Role of special pathogenicity versus prevalence theory in pathogenesis of acute cystitis caused by *Escherichia coli*

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*Escherichia coli* is the most common pathogen causing acute cystitis in sexually active women. Human faeces are generally considered the primary reservoir for infection and the faecal–perineal–urethral pathway is the accepted route of infection. Two theories have been proposed for the pathogenesis of acute cystitis: (1) special pathogenicity, where uropathogenic *E. coli* (UPEC) encoding special virulence factors causes infection; and (2) prevalence, wherein ordinary faecal *E. coli* causes infection by simple mass action. The aim of this study was to compare concurrent urinary *E. coli* isolates from women with acute cystitis with their own dominant faecal, vaginal *E. coli* isolates; thus, these patients served as their own control. *E. coli* isolates from 80 women were analysed by phylotyping, virulence profiling (for 15 putative virulence genes) and enterobacterial repetitive intergenic consensus (ERIC) PCR. A virulence score was calculated for each isolate based on the number of virulence genes detected. Four host ecological groups of *E. coli* were created on the basis of ERIC PCR: group UVF, where vaginal and faecal isolates yielded the infecting urine clone; group UV, where only vaginal isolates yielded the infecting urine clone; group UF, where faecal isolates yielded the infecting urine clone; and group U, where the infecting urine clone was distinct. In the majority of cases the infecting *E. coli* clone from urine was also the dominant faecal clone (66.3 %); groups UVF and UF possessing high virulence scores of 4.6 and 3.9, respectively, indicating that both mechanisms play a role in pathogenesis. Non-dominant yet virulent faecal clones or an external source of *E. coli* seems a possibility in the UV group (13.7 %, VF score 4.8). In 30 % of patients (U group) the infecting urine clone was non-dominant and possessed a low virulence score (2.7); suggesting a possible role for host factors in establishing infection.

INTRODUCTION

*Escherichia coli* causes 70–90 % of community acquired uncomplicated acute cystitis (Foxman, 2002). According to the faecal-perineal-urethral hypothesis the causative *E. coli* strain (derived immediately from the host’s own intestinal flora) ascends to the vagina and then to the bladder (Yamamoto *et al.*, 1997); however, there are reports that suggest an external source of *E. coli* can act as the causative agent (Foxman *et al.*, 2002). As for the mechanism of infection there are two conflicting hypotheses: (1) special pathogenicity, which states that some *E. coli* possess an enhanced ability to cause infection by virtue of specific virulence factors [i.e. uropathogenic *E. coli* (UPEC)]; and (2) prevalence, according to this hypothesis mass action or the high prevalence of *E. coli* at the right place and at the right time causes infection (Plos *et al.*, 1995; Turck & Petersdorf, 1962).

A number of putative virulence factors (VFs) have been described for UPEC, and these can work individually as well as synergistically to cause cystitis (Johnson & Stell, 2000; Agarwal *et al.*, 2012). It is generally believed that *E. coli* strains causing cystitis predominantly belong to phylogroups B2 and D whereas commensal strains belong to phylogroups A and B1 (Johnson & Stell, 2000; Tenaillon *et al.*, 2010). The repertoire of VF belonging to UPEC includes toxins, iron acquisition systems, adhesins and a polysaccharide coat to facilitate bacterial survival and growth in the hostile setting of the host urinary tract. These VFs also help UPEC to colonize, invade and injure host tissues, and stimulate an inflammatory response (Agarwal *et al.*, 2012).

We compared a collection of urinary *E. coli* isolates from a cohort of 80 sexually active women suffering from acute cystitis, with their own concurrent dominant vaginal and faecal *E. coli* isolates; the phylogenetic background of the *E. coli* isolates, along with distribution of various VFs was examined. We further typed the *E. coli* isolates by enterobacterial repetitive intergenic consensus (ERIC)...
PCR to compare distinct clones within the subject’s own urinary, vaginal and faecal collections.

METHODS

**Study site and subjects.** A total of 145 sexually active women in the reproductive age group attending the obstetrics and gynaecology outpatient department at a tertiary care hospital in a developing country, between July 2012 and June 2013, with symptoms suggestive of acute cystitis (i.e. two or more of the following symptoms: dysuria, urine frequency >6 times per day, urgency, suprapubic pain, fever, haematuria/smoky urine, burning sensation during micturition and acute onset incontinence), who gave written informed consent and agreed to provide vaginal, faecal swabs along with urine specimens were enrolled in the study. The swabs were collected by the treating physician. To reduce the risk of contamination of the vaginal specimen with urine, the vaginal swab was obtained prior to the urine specimen, and at the same time, the faecal swab was also collected. Patients were instructed to provide a midstream clean-catch urine sample. Patients with bacteriologically documented *E. coli* as the sole uropathogen in quantities of at least 10⁵ c.f.u. ml⁻¹ (Gieson *et al.*, 2010), wherein we were also able to isolate *E. coli* from concurrent vaginal and faecal swabs, were included in the analysis. Patients with any underlying co-morbidity, pregnancy or having a urinary catheter in place were excluded from the study. The study protocol was approved by the Institutional Ethics Committee (reference code XL.ECM/A-F8).

**Sample processing.** Urine was cultured semiquantitatively on cystine lactose electrolyte-deficient agar plates and incubated overnight at 37 °C; the appropriate colony morphology was subsequently identified as *E. coli* (Agarwal *et al.*, 2013). Vaginal and faecal swabs were cultured on MacConkey agar and lactose-fermenting colonies with appropriate colony morphology were identified as *E. coli*; identification was confirmed by standard conventional biochemical tests (Nataro *et al.*, 2011). All *E. coli* isolates were stored at −70 °C in 15% (v/v) glycerol.

**Phylogenetic classification.** The phylotype (A, B1, B2, and D) of each *E. coli* isolate was determined by a triplex PCR, which uses a combination of three DNA markers (*chuA*, *yjaA*, and DNA fragment TspE4.C2) as described previously (Clermont *et al.*, 2000). All PCRs were performed in duplicate with appropriate positive and negative controls; pyelonephritic isolate J96, human faecal isolate J055 and canine urinary tract infection isolate L31 were used as positive controls for phylogroups B2, D and A, respectively.

**Virulence genotyping.** Fifteen virulence associated genes [adhesins: *papA* (P fimbriae structural subunit), *papG* alleles I, II and III (P adhesin variants), *fimH* (type 1 fimbriae), *afa/draBC* (Dr-binding adhesins), and *sfa/focDE* (S and FIC fimbriae); toxins: *hlyA* (haemolysin) and *cstI* (cytotoxic necrotizing factor 1); siderophores: *iutA* (aerobactin) and *fyuA* (yersiniabactin); capsule synthesis: *kpsMII* (specific for group II K1, K5, K12, etc.); serum resistance associated *traT*; *ibeA* (invasion of brain endothelium) and *malX*, a coding region near the terminus of a pathogenicity-associated island] were detected by multiplex PCR with appropriate positive and negative controls as previously described (Johnson & Stell, 2000). *J96* pyelonephritis isolate, 2H25 urosepsis isolate, V27 urosepsis isolate, L31 canine UTI isolate and 2H16 urosepsis isolate were used as positive controls while human faecal isolate J055 was used as negative control. All PCRs were performed in duplicate. The virulence score was calculated for each isolate as the number of virulence genes detected, with *pap* elements (*papA* alleles I; *papG* alleles I, II, and III) considered as a single *pap* VF. Thus, if a strain was positive for at least one of the studied *pap* alleles, it was regarded as *pap* positive. The results of such *in vitro* testing predict experimental virulence *in vivo* (Johnson *et al.*, 2006).

**ERIC PCR fingerprinting.** ERIC PCR fingerprinting was performed for all sets of *E. coli* isolates (3 in each set; recovered from urine, rectal and vaginal swabs). ERIC PCR patterns were compared within the same set (i.e. concurrent strains from the same patient) because of the limited reproducibility of this method. Isolates with indistinguishable fingerprints by visual inspection or with ≥93% similar ERIC profiles were considered to belong to a single clonal group (Manges *et al.*, 2001). Four distinct host ecological groups of *E. coli* were created for analysis on the basis of the relationship amongst each subject’s urinary and its dominant vaginal and faecal clones based on ERIC PCR (Moreno *et al.*, 2008). In group I, both vaginal and faecal cultures yielded the infecting urine clone (designated the urine-vaginal-faecal group; UVF group); in group II, only the vaginal culture yielded the infecting urine clone (designated the urine-vaginal group; UV group); in group III, only the faecal culture yielded the infecting urine clone (designated the urine-faecal group; UF group) and in group IV, the infecting urine clone was distinct and it did not match either the faecal or vaginal *E. coli* clone (designated the urine-only group; U group).

**Statistical methods.** The data were analysed in Microsoft Excel. Virulence scores were compared by ANOVA among *E. coli* isolates according to site of isolation, phylogenetic group and host ecological group; followed by Bonferroni multiple comparison tests. The results were considered to be significant at the level *P* < 0.05. All analysis was carried out using the SPSS 16.0 statistical software package. The results were presented as mean ± s.d for continuous variables and as percentages for categorical variables. The logistic regression analysis was performed; the resulting odds ratios (OR) and 95% confidence interval (CI) were used to determine the risk of various VFs between the groups.

**RESULTS**

**Isolates**

Of the 145 women enrolled in this study, *E. coli* was isolated in 59.3% of cases (*n* = 86) from all three test sites. Only 80 sets (i.e. urinary, faecal and vaginal samples) of *E. coli* could be revived after isolation and maintenance in glycerol stocks; these were further analysed.

**Host ecological grouping**

The 80 viable sets of samples were group assigned by fingerprinting using ERIC PCR (Fig. 1); 34 were assigned to group UVF, 24 were assigned to group U and 11 each were assigned to group UV and group UF.

**Phylogenetic classification**

Phylogroup B2 was predominant at all the three test sites (although it was not a majority, <50%), followed by group A; however, this association was not statistically significant (OR and 95% CI not shown here). A similar distribution was found within host ecological groups i.e. group B2 was predominant followed by group A [UV (72.7%); UF (63.6%); UVF (47.1%)], except for group U (33.3%) isolates, where phylogroup A was more common than B2 (Table 1).
Virulence genotyping

The site of isolation had no significant bearing on the distribution of virulence genes (OR and 95% CI not shown), whereas amongst the host ecological groups, papG allele II \((P=0.0001)\), fimH \((P=0.01)\), iutA \((P=0.01)\), and hlyA \((P=0.05)\) were significantly distributed among the four groups compared (Table 2, Fig. 2).

Virulence score (VF score)

The VF score was significantly higher in phylogenetic group D (mean VF score = 5.29, \(P=0.0001\)) and B2 (mean VF score = 4.35, \(P=0.01\)) as compared to phylogroup A (mean VF score = 3.35, \(P=0.01\)). The virulence score was significantly higher for host ecological group UVF (mean VF score = 4.68; \(P=0.002\)) and UV (mean VF score = 4.82; \(P=0.02\)) compared to group U (Table 3).

DISCUSSION

There is general agreement that \(E.\ coli\) from faecal flora of the host is the most common cause of cystitis in women. There is less agreement on mechanism of action by \(E.\ coli\) underlying the pathogenesis of cystitis in women, i.e. prevalence versus special pathogenicity. Typically, the infecting urinary strain during cystitis is the predominant faecal and vaginal \(E.\ coli\) strain of the host (Yamamoto et al., 1997; Turck & Petersdorf, 1962); thus, highlighting the rationale behind studying concurrent urinary, vaginal and faecal \(E.\ coli\) isolates from women with acute cystitis. Furthermore, by studying the aforementioned concurrent \(E.\ coli\) isolates each woman also serves as her own control. In this study, amongst \(E.\ coli\) isolated from urine we found a lower prevalence of phylogroup B2 (predominant but not a majority) followed by phylogroup A, compared to reports from developed countries. This is in agreement with the findings from a previous study where authors concluded that isolates from western countries cannot be treated as prototypes for Asian cystitis isolates (Agarwal et al., 2013).

Amongst the 80 sets of \(E.\ coli\) analysed, 34 (42.5%) constituted host ecological group UVF indicating that the infecting urinary clone was also the dominant vaginal and faecal clone at the time of infection, the majority (64.7%) of which belonged to phylogroups B2 and D and had a mean VF score of 4.6. In group UF (11 isolates) the sole dominant faecal clone representing the infecting urinary clone had a mean VF score of 3.9, the majority of which belonged to phylogroup B2. Together, in these two groups (majority 56.3%; \(n=45\)), the predominant faecal isolate represented the cystitis-causing clone; most isolates belonged to the virulence-associated B2 phylogroup and had a higher mean VF score. These results suggest a role for both the prevalence and the special pathogenicity hypotheses in causing infection. In 11 women (host ecological group UV) the infecting urinary clone shared similarities with the dominant vaginal clone but was not the dominant clone in faeces, suggesting the possibility of non-dominant faecal clone or some external source of infection (possibly through sexual transmission) (Foxman et al., 2002). This group had the highest virulence score (4.8) and the majority of isolates belonged to phylogroup B2, thus favouring the role of special pathogenicity in causing infection. Alternatively, Moreno et al. (2006) have raised the possibility that the

<table>
<thead>
<tr>
<th>Phylogroup</th>
<th>Place of isolation</th>
<th>Host ecological groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine ((n=80))</td>
<td>Vaginal ((n=80))</td>
</tr>
<tr>
<td>A</td>
<td>22 (27.5)</td>
<td>18 (22.5)</td>
</tr>
<tr>
<td>B1</td>
<td>11 (13.7)</td>
<td>15 (18.7)</td>
</tr>
<tr>
<td>B2</td>
<td>39 (48.8)</td>
<td>39 (48.8)</td>
</tr>
<tr>
<td>D</td>
<td>8 (10)</td>
<td>8 (10)</td>
</tr>
</tbody>
</table>

Note: values shown in parentheses represent percentages.
Table 2. Distribution of virulence genes according to place of isolation and host ecological groups

<table>
<thead>
<tr>
<th>Functional category</th>
<th>Gene name</th>
<th>Place of isolation</th>
<th>Host ecological groups</th>
<th>Place of isolation</th>
<th>Host ecological groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine (n=80)</td>
<td>Vaginal (n=80)</td>
<td>Faecal (n=80)</td>
<td>UVF (n=34)</td>
</tr>
<tr>
<td>PAI</td>
<td>malX</td>
<td>17 (21.2)</td>
<td>21 (26.2)</td>
<td>17 (21.2)</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>Adhesins</td>
<td>papA</td>
<td>17 (21.2)</td>
<td>21 (26.2)</td>
<td>19 (23.8)</td>
<td>10 (29.4)</td>
</tr>
<tr>
<td></td>
<td>papG I</td>
<td>3 (3.8)</td>
<td>0</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>papG II</td>
<td>35 (43.8)</td>
<td>43 (53.8)</td>
<td>43 (53.8)</td>
<td>17‡ (50.0)</td>
</tr>
<tr>
<td></td>
<td>papG III</td>
<td>14 (17.5)</td>
<td>24 (30.0)</td>
<td>23 (28.8)</td>
<td>8 (23.5)</td>
</tr>
<tr>
<td></td>
<td>fimH</td>
<td>66 (82.5)</td>
<td>66 (82.5)</td>
<td>66 (82.5)</td>
<td>33* (97.1)</td>
</tr>
<tr>
<td></td>
<td>sfa/focDE</td>
<td>16 (20.0)</td>
<td>18 (22.5)</td>
<td>15 (18.8)</td>
<td>6 (17.6)</td>
</tr>
<tr>
<td></td>
<td>afa/draBC</td>
<td>22 (27.5)</td>
<td>20 (25.0)</td>
<td>23 (28.8)</td>
<td>10 (29.4)</td>
</tr>
<tr>
<td>Siderophores</td>
<td>intA</td>
<td>36 (45.0)</td>
<td>31 (38.8)</td>
<td>35 (43.8)</td>
<td>18§ (52.9)</td>
</tr>
<tr>
<td>Toxins</td>
<td>hlyA</td>
<td>3 (3.8)</td>
<td>5 (6.2)</td>
<td>2 (2.5)</td>
<td>1† (2.9)</td>
</tr>
<tr>
<td></td>
<td>fnuA</td>
<td>20 (25.0)</td>
<td>26 (32.5)</td>
<td>28 (35.0)</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td></td>
<td>cnfI</td>
<td>11 (13.8)</td>
<td>10 (12.5)</td>
<td>11 (13.8)</td>
<td>5 (14.7)</td>
</tr>
<tr>
<td>Invasins</td>
<td>kpsmII</td>
<td>28 (35.0)</td>
<td>26 (32.5)</td>
<td>24 (30.0)</td>
<td>14 (41.2)</td>
</tr>
<tr>
<td></td>
<td>traT</td>
<td>48 (60.0)</td>
<td>49 (61.2)</td>
<td>43 (53.8)</td>
<td>27 (79.4)</td>
</tr>
<tr>
<td></td>
<td>ibeA</td>
<td>6 (7.5)</td>
<td>7 (8.8)</td>
<td>7 (8.8)</td>
<td>2 (5.9)</td>
</tr>
</tbody>
</table>

*P=0.01, †P=0.05, ‡P=0.0001 (multiple comparison test). PAI, pathogenicity island.
papG gene alleles I, II and III are denoted papG I, papG I and papG III, respectively.

Fig. 2. Multiplex PCR profiles specific for E. coli virulence genes. (a) Pool 1, malX (930 bp), papA (720 bp), fimH (508 bp). (b) Pool 2, fnuA (880 bp), sfa/foc DE (410 bp), papG allele III (258 bp), intA (300 bp). (c) Pool 3, hlyA (1177 bp), papG allele I (461 bp), kpsmII (272 bp). (d) Pool 4 & Pool 5, traT (290 bp), papG allele II (190 bp), afa/draBC (594 bp), cnfI (498 bp); Lane M, 100 bp DNA ladder.

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dominant faecal clone may migrate to the vaginal reservoir at the time of sampling; this would again indicate a role for both the hypotheses (as for the UVF and UF groups).

Host ecological group U had neither a dominant faecal nor vaginal representative clone. This group was dominated by commensals of phylogenetic group A and had a low virulence score, thus supporting neither of the hypotheses for cystitis pathogenesis. This inconsistency may have been due to sampling only a single dominant isolate or perhaps the number of virulence genes analysed should be expanded to more effectively reflect virulence in vivo.

Another possibility could be the role of host susceptibility, which made up for the lack in virulence determinants in the infecting agent (Moreno et al., 2006).

Amongst vaginal isolates, phylogroup B2 was most common, followed by phylogroup A; this is in contrast to a previous report (Obata-Yasuoka et al., 2002). However, studies on genetic analysis of vaginal E. coli are limited. In this study, among the faecal E. coli isolates, again phylogroup B2 was most prevalent, followed by group A. It has been noted in previous studies that a vast variability exists in phylogenetic background of faecal E. coli isolates due to multiple factors such as host hygiene, dietary habits as well as environment (Tenailion et al., 2010; Bailey et al., 2010). The prevalence of the virulence genes traT, papG allele II and iutA was relatively higher in faecal isolates in our study compared to the published literature (Moreno et al., 2008).

The strengths of this study are the comparison between clonal profiles of concurrent urine, vaginal and faecal E. coli isolates from the same host combined with extensive analysis of the virulence genes and phylotyping of these isolates from a large sample number. Limitations of this study include non-sampling of minor vaginal and faecal clones, and absence of sampling from the sexual partner(s). Future studies should increase the number of virulence genes investigated to include more representative virulence determinants thus reflecting the virulence of UPEC in vivo.

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