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Population structure, virulence potential and antibiotic susceptibility of uropathogenic *Escherichia coli* from Northwest England

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Objectives: Multilocus sequence typing (MLST) has been used to characterize diverse pathogens, including uropathogenic *Escherichia coli* (UPEC). There has been significant interest in the contribution of the O25b:H4-ST131 lineage to UPEC disease, as these isolates are often highly virulent and exhibit multidrug resistance. To reveal the wider impact of sequence type (ST) 131, we have examined its contribution to the overall population structure of UPEC isolates that were not selected on the basis of virulence or antibiotic resistance.

Methods: Three hundred UPEC isolates were recovered from community and hospital urine samples examined by clinical microbiology laboratories in the Northwest region of England in June 2007 and June 2009. They were characterized by susceptibility profiling, MLST and virulence gene PCR. PFGE was used to examine isolates from key clones.

Results: The most common lineage was ST73 (16.6%) followed by ST131 (13.3%), ST69 (9%), ST95 (6.3%), ST10 (4.3%) and ST127 (3.6%). ST131 isolates were significantly more likely to exhibit high levels of antibiotic resistance (35% being CTX-M-15 PCR positive) and those of ST127 were the most widely susceptible but carried the highest number of virulence genes. Only when the CTX-M-15-O25b-positive strains were examined was a high level of virulence observed for ST131 isolates. PFGE indicated ongoing local evolution in ST131.

Conclusions: ST131 isolates are well established in the wider UPEC population. This clone is still evolving and we further support suggestions that it represents a real threat to health. We suggest that ST127 is a recently emerged, community-associated, virulent clone that warrants further study.

Keywords: UPEC, MLST, population genetics, ESBL

Introduction

As an extraintestinal pathogen, *Escherichia coli* is best known for causing urinary tract infection (UTI), bacteraemia and neonatal meningitis.[1] The majority of these infections are due to specialized strains that possess both the genetic background for colonization and survival in a specific niche and an arsenal of virulence factors.[2,3]

Epidemiological investigations of extraintestinal pathogenic strains of *E. coli* (ExPEC) have revealed that most of the distinctive strains of *E. coli* derive from a limited number of clonal groups. Multilocus sequence typing (MLST) has proved to be a reliable epidemiological tool and has more recently been used to describe the emergence of multidrug-resistant *E. coli* producing extended-spectrum β-lactamase (ESBL) enzymes, predominantly CTX-M-15, and frequently belonging to the O25:H4-ST131 lineage.[4–9] The range of antibiotic resistance determinants carried by these organisms significantly reduces the therapeutic options available to clinicians, increasing morbidity and mortality.[10]

MLST, with its expanding online database, provides a powerful epidemiological typing tool that illustrates the relationships between such epidemic uropathogenic *E. coli* (UPEC) strains and endemic strains, but the majority of MLST-based studies analysing UPEC population biology have focused on groups of highly antibiotic-resistant or virulent isolates, or those from

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selected patient groups. To better understand the evolutionary success of expanding clones and their position among the more general UPEC population, we have used MLST to examine the population biology of UPEC from urinary tract infections in patients presenting in the community and hospitals in the Northwest region of England. The isolate collection was not biased by selection for any particular phenotype and this is the first study of this kind.

Materials and methods

Bacterial strains

A total of 300 clinical isolates of *E. coli* from patients with UTI were included in the study. Isolates were recovered from non-duplicate urine samples giving a viable count of $>10^5$ cfu/mL and an elevated white cell count. The first 150 isolates were collected in June 2007. These isolates were recovered from 100 consecutive samples received by the microbiology laboratory at a hospital in Manchester and the first 50 from a hospital laboratory in Preston. Another 150 isolates were collected in the same manner in June 2009. Isolates were designated as hospital acquired when received from inpatients and as community acquired when samples were referred from general practice or outpatient clinics.

DNA extraction

DNA extraction was performed according to the manufacturer's instructions using a PrepMan Ultra sample preparation kit (PrepMan™ Ultra, Applied Biosystems, USA).

Phenotypic and genotypic antimicrobial susceptibility testing

The Vitek 2 compact Automated Expert System (AES; Biomérieux, Basingstoke, UK) was used to confirm the identity and to determine the antibiotic susceptibility profiles of the UPEC isolates. Antibiotics included in the screen were ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefalotin, cefuroxime, cefoxitin, cefotaxime, ceftazidime, cefepime, aztreonam, meropenem, ertapenem, amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, nitrofurantoin and trimethoprim. A resistance score for each isolate was calculated by dividing the number of antibiotic classes to which the isolate was resistant by 5 (the number of antibiotic classes being studied). Analysis of the resistance score based on antibiotic classes, rather than individual antibiotics, avoided any possible effect of cross-resistance to different antibiotics.

Isolates in the collection that were identified as potential ESBL producers using the Vitek 2 AES were examined using double disc diffusion methods.[11] Putative ESBL producers were also examined for carriage of the $bla_{\text{CTX-M}}$ alleles using PCR.[7] CTX-M-15-positive isolates were then screened using PCR[7] for the *rfbO25b* subclone marker.

Quinolone-resistant strains were examined for mutations in quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*, using previously described PCR and sequencing primers.[12,13] Amplified genes were sequenced and compared with native *gyrA* (X06373) and *parC* genes (M58408) in the GenBank database. A PCR-based restriction fragment length polymorphism (PCR-RFLP) assay was used to identify the *aac(6′)-lb-cr* variants using previously described primers and PCR conditions and NdeI and FokI restriction enzymes.[14]

Screening for the three known *qnr* genes was carried out using a previously published multiplex PCR assay.[15]

MLST

Sequence type (ST) determination was performed using the MLST scheme for *E. coli* specified at the University College Cork *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli) as described previously.[16]

eBURST

The Based upon Related Sequence Types (BURST) clustering algorithm (eburst.mlst.net) was used to analyse the allelic profiles and define clonal complexes (CCs). CCs were identified according to the number of single-locus variants (SLVs) and double-locus variants (DLVs) shared between isolates, where only STs that shared six or more loci were assigned to a defined CC.[17]

Clonal frame

The nucleotide sequences of the seven gene fragments of unique STs were concatenated in the order *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA* using START v2.0 software and the resulting 3405 bp sequences were aligned to infer their phylogenetic relatedness. Clonal Frame software[18] was used to construct a majority-rule consensus tree. Ten Clonal Frame runs were computed, each with 100000 iterations after 10000 burn-in iterations. The graphical user interface of the program was used to generate 50% consensus trees and network representations.

PFGE

The genetic relatedness of 32 representative isolates of the common UPEC STs from different phylogenetic groups was determined by XbaI PFGE analysis performed according to the PulseNet standardized PFGE protocol.[19] Generated profiles were compared digitally using BioNumerics v.3.5 (Applied Maths). A dendrogram was generated from the cluster analysis of Dice similarity indices based on the unweighted pair group method with arithmetic mean (UPGMA). Isolates were considered to belong to the same PFGE group if their Dice similarity index was $\geq$85%.

Virulence factor (VF) determination

Carriage of 29 ExPEC-associated VF genes encompassing five categories (adhesins, toxins, siderophores, capsule genes and miscellaneous genes) was assessed using an established multiplex PCR assay.[20]

The adhesin genes investigated were *fimH* (mannose-specific adhesin of type I fimbriae), P fimbriae elements [*papAH*, *papC*, *papEF* and *papG* alleles (*I,II,III*)], *sfaS* (S fimbrial adhesin), *focG* (the putative F1C fimbrial adhesin), *sfa/focDE* (central region of *sfaS* and *focG* operons), *afa/draBC* (Dr antigen-specific adhesin operons), *bmaE* (blood group M-specific adhesion), *nfaE* (non-fimbrial adhesin) and *gafD* (glucosamine-specific G fimbriae). Toxin genes screened were *cnf1* (cytotoxic necrotizing factor), *cdtB* (cytolethal distending toxin) and *hlyA* (α-haemolysin). The siderophore genes studied were *fyuA* (yersiniabactin) and *iutA* (aerobactin). Capsule synthesis-associated genes screened were *kpsMT* (groups II and III) in addition to specifically targeting *K1* and *K5* genes of group II capsules. The miscellaneous VF genes were *cvaC* (colicin V; multifunctional serum resistance-associated plasmids), *traT* (serum resistance associated), *ibeA* (invasion of brain endothelium) and *PAI*/MalX, a pathogenicity island (PAI) marker from the archetypal ExPEC strain CFT073. A virulence score was calculated for each isolate as the sum of positive virulence traits for each isolate.

Statistical analysis

Unless otherwise stated, statistical analyses were performed using Fisher's exact test with a threshold for statistical significance of $P<0.05$.

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Results

MLST reveals the true population structure of UPEC in Northwest England

The 300 UPEC isolates analysed were assigned to 102 STs. Forty-four STs were novel to this study, of which eight contained new alleles, the remainder being novel combinations of previously characterized alleles. The most common STs were ST73 ($n=50$; 16.6% of isolates), ST131 (37; 12.3%), ST69 (27; 9%), ST95 (19; 6.3%), ST10 (13; 4.3%) and ST127 (11; 3.6%). Six STs accounted for >50% of the isolates, demonstrating the diversity of lineages in the sampled area.

Demographic parameters such as patient age and gender, specimen type and geographic origin were retrieved from laboratory records and examined anonymously. Overall, 82% of isolates were from females and patient age ranged from <1 month to 95 years, with a modal age of 64 (mean age 52 years). ST69 isolates were significantly more common in females than in males (11% versus 0%, $P<0.05$). No significant differences were seen between specific STs and patient age. There were nine hospital-associated ST131 isolates recovered in both 2007 and 2009, but five were community associated in 2007 compared with 14 in 2009. Given the relatively low number of isolates studied in each of these groups, the observed differences between sources of acquisition were not significant. More striking was the observation for other STs, with eight out of nine ST127 isolates recovered in the community (Table 1).

Nine STs were consistently detected in both the Manchester and Preston collections (Figure 1). Although the number of isolates was too small to allow examination of the statistical significance of differences, some notable changes in occurrence were seen between 2007 and 2009, with increases in the proportion of ST73 in Preston and ST131 in Manchester, but a decrease in ST69 in Manchester.

Table 1. Comparison of hospital versus community association of UPEC and examination of the degree of antibiotic resistance exhibited by isolates from these groups

		Resistance score			
		hospital associated		community associated	
ST	Isolates	no.	mean, median (range)	no.	mean, median (range)
ST10	13	3	0.3, 0.4 (0.2–0.4)	10	0.16, 0.2 (0–0.4)
ST14	8	2	0.2, 0.2 (0.2)	6	0.25, 0.2 (0–0.6)
ST69	27	7	0.37, 0.4 (0.2–0.6)	20	0.32, 0.4 (0–0.6)
ST73	49	11	0.18, 0 (0–0.8)	39	0.16, 0.2 (0–0.8)
ST88	6	3	0.3, 0.2 (0–0.8)	3	0.3, 0.2 (0–0.2)
ST95	19	4	0.25, 0.3 (0–0.4)	15	0.1, 0 (0–0.6)
ST127	9	1	0.4	8	0.16, 0.2 (0–0.4)
ST131	37	18	0.57, 0.6 (0–0.8)	19	0.41, 0.4 (0–0.8)
ST405	5	1	0.8	4	0.25, 0.2 (0–0.6)

Confirmation of multidrug resistance in ST131 isolates

The susceptibility of isolates of different STs to members of commonly used antibiotic classes was assessed using Fisher's exact test. Overall, STs varied considerably in their antibiotic susceptibilities from the most susceptible, ST95, with the lowest resistance scores (median 0.15 and range 0.0–0.6) to the most resistant, ST131 (median 0.6 and range 0.0–0.8) (Table 2). ST131 showed a distinctive antibiogram that was significantly associated with resistance to most of the tested antibiotic classes. This antibiogram was characterized by the production of ESBL enzymes and AAC(6), as detected using the Vitek AES, in addition to resistance to ciprofloxacin, nitrofurantoin and trimethoprim. These associations, when compared with those of all other STs (Table 2), were all significant ($P\leq0.0004$). Isolates of ST69 were characterized by resistance to trimethoprim ($P\leq0.0001$). When the origin of the isolates from the major STs was examined, there was a correlation between hospital association and increased mean resistance score, as may be expected (Table 1).

Of the 24 isolates in the collection (8% of the total) that exhibited an ESBL phenotype, 15 (5%) contained $bla_{\text{CTX-M}}$ alleles. Thirteen of these isolates were positive for CTX-M-15[7] and were ST131. Ten of these (29% of all ST131) were CTX-M-15-O25b positive. Interestingly, eight of these were recovered in 2009 (four in the community and four associated with hospital inpatients) and only two in 2007 (both hospital associated), though this difference was not significant. When a comparison was made between ST131 isolates that were CTX-M-15-O25b positive or negative, there was a significant

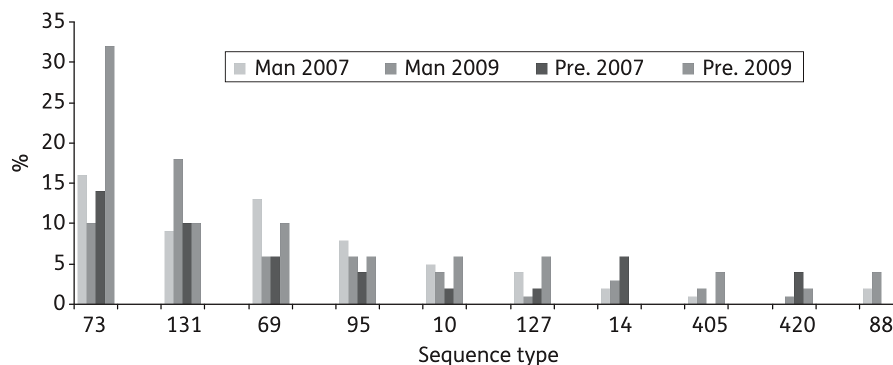

Figure 1. Frequency of consistently detected STs among a collection of 300 UPEC isolates recovered in June of 2007 and 2009 in both Manchester (Man) and Preston (Pre.).

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Table 2. Association of STs with resistance to different antibiotic classes

		β-Lactams		Aminoglycosides			Quinolones		Furanes		Resistance-score
		AP	ESBL	AAC(3)	AAC(3), ANT(2)	AAC(6)	NAL	CIP	NIT	Trimethoprim	[mean, median (range)]
ST	Isolates	(141)	(24)	(2)	(5)	(23)	(31)	(34)	(9)	(112)	
ST10	13	4	2	0	0	0	0	1	0	6	0.2, 0.2 (0.0–0.4)
P value		—	—	—	—	—	—	—	—	—	
ST14	8	4	0	0	0	0	3	0	0	3	0.25, 0.2 (0.0–0.6)
P value		—	—	—	—	—	—	—	—	—	
ST69	27	19	0	0	2	0	3	1	0	20	0.33, 0.4 (0.0–0.6)
P value		—	—	—	—	—	—	—	—	*0.0001*	
ST73	49	25	0	0	0	2	3	1	0	11	0.17, 0.2 (0.0–0.8)
P value		—	—	—	—	—	—	—	—	—	
ST88	6	4	0	0	0	0	2	0	1	2	0.2, 0.2 (0.0–0.8)
P value		—	—	—	—	—	—	—	—	—	
ST95	19	7	0	0	0	0	2	0	0	2	0.15, 0.0 (0.0–0.6)
P value		—	—	—	—	—	—	—	—	—	
ST127	9	5	1	1	0	1	0	0	0	1	0.18, 0.2 (0.0–0.4)
P value		—	—	—	—	—	—	—	—	—	
ST131	37	16	13	1	0	13	3	19	3	24	0.49, 0.6 (0.0–0.8)
P value		—	0.0001	—	—	0.0001	—	0.0001	0.008	0.0004	
ST405	5	1	2	0	0	1	1	1	0	3	0.36, 0.2 (0.0–0.8)
P value		—	—	—	—	—	—	—	—	—	

AP, acquired penicillinase; AAC, *N*-acetyltransferase; ANT, *O*-adenyltransferase; NAL, nalidixic acid; CIP, ciprofloxacin; NIT, nitrofurantoin.
P values (by Fisher's exact test) are shown only where $P \leq 0.005$ and these relate to differences found when susceptibility profiles for isolates of each ST were compared with those of all other STs combined. Data are presented only for isolates that displayed resistance, hence row and column numbers may not add up to the full total in all cases. The median resistance score is displayed for each ST along with the range of resistance scores for individual isolates within each ST and has been calculated based on the expression of resistance to antibiotics of the five classes shown.

Table 3. Characterization of quinolone resistance mechanisms found in ST131 UPEC isolates

		QRDR[b]					
		gyrA		*parC*			
Quinolone profile[a] (*n*)	ESBL production (%)	(S)83(L) (%)	(D)87(N) (%)	(S)80(N) (%)	(E)84(V) (%)	*aac(6′)-lb-cr* (%)	*qnr* genes
Q-S (12)	0	0	0	0	0	0	0
Q-R (1)	0	1 (100)	0	0	0	0	0
FQ-R (19)	11 (58)	19 (100)	19 (100)	19 (100)	19 (100)	9 (47)	0

[a]Q-S, susceptible to quinolones; Q-R, resistant to quinolones alone; FQ-R, resistant to fluoroquinolones.
[b]QRDR, quinolone resistance-determining region; S, serine; L, leucine; D, aspartic acid; N, asparagine; E, glutamic acid; V, valine.

correlation between CTX-M-15-O25b positivity and fluoroquinolone resistance (data not shown).

Twenty out of thirty-two ST131 isolates were resistant to quinolones (nalidixic acid), and 19 (59%) of these expressed additional resistance to fluoroquinolones (ciprofloxacin) (Table 3). Among the quinolone-resistant isolates, 11 showed ESBL production (55%).

All ciprofloxacin-resistant isolates had multiple mutations in both *gyrA* and *parC* genes (Table 3), while the one nalidixic acid-resistant isolate that failed to show resistance to ciprofloxacin had a single *gyrA* mutation at codon 83 (Ser→Leu).

Only nine isolates carried the *aac(6′)-lb-cr* gene, which represented half of the ciprofloxacin-resistant isolates and 54% of the ciprofloxacin-resistant, ESBL-producing isolates. None of the isolates tested carried any of the *qnr* genes (Table 3).

eBURST analysis of STs confirms the recent emergence of ST131

eBURST analysis revealed 17 CCs encompassing 59 STs and representing 259 UPEC isolates with 41 isolates appearing as singletons (Figure 2a). CC73 was the largest and accounted for 60 (20%) isolates and was dominated by ST73, which was identified as the founder of its CC with a bootstrap value of 96%. Eight other major groups comprising more than three STs were found in each CC (bootstrap values ranging between 31% and 98%).

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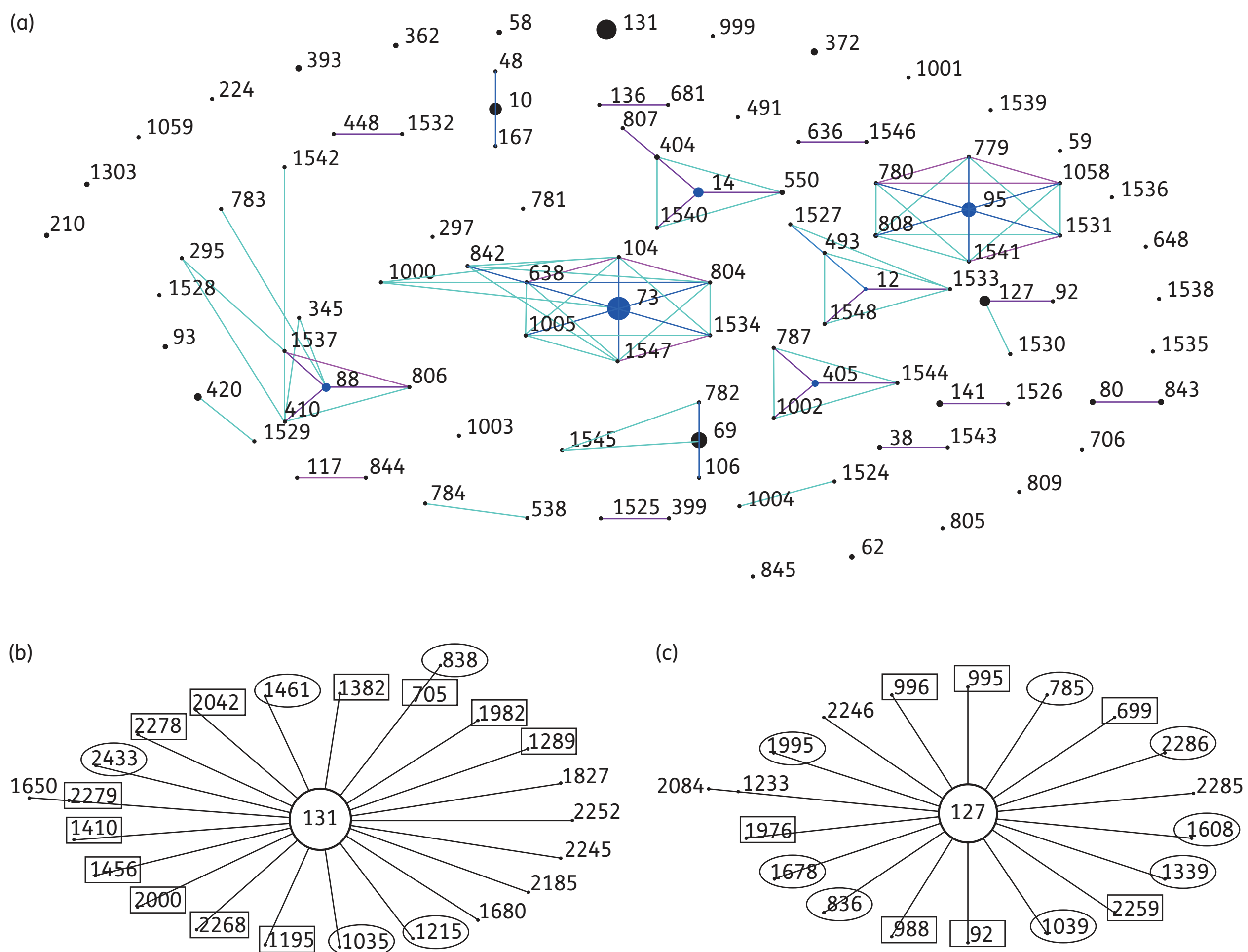

Figure 2. eBURST diagrams (a) of NW-UPEC showing clusters of related STs (CC) and individual (singleton) STs of the 300 UPEC. Each ST is represented by a circle, the size of which correlates to the frequency of the ST. Predicted founders are positioned centrally in each cluster and shown in blue, the subgroup founders are shown in yellow and SLVs and DLVs are shown in pink and blue, respectively. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*. A snapshot of CC131 (b) and CC127 (c) is also shown in an eBURST comparison between the current dataset and all of the information held in the international online database (as at September 2011). STs in the rectangle shapes are those associated with ExPEC and those in oval shapes are those associated with enteric *E. coli*. STs not surrounded by a rectangle or oval are of unknown origin.

Figure 2(a) illustrates eBURST CCs showing, in addition to SLVs, numerous interclonal and intraclonal DLVs. CC73 showed the highest number of DLVs. Interestingly, ST131 was not assigned to a complex, even when less stringent conditions were employed (i.e. STs sharing four or more alleles; data not shown) and ST127 only had two SLVs. We examined the clonality of our dataset in an eBURST comparison with all ExPEC- and enteric-associated STs in the international MLST web database (as at September 2011) and the original founding STs were maintained (data not shown). However, with this wider comparison, both ST131 (21 SLV and 2 DLV) and ST127 (18 SLV and 1 DLV) were recognized as founders of distinct CCs (Figure 2b and c) with bootstrap values of 97% and 94% (data not shown). These findings suggest that isolates of ST131 and ST127 have recently emerged and are able to cause UTI, but they are diversifying and their descendants are present in the wider ExPEC and enteric *E. coli* populations.

The UPEC population studied consists of two major populations, linked by an unidentified common ancestor

Concatenated sequences of all seven loci were then phylogenetically analysed using Clonal Frame software. Clonal Frame analysis is able to reveal genealogies that are not biased by recombination events. A network representation of the Clonal Frame analysis (Figure 3) suggested that all of the isolates fall into one of two groups (GI and GII) with an unidentified common ancestor and that numerous genotypes that have descended from this common ancestor have also not been identified among the studied population. However, almost all STs appeared to have descended from two unknown ancestors, which, in turn, directly arose from the common ancestor. Interestingly ST127, which, based on eBURST analysis, failed to form its own clonal complex in our dataset, appeared as a clonal founder using Clonal Frame analysis.

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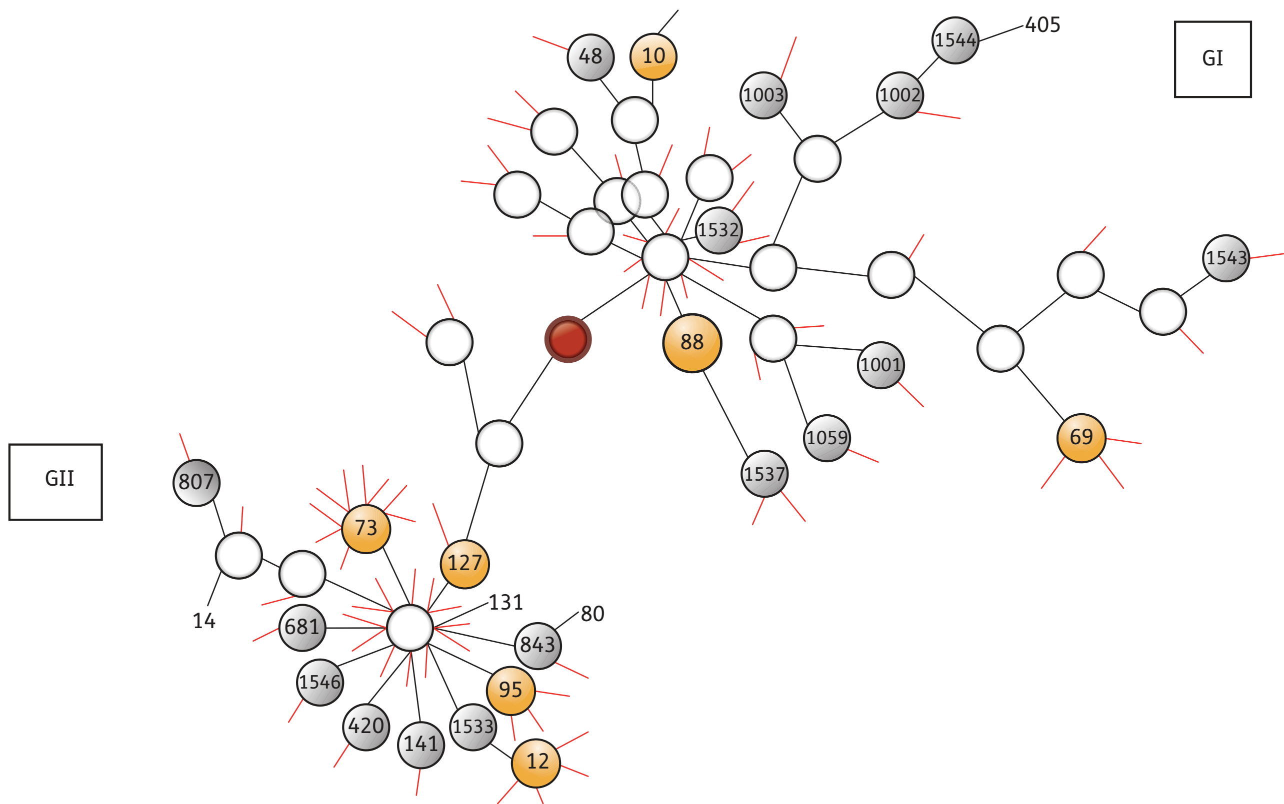

Figure 3. Network representation of 50% consensus of 10 evolutionary trees generated using Clonal Frame v1.1 for all 102 unique STs, disregarding the role of recombination. Clonal complex (CC) founding strains are circled (those from the current study are in yellow) and the ancestral node of the network is indicated by a red circle. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*. Also represented are the major groupings GI and GII, as described in the text.

The contribution of recombination to the diversification of UPEC populations was estimated within CCs using both the Φw test and linkage disequilibrium analysis using established methods.[21] Recombination was observed to be important in evolution at the level of higher groupings, but it appears that mutation contributes significantly to the diversity within individual CCs (data not shown).

PFGE reveals regional subclonal variations in ST131

Cluster analysis of Dice similarity indices using the UPGMA was used to generate a dendrogram describing the relationships among PFGE profiles of 32 representative isolates of the common UPEC STs from different phylogenetic groups: ST69 (n=10 isolates), ST73 (n=9) and ST131 (n=13) (Figure 4).

As expected, finer resolution was obtained by PFGE analysis but each ST constituted a separate cluster. The ST131 cluster (defined at the 65% similarity level) was tied to the two other cluster groups at <60% similarity.

Isolates were considered to belong to the same PFGE group if their Dice similarity index was ≥85%. Using this criterion, ST73 and ST69 clusters comprised eight PFGE profiles each. The ST131 cluster comprised nine different profiles, with most of the isolates collected from Preston forming a subcluster at >85% similarity. There was no evidence for a geographical effect in the subclusters of ST69 (all ST73 isolates were from Manchester).

Prevalence of virulence factors varies significantly in the studied population

Of the 29 virulence genes tested, 26 were detected at least once, with prevalence ranging from 0.3% (*nfaE*) to 97% (*fimH*). Among the adhesin genes, various Pap elements occurred in >40% of the 300 isolates. Although the toxin-associated gene, *hlyA* was as prevalent as *cnf1*, both were significantly more commonly carried than the colicin V production gene, *cvaC* (P<0.001). Of the siderophores, *fyuA* (yersiniabactin) was significantly more frequent than *iut* (aerobactin; P<0.0001). Nearly 60% of the isolates were positive for the group II capsule synthesis gene, with *K5* accounting for 61% of *kpsM II*-positive isolates. The serum resistance-related gene (*traT*) occurred in 72% of the isolates while the pathogenicity island (*PAI*/MalX) marker gene was present in 60% of the isolates.

There was a strong correlation between VF profiles and STs. For frequently detected STs, each one contained closely related VF profiles with similarities ranging from 65% to 100%, suggesting vertical transmission of VFs (Table 4). Overall, the STs varied considerably in VF content, from ST10 with the lowest VF score (mean 3.7) to ST127 with the highest VF score (mean 13.4). Table 4 shows distinctive virulence traits that had a significant positive or negative association with the commonly detected STs. Although different Pap elements were important virulence determinants in many STs, some successful STs, including ST131 and ST10, were notable for their low carriage of Pap

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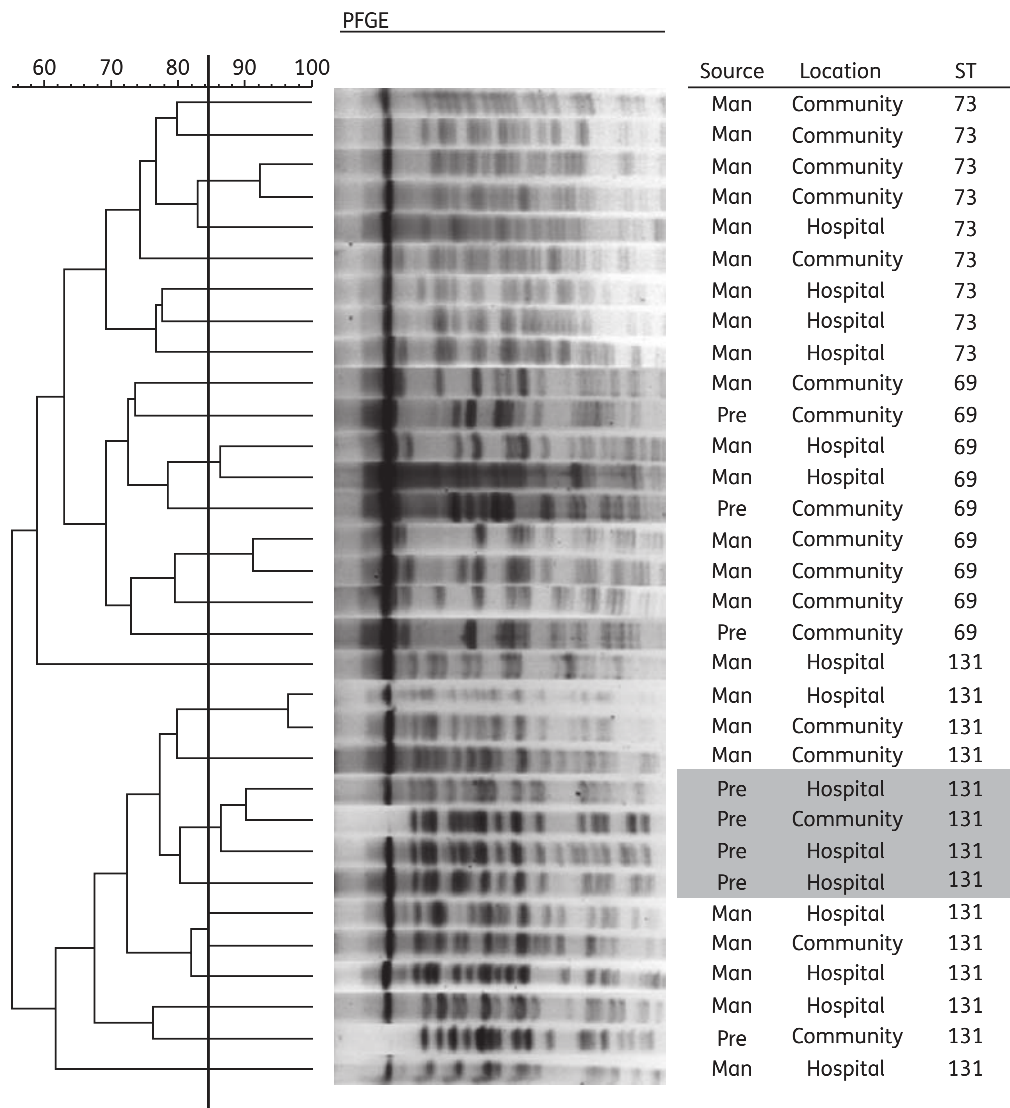

Figure 4. XbaI PFGE dendrogram for 32 UPEC isolates collected in Manchester (Man) and Preston (Pre) as determined using the UPMGA algorithm based on Dice similarity coefficients. The shaded area highlights ST131 isolates collected in Preston. the vertical black line shows the 85% cut-off.

elements. ST131 isolates also showed a significantly low prevalence of the sialosyl-binding adhesion gene (*sfaS*; $P<0.0001$), but were significantly associated with carriage of *ibeA*. ST127 was significantly associated with *papG III* ($P<0.0001$) and ST69 isolates were negatively associated with the *PAI*/MalX marker ($P<0.0001$).

Carriage of a number of virulence-related genes appeared to be higher in the ST131 subset of CTX-M-15-O25b-positive isolates, but again the small number of representatives limited our ability to assess the significance of such differences (data not shown).

Discussion

UPEC have long been recognized as distinct clones of *E. coli* that exhibit specific characteristics, such as specific virulence-associated traits and distinctive O antigens, genotypes and degrees of multidrug resistance.[22] However, despite the increasing use of MLST as a trusted epidemiological tool, the true population structure of UPEC recovered from urine samples has been little studied using this technique. For obvious reasons, many previous studies, including our own, were biased towards collections of isolates with certain characteristics, such as antimicrobial resistance, the presence of specific serogroups or isolation from serious infections or selected patient groups. This has possibly led to a false impression of over-representation of certain STs within the UPEC population.

In this study, we assessed the population structure of UPEC in the Northwest region of England using MLST. Our previous work has demonstrated the concordance of data generated for isolates from a similar region to the overall picture from the UK.[23] We reveal a consistent profile of STs over a 3 year period from a relatively large and demographically diverse geographic area, with isolates primarily from eight lineages (ST73, ST131, ST69,

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Table 4. Virulence factors that were seen to have significant positive or negative associations with UPEC of the most common clonal groups observed in the collection

Category	Specific VF	Total no. 300	Percentage prevalence (*n*) of VF by clonal group: ST10 (*n*=13)	ST69 (*n*=27)	ST73 (*n*=49)	ST95 (*n*=19)	ST127 (*n*=9)	ST131 (*n*=37)
Adhesion	*papA*	120	8 (1)	52 (14)	55 (27)	**89 (17)**	**100 (9)**	**8 (3)**
	papC	127	**0 (0)**	52 (14)	57 (28)	**100 (19)**	**100 (9)**	**5 (2)**
	papEF	129	**8 (1)**	**70 (19)**	55 (27)	**84 (16)**	**100 (9)**	**8 (3)**
	papGII	84	0 (0)	44 (12)	41 (20)	**84 (16)**	11 (1)	**5 (2)**
	papGIII	43	0 (0)	4 (1)	22 (11)	10 (2)	**100 (9)**	3 (1)
	sfa/foc DE	95	0 (0)	**4 (1)**	**84 (41)**	10 (2)	**77 (7)**	**0 (0)**
	afa/draBC	33	23 (3)	4 (1)	**0 (0)**	0 (0)	0 (0)	**43 (16)**
	sfaS	23	0 (0)	0 (0)	8 (4)	10 (2)	**55 (5)**	0 (0)
	focG	55	0 (0)	7 (2)	**61 (30)**	16 (3)	11 (1)	**0 (0)**
Toxins	*hlyA*	78	8 (1)	**0 (0)**	**69 (34)**	5 (1)	**67 (6)**	**8 (3)**
	cnf1	82	**0 (0)**	**0 (0)**	**69 (34)**	10 (2)	**77 (7)**	**8 (3)**
	cvaC	28	8 (1)	4 (1)	**0 (0)**	**42 (8)**	0 (0)	**0 (0)**
Siderophore	*fyuA*	262	**38 (5)**	85 (23)	**96 (47)**	95 (18)	100 (9)	**97 (36)**
	iutA	177	54 (7)	70 (19)	65 (32)	47 (9)	**22 (2)**	**86 (32)**
Capsule	*kpsM* II	177	**8 (1)**	55 (15)	61 (30)	**100 (19)**	**100 (9)**	57 (21)
	kpsM K1	53	0 (0)	7 (2)	**4 (2)**	**100 (19)**	0 (0)	**3 (1)**
	kpsM K5	109	**8 (1)**	51 (14)	43 (21)	**0 (0)**	**89 (8)**	**54 (20)**
Miscellaneous	*ibeA*	32	0 (0)	0 (0)	**0 (0)**	10 (2)	0 (0)	**24 (9)**
	traT	216	54 (7)	**89 (24)**	**57 (28)**	89 (17)	67 (6)	**86 (32)**
	PAI (MalX)	181	**23 (3)**	**11 (3)**	**92 (45)**	**84 (16)**	78 (7)	**92 (34)**
VF score [mean, median (range)]			3.7, 3 (1–8)	7.8, 9 (5–7)	11, 11 (6–16)	11.8, 12 (6–14)	13.4, 13 (9–17)	6.9, 6 (4–14)

Bold values indicate significant associations ($P \leq 0.05$); underlining indicates a negative association.

ST95, ST10, ST127, ST14 and ST405). ST131 and ST69 (CGA) have previously been the focus of extensive reports relating to their role as widely disseminated clones of antimicrobial-resistant UPEC.[4,24–26] The prevalence of ST131 is usually higher in such studies, reflecting the high virulence of the O25b subclone and multidrug resistance, but our data show that ST131 clearly represents a significant proportion of the general UPEC population. Our data support the suggestions of high-level antibiotic resistance in these isolates. CTX-M-15 carriage was demonstrated by 35% of ST131 isolates and was a defining feature of this lineage. Though not significant, the rise in the number of CTX-M-15-positive ST131 isolates during the study is a concern and warrants further surveillance of this lineage. It is difficult to compare these figures with those for populations from previous studies, with isolates selected on the basis of ESBL production or quinolone resistance, but Croxall and colleagues[26] recently reported that approximately 24% of ST131 isolates not selected on the basis of resistance carried CTX-M-15.

Of note was the exclusive association of *aac(6′)-lb-cr* with ESBL-producing ciprofloxacin-resistant UPEC, which agrees with the 2008 report of Pitout and colleagues,[27] and the suggestion of co-habitation of relevant genes on the same plasmids. However, despite their ESBL production status or carriage of plasmid-mediated quinolone resistance markers, all isolates displaying ciprofloxacin resistance showed multiple mutations in the chromosomally located *gyrA* and *parC* genes. This pattern of mutation has been reported frequently and has been associated with high-level fluoroquinolone resistance.[28,29] Although the strong linkage between the ST131 clone and quinolone resistance may be promoted by the high prevalence of plasmid-encoded *aac (6)-lb-cr*, our data support previous suggestions that fluoroquinolone resistance occurs in ST131 UPEC as a result of accumulated point mutations in both *gyrA* and *parC* genes.[13]

Recent reports have associated ST405 with the widespread distribution of CTX-M-15 and other ESBL enzymes in UPEC.[4,14] Previous reports indicate that ST95 is associated with avian pathogenic *E. coli* (APEC),[30,31] and isolates of this lineage have been recovered from food.[32] In addition, the reference UPEC strains UTI89 and NU14 and the neonatal meningitis strain RS218 are ST95,[31] and a recent study used carriage of the *svg* gene to infer the presence of this lineage in a group of APEC and ExPEC isolates, including those causing UTI and neonatal meningitis.[33] The latter study also noted a correlation between organisms presumptively identified as ST95 and carriage of ColV plasmids, a finding echoed in our detection of *cvaC* in nearly half of our ST95 isolates.

Some of the other STs in our study have not been widely reported as significant pathogens, although the reference

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genome-sequenced pyelonephritis strain 536 and several animal isolates have been identified as ST127.[34] We have not previously seen isolates of ST127 in local UPEC populations.[16,23] ST127 isolates were also identified as having particularly high virulence potential by Croxall and colleagues.[26] There are numerous entries in the MLST database for isolates of this ST (human and animal in origin) and our data support the previous suggestion of zoonotic transmission of these virulent organisms.[34]

ST73 has been previously reported as an important and diverse clone within *E. coli* Reference Collection (ECOR) group B2 and is associated with UPEC.[35] Our data emphasize the status of ST73 with its nine related STs forming a large CC accounting for 20% of the population. Spratt and colleagues[17] suggested that the relative age of a CC can be estimated from the number of SLVs it has and the presence of related subgroups. On this basis, ST73 can be considered to be the oldest CC in the population of the current dataset. In comparison, failure to cluster with any other ST in the current population and the formation of only conservative CCs when analysis was expanded to involve data for other ExPEC and enteropathogenic EC isolates suggests that ST131 and ST127 have emerged recently. The recent emergence of ST131 is widely documented. Despite the considerable similarity of PFGE profiles observed among isolates of ST131, we detected clear genetic differences that defined the Preston isolates within the ST131 clone, indicating ongoing evolution of MLST-defined clones.

The evolutionary divergence provided by Clonal Frame analysis resolved the population into two large clusters, which correlates well with previously reported results using other molecular methods[36] and suggests a well-defined structure within the *E. coli* population rather than the vague structures often generated by recombination-sensitive phylogenetic methods such as the neighbour-joining method.[37] Whether this clear-cut segregation within the population holds specific phenotypic relevance for each cluster is not yet clear.

The broad range of VF genes screened revealed VF patterns significantly associated with different STs. Most notably, ST127, which we suggest is a newly evolved clone, gave the highest virulence scores. This correlates with the previous report that ST127 strain 536 causes pyelonephritis,[38] and infections mediated by isolates with such high virulence potential may result in extensive exposure to antibiotics, generating a selective pressure for the acquisition of antibiotic resistance genes. The high correlation of ST127 isolates with a community origin is worrying, given their high virulence, which may contribute to rapidly progressive disease. We suggest that our findings warrant detailed surveillance for ST127 isolates, which may represent an emerging and potentially significant clone.

It has been widely thought that antimicrobial resistance comes at a cost to fitness[20,39–41] and a relatively low virulence score defined some of the multidrug-resistant ST131 isolates. These isolates had distinctive VF profiles that lacked fimbrial adhesins but indicated significant reliance on non-fimbrial adhesins (the Dr family of adhesins). Overall, our ST131 isolates were seen to carry a repertoire of VFs similar to those recently reported in groups of highly virulent ST131 isolates from Spain,[42] the UK[26] and the USA.[43] The O25b-ST131 subclone is known to have a high virulence potential,[42] which is corroborated here, but the number of isolates in our collection may have limited the ability to differentiate significantly between O25b-ST131 and non-O25b-ST131 isolates on the basis of virulence. Clearly, O25b isolates represent a virulent subpopulation of ST131 that are possibly of greater clinical significance than non-O25b isolates. Our data support other recent suggestions that these pathogens concomitantly exhibit high-level virulence and multidrug resistance.[26,42,44]

In conclusion, we have described the population structure of a defined, but unbiased, UPEC population based on MLST and phylogenetic analysis and reveal a consistent profile of STs over a 3 year period. We suggest that ST127 is a recently emerged clone that has a high virulence potential warranting close monitoring. We further support the suggested recent evolution of key clones and present one of the most comprehensive analyses of the virulence potential of isolates of the globally disseminated ST131 clone. Members of this lineage are clearly still evolving at a local level. Continued efforts are required to limit future disease caused by this organism, which can display high virulence and a wide spectrum of drug resistance,[10] including recent reports of NDM-1[44,45] and KPC[46] carriage.

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Transparency declarations

All authors have nothing to declare.

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