple organisms are associated with long-term catheterization or mechanical disorders affecting the urine flow [26].

2.4. Pap, fim, papG and TcpC genotypes

P fimbriae are encoded by the *pap* operon [27]. The *pap* genotype was determined by DNA-DNA hybridization with probes specific for the 5' (*Hind*III) and 3' (*Sma*I) fragments of the *pap* operon and derived from the *pap* gene cluster [22]. The *pap*G adhesin isotypes were defined by PCR, using primer pairs that matched unique regions of the *pap*G_{IA2}, *prs*G_{J96} sequences [28]. Whole bacterial cells provided template DNA and primers did not cross-amplify other *pap*G sequences, as shown by the recombinant strains containing a single known copy of *pap*G_{IA2} or *prs*G_{J96}. The P fimbriated *E.coli* IA2 and *E.coli* J96, and the *pap* positive recombinants *E. coli* HB101 (*pap*G_{IA2}) and *E.coli* HB101 (*prs*G_{J96}) were used as positive controls and *E. coli* HB101, *E.coli* AAEC (pPKL4) as negative controls. The *TcpC* genotype was defined by PCR, using specific primer pairs defining unique regions of the *TcpC* sequences [3].

The *fim*H genotype was defined by PCR, using primer pairs that matched unique regions of the adhesin sequences [28].

2.5. Bacterial phenotypes

Type 1 fimbrial expression was detected by hemagglutination of guinea pig and human erythrocytes after *in vitro* passage in Luria broth. Agglutination was performed both in the presence and absence of α-methyl-D-mannoside. Strains causing mannose-sensitive agglutination were defined as Type 1 fimbriated [15].

The P-fimbrial phenotype was defined by P blood group-dependent hemagglutination [22]. P-fimbrial expression was defined by agglutination of P_1 (receptor positive) but not p (receptor negative) erythrocytes. Class II strains agglutinated A_1P_1, OP_1 but not A_1p erythrocytes and Class III agglutinated only A_1P_1 and not OP_1 erythrocytes. Strains, which agglutinated A_1p erythrocytes were assigned to a group with ‘’other mannose resistant adhesins’’.

Morphotype analysis on Congo red and Calcoflour plates was used to study curli and cellulose expression [17]. After overnight culture, morphotypes were determined at daylight (Congo red) and UV-light (Calcoflour), as previously described. Reference strains were included and all strains were classified as curli+ and cellulose+, curli+ and cellulose-, curli- and cellulose- and curli- and cellulose+.

Biofilm formation was quantified by the crystal violet method [17]. Bacteria diluted in Luria-Bertani broth without salt were seeded into 96-well plates, incubated overnight at 37 °C without shaking, washed, air-dried and stained with crystal violet (3%). The dye was solubilized with ethanol (95%) and the optical density (OD) was measured at 570 nm. Ability to form biofilms was defined at an OD ≥0,5.

2.6. Hemolysin production

Hemolytic strains were identified in nutrient agar with 5% washed horse erythrocytes after overnight incubation. A hemolytic zone larger than the overlying colony was considered positive [4].

2.7. Statistical Analysis

Chi-square test or the Fisher's exact test was used. $p < 0.05$ was considered statistically significant (two-tailed).

3. Results

3.1. Characteristics of the patient population at inclusion

Women with cystitis symptoms and bacteriuria (n=247, mean age 51 years, range 18-91) were included and their infecting *E. coli* strains were saved. All but five patients had bacteriuria defined as $\geq 10^5$ cfu/mL, 98%); the remaining had 10^4 cfu/ml of urine. Most patients (83%) were healthy, except for the ongoing UTI episode, but 39 had hypertension and/or diabetes (Table 1a). The UTI episode was sporadic in 73% while 16% had a history of childhood UTI, indicating UTI susceptibility. Most of the patients (n=215) had only acute cystitis symptoms but a smaller group (n=32, 13%) also had flank pain and/or fever, suggesting upper tract involvement (Table A1). This group had increased circulating CRP levels and white blood cell counts compared to the group with only acute cystitis symptoms (p= 0.01 and p= 0.01 respectively, Table 1b).

3.2. Fim genotype, Type 1 fimbrial and hemolysin expression

As Type 1 fimbriae have been implicated in cystitis pathogenesis and shown to be essential virulence factors in the murine UTI model, we first defined the Type 1 fimbrial genotype by PCR using *fim* specific primers. The expression of Type 1 fimbriae was also detected by mannose-sensitive hemagglutination. Except ten isolates, all were *fim*+ (96%) and Type 1 fimbrial expression was detected in 80% of the isolates (Table 2). There was no significant difference in *fim* frequency between isolates from patients with acute cystitis (81 %) and the subgroup which also had upper tract involvement (71%) (Fig. 1A). Hemolysin expression was only detected in 28% in the total sample and the frequency did not differ between the two

groups (Table 2). The results confirm the high *fim* frequency among cystitis strains, consistent with these adhesins being essential for the pathogenesis of acute cystitis.

3.3. Curli, cellulose and biofilm expression

Curli are bacterial surface organelles that bind several host extracellular matrix and contact phase proteins. These adhesive fibers enhance bacterial biofilm formation on various abiotic surfaces. To analyze curli expression as a virulence factor in acute cystitis isolates, curli expression was examined by morphotype analysis. Curli were detected in seventy-five per cent of the isolates; 73 % in patients with acute cystitis compared to 89 % of patients, who also had upper tract involvement. Only 13% of the strains formed cellulose (Table 2). The curli+ and cellulose– phenotype was more frequent in patients with upper tract symptoms ($p<0.05$) (Fig. 1B). Biofilm, which consists of microorganisms and their extracellular products forming a structured community on a surface, was detected by the crystal violet method in <20% of all strains after growth at 37°C, which was selected to resemble the conditions in the urinary tract. The results suggest that strains causing acute cystitis frequently express curli but biofilm formation was mostly not detected.

3.4.Pap/PapG genotypes and - fimbrial expression

The pap gene cluster is strongly associated with acute pyelonephritis and urosepsis but in acute cystitis strains reported frequencies have been below 50%, suggesting a less strong effect on bladder infections than in the kidneys. The P-fimbrial G adhesin determines the receptor specificity is localized at the tip of the fimbrial organelle and at least 3 isotypes have been distinguished, based on receptor specificity of the G adhesin (Class I PapG, Class II PapG and

Class III PapG or PrsG). Two P-fimbrial isotypes predominate among uropathogenic *E. coli*. Class II G adhesins, encoded by the *pap*G_{IA2} sequences, recognize all P blood group determinants. Class III G adhesins, encoded by the *prs*G_{J96} sequences, recognize P blood group determinants with a terminal blood group A residue [22, 27]. Class I P fimbriae (papG $_{J96)}$ are uncommon in clinical isolates.

To further clarify this question, the P-fimbrial gene cluster was detected by DNA hybridization and adhesin isotypes (*papG/prsG*) were identified by PCR, using specific primers. The *pap* gene cluster was present in 43% of all isolates (Table 3). The *papG* $_{IA2}$ adhesin sequences were present in 24% and *prsG* $_{J96}$ sequences in 20% of all isolates, while 3% of the isolates carried both adhesin genes (Fig. 1C and D).

The P-fimbrial phenotype is defined by hemagglutination, using erythrocytes specifically expressing the P blood group antigens in the presence or absence of the A blood group determinant and with P blood group deficient cells as a negative control. P fimbrial expression (Class II+III) was detected by hemagglutination in 104 (42%) of the isolates (Table 3). Among those, Class II fimbriae (papG $_{IA2}$) were more common (77%) than Class III fimbriae (prsG $_{J96}$) (23%, $p< 0.001$). P blood group independent adhesins were found in 13% of the strains.

P-fimbrial expression was further examined as a function of the *papG* genotype. As expected, most strains expressing Class II P fimbriae were *papG*+ (80%) and isolates expressing Class III P fimbriae were *prsG*+ (96%), 30% of the strains agglutinating A_1p erythrocytes were *prsG*+, suggesting that P-fimbrial expression might be masked in this group.

In patients with upper tract involvement, 56% of isolates were *pap*+ and 50% expressed P fimbriae compared to 41% and 41% of the isolates from patients without upper tract symptoms (p=0.102 and p= 0.332 respectively). There was no difference in Class II distribution among patients with acute cystitis with or without upper tract involvement, however (76% versus 81%, p=0.75).

The results suggest that about half of acute cystitis strains are *pap*+, that the *papG* genotype predominates over *prsG* and that most *pap*+ acute cystitis strains express functional P fimbriae.

3.5. TcpC genotype

TcpC is a TIR domain homologous protein secreted by UPEC, which promotes bacterial survival by inhibiting the innate host response and specifically MyD88 dependent signaling pathways [3]. The *TcpC* genotype of the cystitis isolates was defined by PCR, using specific primers. *TcpC* was detected in 33% of the isolates, in 32 % of patients with acute cystitis compared to 42 % in the subset of patients with upper tract symptoms (Fig. 1E). TcpC was more common in the *papG+/prsG+* subset of the strains than in isolates lacking *papG* and/or *prsG* ($p< 0.01$ and $p= 0.01$, respectively) (Fig. 1F). The results confirmed that *pap*+ uropathogenic strains express *TcpC* more often than *pap*- strains, but showed no significant association with acute cystitis.

3.6. Virulence, UTI history and host compromise

Medical conditions that compromise the host defense have previously been shown to influence the requirements for virulence in strains causing acute pyelonephritis [29]. The virulence factor

profile was therefore compared between isolates from patients with diabetes/hypertension and those who were healthy except for the ongoing UTI episode. Furthermore, genetic predisposition has been shown to influence acute pyelonephritis susceptibility and the frequency of UTI in this group. Isolates from patients with sporadic infections were therefore compared to isolates from patients, who had a history of UTI (Fig. 1G and H). There was no significant difference in overall virulence profile related to these host variables. The frequency of fim+ and curli+ isolates was increased in patients with medical events compared to those with a history of UTI ($p<0.05$).

3.7. Combined virulence profile

The *E. coli* isolates were assigned a virulence profile based on their expression of virulence factors (Fig. 2). The complete virulence profile, comprising the *fim, papG*/*prsG* and *TcpC* genotypes as well as curli was detected in 18 % of the isolates; 15% of the cystitis only and 37% of the group with upper tract involvement ($p<0.01$). 35% of the strains carried the *fim, papG*/*prsG* sequences and expressed curli and this combination was also more common in patients with upper tract involvement ($p=0.001$). There was also a significant difference in the frequency of *fim*+ strains with curli expression between the two group ($p<0.05$). The results showed that strains with the combined virulence profile were significantly more common in patients with acute cystitis who had upper tract involvement than in patients with only lower tract symptoms.

4. Discussion

The molecular basis of acute cystitis has been extensively studied in cellular and experimental infection models [14, 15, 30]. Still, it remains unclear if a specific repertoire of virulence factors distinguishes acute cystitis strains from *E. coli* causing other forms of UTI. The present study examined *E. coli* isolates from 247 women with acute cystitis, using a combination of virulence genes commonly associated with acute pyelonephritis or cystitis. Type 1 fimbrial expression and *fim* sequences were common in the cystitis isolates, supporting their role in bladder infection. Curli, which have been proposed to improve biofilm formation, adhesion to host cells and internalization [31] were expressed by >70% of all isolates. In contrast, P fimbriae and TcpC were expressed by less than half of the cystitis strains, with *papG* being somewhat more common than *prsG.* A subgroup of strains expressed all the tested virulence factors (*fim, papG*, *prsG, TcpC* and curli) but such strains were not abundant in the acute cystitis group. Consistent with a role of these virulence factors in kidney infection, however, strains with the full virulence genotype were most common in patients with acute cystitis and upper tract involvement. The results suggest that Type 1 fimbrial expression is a unifying feature among acute cystitis strains, but provide no evidence that the virulence gene repertoire distinguishes strains causing acute cystitis from other uropathogens. In view of the variable virulence profile and high frequency of Type 1 fimbrial expression, we speculate that characteristic acute cystitis symptoms may be triggered Type 1 fimbrial interactions with the bladder mucosa. The symptoms reflect a different repertoire of host mediators than acute pyelonephritis possibly including bacterial tethering of neuronal circuits in the mucosal compartment.

Type 1 fimbriae are ubiquitously expressed by uropathogenic *E. coli* as well as other Gram-negative bacteria. Due to this high frequency, their role as independent virulence factors has been debated [15]. Recently, strains causing asymptomatic bacteriuria have been shown to carry

fim deletions, suggesting that an intact *fim* gene cluster may be counterproductive and that a loss of functional type 1 fimbriae promotes bacterial adaptation to long-term bacterial carriage in the urinary tract. The high *fim* frequency in the present study is consistent with a contribution of Type 1 fimbriae to acute cystitis pathogenesis, either during the colonization phase or by enhancing inflammation and symptoms [9, 14, 15, 30, 32]. Furthermore, type 1 fimbriae are major virulence factors in the murine cystitis model, where they act by promoting bacterial attachment and by triggering a partially TLR4 dependent innate immune response [33]. FimH has also been shown to suppress NFkB-dependent transcription of pro-inflammatory genes [34, 35] and Type 1 fimbriae have been proposed to enhance *E. coli* uptake into specialized dome cells in the bladder mucosa and promote intracellular bacterial proliferation, thus creating persistent infection and resistance to antibiotic therapy [36, 37]. Binding of the FimH adhesin to uroplakin complexes on the uroepithelial surface mediates bacterial entry into uroepithelial cells [32, 38] through elevated cAMP levels [34]. In addition, Type 1 fimbriae may be involved in eliciting apoptosis in uroepithelial cells [35]. In mucosal mast cells, FimH binding to the CD48 receptor has been proposed to direct bacterial uptake.

Human inoculation studies have provided somewhat contradictory results, regarding Type 1 fimbriae and their contribution to UTI. The prototype ABU strain *E. coli* 83972 fails to express Type 1 fimbriae and gives rise to a weak host response. After transformation of this strain with the *fim* gene cluster followed by human inoculation, the Type 1 fimbriated strain did not trigger a higher innate immune response than the wild type strain and there was no difference in the establishment of bacteriuria, suggesting that Type 1 fimbriae might function differently in the human and murine urinary tracts [15]. In addition to *fim* sequence variation, virulence for the urinary tract is modified by controlled variation in Type 1 fimbrial expression [30, 39, 40]. In a

clinical study of *E. coli* O1K1H7 and acute pyelonephritis in children, disease severity was augmented when the infecting strain expressed both Type 1 and P fimbriae compared to infections caused by the same strain, but having lost Type 1 fimbrial expression [40]. This difference was also observed *in vivo*, where reconstitution with functional *fim* sequences restored virulence in the murine model [30] consistent with Type 1 fimbriae contributing to kidney infection. In the present study, Type 1 fimbrial expression was maintained in the large majority of the strains, suggesting that acute cystitis strains do not loose Type 1 fimbrial expression through phase variation or mutation during the acute phase of infection, consistent with a functional role for these fimbriae in acute cystitis.

The efficiency of the bacterial virulence factors in causing UTI depends on the immune status of the host. Innate immunity controls many aspects of the host response to acute UTI and variation on the efficiency of this response has been shown to affect the degree of tissue damage and the clearance of infection [41]. As a consequence, host genetic variants that modify the innate immune response have been associated with different forms of UTI [42, 43]. In patients with recurrent UTI, which mostly denotes cystitis, several genetic screens have proposed gene associations, including promoter polymorphisms in LTA and TNFα [44], in the coding regions of TLR1, TLR4 and TLR5 [45]. The functional importance of these genetic variants in cystitis is not well understood, however. Several genetic markers of acute pyelonephritis have been established but have shown no association with acute cystitis. Low expression of the chemokine receptor CXCR1 is associated with APN susceptibility and *CXCR1* gene polymorphisms are common in pyelonephritis prone individuals [46]. Other genetic markers of pyelonephritis

susceptibility include IRF 3 polymorphisms [43]. These genetic studies emphasize the difference in pathogenesis and genetic control as well as the symptoms typical of acute pyelonephritis and cystitis. Finally, in ABU, genes like TLR4 may be mutated and promoter polymorphisms have been associated with reduced TLR4 expression and ABU but not with acute cystitis [42]. In future studies, it may be relevant to match bacterial properties against the host immune repertoire, to better understand the pathogenesis of acute cystitis.

It is interesting to speculate that acute cystitis strains may share as yet undefined virulence factors that specifically enhance the attack on the bladder mucosa. The cystitis strains are genetically diverse, however, and it appears less likely that strains of very different clonal origin would share a new, disease-defining cystitis-specific virulence factor. The clinical presentation of disease might instead be determined by the host response pathways, which are activated by the different acute cystitis strains. Innate immunity is crucial for the antimicrobial defense of the urinary tract, and TLR4 dependent signaling pathways have been shown to influence the susceptibility to acute pyelonephritis and asymptomatic bacteriuria. It remains possible that distinct innate response circuits may distinguish cystitis prone patients from patients prone to other forms of UTI. In this case, strains with different virulence profiles may converge on similar host signaling pathways creating the characteristic acute cystitis symptoms. The relevant pathways and host response dynamics need to be further explored.

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Transparency Declaration

None of the authors has a conflict of interest related to this study.

Figure 1. Virulence factor repertoire of *Escherichia coli* isolates from women with acute cystitis

(A) *Fim* genotype and Type 1 fimbrial expression in isolates from 247 patients, all with symptoms of acute cystitis (n=215) and a subgroup, who also had upper tract symptoms (n=32).

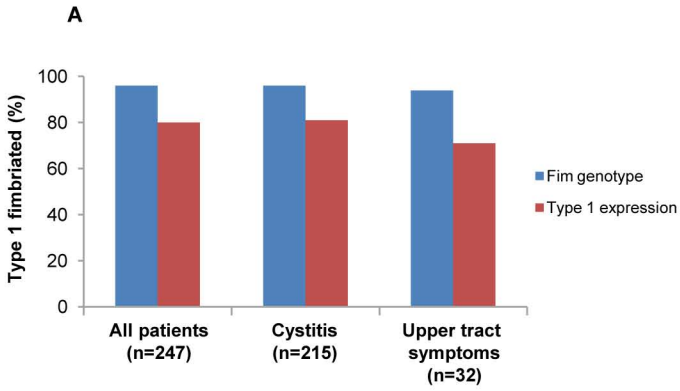
(B) Curli and cellulose expression of *Fim* genotype positive strains in the different patient groups. The curli + and cellulose – phenotype was more frequent in the subset of patients with upper tract symptoms ($p<0.05$).

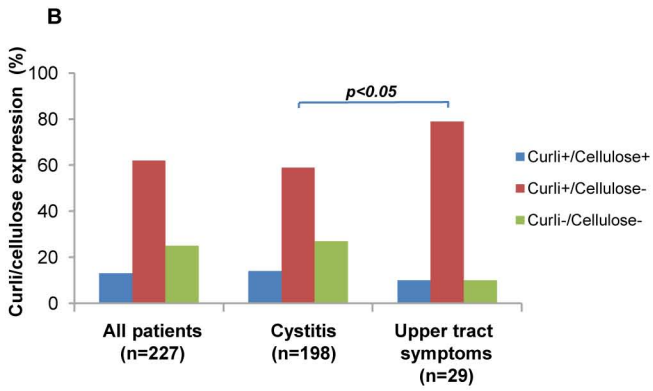
(C) and (D). *Pap* genotype and P fimbrial expression in the different patient groups.

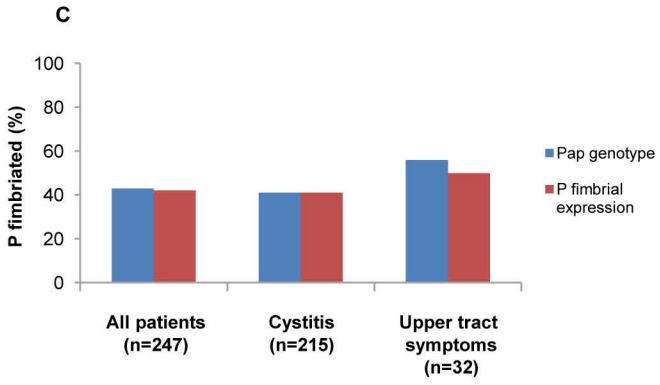
(E). TIR homologous *TcpC* sequences in the different patient groups, and in relation to the *pap* genotype (F). 23 isolates were weakly positive and are not included. Significantly higher *TcpC* frequency in patients with *papG+* and/or *prsG+* strains ($p<0.001$ and $p<0.05$).

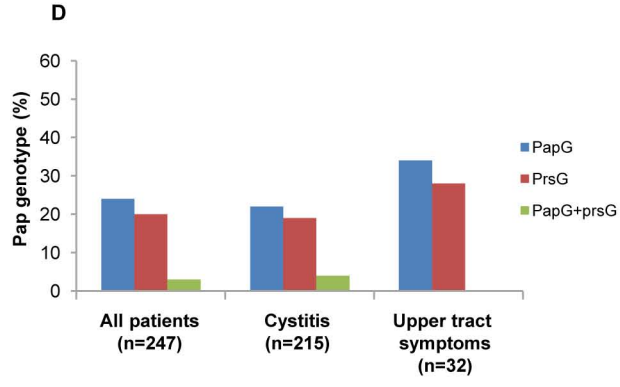
(F) *Fim* genotypes, curli/cellulose expression and *papG* genotypes (G) in patients with no host compromise, patients with history of UTI and patients with medical events. The frequency of fim+ and curli+ isolates was increased in patients with medical events compared to those with a history of UTI ($p<0.05$).

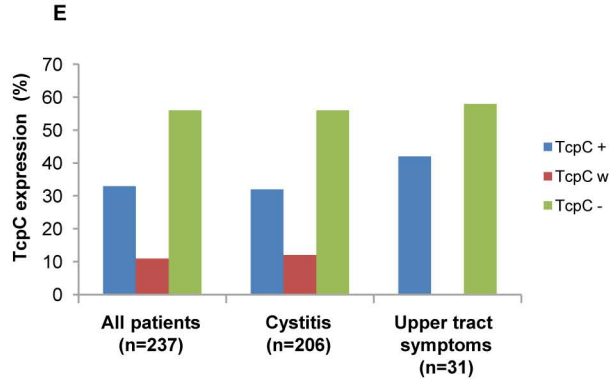
Figure 2. Combined virulence repertoire including the *fim, tcpC, papG/prsG* sequences and curli formation in all patients, those with acute cystitis and upper tract symptoms, respectively. Strains with the combined virulence repertoire were more common in the subgroup of patients with acute cystitis and upper tract involvement compared to patients with acute cystitis alone ($p<0.05$).

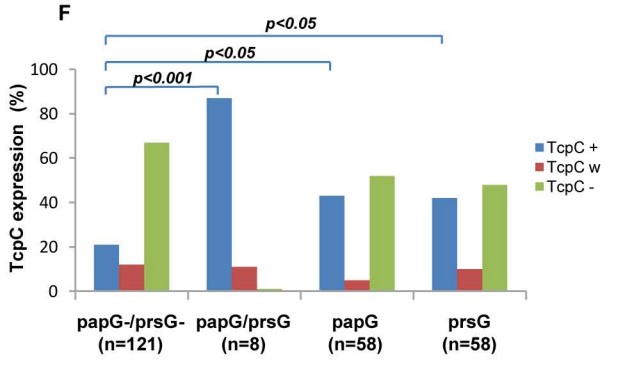

A
Type 1 fimbriated (%)
100
80
60
40
20
0
All patients (n=247)
Cystitis (n=215)
Upper tract symptoms (n=32)
Fim genotype
Type 1 expression

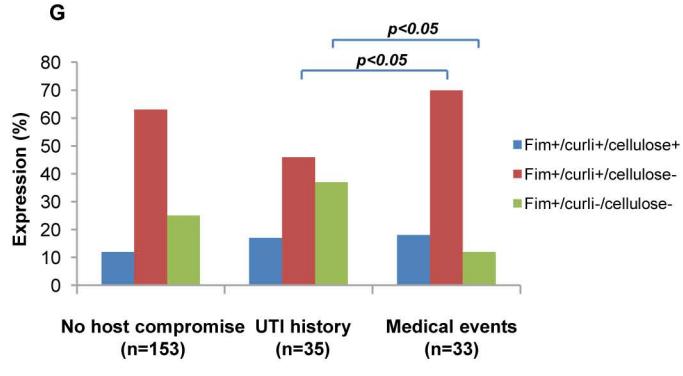

B
Curli/cellulose expression (%)
p<0.05
All patients (n=227)
Cystitis (n=198)
Upper tract symptoms (n=29)
Curli+/Cellulose+
Curli+/Cellulose-
Curli-/Cellulose-

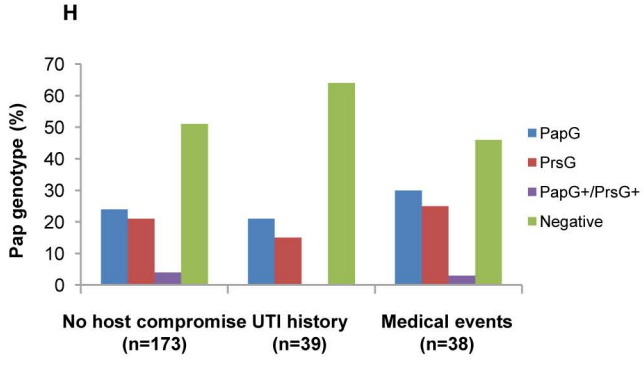

C
P fimbriated (%)
All patients (n=247)
Cystitis (n=215)
Upper tract symptoms (n=32)
Pap genotype
P fimbrial expression


D
Pap genotype (%)
All patients (n=247)
Cystitis (n=215)
Upper tract symptoms (n=32)
PapG
PrsG
PapG+prsG


E
TcpC expression (%)
All patients (n=237)
Cystitis (n=206)
Upper tract symptoms (n=31)
TcpC +
TcpC w
TcpC -


F
p<0.05
p<0.05
p<0.001
TcpC expression (%)
papG-/prsG- (n=121)
papG/prsG (n=8)
papG (n=58)
prsG (n=58)
TcpC +
TcpC w
TcpC -


G
p<0.05
p<0.05
Expression (%)
No host compromise (n=153)
UTI history (n=35)
Medical events (n=33)
Fim+/curli+/cellulose+
Fim+/curli+/cellulose-
Fim+/curli-/cellulose-


H
Pap genotype (%)
No host compromise (n=173)
UTI history (n=39)
Medical events (n=38)
PapG
PrsG
PapG+/PrsG+
Negative

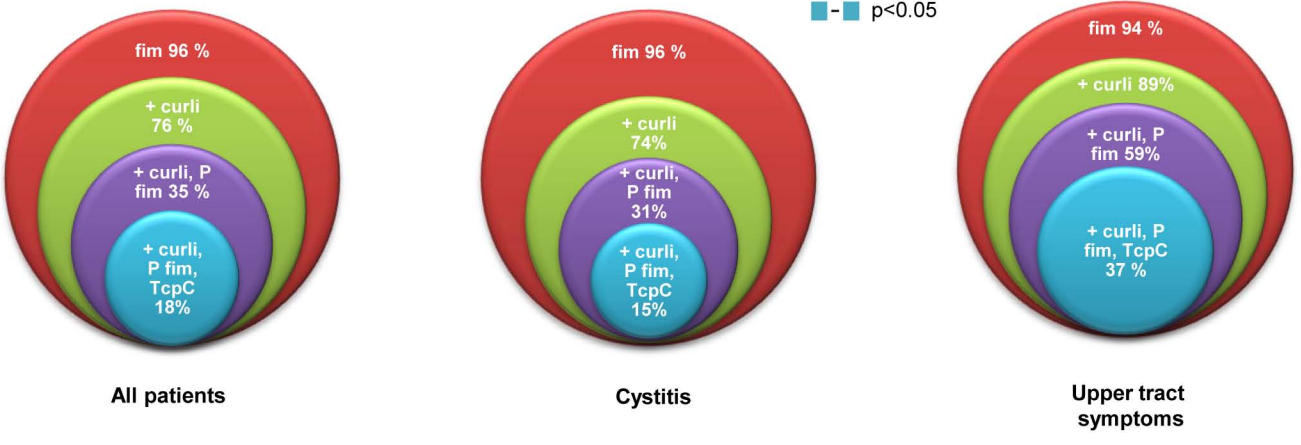

p<0.05
p=0.001
p<0.05
fim 96 %
+ curli 76 %
+ curli, P fim 35 %
+ curli, P fim, TcpC 18%
All patients
fim 96 %
+ curli 74%
+ curli, P fim 31%
+ curli, P fim, TcpC 15%
Cystitis
fim 94 %
+ curli 89%
+ curli, P fim 59%
+ curli, P fim, TcpC 37 %
Upper tract symptoms

Table 1a. Host background variables in women with acute cystitis

Host background variables	Patients No. (%)
Age, years median [range]	51.0 [18-91]
Medical events	
No illness[a]	205 (83)
Hypertension[b]	31 (13)
Diabetes	8 (3)
Diuretics[c]	29 (12)
UTI history	
Childhood UTI	39 (16)
Current UTI	
Cystitis	215 (87)
Upper tract involvement[d]	32 (13)
Type of symptomatic UTI[e, f]	
Sporadic uncomplicated	154 (62)
Sporadic complicated	26 (11)
Recurrent uncomplicated	56 (23)
Recurrent complicated	11 (4)
Total No. of patients	247

[a] Patients without any known illness other than UTI.

[b] One patient had both hypertension and diabetes, 26 patients with hypertension received diuretics and 3 additional patients received diuretic treatment without a diagnosis of hypertension.

[c] Diuretic treatments: tiazides (n=14), loop-diuretics (n=6), K-sparing drugs (n=1), combinations of diuretics (n=8).

[d] Patients with flank pain alone or in combination with cystitis symptoms and/or fever.

[e] Complicated UTI structural or functional abnormalities of the urinary tract including diabetes.

[f] Sporadic UTI < 2 UTI episodes during the last 6 months or <3 during the last 12 months.

Table 1b. **Laboratory parameters in women with acute cystitis**

Laboratory Parameter	Total No. (%)	Symptoms		P values
		Cystitis No. (%)	Upper tract No. (%)	
C-reactive protein	247			
>10 mg/L	67	52 (24)	15 (47)	p = 0.01
White blood cell counts	242			
$>10x10^9$/L	43	32 (15)	11 (35)	p = 0.01
Erythrocyte sedimentation rate	145			
>25 mm/hg	50	45 (21)	5 (16)	n.s.

Table 2. *Fim* genotype, type 1 fimbrial, curli/cellulose expression and biofilm formation

Virulence typing, E. coli isolates	Total No. (%)	Symptoms		P values
		Cystitis No. (%)	Upper tract No. (%)	
Fim genotype[a]	247	215	32	
Positive	237 (96)	207 (96)	30 (94)	n.s.
Type 1 expression[b,c]	226	198	29	
Positive	181 (80)	161 (81)	20 (71)	n.s.
Hemolysin expression[d]	245	213	32	
Positive	68 (28)	60 (28)	8 (25)	n.s.
Morphotypes[e]	227	198	29	
Curli+ and cellulose+	30 (13)	27 (14)	3 (10)	
Curli+ and cellulose-	140 (62)	117 (59)	23 (79)	p = 0.036
Curli- and cellulose-	57 (25)	54 (27)	3 (10)	
Curli- and cellulose+	0	0	0	
Biofilm formation[f]	225	196	29	
0.0 – 0.49	189 (83)	167 (85)	22 (76)	
0.5 ≥ 2	36 (16)	29 (15)	7 (24)	n.s.

[a] Analyzed by PCR

[b] Analyzed by hemagglutination

[c] Information from 21 patients was missing.

[d] 16 strains had weak hemolysin production

[e] Information from 20 patients was missing.

[f] Information from 22 patients was missing.

Table 3. *Pap* genotype and P-fimbrial expression in *E. coli* isolates

***Pap* genotype and P-fimbrial expression**	**No. of isolates (%)**			**P values**
	All isolates	**Cystitis**	**Upper Tract**	
Pap genotype[a], total[b]	247	215	32	
Positive	106 (43)	88 (41)	18 (56)	n.s.
*Pap*G alleles[c], total	247	215	32	
papG_{IA2}	59 (24)	48 (22)	11 (34)	
prsG_{J96}	50 (20)	41 (19)	9 (28)	
papG_{IA2}+*prs*G_{J96}	8 (3)	8 (4)	0 (0)	
P fimbrial expression[d,] total	247	215	32	
Positive[e]	104 (42)	88 (41)	16 (50)	n.s.
P fimbrial subtypes, total	104	88	16	
Class II[f] (PapG)	80 (77)	67 (76)	13 (81)	n.s.
Class III[g] (PrsG)	24 (23)	21 (24)	3 (19)	n.s.

[a] Analysis based on restriction fragment length polymorphism.

[b] Total = number of isolates examined for each parameter.

[c] Analyzed by PCR.

[d] Analyzed by P blood group specific hemagglutination.

[e] Agglutinated human P_1 but not p erythrocytes.

[f] Class II P fimbriated strains defined by agglutination of human A_1P_1, OP_1 but not p erythrocytes. There is a higher frequency of Class II P fimbriae compared to Class III in all three groups $p < .001$.

[g] Class III P fimbriated strains defined by agglutination of human A_1P_1 but not OP_1 or p erythrocytes.

To Appendix

Table A.1 Signs and symptoms of acute cystitis at the time of diagnosis.

Symptoms	Patients No. (%)
Lower tract symptoms only	
Frequency and dysuria	92 (37)
Frequency, dysuria and suprapubic pain	71 (29)
Frequency or dysuria or suprapubic pain	39 (16)
Frequency, suprapubic pain or dysuria, suprapubic pain	13 (5)
Additional upper tract symptoms	
Flank pain and/or fever	32 (13.4)
Total No. of patients	247

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