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Emergence of extended-spectrum β-lactam resistance among *Escherichia coli* at a US academic children's hospital is clonal at the sequence type level for CTX-M-15, but not for CMY-2

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Abstract

Resistance to extended-spectrum β-lactams is increasing worldwide among *Escherichia coli* and has been linked to a small number of emergent clones (e.g. ST38, ST131 and ST405) recovered from extraintestinal infections in community and hospital settings. There are, however, limited data about the relative contributions of bacterial strains, plasmids and β-lactamase genes to extended-spectrum β-lactam resistance in paediatric infections. We performed an extensive molecular analysis of phylogenetic, virulence and antibiotic resistance-related properties of 49 previously reported serial *E. coli* isolates recovered during 1999–2007 at Seattle Children's Hospital (Seattle, WA). Class C enzyme CMY-2 and class A enzyme CTX-M-15 were the most prominent extended-spectrum β-lactam resistance enzymes in the collection, first appearing in this patient population in 2001 and 2003, respectively, and then steadily increasing in frequency over the remainder of the study period. Among 19 CMY-2-positive isolates, 16 distinct STs were detected (D = 98.25%, 95% CI 96–100.25%), indicating that CMY spread is non-clonal at the host strain level. In contrast, among ten CTX-M-15-positive isolates, three STs were detected (D = 37.78%, 95% CI 2.36–73.20%), of which eight represented the worldwide-disseminated ST131 lineage, consistent with clonal spread of CTX-M-15-associated resistance. $fimH_{TR}$ subtyping of ten ST131 isolates (including two CTX-M-negative isolates) revealed that, within ST131, carriage of allele $fimH_{TR}30$ correlated with CTX-M-15 positivity, whilst carriage of non-$fimH_{TR}30$ alleles correlated with carriage of non-CTX-M enzymes. Thus, spread of CMY-2 is non-clonal at the ST level, but clonal spread of CTX-M-15 may be associated with a specific $fimH_{TR}$-defined sublineage of ST131.

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Keywords

Antibiotic resistance; *Escherichia coli*; ST131; Plasmid; CTX-M; CMY

1. Introduction

Antimicrobial resistance is a growing problem in medicine, resulting in limitations in the antimicrobial choices available for treatment of severe infections and increasing the risk of morbidity and mortality in patients. Enterobacteriaceae are important causes both of community-acquired and healthcare-associated infections in adults and children, and production of β-lactamases is a major mechanism of resistance for this family of bacteria. Plasmid-borne, extended-spectrum β-lactam resistance in Enterobacteriaceae was noted to emerge in the late 1980s [1]. In the last decade, dissemination of this type of resistance among Enterobacteriaceae has substantially increased throughout the world [2], in association with specific successful *Escherichia coli* clones, including sequence types ST38, ST131, ST405 and ST648 [3].

Our previous study demonstrated a statistically significant increase in extended-spectrum β-lactam resistance among Enterobacteriaceae at Seattle Children's Hospital (Seattle, WA) over the period 1999–2007 [4]. The emerging resistance was characterised by a significant increase in the prevalence both of AmpC-type and extended-spectrum β-lactamase (ESBL)-type enzymes as well as isolation of three carbapenemase-producing organisms among diverse genera of Enterobacteriaceae. These findings were particularly concerning in relation to paediatric practice, in which β-lactams are the mainstay of empirical therapy for serious infections and where fluoroquinolones are not considered first-line agents. In addition, plasmids are easily shared between organisms, creating a scenario where resistance can spread rapidly. Further study of the epidemiology and molecular mechanisms of extended-spectrum β-lactam resistance in Enterobacteriaceae in paediatrics is sorely needed.

2. Materials and methods

2.1. Strains

In our previous study, we described the initial characterisation of all clinically significant Enterobacteriaceae isolates resistant to extended-spectrum β-lactam agents, including 56 *E. coli*, serially collected at Seattle Children's Hospital between 1999 and 2007 [4]. Of the 56 *E. coli* isolates described previously, 7 were excluded from this study: 3 specimens could not be located for further analysis; 3 failed to produce amplicons of the expected size with *adk* and *fumC* primers, suggesting non-*E. coli* species; and 1 was a second colony-type isolate from a single patient. The 49 remaining *E. coli* were studied in greater detail. Infection types with which these isolates were associated were defined as follows: community-associated (culture obtained from outpatient or <48 h after admission from an otherwise healthy patient not hospitalised in the last year); healthcare-associated (culture obtained from outpatient or <48 h after admission from a patient hospitalised in the last year and/or who has a medical condition requiring frequent contact with the healthcare system); and hospital-associated (culture obtained >48 h after admission, with no signs/symptoms of infection on admission).

2.2. Screening for and sequencing of β-lactamase genes

Antimicrobial susceptibility testing was performed as part of the original analysis as described previously [4]. Briefly, Kirby–Bauer disks containing ceftazidime or cefotaxime were used for ESBL screening, and confirmatory testing was performed using clavulanic acid-containing comparator disks. Results were interpreted according to contemporary

Clinical and Laboratory Standards Institute (CLSI) standards [4]. Clinical isolates with ESBL or AmpC phenotypes had previously been subjected to a five-gene amplification panel for *bla*$_{CMY}$, *bla*$_{DHA}$, *bla*$_{ACC}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ [4]. Sequencing of the positive *bla*$_{CMY}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ amplicons was carried out using the same primers used for detection: CITM-F, 5′-TGG CCA GAA CTG ACA GGC AAA-3′ and CITM-R, 5′-TT CTC CTG AAC GTG GCT GGC-3′ [5]; TEM-A, 5′-GAA AGG GCC TCG TGA TAC GC-3′ and TEM-B, 5′-TCA TCC ATA GTT GCC TGA CTC C-3′ [6]; and CTX-M-F, 5′-CGA TGT GCA GTA CCA GTA A-3′ and CTX-M-R, 5′-TTA GTG ACC AGA ATC AGC GG-3′ [7], respectively. Under published conditions, the following primer pair was used to detect a 475-bp internal fragment of *bla*$_{SHV}$: SHV-F1, 5′-TCA GCG AAA AAC ACC TTG-3′ and SHV-R1, 5′-TCC CGC AGA TAA ATC ACC A-3′ [8]. Then, under published conditions, the following primer pair was used to amplify and sequence *bla*$_{SHV}$ in screen-positive isolates: SHV-F2, 5′-GCC GGG TTA TTC TTA TTT GTC GC-3′ and SHV-R2, 5′-TCT TTC CGA TGC CGC CGC CAG TCA-3′ [9]. Allele identification was accomplished by alignment to a set of reference sequences (http://www.lahey.org/Studies).

2.3. Phylotyping of host strains by multiplex PCR

To assign each *E. coli* isolate to one of the four major phylogenetic groups, a rapid, three-target PCR assay in widespread use was utilised [10].

2.4. Multilocus sequence typing (MLST)

Seven MLST loci were sequenced for all *E. coli* isolates [11]. BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) was used to assemble sequence traces. Allele and ST assignments were made according to the Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli).

2.5. Simpson's index of diversity (D)

The *D* index quantifies the likelihood that two individuals selected randomly from the same population will exhibit different types. The statistic can be used to compare the discriminatory power of two typing methods applied to the same population, or to compare the diversity of two populations characterised by the same typing method. The publicly available script described by Carriço et al. [12] was implemented in BioNumerics.

2.6. Subclonal analysis of same-sequence type isolates

Isolates sharing ST profiles were subjected to pulsed-field gel electrophoresis (PFGE) as well as sequencing of the *fimH* (type 1 fimbrial adhesin) typing region (*fimH*$_{TR}$) and quinolone resistance-determining regions (QRDRs) of the chromosomal *gyrA* and *parC* loci. PFGE was performed using *Xba*I enzyme restriction as described previously [13] to identify epidemic lineages within the STs. Gel images were analysed using BioNumerics. Sequencing of *fimH*$_{TR}$ was carried out, sequences were aligned using BioNumerics and alleles were assigned as previously described [14]. Finally, QRDR sequencing was performed using primers and conditions described previously [15].

2.7. PCR-based virulence factor profiling

Virulence factor carriage was assessed using a protocol described for screening of extraintestinal pathogenic *E. coli* (ExPEC) pathotypes [16]. The five virulence markers (*papA* and/or *papC*, *sfa*/*foc*, *afa*/*dra*, *iutA* and *kpsMT*II) have been validated as independently predictive of ExPEC status by statistical analysis of strain collections wherein ExPEC status could be inferred from epidemiologic source or experimental virulence [17]; the housekeeping gene *uidA* serves as a positive control for bacterial template DNA. Strains

carrying none to one virulence factor are considered of low virulence, and those carrying two or more virulence factors are considered of high virulence.

2.8. Limited PCR-based replicon typing for CMY-2- and CTX-M-15-positive isolates

The plasmid content of qualifying wild-type isolates was evaluated by a subset of replicon typing primers that target essential plasmid replication genes [18]. Specifically, primers designed to identify the major incompatibility groups among extended-spectrum β-lactam resistance-associated plasmids were utilised, including IncI1 and IncA/C for CMY-2-carrying isolates, and IncFIIA, IncFIA and IncFIB for CTX-M-15-carrying isolates.

2.9. Multilocus sequence typing for IncI1 plasmids

MLST analysis for all isolates testing positive for IncI1 plasmids by replicon typing was carried out according to the methodology described by García-Fernández et al. [19]. Allele assignments were made using the online plasmid MLST database (http://pubmlst.org/plasmid).

2.10. Replicon sequence typing for IncF plasmids

IncF plasmid sequence typing (known as replicon sequence typing) was carried out for all isolates testing positive for IncFIIA, IncFIA or IncFIB by PCR-based replicon typing according to the methodology described by Villa et al. [20]. Allele assignments were made using the online plasmid MLST database (http://pubmlst.org/plasmid).

2.11. PCR-based analysis of genetic platforms of CTX-M-15 and CMY-2

To understand the genetic basis for dissemination of the resistance genes identified in this study, specific PCR panels were utilised to characterise the regions flanking the gene of interest. Specifically, the primer sets of Kang et al. were used, which distinguish types I, II and III in the genetic environment of the $bla_{\text{CMY-2}}$ enzyme [21], and the primers of Dhanji et al., which discriminate types 2a through 2g in the genetic environment of $bla_{\text{CTX-M-15}}$ [22].

3. Results

3.1. Patient/isolate description

In 48 patients for whom clinical data were available, 36 (75%) were female and the mean patient age was 7 years (range 0.12–25.4 years). Underlying co-morbidities were urological (25 patients; 52%), oncological (12 patients; 25%), other (5 patients; 10%) or none (9 patients; 19%). Infection types in this cohort were healthcare-associated (33 patients; 69%), community-associated (8 patients; 17%) and hospital-associated (7 patients; 15%). The 49 study isolates were recovered from the following clinical specimens: urine, 40 isolates; blood, 2 isolates; wound, 2 isolates; stool, peritoneal fluid, eye, genital swab, 1 isolate each; and unknown, 1 isolate (Supplementary Table S1).

3.2. Phylogroup and sequence type distribution and virulence properties of extended-spectrum β-lactam-resistant Escherichia coli

Of the 49 isolates, 7 (14%) derived from phylogroup A, 5 (10%) from phylogroup B1, 17 (35%) from phylogroup B2 and 20 (41%) from phylogroup D (Table 1). Thirty-three STs were detected, including 4, 5, 7 and 14 unique STs associated with phylogroups A, B1, B2 and D, respectively. The most prevalent single ST was phylogroup B2-derived ST131 (ST131/B2; 10 isolates), the disseminated clone of serotype O25:H4 that has been associated with emerging extended-spectrum β-lactam and fluoroquinolone resistance worldwide. ExPEC-like virulence profiles (virulence factor score 2) were highly prevalent among phylogroup B2 isolates (16/17; 94%), consistent with the high virulence typical of this

group. However, high virulence profiles were no more common among phylogroup D isolates (3/20; 15%) than among typically low-virulence groups A (1/7; 14%) and B1 (1/5; 20%).

3.3. bla genes conferring extended-spectrum β-lactam resistance

bla gene determinants of extended-spectrum β-lactam resistance were identified in 43/49 isolates, including class A enzymes in 22 isolates and class C enzymes in 21 isolates (Table 1). Among isolates carrying class A enzymes, 17 isolates with CTX-M enzymes (including 10 CTX-M-15, 4 CTX-M-14 and 1 each of CTX-M-1, CTX-M-22 and CTX-M-27), 3 isolates with SHV enzymes (all SHV-12) and 2 isolates with ESBL TEM variants (TEM-10 and TEM-26, 1 each) were identified. Among isolates carrying class C enzymes, 19 isolates with CMY enzymes (all CMY-2) were identified. Upon re-culturing of two previously DHA-positive isolates [4], DHA positivity could no longer be detected by PCR, so amplicon sequencing for variant discrimination could not be performed. No *bla* gene determinant of extended-spectrum β-lactam resistance was identified in six isolates.

3.4. Phylogroup and sequence type (ST) distribution and ST diversity of specific bla determinants

CMY-2 was detected in 19 isolates encompassing 16 ST profiles, spanning the four major phylogroups (Fig. 1). Three lineages (ST10/A, ST68/D and ST362/D) yielded more than one CMY-2-bearing isolate, each with two isolates. The 17 isolates in which CTX-M enzymes were detected spanned nine STs. Specifically, the ten CTX-M-15-positive isolates encompassed three ST profiles (eight isolates from ST131/B2 and one isolate each from ST354/D and ST648/D); CTX-M-14 appeared in four STs, representing phylogroups A, B1 and D; and the remaining CTX-M variants (−1, −22 and −27) were each recovered in single isolates, representing STs from phylogroups A and D. The ST diversity of strains carrying the two most prominent enzymes in this collection was compared using Simpson's diversity index (*D*) values, yielding values of 98.25% [95% confidence interval (CI) 96–100.25%] for CMY-2 and 37.78% (95% CI 2.36–73.20%) for CTX-M-15, indicating greater diversity for CMY-2-positive than for CTX-M-15-positive isolates.

3.5. Plasmid and genetic platform features of CMY-2-positive isolates

Among the 19 CMY-2-positive isolates, IncI1 plasmids were detected in 11 isolates and IncA/C plasmids in 6 isolates (Table 2). All six IncA/C-positive isolates were resistant to at least one of ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole (SXT), whilst only 2/11 IncI1-positive isolates were resistant to any one of these agents. The IncI1-positive isolates were subjected to IncI1 plasmid MLST, revealing six isolates with IncI1-ST23, two with IncI1-ST12 and one each with IncI1-ST2, IncI1-ST17 and IncI1-ST104 (first described here). In two of the six IncA/C-positive isolates, the type I genetic platform configuration that features a tandem $bla_{\text{CMY-2}}$ duplication was detected, as exemplified by model *E. coli* plasmid peH4H [21].

3.6. Plasmid and genetic platform features of CTX-M-15-positive isolates

Serial replicon typing and replicon sequence typing analysis demonstrated that the CTX-M-15-positive isolates from ST131 encompassed two main IncF replicon sequence types: three isolates exhibited type F2:A-:B1 and four isolates exhibited type F2:A1:B- (Fig. 2). A CTX-M-15-positive ST354 isolate exhibited a F-:A1:B1 type profile (as did a second ST354 isolate positive for CTX-M-1). The remaining CTX-M-15-positive isolate (ST648/D) displayed an F1:A1:B1 plasmid profile. Of the ten isolates positive for CTX-M-15, nine exhibited the native IS*Ecp1*-$bla_{\text{CTX-M-15}}$ configuration, known as '2a' [22], that is prevalent worldwide and exemplified by model plasmid pEK516 [23] from UK epidemic strain 'D'

[24]. The sole CTX-M-15 platform variant was ST131 isolate XQ35 that exhibited the configuration known as '2d' [22], seen in model plasmid pEK499 [23] from UK epidemic strain 'A' [24].

3.7. Subclonal analysis of ST131 isolates

Because of diversity in clinical and molecular features among the ten ST131 isolates, subclonal analysis was conducted, including PFGE and sequencing of the *fimH* typing region (*fimH*$_{TR}$) as well as sequencing of the QRDRs in the chromosomal *gyrA* and *parC* loci; two non-ST131 CTX-M-15-positive isolates were included as outgroup controls (Fig. 2). On PFGE analysis, the ten ST131 isolates demonstrated ≥70% similarity by Dice coefficient, including two pairs exhibiting ≥90% similarity; within these two pairs, the individual isolates were collected 21 months and 48 months apart, respectively (Fig. 2). In contrast, two CTX-M-15-positive, non-ST131 isolates (representing two STs from phylogroup D) exhibited ≤60% similarity to the ST131 group.

Then, within ST131, three unique *fimH*$_{TR}$ alleles were detected: *fimH*$_{TR}$30 (eight isolates), *fimH*$_{TR}$27 and *fimH*$_{TR}$22 (1 isolate each). The eight *fimH*$_{TR}$30 isolates, but neither of the non-*fimH*$_{TR}$30 isolates, were CTX-M-15-positive and carried either F2:A1:B- or F2:A-:B1 plasmids. Furthermore, the same eight *fimH*$_{TR}$30 ST131 isolates were resistant to fluoroquinolones and encoded identical QRDR regions of *gyrA* and *parC* (data not shown), with identical QRDR polymorphisms in GyrA and ParC (Fig. 2). Meanwhile, the two remaining ST131 isolates encoding *fimH*$_{TR}$27 and *fimH*$_{TR}$22, carried SHV-12 and CMY-2, respectively, and neither carried F2-related plasmids. In addition, the two non-*fimH*$_{TR}$30 isolates were fluoroquinolone-susceptible, and neither encoded QRDR polymorphisms associated with quinolone resistance. Thus, *fimH*$_{TR}$ alleles were concordant with plasmid- and chromosome-associated antibiotic resistance properties within ST131.

4. Discussion

Among 49 extended-spectrum β-lactam-resistant *E. coli* isolates collected serially from 1999–2007 at a free-standing children's hospital in Seattle, a temporal trend of increasing extended-spectrum β-lactam resistance was driven in near-equal parts by class A and class C enzymes (CTX-M-15 and CMY-2, respectively). Although the class A enzyme CTX-M-15 has attracted widespread attention, the class C enzyme CMY-2 was the most frequently recovered single determinant in this study. The molecular features of the isolates carrying these respective enzymes differed in the extent to which they were associated with particular *E. coli* STs, plasmid incompatibility (Inc) groups and plasmid sequence types (pSTs). Nineteen CMY-2-positive isolates were highly diverse at the ST level, deriving from 16 STs, whilst ten CTX-M-15-positive isolates were highly clonal at the ST level, deriving from 3 STs; the two subsets exhibited non-overlapping values of Simpson's diversity index (*D*). Furthermore, 8 of 10 CTX-M-15-positive isolates derived from a specific subclone of the disseminated ST131 lineage, demonstrating distinctive clonal dynamics for these two prominent enzymes.

CMY-2 was not strongly associated with any single *E. coli* ST, appearing in three same-ST isolate pairs, but mainly appeared in isolates carrying plasmids with either IncI1 (11 isolates) or IncA/C (6 isolates) backbones. Previous reports have identified these two Inc groups in association with CMY-2, but with IncA/C appearing more prevalently than IncI1 [25]. Then, among IncI1-positive isolates in particular, the IncI1-ST23 lineage predominated, appearing in six isolates, whilst IncI1-ST12 plasmids appeared in another two isolates. Of interest, IncI1-ST12 was also detected (data not shown) in two *Salmonella enterica* serotype 4,5,12:i:- isolates recovered at Seattle Children's Hospital during a 2004 outbreak [4], suggesting intergenus host range specificity for this plasmid type. IncI1-

positive isolates were more likely to be susceptible to aminoglycosides, fluoroquinolones and SXT than IncA/C-positive isolates, consistent with previous findings detailing the conserved, multidrug-resistant backbone of IncA/C plasmids [26].

CTX-M-15 carriage was observed in eight isolates of the globally-emergent ST131 lineage that carried IncF plasmids, mainly representing either F2:A1:B- (four isolates) or F2:A-:B1 (three isolates) replicon sequence types. The eight ST131 isolates carrying CTX-M-15 shared additional molecular features that differed from ST131 isolates carrying other enzymes. Specifically, the CTX-M-15 isolates encoded the *fimH*$_{TR}$30 allele as well as specific fluoroquinolone resistance-associated QRDR polymorphisms (GyrA L83/N87, ParC I80/V84), and carried IncF-backbone plasmids of either F2:A-:B1 or F2:A1:B- replicon sequence types. The non-CTX-M-15 isolates carried not *fimH*$_{TR}$30 but *fimH*$_{TR}$27 (SHV-12) or *fimH*$_{TR}$22 (CMY-2), and encoded either wild-type or mutant QRDR polymorphisms in association with fluoroquinolone susceptibility, and IncF plasmids representing non-F2 profiles. Detection of multiple *fimH*$_{TR}$ alleles, representing both phylogenetically-restricted (found in only a single phylogroup) and phylogenetically-dispersed (found in two or more phylogroups) variants [14], has been described for highly successful extraintestinal lineages including ST95/B2 and ST73/B2 [14] and may reflect the importation of promiscuous and adaptive *fim* clusters enabling niche specialisation during clonal expansions or 'blooms'. In ST131, the association of characteristic antibiotic resistance traits (QRDR- and CTX-M-15-associated) with phylogenetically-dispersed allele *fimH*$_{TR}$30 may indicate the emergence of a highly successful subclone from a recent ancestor carrying the phylogenetically-restricted allele *fimH*$_{TR}$22.

Likely enzymatic determinants of extended-spectrum β-lactam resistance were detected in only 43 of 49 *E. coli* studied here. Of the six enzyme-negative isolates, two exhibited >5 mm differences in zone size with clavulanic acid inhibition (data not shown), indicating ESBL-mediated resistance due to an enzyme not included in or not detected by our primer set; the remaining isolates may have undergone derepression/hyperproduction of chromosomal AmpC [27,28] or alteration in expression of outer membrane porin channels [29], a distinction that would be enabled by cloxacillin inhibition testing not performed here. The growing access to rapid, affordable genome sequencing offers the possibility of enhanced detection of novel enzymes without isoelectric focusing, or discovery of sequence variations that might impair PCR detection of *bla* genes by disruption of primer annealing sites.

The limitations of this study include: (a) a relatively small number of isolates collected at a single centre, without comparison with an extended-spectrum β-lactam-susceptible cohort; (b) the use of a clinical microbiological approach for detection of extended-spectrum β-lactam resistance that was more intensive than the contemporary CLSI methodology, thus presenting a challenge to comparison with other centres' experiences; and (c) failure to localise definitively all identified extended-spectrum β-lactam resistance determinants to a plasmid versus chromosome location, or to the specific incompatibility group or pST lineages reported. In addition, the size and nature of this study population precludes further analyses of interest regarding ST131 differences between paediatric and adult isolates, or between extended-spectrum β-lactam-resistant and -susceptible isolates, with regard to traits such as *fimH*$_{TR}$ sublineage distribution, virulence factor carriage and IncF plasmid carriage.

In summary, increasing extended-spectrum β-lactam resistance in this collection was driven by two enzymes with different clonal associations. CMY-2 was non-clonal at the ST level, but exhibited associations with IncI1, specifically the IncI1-ST23 and (to a lesser extent) IncI1-ST12 lineages, and IncA/C plasmid backbones. In contrast, CTX-M-15 was associated with a single ST (the disseminated ST131 clone) as previously described [3,30]. However, CTX-M-15 positivity (and associated carriage of IncF family plasmid variants, including

F2:A1:B- and F2:A-:B1) was observed in a specific *fimH*$_{TR}$30 subclone of ST131 that also displayed chromosomal, QRDR-encoded fluoroquinolone resistance. These findings are based on a molecular typing methodology that accounted for three independent but interconnected levels of bacterial genetic organisation. Molecular typing systems that capture information about bacterial chromosomes, plasmid backbones and resistance genes/platforms may serve as the basis for an integrated global system of surveillance for emerging antibiotic resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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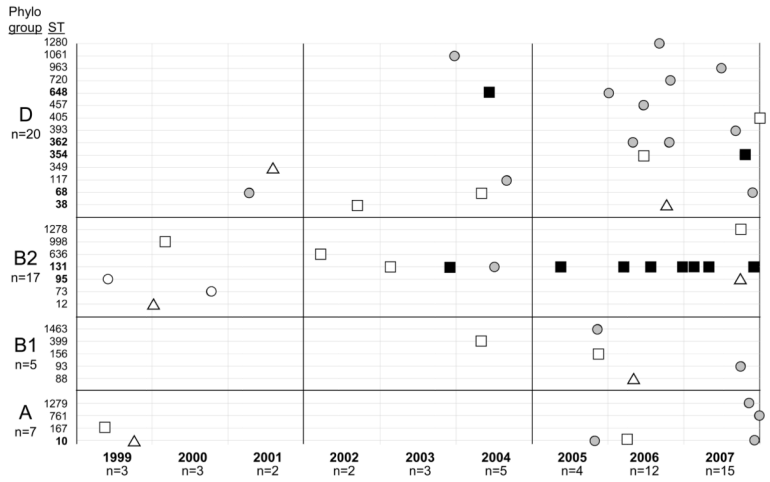
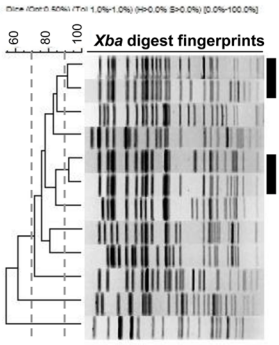


Fig. 1.

Phylogenetic and temporal distribution of 49 *Escherichia coli isolates* resistant to extended-spectrum cephalosporins at Seattle Children's Hospital (Seattle, WA) during 1999–2007. ST, sequence type.

Key: Bold STs have more than 1 representative. Squares: Isolates carrying Class A enzymes. Black squares: Isolates carrying CTX-M-15. Circles: Isolates carrying Class C enzymes. Grey circles: Isolates carrying CMY-2. Triangles: Isolates with no enzyme identified.



Strain	ST/phy	$fimH_{TR}$	GyrA	ParC	CP	GM	T/S	Plasmid type	R gene	IS*Ecp*	VF	Date
XQ18	131/B2	30	L83 N87	I80 V84	R	R	S	F2:A1:B-	CTX-M-15	2a	2	16-May-05
XQ46	131/B2	30	L83 N87	I80 V84	R	R	S	F2:A1:B-	CTX-M-15	2a	2	11-Feb-07
XQ45	131/B2	30	L83 N87	I80 V84	R	S	R	F2:A2:B20	CTX-M-15	2a	2	04-Jan-07
XQ11	131/B2	27	L83	T57	S	S	R	F+:A-:B-	SHV-12	(-)	4	14-Feb-03
XQ12	131/B2	30	L83 N87	I80 V84	R	R	R	F2:A-:B1	CTX-M-15	2a	3	29-Nov-03
XQ43	131/B2	30	L83 N87	I80 V84	R	S	R	F2:A-:B1	CTX-M-15	2a	3	24-Nov-07
XQ23	131/B2	30	L83 N87	I80 V84	R	R	R	F2:A-:B1	CTX-M-15	2a	3	11-Mar-06
XQ29	131/B2	30	L83 N87	I80 V84	R	S	R	F2:A1:B-	CTX-M-15	2a	4	25-Jul-06
XQ35	131/B2	30	L83 N87	I80 V84	R	R	R	F2:A1:B-	CTX-M-15	2d	2	03-May-07
XQ16	131/B2	22	w.t.	w.t.	S	S	S	F4:A-:B1, I1-ST12	CMY-2	(-)	1	03-Jul-04
XQ15	648/D	0	L83 N87	I80	R	R	R	F1:A1:B1	CTX-M-15	2a	1	03-Jun-04
XQ41	354/D	58	n.a.	I80	I	S	R	F-:A1:B1	CTX-M-15	2a	0	15-Oct-07

Fig. 2.

Molecular and antibiotic susceptibility properties of ten sequence type 131 (ST131) and two CTX-M-15-positive, non-ST131 *Escherichia coli* isolates.

ST/phy, sequence type/phylogroup. $fimH_{TR}$, *fimH* typing region allele. GyrA and ParC, amino acid polymorphisms in chromosome-encoded quinolone resistance-determining regions of GyrA and ParC. CP, ciprofloxacin. GM, gentamicin. T/S, trimethoprim-sulfamethoxazole. Plasmid type, incompatibility group and plasmid sequence type. F+, IncFIIA positive by PCR, but sequencing unsuccessful. R gene, resistance gene. IS*Ecp*, CTX-M platform isotype, according Dhanji et al [22]. VF, virulence factor score. Date, date of isolation. Vertical black bars indicate fingerprint pairs with similarity ≥90%. R, resistant. S, sensitive. w.t., wild type. n.a., not able to amplify with study primers. Horizontal line in table divides ST131 (above) from non-ST131 (below) isolates.

Table 1

Phylogenetic and clonal distribution of extended-spectrum β-lactam-resistant *Escherichia coli* isolates from Seattle Children's Hospital (Seattle, WA), 1999–2007

Enzyme variant	*n*	Phylogroup				
		A	B1	B2	D	ST with ≥2 representatives
Class A enzyme-positive	22	2	2	12	6	
CTX-M-15	10	–	–	8	2	ST131/B2 (8)
CTX-M-14	4	1	1	–	2	–
CTX-M-1	1	–	–	–	1	–
CTX-M-22	1	–	1	–	–	–
CTX-M-27	1	–	–	–	1	–
SHV-12	3	1	–	2	–	–
TEM-10	1	–	–	1	–	–
TEM-26	1	–	–	1	–	–
Class C enzyme-positive	21	4	2	3	12	
CMY-2	19	4	2	1	12	ST10/A, ST68/D, ST362/D (2 each)
DHA	2	–	–	2	–	–
No enzyme identified	6	1	1	2	2	
Total	49	7	5	17	20	

ST, sequence type.

Table 2

Molecular and antibiotic susceptibility properties of 19 CMY-2-positive *Escherichia coli* isolates from Seattle Children's Hospital (Seattle, WA), 1999–2007

am Strain	ST/phy.	IncA/C PBRT	IncI1		DsbC- L/CMY-2-R[a]	Antibiotic susceptibility			QRDR polymorphisms [b]	
			PBRT	pMLST		CIP	GEN	SXT	GyrA	ParC
XQ07	68/D	+	−	–	+	S	S	R	w.t.	w.t.
XQ20	1463/B1	+	−	–	−	S	R	R	w.t.	w.t.
XQ30	1280/D	+	−	–	−	S	S	R	w.t.	w.t.
XQ38	393/D	+	−	–	−	R	S	R	L83 N87	I80 T57
XQ40	93/B1	+	−	–	+	I	S	R	L83	I80
XQ50	761/A	+	+	ST104	−	S	R	R	w.t.	w.t.
XQ16	131/B2	−	+	ST12/CC12	n.t.	S	S	S	w.t.	w.t.
XQ56[c]	648/D	−	+	ST12/CC12	n.t.	S	S	S	L83 N87	I80
XQ33	720/D	−	+	ST17/CC2	n.t.	S	S	S	w.t.	T57
XQ55[c]	1061/D	−	+	ST2/CC2	n.t.	S	S	S	w.t.	w.t.
XQ19	10/A	−	+	ST23/CC2	n.t.	S	S	S	w.t.	w.t.
XQ27	457/D	−	+	ST23/CC2	n.t.	S	S	S	n.a.	w.t.
XQ42	1279/A	−	+	ST23/CC2	n.t.	S	S	S	w.t.	w.t.
XQ49	10/A	−	+	ST23/CC2	n.t.	S	S	S	w.t.	w.t.
XQ36	963/D	−	+	ST23/CC2	n.t.	S	S	R	w.t.	w.t.
XQ48	68/D	−	+	ST23/CC2	n.t.	S	S	S	w.t.	w.t.
XQ25	362/D	−	−	–	n.t.	S	S	S	w.t.	w.t.
XQ32	362/D	−	−	–	n.t.	S	S	S	w.t.	w.t.
XQ17	117/D	−	−	–	n.t.	R	S	S	L83 V87	K84

ST, sequence type; phy., phylogenetic group; PBRT, PCR-based replicon typing; pMLST, plasmid multilocus sequence typing, including clonal complex (CC) assignment where appropriate; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; n.t., not tested.

[a] DsbC-L/CMY-2-R PCR primers described by Kang et al. [21].

[b] Quinolone-resistance determining region (QRDR) polymorphisms in GyrA and ParC expressed as wild-type (w.t.) or polymorphic residues. n.a., not amplified with study primers.

[c] Indicates isolates that also carried IMP-4 metallo-β-lactamase.