Uropathogenic *Escherichia coli* from pregnant women in different countries

Running title: Uropathogenic *E. coli* from pregnant women

Nubia L. Ramosa*, Musa Sekikuboa,b*, Dang Thi Ngoc Dzungc, Corinna Kosnopfelâ, Fred Kirondeâ, Florence Mirembeb, Annelie Braunera#

"Department of Microbiology, Tumor and Cell Biology, Division of Clinical Microbiology, Karolinska Institutet and Karolinska University Hospital, 17176 Stockholm, Sweden

bDepartment of Obstetrics and Gynaecology, Makerere University, Uganda

cDepartment of Biochemistry, Hanoi Medical University, Hanoi, Vietnam

dDepartment of Biochemistry, Makerere University, Uganda

*Equal contributors, in alphabetical order.

#Corresponding author: Mailing address: Division of Clinical Microbiology, Karolinska University Hospital Solna, 17176 Stockholm, Sweden. Phone: +46 8 5177 0000 or +46 8 5177 3914. Fax: +46 8 308099. E-mail: Annelie.Brauner@ki.se
Abstract

Urinary tract infection (UTI) is common during pregnancy and can be associated with negative outcomes for both the mother and fetus. Increased risk of infection among these patients has been attributed to physiological changes and less focus has been placed on Escherichia coli, the most frequent causative agent. We investigated virulence properties of isolates causing UTI in pregnant women in Sweden, Uganda and Vietnam as well as non-pregnant women in Sweden. Although phylogenetic group B2 was the most prevalent group, more Ugandan isolates belonged to group B1, associated with commensal strains, compared to isolates from other countries. Adherence to and invasion of urothelial cells, key events in the infection process, was low among group B1 isolates from Swedish pregnant women compared to those from non-pregnant patients. Similar levels of adherence and invasion were seen between isolates from pregnant women in Uganda and Vietnam. More biofilm was formed by group B2 isolates than those belonging to group B1 isolates and by Ugandan group B2 isolates compared to those from pregnant Swedish and Vietnamese women. The antigen 43a-encoding gene, fluA_CFT073, was most prevalent among Ugandan isolates. Expression of the biofilm components, curli and cellulose, was low among all isolates. Multidrug resistance was more common among isolates from Uganda and Vietnam in comparison to those from Swedish patients. We suggest that while bacterial virulence properties play an important role in UTI during pregnancy, physiological changes in the host may contribute more to the incidence of infection caused by less virulent E. coli.
Introduction

Urinary tract infection (UTI) is the predominant type of bacterial infection among pregnant women (7, 18). As many as 90% of UTIs are caused by *Escherichia coli* (5). Hormonal and physiological changes in the urinary tract, including ureteral dilatation and changes in bladder volume and tone may promote infection in pregnant women (18). Interestingly, incidence of UTI during pregnancy is higher among women who have had childhood infections (13). Furthermore, it has been observed that pregnant women have a propensity to develop recurrent UTIs (18).

Overall, UTI can be dangerous for both the mother and fetus. Complications that can arise include pre-term delivery and increased incidence of intra-uterine growth restriction. To a lesser degree, pre-eclampsia, caesarean delivery, anemia, sepsis and septic shock may also be associated with UTI in these patients (14). Among patients suffering acute pyelonephritis, those that are pregnant are more likely to develop renal scars in comparison to non-pregnant women (22).

The possession of virulence factors which enable colonization is important in the pathogenesis of uropathogenic *E. coli* (UPEC). It has been demonstrated that among isolates causing acute pyelonephritis in pregnant women, expression of Type 1, P and Dr fimbriae can vary by gestational age (19). However, little is known about the prevalence of other *E. coli* virulence-associated factors, including those known to play a role in long-term survival in the human host.

The aims of this study were to investigate the impact of virulence properties of *E. coli* isolates causing UTI in pregnant women in different countries. Therefore, ability to cause infection,
the prevalence of virulence factors associated with persistence and survival, as well as antibiotic resistance levels were investigated.

Materials and Methods

Patients

Midstream urine samples from pregnant women with acute UTI caused by \textit{E. coli} were collected at antenatal clinics at Karolinska University Hospital, Stockholm, Sweden; Mulago Hospital, Kampala, Uganda; and the National Hospital of Obstetrics and Gynecology, Hanoi, Vietnam, between September 2009 and January 2012. Patients were aged 17–48 years old and had gestational age ranging from 4–40 weeks (Table 1). Serving as controls, samples from non-pregnant age-matched women with UTI caused by \textit{E. coli} were collected from outpatient clinics in Stockholm, Sweden. Only patients not taking antibiotic treatment at the time of sampling were recruited for the study.

Bacterial species identification and culture

In all, 148 \textit{E. coli} isolates were obtained from urine samples from pregnant patients and 50 isolates were collected from non-pregnant patients (Table 1). Species identification was first performed at the hospitals at which samples were collected. Thereafter, \textit{E. coli} were confirmed using the Vitek 2 Gram-negative Identification Card (BioMérieux, Marcy l’Etoile, France), as previously described (21). Vitek screening and all subsequent tests in this study were performed at the Department of Clinical Microbiology, Karolinska University Hospital.

\textit{E. coli} isolates were grown overnight at 37°C on blood agar or for 24 h on LB agar without salt for infection or biofilm assays, respectively. Colonies were suspended in PBS and centrifuged at 300 x g for 10 min to remove bacterial aggregates. The concentrations of
bacterial suspensions were adjusted in PBS spectrophotometrically and confirmed by viable count.

**In vitro assays**

**Cell culture**

Human bladder T24 (HTB-4; ATCC) cells were grown in McCoys 5A medium supplemented with 10% FBS. For experiments, cells were grown to confluence in 24-well cell culture plates (Costar, Corning, NY, USA) in a humidified incubator with 5% CO₂.

**Infection experiments**

Adherence and invasion were analysed using a modification of the previously described method (12). Cells were infected with 10⁶ CFU/ml of bacteria, centrifuged (600 x g for 5 min) and incubated for 30 min at 37°C with 5% CO₂. For adherence assays, cells were washed with PBS, lysed with 0.1% Triton X-100/0.5% Trypsin in PBS, serially diluted and plated on blood agar plates. To test invasion, cells were infected and washed as per the adherence assay. Thereafter, fresh medium was added and cells were incubated for a further 30 min. Next, cells were washed with PBS and incubated for 30 min in fresh medium containing 100 µg/ml gentamicin. Cells were then washed, lysed and plated as described above. Bacterial colonies were counted following overnight culture at 37°C. Adherence efficiency was calculated as the proportion of total cell-associated bacteria from the viable count of the original bacterial suspension. The invasion efficiency was assessed by calculating the proportion of intracellular bacteria from the total cell-associated bacteria in parallel experiments. Isolates that were gentamicin resistant were excluded due to the use of a gentamicin protection invasion assay.

Therefore, group B1 isolates that were tested included those from pregnant women in Sweden (n=3), Vietnam (n=2) and Uganda (n=12) and non-pregnant Swedish controls (n=3) as well as
group B2 isolates, of similar virulence factor gene profile to group B1 isolates, from each country/patient group.

Biofilm formation

The ability to form biofilm was assessed using a microtiter plate method. All group B1 and B2 isolates that were tested for adhesion and invasion, as well as two more group B1 isolates that were gentamicin resistant (one each from Uganda and Vietnam), were tested for biofilm formation. Bacterial suspensions in PBS (10^8 CFU/ml) were further diluted 1:100 in LB broth without salt. Thereafter, 200 µl of suspension was aliquoted in triplicate into 96-well microtiter plates (Costar, Corning). Plates were incubated for 24 h at 37ºC. Bacterial growth was measured at 630 nm. Bacterial suspensions were aspirated, wells were carefully washed with PBS and air-dried. The biofilm was stained with crystal violet (3%) (BD, Franklin Lakes, USA). Next, the dye was solubilised using 20% acetone/80% ethanol and the optical density was read at 550 nm. Biofilm formation was calculated as the proportion of the optical density of the solubilized crystal violet from the optical density of the bacterial growth.

Expression of type 1 fimbriae

To elucidate the possible effects of type 1 fimbriae during adherence, invasion and biofilm testing, type 1 fimbriae expression was investigated under the specific growth conditions for each respective assay. Briefly, isolates were grown on blood agar or LB agar without salt, as described above. Bacteria were then suspended in PBS (approximately 10^{10} CFU/ml). Mannose-sensitive agglutination was tested by mixing bacteria in equal parts with a suspension of Baker’s yeast (Saccharomyces cerevisiae, 3% in PBS) as previously described (10). Inhibition of agglutination by mannose (5% in PBS) was used to confirm the specificity of the reaction.
PCR amplification of virulence factor genes

A modified boiling method was used to extract whole cell DNA from *E. coli* isolates (21). One to two colonies of bacteria grown on blood agar were suspended in 100 µl sterile deionized water and boiled at 99°C. Following centrifugation, the supernatant, containing the DNA, was stored at –20°C.

A previously described triplex PCR reaction was used to phylogenetically group isolates (1, 21). Genes including *flu*, allelic variants *fluA*<sub>CFT073</sub> and *fluB*<sub>CFT073</sub> (21), *tcpC* (2), and *iroN*<sub>E.coli</sub> (1) were investigated using previously described primers. In uniplex PCRs, amplification reaction mixtures contained DreamTaq Green PCR Master Mix (Fermentas, Thermo Fisher Scientific, Waltham, USA), 0.4 µM of each appropriate forward and reverse primer and 1 µl of DNA template. The amplification conditions for the *flu* gene consisted of initial denaturation at 95°C for 1 min; 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min; and one final cycle of 72°C for 5 min. Amplification conditions for the genes *fluA*<sub>CFT073</sub>, *fluB*<sub>CFT073</sub> and *tcpC*, were as for the *flu* gene except for the annealing temperatures which were 65°C, 63°C and 53°C, respectively. For the *iroN*<sub>E.coli</sub> gene, amplification consisted of heating to 95°C for 1 min; 25 cycles of 95°C for 30 s, 63°C for 30 s, 72°C for 1 min; and one final extension cycle of 72°C for 5 min. Strain UPEC CFT073 was used as the positive control for all virulence factor genes tested. PCR reactions, gel electrophoresis and visualization following GelRed (Biotium, Hayward, USA) or ethidium bromide staining were performed as previously described (21).

Expression of curli and cellulose
The expression of curli and cellulose was determined by inspection of the colony morphology of isolates after 48 h of growth on Congo red and Calcofluor agar as previously described (21).

Antibiotic susceptibility testing

Antibiotic susceptibility testing of all isolates was performed using the Vitek 2 Antimicrobial Susceptibility Tests (AST-N106) (BioMérieux) as previously described (21). Extended spectrum beta lactamase (ESBL)-producing isolates were identified by the Vitek 2 Susceptibility Tests. Isolates that were resistant to antibiotics belonging to three or more different classes were classified as multidrug resistant (MDR) (4).

Statistical analysis

Differences between countries were assessed using a combination of $\chi^2$ and pair-wise Fisher’s exact two-tailed tests as previously described (21); $P<0.01$ was considered significant. For individual countries, correlations between antibiotic resistance, phylogenetic group and virulence factor genes were assessed using Fisher’s exact two-tailed test. Data from in vitro experiments were compared by Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks, as appropriate. $P<0.05$ was considered significant. Statistical analyses were performed using GraphPad Prism, version 5.02 (San Diego, CA, USA) and Statistica Statsoft version 7.0 (Tulsa, OK, USA).

Results

Phylogenetic groups among isolates from different countries

*E. coli* isolates causing UTI in pregnant and non-pregnant women belonged to all four phylogenetic groups; however, group B2 was the most prevalent group among isolates from
Sweden and Vietnam (Fig. 1). Conversely, fewer Ugandan isolates belonged to group B2
(P = 0.03) while more belonged to group B1 (P = 0.02) in comparison to isolates from pregnant
women in Sweden (Fig. 1). However, these differences did not meet the strict criteria for
statistical significance employed in this study. In all, 9 (6%) isolates did not carry any of the
phylogenetic grouping genes and were therefore classified as non-typable. No associations
were found between phylogenetic groups and the trimester of pregnancy from which isolates
were collected (data not shown).

Adhesion and invasion abilities among UPEC

In view of our finding of increased prevalence of group B1 isolates but fewer group B2
isolates from Ugandan patients (Fig. 1), we next investigated isolates’ abilities to cause
infection according to their phylogenetic groups. Interestingly, group B1 isolates from
pregnant women in Sweden adhered and invaded less than corresponding control group B1
isolates from non-pregnant Swedish women (P < 0.05, Fig. 2A & B). While group B1 isolates
from Swedish pregnant women also adhered less than those from Uganda (P < 0.05, Fig. 2A),
no differences in adhesion and invasion efficiencies were seen among group B1 isolates from
Uganda and Vietnam (Fig. 2A & B). Among isolates from pregnant Swedish women, those
belonging to group B2 invaded more than group B1 isolates. However, adhesion and invasion
abilities of isolates from Uganda and Vietnam were similar between the two phylogenetic
groups (Fig. 2A & B). Overall, no differences in adherence and invasion were seen among
group B2 isolates (Fig. 2A & B).

Differences in biofilm formation among UPEC

Ability to form biofilm has been associated with UPEC persistence in the urinary tract (24).
We therefore next investigated biofilm formation among isolates belonging to phylogenetic
groups B1 and B2. Our results revealed that group B1 isolates from Swedish pregnant women formed less biofilm than those from non-pregnant Swedish controls ($P<0.001$), however, this difference was not seen among group B2 isolates (Fig. 2C). Additionally, more biofilm was formed by group B2 isolates from pregnant Ugandan and Swedish patients in comparison to group B1 isolates ($P<0.05$, Fig. 2C). Overall, among group B2 isolates from pregnant women, those from Uganda formed more biofilm than those from Swedish and Vietnamese women ($P<0.05$, Fig. 2C).

Our results revealed that no isolates expressed type 1 fimbriae under the conditions of growth that were employed in the in vitro assays. This therefore suggested that type 1 fimbriae did not play a major role in the differences in adherence, invasion and biofilm formation that were seen in the present study.

The 

The fluACFT073 gene is highly prevalent among Ugandan isolates

Due to differences in biofilm formation, we next investigated the prevalence of the gene encoding antigen 43(Ag43). In particular, one of the allelic variants, Ag43a has been shown to promote biofilm formation, as well as cell aggregation and UPEC persistence (26).

Prevalence of the flu gene which amplifies all 5 Ag43 allelic variants (23) was high among isolates from pregnant women. No differences were seen between countries and non-pregnant controls (Fig. 3). Interestingly, the flu gene was associated with phylogenetic groups which are generally considered virulent (groups B2 and D) (3). More Ugandan isolates belonging to groups B2 or D carried flu compared to those belonging to groups A or B1 (25 [83%] vs. 8 [31%]; $P=0.0001$). Similarly, 31 (91%) Vietnamese isolates belonging to group B2 or D carried the gene compared to 2 (25%) isolates belonging to groups A or B1 ($P=0.0004$).
Gene *fluACFT073*, encoding Ag43a, was carried by more Ugandan isolates (Fig. 3). Among all isolates tested for biofilm formation however, only one group B1 Ugandan isolate and no group B2 isolates carried the gene. This suggests that Ag43a did not play a major role in the differences in biofilm formation that were seen in the present study. Gene *fluB_CFT073*, which is associated with reduced cell aggregation and less biofilm (26), was carried by few isolates (Fig. 3).

Continuing to investigate differences in biofilm formation abilities, we next examined the expression of the biofilm components curli and cellulose. Overall, the morphotype lacking both curli and cellulose expression was the most prevalent among all isolates. Although group B1 isolates generally formed less biofilm than group B2 isolates, no differences in curli expression were seen among the two groups. In all, 9 (47%) group B1 isolates from pregnant patients and 2/3 control isolates expressed curli in comparison to one group B2 isolate each from Uganda, Sweden and Vietnam. Even fewer isolates expressed cellulose. These included 4 (21%) group B1 isolates from pregnant women, 1 group B1 control isolate and one group B2 isolate from Vietnam. The low prevalence of curli and cellulose expression therefore suggested that these biofilm components were not of major importance to the differences in biofilm formation that were seen among isolates.

*Genes tcpC and iroN*<sub>E.coli</sub>* are associated with phylogenetic group B2*

Further exploring other virulence factor genes associated with UPEC survival in the human host, we investigated gene *tcpC*, associated with inhibition of the innate immune response (2, 17), and gene *iroN*<sub>E.coli</sub>, encoding a novel catecholate siderophore (1). Both were less prevalent among Ugandan isolates in comparison to isolates from other countries (Fig. 3). A strong correlation between the *tcpC* and *iroN*<sub>E.coli</sub> genes and phylogenetic group B2 was
observed (Table 2). Overall, no trimester-associated differences in the prevalence of any of the virulence genes and factors were found (data not shown).

Widespread antimicrobial resistance among isolates from Uganda and Vietnam

Antibiotic resistance levels were generally low and similar among isolates from pregnant and non-pregnant patients in Sweden (Fig. 4). However, among isolates from pregnant Ugandan and Vietnamese women, resistance and the prevalence of ESBL-producing and MDR isolates was more common in comparison to pregnant Swedish patients (Fig. 4). Incidence of resistance could not be linked to any of the trimesters of pregnancy (data not shown). Overall, few or no isolates were resistant to other antibiotics including aztreonam, ceftazadime, ertapenem, nitrofurantoin, mecillinam and tobramycin (data not shown).

Interestingly, the presence of the flu gene was associated with antibiotic resistance. More flu-positive isolates from Uganda were resistant to ampicillin compared to flu-negative isolates (29 [88%] vs. 13 [59%], P < 0.05). Similarly, among isolates from non-pregnant controls, 11 (34%) that were flu-positive were ampicillin resistant compared to only 1 (6%) isolate lacking the gene (P<0.05). The flu gene was also associated with cefalexin resistance among Ugandan isolates with 10 (37%) flu-positive isolates resistant compared to just 2 (10%) flu-negative isolates (P<0.05). Among Vietnamese isolates, more that carried the flu gene were trimethoprim resistant compared to those lacking the gene (26 [79%] vs. 3 [38%], respectively, P<0.05).

Discussion

It is commonly accepted that high frequency of UTI during pregnancy is due to physiological changes that the human body undergoes in the pregnant condition (9). In this study, we
demonstrate that differences exist in the virulence properties, including ability to adhere to and invade urothelial cells and form biofilm, among *E. coli* causing UTI in pregnant and non-pregnant women, as well as among isolates from pregnant women in different countries.

Adherence and invasion are key virulence mechanisms employed by UPEC (15). We here found that group B1 isolates from pregnant Swedish women were less efficient in both adherence and invasion in comparison to isolates from non-pregnant Swedish women. It has previously been reported that *E. coli* causing UTI in pregnant women have similar adhesion ability as those causing infection in non-pregnant women (25). Our results among group B2 isolates generally confirm this. However, the differences seen among phylogenetic group B1 isolates suggest that physiological changes in the pregnant condition play a role in facilitating the infection process. The similar ability to adhere and invade that was seen among group B1 and B2 Ugandan isolates suggests that group B1 isolates, which may normally be regarded as commensals, are as well equipped as virulent strains to cause infection in the pregnant host. Furthermore, higher adherence efficiency among Ugandan group B1 isolates compared to those from pregnant Swedish women indicates that Ugandan group B1 isolates may have a higher capacity to cause infection than group B1 isolates from other countries. This may explain the higher prevalence of group B1 among Ugandan isolates compared to other countries (Fig. 1).

Exploring the ability to persist in the human host, we next investigated biofilm formation. Group B1 isolates from pregnant women formed less biofilm than isolates from non-pregnant women. Furthermore, isolates belonging to group B1 formed less biofilm than those belonging to group B2. Collectively, these findings further emphasize the role of physiological changes, rather than bacterial virulence properties, in UTI caused by these less virulent strains.
virulent isolates. While differences in biofilm formation efficiency were seen among isolates, including increased biofilm formation among Ugandan group B2 isolates compared to those from other countries, these results could not be linked to biofilm-associated virulence factors $flu_{CFT073}$, curli or cellulose. Thus it seems likely that alternative virulence factors may play a role in biofilm formation among these isolates.

Our results indicate that isolates from Uganda are equipped to persist in the pregnant host due to higher prevalence of $flu_{CFT073}$ (26). On the other hand, our finding that the tcpC and $iroN_{E.coli}$ genes were more common among group B2 isolates may explain the low prevalence among Ugandan isolates since more belonged to non-B2 phylogenetic groups. A recent study reported that $E. coli$ from the vaginal flora of pregnant women were more virulent than those from non-pregnant women due to higher prevalence of certain virulence factor genes including $iroN_{E.coli}$ (8). However, we saw no differences in virulence factor gene profiles between Swedish pregnant women and non-pregnant controls. This suggests that for both patient groups, only isolates which have a specific repertoire of virulence factors that facilitate colonization of the urinary tract are able to cause UTI.

Treatment of UTI is considerably more challenging if the causative agent is resistant to antibiotics (20). In this study, significantly higher levels of antimicrobial resistance, including ESBL-production and multidrug resistance, were seen among isolates from Uganda and Vietnam. We have previously reported such a finding among isolates causing UTI in children in Vietnam (21). Collectively, these results indicate that resistance among UPEC is common in this country, regardless of patient group or age. It is worthy of note that in Vietnam antibiotics can be obtained without prescription and are often taken inappropriately (6). Similarly, it has been reported that in rural areas of Uganda, antibiotics are available through
private providers and self-treatment is common (11). In contrast, low levels of resistance among isolates from Sweden may be a reflection of clinical practices which are in place to reduce the use of antibiotics and development of resistance (16). Treatment of UTI in pregnancy is crucial for preventing pyelonephritis and complications that can endanger both the mother and fetus (18). In view of this, high prevalence of antibiotic resistance among isolates in the present study is of great concern. Furthermore, our finding of an association between resistance and antigen 43, linked with UPEC persistence, indicates increased potential for development of difficult-to-treat upper UTI.

In conclusion, our results demonstrate that ability to adhere, invade and form biofilm are important in UPEC pathogenesis among isolates causing UTI in pregnant women in different countries. However, where E. coli causing infection in pregnant women have low capacity to colonize the urothelium, physiological changes that occur in the host may play a more dominant role in facilitating the occurrence of UTI during pregnancy.

Acknowledgments

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References


Table 1. Patient and gestational ages of pregnant and non-pregnant women who presented with urinary tract infection caused by *Escherichia coli* in Sweden, Uganda and Vietnam.

<table>
<thead>
<tr>
<th>Patient group and country</th>
<th>Patient age (years), median (range)</th>
<th>Gestational age range (weeks)</th>
<th>No. of Isolates, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnant</strong></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Sweden</td>
<td>32 (19–42)</td>
<td>6–40</td>
<td>50</td>
</tr>
<tr>
<td>Uganda</td>
<td>23 (17–45)</td>
<td>8–32</td>
<td>56</td>
</tr>
<tr>
<td>Vietnam</td>
<td>31 (21–48)</td>
<td>4–39</td>
<td>42</td>
</tr>
<tr>
<td><strong>Non-pregnant</strong></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Sweden control</td>
<td>29 (18–45)</td>
<td>-</td>
<td>50</td>
</tr>
</tbody>
</table>


Table 2. The prevalence of the tcpC and iroNE.coli genes among phylogenetic group B2 and non-B2 Escherichia coli isolates from pregnant and non-pregnant women with urinary tract infection in different countries.

<table>
<thead>
<tr>
<th>Isolates positive, n (%)</th>
<th>B2</th>
<th>Non-B2 (A+B1+D)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden (n=50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tcpC</td>
<td>13 (48)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>iroNE.coli</td>
<td>13 (48)</td>
<td>6 (26)</td>
<td>NS</td>
</tr>
<tr>
<td>Uganda (n=56)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tcpC</td>
<td>5 (28)</td>
<td>1 (3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>iroNE.coli</td>
<td>3 (18)</td>
<td>1 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Vietnam (n=42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tcpC</td>
<td>13 (57)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>iroNE.coli</td>
<td>16 (70)</td>
<td>1 (5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Non-pregnant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden Control (n=50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tcpC</td>
<td>6 (21)</td>
<td>0 (0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>iroNE.coli</td>
<td>14 (50)</td>
<td>3 (14)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data were analyzed using Fisher’s exact two-tailed test with P<0.05 considered significant.

NS: Not significant

20
Fig. 1. Phylogenetic group distribution of *Escherichia coli* isolates causing urinary tract infection in pregnant women in Sweden (n=50), Uganda (n=56) and Vietnam (n=42), and non-pregnant Swedish controls (n=50). Comparisons were made by $\chi^2$ and pair-wise Fisher’s exact two-tailed tests.

Fig. 2. Adherence, invasion and biofilm formation efficiencies of phylogenetic group B1 and B2 *Escherichia coli* isolates causing urinary tract infection in pregnant women from Sweden, Uganda and Vietnam and control isolates from non-pregnant patients in Sweden. (A,B) For adhesion and invasion, group B1 isolates included those from pregnant women in Sweden (n=3), Vietnam (n=2) and Uganda (n=12) and non-pregnant Swedish controls (n=3). Three group B2 isolates, of similar virulence gene profile, from each patient group were also tested. Data are derived from at least two independent experiments. (C) For biofilm formation experiments, Group B1 isolates from Sweden (n=3), Vietnam (n=3), Uganda (n=13) and Swedish controls (n=3) and 3 representative group B2 isolates from each patient group, were tested in triplicate in 2 independent experiments. (A–C) Values shown are mean and standard deviation. Comparisons were made by Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks.

Fig. 3. The prevalence of the virulence factor genes *flu*, *flu*$_{ACFT073}$, *flu*$_{BCFT073}$, *tcpC*, and *iroNE.coli* among *Escherichia coli* isolates causing urinary tract infections in pregnant women in Sweden (n=50), Uganda (n=56) and Vietnam (n=42) and non-pregnant Swedish controls (n=50). Comparisons were made by $\chi^2$ and pair-wise Fisher’s exact two-tailed tests.

Fig. 4. Prevalence of antibiotic resistance among uropathogenic *Escherichia coli* isolates causing urinary tract infection in pregnant patients in Sweden (n=50), Uganda (n=56) and
Vietnam (n=42) and non-pregnant patients (Sweden control, n=50). Comparisons were made by $\chi^2$ and pair-wise Fisher’s exact two-tailed tests. AM: ampicillin, AMC: amoxycillin/clavulanic acid, CIP: ciprofloxacin, CN: cefalexin, CTX: cefotaxime, GM: gentamicin, TMP: trimethoprim, ESBL: extended spectrum beta lactamase producing, MDR: multidrug-resistant.
C

Biofilm (630nm)/Growth (550nm)

Phylogenetic group

B1

B2

Biofilm formation

Sweden: Control
Sweden: Uganda
Vietnam

P < 0.0001
P < 0.05
P < 0.05
P < 0.0001
P < 0.0001
P < 0.0001
P < 0.0001
P < 0.0001

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