Comparative genomic analysis of ESBL-producing Escherichia coli from faecal carriage and febrile urinary tract infection in children: a prospective multicentre study

Philippe Bidet1,2*, André Birgy1,2, Naim Ouldali3,4, Stéphane Béchet3, Corinne Levy3,5,6, Fouad Madhi3,5,6, Elsa Sobral3, Robert Cohen3,5,6 and Stéphane Bonacorsi1,2

1Université Paris Cité, IAME, INSERM, F-75018 Paris, France; 2Service de Microbiologie, Centre National de Référence associé pour Escherichia coli, Hôpital Robert-Debré, AP-HP, Paris, France; 3Association Clinique Thérapeutique Infantile du Val de Marne (ACTIV), Créteil, France; 4Service de Pédiatrie Générale, Hôpital Robert-Debré, AP-HP, Paris, France; 5Université Paris Est, IMRB-GRC GEMINI, Créteil, France; 6GPIP (Groupe de Pathologie Infectieuse Pédiatrique) de la SFP (Société Française de Pédiatrie), Paris, France; 7Service de Pédiatrie Générale, Centre Hospitalier Intercommunal de Créteil, Créteil, France

*Corresponding author. E-mail: philippe.bidet@aphp.fr

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Background: The reliability of ESBL-producing Escherichia coli (ESBL-Ec) faecal carriage monitoring to guide probabilistic treatment of febrile urinary tract infection (FUTI) in children remains unclear.

Objectives: To compare the genomic characteristics of ESBL-Ec isolates from faecal carriage and FUTI to assess their correlation and identify a FUTI-associated virulence profile.

Methods: We conducted a prospective multicentre hospital and ambulatory-based study. We analysed the genotypes and virulence factors of both faecal and FUTI ESBL-Ec by whole genome sequencing. Correlations were assessed by non-parametric Spearman coefficient and virulence factors were assessed by chi-squared tests with Bonferroni correction.

Results: We included 218 ESBL-Ec causing FUTI and 154 ESBL-Ec faecal carriage isolates. The most frequent ST was ST131 (44%) in both collections. We found high correlation between carriage and ESBL-Ec FUTI regarding genes/alleles (rho = 0.88, P < 0.0001) and combinations of virulence genes, MLST and serotypes (rho = 0.79, P = 0.0003, rho = 0.97, P = 0.005 respectively). Beside this strong correlation, we found five genes that were significantly associated with FUTI (papC, papGII, hlyC, hek and traJ). The strongest association with FUTI was found with adhesin gene allele papGII (54% in FUTI versus 16% in carriage) and for papGII and gene traJ alone or in combination (63% versus 24%).

Conclusions: The genomic profile of ESBL-Ec causing FUTI in children strongly correlates with faecal carriage isolates except for a few genes. The presence of papGII and or traJ in a previously identified carriage strain could be used as a marker of uropathogenicity and may guide the empirical antimicrobial choice in subsequent FUTI.

Introduction

Both antimicrobial resistance and extraintestinal virulence factors have increased in Escherichia coli faecal carriage isolates over the last decades. However, the link between virulence and resistance in E. coli has raised many debates and controversy. While several studies have reported that resistance to quinolones was associated with less-virulent strains, or that highly virulent clonal groups harbour fewer resistance determinants, the recent ESBL pandemic is linked to a sequence type ST131 belonging to the virulent phylogenetic group B2. In this study we have focused on the ESBL-producing E. coli (ESBL-Ec) strains isolated in children both in community-acquired febrile urinary tract infections (FUTI) and during asymptomatic faecal carriage to compare their virulomes.

In children, as in adults, E. coli is by far the most frequent aetiological agent of FUTI both in hospitalized patients and in the community. Pathophysiology of FUTI begins with urethral colonization by uropathogenic E. coli (UPEC) strains issuing from the gut microbiota. Strains causing pyelonephritis harbour various virulence factors facilitating ascending colonization of the urinary tract.
Pyelonephritis is particularly harmful in young children, and can be associated with bacteraemia and renal scarring, which may eventually be complicated by chronic high blood pressure or renal failure.7,8 Delayed antimicrobial therapy in FUTI is associated with adverse outcome.8

As about 50% of strains produce class A β-lactamases, empirical antimicrobial therapy of E. coli pyelonephritis mainly consists of either third-generation cephalosporins and/or aminoglycosides.9 Resistance to those antibiotics is increasing worldwide both in adults and children, mainly due to the emergence of ESBL-producing strains.10–12 The increasing percentage of ESBL-producing strains thus questions empirical antimicrobial choice.

However, implementing carbapenems as first-line antibiotics for treatment of this common infection would lead to an increase in carbapenem-resistant strains in children, with adverse consequences for future patients.13 Rapid tests (either phenotypic or genetic) able to screen for ESBL directly in urine can be used to guide antimicrobial choice but have some drawbacks (such as unavailability at the time of diagnosis, or contamination by a few ESBL-producing bacteria not involved in the infectious process).14,15

Faecal carriage monitoring of resistant strains has been proposed to adapt empirical antimicrobial therapy before drug susceptibility testing (DST) results on infecting bacteria are available, especially for patients particularly at risk of invasive infection such as patients with known urologic disorder, premature infants or immunocompromised children.16 This strategy relies on the hypothesis that ESBL-Ec causing infections correlate with ESBL-Ec carriage isolates, a postulate which remains to be further confirmed.

Unfortunately, these patients, who are often hospitalized for long periods of time, are also more frequently carriers of multidrug-resistant bacteria and thus more frequently treated with broad-spectrum β-lactams.17 A method allowing the detection among resistant faecal carriage strains of those more likely to cause FUTI would be helpful to focus effective antibiotics against these strains on patients carrying them.

In this study, we first assessed the genomic correlation between ESBL-Ec faecal carriage and ESBL-Ec causing community-acquired FUTI. We then aimed to identify a FUTI-associated virulence profile by detecting genomic characteristics of E. coli isolates significantly associated with FUTI.

**Whole-genome sequencing and genotyping**

The Nextera XT kit (Illumina, USA) was used to prepare libraries. Sequencing was performed on a HiSeq instrument for 2 × 100 cycles (Illumina Technology). The SPAdes assembler was used to construct assemblies. The quality of the sequencing data was estimated using standard metrics including N50 and coverage. Identification of acquired ESBL genes was performed using ResFinder 4.1 and MLST for E. coli (Warwick scheme).20,21 We also determined serotype, fimH allele and phylogenetic groups (SerotypeFinder 1.1, fimTyper and Enterobase).22–24 Within E. coli of ST131, we distinguished different clades as previously defined.25–28 Clade A is associated with fimH41, clade B with fimH22 and clade C with fimH30. Within clade C, two subclades have been identified. The C1 subclade, also called ST131 H30R, comprises isolates with mutations in the chromosomal gyrA and parC genes, which confer resistance to fluoroquinolones. Subclade C1 was then separated between isolates harbouring blaCTX-M-27 (C1-M27) or not (C1nM27).29 The C2 subclade, also called ST131 H30-Rx, groups isolates with the same gyrA and parC mutations, and the blaCTX-M-15 gene.

Raw reads have been deposited in GenBank under BioProject PRJNA551371 for FUTI isolates31 and under BioProjects PRJNA52236719 for faecal carriage isolates (under completion).

**Virulence factors**

Virulence factor genes were identified using the NCBI BLAST tool (BLAST version 2.2.31) to search for 180 genes and alleles (Table S1, available as Supplementary data at JAC-AMR Online). A threshold of 90% for both coverage and identity was used. The presence of a putative pSS8-like ColV plasmid was defined as co-occurrence of iroD, iucC, sitA, hlyF and mig14.30 Among putative pathogenicity islands (PAIs), PAI-II-J96/PAI-I-C5 was defined by the co-occurrence of hlyC, papC, cnf1 and hek.31 PAI-I-CFT073 (phev inserted) by hlyC, papC, iucC, iha and sat.32 PAI-III-536/PAI-CFT073-serX by sfa5 and iroD.33

**Statistics**

First, we analysed the correlation between genomic characteristics from faecal carriage isolates and ESBL-Ec strains causing FUTI using the non-parametric Spearman correlation coefficient. Correlation was assessed for genes/alleles, and then combination of genes, MLST and serotypes.

Second, we compared the proportion of each gene or allele among FUTI isolates versus carriage isolates using chi-squared tests. Bonferroni correction was used to avoid random false-positive associations among the 180 independent chi-squared tests performed for virulence genes. P values were in this case considered significant if < 0.00028.

**Ethics**

The data collection was approved by the French National Data Protection Commission (CNIL, no. 913582), the Committee on the Processing of Research Information (CCTIRS, no. 13.341) and the Ethics Committee of the Créteil Intercommunal Hospital. All legal guardians of included children provided oral informed consent. The study was registered at ClinicalTrials.gov (registration no. NCT02832258).

**Results**

**Population characteristics**

The median age was 13 months (IQR 10–17) for children from the carriage cohort and 12 months (IQR 4–30) for children from the FUTI cohort. A uropathy was identified in 42/218 (19%) children with FUTI.

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**Material and methods**

**Study design, patients and bacteria**

We conducted a prospective ambulatory and hospital-based multicentre study. We collected paediatric community-acquired FUTI cases caused by ESBL-Ec from March 2014 to March 2017 in a French tertiary hospital as previously described.11,18 From October 2010 to July 2017, eight French ambulatory paediatricians located in three regions (Île de France, Lorraine and Provence-Alpes-Côte d’Azur) took part in a prospective study analysing faecal carriage of ESBL-Ec among children aged 6 to 24 months in the community as previously described.10,19 All isolates from faecal carriage and FUTI were whole genome sequenced. Some of the isolates found in FUTI have been previously described globally to objectify the diversity and trends in ESBL-producing Enterobacteriaceae found in FUTI in children. However, their virulence content has not been studied exhaustively and they had not been compared with faecal isolates.11
The sex ratio was 0.80 (44.5% of male children) in the FUTI population and 0.92 (48.0% of male children) in the carriage population.

**Sequencing results**
We included and whole genome sequenced 218 FUTI ESBL-Ec and 154 faecal carriage isolates.

Sequencing data are presented in Table S2. Mean coverage was $10^4 \times$ and mean N50 was 173,714 bp.

**Sequence types, serotypes and fimH types**
The most frequent molecular features of the strains are presented in Table 1, detailed molecular typing is presented in Table S1. The most frequent phylogroup was B2 both in FUTI (64%) and carriage isolates (60%) whereas the most frequent STs were ST131 (44%), ST38 (9%), ST69 (6%), ST73 (3%) and ST95 (3%) in both collections with similar rates. The most frequent serotypes were O25:H4 (38%), O16:H5 (8%), both of them associated with ST-complex (Stc) 131, O6:H1 (2%), O75: H5 (3%) and O86:H18 (5%). Among the different clades and subclades of ST131, subclade C2H30Rx was associated with FUTI (27% versus 16%, $P < 0.05$) while clade C1 and subclade C1-M27 were associated with carriage isolates (21 and 18% versus 7 and 5%, respectively, $P < 0.05$). The most frequent fimH types encountered were fimH30 (40%), fimH27 (14%) and fimH41 (9%). The percentage of fimH27 strains was higher among FUTI strains than among carriage strains (18% versus 8%, $P = 0.007$). This particular fimH27 was associated with various STs, the most frequent being ST69 (34%).

**Genomic correlation between E. coli faecal carriage and ESBL-Ec causing FUTI**
We found a very high correlation between carriage and ESBL-Ec FUTI regarding the 180 genes/alleles associated with E. coli virulence (Table S1) ($r_h = 0.88$, $P < 0.0001$, Figure 1a).

Then, we confirmed this highly significant correlation between carriage and ESBL-Ec FUTI when analysing combinations of virulence genes ($r_h = 0.90$, $P < 0.0001$), MLST ($r_h = 0.99$, $P = 0.0003$), and serotypes ($r_h = 0.97$, $P = 0.005$ respectively, Figure 1b–d).

**Putative virulence genes**
Among the 180 genes associated with E. coli virulence, 27 were found in more than 20% of FUTI isolates (Table 1). Most of them were genes involved in iron capture (yersiniabactin: yfuA and irp2; salmochelin: iroD; aerobactin: iucC and iutA, chuA, sitA); capsule biosynthesis (kpsM, KpaA); adhesion (type P-pili: papC and papGII, iha, eIIa, nfaE, aacC); invasion (hek) or cell toxicity (hlyC, cnf1, sat, senB).

After Bonferroni correction, only 5 of the 180 putative or known virulence genes and alleles searched (papC, papGII, hlyC, hek and traJ) remained significantly associated with FUTI (Table 1 and Table S1). All papGII-positive isolates were also papC positive, both genes belonging to the pap operon. The strongest association with FUTI was found for PapG adhesin gene allele papGII (54% versus 16%, $P = 8.8079E-14$). Among FUTI cases papGII was present in 48% of those with a known urolologic disorder versus 55% of those without any ($P = 0.3597$, non-significant). Adhesin papGII was found in 75% of fimH27 FUTI isolates versus 23% of fimH27 carriage isolates.

We tested the possibility to combine papGII with the other genes (except papC) also associated with urinary virulence (Table 1 and Figure 1b) in order to increase the percentage of FUTI isolates categorized with a risk genotype, a low-risk isolate being an isolate without any of the markers.

The strongest association of gene combinations with FUTI was found with the combination of papGII and/or traJ isolates harbouring at least one of these two genes represented 63% of FUTI cases versus 24% of healthy carriage cases. This dual detection used as a screening test would thus have a sensitivity of 63%, a specificity of 76%, a positive predictive value of 79% and a negative predictive value of 59% (Figure S1).

**Combinations of genes suggesting the presence of putative PAIs and virulence plasmid**
The presence of a putative pS88-like ColV plasmid (defined as co-occurrence of iroD, iucC, sitA, hlyF and mlg14) was found in about 10% of strains without significant difference between FUTI and carriage. Among putative PAIs, PAI-1-CFT073 (defined as the co-occurrence of hlyC, papC, iucC, iha-like and sat) was significantly more frequent among FUTI isolates than among carriage isolates (41% versus 14%, $P < 0.01$). PAI-II-J96/PAI-1-C5 (defined as the co-occurrence of hlyC, papC, cnf1 and hek) was found in 19% of FUTI isolates versus 8% of carriage isolates, however this difference did not remain significant after Bonferroni correction.

**ESBL genes**
The most frequent ESBL genes encountered among the whole collection of isolates (FUTI and carriage) were $\text{bla}_{CTX-M-15}$ ($n = 161$, 43%), $\text{bla}_{CTX-M-14}$ ($n = 72$, 19%), $\text{bla}_{CTX-M-27}$ ($n = 71$, 19%) and $\text{bla}_{CTX-M-1}$ ($n = 44$, 12%).

$\text{bla}_{CTX-M-15}$ was more frequent among FUTI isolates (52% versus 31%, $P < 0.01$), while $\text{bla}_{CTX-M-27}$ and $\text{bla}_{CTX-M-1}$ were less frequently encountered among carriage isolates (8% versus 18 and 14% versus 26%, respectively, $P < 0.01$).

Whatever the origin, isolates carrying $\text{bla}_{CTX-M-15}$ were more frequently equipped with PapGII adhesin (55%) than those carrying either $\text{bla}_{CTX-M-1}$ (11%) or $\text{bla}_{CTX-M-27}$ (18%), $P < 0.01$.

The gene $\text{traJ}$ was present in 36.4% of $\text{bla}_{CTX-M-15}$, 19.4% of $\text{bla}_{CTX-M-14}$, 26.3% of $\text{bla}_{CTX-M-1}$, and 1.4% of $\text{bla}_{CTX-M-27}$ isolates.

**Comparison of O25:H4 and O16:H5 subclones among Stc-131 isolates**
Among STc131 isolates of the whole collection, those belonging to O25:H4 serotype ($n = 141$) more frequently harboured PapGII adhesin and $\text{bla}_{CTX-M-15}$ $\beta$-lactamase gene than O16:H5 serotype ($n = 31$) (50% versus 26%, $P < 0.05$ and 63% versus 26%, $P < 0.05$, respectively) (Table 2).

**Comparison of St131 clades and subclades**
$\text{bla}_{CTX-M-15}$ was present in 32% of clade A isolates, none of the C1 isolates, and 100% of C2 isolates. The gene papGII was present in 28% of clade A isolates, 16.7% of C1 isolates, and 68.7% of C2 isolates.
Table 1. Most frequent virulence-associated genes/alleles, genotypes and serotypes among febrile urinary tract infection (FUTI) and faecal carriage ESBL-producing *E. coli* isolates in children

| Characteristic | All isolates | FUTI isolates | Carriage isolates | Delta % (FUTI–carriage) | P value | $\chi^2$ with Bonferroni correction |
|----------------|--------------|---------------|------------------|-------------------------|---------|-----------------------------------|
| **Single gene(s)/allele(s)** | | | | | | |
| *gad* | 351 | 94% | 205 | 94% | 146 | 95% | −1% | NS | NS | |
| *fyuA* | 333 | 90% | 202 | 93% | 131 | 85% | 8% | <0.05 | NS | |
| *chuA* | 331 | 89% | 200 | 92% | 131 | 85% | 7% | <0.05 | NS | |
| *sitA* | 321 | 86% | 196 | 90% | 125 | 81% | 9% | <0.05 | NS | |
| *kpsF* | 312 | 84% | 190 | 87% | 122 | 79% | 8% | <0.05 | NS | |
| *irp2* | 287 | 77% | 175 | 80% | 112 | 73% | 8% | NS | NS | |
| *iss* | 294 | 79% | 175 | 80% | 119 | 77% | 3% | NS | NS | |
| *iucC* | 289 | 78% | 174 | 80% | 115 | 75% | 5% | NS | NS | |
| *iutA* | 291 | 78% | 174 | 80% | 117 | 76% | 4% | NS | NS | |
| *shF* | 290 | 78% | 174 | 80% | 116 | 75% | 4% | NS | NS | |
| *ihA-like* | 233 | 63% | 144 | 66% | 89 | 58% | 8% | NS | NS | |
| *sat* | 225 | 60% | 137 | 63% | 88 | 57% | 6% | NS | NS | |
| *papC* | 157 | 42% | 126 | 58% | 31 | 20% | 38% | <0.05 | <0.00028 | |
| *papGII* | 141 | 38% | 117 | 54% | 24 | 16% | 38% | <0.05 | <0.00028 | |
| *imm* | 194 | 52% | 116 | 53% | 78 | 51% | 3% | NS | NS | |
| *senB* | 175 | 47% | 105 | 48% | 70 | 45% | 3% | NS | NS | |
| *hek* | 134 | 36% | 97 | 44% | 37 | 24% | 20% | <0.05 | <0.00028 | |
| *hlyC* | 93 | 25% | 74 | 34% | 19 | 12% | 22% | <0.05 | <0.00028 | |
| *traJ* | 80 | 22% | 62 | 28% | 18 | 12% | 17% | <0.05 | <0.00028 | |
| K2-type *kfIA* | 75 | 20% | 57 | 26% | 18 | 12% | 14% | <0.05 | NS | |
| *elIA* | 88 | 24% | 56 | 26% | 32 | 21% | 5% | NS | NS | |
| *cnf1* | 70 | 19% | 52 | 24% | 18 | 12% | 12% | <0.05 | NS | |
| *nfoE* | 74 | 20% | 50 | 23% | 24 | 16% | 7% | NS | NS | |
| *aatC* | 72 | 19% | 48 | 22% | 24 | 16% | 6% | NS | NS | |
| *iroD* | 74 | 20% | 45 | 21% | 29 | 19% | 2% | NS | NS | |
| *astA* | 66 | 18% | 44 | 20% | 22 | 14% | 6% | NS | NS | |
| K5-type *kfIA* | 87 | 23% | 43 | 20% | 44 | 29% | −9% | <0.05 | NS | |
| **Combinations** | | | | | | | |
| *papGII and/or hlyC and/or traJ* | 187 | 50% | 143 | 66% | 44 | 29% | 37% | <0.05 | <0.00028 | |
| *papC and/or traJ* | 185 | 50% | 142 | 65% | 43 | 28% | 37% | <0.05 | <0.00028 | |
| *papC and/or hek* | 194 | 52% | 141 | 65% | 53 | 34% | 30% | <0.05 | <0.00028 | |
| *papGII and/or hek* | 192 | 52% | 139 | 64% | 53 | 34% | 29% | <0.05 | <0.00028 | |
| *papGII and/or hlyC and/or hek* | 192 | 52% | 139 | 64% | 53 | 34% | 29% | <0.05 | <0.00028 | |
| *papGII and/or traJ* | 174 | 47% | 137 | 63% | 37 | 24% | 39% | <0.05 | <0.00028 | |
| *papC and/or K2* | 175 | 47% | 133 | 61% | 42 | 27% | 34% | <0.05 | <0.00028 | |
| *papGII and/or hlyC and/or K2* | 172 | 46% | 130 | 60% | 42 | 27% | 32% | <0.05 | <0.00028 | |
| *papC and/or hlyC* | 160 | 43% | 128 | 59% | 32 | 21% | 38% | <0.05 | <0.00028 | |
| *papC and/or cnf1* | 160 | 43% | 128 | 59% | 32 | 21% | 38% | <0.05 | <0.00028 | |
| *papGII and/or K2* | 160 | 43% | 125 | 57% | 35 | 23% | 35% | <0.05 | <0.00028 | |
| *papGII and/or hlyC* | 154 | 41% | 123 | 56% | 31 | 20% | 36% | <0.05 | <0.00028 | |
| *papGII and/or cnf1* | 154 | 41% | 123 | 56% | 31 | 20% | 36% | <0.05 | <0.00028 | |
| *papGII and/or hlyC and/or cnf1* | 154 | 41% | 123 | 56% | 31 | 20% | 36% | <0.05 | <0.00028 | |
| **Putative pathogenicity island/plasmid pS88** | | | | | | | |
| PAI I CFT073 (hlyC, papC, iucC, iha-like, sat) | 110 | 30% | 89 | 41% | 21 | 14% | 27% | <0.05 | <0.00028 | |
| PAI IIJ96/PAI ICS (hlyC, papC, cnf1, hek) | 54 | 15% | 42 | 19% | 12 | 8% | 11% | <0.05 | NS | |
| PAI III536 (sfos, iroD) | 9 | 2% | 5 | 2% | 4 | 3% | 0% | NS | NS | |
| pS88 (iroD, iucC, sitA, hlyF, mig14, traJ) | 27 | 7% | 16 | 7% | 11 | 7% | 0% | NS | NS | |

Continued
isolates. The gene \textit{traJ} was absent in clade A and C1 isolates but present in 36.1% of C2 isolates.

**Discussion**

In this study we have compared the genomes of ESBL-Ec isolates from children with either healthy gut carriage or FUTI. We first found a strong correlation between the genomics of ESBL-Ec faecal carriage and ESBL-Ec causing FUTI. This similar genomic profile between carriage and infection has been recently suggested by Verschuuren et al.,\textsuperscript{34} who found that the distributions of the 10 most prevalent genes from ESBL-Ec faecal carriage and extraintestinal infection often overlapped. Taken together, these findings suggest that faecal carriage monitoring may be a valuable tool to monitor ESBL-Ec strains involved in FUTI.

Beside this high correlation, we identified some particular bacterial traits associated with invasive infection. A few genes were significantly associated with ESBL-Ec FUTI. After Bonferroni’s adjustment, \textit{papGII}, \textit{papC}, \textit{hlyC}, \textit{hek} and \textit{traJ} were positively associated with a risk of FUTI among ESBL-Ec isolates. All those five genes or gene alleles have been previously associated with \textit{E. coli} extraintestinal virulence, implicated at different steps of the pathogenesis process.

\textit{Gene \textit{papC} and the \textit{papGII} allele of gene \textit{papG} belong to the same operon encoding type P pilus. Thus, both genes are physically linked and all \textit{papGII}-positive strains are also \textit{papC} positive. PapC is a conserved outer-membrane protein acting as a molecular usher in type P pilus assembly while \textit{PapG} mediates adhesion to urothelial globoside’s (α-gal-1-4 β-gal) disaccharide.\textsuperscript{35} Several alleles of adhesin \textit{PapG} exist with different tropisms. \textit{PapGII} is the allele specifically involved in the pathogenesis of pyelonephritis. \textit{E. coli} strains lacking this allele rarely cause pyelonephritis except in case of pre-existing urinary tract abnormalities such as urinary tract obstruction, anatomical abnormalities or vesicoureteral

### Table 1. Continued

| Characteristic                                      | All isolates (n = 372) | FUTI isolates (n = 218) | Carriage isolates (n = 154) | Delta % (FUTI−carriage) | \( \chi^2 \) with Bonferroni correction |
|-----------------------------------------------------|------------------------|-------------------------|-----------------------------|--------------------------|----------------------------------------|
| FimH types                                          |                        |                         |                             |                          |                                        |
| \textit{fimH30}                                      | 147 40%                | 84 39%                  | 63 41%                      | −2%                      | NS                                     |
| \textit{fimH27}                                      | 53 14%                 | 40 18%                  | 13 8%                       | 10%                      | <0.05                                  |
| \textit{fimH41}                                      | 34 9%                  | 17 8%                   | 17 11%                      | −3%                      | NS                                     |
| MLST (Warwick scheme)                               |                        |                         |                             |                          |                                        |
| ST131                                               | 164 44%                | 92 42%                  | 72 47%                      | −5%                      | NS                                     |
| ST38                                                | 32 9%                  | 23 11%                  | 9 6%                        | 5%                       | NS                                     |
| ST69                                                | 21 6%                  | 15 7%                   | 6 4%                        | 3%                       | NS                                     |
| ST73                                                | 13 3%                  | 9 4%                    | 4 3%                        | 2%                       | NS                                     |
| ST95                                                | 12 3%                  | 9 4%                    | 3 2%                        | 2%                       | NS                                     |
| phylogroup B2                                       | 232 62%                | 140 64%                 | 92 60%                      | 4%                       | NS                                     |
| Serotype                                            |                        |                         |                             |                          |                                        |
| \textit{O25:H4}                                      | 141 38%                | 80 37%                  | 61 40%                      | −3%                      | NS                                     |
| \textit{O16:H5}                                      | 31 8%                  | 17 8%                   | 14 9%                       | −1%                      | NS                                     |
| \textit{O86:H18}                                     | 20 5%                  | 11 5%                   | 9 6%                        | −1%                      | NS                                     |
| \textit{O75:H5}                                      | 10 3%                  | 7 3%                    | 3 2%                        | 1%                       | NS                                     |
| \textit{O6:H1}                                       | 9 2%                   | 6 3%                    | 3 2%                        | 1%                       | NS                                     |
| ESBL genes                                           |                        |                         |                             |                          |                                        |
| \textit{bla\textsubscript{CTX-M-15}}                | 161 43%                | 114 52%                 | 47 31%                      | 21%                      | <0.05                                  |
| \textit{bla\textsubscript{CTX-M-14}}                | 72 19%                 | 39 18%                  | 33 21%                      | −3%                      | NS                                     |
| \textit{bla\textsubscript{CTX-M-27}}                | 71 19%                 | 31 14%                  | 40 26%                      | −12%                     | <0.05                                  |
| \textit{bla\textsubscript{CTX-M-1}}                 | 44 12%                 | 17 8%                   | 27 18%                      | −10%                     | <0.05                                  |
| Subclades of ST131                                   |                        |                         |                             |                          |                                        |
| Clade A                                              | 25 7%                  | 15 7%                   | 10 6%                       | 1%                       | NS                                     |
| C1                                                   | 48 13%                 | 15 7%                   | 33 21%                      | −14%                     | <0.05                                  |
| C1-M27                                               | 37 10%                 | 10 5%                   | 27 18%                      | −13%                     | <0.05                                  |
| C1-nM27                                              | 11 3%                  | 5 2%                    | 6 4%                        | −2%                      | NS                                     |
| C2/H30Rx                                             | 83 22%                 | 58 27%                  | 25 16%                      | 11%                      | <0.05                                  |

\footnote{For each gene/allele or combination of genes, the number and percentage of isolates harbouring this attribute is indicated. The Delta % is calculated as the percentage in FUTI isolates minus the percentage in faecal carriage isolates. Chi squared \( \chi^2 \) P value is indicated without and with Bonferroni correction. NS, non-significant.}
Catheter-associated bacteriuria isolates are also less likely to harbour this virulence factor. In our population, a urologic disorder was documented in 23% of FUTI cases and $papGII$ was slightly less frequent in those children (48%) than in those without known abnormality (55%), but this difference was not significant. Thus, the low percentage of $papGII$-positive isolates in our collection of ESBL-Ec isolates causing FUTI, contrasting with the higher percentages (70%–80%) usually observed in other studies on pyelonephritis in children, could not be explained by a higher rate of urinary tract impairment. However, the clinical data that were collected concerned only already known disorders at the time of infection and not those that could have been discovered following this episode of FUTI, and the actual percentage of urologic disorders may in fact have been higher.

The presence of $papGII$ was significantly more frequent among ESBL-Ec isolates carrying the $bla_{CTX-M-15}$ gene than among those carrying $bla_{CTX-M-1}$ or $bla_{CTX-M-27}$, thus explaining the association of $bla_{CTX-M-15}$ with FUTI. Among STc-131 isolates, this link between $papGII$ and $bla_{CTX-M-15}$ was related to the emergent O25:H4 serotype frequently harbouring both attributes.

**Table 2.** Comparison of O16:H5 and O25:H4 among STc-131 isolates

| Number and percentage of isolates | O16:H5 | O25:H4 | Delta % | $\chi^2$ | P value |
|-----------------------------------|--------|--------|---------|---------|---------|
| All STc-131 isolates              |        |        |         |         |         |
| $(n = 31)$                        |        |        |         |         |         |
| $(n = 141)$                       |        |        |         |         |         |
| $papGII$                          | 8      | 26%    | 70      | 50%     | $-24\%$ | $<0.05$ |
| $bla_{CTX-M-14}$                  | 6      | 19%    | 11      | 8%      | $12\%$  | NS      |
| $bla_{CTX-M-15}$                  | 8      | 26%    | 89      | 63%     | $-37\%$ | $<0.05$ |
| $bla_{CTX-M-27}$                  | 13     | 42%    | 40      | 28%     | $14\%$  | NS      |

**Figure 1.** Correlation between ESBL-producing E. coli from faecal carriage and febrile urinary tract infection (FUTI) in children ($N = 372$). (a) Depending on genes. (b) Depending on gene combinations. (c) Depending on MLST. (d) Depending on serotypes.
and more precisely subclade C2/H30Rx associated with FUTI isolates (Table 1).

Gene hlyC is part of the hlyCABD operon involved in α-haemolysin synthesis. Its exact role in FUTI is still unclear and probably multifactorial, including lysis of white blood cells (such as natural-killer cells) and epithelial cells. The hly operon is frequently collocated with pap operon within pathogenicity islands specific for UPEC strains. Thus, the combinations including hly and pap operons such as those suggesting the presence of a putative PAI ICFT073 or PAI II J96/PAI IC5 (Table 1) were also significantly associated with FUTI isolates. This fact may explain why combining the detection of the hlyC gene with either papC or papGII did not significantly increase the number of strains carrying at least one of those genes.

Hek outer membrane protein is an auto-aggregating adhesin and invasin initially described in neonatal meningitis E. coli (NMEC) isolates of capsular serogroup K1. However, Hek is not restricted to NMEC isolates and has been found in about one-half of urinary tract isolates, suggesting a role in urothelial barrier interaction. Indeed, in our collection of FUTI isolates, the gene hek was mainly present in non-K1 strains (94 non-K1 versus 3 K1 strains), most of them (n = 47) belonging to sequence type ST131 and O25b:H4 serotype.

TraJ is an activator of the transfer (tra) operon in the F plasmid that counteracts histone-like nucleoid-structuring protein (H-NS) silencing at the main transfer promoter and has been implicated in NMEC pathogenesis via specific TraJ-dependent bacterial interactions with macrophages. To our knowledge, TraJ protein has not been directly implicated in the pathogenesis of FUTI. Thus, its statistical association with FUTI in our study may be linked with a higher frequency of F-plasmids carrying other genes implicated in extra-intestinal virulence.

Although none of these five genes (papGII, papC, hlyC, hek and traJ) was present in more than 58% of ESBL-producing E. coli FUTI isolates, combining the detection of two genes (papGII and traJ) would permit the attribution of a risk-associated genetic profile (if at least one is present) to 63% of FUTI isolates versus only 24% of healthy carriage isolates. This detection can easily be performed by PCR.

The double detection of either papGII or traJ used as a screening test for high-risk ESBL-producing E. coli carriage has a sensitivity of 63%, a specificity of 76%, a positive predictive value of 79% and a negative predictive value of 59% (Figure S1). This means that in about one-third of cases of FUTI caused by ESBL-producing E. coli the carriage isolate would have been previously categorized as a ‘low-risk’ strain and the clinician incited not to use carbapenems. Thus, this low sensitivity should lead to great caution in implementing the genotyping test for the management of particularly frail patients such as young infants or the highly immunocompromised. Moreover, patients with known urologic disorder would be more likely to have a FUTI with papGII-negative strains.

In conclusion, ESBL-Ec strains causing FUTI in children have a genetic background similar to those found in faecal carriage; however, a few genes are not equally distributed in those two populations. Combining the detection of two genes (papGII and/or traJ) would permit the attribution of a risk-associated genetic profile to 63% of FUTI isolates versus only 24% of healthy carriage isolates. However, we believe that the empirical choice of antimicrobial in children with FUTI should result from a confrontation of clinical elements (severity of the disease, impairment of the patient) with microbiological testing such as rapid screening tests for ESBL if available and/or the proposed previous genotypng of faecal carriage isolate.

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Supplementary data
Figure S1 and Tables S1 and S2 are available as Supplementary data at JAC-AMR Online.

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