Interactions of uroseptic *Escherichia coli* with renal (A-498) and gastrointestinal (HT-29) cell lines

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We investigated the ability of *Escherichia coli* isolated from septic patients with urinary tract infection (UTI) to translocate through the gastrointestinal (GI) tract of the same patients using cell-culture models. Forty-seven hospitalized patients with urosepsis were included in this study. *E. coli* was isolated from their urine and blood (total 94 isolates) and investigated for genetic relatedness and interaction with the cell lines A-498 and HT-29. An initial comparison of the strains isolated from urine and blood showed that 44 out of 47 patients (94 %) had identical strains in their blood and urine. The blood isolates adhered to both cell lines, although their rate of adherence to A-498 cells was significantly higher than that to HT-29 cells (5.8 ± 3.8 per cell vs 2.8 ± 1.9; *P*<0.0001). The rate of translocation in A-498 cells was also significantly higher after 120 min (8.7×10⁵ vs 2.9×10⁵; *P* = 0.0006). Three non-identical blood isolates were unable to translocate in HT-29 cells, indicating that host immune factors might be more important than bacterial ability to translocate the GI epithelium in these patients. Our data showed that blood isolates from uroseptic patients are able to adhere to and translocate through both cell lines. This suggests that *E. coli* in patients with UTI may translocate from either the GI tract or the urinary tract, hence questioning the assumption that the urinary tract is the only source of septicemia in these patients.

**INTRODUCTION**

*Escherichia coli* is a common inhabitant of the large intestine of humans and can cause life-threatening intestinal and extra-intestinal infections (Johnson, 1991; Wilson & Blitchington, 1996). *E. coli* has been shown to translocate through the gastrointestinal (GI) epithelium to cause septicemia (Berg, 1980; MacFie et al., 1999; O’Boyle et al., 1998). The disruption of gut flora equilibrium, such as during enteric bacterial overgrowth (Guarner et al., 1997; Katouli et al., 1994), physical disruption of the epithelial barrier (Mucutkiewicz et al., 2008; Parks et al., 2000; Yu & Martin, 2000) or impairment of the host immune system (Xu et al., 1998), can lead to the development of bacterial translocation. *α*-Haemolysin-producing *E. coli* has also been shown to create small openings within the GI epithelium, known as focal leaks, as a novel mechanism to allow translocation via a paracellular pathway (Troeger et al., 2007). The gut origin hypothesis for septicemia implies that the GI tract forms the source of septicaeic events in patients due to bacterial translocation from this site (Alexander et al., 1990; Berg & Garlington, 1979; Katouli et al., 1994; Sedman et al., 1994). The urinary tract is also an important site for bacterial translocation and has been linked to the passage of pathogenic *E. coli* into the bloodstream, a process known as urosepsis (Grandsen et al., 1990; Leblebicioglu et al., 2003; Yamamoto et al., 1997).

Bacterial adherence to the host epithelium is an important step in translocation. There are a number of adhesins that help *E. coli* to attach to the epithelium, including type 1, P, DR, S and F1C fimbriae (Johnson & Stell, 2000). In view of the GI tract being a common reservoir for uropathogenic *E. coli* in patients with urinary tract infection (UTI), identification of the true source of bacterial translocation in septicaeic patients with a UTI is difficult. It has been shown that certain clonal groups of *E. coli* in the GI tract have a better ability to adhere to and colonize both the urinary tract and the GI epithelium (Katouli et al., 1997; Ramos et al., 2010; Tullus et al., 1992). In the present study, we hypothesized that, in hospitalized patients, *E. coli* may cause UTIs but does not necessarily translocate through kidney cells; instead, it may adhere to the GI epithelium and translocate through these cells into the bloodstream to cause a gut-associated septicemia. In view of the above,
the present study was undertaken to investigate the interaction of a collection of *E. coli* isolates from hospitalized patients having both UTIs and septicaemia, using cell-culture models of renal (A-498) and GI (HT-29) epithelium to determine whether *E. coli* in urosepsis patients can translocate through the GI epithelium. We also investigated the prevalence of different virulence genes (VGs) associated with the adherence of *E. coli* strains causing UTIs among these isolates in order to identify whether there was any correlation between the presence of any of these VGs, adherence and/or translocation of these bacteria in either cell line.

**METHODS**

**Patients and strains.** *E. coli* isolates were obtained from the blood and urine of 47 hospitalized patients (73% female, median age 67 years, range 18–97 years) suffering simultaneously from septicaemia and a symptomatic UTI at the Princess Alexandra Hospital, Brisbane, Australia, between September 2006 and August 2007. All patients developed UTI and septicaemia after admission, and a symptomatic UTI (termed urosepsis) at the Princess Alexandra Hospital, Brisbane, Australia, between September 2006 and August 2007. All patients developed UTI and septicaemia after admission, and none of the patients was diagnosed with pyelonephritis. Only two patients (patient nos PA29 and PA31) had urinary catheters at the time of the UTI. The characteristics of the patients and urine upon examination are outlined in Table 1. There was no mixed infection among any samples from blood or urine. All strains were transferred to the laboratory and purified on MacConkey agar no. 3 (Oxoid) containing 20 % glycerol.

**DNA extraction and confirmation of *E. coli*.** Strains were recovered onto nutrient agar (Oxoid) grown overnight at 37 °C. DNA extraction was performed by boiling a single colony in sterile water at 95 °C for 10 min. All strains were confirmed as *E. coli* by screening for the presence of the universal stress protein (upA) gene, as described previously (Chen & Griffiths, 1998).

**Typing of isolates.** All strains were typed using a high-resolution biochemical fingerprinting method specifically developed for typing of *E. coli* strains (PhP-RE; PhPlate AB) (Kühn & Möllby, 1993), as described previously (Vollmerhausen et al., 2011). The fingerprinting method was performed according to the manufacturer's instructions. The biochemical fingerprints of blood and urine isolates were then compared pairwise and the similarity between each pair of strains was calculated as the correlation coefficient and clustered according to the unweighted pair group mathematical average method to yield a dendrogram (Saeedi et al., 2005; Sneath & Sokal, 1973). The identity (ID) level of 0.975 was established based on reproducibility of control strains tested in duplicate. Isolates showing similarity to each other above the ID level were regarded as identical and assigned to the same biochemical phenotype. All data handling, including calculations of correlations and coefficients and clustering, were performed using the PhPWin software.

**Phylogenetic grouping and PCR detection of VGs.** A multiplex PCR screening for the chuA and yjaA genes, and the TSPE4.C2 DNA fragment, was used to determine the phylogenetic grouping of the isolates, as described previously (Clermont et al., 2000).

Isolates were tested for the presence of adhesin genes known to be associated with *E. coli* causing UTIs. These included genes coding for the production of P fimbriae (*papAH, papEF, papC, papG* allele II and *papG* allele III), type 1 fimbriae (*fimH*), the central region of DR antigen-specific fimbrial and afimbrial adhesion operons (*afa/draABC*), and the central region of S fimbriae and FIC fimbrial operons (*sfa/focDE*). Genes were grouped into two multiplex primer sets and three uniplex sets in a method modified from that previously described by Johnson & Stell (2000), with appropriate positive and negative controls.

The multiplex PCR volume of 25 μl consisted of 2 μl template DNA, 1 × PCR buffer (Qiagen), 0.4 mM dNTPs (Fisher Biotec), 2.5 mM MgCl<sub>2</sub>, 0.6 μM primer (Invitrogen) for *papAH, papEF, fimH*, *papG* allele III and *sfa/focDE*, and 1 U HotStartTaq DNA polymerase (Qiagen). Amplification was conducted in a Mastercycler gradient (Eppendorf) using the following cycling conditions: denaturation at 95 °C for 15 min; 25 cycles of 94 °C for 30 s, 63 °C for 30 s and 68 °C for 3 min; and a final extension at 72 °C for 10 min.

The uniplex PCR was performed in a final volume of 25 μl consisting of 2 μl template DNA, 1 × PCR buffer (Bioline), 0.4 μM dNTPs (Fisher Biotec), 0.5 mM MgCl<sub>2</sub>, 0.6 μM each primer (Invitrogen) for *papG* allele II and *afa/draBC* (0.3 μM for *papC*), and 1 U BIOTAQ DNA polymerase (Bioline). Amplification was conducted in a Mastercycler gradient (Eppendorf) using the following cycling conditions: denaturation at 95 °C for 4 min; 25 cycles of 94 °C for 30 s, 63 °C for 30 s and 68 °C for 3 min; and a final extension at 72 °C for 10 min. All amplified PCR products were separated on a 2 % agarose gel (Amresco, Astral Scientific) in 0.6 × TBE buffer. PCR products were visualized following ethidium bromide staining using a Syngene GeneGenius Gel Light Imaging System.

**Cell-culture conditions and adherence assay.** The human colonic adenocarcinoma cell line HT-29 [HTB-38; American Type Culture Collection (ATCC)] was used to model the GI epithelium. This cell line has been used previously as a model for bacterial interactions with the human GI epithelium (Couto et al., 2007; Wold et al., 1992). HT-29 cells were grown in RPMI 1640 with 25 mM HEPES and L-glutamine (Gibco), supplemented with 10 % FBS (Gibco) and 1 % penicillin/streptomycin (Thermofisher, Australia). The human kidney carcinoma cell line A-498 (HTB-44; ATCC) was used to model the kidney epithelium. A-498 cells have been used previously as a model for bacterial interaction with the kidney epithelium (Hedlund et al., 2001; Kai-Larsen et al., 2010). A-498 cells were grown in Eagle’s minimum essential medium (Gibco), supplemented with 10 % FBS and 1 % penicillin/streptomycin. Cell-culture medium was replaced every 48 h.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tr>
<td>Age (years)</td>
<td>66 ± 18</td>
<td>18–97</td>
</tr>
<tr>
<td>No. of bacteria in urine (c.f.u. ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>9.4 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1 × 10&lt;sup&gt;6&lt;/sup&gt;–1 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of leukocytes (cells l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.5 × 10&lt;sup&gt;5&lt;/sup&gt; ± 1.9 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>2 × 10&lt;sup&gt;5&lt;/sup&gt;–5 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of erythrocytes (cells l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>7.2 × 10&lt;sup&gt;7&lt;/sup&gt; ± 1.2 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>1 × 10&lt;sup&gt;7&lt;/sup&gt;–1.8 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of epithelial cells (cells l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.2 × 10&lt;sup&gt;7&lt;/sup&gt; ± 7.9 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1.0 × 10&lt;sup&gt;7&lt;/sup&gt;–4.0 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
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with antibiotic-free medium prior to the infection experiments. For the adherence assays, cells were seeded onto round 12 mm diameter glass coverslips in 24-well tissue-culture plates (Nunc) and grown to semi-confluence. *E. coli* isolates were grown in TSB in 100 ml flasks at 37 °C with agitation for 4 h, centrifuged, washed three times in 5 ml PBS (pH 7.4) and resuspended in PBS, and 100 μl of the suspension (~1.0 × 10^8 c.f.u.) was inoculated into each well. The plates were incubated for 90 min at 37 °C after which non-adherent bacteria were washed from the coverslips. The cells were then fixed for 5 min in 95% ethanol (for HT-29 cells) or methanol (for A-498 cells), stained with Giemsa stain and observed by light microscopy. Adherence was determined by counting the number of adherent bacteria on 100 randomly selected cells per bacterial isolate, from which the mean number of bacteria per cell was calculated. Isolates were grouped into low- (<3 bacteria per cell), medium-(4–7 bacteria per cell) and high-(>8 bacteria per cell) adherence groups using a modified method by Grey & Kirov (1993). *E. coli* strains HMLN-1 and JM109 were used as positive controls (Ljungdahl et al., 2006). *E. coli* isolates from the blood and urine of these three patients were tested as two separate non-replicate isolates.

**Translocation assay.** Bacterial translocation was assessed using a two-compartment system with cells grown in Millicell Inserts (Millipore), each with a permeable base containing 0.8 μm diameter pores in a 24-well tissue-culture plate. Inserts wereseeded with 400 μl growth medium containing 10^6 HT-29 cells and 10^6 A-498 cells and were then incubated at 37 °C in 5% CO_2_. Transepithelial electrical resistance (TEER) between the inner and outer wells was measured and calculated using the following equation: measured monolayer resistance – measured blank resistance (filter without cells) × area of the filter (0.6 cm^2) = transmonolayer resistance (Ω cm^-2) (Maher & McClean, 2006; Snowden et al., 2006). A stable TEER was achieved after 4 days for HT-29 cells (45–85 Ω cm^-2) and after 6 days for A-498 cells (45–95 Ω cm^-2). One hundred microlitres of bacterial suspension was then inoculated into each inner well (final concentration ~1.0 × 10^8 c.f.u. per well) and the cells were incubated at 37 °C in 5% CO_2_. After 15, 30, 60 and 120 min, samples were collected from the outer chamber, serially diluted in PBS, plated onto MacConkey agar no. 3 and grown overnight at 37 °C to determine the number of translocated bacteria by c.f.u. In all assays, *E. coli* HMLN-1, which was isolated from the mesenteric lymph nodes and blood of a patient with fatal haemorrhagic pancreatitis, was used as the positive control (Ljungdahl et al., 2006; Nettelbladt et al., 2000). *E. coli* JM109 was used as a negative control due to its previous demonstrated inability to translocate (Snowden et al., 2006).

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism version 6.02 for Windows software (GraphPad Software). A χ^2 test was used to compare low, medium and high adherence for HT-29 and A-498 cells. Fisher's exact test was used when comparing the difference between the numbers of bacteria or VGs in two groups. The degree of translocation of strains was compared using an unpaired t-test with Welch correction. Differences were considered significant if P<0.05.

**RESULTS**

**Typing of blood and urine isolates**

Typing of the 47 pairs of isolates from blood and urine from the same patients showed that 44 pairs (94%) had identical biochemical phenotypes (Fig. 1). Three non-identical pairs (patient nos PA30, PA43 and PA48) were found to be distantly related to each other with similarity levels below 0.681, indicating that the *E. coli* isolated from the blood and urine of these patients originated from different sources (Fig. 1). As a result, in all subsequent experiments, *E. coli* strains isolated from both the blood and urine of these three patients were tested as two separate non-replicate isolates.

**Blood isolates have higher adherence to A-498 cells**

To investigate whether *E. coli* isolated from the blood of patients with urosepsis possessed the ability to translocate through the GI epithelium, we tested their interaction with gut (HT-29) and kidney (A-498) cells. Variations were seen in the adherence rate of *E. coli* to both cell lines and therefore isolates were grouped into low (<3 bacteria per cell), medium (4–7 bacteria per cell) and high (≥8 bacteria per cell) adherence. The number of blood isolates adhering to A-498 cells was significantly (P<0.0001) higher for high (11 vs 0) and medium (20 vs 6) adherence than those adhering to HT-29 cells (data not shown). The number of blood isolates adhering to HT-29 cells with low adherence was significantly (41 vs 16) higher than the number adhering to A-498 cells.

**Blood isolates have a higher translocation rate with A-498 cells**

The ability of blood isolates from 46 patients to translocate through HT-29 and A-498 cells was measured at intervals over 120 min. The TEER value of both cell lines during this time remained relatively constant throughout all experiments, demonstrating the integrity of the monolayer (Fig. 2a, b). The degree of translocation of the blood isolates varied between cell lines, ranging from 1.8 × 10^4 to 4.2 × 10^6 c.f.u. ml^-1 for A-498 and from 0 to 3.2 × 10^6 c.f.u. ml^-1 for HT-29 cells. Whilst the mean rate of translocation for all 46 blood isolates in the two cell lines did not differ after the first 30 min, their mean rate of translocation in A-498 cells after 120 min increased by 1 log yielding a significantly (P=0.0006) higher number of translocating bacteria compared with HT-29 cells (8.7 × 10^5 and 2.9 × 10^5 c.f.u. ml^-1 for each cell line, respectively; Fig. 2c). The higher rate of translocation of blood isolates in A-498 cells was also associated with their higher level of adherence to A-498 cells (5.8 ± 3.8, mean ± sd) compared with HT-29 cells (2.8 ± 1.9; P<0.0001) after 90 min. Six blood isolates showed higher translocation in HT-29 cells compared with A-498 cells.

**Non-identical blood isolates did not translocate through HT-29 cells**

Typing of *E. coli* isolates from the blood and urine showed non-identical strains in three patients. To determine whether the GI tract was a potential site for translocation of these strains, we investigated their ability to adhere to and translocate through HT-29 cells. Whilst the strains isolated from the urine of these patients belonged to phylogenetic group B2, only one blood isolate belonged to...
this phylogenetic group, and the blood isolates from the other two patients belonged to phylogenetic groups A and B1 (Table 2). The blood isolates from these three patients showed low adherence to HT-29 cells, and all three blood isolates failed to translocate HT-29 cells at all tested time points (Table 2). The three non-identical blood isolates varied in their adherence rate (one each had low, medium and high adherence) to A-498 cells. All non-identical blood and urine isolates were able to translocate through A-498 cells after 120 min.

**Adherence does not correlate with translocation of HT-29 cells or VGs**

The majority of blood isolates showed higher adherence and translocation in A-498 cells (Fig. 2c). There was no correlation between adherence of the strains and their translocation in A-498 and HT-29 cells (data not shown). There was also no correlation between the prevalence of any of the VGs tested and the adherence of the strains to A-498 and HT-29 cells (Table 3).

**DISCUSSION**

Bacteria that translocate through the epithelial layer to reach underlying tissues have a greater opportunity to cause severe diseases such as septicemia (MacFie, 2004). Patients experiencing bacterial translocation are twice as likely to become septicemic (MacFie et al., 2006; O’Boyle et al., 1998). Bacterial overgrowth is one mechanism that can yield high numbers of bacteria in the mesenteric lymph nodes, promoting bacterial translocation (Berg, 1981; Katouli et al., 1994). The GI tract is also a major reservoir of *E. coli* causing UTIs, and the dominance of uropathogenic *E. coli* in the gut microflora is associated with incidences of UTI (Moreno et al., 2008). Overall, 32% of cases of severe sepsis have been attributed to infection from genito-urinary sources, compared with 22% from GI sources (Moss, 2005). Previously, identical strains in the blood, urine and stools of patients with bacteraemia have been identified, suggesting the spread of these strains from the GI tract, possibly also via the blood, to the urine (Brauner et al., 1991). A recent study using genome
sequencing identified the presence of non-identical blood and urine *E. coli* strains in a patient with urosepsis (McNally et al., 2013). To understand the routes of infection in urosepsis, our study employed a two-compartment model of translocation using renal and GI epithelial cell lines. In this study, the majority of *E. coli* isolates causing urosepsis were able to translocate in our GI epithelial cell model. This laboratory model of translocation enabled an insight into the understanding of the routes in which *E. coli* strains enter the bloodstream, making the GI tract, in addition to the kidney, a potential site for translocation.

Using a high-resolution biochemical fingerprinting method, the uroseptic strains were shown to be identical between blood and urine samples for the majority of patients, suggesting the urinary tract as the source of bacterial translocation in 94% of uroseptic patients. It has been shown previously that this typing method is as powerful as molecular typing methods including randomly amplified polymorphic DNA PCR (Ramos et al., 2010; Vollmerhausen et al., 2011) and enterobacterial repetitive intergenic consensus PCR fingerprinting (Ansaruzzaman et al., 2000). Two of the three non-identical pairs were shown to belong to different phylogenetic groups, and all three pairs varied in their ability to adhere to and translocate through the kidney and GI epithelial cell lines. The fact that, apart from infection of the urinary tract, there was no other focus of infection in these patients suggests that the presence of these isolates in the blood may be due to their translocation from the gut of these patients. *E. coli* strains isolated from the blood and urine in this study were identified clinically as monoclonal using classical culture techniques. Recent studies have highlighted the limitations of classical culture-based diagnosis of infectious disease (Levert et al., 2010; McNally et al., 2013), and therefore the possibility of polyclonal infections in our study cannot be ruled out. Up to 58% of patients experiencing extra-intestinal *E. coli* infections have been shown to be infected by either several distinct clones or a single clone with microheterogeneity (Levert et al., 2010). Future phenotypic and genotypic investigation of multiple colonies from the site of infection may provide greater insight into the complexity of polyclonal extra-intestinal infections. Despite these limitations, our study highlights

### Table 2. Adherence to (bacteria per cell) and translocation (c.f.u.) through A-498 and HT-29 cells of non-identical *E. coli* blood and urine isolates

The source of isolation and the phylogenetic group (PGG) membership of each isolate are shown.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Source</th>
<th>PGG</th>
<th>Adherence (mean no. bacteria per cell ± SD)</th>
<th>Translocation (c.f.u. ml⁻¹×10⁴)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>HT-29</strong></td>
<td><strong>A-498</strong></td>
</tr>
<tr>
<td>PA30</td>
<td>Blood</td>
<td>B1</td>
<td>1.86 ± 1.04</td>
<td>3.82 ± 2.41</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>B2</td>
<td>1.49 ± 0.95</td>
<td>4.13 ± 2.72</td>
</tr>
<tr>
<td>PA43</td>
<td>Blood</td>
<td>B2</td>
<td>1.99 ± 1.24</td>
<td>8.91 ± 9.47</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>B2</td>
<td>2.76 ± 1.94</td>
<td>2.29 ± 1.52</td>
</tr>
<tr>
<td>PA48</td>
<td>Blood</td>
<td>A</td>
<td>2.12 ± 1.35</td>
<td>3.14 ± 2.17</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>B2</td>
<td>2.76 ± 2.1</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3. Prevalence of eight VGs among 47 *E. coli* strains isolated from uroseptic patients and their relationship with adherence to A-498 and HT-29 cells

Isolates were grouped according to the mean number of bacteria adhering to cells into low (<3 bacteria per cell), medium (4–7 bacteria per cell) and high (>8 bacteria per cell) adherence.

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th>A-498 adherence (%)</th>
<th>HT-29 adherence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (<em>n=16</em>) Medium (<em>n=20</em>) High (<em>n=11</em>)</td>
<td>Low (<em>n=41</em>) Medium (<em>n=6</em>)</td>
</tr>
<tr>
<td>papAH</td>
<td>12 (75) 16 (80) 8 (73)</td>
<td>31 (76) 5 (83)</td>
</tr>
<tr>
<td>papEF</td>
<td>11 (69) 13 (65) 9 (81)</td>
<td>28 (68) 5 (83)</td>
</tr>
<tr>
<td>papC</td>
<td>9 (56) 12 (60) 7 (64)</td>
<td>25 (61) 3 (50)</td>
</tr>
<tr>
<td>papG allele III</td>
<td>5 (31) 3 (15) 2 (18)</td>
<td>9 (22) 1 (17)</td>
</tr>
<tr>
<td>papG allele II</td>
<td>9 (56) 12 (60) 8 (73)</td>
<td>25 (61) 4 (67)</td>
</tr>
<tr>
<td>fimH</td>
<td>16 (100) 20 (100) 11 (100)</td>
<td>41 (100) 6 (100)</td>
</tr>
<tr>
<td>afa/draABC</td>
<td>4 (25) 6 (30) 3 (27)</td>
<td>12 (29) 1 (17)</td>
</tr>
<tr>
<td>sfa/focDE</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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Journal of Medical Microbiology 63
the potential for \textit{E. coli} isolated from the blood of patients with urosepsis to enter the blood from either the kidney or the GI tract in the majority of patients.

Alternatively, these isolates may contain adhesin molecules, such as P fimbriae, which has been shown to promote adherence of \textit{E. coli} to the urinary tract epithelium better than the GI epithelium. Using animal models, many studies have shown that adherence is an important step and even a predictor of translocation (Katayama et al., 1997; Katouli et al., 1997; Nettelbladt et al., 1997). Contrary to previous findings, this study identified no correlation between the number of adhering bacteria and their rate of translocation in A-498 and HT-29 cells (Katayama et al., 1997; Katouli et al., 1994; Nettelbladt et al., 1997).

Although the prevalence of the adhesive tip, FimH, has no correlation with uropathogenic strains (Johnson, 1991), its expression is crucial during the initial stages of infection to mediate binding to uroepithelial cells (Arthur et al., 1989; Holden & Gally, 2004) and plays an important role mediating invasion of host cells for the formation of intracellular bacterial communities. Type 1-piliated \textit{E. coli} can mediate host-cell invasion through the recognition of integrins (Eto et al., 2007) to allow bacteria to colonize, multiply and persist as intracellular bacterial communities (Berry et al., 2009). From the urinary tract, bacteria can gain entry into the bloodstream via the kidney, with no evidence that bacterial translocation can occur from the bladder. The patients in our study were suffering from cystitis, with no clinical data to support a diagnosis of pyelonephritis. Clinically, diagnosis between cystitis and pyelonephritis based on symptoms, especially among elderly patients with septicaemia, might be difficult. In view of the GI tract being a major reservoir for \textit{E. coli}, the potential exists for bacterial translocation across the GI epithelium. From the findings in this study, it is proposed that, in hospitalized patients with concurrent septicaemia and UTI, the source of translocation may in some cases be the GI tract. This is further supported by the fact that most septicaemic patients in this study were of a higher age group (median age 67 years), who often have a decreased immune system, a factor that has been linked with increased bacterial translocation (Grubeck-Loebenstein, 1997; MacFie et al., 1999; O’Boyle et al., 1998). We have previously shown a higher prevalence of uropathogenic VGs among \textit{E. coli} isolated from the gut of healthy elderly adults compared with young adults (Vollmerhausen et al., 2011), which may be a risk factor for extra-intestinal infections among hospitalized elderly patients. In the absence of any study characterizing the \textit{E. coli} population within the GI tract of such uroseptic patients, the translocation of \textit{E. coli} from the GI tract of patients with urosepsis cannot be fully justified.

In conclusion, we found that, whilst the majority of \textit{E. coli} strains isolated from the blood and urine of the same patient were identical, it is possible that some patients suffered infection by two different \textit{E. coli} strains in the urinary tract and in their bloodstream. The fact that non-identical pairs of isolates did not show the ability to translocate HT-29 cells may suggest that either this cell line is not as suitable as other cells such as Caco-2 for assessing translocation or as-yet-unidentified host factors may play a role in translocation from the GI tract in these patients. The fact that adherence to HT-29 cells was not correlated with translocation further suggests that factors other than adhesion are necessary for translocation of uropathogenic \textit{E. coli}. Based on these results, it is suggested that future studies on the source of bacterial translocation in patients with urosepsis include testing of additional cell lines as well as characterizing \textit{E. coli} populations of the gut at the time of hospitalization.

**REFERENCES**


