Faecal Escherichia coli from patients with E. coli urinary tract infection and healthy controls who have never had a urinary tract infection

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Received 27 September 2013
Accepted 24 January 2014

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Urinary tract infections (UTIs) are primarily caused by Escherichia coli with the patient’s own faecal flora acting as a reservoir for the infecting E. coli. Here we sought to characterize the E. coli faecal flora of UTI patients and healthy controls who had never had a UTI. Up to 20 E. coli colonies from each rectal swab were random amplified polymorphic DNA (RAPD) typed for clonality, dominance in the sample and correlation to the infecting UTI isolate in patients. Each distinct clone was phylotyped and tested for antimicrobial susceptibility. Eighty-seven per cent of the UTI patients carried the infecting strain in their faecal flora, and faecal clones causing UTI were more often dominant in the faecal flora. Patients had a larger diversity of E. coli in their gut flora by carrying more unique E. coli clones compared to controls, and patient faecal clones were more often associated with multidrug resistance compared to controls. We found a similar phylotype distribution of faecal clones from UTI patients and healthy controls, including a large proportion of B2 isolates in the control group. Faecal-UTI isolates from patients were more often associated with multidrug resistance compared to faecal-only clones, indicating a link between UTI virulence and antimicrobial resistance. Intake of any antibiotic less than 6 months prior to inclusion in the experiment occurred significantly more in patients with UTI than in controls. In contrast, presence of an intrauterine device was significantly more common in controls indicating a protective effect against UTI. In conclusion, healthy controls have a large proportion of potentially pathogenic E. coli phylotypes in their faecal flora without this causing infection.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections with approximately 60% of all women being diagnosed with a UTI at least once during their lifetime (Foxman et al., 2000), and 20–30% of women with a first UTI will have recurring infection (Foxman, 1990). Interestingly, the majority of these cases cannot be explained by anatomical or functional abnormalities (Finer & Landau, 2004; Sobel, 1997). UTI is primarily caused by Escherichia coli (Russo & Johnson, 2003) with the woman’s own faecal flora acting as an immediate reservoir for the infecting E. coli, indicating a faecal–perineal–urethral route of infection (Yamamoto et al., 1997). The infecting E. coli most often belongs to phylotype B2 followed by phylotype D, although all of the four phylotypes are found in the gut of healthy humans (Moreno et al., 2009; Vollmerhausen et al., 2011). Some studies indicate that phylotype B2 strains are rare in faecal samples, while others report the opposite (Duriez et al., 2001; Moreno et al., 2009; Zhang et al., 2002). It has been shown that E. coli from faecal samples vary with respect to phylotypes depending on geography (Escobar-Paramo et al., 2004), which perhaps, along with various sampling methods, can explain the varying reports on E. coli in the gut. Characterizing the phylogenetic distribution of faecal...
E. coli of UTI patients and healthy controls who have never had a UTI can provide important knowledge regarding the risk of infection.

It has been shown that the UTI isolate is the most dominant isolate among E. coli clones from the faeces of patients (Yamamoto et al., 1997). Additionally, UTI isolates on average contain more virulence factors and more often belong to phylogenotype B2 and D compared to the remaining E. coli faecal flora (Moreno et al., 2008; Zhang et al., 2002). At this stage, it is not clear whether the infecting E. coli is the most prevalent faecal/vaginal clone (the prevalence hypothesis) or a subset of the population having achieved virulence compared to other faecal E. coli (special-pathogenicity hypothesis) (Moreno et al., 2008). It has been proposed that these two hypotheses are not mutually exclusive and in reality a combination of the two may exist (Moreno et al., 2008).

Alterations in the balance between human hosts and their microbiota can give rise to infections, and antimicrobial agents are known to disturb this delicate balance, leaving options for colonization by pathogens with infections occurring as a possible result (Sullivan et al., 2001). Additionally, antimicrobial treatment can select for antimicrobial resistance of the commensal bacteria, which could then disseminate as potential pathogens in the intestine or cause colonization with antimicrobial resistant clones. This would increase the likelihood of being infected with an antimicrobial resistant pathogen. Several studies have investigated levels of antimicrobial resistance in commensal E. coli (Linton et al., 1972; Vinué et al., 2008). However, some studies only sampled one or a few E. coli colonies per sample, increasing the risk of overlooking minor E. coli clones.

The aim of this study was to characterize and compare the E. coli faecal flora of patients with a UTI, and the UTI isolates, to the faecal flora of control individuals who had never had a UTI, with respect to the number of clones, phylotypes, the dominance of clones and phylotypes, and their susceptibility towards a broad range of antimicrobials. Additionally, we wanted to compare the epidemiological data of patients and controls in order to further describe the risk factors for UTI. These studies could clarify whether UTI patients have a different faecal flora, and hence UTI reservoir, than healthy controls.

**METHODS**

**Study participants and samples.** The study was approved by The National Committee on Health Research Ethics, Denmark, and informed consent was obtained for all participants. UTI patients and controls were recruited from two general practices in Zealand, Denmark, serving approximately 14 000 and 13 000 inhabitants, respectively. Inclusion criteria for patients were: otherwise healthy, non-related, premenopausal women presenting with > 10^3 E. coli ml^-1 and an increased number of leukocytes in their urine, as well as UTI symptoms. Inclusion criteria for controls were: healthy, non-related, premenopausal women who had never had a UTI, and whose urine samples (collected for study purposes only) were negative for urinary pathogens and leukocytes. Exclusion criteria for both patients and controls were those who were postmenopausal, diabetic, pregnant, and those who had recently had surgery in the urogenital area, or who had cancer.

UTI patients delivered a urine sample and a rectal swab upon inclusion in the study, and a urine sample again more than 1 week post conclusion of antibiotic treatment. Controls delivered a urine sample and a rectal swab at inclusion. Recurrence was defined as two episodes within 30 days of sampling.

Fifty UTI patients and 53 controls were enrolled in the study. The following epidemiological data were collected from the patient’s own medical record: age, total number and recurrence of infections, number of children, previous antibiotic treatment 6 months prior to infection, underlying illness and birth control.

**Bacterial isolates.** Rectal swabs were plated within 48 h on Chromogenic ID agar plates (SSI Diagnostica), on which E. coli could be distinguished by their size and pink colour from other species. In order to confirm the presence of E. coli, colonies were streaked onto p-nitrophenyl β-D-glucopyranosiduronic acid (PGUA) plates (SSI Diagnostica). In case of discrepancies, additional plating was performed on bromothymol blue sucrose agar (SSI Diagnostica) followed by identification by the analytical profile index (API) system, API 20E (bioMérieux), if necessary. From each patient, up to 20 E. coli isolates (if available) were frozen and stored at −80 °C. Selecting 20 E. coli colonies from each sample will with approximately 88 % certainty reveal all E. coli clones with as low as 10 % prevalence in the sample (Lautenbach et al., 2008). UTI isolates were isolated from Flexicult (SSI Diagnostica) agar plates, which was the diagnostic procedure used by the general practitioner, and confirmed as E. coli by plating onto PGUA plates as described above.

**Random amplified polymorphic DNA (RAPD) typing.** In order to distinguish whether up to 20 isolates from each sample were identical or different E. coli clones, a RAPD PCR typing assay was developed for this study. The assay consisted of two PCRs, each containing one primer; 1247 (5’-AAAGGCCCCGTT-3’) or 1283 (5’-GGGATCCCTCCCA-3’), respectively. Qiagen Multiplex Master Mix was used as recommended by Qiagen without Q-solution, and each 25 µl reaction contained a 2 µM concentration of one of the two primers and 2.5 µl of template DNA. Cycling conditions for 1247 PCR were: 95 °C for 15 min, 35 cycles of 94 °C for 1 min, 38 °C for 1 min and 72 °C for 2 min, and finally, 72 °C for 10 min. Cycling conditions for 1283 PCR were: 95 °C for 15 min, 35 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min, and finally, 72 °C for 10 min. Template DNA was prepared by adding a loopful (1 µl inoculation needle) of bacteria from a plate to 300 µl of DNase-free water followed by incubation at 95 °C for 10 min.

All isolates from the same person were included in the same PCR run and gel; this procedure was also followed when comparing a UTI isolate of a patient with its respective faecal clones. The criterion for distinguishing clones was more than one band difference in at least one of the PCRs (example of gel in Fig. S1, available in the online Supplementary Material). Classification of clones was performed in order to describe the prevalence of the clone in a sample, although this status could change shortly after sampling. Dominant clones were defined as >50% of the isolates in the sampling of 20 E. coli colonies from each swab, minor clones as <10% of the isolates, and intermediate clones were defined as >10% but <50% of the isolates. Faecal isolates determined as being identical to the infected urine isolate were defined as faecal-UTI isolates, whereas solely faecal isolates found in the faecal swab were defined as faecal-only isolates.

**Phylotyping.** Phylotyping into the four phylogenetic groups (A, B1, B2 and D) plus non-typable (NT) strains was performed by multiplex PCR modified from the method of Clermont et al. (2000). Briefly,
each 25 µl reaction contained Multiplex Master Mix and Q-solution (Qiagen) according to manufacturer recommendations, 0.2 µM of each primer and 1 µl of template DNA. Cycling conditions were as follows: 15 min at 95 °C, 29 cycles of 30 s at 94 °C, 90 s at 58 °C and 60 s at 72 °C, followed by 10 min at 72 °C. As recommended by Gordon et al. (2008), an additional PCR for iheA was performed on isolates positive for chuA and Tspe4.c2. The PCR for iheA was performed with forward primer 5’-TGAACCCGGCTGTAAATAC-3’ and reverse 5’-CTGCCGTTTCAAGATTGCA-3’ as follows: Multiplex Master Mix (Qiagen) with Q-solution was used as recommended by the manufacturer. Each 25 µl reaction contained 0.4 µM of each primer and 1 µl template DNA. Cycling conditions were 15 min at 95 °C, 29 cycles of 30 s at 94 °C, 30 s at 56 °C and 60 s at 72 °C, followed by 10 min at 72 °C.

**Antimicrobial susceptibility testing.** MIC values for E. coli faecal clones identified by RAPD PCR were determined for amoxicillin–clavulanic acid, ampicillin, apramycin, cefotaxime, cefotiofur, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin, nalidixic acid, neomycin, spectinomycin, streptomycin, sulphonamethoxazole, tetracycline and trimethoprim using the DKMVN4 Sensititre plate (TREK Diagnostic Systems). Additionally, all faecal clones were tested for susceptibility towards nitrofurantoin and mecillinam by disc diffusion using Mueller–Hinton agar plates (SSI Diagnostica) with confluent growth and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for antimicrobial susceptibility testing with paper discs (Oxoid). Quality control was assured by including E. coli ATCC 25922 in each run. Interpretations of both assays were based on EUCAST breakpoints where applicable; alternatively, Clinical and Laboratory Standards Institute (CLSI) breakpoints were applied. Epidemiological cut-off values were applied when clinical breakpoints were not available. Multidrug resistance was defined as resistance towards three or more of the investigated antimicrobials.

**Statistics.** GraphPad Prism 5 and GraphPad InStat 3 (GraphPad Software) were used for all statistical analyses, and a P value of <0.05 was used unless otherwise stated. RAPD typing was evaluated by the two-tailed Mann–Whitney test. Linear regression was performed to correlate the age of the participant and the number of faecal clones. Dominance of clones and antimicrobial resistance correlations to antimicrobial consumption, respectively, were evaluated by the two-tailed Fisher’s exact test. The number of antimicrobial resistances was compared using a two-tailed Mann–Whitney test. Correlation of phylotypes to the source of the isolate was evaluated by the χ² test for independence followed by the relevant post-test; phylotype B2 and NT isolates of patients and controls were evaluated by the two-tailed Fisher’s exact test for correlation to the source of the isolate (P<0.008, Bonferroni corrected). Correlation of phylotypes to the number of antimicrobial resistances was evaluated by the Kruskal–Wallis test followed by two-tailed Mann–Whitney tests comparing the number of antimicrobial resistances carried by phylotype A and NT isolates in comparison to B2 and D, respectively (P<0.0125, Bonferroni corrected for multiple comparisons). Multidrug resistance and the difference in antimicrobial resistance prevalence between the two groups were evaluated by the two-tailed Fisher’s exact test. Univariate analysis of epidemiological data was performed using Fisher’s two-tailed exact test and the two-tailed Mann–Whitney test. Stepwise logistic regression on the epidemiological data was performed with SAS 9.1 (SAS Institute) in order to analyse independent risk factors and confounding variables. The stepwise logistic regression included the following parameters in age-adjusted analyses: antibiotics within 6 months of sampling, antibiotics within 6 months of sampling excluding UTI treatments, having an intrauterine device (IUD) and children (possible confounder for an IUD).

**RESULTS**

**Study participants**

Of the 50 UTI patients and 53 controls initially enrolled in the study, 47 patients and 50 controls were retained after exclusions. Reasons for exclusions were as follows: two controls proved to be close family relatives (the second one included in the study was excluded), two controls had a UTI previously according to medical records, two patients were postmenopausal according to medical records and one case was recruited, but proved to have only 10⁵ E. coli ml⁻¹. The mean ages of patients and controls did not differ significantly (average ± SD of 35.2 ± 7.3 and 37.4 ± 8.5 years, respectively, unpaired t-test, P=0.2). Twenty-seven patients had a UTI history with a range of 1–31 episodes (median=5) per subject. Eighteen subjects had had a UTI less than 1 year prior to sampling.

**Faecal and UTI clones**

RAPD typing was applied in order to determine the number of unique faecal clones and how often the urine isolate was found in faeces. The number of UTI isolates was 48 from 47 different patients. From the included participants, a total of 1820 faecal E. coli colonies were picked and analysed by RAPD PCR. On average, 17.6 isolates were obtained per control and 19.6 isolates per patient. RAPD revealed 160 unique faecal E. coli clones; 71 clones were from controls and 89 clones were from patients. Comparing the respective faecal clones from the patients to the UTI isolate from the same individual we found that 42 faecal isolates were identical to the UTI isolate (faecal-UTI) as well as 47 isolates which were in faeces only (faecal-only). Five patients were found not to carry the infecting isolate in their rectum; however, they all carried other E. coli. E. coli faecal flora of patients and controls collectively consisted of anything from 0 to 5 clones per participant.

![Fig. 1. E. coli diversity in faecal flora of UTI patients and healthy controls illustrated by number of E. coli clones, identified by RAPD typing of up to 20 E. coli isolates from each rectal swab (P=0.02). Lines indicate medians.](image-url)
with medians of 1–2 clones per participant (Fig. 1). UTI patients on average had significantly more *E. coli* clones in their faecal flora compared to controls (*P*= 0.02) (Fig. 1). There was no correlation between the number of clones and age (data not shown). The *E. coli* clones were characterized according to dominance of a particular clone in the faecal flora composition. 54% (26) of the infecting *E. coli* were dominant in the faecal flora of the patient, 23% (11) were intermediate clones and 10% (5) were minor clones (*P*= 0.01). Hence, UTI was caused by a dominant clone in the majority of the patients. By applying a definition of dominant as the most prevalent clone in the sample as proposed by Moreno *et al.* (2008), this yielded the same conclusion of 54% of the infecting *E. coli* being dominant.

**Phylotype distribution of clones from patients and controls**

The faecal phylotype distribution of patients and controls was similarly distributed between the four phylotypes and non-typable isolates (Table 1). The phylotypes of faecal and UTI isolates differed significantly (*P*= 0.03). From these data it is evident that controls have the same phylotype distribution in their faeces as patients, without this causing infection. Comparing faecal-only isolates with the UTI isolates or faecal-UTI isolates (a subpopulation of the UTI isolates), we found significantly more B2 isolates in the faecal-UTI and UTI isolate group (*P*< 0.001, Table 1).

**Phylotypes and faecal dominance**

From the stratifying dominance of the clones in correlation to phylotypes (Table 2), it is evident that among controls, B2 is significantly more common as the dominant faecal clone compared to the other phylotypes (Table 2, *P*= 0.002). The same result was not found for faecal clones of UTI patients. There was no correlation between specific phylotypes being more dominant in the flora of UTI patients as all four phylotypes were found to be dominant ≥50% of the times that they were isolated as a faecal clone (Table 2).

**Antimicrobial resistances of *E. coli* faecal flora**

There was a significant difference in number of antimicrobial resistance markers carried by the different phylotypes (*P*= 0.005). Phylotype D carried the highest number of antimicrobial resistance markers (*X* resistance markers ± SD: 2.7±0.4) followed by B1 (2.0±0.8), B2 (1.8±0.3), NT (0.8±0.3) and A (0.6±0.3). Phylotype D carried significantly more antimicrobial resistance markers when compared to phylotype A (*P*< 0.001).

Patient and control faecal clones did not differ significantly with respect to the number of antimicrobial resistance markers (mean 2.0 and 1.3, respectively, *P*> 0.05). However, faecal *E. coli* isolates of UTI patients were more resistant than those of controls with respect to spectinomycin and streptomycin (Table 3). Multidrug resistance was more common in patient faecal clones (*n*= 30, 34%) compared to control faecal clones (*n*= 14, 20%, *P*= 0.05) and in faecal-UTI clones (*n*= 19, 45%) compared to faecal-only clones (*n*= 11, 23%, *P*= 0.04).

There was no significant correlation between receiving antimicrobials up to 6 months prior to sampling and carrying drug-resistant faecal clones (data not shown).

**Prediction of recurrence**

Sixteen patients (34%) developed recurring infections, and we investigated whether recurrence could be predicted from characteristics of the *E. coli* faecal flora. There was no correlation between the 16 initial UTI isolates of patients with recurring infection and their clonal status in the faecal flora; three were not found in the patient’s faecal flora, one isolate was a minor clone, seven isolates were intermediate clones and five isolates were dominant in the faecal flora. We found no correlation between the number of faecal clones and recurrence of infection (data not shown).

Finally, we found no difference in phylotype distribution of either faecal or UTI *E. coli* of individuals with recurrence compared to individuals experiencing just one episode of UTI (data not shown).

**Risk factors for UTI**

In order to analyse whether specific host-behavioural factors were associated with UTI, we performed univariate and logistic regression on clinical data from the medical records of the study participants. Univariate analyses showed no significant difference between UTI patients and controls with respect to age, oral contraceptives and children (logistic variable) (Table 4). However, there was a significant difference between UTI patients and controls when analysing an IUD and previous antibiotic treatment within 6 months (*P*= 0.04 and *P*= 0.002, Table 4). The stepwise logistic regression examined factors from the

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**Table 1.** Phylotype distribution of UTI isolates and faecal isolates representing unique clones from patients and controls, respectively

<table>
<thead>
<tr>
<th>Type of clone</th>
<th>A (%)</th>
<th>B1 (%)</th>
<th>B2 (%)</th>
<th>D (%)</th>
<th>NT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, faecal</td>
<td>8 (11)</td>
<td>2 (3)</td>
<td>32 (45)</td>
<td>19 (27)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>Patients, faecal, total</td>
<td>8 (9)</td>
<td>6 (7)</td>
<td>40 (45)</td>
<td>21 (23)</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Patients, faecal-only</td>
<td>6 (13)</td>
<td>4 (8)</td>
<td>14 (30)</td>
<td>10 (21)</td>
<td>13 (28)</td>
</tr>
<tr>
<td>Patients, faecal-UTI</td>
<td>2 (5)</td>
<td>2 (5)</td>
<td>26 (62)</td>
<td>11 (26)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>UTI*</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>29 (60)</td>
<td>13 (27)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

*UTI* includes the faecal-UTI group, six UTI isolates which were not found in faeces.

†Significantly different compared to faecal-UTI and UTI group, *P*< 0.001.
univariate analysis with $P \leq 0.2$; these were age, antibiotics within 6 months, antibiotics within 6 months excluding UTI treatments, IUD use and children (logistic parameter) (Table 4). We analysed two different parameters with respect to previous antibiotic treatment; one including all antibiotics given and one excluding antibiotics given for UTI, as such treatment would be found in the patient group only, and hence, could bias the analyses. By logistic regression, we found a significant positive correlation between previous antibiotic treatment (less than 6 months from sampling) and UTI (odds ratio (OR) = 7.14, 95% confidence interval (CI) 2.12–24.1, $P = 0.002$) and a significant negative correlation between an IUD and UTI (OR = 0.18, 95% CI 0.04–0.83, $P = 0.03$). The analysis of antibiotic treatment excluding UTI treatments did not alter the conclusions; antibiotics within 6 months excluding UTI

### Table 2. Phylotype distribution of patient faecal clones and control faecal clones

<table>
<thead>
<tr>
<th>Phylotype</th>
<th>Control faecal clones ($N=71$), $n$ (%)</th>
<th>Patient faecal clones ($N=89$), $n$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dominant*</td>
<td>Intermediate†</td>
</tr>
<tr>
<td>A</td>
<td>43 (60)</td>
<td>19 (27)</td>
</tr>
<tr>
<td>B1</td>
<td>1 (50)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>B2</td>
<td>26 (81)§</td>
<td>6 (19)</td>
</tr>
<tr>
<td>D</td>
<td>10 (53)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>NT</td>
<td>4 (40)</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

*Dominant clone was defined as being $\geq 50\%$ of the isolates in the sampling of up to 20 $E. coli$ colonies from each swab.
†Intermediate clone was defined as being $\geq 10\%$ and $<50\%$ of the isolates in the sampling of up to 20 $E. coli$ colonies from each swab.
‡Minor clone was defined as being $<10\%$ of the isolates in the sampling of up to 20 $E. coli$ colonies from each swab.
§For faecal clones of the control group, phylotype B2 was significantly more common as a dominant clone when compared to the other phylotypes ($P = 0.002$).

### Table 3. Number of $E. coli$ faecal clones resistant to selected antimicrobials and respective clinical breakpoints/epidemiological cut-off values (ECOFF)

<table>
<thead>
<tr>
<th>Antimicrobial Agent*</th>
<th>Breakpoint or ECOFF† (mg l$^{-1}$)</th>
<th>Control $N=71$, $n$ (%)</th>
<th>Patient $N=89$, $n$ (%)</th>
<th>$P$ value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin–clavulanic acid</td>
<td>$\geq 16$</td>
<td>4 (6)</td>
<td>9 (10)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>$\geq 16$</td>
<td>16 (23)</td>
<td>26 (29)</td>
<td>0.7</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>$\geq 16$</td>
<td>17 (24)</td>
<td>24 (27)</td>
<td>0.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>$\geq 2$</td>
<td>2 (3)</td>
<td>1 (1)</td>
<td>0.6</td>
</tr>
<tr>
<td>Spectinomycin wt</td>
<td>$\leq 64$</td>
<td>3 (4)</td>
<td>13 (15)</td>
<td>0.03</td>
</tr>
<tr>
<td>Streptomycin wt</td>
<td>$\leq 16$</td>
<td>12 (17)</td>
<td>28 (31)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>$\geq 512$</td>
<td>12 (17)</td>
<td>24 (27)</td>
<td>0.1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>$\geq 16$</td>
<td>14 (20)</td>
<td>20 (23)</td>
<td>0.7</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>$\geq 8$</td>
<td>7 (10)</td>
<td>19 (21)</td>
<td>0.06</td>
</tr>
<tr>
<td>Mecillinam wt</td>
<td>$&lt;15$ mm§</td>
<td>1 (1)</td>
<td>7 (8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Nitrofurantoin wt</td>
<td>$&lt;11$ mm§</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>$\geq 8$</td>
<td>0 (0)</td>
<td>2 (2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Nalidixic acid wt</td>
<td>$\leq 8$</td>
<td>8 (11)</td>
<td>8 (9)</td>
<td>1.0</td>
</tr>
<tr>
<td>Neomycin</td>
<td>$\leq 8$</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>$\geq 2$</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.4</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>$\geq 4$</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

wt, Wild-type.
*Apramycin, colistin and florfenicol resistance were not found in any of the isolates and were therefore not listed in the table.
†EUCAST breakpoints. CLSI breakpoints were applied in cases where EUCAST breakpoints were not available. In cases where only epidemiological cut-off values were available, these were used instead of clinical breakpoints.
‡$P$ values are based on two-tailed Fischer’s exact test comparing faecal clones of patients with faecal clones of controls.
§$P$ values are based on two-tailed Fischer’s exact test comparing faecal clones of patients with faecal clones of controls.
§§Refers to zone diameters measured using EUCAST susceptibility testing.
DISCUSSION

With this study, we wanted to compare the faecal flora of otherwise healthy premenopausal women with UTI with the faecal flora of a similar control group who have never had a UTI previously. Additionally, we wanted to analyse the epidemiological data of the participants in order to further describe the risk factors for UTI. We have characterized the faecal flora of premenopausal women with and without a UTI by sampling up to 20 E. coli colonies from each participant. The number of E. coli clones per participant in this study is similar to that found by Bailey et al. (2010) who sampled 100 E. coli colonies from each sample, indicating that 20 colonies is sufficient in order to identify both major and minor clones.

We have shown that UTI patients carry more unique E. coli clones in their faecal flora compared to control subjects and that these isolates are more often multiresistant. To our knowledge, this is the first study to indicate that more E. coli faecal clones in the intestinal flora and multidrug resistance of these could be correlated with UTI. On the other hand, a large diversity of commensal E. coli could have a protective effect and prevent colonization by pathogens. Whether a large number of E. coli clones decreases or increases UTI risk depends on the nature of the clone. It has previously been described that the elderly carry more clones than young individuals (Vollmerhausen et al., 2011). This could not be confirmed in this study on premenopausal women.

The high prevalence of multidrug resistance in patient faecal clones, a relatively high level of antimicrobial resistance in phyotype B2 and D compared to the other phyotypes, and a higher level of multidrug resistance in faecal-UTI isolates compared to faecal-only isolates indicate that antimicrobial resistance could cause treatment problems for an otherwise uncomplicated UTI. Whether there is a link between virulence and antimicrobial resistance in this study should be investigated further.

The data in this study confirms previous studies indicating that the faecal flora is a reservoir for infecting E. coli of the urinary tract; 42 out of 48 UTI isolates were found in the faecal flora of the patient. The six patients where we were not able to identify the infecting clone in the faecal flora can be explained by the detection limit of the sampling method or perhaps the vagina served as a reservoir on its own in these cases. More surprisingly, and in contrast to what was expected, we found a similar proportion of B2 isolates in the faecal flora of controls as in UTI patients, without this causing infection in the individual control women. This indicates that acquisition of a B2 isolate is not in itself associated with UTI risk. The authors acknowledge that this finding should be elaborated by virulence testing and further typing.

The faecal flora of healthy individuals without a UTI has only been described and characterized in a few studies (Moreno et al., 2009; Yamamoto et al., 1997). Moreno et al. (2009) found a significantly higher proportion of B2 isolates in the faecal flora of UTI patients compared to controls. Phyotypes have been closely correlated with virulence properties, with B2 being the most virulent

Table 4. Evaluation of epidemiological data based on univariate and multivariate analyses

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<th>Table 4. Evaluation of epidemiological data based on univariate and multivariate analyses</th>
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<tbody>
<tr>
<td><strong>Univariate analyses</strong></td>
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<tr>
<td>Age</td>
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<tr>
<td>Antibiotics ≤ 6 months</td>
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<tr>
<td>Antibiotics ≤ 6 months excluding UTI treatments</td>
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<tr>
<td>Children (logistic)</td>
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<tr>
<td>Intrauterine device</td>
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<td>Oral contraceptives</td>
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<table>
<thead>
<tr>
<th>Multivariate analyses</th>
<th>OR</th>
<th>95 % CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.98</td>
<td>0.93–1.03</td>
<td>0.4</td>
</tr>
<tr>
<td>Antibiotics ≤ 6 months</td>
<td>7.14</td>
<td>2.12–24.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Antibiotics ≤ 6 months excluding UTI treatments</td>
<td>4.19</td>
<td>1.18–14.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Children (logistic)</td>
<td>2.19</td>
<td>0.67–7.14</td>
<td>0.2</td>
</tr>
<tr>
<td>Intrauterine device</td>
<td>0.18</td>
<td>0.04–0.83</td>
<td>0.03</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, Confidence interval.

*Two-tailed Fisher’s exact test or Mann–Whitney test as applicable.

†Stepwise logistic regression on parameters from univariate analyses with P≤0.2.
phytotype (Moreno et al., 2008). This indicates that cases of that study, on average, had more UTI pathogenic *E. coli* in the faecal flora, and hence, could be more prone to infection. The present study, in contrast, shows similar trends in phylotype distribution of faecal *E. coli* from patients and controls in correlation with the study by Yamamoto et al. (1997). Discrepancies between the studies indicate that phylotypes in otherwise healthy individuals vary geographically, and that sampling methods as well as inclusion/exclusion criteria could influence the outcome of the study. In the study by Moreno et al. (2009), the controls had not had a UTI in the 2 months prior to sampling, whereas the study by Yamamoto et al. (1997) and the present study included female subjects who had never had a UTI, which could in part explain discrepancies between the three studies.

The dominant clone in the faecal flora of patients was significantly associated to UTI as also found by Moreno et al. (2008) and dominant clones caused infection in 54% of the patients in this study. On the other hand, B2 was found also to dominate the faecal flora of controls compared to the other phylotypes. It is possible that control B2 isolates are more similar to faecal-only isolates from patients compared to faecal-UTI isolates. This could explain why these isolates do not cause infection in the controls. This would need an investigation of virulence properties and further typing by, for example, whole genome sequencing in order to provide high-resolution typing. We have, however, shown that such faecal clones from controls were able to cause infection in both the bladder and the kidneys in a murine UTI model (Nielsen et al., 2014). Taken together, our results and previous results indicate that it takes more than the presence of a pathogenic *E. coli* phylotype in the faecal flora in order to develop a UTI. This is probably due to a complex interplay between the immune defence mechanisms of the host and the optimal virulence properties of the pathogen in order to cause infection.

We found no correlation between recurrence and faecal clone status or phylotype of the infecting UTI clone, respectively. This indicates that recurrence cannot be predicted from the clonal status in the gut flora.

Concerning risk factors for UTI, previous studies have found that, for example, sexual intercourse, a history of recurrent UTI, diaphragm-spermicide use (Hooton et al., 1996), spermicide-coated condom use (Fihn et al., 1996) and recent antimicrobial consumption (Smith et al., 1997) act as independent host-behavioural risk factors for UTI. Previous antimicrobial consumption was included, as this has previously been correlated with UTI as well as intrauterine device (IUD) use, as we would expect an object in the uterus to affect the microflora, and hence, possibly influence UTI development. IUDs are more often given to women who have given birth, and children are therefore a possible confounder for having an IUD. In the present study, we could confirm that antimicrobial consumption within 6 months was associated with UTI, whereas it is a novel finding that an IUD had a protective effect towards UTI. The participating study groups of UTI patients and controls in this study were very homogeneous, as several underlying factors predisposing to UTI were eliminated due to inclusion and exclusion criteria, for example, diabetes, pregnancy, changes of microflora postmenopause and immunosuppression due to recent surgery in the urogenital area or cancer.

IUDs in this study were primarily hormonal IUDs for both patients and controls. We have no obvious explanation why an IUD should lower the risk of UTI. It could be argued that these participants were not being treated with oral hormonal contraceptives, which have been shown to increase the risk of UTI (Evans et al., 1978), possibly lowering the UTI risk. Hormonal contraceptives were, however, included in this analysis without having a significant effect on the outcome of the analyses. The correlation of IUD use and UTI should be verified in a larger study.

In conclusion, we have found that healthy premenopausal women who have never had a UTI carried pathogenic *E. coli* phylotypes in their faecal flora, and that B2 isolates dominated the flora of control subjects compared to the other phylotypes. This indicates a complex interplay involving host and behavioural risk factors, the immune system and pathogenic bacteria in order for a UTI to develop. Patients carried a significantly higher number of different *E. coli* clones and these were more often multidrug resistant compared to control clones. Faecal-UTI isolates from patients were more antimicrobial resistant compared to faecal-only isolates, indicating a possible link between virulence and antimicrobial resistance. Finally, we found a larger proportion of antimicrobial-resistant isolates in patient faecal flora compared to controls. Receiving antibiotics within 6 months of sampling increased the risk of UTI, while presence of an IUD protected against UTI.

**ACKNOWLEDGEMENTS**

The authors would like to thank the technical staff and doctors at Haslev Lægecenter and Lægehused Ellemarksvej for excellent technical assistance and a fruitful collaboration. This work was a part of Predicting Antibiotic Resistance (PAR), an EU FP7-Health-2009-Single-Stage project (grant 241476). Additionally, the work was supported by The SanCop Foundation, Fonden til Lægevidenskabens Fremme and The Danish Council for Strategic Research (DanCARD project 09-067075/DSF).

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