Journal of Antimicrobial Chemotherapy (2008) **61**, 273–281
doi:10.1093/jac/dkm464
Advance Access publication 11 December 2007

JAC

Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15

Marie-Hélène Nicolas-Chanoine[1,2]*, Jorge Blanco[3], Véronique Leflon-Guibout[1], Raphael Demarty[1], Maria Pilar Alonso[4], Maria Manuela Caniça[5], Yeon-Joon Park[6], Jean-Philippe Lavigne[7], Johann Pitout[8] and James R. Johnson[9]

[1]Service de Microbiologie, Hôpital AP-HP Beaujon, 92110 Clichy, France; [2]Inserm, U-773, Faculté de Médecine D. Diderot, Université Paris 7, Paris, France; [3]E. coli Reference Laboratory, Department of Microbiology and Parasitology, Faculty of Veterinary Science, University of Santiago de Compostela, Lugo, Spain; [4]Laboratory of Clinical Microbiology, Complejo Hospitalario Xeral-Calde, Lugo, Spain; [5]Antibiotic Resistance Unit, National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal; [6]Department of Clinical Pathology, College of Medicine, The Catholic University of Korea, Kangnam St Mary's Hospital, Seoul, South Korea; [7]Laboratoire de Bactériologie, Virologie et Parasitologie, CHU de Nîmes, Nîmes, France; [8]Calgary Laboratory Services and Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada; [9]Veterans Affairs Medical Center and University of Minnesota, Minneapolis, MN, USA

Received 29 August 2007; returned 12 October 2007; revised 19 October 2007; accepted 6 November 2007

***Background*: Concomitant with the recent emergence of CTX-M-type extended-spectrum β-lactamases (ESBLs), *Escherichia coli* has become the enterobacterial species most affected by ESBLs. Multiple locales are encountering CTX-M-positive *E. coli*, including specifically CTX-M-15. To gain insights into the mechanism underlying this phenomenon, we assessed clonality and diversity of virulence profiles within an international collection of CTX-M-15-positive *E. coli*.**

***Methods*: Forty-one ESBL-positive *E. coli* isolates from eight countries and three continents (Europe, Asia and North America) were selected for study based on suspected clonality. Phylogenetic group, ERIC2 PCR profile, O H serotype, AmpC variant and antibiotic susceptibility were determined. Multilocus sequence typing (MLST) and PFGE provided additional discrimination. Virulence potential was inferred by detection of 46 virulence factor (VF) genes.**

***Results*: Thirty-six (88%) of the 41 *E. coli* isolates exhibited the same set of core characteristics: phylogenetic group B2, ERIC2 PCR profile 1, serotype O25:H4, AmpC EC6, ciprofloxacin resistance and MLST profile ST131. By PFGE, the 36 isolates constituted one large cluster at the 68% similarity level; this comprised 17 PFGE groups (defined at 85% similarity), some of which included strains from different countries. The 36 isolates exhibited highly (91% to 100%) similar VF profiles.**

***Conclusions*: We describe a broadly disseminated, CTX-M-15-positive and virulent *E. coli* clonal group with highly homogeneous virulence genotypes and subgroups exhibiting highly similar PFGE profiles, suggesting recent emergence. Understanding how this clone has emerged and successfully disseminated within the hospital and community, including across national boundaries, should be a public health priority.**

Keywords: enterobacteria, *E. coli*, multidrug resistance

Introduction

Escherichia coli, a universal commensal of humans and several animal species, is also one of the most common enterobacterial species causing extraintestinal infections in these same hosts. *E. coli* infections are becoming increasingly difficult to treat because of emerging antimicrobial resistance, most recently to expanded-spectrum cephalosporins, which is usually due to the

*Corresponding author. Tel: +33-1-40-87-56-06; Fax: +33-1-40-87-05-50; E-mail: mhn.chanoine@bjn.aphp.fr

production of extended-spectrum β-lactamases (ESBLs).[1] The earliest ESBLs, which were first reported in 1985, consisted of plasmid-mediated TEM-1, TEM-2 and SHV-1 derivatives and were primarily a hospital-based problem.[1] However, since 2000, ESBLs increasingly have also appeared in the community.[2] This phenomenon coincided with the emergence of a new group of plasmid-mediated ESBLs, namely the CTX-M enzymes, which seem to be taking over as the main ESBL type in some locales.[3,4]

Multiple locales are encountering CTX-M-positive *E. coli* clinical isolates, including specifically CTX-M-15, which is one of the more than 60 variants described in this enzyme group and is able to efficiently hydrolyse not only cefotaxime but also ceftazidime.[3–8] The widespread occurrence of CTX-M-15-positive *E. coli* could have two alternative explanations. That is, the corresponding plasmids or other mobile genetic elements surrounding the plasmid-mediated $bla_{\text{CTX-M-15}}$ gene may be moving from strain to strain through the *E. coli* population.[3,9] Alternatively, the strains themselves may be spreading in a clonal fashion, as has been described for methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae* and certain clonal groups of trimethoprim/sulfamethoxazole-resistant *E. coli* (i.e. 'clonal group A' and *E. coli* O15:K52:H1).[10–13]

While some articles have reported on the similarity of CTX-M-15-encoding plasmids harboured by strains in different locations, in the present study, we focused on strain genetic background to assess the extent of clonality within a collection of international CTX-M-positive *E. coli* isolates that we suspected were clonally related to a group of French CTX-M-15 isolates of serogroup O25 previously studied in our laboratory.[6,14,15] We also sought to assess these strains' molecularly inferred virulence potential, a possible contributor (in addition to antimicrobial resistance) to their recent emergence and dissemination as successful pathogens.

Materials and methods

Isolate collection

While we observed in France that CTX-M-15 was the most common CTX-M enzyme and that the great majority of epidemiologically unrelated, CTX-M-15-positive *E. coli* isolates displayed an identical genetic background, we also observed that different studies performed in different countries found CTX-M-15 as the most common CTX-M-type enzyme in *E coli*. These concomitant observations pushed us to see whether this worldwide outbreak of CTX-M-15-producing *E. coli* was due to the spread of a clonal strain as found in France. Therefore, a total of 41 recent human *E. coli* isolates that were known ($n = 34$) or presumed ($n = 7$) to produce CTX-M-15, from three continents (Europe, Asia and North America) and eight countries (France, Portugal, Spain, Switzerland, Lebanon, India, Korea and Canada) were studied. They were selected because they either were known to be clonal (the 13 French isolates) or, if from outside France, were suspected of being related to the French isolates based on CTX-M-15 production, the O25 antigen, the phylogenetic group B2 and/or ciprofloxacin resistance. Previously published isolates included those from Canada ($n = 6$), India ($n = 2$), Korea ($n = 2$), Lebanon ($n = 4$) and Portugal ($n = 5$).[16–20] As indicated in Table 1, they included community-, hospital- and nursing-home-acquired isolates. Except for the Lebanese and two French strains that were digestive tract colonizers, the isolates were obtained from clinical samples: primarily urine but also blood, sputum, intra-abdominal pus and ascites (Table 1).

Relevant characteristics of the isolates that were known prior to this study are italicized in Table 1. Notably, the French isolates [including a strain (TE1) previously reported as responsible for an outbreak in a long-term care facility] were previously shown to produce CTX-M-15 and to exhibit the same genomic PCR profile, O antigen (O25), chromosomal cephalosporinase variant (AmpC EC6) and ciprofloxacin phenotype (resistant).[15,21]

β-Lactamase determination

CTX-M-type β-lactamase genes were identified as previously described.[21] Briefly, two sets of primers were used, allowing amplification and sequencing of any type of $bla_{\text{CTX-M}}$ gene (primer set 1) versus specific $bla_{\text{CTX-M}}$ variants (primer set 2). To determine an isolate's *ampC* variant, DNA amplification was done using primers VL1A (5′-TGCACGATCTGAAAATCCAC-3′) and VL2A (5′-AGCAGGCGCATAAATGTTTC-3′) under standard PCR conditions, with a T_m of 42°C, which yielded a fragment of 1398 bp. Direct sequencing of the PCR product was performed using these PCR primers and two additional primers, VL1S (5′-TATCTTCA
ATGGTCG-3′) and VL2S (5′-TGCATGGGCTCCAGG-3′). The *ampC* nucleotide sequences and deduced protein sequences were analysed by using software available at the Biosupport web site (http://bioinfo.hku.hk/). These were then compared with those available in GenBank by using Blast sequence software (htpp://www.ncbi.nlm.nih.gov). The new AmpC peptide sequences were named EC66 and EC68 and their corresponding genes were deposited in GenBank under accession numbers EF507686 and EF507687, respectively.

ERIC2 PCR profiles

ERIC2 PCR profiles, which are strain-specific banding patterns obtained by amplifying multiple anonymous regions of the genome using repetitive element-based primers, were generated as previously described, with bacterial lysates used as template DNA.[22] Profiles were defined as different when they exhibited at least one high intensity band difference according to visual inspection.

Phylogenetic group

Determination of major *E. coli* phylogenetic group (A, B1, B2 and D) was done by multiplex PCR.[23]

Serotyping

The determination of O and H antigens was carried out by using the method previously described by Guinée *et al.*,[24] in which all available O (O1–O185) and H (H1–H56) antisera were tested. All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove the non-specific agglutinins. The O25 antigen was also determined by a PCR-based method.[25]

Table 1. Clinical, bacterial and molecular characteristics of the 41 studied isolates of ESBL-producing *E. coli*

Country/ isolate	Sample/ acquisition site	Epidemic (Ep) or sporadic (Sp)	CTX-M-type	ERIC2 PCR profile	Phylogenetic group	AmpC type	Serotype	Antimicrobial susceptibility					
								CIP	GEN	AMK	TET	CHL	SXT
France													
MECB5	urine/C	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	R	R	S	R
Vlab2	urine/C	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	S	R	S	R
VA1	catheter/C	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	R	R	S	R
VB6	urine/C	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	S	R	R	S	R
VB8	rectal swab/C	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	R	S	S	S
VB9	rectal swab/C	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	S	R	S	R
HBS1	urine/H	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	R	R	S	R
HBS4	ascites/H	Sp	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	S	S	S	S	S
TNN (TE2)	urine/H	Ep-AC-1	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	S	R	S	S
TE1	urine/H	Ep-LTC-1	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	*R*	*R*	*R*	*S*	*S*
HDE1	urine/H	Ep-LTC 2a	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	S	S	S	S	S
HDE2	urine/H	Ep-LTC-2b	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	R	R	S	S	R
HDE3	urine/H	Ep-LTC-2c	*M15*	*1*	*B2*	*EC6*	*O25*:H4	*R*	S	R	S	S	R
Switzerland													
EcS1	urine/NA	Sp	*M15*	5	B2	EC30	NT	S	S	S	S	S	S
3756 EcS2	urine/NA	Sp	*M15*	1	B2	EC6	O25:H4	R	R	R	R	S	S
Spain													
FV7561	urine, blood/H	Sp	M15	1	B2	EC6	*O25*:H4	R	S	R	R	S	R
FV7563	urine/C	Sp	M15	1	B2	EC6	*O25*:H4	R	S	R	R	S	R
FV7569, FV7588, FV7593, FV7595	urine/H	Sp	M15	1	B2	EC6	*O25*:H4	R	S	R	R	S	R
FV7591	urine/H	Sp	M1	2	D	EC68	*O25*:H4	S	R	S	R	S	S
Portugal													
5753	urine/C	Sp	*M15*	1	B2	EC6	O25:H4	R	S	R	R	S	S
5754	blood/C	Sp	*M15*	1	B2	EC6	O25:H4	R	R	R	R	S	S
5800	sputum/H	Sp	*M15*	1	B2	EC6	O25:H4	R	R	R	R	S	S
5936	urine/C	Sp	*M15*	1	B2	EC6	O25:H4	R	R	R	R	S	S
6373	urine/C	Sp	*M15*	1	B2	EC6	O25:H4	R	R	R	R	S	R
Korea													
KUMC KN1604	urine/H	Sp	*M15*	1	B2	EC6	O25:H4	R	R	R	R	R	R
KUMC KN1608	urine/H	Sp	*M15*	6	A	EC68	NT	R	R	R	R	R	R
India													
E. coli 1	urine/H	Sp	*M15*	3	A	EC66	O101	R	S	R	R	S	S
E. coli 2	urine/H	Sp	*M15*	4	B1	EC74	NT	R	R	R	R	R	R
Lebanon													
AH8, AH9, AH10, AH15	faeces/C	Sp	*M15*	1	B2	EC6	O25:H4	R	S	R	R	S	S
Canada													
1100	urine/C	Sp	*M15*	1	*B2*	EC6	O25:H4	*R*	R	R	R	S	S
17102	urine/C	Sp	*M15*	1	*B2*	EC6	O25:H4	*R*	S	R	R	S	R

Continued

Table 1. *Continued*

Country/ isolate	Sample/ acquisition site	Epidemic (Ep) or sporadic (Sp)	CTX-M-type	ERIC2 PCR profile	Phylogenetic group	AmpC type	Serotype	Antimicrobial susceptibility					
								CIP	GEN	AMK	TET	CHL	SXT
15802	intra abd pus/H	Sp	*M15*	1	*B2*	EC6	O25:H4	*R*	S	S	R	S	R
19502	urine/NH	Sp	*M15*	1	*B2*	EC6	O25:H4	*R*	R	S	R	S	R
8501	urine/NH	Sp	*M15*	1	*B2*	EC6	O25:H4	*R*	R	R	R	S	S
16102	urine/C	Sp	*M15*	1	*B2*	EC6	O25:H4	*R*	S	S	S	S	S

R, resistant; S, susceptible; C, community-acquired; H, hospital-acquired; NH, nursing home; intra abd, intra-abdominal; AC, acute care; LTC, long-term care; NA, not available; NT, non-typeable; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin; TET, tetracycline; CHL, chloramphenicol; SXT, co-trimoxazole. Italic font indicates the bacteriological characteristics that were already known when the strains were selected for this study.

Sequence type (ST) determination

Multilocus sequence typing (MLST) was carried out as previously described.[26] Gene amplification and sequencing were performed by using the primers specified at the *E. coli* MLST web site (http://web.mpiib-berlin.mpg.de/mlst/dbs/Ecoli) except for *mdh*, *icd* and *recA*. Both the forward and reverse strands of *mdh* were sequenced using primers mdh SF: 5′-CCAGGCGCTTGCACTACTGTTAA-3′ and mdh SR: 5′-GCGATATCTTTCTTCAGCGTATC-3′, respectively, whereas the forward strand of *icd* and the reverse strand of *recA* were sequenced with the primers icd SF: 5′-CGGCAAACTCAAC GTTCC-3′ and recA SR: 5′-CTGACGCTGCAGGTGAT-3′, respectively. Allelic profile and ST determinations were as per the *E. coli* MLST web site scheme.

PFGE profiles

*Xba*I PFGE analysis was performed as previously described.[27] Profiles were compared digitally using BioNumerics software (Applied Maths). Cluster analysis of Dice similarity indices based on the unweighted pair group method with arithmetic mean (UPGMA) was used to generate a dendrogram describing the relationships among PFGE profiles. Isolates were considered to belong to the same PFGE group if their Dice similarity index was $\geq$85%.[28]

Virulence genotypes

Forty-six extraintestinal virulence-associated genes were detected by multiplex PCR, as previously described.[29] These included 16 adhesin-encoding genes (*papAH*, *papC*, *papEF*, *papG* and its 3 alleles, *sfa/focDE*, *sfaS*, *focG*, *afa/draBC*, *afaE8*, *iha*, *bmaE*, *gafD*, *F17*, *clpG*, *fimH* and *hra*), 8 toxin-encoding genes (*hlyA*, *hlyF*, *cnf1*, *cdtB*, *sat*, *pic*, *tsh* and *astA*) and 4 siderophore-related genes (*iroN*, *fyuA*, *ireA* and *iutA*). They also included 10 protectin/invasin-encoding genes (*kpsM* II, *kpsMT* III, the K1, K2, K5 and K15 *kps* variants, *rfc*, *traT*, *ibeA* and *iss*) and 7 pathogenicity island markers and miscellaneous genes (*cvaC*, *usp*, *ompT*, *clbB*, *clbN*, *fliC* H7 and *malX*). A UPGMA-based dendrogram was constructed depicting similarity relationships among the isolates according to composite virulence gene profiles.

Antibiotic susceptibility

Susceptibility to the following non-β-lactam molecules was determined by disc diffusion: ciprofloxacin, gentamicin, amikacin, tetracycline, chloramphenicol and co-trimoxazole. Isolates were defined as resistant or susceptible according to the standards of the French Antibiogram Committee.[30]

Results

The primary strain set comprised 13 epidemiologically diverse French *E. coli* isolates, all from the Paris area except one (strain MECB5, from the south of France). All were known to be characterized in terms of CTX-M-15 production, phylogenetic group B2, ERIC2 PCR profile 1, serogroup O25, AmpC variant 6 and ciprofloxacin resistance (Table 1). H antigen determined in this study was found to be H4.

These characteristics (when unknown) were newly assessed for 28 other ESBL-positive *E. coli* isolates from seven other countries, representing three continents. Twenty-three (82%) of these 28 isolates were found to be identical to the 13 French isolates with respect to all 6 core characteristics (CTX-M-15, group B2, ERIC2 PCR profile 1, serotype O25:H4, AmpC EC6 and ciprofloxacin resistance). These 23 isolates included 6 (86%) of 7 from Spain and 17 (81%) of 21 from the other six countries, including all 15 from Lebanon, Portugal and Canada, plus 1 each from Switzerland and Korea (Table 1).

In contrast, five of the isolates (both isolates from India, and one each from Spain, Switzerland and Korea) were found to exhibit non-1 ERIC profiles (Figure 1) and non-EC6 AmpC types, and proved to be mostly non-O25 and non-B2 (Table 1). Moreover, the Spanish isolate (FV7591) exhibited CTX-M-1 rather than CTX-M-15 (Table 1).

These findings suggested that most (82%) of the non-French isolates, like the 13 French isolates, represented a geographically dispersed, group B2-derived, serotype O25:H4, AmpC variant EC 6 clonal group of *E. coli*, characterized by CTX-M-15 and ciprofloxacin resistance.

MLST results

To more rigorously assess phylogenetic relationships within this collection, all 37 O25:H4 isolates (including the 36

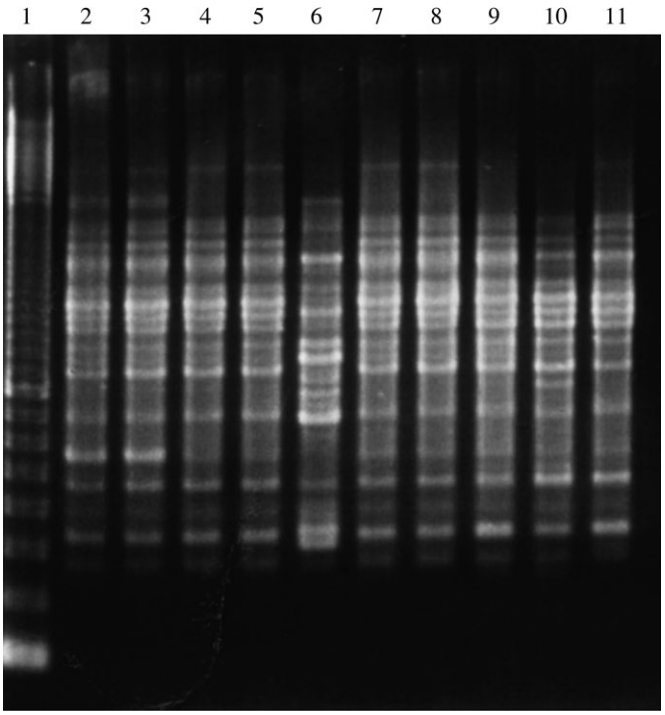

Figure 1. ERIC2 PCR profiles of seven Spanish and three French isolates of ESBL-producing *E. coli*. Lane 1, molecular weight marker. Lanes 2–8, Spanish isolates with strain FV7591 in lane 6. Lanes 9–11, French isolates [strain HBS1 (lane 9), strain HDE2 (lane 10) and strain TE1 (lane 11)]. A uniform profile was found among these isolates except for the Spanish strain FV7591 (CTX-M-1).

CTX-M-15-positive isolates and the single CTX-M-1 isolate from Spain) underwent seven-locus MLST. Irrespective of geographical origin, the 36 CTX-M-15-positive O25:H4 isolates exhibited the same combination of alleles across the seven sequenced loci, corresponding to an established ST, ST131. In contrast, the Spanish CTX-M-1-positive O25:H4 isolate (phylogenetic group D, ERIC2 PCR profile 2, ciprofloxacin-susceptible) exhibited a novel combination of alleles and was assigned to a new ST, ST648. This confirmed the clonality and distinctness of the CTX-M-15 isolates.

PFGE profiles

Finer resolution of clonal relationships was obtained by PFGE analysis. Figure 2 shows PFGE analysis results for the 36 ST131 strains and the ST648 Spanish strain FV7591. The 36 ST131 strains constituted one large cluster (defined at the 68% similarity level), which was tied to the 'outgroup' strain FV7591 at $<$40% similarity. The ST131 cluster, in turn, comprised 17 separate PFGE groups, as defined at the 85% similarity level. These PFGE groups corresponded inconsistently with geographical origin. That is, the 13 French strains were classified into seven PFGE groups, the 6 Canadian strains into five groups, the 5 Portuguese strains into four groups and the 4 Lebanese strains into two groups. The six Spanish CTX-M-15-producing strains represented the only example of all isolates from a given country being classified into the same PFGE group. (Of note, the single Korean strain and the single Swiss strain were the sole representatives of their respective PFGE groups.) Likewise, multiple countries were represented within certain PFGE groups, including PFGE group I (France and Canada), group V (France, Canada and Portugal) and group XIII (France and Portugal). Nonetheless, frankly indistinguishable PFGE profiles were encountered only among strains from the same country, including two strains each from France (VB6 and HBS1), Lebanon (AH15 and AH10) and Spain (FV7569 and FV7595).

Virulence profiles

Extended virulence profiles were determined for the 36 ST131 isolates to assess the extent of within-group diversity and the virulence potential of the clonal group. Of the 46 virulence genes tested, 16 (35%) were detected in at least 1 isolate each. Isolates contained from 7 to 14 genes each (Table 2). Five different virulence genes were uniformly present in all 36 isolates, including *fimH* (type I fimbriae), *sat* (secreted autotransporter toxin), *fyuA* (yersiniabactin receptor), *usp* (uropathogenic specific protein) and *malX* (pathogenicity island marker) (Table 2). Four other genes were present in $>$90% of the isolates, including *iha* (adhesin-siderophore receptor: 91%), *kpsM* II (group 2 capsule synthesis: 94%), *iutA* (aerobactin receptor: 97%) and *ompT* (outer membrane protease T: 97%) (Table 2), with the K5 and K2 *kpsM* II variants being detected in 53% and 39% of the isolates, respectively. Intermediate prevalence virulence genes included *traT* (serum resistance associated: 75%) and *afa/draBC* (afimbrial Dr-binding adhesins: 22%). In contrast, three genes occurred in $<$12% of strains each, typically together. These included *hlyF* (haemolysin F: 8%), *iss* (increased serum survival: 8%) and *iroN* (siderophore receptor: 11%) (Table 2).

Overall, virulence profile similarity among the 36 isolates was high, ranging from 91% to 100% (Figure 3). Only eight isolates exhibited a unique virulence profile. Indeed, 14 isolates (from Canada, France, Portugal, Korea and Switzerland) had an identical 11-gene virulence profile and four other groups of 2–6 strains each exhibited uniform VF profiles (Figure 3 and Table 2). Although some geographical segregation of virulence profiles was evident, virulence profiles corresponded inconsistently with PFGE type or locale (Figure 3), suggesting ongoing evolution of virulence genotypes.

Antimicrobial susceptibility patterns

To assess the multidrug resistance of the 36 ST131 isolates, susceptibility to non-β-lactam antimicrobials was tested (Table 1). Eighty-three per cent of the isolates were resistant to tetracycline, 77% to amikacin, 53% to co-trimoxazole and 50% to gentamicin, but only 0.3% to chloramphenicol.

Discussion

Our findings provide novel evidence of a recently emerged, broadly disseminated, CTX-M-15-positive *E. coli* clonal group as a cause of multidrug-resistant extraintestinal infections on at least three continents. This lineage exhibits a fairly robust virulence gene profile, implying substantial extraintestinal pathogenic potential. In most study locales, it accounted for a large proportion of ESBL-positive *E. coli* that were CTX-M-15 and/or O25-positive. The emergence of a new multidrug-resistant

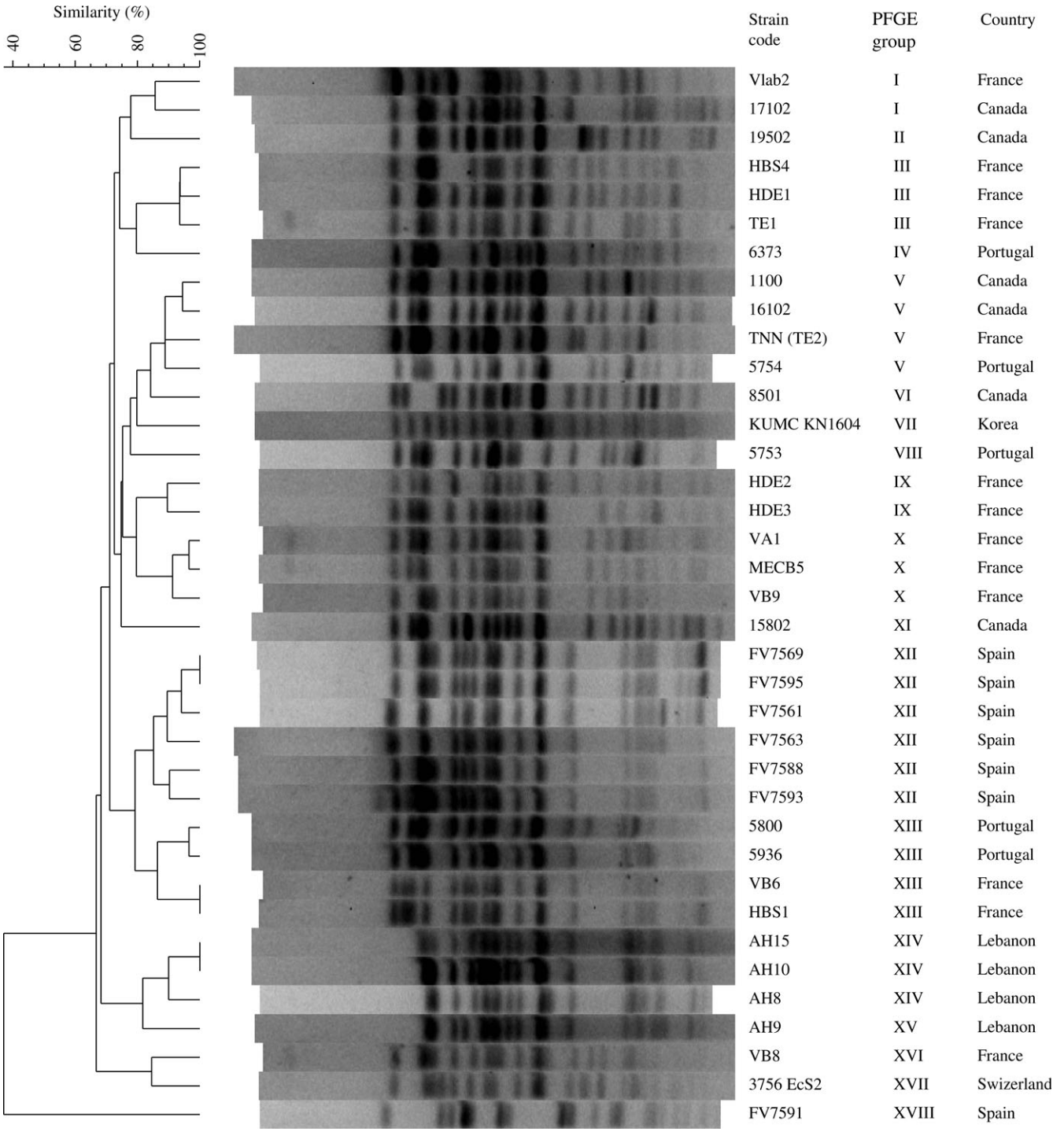

Figure 2. *Xba*I-PFGE dendrogram for 36 CTX-M-15-positive *E. coli* isolates from ST131 and a Spanish strain from ST648. The dendrogram for the 37 isolates, as produced by the UPGMA algorithm based on Dice similarity coefficients, included 18 PFGE groups, as defined based on ≥85% similarity of PFGE profiles.

extraintestinal pathogen that may be spreading rapidly through the population while continuing to evolve appears to pose a significant public health threat in need of urgent attention.

The clonality of the ST131 strains is evident from their homogeneity with respect to phylogenetic group, seven-gene MLST allele combination and ERIC2 PCR profile. Clonality is further supported by the isolates' uniform serotype (O25:H4), β-lactamase repertoire (CTX-M-15 and AmpC EC6) and ciprofloxacin phenotype, and their >90% similar virulence gene profiles. Furthermore, the considerable similarity of PFGE profiles observed among certain isolates indicates quite recent divergence from a common ancestor, whereas the occurrence in different locales of isolates with similar PFGE profiles suggests recent or ongoing transmission.

Table 2. Virulence genotype of 36 CTX-M-15-producing *Escherichia coli* strains of clone ST131

	Adhesin			Toxin		Siderophore			Protectin, invasin, pathogenicity island marker, miscellaneous							
Country/isolate	*afa/ draBC*	*iha*	*fimH*	*hlyF*	*sat*	*iroN*	*fyuA*	*iutA*	*kpsM* II	K5 *kps* variant	K2 *kps* variant	*usp*	*traT*	*ompT*	*iss*	*malX*
France																
MECB5	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
VA1	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+
Vlab2, VB8, HBS4, TNN (TE2), TE1, HDE1	−	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+
VB9	−	+	+	−	+	−	+	+	+	+	−	+	−	−	−	+
HDE2	−	+	+	−	+	−	+	+	+	−	−	+	+	+	−	+
HDE3	−	−	+	−	+	−	+	+	+	−	+	+	+	+	−	+
VB6, HBS1	+	+	+	−	+	−	+	+	+	−	+	+	−	+	−	+
Switzerland																
3756 EcS2	−	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+
Spain																
FV7561, FV7563	+	+	+	−	+	−	+	+	+	−	+	+	+	+	−	+
FV7569, FV7588, FV7593, FV7595	+	+	+	−	+	−	+	+	+	−	+	+	−	+	−	+
Portugal																
5753	−	−	+	−	+	−	+	−	−	−	−	+	+	+	−	+
5936	−	−	+	−	+	−	+	+	−	−	−	+	+	+	−	+
5754, 5800, 6373	−	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+
Korea																
KUMC KN1604	−	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+
Lebanon																
AH9, AH8, AH15, AH10	−	+	+	−	+	−	+	+	+	−	+	+	+	+	−	+
Canada																
1100, 8501, 16102	−	+	+	−	+	−	+	+	+	+	−	+	+	+	−	+
17102	−	+	+	−	+	−	+	+	+	−	+	+	−	+	−	+
15802	−	+	+	−	+	+	+	+	+	+	−	+	−	+	+	+
19502	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+

afa/draBC, afimbrial Dr-binding adhesins; *iha*, adhesin-siderophore receptor; *fimH*, type I fimbriae; *hlyF*, haemolysin F; *sat*, secreted auto-transporter toxin; *iroN*, siderophore receptor; *fyuA*, yersiniabactin receptor; *iutA*, aerobactin receptor; *kpsM* II, group 2 capsule synthesis (variant K5 and K2); *usp*, uropathogenic specific protein; *traT*, serum resistance associated; *ompT*, outer membrane protease; *iss*, increased serum survival; *malX*, pathogenicity island marker.

The virulence of the O25:H4-ST131 isolates can be inferred from two lines of evidence. First, most isolates were from samples submitted to clinical microbiology laboratories from inpatients and outpatients, so likely caused extraintestinal infections; this certainly was true for the blood and ascites isolates. Second, the number and types of virulence genes present in these strains (7–14 per isolate; coding for adhesins, siderophores, toxins, protectins and pathogenicity island markers) imply a robust virulence capability.[29,31] Although these virulence profiles are not so extensive as those of typical antimicrobial-susceptible pathogenic *E. coli* from phylogenetic group B2, they nonetheless are more extensive than is usually observed among fluoroquinolone- or extended-spectrum cephalosporin-resistant *E. coli*, including human clinical isolates.[32] Thus, these strains appear to pose the double threat of multidrug resistance (including to first-line therapeutic agents for Gram-negative infections) and substantial extraintestinal virulence capability. This makes their emergence and dissemination particularly concerning and suggests a need to identify their origins, reservoirs and transmission pathways so that appropriate interventions can be implemented. Better definition of the extent of this problem is needed, to clarify how great a public health threat these strains actually pose, so that resources can be allocated accordingly.

Dissemination of antimicrobial resistance genes by spread of the particular clone(s) in which they reside differs from the established paradigm for the emergence of ESBLs, which involves transfer of resistance-encoding plasmids rather than the host bacteria *per se*.[9] However, clonal dispersal of drug-resistant pathogens has precedent in other species, such as methicillin-resistant *S. aureus* and penicillin-resistant *S. pneumoniae*.[11,12] It also has been documented in *E. coli*, as exemplified by the localized outbreaks and international dissemination observed with multidrug-resistant clonal groups such as (group D-derived) 'clonal group A' and serotype O15:K52:H1, including the recent detection of clonal group A isolates in wastewater effluents from

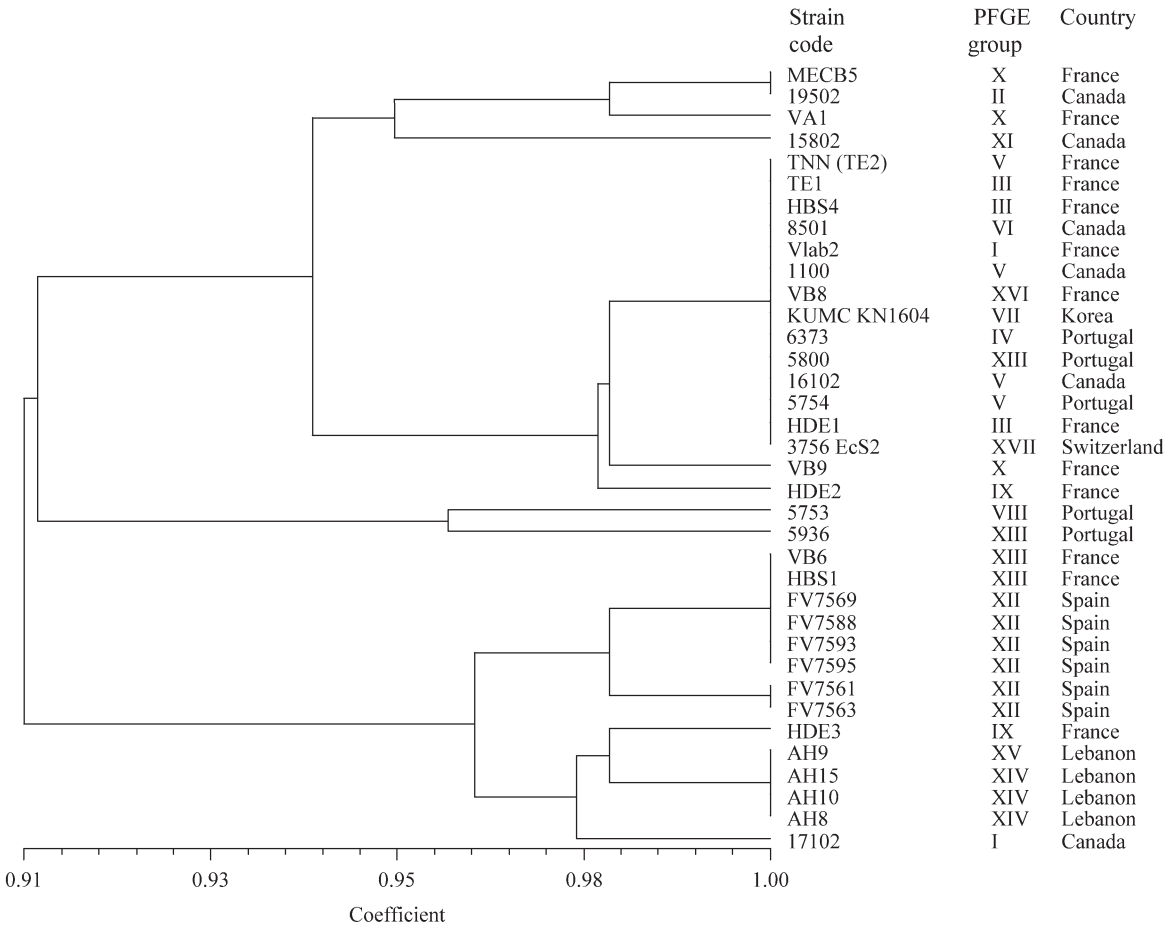

Figure 3. Virulence profile dendrogram for 36 CTX-M-15-positive *E. coli* isolates from ST131. The dendrogram was produced by the UPGMA algorithm based on extended virulence gene profiles for the 36 strains from ST131. Virulence profile similarity varied from 91% to 100%.

geographically dispersed areas of the United States.[10,13,33] The present CTX-M-15-positive *E. coli* clonal group was previously shown to have caused what appeared to be localized outbreaks involving specific healthcare institutions (France) or geographical regions (Calgary).[20,21] Our findings suggest that some of these seemingly isolated occurrences are actually linked, a principle that may apply broadly to drug-resistant extraintestinal infections. From this point of view, it would be relevant to determine whether the serogroup O25, CTX-M-15-positive *E. coli* previously published, notably in the UK, and not included in this study also belong to clone ST131.[34] Recognition that geographically distant infection episodes may be caused by the same bacterial clone, arising from a common source, is the basis for the CDC's PulseNet surveillance system.[35] Whereas that system focuses mainly on diarrhoeal pathogens, a similar system may be needed for extraintestinal infections.

In summary, we have characterized a broadly disseminated, CTX-M-15-positive, multidrug-resistant, virulent *E. coli* clonal group with highly homogeneous virulence genotypes and subgroups exhibiting highly similar PFGE profiles, suggesting recent emergence. Understanding how this clone has emerged and successfully disseminated within the hospital and community, including across national boundaries, should be a public health priority.

Acknowledgements

We are indebted to Professor Patrice Nordmann, Professor Guillaume Arlet and Dr Florence Doucet-Populaire for providing us with the Indian and Swiss strains, strain TNN (TE2) and the Lebanese strains, respectively. We also are grateful to Dr Azucena Mora, Dr Jesus Blanco, Dr Miguel Blanco, Mrs Ghizlane Dahbi and Mrs Cecilia Lopez for their contribution to this study.

Funding

This study was supported by a grant (AOR 04016) from La Direction de le Recherche Clinique de l'Assistance Publique-Hôpitaux de Paris (M.-H. N.-C.), a grant (PI052023-PI051481) from Fondo de Investigacion Sanitaria (FIS), Instituto de Salud Carlos III, Spanish Ministerio de Sanidad y Consumo (J. B.) and Office of Research and Development, Medical Research Service, Department of Veterans Affairs (J. R. J.).

Transparency declarations

All of the authors except one have none to declare. J. R. J. is a consultant for the following companies: Bayer, Ortho-McNeil, Merck, Wyeth-Ayerst, and Procter and Gamble.

References

1. Paterson D, Bonomo R. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; **18**: 657–86.

2. Pitout JD, Nordmann P, Laupland KB *et al*. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *J Antimicrob Chemother* 2005; **56**: 52–9.

3. Canton R, Coque TM. The CTX-M β-lactamase pandemic. *Curr Opin Microbiol* 2006; **9**: 466–75.

4. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 2004; **48**: 1–14.

5. Gangoue-Pieboji J, Miriagou V, Vourli S *et al*. Emergence of CTX-M-15-producing enterobacteria in Cameroon and characterization of a $bla_{CTX\text{-}M\text{-}15}$ carrying element. *Antimicrob Agents Chemother* 2005; **49**: 441–5.

6. Lavollay M, Mamlouk K, Frank T *et al*. Clonal dissemination of a CTX-M-15 β-lactamase-producing *Escherichia coli* strain in the Paris area, Tunis and Bangui. *Antimicrob Agents Chemother* 2006; **50**: 2433–8.

7. Livermore DM, Canton R, Gniadkowski M *et al*. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007; **59**: 165–74.

8. Pallecchi L, Malossi M, Mantella A *et al*. Detection of CTX-M-type β-lactamase genes in fecal *Escherichia coli* isolates from healthy children in Bolivia and Peru. *Antimicrob Agents Chemother* 2004; **48**: 4556–61.

9. Fierer J, Guiney D. Extended-spectrum β-lactamases: a plague of plasmids. *JAMA* 1999; **281**: 563–4.

10. Manges AR, Johnson JR, Foxman B *et al*. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *N Engl J Med* 2001; **345**: 1007–13.

11. McGee L, McDougal L, Zhou J *et al*. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J Clin Microbiol* 2001; **39**: 2565–71.

12. Oliveira DC, Alexander T, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* 2002; **2**: 180–9.

13. Prats G, Navarro F, Mirelis B *et al*. *Escherichia coli* serotype O15:K52:H1 as a uropathogenic clone. *J Clin Microbiol* 2000; **38**: 201–9.

14. Arpin C, Coulange L, Dubois V *et al*. Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in various types of private health care centers. *Antimicrob Agents Chemother* 2007; **51**: 3040–4.

15. Prince N, Leflon-Guibout V, Doit C *et al*. Genetic background of CTX-M-producing *Escherichia coli* isolates reveals a great strain diversity and the emergence of a clone in the community and the hospital. In: *Abstracts of the Forty-sixth Interscience Conference on Antimicriobial Agents Chemotherapy, San Francisco, CA, 2006*. Abstract K-1508, p. 357. American Society for Microbiology, Washington, DC, USA.

16. Karim A, Poirel L, Nagarajan S *et al*. Plasmid-mediated extended-spectrum β-lactamase (CTX-M-3 like) from India and gene association with insertion sequence IS*Ecp1*. *FEMS Microbiol Lett* 2001; **201**: 237–41.

17. Kim Y, Kim S, Lee J *et al*. Nosocomial transmission of CTX-M-15 and OXA-30 β-lactamase-producing *Escherichia coli* in a neurosurgical intensive care unit. *Ann Clin Lab Sci* 2005; **35**: 297–301.

18. Mendonça N, Leitao J, Manageiro V *et al*. Spread of clinical extended-spectrum β-lactamase (CTX-M)-producing *Escherichia coli* isolates in community and nosocomial environments in Portugal. *Antimicrob Agents Chemother* 2007; **51**: 1946–55.

19. Moubareck C, Daoud Z, Hakimé NI *et al*. Countrywide spread of community- and hospital-acquired extended-spectrum β-lactamase (CTX-M-15)-producing Enterobactericeae in Lebanon. *J Clin Microbiol* 2005; **43**: 3309–13.

20. Pitout JDD, Laupland KB, Church DL *et al*. Virulence factors of *Escherichia coli* isolates that produce CTX-M-type extended-spectrum β-lactamases. *Antimicrob Agents Chemother* 2005; **49**: 4667–70.

21. Leflon-Guibout V, Jurand C, Bonacorsi S *et al*. Emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. *Antimicrob Agents Chemother* 2004; **48**: 3736–42.

22. Johnson JR, O'Bryan TT. Improved repetitive-element PCR fingerprinting for resolving pathogenic and nonpathogenic phylogenetic groups within *Escherichia coli*. *Clin Diagn Lab Immunol* 2000; **7**: 265–73.

23. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; **66**: 4555–8.

24. Guinée PAM, Jansen WH, Wadström T *et al*. *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. In: Leeww PW, Guinée PAM, eds. *Laboratory Diagnosis in Neonatal Calf and Pig Diarrhoea: Current Topics in Veterinary and Animal Science*. The Hague, Netherlands: Martinus-Nijhoff, 1981; 126–62.

25. Clermont O, Johnson JR, Menard M *et al*. Determination of *Escherichia coli* O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia. *Diagn Microbiol Infect Dis* 2007; **57**: 214–23.

26. Tartof SY, Solberg OD, Manges AR *et al*. Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. *J Clin Microbiol* 2005; **43**: 5860–4.

27. Blanco M, Blanco JE, Dahbi G *et al*. Typing of intimin (*eae*) genes from enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhoea in Montevideo, Uruguay: identification of two novel intimin variants (μB and ξR/β2B). *J Med Microbiol* 2006; **55**: 1165–74.

28. Carrico JA, Pinto FR, Simas C *et al*. Assessment of band-based similarity coefficients for automatic type and subtype classification of microbial isolates analyzed by pulsed-field gel electrophoresis. *J Clin Microbiol* 2005; **43**: 5483–90.

29. Johnson JR, Kuskowski MA, O'Bryan TT *et al*. Epidemiological correlates of virulence genotype and phylogenetic background among *Escherichia coli* blood isolates from adults with diverse-source bacteremia. *J Infect Dis* 2002; **185**: 1439–47.

30. Anonymous. Comité de l'Antibiogramme de la Société Française de Microbiologie report 2003. *Int J Antimicrob Agents* 2003; **21**: 364–91.

31. Johnson JR, Clermont O, Menard M *et al*. Experimental mouse lethality of *Escherichia coli* isolates in relation to accessory traits, phylogenetic group, and ecological source. *J Infect Dis* 2006; **194**: 1141–50.

32. Johnson JR, Kuskowski MA, Owens K *et al*. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. *J Infect Dis* 2003; **188**: 759–68.