The Molecular Epidemiology of Resistance in Cefotaximase-Producing Escherichia coli Clinical Isolates from Dublin, Ireland.

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Title:

The molecular epidemiology of resistance in cefotaximase (CTX-M)-producing *Escherichia coli* clinical isolates from Dublin, Ireland

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Introduction

Extended-spectrum β-lactamases (ESBLs) are amongst the most important resistance determinants in Enterobacteriaceae, conferring resistance to oxyimino-cephalosporins. ESBLs have increased remarkably in diversity and range recently, predominantly due to the evolution of cefotaximase (CTX-M)-type enzymes, which are now the most common ESBLs worldwide, and are often found on widespread transferable plasmids in *Escherichia coli* responsible for infections in both the community and hospitals[4]. In addition to human-to-human transmission, the contribution of zoonotic transmission by direct contact and through the food chain, to the diversity and mobilisation of $bla_{\text{CTX-M}}$ genes is increasingly recognised.[14,18,21] CTX-Ms are organized into five clusters (groups 1, 2, 8, 9, and 25) based on their amino acid sequences (www.lahey.org/studies/webt.stm), with group 1 (including CTX-M-1, -3 and -15) and group 9 enzymes (including CTX-M-9, -14 and -27) widespread in Europe. The insertion sequences (ISs), IS*Ecp1* and IS*CR1* are responsible for the mobilization of $bla_{\text{CTX-M}}$ and drive their expression. CTX-M genes are frequently found downstream of IS*Ecp1* within modular multidrug-resistance regions (MRRs) on ESBL plasmids[9,29] or are linked to IS*CR1* on complex class 1 integrons which also bear highly variable gene cassette regions encoding resistance to multiple antimicrobial classes. The surveillance of non $bla_{\text{CTX-M}}$ - associated integrons in nosocomial ESBL is important as ESBL genes co-localised on the same plasmid may be co-selected in the gut flora of animals where these antibiotics are used and may be a source of zoonotic transfer to humans through the food chain. Multiple copies of IS*26* are also found on MRRs of ESBL plasmids and have contributed to the spread of $bla_{\text{CTX-M}}$ by facilitating genetic rearrangements of these regions between ESBL plasmids.[22,29] The staggering propensity for recombination and transposition, afforded by this genetic arrangement, has resulted in the evolution of mosaic ESBL plasmids of narrow and broad host ranges that confer multidrug-resistance, including to the carbapenems[2]. In the healthcare setting, these plasmids are likely to be vertically and horizontally disseminated, as the multi-drug resistant (MDR) phenotype they confer provides a survival advantage to bacteria in the antimicrobial laden environment. Their association with widespread successful *E. coli* clones, such as the pandemic O25b-ST131 clone, further facilitates their dissemination by clonal expansion[2].

We previously investigated the genetic relatedness of 100 ESBL-producing *E. coli* (ESBL-EC) from North Dublin[3] revealing the widespread dissemination of ST131 and other clones within Beaumont Hospital, Dublin and the local community. The aims of the present study were: (1) to characterise the mobile genetic elements, including the ISs, integrons and plasmids to which $bla_{\text{CTX-M}}$ and other resistance genes were associated in this collection with reference to the published literature from human and animal *E. coli* and (2) to determine the potential for horizontal transfer of CTX-M plasmids from clinical *E. coli* to laboratory strains.

Materials and Methods

Bacterial strains and culture conditions

One hundred ESBL-EC clinical isolates were collected as part of a previous study between January 2009 and December 2010 in Beaumont Hospital, Dublin, Ireland and were previously subjected to routine diagnostic antimicrobial susceptibility tests and PFGE, which identified 12 clusters A-L.[3] Appropriate control strains for beta-lactamase negative, bla_{TEM}, bla_{SHV} and each of the five $bla_{\text{CTX-M}}$ groups and epidemic UK strain A were sourced from the American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC). A sodium azide-resistant *E.coli* strain J53 was the recipient for conjugations and was a gift from Prof. Martin Cormican, Department of Bacteriology, National University of Ireland, Galway. *E. coli* ElectroMAX™ DH5α-E™ (Invitrogen) was the recipient for transformations. All isolates were routinely grown on Mueller-Hinton (MH) agar.

Characterisation of resistance genes and their associated genetic elements

DNA was prepared from overnight cultures using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The carriage of bla_{TEM}, bla_{SHV} and the five $bla_{\text{CTX-M}}$ gene groups in the isolates was investigated by multiplex PCR using previously described primers and cycling conditions.[17,30] Specific $bla_{\text{CTX-M}}$ alleles and their upstream genetic environments were identified by

PCR mapping and were fully sequenced. The genes, *bla*$_{SHV}$ and *bla*$_{TEM}$ were sequenced in isolates that were*bla*$_{CTX-M}$-negative. However, resource restraints prevented sequencing of the upstream genetic environments of *bla*$_{TEM}$ and *bla*$_{SHV}$ alleles in favour of the more widely disseminated *bla*$_{CTX}$ alleles. PCR and sequencing primers used to detect class 1 and 2 integron motifs, IS*Ecp1*, IS*26*, and IS*CR1* and their arrangement in relation to *bla*$_{CTX-M}$ are listed in Table S1. Sequencing was performed by Source BioScience (Dublin, Ireland) and GATC Biotech (Köln, Germany) and sequence analysis was carried out using CLC Main Workbench 6.6.2 (CLC Bio, Aarhus, Denmark). Representative integron variable region amplicons of each different size were sequenced and used to perform BLAST searches on the National Centre for Biotechnology Information (NCBI) nucleotide databases.

Transfer of CTX-M plasmids by conjugation and transformation

Conjugation was attempted by broth mating following the method of Gray *et al.* with modifications[10] for all ESBL-EC strains except the 33 strains that clustered with the single reference UK strain A (PFGE cluster A), whose ESBL plasmids lack the necessary conjugation machinery.[29] Briefly, after 4 h aerobic growth in tryptone soya broth (TSB) cultures of donor and recipient strains were mixed at 1:1 ratio and incubated at 37°C overnight. Mating mixtures were pelleted, rinsed and serially diluted in PBS. Transconjugants were selected using MH agar containing 100 µg/ml sodium azide and 1 µg/ml cefotaxime. Presumptive transconjugants were confirmed by PCR[5] and *bla* multiplex PCR[17] with reference to donor and recipient strains, using 10 µl of cleared cell lysate as the template. Where conjugation was unsuccessful, transformation of ESBL plasmids into *E. coli* DH5α was attempted by electroporation performed at 1700 V and at a time constant of 4.8-5.0 ms using an electroporator (Eporator®, Eppendorf, UK).

Characterisation of CTX-M plasmids

Cefotaxime (CTX) minimum inhibitory concentration (MIC) assays were performed and interpreted using the guidelines of the Clinical and Laboratory Standards Institue (CLSI) for all clinical isolates and their transconjugants/transformants using Etest® strips (bioMérieux).[6] High-level cefotaxime resistance was defined as MIC ≥128 µg/ml. Plasmid DNA was isolated from clinical isolates and their transconjugants/transformants using the phenol-chloroform extraction method of Kado and Liu.[12] The presence of *bla*$_{CTX-M}$ genes, associated IS elements and integrons in plasmid extracts of transconjugants/transformants was investigated by PCR as described for clinical isolates. CTX-M-containing plasmids were sized by S1 PFGE.[1] Plasmid incompatibility typing was carried out on transconjugant/transformant plasmid preparations and clinical isolates using PCR-based replicon typing (PBRT). Transferable IncF plasmids were further characterised by replicon sequence typing (RST) and IncN and IncI plasmids were further characterised by plasmid multi-locus sequence typing (pMLST). All typing schemes were performed and interpreted using the methods and databases available at http://pubmlst.org/plasmid/.

Results

Genotypic characterisation of ESBL genes in *E. coli* clinical isolates

Investigation of the carriage of *bla*$_{CTX-M/TEM/SHV}$ genes revealed that 94% of isolates were *bla*$_{CTX-M}$ gene positive. *Bla*$_{TEM}$ genes were the second most common (46%) and 5% of clinical isolates carried a *bla*$_{SHV}$ gene. Group 1 was the most common cefotaximase gene cluster (80/94; 85%) and comprised 66 *bla*$_{CTX-M-15}$, 7 *bla*$_{CTX-M-1}$, 4 *bla*$_{CTX-M-3}$, 2 *bla*$_{CTX-M-55}$ and 1 *bla*$_{CTX-M-32.}$ Thirteen group 9 (13/94; 14%) were identified, comprising 8 *bla*$_{CTX-M-14}$, 3 *bla*$_{CTX-M-9}$ and 2 *bla*$_{CTX-M-27}$. A single isolate contained a *bla*$_{CTX-M-2}$ gene, but no group 8 or group 25 CTX-M genes were detected.

Specific *bla*$_{CTX-M}$ genes and their upstream genetic environments

The arrangement of *bla*$_{\text{CTX-M}}$ genes and associated upstream IS elements are summarised in Table 1, with reference to identical previous GenBank entries. A number of common genetic arrangements were identified within and between the PFGE cluster groups and these arrangements were grouped together for comparison with arrangements previously described in the literature. An IS*Ecp1* promoter was located upstream of *bla*$_{\text{CTX-M}}$ in 53 isolates (56%). In 35 (66%) of these isolates, PCR detected the full length IS*Ecp1* element (1.7 kb) including the *tnpA* transposase gene (0.8 kb). However, PCR results suggested truncation within IS*Ecp1* in the other 18 isolates (no IS*Ecp1* amplicon was detected), which apparently occurred within *tnpA* for 10 isolates where no *tnpA* amplicon was detected either. Many of the genetic environments identified between IS*Ecp1* and *bla*$_{\text{CTX-M}}$ matched the frequently described "W", "X" and "V" common regions first described by Eckert *et al*[9] the most common of which was the 48 bp W spacer region typical of UK strains B to E[31], which was present in 32 isolates in combination with *bla*$_{\text{CTX-M-15}}$ and generally associated with high-level cefotaxime resistance (MICs ≥128 μg/ml). PCR and sequencing confirmed reversely oriented IS*26* inserted within the terminal inverted repeat of IS*Ecp1* (24 bp before the 3' end of IS*Ecp1*) and upstream of *bla*$_{\text{CTX-M-15}}$, as described previously for UK strain A[31] in all but one (33/34) PFGE cluster A isolates and an ST131 isolate from cluster J. These 34 isolates also contained the alternative 'promoter X' region with -35 TTCATG and -10 GGGGATGAT sequences positioned 140 bp and 115 bp, respectively, upstream of the *bla*$_{\text{CTX-M-15}}$ start codon within IS*26*, conferring variable levels of cefotaxime resistance as described previously.[7] The remaining PFGE cluster A isolate contained *bla*$_{\text{CTX-M-14}}$ downstream of IS*Ecp1*. The IS*CR1* element was located upstream of *bla*$_{\text{CTX-M-9}}$ as part of a complex class 1 integron in all three PFGE cluster G isolates, which upon sequencing resembled the *sul1*-type integron In60-D.[20] IS*CR1* was also detected upstream of a *bla*$_{\text{CTX-M-2}}$ gene as part of an In35-like complex class 1 integron.

Integron content of ESBL *E. coli* clinical isolates

Class 1 integrons were detected in 66% of isolates. Four isolates contained complex class 1 integrons bearing $bla_{\text{CTX-M}}$ as described above and these are indicated in Table 2. The remaining isolates contained class 1 integrons associated with resistance to agents such as trimethoprim, sulphonamides and aminoglycosides. Nine distinct class 1 integron variable region amplicons of different sizes were detected and their distribution amongst PFGE clusters is summarised in Table 2 and details of each individual isolate are given in supplementary Table 2. Seven isolates produced more than one amplicon, indicating multiple class 1 integrons were present. Six isolates contained class 2 integron variable regions of 2.2 kb (*dfrA1-sat1-aadA1*), five of which also contained a class 1 integron. The most common class 1 integron variable region was 1.7 kb (*dfrA17-aadA5*), present in 54% of isolates. Most integron variable regions identified in this study contained genes for trimethoprim (*dfr*) and streptomycin/spectinomycin (*aad*) resistance. The presence of dihydrofolate reductase-containing integrons correlated with resistance to trimethoprim and/or trimethoprim/sulfamethoxazole in all clinical *E. coli* isolates. However, streptomycin or spectinomycin susceptibility was unknown for ESBL-EC as they were not routinely tested for. Gene cassettes conferring resistance to gentamicin and tobramycin were detected infrequently, were co-localised with a chloramphenicol resistance gene: either *cmlA1*, *cmlA6* (sporadic isolates) or *catB8* (cluster G isolates). Although susceptibility patterns to chloramphenicol were untested, the presence of either *aacA4* or *aadB* correlated with phenotypic resistance to gentamicin in all five strains with these gene cassettes.

Characterization of transferable CTX-M plasmids

PBRT of all clinical isolates detected IncF plasmids in 90% of clinical isolates, IncI1 plasmids in 30%, IncN plasmids in 3% and two isolates carrying L/M and B/O plasmids (Table S2). Transfer of ESBL plasmids was successful for 33 strains, 28 of which transferred a CTX-M-producing plasmid. The remaining 5 plasmids conferred an ESBL phenotype through expression of bla_{TEM} or bla_{SHV}. The CTX-M plasmids were transferred by conjugation (18) and transformation (10). The replicon types identified in these plasmids were IncF (12), IncI1 (11) and IncN (2). The remaining three CTX-M

plasmids were untypeable by PBRT. Characteristics of the transferable CTX-M plasmids are detailed in Table 3. Individual RSTs were indeterminable for two CTX-M bearing plasmids in recipient strains that harboured multiple IncF plasmids; pBHEC48 and pBHEC12. Six different RSTs were identified amongst the remaining ten IncF CTX-M plasmids. IncF plasmids carried group 1 CTX-M genes except for the 27.5 kb plasmid pBHEC91, which carried a group 9 CTX-M gene (CTX-M-27). The 1.7 kb *dfrA17-aadA5* integron was commonly transferred by IncF plasmids. Among 11 Incl1 CTX-M plasmids identified, six different sequence type (ST)s were identified by IncI1 pMLST. These were ST3 (n=3, size range 107-112.5 kb), ST7 (n=3, size range 104.5-113 kb), ST 16 (n=2, 94 kb), ST31 (n=1, 93 kb), ST 57 (n=1,84 kb) and the previously undefined ST159, 100 kb with repI1/ardA/trbA/sogS/pilL alleles 1/2/9/1/7 (pBHEC16). The 1.5 kb *dfrA1-aadA1* integron was transferred on two very similar IncI1-ST7 plasmids, pBHEC20 and pBHEC90. Two IncN type plasmids of ~30 kb were identified which belonged to the previously identified ST1 and ST6 types. CTX-M plasmids untypeable by PBRT comprised pBHEC66 (CTX-M-55), pBHEC54 (CTX-M-15, TEM) and pBHEC76 (CTX-M-15). Cefotaxime MICs for recipient strains bearing CTX-M plasmids were often less (by between 2 and 5 doubling dilutions) than those of their corresponding clinical isolate donor strains. Data for all CTX-M plasmids were deposited in the relevant plasmid MLST database at http://pubmlst.org/plasmid/.

Discussion

Similar to the situation reported in Europe and globally, our study identified *bla*$_{\text{CTX-M}}$ as the most common ESBL gene in clinical *E. coli* isolates collected during 2009-2010 with group 1 alleles the most common (80/94, 85%), especially *bla*$_{\text{CTX-M-15}}$, which was found in two thirds (66%) of ESBL-EC. A previous nationwide 11 year study (1997-2007) reported a prevalence of 59% group 1 *bla*$_{\text{CTX-M}}$ among *bla*$_{\text{CTX-M}}$ producing *E. coli*. Group 9 CTX-M prevalence among *E. coli* was 144/348 (41.4%) compared to 13/94 (14%) in this study, however, this may reflect regional differences in prevalence or changes during the intervening period.[19] The increased prevalence of group 1 genes may be partly

due to *E. coli* ST131 dissemination in Ireland, associated with $bla_{\text{CTX-M-15}}$.[3,24] The endemic nature of CTX-M-15-producing *E. coli* is evident in Dublin, as indicated by the detection of $bla_{\text{CTX-M-15}}$ in clonal and sporadically-occurring isolates. Isolates from the endemic PFGE cluster A were confirmed as genetically indistinguishable from UK strain A and contain $bla_{\text{CTX-M-15}}$ under the control of "promoter X", as described previously.[7] Despite the local dominance of CTX-M-15, four other group 1 genes were detected amongst 14 isolates; $bla_{\text{CTX-M-1, -3, -32}}$ and $bla_{\text{CTX-M-55}}$. To the best of our knowledge, this represents the first detection of the latter two genes, or indeed the group 2 gene $bla_{\text{CTX-M-2}}$, amongst human *E. coli* isolates in Ireland. Molecular investigation of the promoter regions by PCR mapping enabled the identification of a number of allele-specific associations. Amongst group 9 CTX-M genes $bla_{\text{CTX-M-14}}$ was associated with IS*Ecp1*, $bla_{\text{CTX-M-27}}$ with IS*26* and $bla_{\text{CTX-M-9}}$ with IS*CR1* as part of the In60-D integron.[20] Group 1 alleles were usually associated with IS*Ecp1*, likely controlling *bla* expression and driving high-level cefotaxime resistance when associated with $bla_{\text{CTX-M-15}}$ as previously described.[23]

Class 1 integron carriage in our Dublin ESBL-EC collection (66%) was similar to that recorded in a longitudinal study of ESBL-EC isolates from Madrid (67%)[16] but lower than recorded in clinical *E. coli* isolates collected from 1998-2004 in Guangzhou, China (86%).[28] As previously reported, a low prevalence of complex class 1 integrons containing $bla_{\text{CTX-M}}$ was found[16]. Interestingly, *dfrA17-aadA5* was the most common integron array in the present study (54%) and the Chinese study (36%). This integron was also detected in Madrid and is globally disseminated in *E. coli*.[11,16,22,28] Class 1 integrons are frequently associated with Tn*21*-like transposons[15] or multiple copies of IS*26* on large conjugative plasmids in clinical Enterobacteriaceae, which facilitate the mobilisation of drug resistance elements among plasmids by homologous recombination.[22,29] This may explain the presence of the *dfrA17-aadA5* integron on at least four different IncF type plasmids in our *E. coli* collection. The epidemic distribution of host strains, as exemplified here by UK strain A, may account for its high prevalence.

Six of the nine distinct integron variable regions identified in the present study, including all those identified in multiple isolates, were documented previously in a study carried out at the

Veterinary Hospital, University College Dublin during 2007.[13] The authors characterised consecutive MDR *E. coli* isolates from predominantly faecal samples of horses (44), cattle (17), pigs (9), dogs (3) and a sheep, reporting high carriage rates of class 1 integron gene cassette regions (76%). None of the veterinary *E. coli* isolates belonged to the B2 phylogenetic lineage which is mainly associated with infections in humans.[13] Nonetheless, the identification of matching class 1 integron variable regions exemplifies a possible reservoir for these antimicrobial resistance determinants, which may evolve in the commensal *E. coli* of companion and food animals amidst the selective pressure of veterinary antimicrobial use and transfer to strains causing human disease.[8,18] The widespread and lengthy use of trimethoprim and sulphonamides in veterinary medicine has been implicated in the evolution and persistence of integron-bound resistance genes of the *dfr* and *sul* families.[25] Likewise, the *aad* genes for streptomycin resistance are positively selected for in animals, where it is used as a first-line drug for Gram-negative infections. One can speculate that co-carriage of ESBL genes on integrons or indeed plasmids containing these gene cassettes may drive their co-selection and propagation in the gut flora of animals treated with veterinary antimicrobials.

We noted a local dominance of multi-replicon IncF and IncI1 plasmids amongst CTX-M producing ESBL-EC in Dublin and identified four new RSTs. The range of IncF replicon sequences identified in this small cross section of ESBL-EC isolates from the same geographical location demonstrates the diversity in ESBL-bearing IncF plasmids. The diversity of STs amongst the ten transferred IncF CTX-M plasmids contrasts with the phylogenetic homogeneity of the host isolates, 7/10 (70%) of which belonged to the ST131 pandemic clone. This reflects the plasticity of IncF plasmids, which contain multiple hotspots for genetic recombination.[22] The success of conjugation (18) and transformation (10) of CTX-M plasmids was limited. Low success of conjugation may be explained by assuming that the replicon type FII-FIA (pEK499-like) plasmids detected here in 41 strains, including PFGE cluster A, lack the requisite *traW* to *traX* genes for conjugation, as has been shown previously. [29] Nonetheless, the importance of horizontal transfer in the dissemination of $bla_{\text{CTX-M}}$ and of trimethoprim and aminoglycoside resistance genes on class 1 integrons is evident, given their frequent location on conjugative plasmids.

Many of the 28 CTX-M plasmids characterised had RSTs and *bla*$_{CTX-M}$ alleles in common with those previously identified throughout Europe in both human and animal *E. coli* isolates. Identical ESBL genes, plasmids and strains of *E. coli* have been identified in Dutch poultry, chicken meat and humans.[14,21] There are relatively few studies on the prevalence of ESBLs in Irish animals and animal food products. However, it can be speculated based on the similarity of mobile genetic elements between commensal *E. coli* of animals and clinical ESBL-E from humans that they may be a reservoir for MDR plasmids. However, further investigations in this area are warranted. Ireland is a major exporter of animal meats with 75 % exported to UK and European markets and the remainder going to the rest of the world. Studies investigating epidemiological links between agricultural and human isolates of MDR Enterobacteriaceae, particularly in relation to antimicrobial resistance platforms should be investigated at least at a European level to provide an evidence base for informed policy in relation to antibiotic use in agriculture.

In conclusion, this study reveals the complex array of tools for the mobilization and expression of *bla*$_{CTX-M}$ and other antibiotic resistance genes within ESBL-EC circulating in Dublin and highlights the importance of group 1 and 9 CTX-M genes and specifically *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-14}$. Our data supports significant roles for both horizontal transfer of ESBL and integron-bound resistance genes via conjugative IncF, I1 and N plasmids and vertical transfer via clonal spread of the pandemic ST131 clone. Zoonotic transfer of both integrons and ESBL plasmids to human-associated *E. coli* may occur through contact with animals or through the food chain.

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Disclosure Statement

No competing financial interests exist.

Table 1. *bla*$_{CTX-M}$ genes and their genetic environments in 94 ESBL-*E. coli* clinical isolates

N	PFGE clusters	*bla*$_{CTX-M}$ allele	Upstream IS	Spacer Region[a]	CTX MIC[b]	GenBank ID	Integrons (size in kb)
34	A, J	15	IS*26* (rev)	24 bp IS*Ecp1* + W	2-256 (8)	GU264003	32 x *dfrA17-aadA5* (1.7) 1 x *dfrA1-sat1-aadA1* (2.2)
32	B-E, I, K, L, sporadic	15	IS*Ecp1*	W	16-256 (256)	AY463958	13 x *dfrA17-aadA5* (1.7) 2 x *dfrA1-sat1-aadA1* (2.2)
7	H, L, sporadic	1	IS*Ecp1*	XW	12-256 (256)	AM003904	2 x *dfrA1-aadA1* (1.6)
3	E, sporadic	3	IS*Ecp1*	W	12-256	HF549092	2 x *dfrA17-aadA5* (1.7)
1	sporadic	3	IS*Ecp1*	VW	12	EU935740	none
1	sporadic	55	IS*Ecp1*	45 bp	256	KC576516	none
1	sporadic	55	IS*Ecp1*	W	256	GQ456159	none
1	sporadic	32	ND	NS	256	AJ557142	none
8	I, F, sporadic	14	IS*Ecp1*	NS	32-256	AF252622	6 x *dfrA17-aadA5* (1.7) 1 x *aadA1* (1.0)
2	B	27	IS*26*	NS	48-128	AY156923	1 x *dfrA17-aadA5* (1.7)
3	G	9	IS*CR1*	326 bp	8	AM040708	3 x *drfA12-orfF-aadA8b* (1.8) + [*orf513-bla*$_{CTX-M-9}$]
1	sporadic	2	IS*CR1*	498 bp	256	EF592570	1 x *dfrA1-aadA1* (1.6) +

[a] Spacer region size or letter-coded description: W = 48 bp, X = 32 bp, V = 79 bp (see text and [7]). [b] Cefotaxime MIC range in mg/L (mode, where distinguishable). ND: None Detected; NS: Not Sequenced.

Table 2. Characteristics of 28 transferable CTX-M plasmids

Plasmid ID	Replicon Sequence	Previously found	Size (kb)	Transfer	Insertion	ESBLs	Integron transferre	Donor	Recipient CTX
IncF plasmids									
pBHEC9	F1:A-:B-	Novel	27.5	T	IS*26*	CTX-	No transfer	128	12
pBHEC8	F2:A-:B-	H/A WW	78.5	C	IS*Ecp1*	CTX-	*dfrA17-*	>256	8
pBHEC8	F2:A1:B-	H UK	95	T	IS*26*	CTX-	*dfrA17-*	32	6
pBHEC8	F2:A1:B-	H UK	115	T	IS*26*	CTX-	*dfrA17-*	3	3
pBHEC3	F45:A1:B-	Novel	100	C	IS*Ecp1*	CTX-	N/A	>256	>256
pBHEC9	F45:A4:B-	Novel	151	C	IS*Ecp1*	CTX-	*dfrA17-*	>256	32 [d]
pBHEC4	FII, FIA	N/A	137	T	IS*26*	CTX-	*dfrA17-*	12	8[d]
pBHEC1	FII, FIB	N/A	122	C	IS*Ecp1*	CTX-	N/A	>256	32[d]
pBHEC8	F22:A1:B2	H/A EU	157	C	IS*Ecp1*	CTX-	*dfrA7*	>256	64[d]
pBHEC3	F31:A4:B1	H/A EU	157	C	IS*Ecp1*	CTX-	*dfrA17-*	192	32
pBHEC5	F31:A4:B1	H/A EU	162	T	IS*Ecp1*	CTX-	*dfrA17-*	>256	32
pBHEC9	F31:A4:B1	H/A EU	146.	C	IS*Ecp1*	CTX-	*dfrA17-*	>256	32
IncI1 plasmids									
pBHEC0	I1-ST3	H/A EU	107	T	IS*Ecp1*	CTX-	N/A	>256	>256
pBHEC1	I1-ST3	H/A EU	112.	C	IS*Ecp1*	CTX-	N/A	12	8
pBHEC2	I1-ST3	H/A EU	112.	C	IS*Ecp1*	CTX-	N/A	>256	64
pBHEC5	I1-ST7	H/A EU	104.	T	IS*Ecp1*	CTX-	No transfer	>256	128
pBHEC2	I1-ST7	H/A EU	113	C	IS*Ecp1*	CTX-	*dfrA1-*	16	6
pBHEC9	I1-ST7	H/A EU	113	C	IS*Ecp1*	CTX-	*dfrA1-*	48	6
pBHEC1	I1-ST16	H UK	94	C	IS*Ecp1*	CTX-	No transfer	12	4
pBHEC5	I1-ST16	A UK	94	C	IS*Ecp1*	CTX-	N/A	>256	64
pBHEC3	I1-ST31	H/A EU	93	C	IS*Ecp1*	CTX-	No transfer	>256	64
pBHEC7	I1-ST57	H EU	84	C	IS*Ecp1*	CTX-	N/A	64	24
pBHEC1	I1-ST159	Novel	100	T	IS*Ecp1*	CTX-	N/A	48	32
Other plasmids									
pBHEC3	N-ST1	H/A EU	30	C	ND	CTX-	N/A	>256	>256
pBHEC7	N-ST6	H UK	31	C	ND	CTX-	No transfer	>256	16
pBHEC6	N/D	N/A	62	C	IS*Ecp1*	CTX-	N/A	>256	>256
pBHEC5	N/D	N/A	116	T	IS*Ecp1*	CTX-	N/A	>256	24
pBHEC7	N/D	N/A	115	T	IS*Ecp1*	CTX-	N/A	>256	32

Abbreviations: ND = none detected; N/A = not applicable; UTD = unable to determine; T = transformation; C = conjugation; sporadic = sporadically occurring strain; IS = insertion sequence; CTX = cefotaxime; MIC = minimum inhibitory concentration (mg/L).[a] Plasmid with same RST and CTX-M allele previously found in human (H) or animal (A) isolates worldwide (WW), in the UK (UK) or in Europe (EU), see http://pubmlst.org/plasmid/; [b] Transfer Method = Transformation (T) or Conjugation (C); [c] ESBLs: Specific CTX-M alleles detected. [d] Integrons transferred on plasmid [d] recipient contains >1 β-lactamase plasmid

Table S1. Primers for analysis of bla_{CTX-M} genetic environment

Primer name	S e q u e n ☐ (5 ô	Target	Reference
tnpA IS*Ecp1*	AATACTACCTTGCTTTCTGA	*tnpA* of IS*Ecp1*	[2]
IS*Ecp1* 5’	TTCAAAAAGCATAATCAAAGCC	IS*Ecp1* 5’-3’	[2]
IS*Ecp1* reverse	CAACCACCTTTCAATCATTTTT	IS*Ecp1*	[2]
Orf513	TGGAAGAGGGCGAAGACGAT	Orf513 of IS*CR1*	[2]
Orf513 rev	GCGTTTTATCGGTAGTCGTC	Orf513 of IS*CR1*	[2]
IntI1-F	GGTCAAGGATCTGGATTTCG	*Int1*	[3]
IntI1-R	ACATGCGTGTAAATCATCGTC	*Int1*	[3]
5’CS	GGCATCCAAGCAGCAAG	Class 1 integron	[3]
3’CS	AAGCAGACTTGACCTGA	Class 1 integron	[3]
IntI2-F	CACGGATATGCGACAAAAAGGT	*Int2*	[3]
IntI2-R	GTAGCAAACGAGTGACGAAATG	*Int2*	[3]
attI2-F	GACGGCATGCACGATTTGTA	Class 2 integron	[3]
orfX-R	GATGCCATCGCAAGTACGAG	Class 2 integron	[3]
PROM+	TGCTCTGTGGATAACTTGC	bla_{CTX-M} +	[4]
PRE-CTX-M-3B	CCGTTTCCGCTATTACAAAC	$bla_{CTX-M-1}$ +	[4]
IS*26*	AGCGGTAAATGCTGGAGTGA	IS*26*	[5]
CTX15 rev	ATTCGGCAAGTTTTTGCTGT	IS*26* + $bla_{CTX-M-1/3}$	[5]
M9 upper	ATGGTGACAAAGAGAGTGCA	$bla_{CTX-M-9}$	[1]
M9 lower	CCCTTCGGCGATGATTCTC	IS*Ecp1*/IS*26* +	[1]

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323

Table S2. Summary of antimicrobial resistance platforms for 100 ESBL-producing *E. coli* collected in Dublin

CG[a]	Strain	PG	IS[b]	ESBL	β-L[c]	Integron gene cassette arrays	CTX MIC	Replicons	Transfer Method	Plasmid ST (size kb)[d]
A	BHEC44	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	3	F2, F1A	N/A	N/A
A	BHEC45	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	16	F2, F1A	N/A	N/A
A	BHEC48	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	12	F2, F1A	Trans	F-UT (137)
A	BHEC26	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	4	F2, F1A	N/A	N/A
A	BHEC27	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	3	F2, F1A, I1	N/A	N/A
A	BHEC28	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	4	F2, F1A	N/A	N/A
A	BHEC34	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	4	F2, F1A	N/A	N/A
A	BHEC38	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	64	F2, F1A	N/A	N/A
A	BHEC40	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	2	F2, F1A	N/A	N/A
A	BHEC74	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	24	F2, F1A	N/A	N/A
A	BHEC85	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	>256	F2, F1A	No	N/A
A	BHEC96	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	6	F2, F1A	No	N/A
A	BHEC86	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	8	F2, F1A	No	N/A
A	BHEC63	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	48	F2, F1A	No	N/A
A	BHEC92	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	16	F2, F1A	N/A	N/A
A	BHEC94	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	8	F2, F1A	N/A	N/A
A	BHEC65	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	6	F2, F1A	N/A	N/A
A	BHEC67	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	4	F2, F1A, I1	N/A	N/A
A	BHEC21	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	96	F2, F1A, I1	N/A	N/A

A	BHEC60	B2-ST131	IS26	CTX-M-15	TEM	ND	6	F2, F1A	N/A	N/A
A	BHEC46	B2-ST131	IS26	CTX-M-15	TEM	*dfrA17-aadA5*	8	F2, F1A	N/A	N/A
A	BHEC4	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	8	F2, F1A	N/A	N/A
A	BHEC9	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	16	F2, F1A	N/A	N/A
A	BHEC50	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	24	F2, F1A	N/A	N/A
A	BHEC61	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	8	F2, F1A	N/A	N/A
A	BHEC77	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	>256	F2, F1A	N/A	N/A
A	BHEC50	B2-ST131	IS26	CTX-M-15	TEM	*dfrA17-aadA5*	1.5	F2, F1A	N/A	N/A
A	BHEC55	B2-ST131	IS26	CTX-M-15	ND	*dfrA7, dfrA17-aadA5*	6	F2, F1A, I1	N/A	N/A
A	BHEC35	B2-ST131	IS26	CTX-M-15	TEM	*dfrA17-aadA5*	2	F2, F1A, I1	No	N/A
A	BHEC7	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	8	F2, F1A	N/A	N/A
A	BHEC18	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	2	F2, F1A, I1	N/A	N/A
A	BHEC23	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	64	F2, F1A	N/A	N/A
A	BHEC82	B2-ST131	IS26	CTX-M-15	ND	*dfrA17-aadA5*	32	F2, F1A	Trans	F2:A1:B- (95)
A	BHEC16	B2-ST131	ISEcp1	CTX-M-14	TEM	ND	48	F2, F1A, F1B, I1	Trans	I1-ST159 (100)
B	BHEC43	B2-ST131	ISEcp1	CTX-M-15	TEM	*dfrA17-aadA5*	>256	F1A, F1B, I1	No	N/A
B	BHEC57	B2-ST131	ISEcp1	CTX-M-15	TEM	ND	>256	F1A, F1B, I1	No	N/A
B	BHEC56	B2-ST131	ISEcp1	CTX-M-15	ND	*dfrA17-aadA5*	>256	F2, F1A, F1B	Trans	F31:A4:B1 (162)
B	BHEC15	B2-ST131	IS26	CTX-M-27	ND	ND	48	F2, F1A, F1B	No	N/A
B	BHEC91	B2-ST131	IS26	CTX-M-27	ND	*dfrA17-aadA5*	128	F2, F1A, F1B	Trans	F1:A-:B- (27.5)
C	BHEC32	B2-ST131	ISEcp1	CTX-M-15	TEM	ND	>256	F2, F1A	Conj	F45:A1:B- (100)
C	BHEC47	B2-ST131	ISEcp1	CTX-M-15	TEM	ND	>256	F2, F1A	No	N/A

C	BHEC84	B2-ST131	ISEcp1	CTX-M-15	TEM	ND	>256	F2, F1A, F1B	No	N/A
C	BHEC1	B2-ST131	ISEcp1	CTX-M-15	ND	ND	>256	F2, F1A	No	N/A
C	BHEC33	B2-ST131	ISEcp1	CTX-M-15	TEM	*dfrA17-aadA5*	>256	F2, F1A, F1B	No	N/A
C	BHEC10	B2-ST131	ISEcp1	CTX-M-15	TEM	*dfrA17-aadA5*	>256	F2, F1A, F1B, B/O	No	N/A
D	BHEC58	B2-ST131	ISEcp1	CTX-M-15	ND	ND	>256	F2, F1A	No	N/A
D	BHEC88	B2-ST131	ISEcp1	CTX-M-15	TEM	*dfrA7*	>256	F2, F1A, F1B, I1	Conj	F22:A1:B20 (157)
E	BHEC3	B2	ISEcp1	CTX-M-3	ND	ND	12	F2, F1A, F1B	No	N/A
E	BHEC62	B2	ISEcp1	CTX-M-15	TEM	ND	>256	F2, F1A, F1B	No	N/A
F	BHEC41	B2	ISEcp1	CTX-M-14	TEM	*dfrA17-aadA5*	64	F2, F1A, F1B, L/M	No	N/A
F	BHEC42	B2	ISEcp1	CTX-M-14	TEM	*dfrA17-aadA5*	32	F2, F1A, F1B, L/M	No	N/A
G	BHEC2	D	ISCR1	CTX-M-9	TEM	*drfA12-orfF-aadA8b, orfD-aacA4-orf105-catB8*	8	F2, F1B	No	N/A
G	BHEC11	D	ISCR1	CTX-M-9	TEM	*drfA12-orfF-aadA8b, orfD-aacA4-orf105-catB8*	8	F2, F1B	No	N/A
G	BHEC13	D	ISCR1	CTX-M-9	TEM	*drfA12-orfF-aadA8b, orfD-aacA4-orf105-catB8*	8	F2, F1B	No	N/A
H	BHEC31	D	N/A	SHV	SHV	ND	0.75	I1	Conj	ND (32.5)
H	BHEC90	D	ISEcp1	CTX-M-1	TEM	*dfrA1-aadA1, dfrA1-sat1-aadA1*	48	F2, F1A, F1B, I1	Conj	I1-ST7 (113)

I	BHEC14	D	ISEcp1	CTX-M-14	TEM	*aadA1*, *dfrA17-aadA5*	>256	F2, F1A, F1B, I1	No	N/A
I	BHEC37	D	ISEcp1	CTX-M-15	TEM	*aadA1*, *dfrA17-aadA5*	>256	F2, F1B, I1	Conj	I1-ST31 (93)
J	BHEC24	B2	N/A	TEM	TEM	*aadA1*, *dfrA1-sat1-aadA1*	2	F2	Conj	F-UT (15), F-UT (48.5), F-UT (66)
J	BHEC87	B2-ST131	IS26	CTX-M-15	TEM	*aadA1*, *dfrA1-sat1-aadA1*	3	F2, F1A	Trans	F2:A1:B- (115)
K	BHEC36	A	ISEcp1	CTX-M-15	ND	*dfrA17-aadA5*	192	F2, F1A, F1B, I1	Conj	F31:A4:B1 (157)
K	BHEC78	A	ND	CTX-M-15	ND	*dfrA17-aadA5*	96	F2, F1A, F1B	No	N/A
L	BHEC22	B1	ISEcp1	CTX-M-1	ND	ND	>256	F2, F1B, I1	Conj	I1-ST3 (112.5)
L	BHEC76	B1	ISEcp1	CTX-M-15	ND	ND	>256	ND	Trans	ND (115)
none	BHEC25	B2-ST131	ISCR1	CTX-M-2	TEM	*dfrA1-aadA1*	>256	F2, F1B, I1	No	N/A
none	BHEC72	A	ISEcp1	CTX-M-14	TEM	*dfrA17-aadA5*, *aacA4-cmlA1*	96	F2, F1B	No	N/A
none	BHEC81	A	ISEcp1	CTX-M-14	TEM	*dfrA12-orfF-aadA2*	>256	F2, F1B	No	N/A
none	BHEC64	B2-ST131	ISEcp1	CTX-M-14	TEM	*dfrA17-aadA5*	48	F2, F1A	No	N/A
none	BHEC49	D	ISEcp1	CTX-M-14	TEM	*dfrA17-aadA5*	96	F2, F1A, F1B	No	N/A
none	BHEC51	A	ISEcp1	CTX-M-15	ND	*dfrA17-aadA5*	>256	F2, F1A, F1B	No	N/A
none	BHEC80	A	ISEcp1	CTX-M-15	TEM	*dfrA17-aadA5*, *dfrA1-sat1-aadA1*	>256	F2	Conj	F2:A-:B- (78.5)
none	BHEC99	A	ISEcp1	CTX-M-15	TEM	*dfrA17-aadA5*, *dfrA1-sat1-aadA1*	>256	F2, F1A, F1B, B/O	Conj	F45:A4:B- (151)
none	BHEC17	B1	ISEcp1	CTX-M-1	ND	ND	12	I1	Conj	I1-ST3 (112.5)
none	BHEC83	B1	ISEcp1	CTX-M-15	ND	ND	>256	ND	No	N/A

none	BHEC95	B1	ISEcp1	CTX-M-15	TEM	ND	>256	F1A	No	N/A
none	BHEC6	B2	ISEcp1	CTX-M-1	ND	ND	>256	F2, F1B, I1	Trans	I1-ST3 (107)
none	BHEC66	B2	ISEcp1	CTX-M-55	ND	ND	>256	ND	Conj	ND (62)
none	BHEC70	B2	ISEcp1	CTX-M-3	ND	ND	64	F2, F1B, I1	Conj	I1-ST57 (84)
none	BHEC93	B2-ST131	ISEcp1	CTX-M-15	ND	ND	>256	F2, F1A, F1B	No	N/A
none	BHEC97	B2-ST131	ISEcp1	CTX-M-15	ND	*dfrA17-aadA5*	>256	F2, F1A, F1B	Conj	F31:A4:B1 (146.5)
none	BHEC100	B2-ST131	ISEcp1	CTX-M-15	TEM	*dfrA17-aadA5*	192	F2, F1A	No	N/A
none	BHEC8	D	ISEcp1	CTX-M-15	TEM	ND	64	F2, F1A, F1B	No	N/A
none	BHEC12	D	ISEcp1	CTX-M-55	TEM	ND	>256	F2, F1B	Conj	F-UT (122)
none	BHEC19	D	ISEcp1	CTX-M-3	TEM	*dfrA17-aadA5*	12	I1	Conj	I1-ST16 (94)
none	BHEC29	D	ISEcp1	CTX-M-1	TEM	ND	64	F2, F1B, I1	No	N/A
none	BHEC30	D	ISEcp1	CTX-M-15	TEM	ND	>256	F2, F1A, F1B	No	N/A
none	BHEC53	D	ISEcp1	CTX-M-1	ND	*dfrA1-sat1-aadA1*	>256	I1	Trans	I1-ST7 (104.5)
none	BHEC54	D	ISEcp1	CTX-M-15	TEM	ND	>256	F2, F1B	Trans	ND (116)
none	BHEC98	D	ISEcp1	CTX-M-15	ND	*dfrA17-aadA5*	>256	F2, F1A, F1B	No	N/A
none	BHEC20	U	ISEcp1	CTX-M-1	ND	*dfrA1-aadA1*	16	I1	Conj	I1-ST7 (113)
none	BHEC52	U	ISEcp1	CTX-M-15	TEM	ND	>256	I1	Conj	I1-ST16 (94)
none	BHEC89	U	ISEcp1	CTX-M-15	SHV	ND	16	ND	No	N/A
none	BHEC59	A	N/A	SHV	TEM	ND	1.5	F2, F1B, I1	Conj	I1-ST3 (118)
none	BHEC69	A	N/A	SHV	TEM	*dfrA1-aadA1*	4	F2, I1, N	No	N/A
none	BHEC73	A	N/A	SHV	TEM	ND	4	F1B, I1	Conj	I1-ST3 (101.5)

none	BHEC68	B1	N/A	TEM	TEM	ND	32	F2, F1B, I1	Trans	ND (31.5), ND (21.5)
none	BHEC39	B1	ND	CTX-M-32	ND	ND	>256	F2, F1B, N	Conj	N-ST1 (30)
none	BHEC79	B2-ST131	ISEcp1 [b]	CTX-M-15	TEM	ND	>256	F2, F1A, F1B, I1	No	N/A
none	BHEC71	D	ND	CTX-M-3	TEM	*dfrA17-aadA5*	>256	F2, F1B	Conj	N-ST6 (31)
none	BHEC75	D	ISEcp1 [b]	CTX-M-15	TEM	*aadB-aadA1-cmlA6*	>256	F2, F1A	No	N/A

Abbreviations: ND = none detected; N/A = not applicable; UTD = unable to determine; Trans = transformation; Conj = conjugation; PG = phylogenetic group; B2-ST131 = O25B-ST131 pandemic clone; CG = clonal group, none = sporadically occurring strain; IS = insertion sequence; CTX = cefotaxime; MIC = minimum inhibitory concentration (mg/L). [a] Clonal groups as identified previously [1]. [b] IS not linked directly to CTX-M gene in indicated isolates. [C] Beta-lactamase gene families detected by PCR [d] Plasmid ST = plasmid sequence type; F:A:B formulae given for IncF plasmids; pMLST formulae given for I1 (I1-STx) and N (N-STx) plasmids; F-UT = F plasmid untypable by RST; approximate plasmid size (kb) is given in parentheses.

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433

434