Population structure and uropathogenic virulence-associated genes of faecal *Escherichia coli* from healthy young and elderly adults

Tara L. Vollmerhausen,¹ Nubia L. Ramos,¹ Aycan Gündoğdu,¹ Wayne Robinson,² Annelie Brauner³ and Mohammad Katouli¹

Correspondence

Mohammad Katouli
mkatouli@usc.edu.au

¹Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore DC, QLD 4558, Australia

²School of Environmental Sciences, Charles Sturt University, Thurgoona, NSW 2641, Australia

³Department of Clinical Microbiology, MTC, Karolinska University Hospital, S-171 76 Stockholm, Sweden

We investigated the population structures of faecal *Escherichia coli* in 30 healthy young adults (13 males and 17 females) aged between 20 and 45 years and 29 elderly adults (14 females and 15 males) aged between 65 and 77 years. In all, 1566 strains were typed with the PhPlate system and grouped into biochemical phenotypes (BPTs). Strains with shared BPTs were further typed using randomly amplified polymorphic DNA analysis. Forty-four per cent of the strains were shared between two or more age and gender groups. Elders had a significantly higher ($P<0.001$) number of BPTs (mean $\pm$ standard error 3.3 ± 0.27) than younger groups (1.82 ± 0.27). Phylogenetic affiliation and virulence-associated genes (VAGs) of the strains showed that more than 80 % of the strains belonging to dominant types belonged to phylogroups B2 and D. Amongst dominant BPTs, phylogenetic group A was significantly associated with females ($P<0.0001$), and elders were more likely to carry group D ($P<0.0124$). Elderly males had a higher prevalence of VAGs than young males ($P<0.0001$) and young females ($P<0.0005$). We conclude that there is a lower prevalence of *E. coli* with uropathogenic properties in healthy young adults than in elders.

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common diseases of young women worldwide, with 60 % of women experiencing at least one episode during their lifetime (Foxman *et al.*, 2000). Young women are more susceptible to UTI than men, with the shortness of the female urethra a predisposing factor to infection (Foxman, 2003). Other factors also increase a woman's likelihood of infection, including sexual activity, oral contraceptives, oestrogen deficiency, diabetes, obstructing lesions and genetic factors such as blood group secretor status (Harrington & Hooton, 2000; Scholes *et al.*, 2000). Among the causative agents of UTI, *Escherichia coli* is responsible for >90 % of cases and is almost always found amongst the faecal flora of the same host, although these bacteria may be harboured in the

vagina or periurethra (Czaja *et al.*, 2009; Moreno *et al.*, 2008).

The intestinal niche can harbour a range of commensal and pathogenic *E. coli* strains and has been elucidated as a reservoir of strains capable of causing extraintestinal infection. It is widely accepted that uropathogenic *E. coli* (UPEC) strains largely belong to phylogenetic group B2, and to a lesser extent group D, and intrinsically harbour a range of virulence genes (Johnson *et al.*, 2005; Moreno *et al.*, 2008; Picard *et al.*, 1999; Sannes *et al.*, 2004). Commensal strains, on the other hand, generally belong to phylogenetic groups A and B1, and are associated less frequently with disease (Bingen *et al.*, 1998; Boyd & Hartl, 1998; Duriez *et al.*, 2001; Johnson & Stell, 2000). All of these groups are commonly isolated from the gut of healthy humans, with groups B2 and D commonly associated with dominant populations (Moreno *et al.*, 2009; Zhang *et al.*, 2002).

UPEC strains have virulence-associated genes (VAGs) that facilitate their adherence to the opening of the urinary tract and allow them to ascend to the bladder to cause UTI. Type 1 fimbriae are important for initiating adhesion, although it is the P-pili that are regarded as an essential

Abbreviations: BPT, biochemical phenotype; C-BPT, common BPT; CT, common type; RAPD, randomly amplified polymorphic DNA; S-BPT, single BPT; UPEC, uropathogenic *E. coli*; UTI, urinary tract infection; VAGs, virulence-associated genes.

A supplementary figure showing RAPD-PCR comparison of the *E. coli* strains is available with the online version of this paper.

virulence factor for adhesion of *E. coli* to the uroepithelium (Abraham *et al.*, 2001; Arthur *et al.*, 1989; Wright *et al.*, 2007). Nonetheless, these VAGs also have an important role in survival of these strains within the gut, enhancing their colonization as dominant strains (Tullus *et al.*, 1992; Wold *et al.*, 1988, 1992). We hypothesized that in addition to the predisposing factors for UTI mentioned above, UPEC strains may be more dominant in the intestinal *E. coli* populations of healthy young and elderly females than in those of males in the same age groups, making females more vulnerable to UTI. Furthermore, the population structure, diversity and prevalence of specific VAGs associated with UPEC among *E. coli* from healthy young and elderly adults is not well understood. The aim of this study was therefore to investigate the prevalence of uropathogenic VAGs among faecal *E. coli* from healthy young and elderly adults and to determine whether differences in *E. coli* population structures in the gastrointestinal tract could indicate predisposition to UTI.

METHODS

Subjects. This study was approved by the University of the Sunshine Coast Human Research Ethics Committee and participation entailed providing informed consent and returning a self-collected faecal swab. Between August and November 2009, a total of 59 healthy community-dwelling individuals were registered for this study. These included 17 healthy young females aged between 21 and 45 years (mean age $\pm$ standard deviation, 33.2 ± 9.7), 13 healthy young males aged between 20 and 45 years (26.5 ± 8.9), 14 healthy elderly females aged between 65 and 77 years (67.9 ± 3.5) and 15 healthy elderly males aged between 65 and 79 years (72.1 ± 5.2). Young subjects were recruited after an invitation email to staff and students at the University of the Sunshine Coast community. Elderly subjects were recruited via the University of the 3rd Age Community Group at University of the Sunshine Coast. Care was taken to recruit subjects that were not from the same household but lived in the Sunshine Coast region of Queensland, Australia. None of the subjects had a symptomatic UTI in the preceding 6 months, or had taken any antibiotics or commercially available probiotics in the 3 months prior to participating in the study. In female groups, an additional criterion was the lack of known pregnancy.

Isolation and confirmation of *E. coli* strains. Each volunteer returned a self-collected faecal swab inserted into Amies transport medium containing charcoal. Swabs were kept at 4 °C and streaked directly onto MacConkey agar no. 3 and membrane faecal coliform agar within 24 h of collection. Plates were incubated for 24 h at 37 °C and 28 *E. coli*-like colonies (where possible) were randomly selected and tested. This number of colonies provide >90% chance of detecting minor clones amongst the host *E. coli* faecal population as previously described (Schlager *et al.*, 2002). Extraction of chromosomal DNA was done by growing a single colony of the isolates in Luria-Bertani broth overnight and collecting pellet in 200 μ l sterile MilliQ water and boiling at 100 °C for 15 min. In all, 1566 putative *E. coli* strains were collected and subjected to confirmatory tests using PCR amplification of the universal stress protein (*uspA*) gene as previously described (Chen & Griffiths, 1998).

Biochemical fingerprinting. All strains were typed using a high-resolution biochemical fingerprinting method (PhPlate system), specifically developed for *E. coli* strains (PhP-RE; Bactus). The fingerprinting method was performed according to the manufacturer's

instructions. Briefly, *E. coli* colonies were suspended in the first well of each row containing 325 μ l growth medium comprised of 0.011% (w/v) bromothymol blue and 0.1% (w/v) proteose peptone. Aliquots of 25 μ l of bacterial suspensions were transferred into each of the other 11 wells containing 150 μ l growth medium. Plates were then incubated at 37 °C and read at intervals of 7, 24 and 48 h. Images of plates at corresponding times were scanned using a HP Scanjet 4890 scanner. After the final scan, the PhPlate software (PhPWin4.2) was used to create absorbance data from the scanned PhP-RE plate images. After the final reading of the plate images, the mean of the absorbance values from all individual readings was calculated for each reagent, creating the biochemical fingerprint for each isolate (Landgren *et al.*, 2005). Similarity among the isolates was calculated as a correlation coefficient, and clustered according to the unweighted pair-group method using arithmetical averages to yield a dendrogram. An identity level of 0.965 was established based on the reproducibility of the system after testing 60 isolates in duplicate. Isolates showing similarity to each other above the identity level were regarded as identical and assigned to the same biochemical phenotype (BPT). BPTs containing more than one isolate were called common (C) BPTs and those with one isolate were named single (S) BPTs. Diversity among the isolates was calculated as a diversity index using Simpson's index of diversity. All data handling, including calculations of correlations and coefficients, diversity indices as well as clustering were performed using the PhPlate software version 4002 (PhPlate).

Randomly amplified polymorphic DNA (RAPD) analysis. Strains that were identical in two or more individuals or groups as determined by the biochemical fingerprinting method were further typed by RAPD-PCR. The RAPD analysis was performed with the KG (5'-ACACGCACACGGAAGAA-3') primer (Ramos *et al.*, 2010). The PCR was carried out in a 50 μ l volume containing 31.3 μ l sterile MilliQ water, 6 μ l 10 $\times$ PCR buffer, 3.8 μ l 50 mM MgCl₂, 1 μ l 10 mM deoxynucleoside triphosphates (dNTPs) (Fisher Biotec), 0.4 μ l *Taq* polymerase (Bioline), 6 μ l primer (20 pmol μ l⁻¹) (Invitrogen) and 1.5 μ l DNA. PCRs were amplified as previously described (Ramos *et al.*, 2010) and products were size separated by electrophoresis in 2.2% agarose stained with ethidium bromide at 90 V for 180 min. Molecular mass markers (100 bp and 1 kb) were used in all of the experiments. Similarity between the banding patterns was compared visually and divided into similarity groups (RAPD types). Strains belonging to the same C-BPT and sharing the same RAPD pattern were regarded as common types (CTs) while those with different RAPD patterns but an identical BPT or identical RAPD pattern but different BPTs were regarded as single types.

Phylogenetic grouping and VAGs. All confirmed *E. coli* strains were tested for their phylogenetic groups (A, B1, B2 and D) using a triplex PCR method for *chuA*, *yjaA* and TSPE4.C2 as previously described (Clermont *et al.*, 2000). The presence of the *ibeA* gene among Clermont phylogenetic group D (i.e. *chuA*- and TSPE4.C2-positive and *yjaA*-negative) was also investigated and those strains harbouring *ibeA* genes were regarded as belonging to phylogenetic group B2 as described before (Gordon *et al.*, 2008).

Strains were also tested for 12 VAGs known to be associated with UTI. These included adhesin genes for P-pili (*papAH*, *papEF*, *papC*, *papG* allele II and *papG* allele III) and type 1 fimbriae (*fimH*); toxin genes α -haemolysin (*hlyA*) and cytotoxic necrotizing factor 1 (*cnf1*); siderophore genes ferric aerobactin receptor (*iutA*) and a catecholate siderophore (*iroN_{E. coli}*); K1 capsule gene (*kpsMT* K1) and group II capsular polysaccharide synthesis gene, e.g. K1, K5 and K12 (*kpsMT* II). Genes were grouped into four multiplex primer sets and two uniplex sets in a modified method to that previously described (Johnson & Stell, 2000), with appropriate positive and negative controls.

The multiplex PCRs consisted of 2.5 μ l 10 $\times$ reaction buffer, 5 μ l 2 mM dNTPs, 2.5 μ l 25 mM MgCl₂, 0.3 μ l of each primer from

50 pmol μl^{-1} stock solution for *papAH*, *papEF*, *fimH*, *iutA*, *papG* allele III, *kpsMT* K1 and *papG* allele II and III (0.15 μl for *kpsMT* II, *papC* and *cnf1*), 0.1 μl HotStar *Taq* polymerase (Qiagen) (0.2 μl for *papG* allele II and III and *cnf1*), 2 μl DNA and sterile MilliQ water to make up 25 μl reaction volume. Amplification used the following cycles: denaturation for 15 min at 95 °C; 25 cycles at 94 °C for 30 s, 63 °C for 30 s, 68 °C for 3 min; and final extension at 72 °C for 10 min.

The uniplex PCRs consisted of 2.5 μl 10 $\times$ reaction buffer (3.0 μl for *iroN_{E. coli}*), 5 μl 2 mM dNTPs, 1.0 μl 50 mM MgCl_2 , 0.3 μl of each primer from 50 pmol μl^{-1} stock solution for *papG* allele II (0.4 μl for *iroN_{E. coli}*), 0.15 μl *Taq* polymerase (BioLine) (0.1 μl for *iroN_{E. coli}*), 2 μl DNA and sterile MilliQ water to make up 25 μl reaction volume (28 μl for *iroN_{E. coli}*). Amplification used the following cycles: denaturation for 4 min at 95 °C; 25 cycles at 94 °C for 30 s, 63 °C for 30 s, 68 °C for 3 min (1 min for *iroN_{E. coli}*); and final extension at 72 °C for 10 min. All PCR products were size separated by electrophoresis in 2% agarose stained with ethidium bromide at 90 V for 90 min.

Definitions. C-BPTs containing more than 80% of the tested isolates in each subject were regarded as dominant BPTs. Some individuals harboured two C-BPTs. To evaluate whether these two C-BPTs were true dominant populations and in order to do a quality control of the ability of the biochemical fingerprinting to distinguish strains from each individual, we confirmed the presence of two populations in these individuals with RAPD-PCR (see Supplementary Fig. S1 available in JMM Online). Under these conditions, both BPTs/RAPD-PCR were regarded as dominant if their population sizes did not differ from each other by more 15% and together their population size exceeded 90% of total isolates tested in each subject. All other BPTs were regarded as minor BPTs.

A VAG score was first established for each BPT. Each VAG was given a score of one (adjusted for multiple detection of *pap* genes, i.e. *papAH*, *papC*, *papEF* and *papG* allele II and III, and capsule genes, i.e. *kpsMT* II and *kpsMT* K1). VAG score for shared BPTs was the VAG score for each BPT multiplied by the number of isolates it represented, divided by the total number of isolates represented in each shared BPT/RAPD type.

Statistical analysis. Factorial analysis of variance was used to compare diversity and abundance of BPTs between age and gender groups and residual analyses were used to investigate the assumptions of normality and homogeneity of variances. A square root transformation was applied to meet the assumption of homogeneity of variances for the number of BPTs. The numbers of VAGs per dominant and minor BPT were compared using the Wilcoxon Rank Sum test with a normal approximation correction for large sample sizes.

A log-linear model was used to compare the distribution of dominant and minor BPTs between the phylogenetic groups and gender and age

categories. When there was a significant interaction of effects, we partitioned the analysis and applied the Benjamini–Hochberg method to moderate the false discovery rate (Waite & Campbell, 2006).

RESULTS

BPT diversity amongst groups

Of the 1566 putative *E. coli* strains, 1541 were confirmed as *E. coli* using the *uspA* gene. Biochemical fingerprinting of these isolates yielded 112 C-BPTs comprising 1502 isolates and 39 S-BPTs. The number of BPTs found in each subject ranged from 1.7 ± 0.9 in young males to 3.8 ± 2.0 in elderly males (Table 1). There was a significant difference between elderly (3.3 ± 0.27 mean $\pm$ standard error) and younger (1.82 ± 0.27) subjects ($F_{\text{agegroup}} = 12.3$, $\text{df} = 1.55$, $P < 0.001$). There were, however, no differences when the numbers of BPTs in the two genders were compared. Similarly, the diversity of *E. coli* strains found among elderly (0.35 ± 0.046) subjects was significantly higher than that found in younger subjects (0.20 ± 0.045) ($F_{\text{agegroup}} = 4.97$, $\text{df} = 1.55$, $P < 0.05$) (Table 1). Again, there was no difference between the diversity of *E. coli* strains among genders.

Phylogenetic groups

A total of 63 dominant BPTs were identified among all 59 subjects (four subjects had two dominant BPTs). Of these, 80% belonged to phylogenetic groups B2 and D (Table 2). The prevalence of phylogenetic group was dependent on age and gender ($\chi^2 = 8.19$, $\text{df} = 3$, $P < 0.0423$). Partitioning the analysis indicated that gender was significantly associated with phylogenetic group A ($\chi^2 = 16$, $\text{df} = 1$, $P < 0.0001$) (Table 2) and that only females carried *E. coli* belonging to this phylogenetic group. Elderly were more likely than young groups to be carriers of *E. coli* belonging to phylogenetic group D ($\chi^2 = 6.25$, $\text{df} = 1$, $P < 0.0124$) (Table 2). There were no differences for phylogenetic groups B1 and B2 between different age and gender groups. Elderly subjects had significantly more minor BPTs than younger subjects ($\chi^2 = 121.0$, $\text{df} = 1$, $P < 0.0001$) and phylogenetic group D had significantly more minor BPTs than

Table 1. Descriptive data of participants and the number of biochemical phenotypes (BPTs) of *E. coli* isolates found in each age and gender group

Parameter	Young females (range)	Elderly females (range)	Young males (range)	Elderly males (range)
No. of participants	17	14	13	15
Mean age	33.2 ± 9.7 (21–45)	67.9 ± 3.5 (65–77)	26.5 ± 8.9 (20–45)	72.1 ± 5.2 (65–79)
No. of isolates tested per person	25.3 ± 5.6 (10–28)	25.7 ± 4.1 (15–28)	27.4 ± 1.9 (21–28)	25.8 ± 5.7 (24–28)
No. of BPTs per person	1.9 ± 1.0 (1–4)	2.8 ± 1.7 (1–7)	1.7 ± 0.9 (1–3)	3.8 ± 2.0 (1–7)
Diversity index	0.22 ± 0.24 (0.0–0.62)	0.26 ± 0.22 (0.00–0.49)	0.18 ± 0.23 (0.00–0.61)	0.43 ± 0.28 (0.00–0.80)
No. of dominant BPTs	19*	14	14*	16*
No. of minor BPTs	14	25	8	41

*Two dominant BPTs were found in two young females, one young male and one elderly male.

Table 2. Distribution of different phylogenetic groups and VAG score for dominant ($n=63$) and minor ($n=88$) *E. coli* types found in 59 healthy adults: 17 young females, 14 elderly females, 13 young males and 15 elderly males

Four participants carried two dominant types.

Phylogenetic group	Number of <i>E. coli</i> types (mean VAG score $\pm$ SD)				
	Young females ($n=33$)	Elderly females ($n=39$)	Young males ($n=22$)	Elderly males ($n=57$)	Total ($n=151$)
Dominant types					
A	6 (1.8 ± 1.5)	2 (2.5 ± 1.7)	0	0	8 (2 ± 1.3)
B1	1 (1.0)	0	3 (1.3 ± 0.6)	0	4 (1.3 ± 0.5)
B2	5 (3.6 ± 1.8)	5 (4.0 ± 1.0)	6 (3.7 ± 1.2)	6 (4.2 ± 2.1)	22 (3.9 ± 1.5)
D	7 (2.3 ± 1.7)	7 (2.0 ± 1.5)	5 (3.2 ± 2.2)	10 (3.0 ± 1.9)	29 (2.6 ± 1.8)
Minor types					
A	4 (2.3 ± 2.5)	2 (2.5 ± 0.7)	1 (1.0)	8 (1.9 ± 1.4)	15 (2.0 ± 1.6)
B1	2 (1.0 ± 1.0)	5 (1.4 ± 0.9)	0	5 (1.2 ± 0.4)	12 (1.3 ± 0.6)
B2	3 (3.3 ± 1.5)	5 (3.2 ± 1.1)	2 (4.5 ± 0.7)	11 (2.8 ± 1.9)	21 (3.1 ± 1.6)
D	5 (1.8 ± 1.1)	13 (2.7 ± 1.2)	5 (1.6 ± 1.3)	17 (1.7 ± 1.4)	40 (2.0 ± 1.3)

the other groups ($\chi^2=118.5$, $df=3$, $P<0.0001$). The tendency for *E. coli* to belong to group D was even more pronounced in elderly subjects ($\chi^2=31.5$, $df=1$, $P<0.0001$).

Distribution of strains among individuals

Biochemical fingerprinting identified 63 dominant BPTs and 88 minor BPTs. These 151 BPTs were further typed using RAPD-PCR to confirm the clonality of the isolates by two typing methods (i.e. BPT/RAPD types). It was found that 50 BPTs, representing 44 % of isolates ($n=674$) that were shared between two or more age and gender groups, belonged to 18 BPT/RAPD types (Fig. 1). These BPT/RAPD types were designated CTs. Of these, CT5 and CT14 constituted more than 24 % of the shared isolates and all belonged to phylogenetic groups B2 and D. These CTs contained strains with the highest VAG scores (Fig. 1).

Virulence traits

Comparison of dominant and minor BPTs found that dominant BPTs (2.48 ± 0.185) had significantly ($z=2.25$, $n=63$, 88 , $P=0.0244$) higher VAG scores than minor ones (1.98 ± 0.137). There was a significant difference in prevalence between the age groups ($\chi^2=22.13$, $df=1$, $P<0.0001$) and some VAGs occurred more frequently than others ($\chi^2=250.23$, $df=12$, $P<0.0001$) (Table 3). There was a slight, not significant, interaction between age and gender of subjects ($\chi^2=2.97$, $df=1$, $P<0.10$) (Table 3). A follow-up analysis found that the prevalence of VAGs was significantly higher in elderly males than in either young males ($P<0.0001$) or young females ($P<0.0005$) and higher in elderly females than in young males ($P<0.05$).

DISCUSSION

In this study, we used a high-resolution biochemical fingerprinting method specifically developed for typing of

E. coli strains. The system has been shown to be as powerful as other molecular typing methods such as RAPD-PCR (Ramos *et al.*, 2010) and ERIC-PCR (Ansaruzzaman *et al.*, 2000) for typing of *E. coli* strains. Up to 28 colonies from each participant were tested, providing a greater than 90 % chance of detecting minor clones amongst the host *E. coli* faecal population (Schlager *et al.*, 2002). Based on this number of isolates, we found that elderly males had a greater diversity of *E. coli* strains than both young males and females. Increased diversity amongst dominant bacterial species has been observed in healthy elderly subjects (Saunier & Doré, 2002). One possible reason could be changes in the mucosal surfaces in elderly subjects as also suggested by others (Ouwehand *et al.*, 1999). It has also been postulated that mucus in elderly subjects differs from that in young adults, containing more carbohydrates and less protein (Ouwehand *et al.*, 1999). Furthermore, decreased mucus production amongst elderly subjects may expose more receptors on the gut epithelium as postulated before (Ouwehand *et al.*, 1999).

All subject groups, except young males, carried populations of *E. coli* belonging to phylogenetic group D as the major gut population. Phylogenetic group B2 was the second dominant group for elders. Young females carried strains belonging to phylogenetic group D as their largest population size, followed closely by group A, as found previously (Moreno *et al.*, 2009). Amongst dominant types belonging to phylogenetic group A, young females had the largest population; these intrinsically carry fewer extra-intestinal virulence factors than those of groups B2 and D (Clermont *et al.*, 2000; Johnson *et al.*, 2001). Similarly, young males and, to a lesser extent, young females also carried strains belonging to group B1, which is also less associated with UTI. These results suggest that faecal dominance of an *E. coli* strain in healthy young females is not a predictor of UTI. Similar to this finding, Schlager *et al.* (2003) identified that amongst girls aged 3–6 years,

Fig. 1. An unweighted pair-group method using arithmetical averages dendrogram showing comparison of 50 *E. coli* types shared between two or more individual volunteers of different age and gender groups as determined by biochemical fingerprinting and RAPD analysis. The 18 common types (CTs) are shown as CT1–CT18. The RAPD pattern of each CT is also shown. Strains that were highly similar but showed separate RAPD patterns were designated subtypes using letters. Percentage of the shared population and VAG scores are also shown. PGG, Phylogenetic groups; A/G, age and gender group (YF, young female; YM, young male; EF, elderly female; EM, elderly male).

Table 3. Prevalence of VAGs among 151 biochemical phenotypes (BPTs; 63 dominant and 88 minor) found in all four age and gender groups studied

These include 33 BPTs found in young females, 39 BPTs found in elderly females, 22 BPTs found in young males and 57 BPTs found in elderly males.

VAG tested	No. of dominant BPTs (%)	No. of minor BPTs (%)	No. of young female BPTs (%)	No. of elderly female BPTs (%)	No. of young male BPTs (%)	No. of elderly male BPTs (%)
Adhesins						
<i>papAH</i>	14 (22)	7 (8)	6 (18)	4 (10)	5 (23)	6 (11)
<i>papEF</i>	21 (33)	19 (22)	7 (21)	11 (28)	6 (27)	16 (28)
<i>papC</i>	20 (32)	13 (15)	7 (21)	8 (21)	6 (27)	12 (21)
<i>papG</i> allele II	11 (18)	7 (8)	1 (3)	7 (18)	3 (14)	7 (12)
<i>papG</i> allele III	7 (11)	4 (5)	2 (6)	2 (5)	2 (9)	5 (9)
<i>papG</i> allele II and III	16 (25)	10 (11)	5 (15)	6 (15)	4 (18)	11 (19)
<i>fimH</i>	59 (94)	85 (97)	32 (97)	38 (97)	20 (91)	54 (95)
Toxins						
<i>hlyA</i>	9 (14)	5 (6)	3 (9)	3 (8)	1 (5)	7 (12)
<i>cnf1</i>	11 (18)	7 (8)	3 (9)	4 (10)	4 (18)	7 (12)
Siderophores						
<i>iutA</i>	18 (29)	13 (15)	6 (18)	10 (26)	6 (27)	9 (16)
<i>iroN</i> _{<i>E. coli</i>}	25 (40)	18 (21)	9 (9)	8 (21)	11 (50)	15 (26)
Protectins						
<i>kpsMT</i> K1	20 (32)	22 (25)	10 (30)	19 (49)	5 (23)	8 (14)
<i>kpsMT</i> II	35 (56)	45 (51)	13 (39)	28 (72)	12 (55)	27 (47)

dominant faecal clones with P-pili were less likely to be found in the urinary tract than minor clones. Interestingly, young females in our study carried lower numbers of *E. coli* with uropathogenic properties and lower VAG scores, although this was not a significant result due to the low sample size. This may indicate that other factors, such as shortness of urethra, sexual activity or oral contraceptives, are more important than the structure of dominant *E. coli* populations in young females for their susceptibility to UTI. Capsule antigen genes were the only genes found more frequently in females, although this was not a significant difference in this study. A larger sample size is needed to determine whether females carry more capsular antigen K1 strains than males. This was an interesting observation as the K1 antigen gene is a key VAG in extraintestinal pathogenic *E. coli* associated with neonatal meningitis (Öhman *et al.*, 1995) and perhaps a higher number of faecal K1 strains in this group is associated with contamination and infection of neonates after birth.

Comparison of the *E. coli* types among the four groups showed that a high proportion, 44 % of the *E. coli* tested in this study, were common among the groups with two major groups accounting for more than 24 % of the shared populations. The fact that these CTs belonged to pathogenic groups B2 and D and had the highest VAG scores suggests that certain *E. coli* clones of the dominant flora of the gut of healthy individuals are better equipped to cause UTI. Alternatively, these strains may be better adapted for the intestinal niche (Diard *et al.*, 2010). Contrary to our expectation, we found that clones with increased virulence factors were more prevalent among

elderly males than among young females. It has to be noted that in our study we tested *E. coli* from a small number of individuals (between 13 and 17 individuals in each group) and therefore confirmation of such a finding requires testing more individuals.

In this study, elderly males had a significantly higher prevalence of the tested VAGs. Contrary to our initial hypothesis that young females might have highly virulent dominant *E. coli* as a predisposition to UTI, the elderly groups, especially males, showed a significantly higher prevalence of VAGs. This finding not only indicates that dominant *E. coli* populations in healthy young females may not be the main cause of UTI in this group, but helps to explain the reported higher prevalence of extraintestinal infections such as UTI and septicemia in hospitalized elderly patients, especially males, as more virulent strains were carried by these groups (Kaye, 1980).

Our results showed that dominant *E. coli* strains have more VAGs than minor strains as previously reported (Moreno *et al.*, 2009). Apart from their role in adhering to the urinary tract cells, P-pili have also been shown to bind to the intestinal tract, which enhances their colonization of the gut (Tullus *et al.*, 1992; Wold *et al.*, 1988, 1992). Pathogenicity-associated islands, which carry many extra-intestinal virulence factors, have been shown to enhance fitness of these strains to survive in the intestinal tract, with the ability to cause extraintestinal infections being a coincidental by-product (Diard *et al.*, 2010). Our unpublished data also indicate that *E. coli* strains carrying these adhesins bind with almost the same rate to Caco-2 cells (an intestinal epithelial cell line) as they do to the renal cell line

A-489 (M. Katouli). These findings explain why dominant BPTs in our study carried more *pap* genes than minor ones.

Zhang *et al.* (2002) found that *E. coli* strains carrying P-pili genes in young women (aged 18–39 years) were strongly associated with phylogenetic groups B2 and D. However, group B2 had two distinct subgroups with differing levels of pathogenicity. These researchers suggested that healthy adults are capable of carrying B2 strains but with less virulent subclasses than UTI isolates (Zhang *et al.*, 2002). In our study, B2 was the second major phylogenetic group after group D (except in young adults), suggesting that the B2 strains may be a subset of pathogenic strains that only increase in number and become dominant when there is a major upset of the colonic *E. coli* leading to UTI. Furthermore, these results indicate that under healthy conditions, and with regard to the *E. coli* population structure of the gastrointestinal tract, elderly females may be at a greater risk than younger females for UTI from their faecal intestinal flora. Despite this, other factors such as sexual activity increase the likelihood of UTI amongst young women (Hooton *et al.*, 1996). Additionally, healthy males may serve as a reservoir of uropathogens transmitted directly via sexual transmission (Foxman *et al.*, 1997). The low prevalence of uropathogenic virulence factors amongst young women in our study is somewhat contradictory to previous reports of females with UTI which have found that pathogenic urinary strains are often associated with dominant faecal strains (Moreno *et al.*, 2008). These results suggest that a dramatic change may occur in the intestinal *E. coli* populations of young females before developing UTI. If this hypothesis is correct, then we postulate that a subset of the intestinal *E. coli* population, probably a minor clone, in young females may find an opportunity to increase in population size, colonize the opening of the urinary tract and become a short-term dominant clone during the course of UTI.

In conclusion, our findings indicate that the *E. coli* population of healthy young adults has a lower prevalence of VAGs than that of elderly males, and to a lesser extent, elderly females. Despite this, uropathogenic strains are commonly reported as the dominant clones amongst the faecal population in young women with UTI. We postulate that in young females a subset of *E. coli* populations may increase in size to become a short-term dominant flora during the course of UTI. We also conclude that the presence and dominance of VAGs in *E. coli* strains in elderly males and females may be a risk factor for developing extraintestinal infections in these groups.

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