Commentary

Genomes of *Escherichia coli* bacteraemia isolates originating from urinary tract foci contain more virulence-associated genes than those from non-urinary foci and neutropaenic hosts

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**S U M M A R Y**

Objectives: *Escherichia coli* is the leading cause of bacteraemia. In an era of emerging multi-drug-resistant strains, development of effective preventative strategies will be informed by knowledge of strain diversity associated with specific infective syndromes/patient groups. We hypothesised that the number of virulence factor (VF) genes amongst bacteraemia isolates from neutropaenic patients would be lower than isolates from immunocompetent patients.

Methods: Immunocompetent and neutropaenic adults with *E. coli* bacteraemia were recruited prospectively and the source of bacteraemia determined. VF gene profiles were established in *silico* following whole genome sequencing.

Results: Isolates from individual patients were monoclonal. Strains from immunocompetent patients with urinary tract infective foci (UTIF) harboured more VF genes (median number of VF genes 16, range 8–24) than isolates from both immunocompetent patients with non-UTIF (10, 2–22, \(p = 0.0058\)) and neutropaenic patients with unknown focus of infection (NPUFI) (8, 3–13, \(p < 0.0001\)). Number of VF genes (OR 1.21, 95% CI 1.01–1.46, \(p = 0.039\)) and urinary catheter/recurrent urinary tract infection (OR 12.82, 95% CI 1.24–132.65, \(p = 0.032\)) were independent predictors of bacteraemia secondary to UTIF vs. non-UTIF in immunocompetent patients. *papA, papC, papE/F, papG, agn43, tia, iut, fyuA, kpsM* and *sat* were significantly more prevalent amongst UTIF- vs non-UTIF-originating isolates amongst immunocompetent patients, while *papC, papE/F, papG, agn43, tia, fyuA, hlyA, usp* and *clf* were significantly more prevalent amongst UTIF- vs NPUFI-associated isolates.

Conclusions: Bacteraemia-associated *E. coli* strains originating from UTIF have distinct VF gene profiles from strains associated with non-UTIF- and NPUFI. This diversity must be addressed in the design of future vaccines to ensure adequate coverage of strains responsible for site-specific disease.

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**Introduction**

Extra-intestinal pathogenic *E. coli* (ExPEC) are the leading cause of bacteraemia world-wide and are associated with urinary tract, hepatobiliary/gastro-intestinal tract, skin/soft tissue and respiratory tract infections, as well as neonatal meningoencephalitis and febrile neutropaenia. The scale of the ExPEC problem is large, particularly in the context of increasing antimicrobial resistance and the current dominance of multi-drug-resistant (MDR) sequence types (STs), e.g. ST 131.\(^1\)

ExPEC possess multiple virulence factor (VF) genes encoding adhesins, iron-acquisition systems, protectins/invasin, and toxins, and are gut colonisers in >10% of individuals.\(^4\) ExPEC have previously been defined as those that contain at least two of the following VF-encoding genes: *papA* and/or *papC* (P fimbiae), *sfa/foc* (S fimbiae), *afa/draBC* (Dr binding adhesins), *kpsM II* (group 2 capsule) and *iutA* (aerobactin receptor).\(^5\) ExPEC VFs (herein referred to as VFs) have been associated with site-specific disease, e.g. pyelonephritis (*pap, afa/draBC* and *sfa* adhesin genes, *iha* adhesin siderophore gene, and the *ibeA* protectin invasin gene)\(^6\).
and neonatal meningitis (kps capsule gene, ompA and the protective/invasin genes, fimH1 adhesive gene, and cnf1 toxin gene).\(^1,7\)

A broad range of STs can cause disease but 50–70% of disease-associated isolates belong to STs 69, 73, 95, 127 and 131.\(^8\)

In severely immunocompromised patients, e.g. those with haematological malignancy and neutropaenia, *E. coli* bacteraemia often occurs in the absence of any clinically-identifiable focus as a consequence of direct translocation from the gut.\(^3\) This process likely occurs secondary to damage to the structural integrity of the intestinal mucosa, as a result of compromised mucosal/systemic immunity, or due to bacterial overgrowth.\(^10\) The contribution of VFs in this context is undefined.

We hypothesised that, in severe immunocompromise, *E. coli* strains with fewer VFs would be able to translocate across the bowel and survive haematogenously compared with bacteraemia strains from immunocompetent patients. Additionally, we posited that *E. coli* bacteraemia was more likely to be polycylnal in patients with severe immunocompromise given that humans often carry multiple *E. coli* strains simultaneously.\(^1\)

We assembled a prospective cohort of immunocompetent and neutropaenic patients with *E. coli* bacteraemia. Whole genome sequencing (WGS) was performed on isolates and VF gene profiles, ST distribution, and isolate antibiogram data compared between patient groups.

**Methods**

**Patients and study design**

Adults admitted to University Hospital Southampton (UHS), UK, with *E. coli* bacteraemia were recruited prospectively within 2 weeks of the positive blood culture (BC) and allocated into two groups: (1) immunocompetent patients and; (2) neutropaenic patients (neutrophil count \(<1.0 \times 10^9/L\) within 24 h of BC sampling). Haematological malignancy, metastatic solid organ tumour/other immunocompromising conditions (e.g. inherent immunodeficiency syndromes or infection with human immunodeficiency virus), and immunosuppressant medications (oral/intra-venous steroids, disease modifying anti-rheumatic drugs, immunological therapies or chemotherapy) were exclusion criteria for admission to group 1. Patients who were discharged or deceased prior to screening were excluded. Charlson Comorbidity Index\(^15\) and severity of sepsis (severe inflammatory response syndrome scoring system)\(^15\) were calculated on admission. Presence of a urinary catheter and history of recurrent urinary tract infection (UTI) (defined as \(\geq 2\) episodes of UTI in last 6 months or \(\geq 3\) episodes of UTI in last 12 months),\(^13\) as well as date of discharge and in hospital death were recorded.

**Infection focus definitions**

Infective foci were determined by the study physician following direct clinical consultation/review of laboratory and radiological data. Urinary tract infective foci (UTIF) were defined microbiologically (localised symptoms/signs with urinary *E. coli* culture – same antibiogram as bacteraemia isolate), radiologically (localised symptoms/signs with radiological findings suggesting UTIF), or clinically (localised symptoms/signs, microbiological/radiological investigations not performed or culture negative despite presence of urinary pyuria). In the neutropaenic group, ‘unknown infective foci’ was assigned when no clinical/radiological/microbiological evidence identified a focus. When performed, urine culture was *E. coli* culture negative in these patients.

**Bacterial strains and antimicrobial susceptibility testing**

BCs were incubated (BacTAlert\(^6\) 3D microbial detection system, Biomérieux) and *E. coli* colonies identified by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (Microflex, Bruker) following growth on cysteine lactose electrolyte deficient (CLED) agar (Oxoid). Antimicrobial susceptibilities were determined using Metascan Elite (MAST) with British Society for Antimicrobial Chemotherapy (BSAC) breakpoints.\(^14\) Isolates resistant to amoxicillin/piperacillin plus cefotaxime were screened for extended-spectrum beta-lactamase (ESBL) production utilising antimicrobial/inhibitor discs (Rosco).

Antimicrobial resistance scores comprised the number of antimicrobial agents to which the isolate was resistant. MDR was defined in line with international guidelines (non-susceptible to \(\geq 1\) agent in \(\geq 3\) antimicrobial categories).\(^15\)

Urine microscopy (Sedimax platform, Menarini Diagnostics), culture and sensitivity testing (Metascan Elite) was performed. A urinary WCC > 10/μl was considered elevated. Urinary isolates were confirmed as *E. coli* using MALDI-TOF mass spectrometry.

Bacteraemia and, where available, linked urinary isolates were sequenced.

**Determination of *E. coli* bacteraemia clonality**

Random amplified polymorphic DNA (RAPD) fingerprinting was performed on isolates using a previously validated method.\(^16\) BC broths were sub-cultured onto CLED agar and incubated (5% CO2, 37 °C, 24 h). Following confirmation of *E. coli* growth, between 8 and 9 colonies per patient were randomly selected for RAPD. Two polymerase chain reactions (PCRs) were performed per colony (primers 1247\(^17\) [AAGACCGGCT] and 1283\(^18\) [GCGATCCCCA]). Each 20 μl PCR reaction contained 1 μl of primer (final concentration 2 μM), 10 μl MyTaq Red Mix (BioLine) master mix, 6.5 μl PCR-grade water (Thermofisher) and 2.5 μl of DNA template (prepared by placing a 1 μl loop of colony into 50 μl of PCR-grade water and heating at 90 °C, 10 min). Cycling conditions for primers 1247 and 1283 were as follows: 95 °C for 10 min; 35 cycles of: 94 °C for 30 s, 38/36 °C for 30 s and 72 °C for 2 min; followed by 72 °C for 10 min (final elongation step). Amplification products were run on 0.7% agarose gels containing midori green (Geneflow) (90 V for 90 min) prior to image capture of PCR amplification products using a UV transilluminator linked to a digital camera.

**WGS and analyses**

*E. coli* genomes were sequenced by Public Health England (PHE), Colindale (UK), using the Nextera sample preparation method with the standard 2× base sequencing protocol on a HiSeq instrument (Illumina, San Diego, CA, USA), as described previously.\(^19\) This resulted in 2× paired-ended 100 bp length sequencing reads. SRST2 was used with standard parameters\(^20\) in conjunction with the VF (DoA: 05/08/2017)\(^21\) and *Escherichia coli* #1 multi-locus sequence typing (MLST)\(^22\) databases to determine VF gene profiles and STs, respectively. VF genes (31 in total) were included in the analysis if they were listed in the VF database\(^21\) and previously outlined as ExPEC-associated VFs in the literature.\(^1,2\) Genomes were assembled and error-corrected using the AS pipeline V20160825.\(^24\) Assembly metrics were generated using QUAST V4.6.3.\(^25\) Genome assemblies were annotated using Prokka V1.12.26 using the – use_genus and a list of proteins derived from sequenced reference urinary pathogenic *E. coli* (UPEC) isolates with the – proteins flag. GFF annotations were used in conjunction with Prank\(^27\) as part of the Roary pipeline V3.8.0\(^28\) to generate core genome alignment. This utilised 1451 core genes out of a total 20,461 genes. The alignment was used in conjunction with FastTree V2.\(^29\) and recomopiled with duse_double to generate a maximum likelihood tree in .newick format using the gtr nt model. Phylogenetic tree visualisation and node editing was performed using FigTree V1.4.2.\(^30\) Paired sequencing reads utilised in the meth-
For patients on admission non-UTIF Table parameters, unknown respectively), were available (Student's t test and Mann Whitney tests were used to compare parametrically and non-parametrically-distributed continuous data, respectively. Comparison of proportions across two groups was performed using Fisher's exact test. Chi squared ($\chi^2$) test for trend was used to compare proportions across three groups. In these analyses, no corrections were made for multiple comparisons.

Binomial logistic regression analysis was utilised to determine independent risk factors associated with UTIF vs. non-UTIF bacteraemia in immunocompetent subgroup analysis. Statistical analyses were performed in GraphPad Prism (version 7.0a) and SPSS (version 25.0).

**Results**

**Study population and E. coli isolates**

147 consecutive patients with E. coli bacteraemia were screened between August 2015 and April 2016. 50 immunocompetent patients were enrolled representing 51 bacteraemia episodes (one patient had 2 bacteraemia episodes of different ST, separated by 46 days. Both isolates were included in inter-group VF gene comparison). 10 neutropenic patients were enrolled representing 10 bacteraemia episodes (for causes of neutropenia see Supplementary Table 1). Following withdrawals (Fig. 1), data from 49 immunocompetent (50 isolates) and 8 neutropenic (8 isolates) patients were available for inter-group VF gene analysis.

Foci of E. coli bacteraemia included UTIF ($n=23$; 70%, 17% and 13% proven microbiologically, radiologically and clinically, respectively), non-UTIF ($n=26$) and neutropenic patients with unknown focus of infection (NPUI) ($n=8$). Analysis of WGS data demonstrated that 15/16 linked urinary isolates from patients with microbiologically-proven UTI shared the same ST as the bacteraemia strain. Baseline characteristics, admission sepsis severity parameters, and mortality/length of stay data, are outlined in Table 1. Significantly more immunocompetent patients with UTIF vs. non-UTIF had a history of recurrent UTI, while patients with non-UTIF vs. UTIF were more likely to have severe sepsis on admission (because of hyperbilirubininaemia and coagulopathy in patients with cholangitis/cholecystitis). WCC and platelet counts were significantly lower in neutropenic patients as expected.

**E. coli bacteraemia is monoclonal in neutropaenic and non-neutropaenic patients**

RAPD analysis was performed on 8–9 E. coli colonies (growing on CLED agar) for 14/23, 20/26 and 8/8 bacteraemia isolates from patients with UTIF, non-UTIF, and NPUI, respectively (representative example for isolate 43 demonstrated in Supplementary Fig. 1). For all patients, intra-patient E. coli colonies differed by $\leq1$ band across the 2 RAPD primers utilised, consistent with a low probability of genomic differences (when compared to WGS) as previously described. The possibility of polyclonal E. coli bacteraemia was thus excluded prior to selection of a single colony per patient for WGS.

**Bacteraemia isolates originating from the urinary tract have distinct VF gene profiles compared with isolates from non-urinary foci**

Univariate analysis demonstrated that the median number of VF genes was significantly higher amongst isolates from immunocompetent patients ($n=50$, all infective foci) compared to NPUI ($n=8$) (median number of VF genes 15.5, range 2–24, and 8, 3–13, respectively, $p=0.0076$). Within the immunocompetent group, the median number of VF genes was significantly higher amongst isolates derived from UTIF ($n=23$) vs. non-UTIF ($n=27$) (16, range 8–24, and 10, 2–22, respectively, $p=0.0058$). Isolates originating from NPUI had a significantly lower median number of VF genes (8, 3–13) compared with isolates from immunocompetent patients originating from UTIF (16, 8–24, $p<0.0001$ (Fig. 2).

Binomial logistic regression analysis demonstrated that number of VF genes (OR 1.21, 95% CIs 1.01–1.46, $p=0.039$) and recurrent UTI history/presence of urinary catheter (OR 12.82, 95% CIs 1.24–132.65, $p=0.032$) were independent predictors of bacteraemia originating from UTIF in a model inclusive of number of VF genes present within the E. coli isolate, and host variables associated with susceptibility to bacteraemia and UTI including gender, age (years), Charlson Comorbidity Index, history of recent antimicrobials (28 days prior to bacteraemia), and recurrent UTI history/presence of a urinary catheter (Table 2) [31–33]. For every unit increase in the VF gene number, the odds of a bacteraemia isolate being derived from a urinary focus increased by 1.21 times.

Univariate analysis demonstrated that the prevalence of papA, papC, papE/F, papG, agm43, tia, iut, fyuA, kpsM and sat genes was significantly higher amongst isolates originating from UTIF vs. non-UTIF, while prevalence of papC, papE/F, papG, agm43, tia, fyuA, hlyA, usp and clb was significantly higher amongst isolates originating from UTIF vs. NPUI (Table 3). Full VF gene profiles for each isolate are outlined in Supplementary Table 2.

Strains belonging to MLST STs 12 and 69 were more frequent in immunocompetent bacteraemia originating from UTIF vs. non-UTIF (17.4% vs 0%, $p=0.04$, and 21.7% vs. 0%, $p=0.02$, respectively, See Table 4). Antimicrobial resistance scores and the proportion of MDR isolates were not significantly different between isolates from UTIF and non-UTIF in immunocompetent patients. Ciprofloxacin resistance was significantly more prevalent in NPUI vs. isolates from immunocompetent patients with UTIF (75% vs 21.7%, $p=0.012$) (Table 4), reflecting the use of ciprofloxacin prophylaxis in patients with haematological malignancy.

**Bacteraemia isolates originating from NPUI are similar to those originating from non-urinary foci in immunocompetent patients**

Bacteraemia isolates from immunocompetent patients originating from non-UTIF had similar numbers of VF genes to those from NPUI (median number of VF genes 10, range 2–22, and 8, 3–13, respectively, $p=0.28$). In addition, no significant differences in the prevalence of individual VF genes (Table 3), groups of VF genes, or in distribution of common STs were observed between these groups (Table 4). The proportion of non-UTIF- and NPUI-derived bacteraemia isolates meeting the previously-defined ExPEC definition [5] was 56% and 38%, respectively ($p=0.44$) demonstrating that strains that did not meet the ExPEC definition were responsible for a large proportion of disease amongst these patient groups. By comparison, 100% of bacteraemia isolates derived from UTIF...
met the ExPEC definition \( n = 23/23 \) UTI vs \( n = 15/27 \) non-UTI, \( p < 0.001; n = 23/23 \) UTI vs \( n = 3/8 \) NPUIF, \( p < 0.001 \) (Table 4).

Total antimicrobial resistance scores and the proportion of MDR isolates were not significantly higher in NPUIF-associated isolates compared with non-UTI isolates from immunocompetent patients. Ciprofloxacin resistance was significantly more frequent amongst NPUIF-associated isolates compared with non-UTI isolates from immunocompetent patients (75% vs. 18.5%, \( p = 0.006 \)) (Table 4).

**Table 2**

| Variable | \( B \) | S.E. | Odds ratio | Wald p value | 95% CIs |
|----------|--------|------|------------|--------------|--------|
| Number of virulence factors | 0.193 | 0.093 | 1.21 | 0.039 | 1.01–1.46 |
| Age (years) | -0.014 | 0.042 | 0.99 | 0.735 | 0.91–1.07 |
| Charlson Comorbidity Index | 0.466 | 0.357 | 1.49 | 0.281 | 0.79–3.21 |
| Antimicrobials in last 28 days | 1.622 | 0.879 | 5.06 | 0.035 | 1.01–1.46 |
| Recurrent UTI history or urinary catheter | 2.551 | 1.192 | 2.30 | 0.220 | 1.24–4.62 |
| Female gender | 0.797 | 0.773 | 1.01 | 0.303 | 0.49–10.10 |
| Constant | -4.722 | 2.881 | 0.01 | 0.101 | 0.01 |

\[ x^2 = 22.32, df = 6, p = 0.001. \] Nagelkerke R2 49.7%. Hosmer and Lemeshow test p = 0.336 Classification accuracy 78%.

**Table 1**

| Characteristic | Immunocompetent | P value |
|---------------|-----------------|---------|
| 1. All (n = 49) | 70.1 (19.6–96.4) | 0.54 (a) 0.93 (a) |
| 2. Systolic | 23.1 (18.5–28.7) | 0.30 (a) 0.58 (a) |
| 3. Diuretic | 10.7 (7.5–14.3) | 0.33 (a) 0.58 (a) |
| 4. Neutropenic | 6.2 (3.8–9.1) | 0.33 (a) 0.58 (a) |

**Table 2**

| Method | DF | p value | F-value | R2 |
|--------|----|---------|---------|----|
| Chi-square | 4 | 0.001 | 0.49 | 0.78 |
| Logistic regression | 4 | 0.001 | 0.49 | 0.78 |

**Table 3**

| Variable | DF | p value | F-value | R2 |
|----------|----|---------|---------|----|
| Chi-square | 4 | 0.001 | 0.49 | 0.78 |
| Logistic regression | 4 | 0.001 | 0.49 | 0.78 |

**Table 4**

| ST | Frequency | Isolated From | Resistance Profile |
|----|-----------|---------------|--------------------|
| 1 | 10 | UTI | MDR (Extended Spectrum Beta-Lactamase, AmpC and Aminoglycoside Resistant) |
| 2 | 5 | NPUIF | MDR (Extended Spectrum Beta-Lactamase, AmpC and Aminoglycoside Resistant) |
| 3 | 2 | UTI | MDR (Extended Spectrum Beta-Lactamase, AmpC and Aminoglycoside Resistant) |
| 4 | 1 | NPUIF | MDR (Extended Spectrum Beta-Lactamase, AmpC and Aminoglycoside Resistant) |
| 5 | 1 | UTI | MDR (Extended Spectrum Beta-Lactamase, AmpC and Aminoglycoside Resistant) |

**Table 5**

| Variable | DF | p value | F-value | R2 |
|----------|----|---------|---------|----|
| Chi-square | 4 | 0.001 | 0.49 | 0.78 |
| Logistic regression | 4 | 0.001 | 0.49 | 0.78 |

**Table 6**

| Variable | DF | p value | F-value | R2 |
|----------|----|---------|---------|----|
| Chi-square | 4 | 0.001 | 0.49 | 0.78 |
| Logistic regression | 4 | 0.001 | 0.49 | 0.78 |
Assessed for eligibility (n=147)

Not neutropenic (neutrophil count >2.0x10^9/L) (n=137)

Excluded (n=87)
- Lacked capacity (33)
- Hospital discharge prior to eligibility assessment (13)
- Metastatic cancer/haematological malignancy (9)
- Deceased prior to screening (8)
- Liver cirrhosis (7)
- Non consent (7)
- Active chemotherapy (5)
- Oral/intravenous steroids (3)
- Disease-modifying anti-rheumatic drugs (2)

Enrolled (n=50)
- Included in comparative E. coli virulence factor profile analysis (n=49)
- Excluded from comparative E. coli virulence factor profile analysis (n=1).

Neutropaenic (neutrophil count <1.0x10^9/L) (n=10)

Excluded (n=10)
- Included in comparative E. coli virulence factor profile analysis (n=8)
- Excluded from comparative E. coli virulence factor profile analysis (n=2). Excluded as clinically-evident source of E. coli infection subsequently demonstrated. 1x urinary tract infection; 1x pleural empyema.

Enrolled (n=10)

Fig. 1. E. coli bacteraemia screening and recruitment chart.

Total median number of VF genes of isolates within STs 12, 73, 127, 131 and 69 were: 20 (range 19–23), 20.5 (19–23), 17 (16–20), 15 (10–18) and 13 (7–14), respectively. Significant differences in numbers of VF genes (Supplementary Fig. 2) and subgroups of VF genes (particularly pronounced across adhesin and toxin categories – Fig. 4) were evident on comparing isolates belonging to certain STs. Median antimicrobial resistance scores were highest amongst isolates belonging to ST131 (7, range 0–11.5). Antimicrobial resistance scores did not correlate with number of VF genes across STs (Fig. 4). Of the isolates, 7/61 (11.5%) carried ESBL enzymes (6/7 ST131 and 1/7 ST648) and 19/61 (31.1%) were MDR (Table 4 and Supplementary Table 3).

Discussion

In our study, bacteraemia-associated E. coli strains originating from UTIF harboured significantly more VF genes than non-UTIF- and NPUFI-associated strains. Number of VF genes was an independent predictor of bacteraemia derived from UTIF in immunocompetent patients with the odds of bacteraemia secondary to UTIF increasing by 1.21 times for every unit increase in VF gene number. A broad range of STs were identified with STs 12, 69, 73, 127 and 131 accounting for 51% of isolates, a finding that is in keeping with recently published UK data.\(^3\)

VFs associated with UTI-associated E. coli strains are well described\(^3,4,5,6,7\) but analyses comparing VF gene profiles of bacteraemia strains originating from well-defined infective foci are rare. Like us, Micenková et al. found more VF genes amongst UTIF-compared with non-UTIF bacteraemia isolates.\(^7\) In our study, univariate analysis of VF genes demonstrated that UTIF-associated isolates more frequently harbour ed papA, papC, papE/F, papG (P fimbriae), agn43 and tia (adhesins), iutA, fyuA (iron-acquisition-related genes), kpsM (capsule) and the sat toxin compared to non-UTIF isolates, and more frequently harbour ed papC, papE/F, papG, agn43, tia, fyuA, hlyA (haemolysin A), usp (urotrophogenic-specific protein) and clb (colibactin synthesis gene) compared to NPUFI-associated isolates. These findings strengthen previously described associations between P fimbriae-encoding genes and uroepithelial adhesion/associations with cystitis or pyelonephritis-causing strains,\(^6,7,35,36,38\) iutA/hlyA and pyelonephritis-causing strains,\(^4,6,7\) and kpsM (capsule)/P fimbriae and their relationships with UTI-associated bacteraemia.\(^4,7\) Although previously associated with UTI/pyelonephritis,\(^3,5,7,35\) qfA/drBC (Dr-binding adhesins), ibeA (invasion of brain endothelium) and sfa/R (S fimbriae) were not more

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prevalent amongst UTIF-associated isolates in our study. Our data strengthen previous observations relating to UTIF-specific VF genes but also reveal that, in UTIF-associated bacteraemia, ogn43, tio, fyuA and usp may be of significance.

Isolates from immunocompetent patients originating from non-UTIF were not significantly dissimilar to isolates from NPUFI in relation to total number of VF genes or distribution of individual VF genes. Only 56% and 33% of isolates from immunocompetent patients with non-UTIF and NPUFI, respectively, met the utilised genomic definition for ExPEC compared to 100% of isolates from urinary foci. These data demonstrate the broad diversity of strains associated with invasive disease outside of the context of UTI and support the hypothesis that non-UTIF and NPUFI-derived isolates likely originated from the same location, i.e. the gastro-intestinal tract.

The number of VF genes amongst isolates from NPUFI were low and 11/31 VF genes (focA, sfaA, ireA, ibeA, tcpC, cnf1, astA, hlyA, clb, pic and flic) were completely absent. Recently published data comparing the VF gene profiles of bowel translocation-associated bacteraemia isolates to faecal controls in patients with haematological malignancy demonstrated that specific clusters of VF genes may be associated with increased translocation potential. In our study which focused specifically on bacteraemia-associated strains, no individual VF genes were more frequent amongst isolates derived from NPUFI compared with UTIF or non-UTIF. Put together, these findings suggest that although bowel translocation-associated isolates derived from immunocompromised patients may possess specific VFs that enable this process, these isolates generally harbour fewer ExPEC-associated VF genes compared with bacteraemia-associated isolates derived from immunocompetent hosts.

It seems likely that translocation events occur secondary to damage to the structural integrity of the intestinal mucosa or as a result of compromised mucosal or systemic immunity, or both. Interestingly, a large proportion of isolates associated with bacteraemia secondary to non-UTIF in immunocompetent patients were caused by isolates with low numbers of VF genes. The majority of these isolates were associated with intra-abdominal pathologies where the physical integrity of visera and associated structures is often compromised due to the underlying pathology, e.g. severe inflammation +/- mechanical obstruction in cholecystitis/cholangitis. Under these circumstances E. coli isolates with low numbers of VFs may be able to translocate easily into the vascular system.

Key strengths of this study include its prospective design, the distinction between immunocompetent/neutropaenic groups, the rigorous methods utilised to assign infective foci, the use of logistic regression, and the application of WGS to determine VF gene profiles. The small NPUFI group (a group that was difficult to recruit) was the main limitation and likely reduced the power to detect differences in VF gene distribution between isolates derived from neutropaenic and immunocompetent sub-groups. Additionally, the mode of infecting strain acquisition was not determined and thus a comparative analysis of community vs. nosocomially-acquired strains was not possible in this study.

In conclusion, E. coli bacteraemia strains associated with UTIF have enriched VF gene profiles compared to those from non-UTIF
Fig. 3. Core-genome maximum-likelihood phylogenetic tree of E. coli bacteraemia isolates. Tree constructed with the generalised time-reversible model using FastTree V2.1 and features 56/61 isolates. Isolate numbers and associated sequence type (ST) data are presented. Bacteraemia isolates associated with urinary tract foci, non-urinary tract foci (immunocompetent patients) and unknown foci (neutropaenic patients) are indicated in purple (01–23), red (24–51) and black (52–59), respectively. Isolates excluded from the inter-patient VF gene analysis are indicated in green (01–23) and immunocompetent patient with cirrhosis (25) and neutropaenic patients with demonstrable focus of infection (60–61). Novel STs indicate the emergence of a new sequence type (to be classified) due to unambiguous, multi-locus ST-allelic variation. Reads from Isolates 9, 14, 43, 50 and 56 were unable to be resolved into draft genome assemblies using the A5 pipeline and were excluded from phylogenetic inference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 3
Distribution of virulence factor genes amongst 58 E. coli isolates from immunocompetent (urinary vs. non-urinary infective focus) and neutropaenic (unknown focus) patient groups. Proportions expressed as number of isolates with virulence factor gene detected (percentage in brackets). Proportions across groups compared with Chi-squared test for trend (a) and Fisher’s exact test (b) as indicated. na (not applicable).

| Virulence factor gene | Immunocompetent | Neutropaenic (unknown focus, n=8) | P value |
|-----------------------|-----------------|-----------------------------------|---------|
|                       | 1. Urinary (n=32) | 2. Non-urinary (n=27) | 3. Neutropaenic (unknown focus, n=8) | 1 × 2a | 1 × 3b | 2 × 3b |
| **Adhesins**           |                 |                                   |         |
| afu/druBC (afimbrial adhesins) | 0 (0) | 1 (4) | 1 (13) | 1.00 | — | — |
| ecPA (E. coli common pilus) | 23 (100) | 24 (89) | 7 (88) | 0.12 | 0.24 | 0.26 | < 0.001 |
| focA (FIC fimbriae) | 3 (13) | 3 (11) | 0 (0) | 0.36 | 0.55 | 0.99 | < 0.001 |
| **P fimbriae genes**  |                 |                                   |         |
| papA                  | 1 (4) | 9 (33) | 3 (38) | 0.01 | 0.07 | 0.001 | < 0.001 |
| papC                  | 22 (96) | 12 (44) | 1 (13) | 0.0001 | 0.003 | 0.003 | 0.01 |
| papEF                 | 22 (96) | 13 (48) | 1 (13) | 0.0001 | 0.001 | 0.001 | 0.11 |
| papG                  | 22 (96) | 11 (41) | 1 (13) | 0.0001 | 0.001 | 0.001 | 0.22 |
| sfaA (S fimbriae)     | 1 (4) | 2 (7) | 0 (0) | 0.62 | 0.99 | 0.99 | 0.99 |
| **tsh (temperature sensitive hamagglutinin)** | 0 (0) | 1 (4) | 1 (13) | 0.11 | 0.26 | 0.42 | < 0.001 |
| fimH (type 1 fimbriae) | 23 (100) | 24 (89) | 7 (88) | 0.12 | 0.24 | 0.26 | < 0.001 |
| **agdA (antigen 43)** | 20 (87) | 13 (48) | 3 (38) | < 0.01 | < 0.01 | 0.01 | < 0.001 |
| **toA (hek/tia adhesion and invasin)** | 18 (78) | 13 (48) | 2 (25) | < 0.01 | 0.04 | 0.01 | 0.42 |
| **Iron-acquisition systems** |                 |                                   |         |
| istA (aerobactin receptor) | 19 (83) | 10 (37) | 4 (50) | 0.01 | 0.15 | 0.69 | < 0.001 |
| sitB (peri-plasmic iron binding protein) | 22 (96) | 22 (81) | 6 (75) | 0.09 | 0.19 | 0.16 | < 0.001 |
| isiA (salmochelin receptor) | 12 (52) | 11 (41) | 2 (25) | 0.15 | 0.24 | 0.68 | < 0.001 |
| isiE (siderophore receptor) | 5 (22) | 4 (15) | 0 (0) | 0.17 | 0.29 | 0.55 | < 0.001 |
| fyuA (yersiniabactin receptor) | 23 (100) | 20 (74) | 5 (63) | < 0.01 | 0.01 | 0.67 | < 0.001 |
| **Protectins and Invasins** |                 |                                   |         |
| kpfM (group 2 capsule) | 22 (96) | 17 (63) | 6 (75) | 0.04 | 0.16 | 0.68 | < 0.001 |
| ompA (outer membrane protein A) | 23 (100) | 27 (100) | 8 (100) | na | < 0.99 | < 0.99 | < 0.99 |
| ibeA (invasion of brain endothelium A) | 1 (4) | 4 (15) | 0 (0) | 0.83 | 0.99 | 0.55 | < 0.001 |
| tcpC (toll receptor inhibitor) | 9 (39) | 6 (22) | 0 (0) | 0.02 | 0.20 | 0.07 | 0.31 |
| **Toxins**            |                 |                                   |         |
| cdtB (cytolethal distending toxin) | 2 (9) | 4 (15) | 1 (13) | 0.62 | 0.67 | < 0.001 | < 0.001 |
| catF (cytotoxic necrotising factor) | 8 (35) | 7 (26) | 0 (0) | 0.08 | 0.76 | 0.08 | 0.16 |
| astA (heat stable enterotoxin 1) | 3 (13) | 1 (4) | 0 (0) | 0.14 | 0.55 | 0.99 | < 0.001 |
| hlyA (haemolysin A) | 12 (52) | 7 (26) | 0 (0) | < 0.01 | 0.09 | 0.01 | 0.16 |
| sit (secreted autotransporter toxin) | 15 (65) | 7 (26) | 4 (50) | 0.11 | 0.68 | 0.39 | < 0.001 |
| usp (uropathogen-specific protein) | 17 (74) | 13 (48) | 2 (25) | < 0.01 | 0.08 | 0.03 | 0.42 |
| clb (colibactin synthesis) | 9 (39) | 8 (30) | 0 (0) | < 0.01 | 0.20 | 0.02 | 0.16 |
| puc (serine protease) | 4 (17) | 5 (19) | 0 (0) | 0.39 | 0.55 | 0.31 | < 0.001 |
| vat (vacuolating toxin) | 10 (43) | 11 (41) | 2 (25) | 0.38 | 0.78 | 0.43 | 0.68 |
| **Others**            |                 |                                   |         |
| βIC (flagellin variant) | 1 (4) | 1 (4) | 0 (0) | 0.62 | 0.99 | 0.99 | < 0.001 |

Fig. 4. Virulence factor gene subgroups and antimicrobial resistance scores amongst most prevalent E. coli sequence types (STs). Bars represent median values with interquartile range (error bars). Distribution of virulence factor numbers between STs compared with Mann–Whitney test. Significant indicated ('p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).
Table 4

Distribution of E. coli virulence factor genes (including subgroups), common STs, and antimicrobial resistance among isolates from clinical groups. Proportions compared using Fisher’s exact test. ESBL (extended-spectrum beta-lactamase); ExPEC (extra-intestinal pathogenic E. coli); ST (sequence type); MDR (multi-drug-resistant). Isolates classified as ‘intermediate’ or ‘non-susceptible’ to a ≥1 agent in ≥3 antimicrobial categories including aminoglycosides, anti-MRSA cefalosporins, anti-pseudomonal penicillins with beta-lactamase inhibitors, carbapenems, non-extended spectrum cefalosporins (i.e. 1st and 2nd generation), cephalosporins, fluoroquinolones, trimethoprim-sulphamethoxazole, glycolycins, monobactams, penicillins, penicillins with beta-lactamase inhibitors, chloramphenicol, phosphonic acids and colistin.

| Characteristic | 1a. All (n = 50) | 1b. Urinary focus (n = 23) | 1c. Non-urinary focus (n = 27) | 2. Neutropenic (n = 8) | 1a × 2 | 1b × 1c | 1b × 2 | 1c × 2 | P value |
|----------------|-----------------|--------------------------|-----------------------------|----------------------|-------|--------|--------|--------|--------|
| VF gene number, median (range) | | | | | | | | | |
| Total VF genes | 15.5 (2–24) | 16 (8–24) | 10 (2–22) | 8 (3–13) | 0.01 | 0.01 | <0.0001 | 0.28 |
| Adhesin VF genes | 7 (1–9) | 8 (3–9) | 4 (1–9) | 3 (1–7) | 0.01 | <0.001 | <0.0001 | 0.37 |
| Iron-acquisition VF genes | 3 (0–5) | 3 (2–5) | 3 (0–5) | 3 (1–3) | 0.18 | <0.01 | 0.02 | 0.73 |
| Proteus/immovin VF genes | 2 (1–3) | 2 (1–3) | 2 (1–3) | 2 (1–2) | 0.09 | >0.08 | 0.01 | 0.50 |
| Toxins VF genes | 3 (0–7) | 3 (1–7) | 2 (0–6) | 1 (0–2) | 0.02 | 0.05 | 0.001 | 0.21 |
| Distribution of most frequent STs, n (%) | | | | | | | | | |
| ST 73 | 6 (12) | 3 (13) | 3 (11) | 0 (0) | 0.58 | >0.99 | 0.55 | >0.99 |
| ST 12 | 4 (8) | 4 (17) | 0 (0) | 0 (0) | >0.99 | 0.04 | 0.55 | >0.99 |
| ST 127 | 5 (10) | 2 (9) | 3 (11) | 0 (0) | >0.99 | >0.99 | >0.99 | >0.99 |
| ST 131 | 13 (26) | 6 (26) | 5 (19) | 2 (25) | >0.99 | 0.73 | >0.99 | 0.65 |
| ST 69 | 5 (10) | 5 (22) | 0 (0) | 0 (0) | >0.99 | 0.02 | 0.29 | >0.99 |
| ST648 | 3 (6) | 0 (0) | 3 (11) | 0 (0) | >0.99 | 0.24 | >0.99 | >0.99 |
| Antimicrobial resistance score, median (range) | | | | | | | | | |
| Meet MDR definition, n (%) | 15 (30) | 8 (35) | 7 (26) | 4 (50) | 0.42 | 0.55 | 0.68 | 0.23 |
| Resistant to, n (%) | | | | | | | | | |
| Amoxicillin | 27 (54) | 14 (61) | 13 (48) | 5 (63) | 0.72 | 0.41 | >0.99 | 0.69 |
| Piperacillin | 21 (42) | 12 (52) | 9 (30) | 5 (63) | 0.45 | 0.25 | 0.70 | 0.22 |
| Cefoxitin | 10 (20) | 6 (26) | 4 (15) | 2 (25) | 0.66 | 0.48 | >0.99 | 0.60 |
| Piperacillin-tazobactam | 5 (10) | 3 (13) | 2 (7) | 2 (25) | 0.25 | 0.65 | 0.58 | 0.22 |
| Cefuroxime | 10 (20) | 5 (21) | 5 (19) | 2 (25) | 0.65 | >0.99 | >0.99 | 0.65 |
| Cefotaxime | 8 (16) | 4 (17) | 4 (15) | 0 (0) | 0.58 | >0.99 | 0.55 | 0.55 |
| Cefazidime | 6 (12) | 4 (17) | 2 (7) | 0 (0) | 0.58 | 0.39 | 0.55 | >0.99 |
| Cephaloridine | 10 (20) | 5 (22) | 5 (19) | 6 (75) | >0.99 | 0.99 | 0.01 | >0.99 |
| Gentamicin | 6 (12) | 4 (17) | 2 (7) | 2 (25) | 0.30 | 0.39 | 0.63 | 0.22 |
| Meropenem | 0 (0) | 0 (0) | 0 (0) | 0 (0) | >0.99 | >0.99 | >0.99 | >0.99 |
| Ertapenem | 0 (0) | 0 (0) | 0 (0) | 0 (0) | >0.99 | >0.99 | >0.99 | >0.99 |
| Chloramphenicol | 9 (18) | 4 (17) | 5 (9) | 2 (25) | 0.64 | >0.99 | 0.63 | 0.65 |
| Trimethoprim-sulfamethoxazole | 13 (26) | 7 (30) | 6 (20) | 4 (50) | 0.22 | 0.54 | 0.41 | 0.19 |
| Colistin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | >0.99 | >0.99 | >0.99 | >0.99 |
| Tetracycline | 4 (8) | 2 (9) | 2 (7) | 1 (13) | 0.54 | >0.99 | >0.99 | 0.55 |
| n (%) meeting ExPEC definition | 38 (76) | 23 (100) | 15 (56) | 3 (38) | 0.04 | <0.001 | <0.0001 | 0.44 |
| Phenotypic +/- Genotypic detection of ESBL, n (%) | 7 (14) | 4 (17) | 3 (11) | 0 (0) | 0.18 | 0.69 | 0.55 | >0.99 |

and NPUFI. Strains are genomically diverse and in this study non-UTIF-associated bacteraemia in immunocompetent patients was frequently caused by strains that did not meet the utilised genomic definition for ExPEC. Mapping the diversity of bacteraemia-causing strains will inform targeted or universal preventative strategies. Future vaccine development will depend upon these data to ensure adequate coverage of strains associated with site-specific disease.

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Conflicts of interest

APD, AKP, RJH, KB, JRL, KCS and RCR: No reported conflicts.

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Supplementary materials

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References

1. Dale AP, Woodford N. Extra-intestinal pathogenic Escherichia coli (ExPEC): disease, carriage and clones. J Infect 2015;71:615–62.
2. Public Health England. Thirty-day all-cause fatality subsequent to MRSA, MSSA and E. coli bacteraemia and C. difficile infection, 2010/17. Available at:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/637436/HCA1_thirty_day_all_cause_fatality_report_2016_2017.pdf. Accessed 02 April 2018.

3. Johnson JR, Porter S, Thrusa P, Castanheira M. Epidemic emergence in the United States of Escherichia coli sequence type 131-H30 (ST131-H30), 2000 to 2009. Antimicrob Agents Chemother 2017;61(8).

4. Starčič Erjavac M, Žgur-Bertok D. Virulence potential for extraintestinal infections among commensal Escherichia coli isolated from healthy humans—the Trojan horse within our gut. FEMS Microbiol Lett 2015;362(5).

5. Peirano G, Mulvey GL, Armstrong GD, Pitout JD. Virulence potential and adherence properties of Escherichia coli that produce CTX-A and NDM β-lactamases. J Med Microbiol 2013;62:525–30.

6. Croxon MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Microbiol 2010;8:26–38.

7. Kini KS. Mechanisms of microbial traversal of the blood-brain barrier. Nat Rev Microbiol 2008;6:625–34.

8. Abernethy G, Guy R, Sheridan EA, Hopkins S, Kiernan M, Wilcox M, et al. Epidemiology of Escherichia coli bacteraemia in England: results of an enhanced sentinel surveillance programme. J Hosp Infect 2017;95:365–75.

9. Samet A, Śledzińska A, Krawczyk B, Hellmann A, Nowicki S, Kur J, et al. Leukemia and risk of recurrent Escherichia coli bacteriuria: genotyping implicates E. coli translocation from the colon to the bloodstream. J Clin Microbiol Inf Dis 2013;32:1393–400.

10. Berg RD. Bacterial translocation from the gastrointestinal tract. Adv Exp Med Biol 1999;473:11–30.

11. Charlson M, Sztatrowski T, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1995;48:1245–51.

12. Levy MM, Frisk MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACP/ATS/SIS international sepsis definitions conference. Crit Care Med 2003;31:1250–6.

13. Grabe M, Bjerklund-Johansen T, Botto H, Ček M, Naber KG, Tonké P. Guidelines on urological infection. European Associations of Urology; 2015. Available at: https://uroweb.org/wp-content/uploads/Urological-Infections-2010-1.pdf. Accessed 09 March 2018.

14. British Society for Antimicrobial Chemotherapy Standing committee on susceptibility testing (version 14.0, 05.01.2015). http://bsac.org.uk/wp-content/uploads/2012/02/BSAC-Susceptibility-testing-version-14.pdf. Accessed 01 March 2018.

15. Magorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.

16. Nielsen KL, Godtfred K, Stegger M, Andersen PS, Feldgarden M, Frimodt-Møller N. Selection of unique Escherichia coli clones by random amplified polymorphic DNA (RAPD): evaluation by whole genome sequencing. J Microbiol Methods 2014;103:101–3.

17. Venier D, Vantarakis A, Kominou G, Papapetropoulos M. Differentiation of faecal Escherichia coli from human and animal sources by random amplified polymorphic DNA-PCR (RAPD-PCR). Water Sci Technol 2004;49:193–8.

18. Madico G, Akopyants NS, Berg DE. Arbitrarily primed PCR DNA fingerprinting of Escherichia coli O157:H7 strains by using templates from boiled cultures. J Clin Microbiol 1995;33:1514–6.

19. Doumith M, Day M, Ciesielczuk H, Hope R, Underwood A, Reynolds R, et al. Rapid identification of major Escherichia coli sequence types causing urinary tract and bloodstream infections. J Clin Microbiol 2015;53:160–6.

20. Inouye M, Dashnow H, Raven LA, Schultz MB, Pope BJ, Tomita T, et al. SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. Genome Med 2014;6:50.

21. Chen L, Zheng D, Liu B, Yang J, Jin Q. VITDR 2016: hierarchical and refined dataset for big data analysis—10 years on. Nucleic Acids Res 2016;44:D694–7.

22. Achtmann M, Vellayudan V, Zhou Z. Multi Locus Sequence Typing (MLST). University of Warwick and Development funded by the BBSRC. Available at: http://mlst.warwick.ac.uk/mlst/dbs/Ecoli. Accessed 05 August 2017.

23. Johnson JR, Stell AE. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 2000;181:261–72.

24. Cool D, Josip G, Darling AE. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 2015;31:587–9.

25. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics 2013;29:1072–5.

26. Seemann T, Pourkashanian M, Holzapfel M, Royle B. Rapid large-scale prokaryote genome annotation. Bioinformatics 2014;30:2068–9.

27. Löstynoja A. Phenylogeny-aware alignment with PRANK. Methods Mol Biol 2014;1079:155–70.

28. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, et al. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 2015;31:3691–3.

29. Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 2010;5:e9490.

30. A Rambaut, ‘Figtree, a graphical viewer of phylogenetic trees’. Institute of Evolutionary Biology, University of Edinburgh, 2016. Available at: http://tree.bio.ed.ac.uk/software/figtree/. Accessed 05 August 2017.

31. Laupland KB, Gregson DR, Church DL, Ross T, Pitout JD. Incidence, risk factors and outcomes of Escherichia coli bloodstream infections in a large Canadian region. Clin Microbiol Infect 2008;14:1041–7.

32. Marschall J, Zhang L, Foxman B, Warren DK, Henderson JP. CDC Prevention Epicenters Program. Both host and pathogen factors predispose to Escherichia coli urinary-source bacteriuria in hospitalized patients. Clin Infect Dis 2012;54:1692–8.

33. Gudčik D, Ševo M, Rase S, Vavčar P, Křížová V, Pavlíček J, et al. Bacteriemia due to multidrug-resistant Gram-negative bacilli in cancer patients: risk factors, antibiotic therapy and outcomes. J Antimicrob Chemother 2011;66:657–63.

34. Firoozeh F, Safiari M, Neamati F, Zibaei M. Detection of virulence genes in Escherichia coli isolated from patients with cystitis and pyelonephritis. Int J Infect Dis 2014;29:219–22.

35. Er DK, Dundar D, Uzuner H, Osmanli A. Relationship between phylogenetic groups, antibiotic resistance and patient characteristics in terms of adhesion genes in cystitis and pyelonephritis isolates of Escherichia coli. Microb Pathog 2015;89:188–94.

36. Johnson JR, Owens K, Gajewski A, Kuskowski MA. Bacterial characteristics in relation to clinical source of Escherichia coli isolates from women with acute cystitis or pyelonephritis and infected women. J Clin Microbiol 2005;43:6064–6072.

37. Mincéková L, Beňová A, Frankovcová L, Bosák J, Vrbá M, Švečková A, et al. Human Escherichia coli isolates from hemocultures: Septicemia linked to urogenital tract infections is caused by isolates harboring more virulence genes than bacteriemia linked to other conditions. Int J Med Microbiol 2017;307:182–9.

38. Lane MC, Molloy HLT. Role of Fimbrial-mediated adherence in pyelonephritis and persistence of uropathogenic Escherichia coli (UPEC) in the mammalian kidney. Kidney Int 2007;72:19–25.

39. Krawczyk B, Śledzińska A, Szeniak K, Samet A, Nowicki B, Kur J. Characterisation of Escherichia coli isolates from the blood of haematological adult patients with bacteremia: translocation from gut to blood requires the cooperation of multiple virulence factors. Eur J Clin Microbiol Infect Dis 2015;34:1135–1143.

40. Green SL, Ajami NJ, Ma L, Poole NM, Price RE, Petrosino JF, et al. Mouse model of chemotherapy-induced extraintestinal pathogenic Escherichia coli translocation. Infect Immun 2015;83:3243–3256.

41. Samel S, Reese M, Kleczka M, Laniš G, Groetz N, Hafner M, et al. Microscopy of bacterial translocation during small bowel obstruction and ischemia in a new animal model. BMC Surg 2002:2:6.