Characterization of *Escherichia coli* bloodstream isolates associated with mortality

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**INTRODUCTION**

Rates of *Escherichia coli* bacteraemia have increased steadily in recent years (de Kraker *et al.*, 2013). Concurrently, rates of multidrug resistant (MDR) *E. coli* infections are on the rise. In particular, the predominant extra-intestinal pathogenic *E. coli* (ExPEC) global lineage sequence type (ST) 131 is frequently associated with fluoroquinolone resistance and production of extended-spectrum \(\beta\)-lactamases (ESBLs) (Nicolas-Chanoine *et al.*, 2014).

Unlike other commensals found in the gastrointestinal tract (GT), ExPEC have an associated array of virulence factors that allow survival outside of this setting. Many of these virulence factors have been horizontally acquired and vary from strain to strain (Dobrindt *et al.*, 2003). They can be classed into adhesins, protectins, iron acquisition systems and toxins (Köhler & Dobrindt, 2011). Adhesins, such as the type 1 fimbriae, contribute to colonization of the urinary tract (UT) epithelium (Kaper *et al.*, 2004; Mulvey *et al.*, 2000; Ulett *et al.*, 2013). The UT is the most common source of infection in cases of bacteraemia (Russo & Johnson, 2003). Access to the bloodstream follows an ascending infection in which the F1C and P fimbriae play a role (Kaper *et al.*, 2004; Mulvey *et al.*, 2000). Many other adhesins have been associated with pathogenesis of *E. coli*, including S fimbriae, the Hek adhesin, antigen 43, the temperature-sensitive haemagglutinin (Tsh), F9 fimbriae and the Iha adhesin (Fagan & Smith, 2007; Köhler & Dobrindt, 2011; Ulett *et al.*, 2007; Wurpel *et al.*, 2014).

ExPEC that cross the tubular epithelial cell barrier and enter the bloodstream can induce a powerful host inflammatory response, resulting in sepsis. Sepsis is associated with high rates of morbidity and mortality (Laupland *et al.*, 2008; Russo & Johnson, 2003). To survive in the bloodstream, *E. coli* have to resist immune defences of the host, including the complement system and phagocytosis. Resistance to serum complement is strongly associated with *E. coli* that cause bacteraemia and with a higher incidence of sepsis and mortality (Johnson, 1991; Sarma & Ward, 2011). Surface-associated polysaccharides have been shown to be important in the defence against serum complement. Polysaccharides such as the K2 capsule, colanic acid and LPS promote survival in serum (Buckles *et al.*, 2009; Miajlovic *et al.*, 2014; Phan *et al.*, 2013). Other factors
associated with immune evasion include the outer-
membrane proteins A and T (OmpA and OmpT), TraT,
the increased serum survival protein (Iss), and the TIR
homologous protein TcpC (Cirl et al., 2008; Hui et al.,
2010; Mialjlovic & Smith, 2014).

An important defence against bacterial survival and growth
in the bloodstream is the lack of available iron, the majority
of which is sequestered intracellularly (Bullen et al., 2005;
Skaar, 2010). Acquisition of iron is facilitated by expression
of iron acquisition systems that are crucial to the fitness of
ExPEC (Subashchandrabose et al., 2013). The siderophore
receptor A (IreA) and the iron-binding protein SitA are
frequently identified in ExPEC, as are the siderophores
aerobactin, yersiniabactin and salmochelin (Köhler &
Dobrindt, 2011).

Many of the toxins produced by ExPEC contribute to dis-
dease pathogenesis and host tissue damage. Haemolysin A
and cytotoxic necrotizing factor 1 (CNF-1) play a role in
the pathogenesis of UT infections (Smith et al., 2008;
Mills et al., 2000). The genotoxin encoded by usp is associ-
ated with pyelonephritis, prostatitis and bacteraemia
(Nipić et al., 2013). Other toxins frequently identified in
ExPEC include the genotoxin colibactin, the autotranspor-
ter toxins Sat and Pic, and cytolethal distending toxin
(CDT-1) (Köhler & Dobrindt, 2011).

In this study, 20 ExPEC isolates that caused bloodstream
infection (BSI) and subsequent mortality in an Irish hospi-
tal were characterized. Whole-genome sequencing (WGS)
analysis was used to determine the predominant STs and
to identify ExPEC virulence factors. Phenotypic assays
determined resistance to the bactericidal action of serum and
pmorphonuclear leukocytes (PMNLs). The influ-
ence of bacterial and host factors on disease severity indi-
cated by early and late mortality was also assessed.

METHODS

Bacterial isolates and culture conditions. Bacterial isolates were
routinely cultured at 37 °C in Luria broth (LB) or agar. The uro-
pathogenic E. coli strain CFT073, which was used as a control in this
study, was originally isolated from the blood of a patient with acute
pyelonephritis (Mobley et al., 1990). Strain Nüssle 1917 is considered
non-pathogenic and was used as a negative control in serum and
neutrophil killing assays (Lodinová-Žádníková & Sonnenborn, 1997).

Data collection. Patient data were obtained from the laboratory BSI
database, the hospital information systems and the blood culture
clinical record book. These data included the age of the patient, sex,
present risk factors, co-morbidities, time to mortality and the
recorded source of infection. Ethical approval for this study was
granted by the SJH/AMNCH ethics committee (REC reference 2010/
07/02).

WGS. Chromosomal DNA was extracted from E. coli using an
ArchivePure DNA isolation kit (5 Prime). Samples were prepared for
sequencing using the Nextera XT DNA sample preparation kit
(Illumina). Resultant libraries were sequenced on an Illumina MiSeq
platform at the TrinSeq sequencing facility (Trinity College Dublin,
Ireland). Raw short-read data from sequenced strains have been
deposited at the European Nucleotide Archive (http://www.ebi.ac.uk/
enia), with study accession number PRJEB9927.

WGS analysis of bacteraemia isolates. Raw sequencing reads
were trimmed with Trimmomatic and assembled de novo using the
SPAdes assembler with default parameters for paired end data
(Bankevich et al., 2012; Bolger et al., 2014). The presence of virulence
genes (Table S1, available in the online Supplementary Material) in
each isolate was determined using BLASTN to query genome assembly
databases (Altschul et al., 1990). An enforced cut-off of 70 % identity
over at least 70 % of the virulence gene sequences queried was utilized
to distinguish between true and false matches. Multi-locus sequence
typing (MLST) was performed according to the Achtman E. coli
typing scheme using the MLST website (http://pubmlst.org/mlst/
developed by Keith Jolley at the University of Oxford (Jolley &
Maiden, 2010; Wirth et al., 2006). Subclones within the ST131 clonal
group were determined based on fimH allele sequence variation
(Weissman et al., 2012).

Antimicrobial susceptibility. Antimicrobial susceptibilities were
determined by the Vitek 2 system using Clinical and Laboratory
Standards Institute interpretive criteria (CLSI, 2015). Confirmation of
predicted ESBL-positive status was carried out using E-test strips
(bioMérieux) and EUCAST interpretative criteria.

Preparation of serum. The serum used in this study was collected
from four healthy volunteers into serum clot activator VACUETTE
tubes (Greiner Bio-One). Ethical approval for the use of human
bloods was sought and granted from the St James Hospital Ethics
Committee. Serum was prepared as described previously (Mialjlovic
et al., 2014). Serum was isolated by centrifugation of whole blood at
1000 g for 10 min, pooled and stored in at −80 °C.

Bactericidal serum assay. Serum resistance of E. coli bloodstream
isolates was assessed as follows. Exponential-phase bacteria were
collected by centrifugation and adjusted to 2.5 × 10^7 cfu ml^-1
in HBSS (Sigma Aldrich) supplemented with 10 % (v/v) normal human
serum (NHS). Samples were incubated at 37 °C for 110 min. Tenfold
serial dilutions of each sample were prepared and c.f.u. ml^-1
were quantified by the Miles and Misra method (Miles et al., 1938).

PMNL isolation. Blood was collected from healthy volunteers into
EDTA VACUETTE tubes. PMNLs were isolated from whole blood as
described previously (Oh et al., 2008). Briefly, 5 ml whole blood was
layered onto a Histopaque gradient (H1119 and H1077; Sigma
Aldrich) and centrifuged at 300 g for 10 min, red blood cells were
eliminated from the granulocyte pellet by resuspension in 2 ml red
cell lysis buffer (Sigma Aldrich). Granulocytes were washed in HBSS
before final resuspension in 2 ml HBSS (with CaCl2 and MgSO4;
Sigma Aldrich) containing 2 % human serum albumin (Sigma
Aldrich). Granulocytes were counted in a haemocytometer and
adjusted to 5 × 10^6 cells ml^-1.

Resistance to PMNL killing. Exponential-phase bacteria were
adjusted to 5 × 10^5 cells ml^-1 and incubated with 10 % NHS in HBSS
for 20 min at room temperature with shaking. Following opsoniza-
tion of bacterial cells, an equal volume of PMNLs was added to the
bacteria, resulting in an m.o.i. of 0.1. Opsonized bacteria and PMNLs
were incubated for 90 min at 37 °C with shaking, after which sur-
viving bacteria were quantified by the Miles and Misra method (Miles
et al., 1938).

Statistical analysis. The data presented in this study represent the
mean of three experiments ± SE unless otherwise stated. An unpaired
-test and Fisher’s exact test were used to determine statistical significance, with significance defined as \( P < 0.05 \).

**RESULTS**

**Bacteraemia isolates used in this study**

Twenty *E. coli* bloodstream isolates that were collected between 2004 and 2010, and that were associated with mortality, were selected for analysis. Severe initial bacteraemia was indicated by early mortality, which was classed as death that occurred within 7 days of hospital admission. Late mortality was defined as death occurring from 8 to 30 days following admission. Of the 20 selected strains, eight (40 %) were isolated from patients in whom early mortality occurred (Table 1). The mean age of these eight patients was 54 years, which is significantly lower than the mean age of patients with late mortality (86 years, \( P < 0.0001 \), unpaired \( t \)-test).

Other host factors, including gender, the source of origin of the bacteraemia and the existence of co-morbidities, were assessed to determine any association with early mortality. Early mortality was more common in patients whose infection originated in the GT (\( P < 0.0001 \) by Fisher’s exact test). Observationally, males were more likely to have bacteraemias that originated in the GT (80 %, Table 1), while the UT was the predominant source in females (80 %, Table 1). This difference was not quite significant when assessed by Fisher’s exact test (\( P = 0.06 \)). Alcoholic liver disease, hepatitis C cirrhosis and liver complications were recorded in 62.5 % of patients with early mortality compared with 16.7 % of patients with late mortality. This difference was not quite significant when assessed by Fisher’s exact test (\( P = 0.06 \)).

**Antibiotic resistance of *E. coli* bacteraemia isolates**

A high prevalence of antibiotic resistance was detected among the bacteraemia isolates. Forty per cent were resistant to one or more of the \( \beta \)-lactam class of antibiotics (Table 1). A further 40 % of the isolates were resistant to two or more classes of antibiotics and were categorized as MDR (Table 1). Of the MDR isolates, two (nos 13 and 14) encoded ESBLs. Only 20 % of the isolates were sensitive to all the antibiotics they were screened against. Detailed antibiotic resistance profiles for each isolate are presented in Table S2.

**MLSTs of *E. coli* bacteraemia isolates**

WGS data were analysed to determine the MLSTs of the *E. coli* used in this study. The allelic profiles of seven housekeeping genes were used to assign a defined MLST to each of the isolates. The ST of each isolate is indicated in Table 2. A total of nine different MLSTs were identified among the bacteraemia isolates. The most common ST, representing 25 % of the bacteraemia isolates, was ST131 (Table 2). All ST131 isolates were MDR and two encoded

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**Table 1. Bacteraemia isolates used in this study**

F, female; M, male.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Gender</th>
<th>Patient age (years)</th>
<th>Source*</th>
<th>Liver disease</th>
<th>Early death (within 7 days)</th>
<th>Late death (8–30 days)</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>88</td>
<td>UT</td>
<td>++</td>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>80</td>
<td>UT</td>
<td>++</td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>42</td>
<td>GT</td>
<td>++</td>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>52</td>
<td>GT</td>
<td>++</td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>80</td>
<td>Cath</td>
<td></td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>89</td>
<td>Cath</td>
<td></td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>38</td>
<td>GT</td>
<td>++</td>
<td></td>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>94</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>83</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>75</td>
<td>UT</td>
<td>++</td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>90</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>70</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>81</td>
<td>GT</td>
<td></td>
<td></td>
<td></td>
<td>MDR (ESBL)</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>93</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>MDR (ESBL)</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>85</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>44</td>
<td>GT</td>
<td></td>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>86</td>
<td>UT</td>
<td></td>
<td></td>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>89</td>
<td>Cath</td>
<td></td>
<td></td>
<td></td>
<td>MDR</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>74</td>
<td>GT</td>
<td></td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>31</td>
<td>GT</td>
<td></td>
<td></td>
<td></td>
<td>( \beta )-Lactam</td>
</tr>
</tbody>
</table>

* Cath, urinary catheter.
ESBLs (Table 1). These two isolates, numbers 13 and 14, harboured CTX-M-15 and CTX-M-27 enzymes respectively. The second most common ST (20%) was ST73, followed by ST69 (15%). Two isolates each were identified as ST393 and ST405 (Table 2). ST62, ST130, ST127 and ST453 each occurred once.

Presence of EXPEC-associated virulence factors

Data obtained from WGS were analysed to detect the presence of virulence factors associated with ExPEC. Adhesin genes included in the analysis were fimH, fimA, lha, papG, flu, hek, focA, sfaA and tsh. The fimH gene of the ST131 isolates was also analysed to ascertain the predominant subclones within this group (Weissman et al., 2012). The fimH30 allele was dominant, with only one of the five ST131 isolates belonging to a different subclone (H41, isolate 17). Although fluoroquinolone resistance is less common among the H41 than the H30 lineage, all the ST131 isolates in this study were ciprofloxacin resistant (Dahbi et al., 2014).

As well as adhesin genes, the presence of iron acquisition systems (iutA, sitA, fyuA, ireA and ireN), protectins (wca, kpsM, ompA, ompT, traT, tcpC and iss), toxins (cdt locus, sat, clb locus, pic, cnf-1, hlyA and usp) and other genes associated with virulence (malX and dsdA) was ascertained.

**Table 2. Virulence factors detected in E. coli bacteraemia isolates**

Presence of virulence factor (VF) is indicated by a ‘+’.

<table>
<thead>
<tr>
<th>Bacteraemia isolate</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ST</td>
<td>62</td>
</tr>
<tr>
<td><strong>Adhesin</strong></td>
<td></td>
</tr>
<tr>
<td>Type 1 fimbriae</td>
<td>+</td>
</tr>
<tr>
<td>F9 fimbriae</td>
<td>+</td>
</tr>
<tr>
<td>lha adhesin</td>
<td>+</td>
</tr>
<tr>
<td>P fimbriae</td>
<td>+</td>
</tr>
<tr>
<td>Antigen 43</td>
<td>+</td>
</tr>
<tr>
<td>Hek</td>
<td>+</td>
</tr>
<tr>
<td>FIC fimbrae</td>
<td>+</td>
</tr>
<tr>
<td>S fimbrae</td>
<td>+</td>
</tr>
<tr>
<td>Tsh</td>
<td>+</td>
</tr>
<tr>
<td><strong>Iron aquisition</strong></td>
<td></td>
</tr>
<tr>
<td>SitA</td>
<td>+</td>
</tr>
<tr>
<td>Salmochelin</td>
<td>+</td>
</tr>
<tr>
<td>Yersiniabactin</td>
<td>+</td>
</tr>
<tr>
<td>Aerobactin</td>
<td>+</td>
</tr>
<tr>
<td>IreA</td>
<td>+</td>
</tr>
<tr>
<td><strong>Protectins</strong></td>
<td></td>
</tr>
<tr>
<td>Colanic acid</td>
<td>+</td>
</tr>
<tr>
<td>OmpA</td>
<td>+</td>
</tr>
<tr>
<td>OmpT</td>
<td>+</td>
</tr>
<tr>
<td>Las</td>
<td>+</td>
</tr>
<tr>
<td>Capsule</td>
<td>+</td>
</tr>
<tr>
<td>TraT</td>
<td>+</td>
</tr>
<tr>
<td>TcpC</td>
<td>+</td>
</tr>
<tr>
<td><strong>Toxins</strong></td>
<td></td>
</tr>
<tr>
<td>CDT-1</td>
<td>+</td>
</tr>
<tr>
<td>Sat</td>
<td>+</td>
</tr>
<tr>
<td>Usp</td>
<td>+</td>
</tr>
<tr>
<td>Colibactin</td>
<td>+</td>
</tr>
<tr>
<td>Pic</td>
<td>+</td>
</tr>
<tr>
<td>CNF-1</td>
<td>+</td>
</tr>
<tr>
<td>HlyA</td>
<td>+</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>MalX</td>
<td>+</td>
</tr>
<tr>
<td>DsdA</td>
<td>+</td>
</tr>
<tr>
<td><strong>VF score</strong></td>
<td>16</td>
</tr>
</tbody>
</table>
in the bloodstream isolates. The sum of all virulence factors present in each isolate is represented as a virulence factor score in Table 2. Interrogation of the genomic sequence revealed that each isolate encoded multiple virulence factors, ranging in number from 14 to 29. The genes/loci fimH, sitA, iroN, fyuA, wca, ompA, cdt and malX were present in all 20 isolates. ST73 isolates had the highest virulence factor scores. The mean virulence factor score of ST73 isolates was 26.5 compared with 17.3 for all other STs (unpaired t-test, P < 0.0001). In particular, ST73 isolates had more genes encoding toxins and adhesins (Fig. 1).

Resistance of bloodstream isolates to killing by serum

Serum resistance is associated with E. coli that cause BSIs and has also been related to increased incidence of septic shock and mortality (Johnson, 1991). The ability of the bacteraemia isolates to survive in the presence of human serum was therefore assessed. Bacteria were incubated with serum over a 110 min time period, after which surviving bacteria were quantified as a percentage of the input number of cells (2.5 × 10⁵ c.f.u. ml⁻¹). Survival was compared with a known serum-resistant strain, CFT073, and the non-pathogenic strain Nissle 1917. Bacteria were classed as sensitive if survival was equal to or less than that of strain Nissle 1917 (Fig. 2).

Seventy-five per cent of the bloodstream isolates were classed as serum resistant (Fig. 2). Like CFT073, 55 % of the isolates were able not only to survive but to multiply...
in the presence of serum. These included isolates 1, 2, 3, 5, 6, 8, 9, 11, 13, 16 and 17. Serum exposure reduced survival of 20 % of the isolates, including isolates 7, 12, 15 and 18. However, they still survived in greater numbers than Nissle 1917 and were considered serum resistant. Five strains (25 %) were classed as serum sensitive. Isolates 4, 10 and 14 survived at very low levels, while isolates 19 and 20 were completely eradicated by serum exposure (Fig. 2).

Resistance of bloodstream isolates to killing by PMNLs

Deposition of complement components on the bacterial surface marks cells for clearance by phagocytes. In the absence of an active complement system, efficient phagocytosis cannot occur (Buckles et al., 2009). The ability of the bacteremia isolates to survive in the presence of PMNLs was compared with that of CFT073 and Nissle 1917. Following a 20 min incubation with serum (to promote opsonization), PMNLs were added for 90 min. Survival of bacteria in serum alone was compared with survival in serum with added PMNLs (Fig. 2). The fold reductions in survival are presented in Table S3. The addition of PMNLs reduced survival of the majority of bacteremia isolates to a small degree. This ranged from a 1.3-fold to a 33-fold reduction in survival (Table S3). Fifty per cent of the isolates (1, 3, 5, 6, 8, 9, 11, 13, 16 and 17), which included all but one of the highly serum-resistant isolates, also survived in high numbers in the presence of PMNLs (Fig. 2). While 25 % of isolates (2, 7, 15, 16 and 18) were reduced in numbers, they still survived at greater levels than the Nissle 1917 control. The serum-sensitive strains were either further reduced (isolates 4 and 14) by addition of PMNLs, or completely eradicated (isolate 10; Fig. 2). Since isolates 19 and 20 were completely eliminated by serum exposure alone, no conclusions can be drawn about their resistance to PMNLs.

DISCUSSION

Across Europe, rates of E. coli BSIs are on the rise (de Kraker et al., 2013). In this study, we characterized a number of ExPEC isolates that were the cause of bacteremia and subsequent mortality in an Irish hospital. ExPEC can gain access to and survive in the bloodstream owing to the possession of virulence factors that distinguish this group from other commensals of the gut. These virulence factors were well represented in E. coli bacteremia isolates that caused mortality. Isolates encoded varying numbers of these factors and even those with the lowest virulence scores possessed representatives of adhesins, iron acquisition factors, protecins and toxins. Early mortality was not significantly associated with any specific virulence factor of E. coli (assessed by Fisher’s exact test). This may be the result of the significant genome plasticity of ExPEC and expression of virulence factors whose functions overlap (Dobrindt et al., 2003; Skjøt-Rasmussen et al., 2012).

The most prevalent adhesins among the isolates were the type I and F9 fimbriae, which have been implicated in colonization of the UT and biofilm formation, respectively (Antão et al., 2009; Wurpel et al., 2014). In cases of bacteremia, access to the bloodstream generally follows ascending infection of the bladder and kidney. P fimbiae, which were detected in 55 % of isolates, play a role in this process. The predominant variant identified was PapGII, which has been associated with colonization of the mammalian kidney (Antão et al., 2009). Overall, adhesins were encoded in greatest numbers by ST73.

The genes encoding salmochelin, yersiniabactin and the iron-binding protein SitA were identified in all isolates. Iron acquisition systems are recognized as important factors for colonization of the UT, which is an iron-limited environment. Clinical isolates tend to encode multiple siderophores and express combinations of these depending on environmental factors such as pH and carbon source (Watts et al., 2012). Preferential expression of salmochelin and yersiniabactin has been reported in urinary strains (Henderson et al., 2009). Since the majority of bloodstream isolates used in this study originated from the UT, the prevalence of iron acquisition systems is not surprising. The bloodstream itself is an iron-limited environment, with the majority of iron sequestered intracellularly in haem-containing proteins. Studies have shown that haem receptors such as Hma, the Sit system, salmochelin, enterobactin and yersiniabactin contribute to fitness during systemic models of infection (Smith et al., 2010; Subashchandrabose et al., 2013).

Survival is also dependent on evasion of the immune defences of the host. The bloodstream isolates were well equipped to evade these defences. With one exception (TcpC), the protecins were detected in high numbers throughout all the STs. The same cannot be said of the toxins, which only occurred in high numbers in ST73. The consistently high numbers of protectins and iron chelators among the isolates suggest that iron acquisition and immune evasion are prioritized by ExPEC causing bacteremia.

The predominant STs identified in this study are in line with international epidemiological trends (Gibreel et al., 2012, b; Horner et al., 2014). ST131 was the most common ST identified, followed by ST73 and ST69. Along with ST95, these three STs predominate among large collections of E. coli bacteremia isolates (Alhashash et al., 2013; Gibreel et al., 2012, b; Horner et al., 2014). ST131 has emerged worldwide as a frequent cause of both community- and healthcare-associated BSIs (Nicolas-Chanoine et al., 2008, 2014). Treatment is complicated by the fact that MDR is frequently associated with ST131 (Coque et al., 2008; Johnson et al., 2010). All the ST131 bacteremia isolates characterized in this study were MDR and two encoded ESBLs. The overall prevalence of MDR in this study was 40 %, which is consistent with the high levels of
MDR that have been reported in E. coli causing bacteraemia (Alhashash et al., 2013).

Although a predominant cause of BSIs, ST131 had a moderate virulence factor score (mean score of 17.6) and was particularly lacking in adhesins compared with ST73. This corresponds with a previous report, which found the P fimbriae are absent in ST131 (Nicolas-Chanoine et al., 2008). Although the success of ST131 has not been related to any particular virulence factor, initial studies suggested malX, ompT and usp were more common in this ST (Johnson et al., 2009; Pitout et al., 2005). These three virulence factors were present in all ST131 isolates and were also present in ST73. A high metabolic rate may contribute to the fitness of this clone and compensate for moderate virulence capacity, although this is a matter of dispute (Alqasim et al., 2014; Gibreel et al., 2012a; Vimont et al., 2012).

Serum resistance is associated with E. coli causing BSIs and has also been linked to increased incidence of sepsis and mortality (Johnson, 1991). Of the bloodstream isolates, 75% were resistant to serum. The same isolates were also resistant to killing by phagocytes. All ST73 E. coli used in this study could be classed as resistant to both serum and PMNL killing. Interestingly, this ST also had the highest mean number of virulence factors, including the protectins, which defend against the host immune response. Despite this apparently high pathogenic potential, early mortality was not more common in patients with ST73 bacteraemias, nor was it associated with serum and PMNL resistance.

The majority of ST131 isolates were serum resistant. The serum resistome of an ST131 representative strain has been determined in a previous study (Phan et al., 2013). Genes involved in lipopolysaccharide and O-antigen chain synthesis were implicated in serum survival (Phan et al., 2013). Sequence comparisons revealed that these genes were highly conserved in the ST131 bacteraemia isolates, with one exception. Isolate 14 had five non-synonymous changes in the amino acid sequence of HyxA. This isolate was the only serum-sensitive ST131 strain. Deletion of the hyxA gene in EC985 resulted in modulation of O-antigen chain length and a 1000-fold reduction in serum survival (Phan et al., 2013). O-antigens are believed to protect bacteria by activating complement away from the outer membrane or by blocking antibody-binding sites (Miajlovic & Smith, 2014). It is possible that the differences in HyxA of isolate 14 resulted in expression of O-antigens that are less protective against the action of complement.

It has been reported that host factors outweigh bacterial factors when predicting morbidity and mortality (Hekker et al., 2000; Lefort et al., 2011). Surprisingly, in this study, early mortality was significantly associated with a younger patient age group (mean age 54). Upon further investigation, this group of patients had a high incidence of alcoholic liver disease and hepatitis C cirrhosis (62.5%). This was corroborated by liver function tests of early-mortality patients, which showed significantly elevated levels of bilirubin, y-glutamyltransferase, aspartate aminotransferase and alkaline phosphatase compared with late-mortality patients (Table S4). The mean age of early-mortality patients was increased to 75 years when those with alcoholism and liver disease were excluded from the analysis. However, this was still significantly lower than the mean age of late-mortality patients (86 years, P=0.01).

Synthesis of 90% of plasma complement components occurs in the liver (Qin & Gao, 2006). In cases of alcoholic liver disease, serum levels of complement components are decreased compared with healthy individuals, resulting in a greater risk of bacterial and fungal infection (Homann et al., 1997). Four out of five of the serum-sensitive isolates (4, 10, 19 and 20) originated from patients in whom liver disease was recorded as a risk factor or liver function tests were indicative of liver disease (Table S4). It is likely that this contributed to survival of serum-sensitive isolates in the bloodstream, since they would otherwise be eliminated by a fully functioning complement system. Only one of the serum-sensitive E. coli (no. 14) was isolated from a patient in whom no indications of liver disease were apparent. However, the advanced age of this patient (93 years) may have been a contributing factor, since extremes of age are known to adversely affect immunity. Pneumonia, neutropaenia, solid-organ cancer and chronic renal insufficiency, which also negatively affect host immunity, have also been implicated in BSI mortality (Ortega et al., 2009).

Another host factor that was linked to early mortality was the source of origin of the infection. Early mortality was more common in bacteraemia originating from the GT. Observationally, patients with bacteraemias originating in the GT were predominantly male (86%). Previous studies have reported that severe initial bacteraemia originating from the digestive tract was associated with male gender (Jauregy et al., 2007). Later mortality was more frequently seen in females where the UT was the source of origin. Lower mortality has been noted previously in patients whose bacteraemia originated from the UT, and it has been suggested that concentrations of bacteria in the blood may be lower in these cases (Jauregy et al., 2007; Lefort et al., 2011; Peralta et al., 2007).

As a group, ExPEC encode a wide range of virulence factors that allow these bacteria to thrive as both commensals and pathogens. While these are important in establishing infection and contributing to disease pathogenesis, the importance of host immunity should not be overlooked. Factors affecting immunity, such as liver disease, can undermine the functionality of the innate immune system, allowing survival of bacteria that would be killed in healthy individuals.

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