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Replicon Typing of Plasmids Carrying CTX-M or CMY β-Lactamases Circulating among *Salmonella* and *Escherichia coli* Isolates†

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Replicon typing of plasmids carrying bla_{CTX-M} or bla_{CMY} β-lactamase genes indicates a predominance of I1 and A/C replicons among bla_{CMY}-carrying plasmids and five different plasmid scaffolds associated with the different types of bla_{CTX-M} genes (I1, FII, HI2, K, and N). These results demonstrate the association of certain β-lactamase genes with specific plasmid backbones.

Understanding the molecular epidemiology of resistance plasmids has proven to be a complex task due to the diversity and promiscuity of these elements. The relatedness of plasmids can be demonstrated either by restriction fragment pattern analysis or by classification into incompatibility groups (Inc) and replicon (rep) typing (8). These analyses require laborious hands-on work, multiple conjugation or transformation assays, or hybridization experiments (8). A PCR-based replicon typing (PBRT) method has been recently developed and can be easily applied to a larger number of strains (6).

The aim of this work was to investigate phylogenetic relatedness among plasmids carrying extended-spectrum cephalosporin (ESC) resistance that are emerging in the United Kingdom and tracing, by PBRT, the diffusion of prevalent plasmids in association with specific ESC resistance gene variants.

The PBRT was applied to transformants or transconjugants obtained from 29 *Salmonella enterica* and 38 *Escherichia coli* isolates, representing a collection of plasmid-mediated CTX-M or AmpC producers isolated in the United Kingdom between 1995 and 2003. Strains were received from different laboratories in the country or were isolated at the Health Protection Agency Centre for Infections, London, United Kingdom, or at the Veterinary Laboratory Agency, Addlestone, Surrey, United Kingdom, and archived with antimicrobial susceptibility tests, β-lactamase gene identification, pulsed-field gel electrophoresis (PFGE), and plasmid profiling, as previously described (1, 2, 3, 10, 11). These strains were not repetitive (one from each patient), and only one strain for each cluster (100% identity by PFGE) was included in the study. Sixty strains were isolated from human urine, blood, feces, sputum, and surgical wounds, and seven strains were from animals (poultry, turkey, horse, and cattle). The ESC resistance genes in this collection were the following: $bla_{CTX-M-9}$, $bla_{CTX-M-15}$, $bla_{CTX-M-2}$, $bla_{CTX-M-14}$, $bla_{CTX-M-3}$, $bla_{CTX-M-40}$, bla_{CMY-2}, bla_{CMY-4}, bla_{CMY-7}, and bla_{CMY-21} (Tables 1 and 2). *Salmonella* strains were genetically unrelated by PFGE and belonged to several different serotypes or were isolated in different years. Using a ≥85% similarity cutoff point, PFGE of the *E. coli* strains identified 14 and 12 different XbaI digest profiles among the 20 bla_{CTX-M}- and 18 bla_{CMY}-producing isolates, respectively, thus indicating that these strains, with the exception of a few clusters of two to three strains each, were genetically unrelated.

Thirty-one transformants/transconjugants carried plasmid-located AmpC-derivative β-lactamases. All plasmids were from epidemiologically unrelated isolates: 27 were from human cases, and 7 of these strains were isolated in other countries or were from patients who had recently traveled abroad; the remaining 4 were from animals (Table 1). All the transformants/transconjugants except for two were successfully typed by PBRT, and four strains showed positive results for two different replicons (Table 1). The latter strains were further examined by Southern blot hybridization experiments performed on purified undigested plasmid DNA using the two amplified replicons as probes (data not shown). This experiment indicated that strains A2-VLA and E108137-HPA carried multireplicon plasmids positive for both the FII-FIA and FIA-FIB replicons, respectively, while the E134708-HPA and E115837-HPA strains carried an IncI1 plasmid plus an IncN or IncA/C plasmid, respectively, cotransferred by conjugation. By Southern blot hybridization, the bla_{CMY-2} gene was associated with the IncI1 plasmid in both strains (data not shown) (Table 1). Thus, with the exception of three IncF plasmids (two of them carrying the bla_{DHA-1} gene) and two untypeable plasmids, all the bla_{CMY}-positive plasmids of this collection were located on repI1 or repA/C plasmids.

Among the nine bla_{CMY-2} repA/C plasmids, seven of them were from *Salmonella* of different serotypes and two were from *E. coli* showing 53% genetic similarity index by PFGE (10). The DNA sequence of the *repA* gene obtained by sequencing the entire repA/C amplicon (465 bp) from these nine plasmids showed 100% identity to the *repA* gene of the previously characterized 2039 plasmid (EMBL accession no. AM087198), originating from the ceftriaxone-resistant *S. enterica* serovar

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† Supplemental material for this article may be found at http://aac.asm.org/.

TABLE 1. Characteristics of the AmpC-carrying plasmids analyzed in this study

AmpC derivative	Strain(s)	Yr of isolation	Species	Source	Origin	Extra resistance in plasmid[c]	Self transfer[d]	Replicon typing
DHA-1	A2-VLA	1996	*S. enterica* serovar Senftenberg	Human	United Kingdom	CHL, SUL, TET	Yes	FII(2), FIA[e]
	A3-VLA	1999	*S. enterica* serovar Senftenberg	Human	United Kingdom	CHL, GEN, NET, SUL, TET, TMP	No	FII(2)
CMY-2	EC30-VLA	2003	*E. coli*	Cattle	United Kingdom	SUL	Yes	I1
	EC44-VLA	2003	*E. coli*	Cattle	United Kingdom	SUL	Yes	I1
	A4-VLA	1999	*S. enterica* serovar 4,5,12:I:-	Human	Gambia	None	Yes	I1
	A5-VLA	2001	*S. enterica* serovar Ajiobo	Human	United Kingdom	None	Yes	I1
	E108141-HPA	1995	*E. coli*	Human	United Kingdom	CHL, GEN, KAN, SUL, TET, TMP	No	A/C
	E127226-HPA	1997	*E. coli*	Human	United Kingdom	SUL, TET	No	A/C
	A10-VLA	2003	*S. enterica* serovar Anatum	Human	United States	CHL, SUL, TET	Yes	A/C
	CEF9-VLA	1999	*S. enterica* serovar Bredeney	Turkey	Canada	SUL, TET	No	A/C
	A7-VLA	2002	*S. enterica* serovar Heidelberg	Human	Greece	CHL, GEN, SUL, TET	No	A/C
	UCM267-VLA	2002	*S. enterica* serovar Infantis	Human	Honduras	AMK, CHL, GEN, SUL, TMP	No	A/C
	C1-VLA[a]	2000	*S. enterica* serovar Ohio	Human	United Kingdom	AMK, GEN, SUL, TMP	Yes	A/C
	A12-VLA	Unknown	*S. enterica* serovar Typhimurium	Horse	Ireland	CHL, GEN, NET, SUL, TET, TMP	Yes	A/C
	A11-VLA	2003	*S. enterica* serovar Worthington	Human	Iraq	CHL, SUL, TET	No	A/C
	E108137-HPA	1995	*E. coli*	Human	United Kingdom	KAN, GEN, SUL, TET	No	FIA, FIB[e]
	A9-VLA	2003	*S. enterica* serovar Typhimurium	Human	Mexico	None	No	Negative
	E128986-HPA	1997	*E. coli*	Human	United Kingdom	None	No	Negative
CMY-4	A6-VLA	2001	*S. enterica* serovar Senftenberg	Human	United Kingdom	None	No	A/C
CMY-7	E127346-HPA	1997	*E. coli*	Human	United Kingdom	None	No	I1
	E132587-HPA	1998	*E. coli*	Human	United Kingdom	None	No	I1
	E133554-HPA*[b]	1998	*E. coli*	Human	United Kingdom	None	No	I1
	E133761-HPA*	1998	*E. coli*	Human	United Kingdom	None	No	I1
	E133003-HPA	1998	*E. coli*	Human	United Kingdom	None	No	I1
	E146836-HPA	1999	*E. coli*	Human	United Kingdom	None	No	I1
	E162599-HPA	2001	*E. coli*	Human	United Kingdom	None	No	I1
	E154371-HPA**	2000	*E. coli*	Human	Pakistan	TMP	Yes	I1
	E159045-HPA**	2001	*E. coli*	Human	United Kingdom	None	No	I1
	E134708-HPA**	1998	*E. coli*	Human	United Kingdom	TMP	Yes	I1, N[f]
	E115837-HPA	1996	*E. coli*	Human	United Kingdom	KAN, NET, SUL, TET	Yes	I1, A/C[f]
CMY-21	E111726-HPA	1996	*E. coli*	Human	United Kingdom	None	No	I1

[a] This strain carries the $bla_{\text{CMY-2}}$ gene and an additional $bla_{\text{CTX-M-15}}$ gene.
[b] Two clusters of strains (indicated with single and double asterisks) showed a genetic similarity index of 90% by PFGE (10).
[c] Antimicrobial drugs used were the following: amikacin (AMK), chloramphenicol (CHL), gentamicin (GEN), kanamycin (KAN), netilmicin (NET), sulphonamides (SUL), tetracycline (TET), and trimethoprim (TMP).
[d] For the strains where transfer was not achieved by conjugation, transformation experiments were conducted in ElectroMAX streptomycin-resistant DH10B cells (Invitrogen, Paisley, United Kingdom) selecting on cefoxitin (4 μg/ml) MacConkey agar plates.
[e] Multireplicon plasmid; each single replicon was used as a probe in Southern blot hybridization experiments performed on purified plasmids, demonstrating their colocalization on the same plasmid.
[f] Two different plasmids simultaneously detected in the same strain.

Typhimurium strain isolated in the United States in 1997 (7). This *repA* gene is slightly different (94% identity, 26 nucleotide substitutions) from the *repA* gene of the RA1 IncA/C reference plasmid (GenBank accession no. X73674). The detection of the same resistance gene ($bla_{\text{CMY-2}}$) on an IncA/C plasmid carrying the same *repA* gene variant strongly indicates that the bla_{CMY}-A/C plasmid could be a successful and widely distributed plasmid circulating on different continents.

The 11 plasmids carrying the $bla_{\text{CMY-7}}$ gene were all associated with the repI1 replicon as well as 4 plasmids carrying the $bla_{\text{CMY-2}}$ and 1 plasmid carrying the $bla_{\text{CMY-21}}$ variant. Thirteen of these plasmids were from *E. coli* isolated in the United Kingdom. Two clusters, of two and three strains, respectively, showed a genetic similarity index of 90% by PFGE (Table 1), but the other strains showed an index lower than 68%, indicating that they were genetically unrelated (10). These results suggest the circulation of a prevalent IncI1 plasmid carrying the different bla_{CMY} gene variants. It is interesting to note that the IncI1 plasmids carrying the bla_{CMY} gene show some difference with respect to the additional plasmid-located resistance (mainly sulfonamide or trimethoprim resistance; Table 1) or to a capability of self transferability. This observation suggests interesting clues on the evolution of related plasmids that can gain additional resistance determinants and/or lose the transferability gene-associated functions, evolving in different plasmid variants. Furthermore, since the $bla_{\text{CMY-2}}$, $bla_{\text{CMY-4}}$, and $bla_{\text{CMY-21}}$ genes differ from each other by only one or a few nucleotide substitutions, they could therefore simply be variants of one another, and this evolution could have occurred within the same IncI1 or IncA/C plasmid scaffold by accumulation of single point mutations.

In contrast with the previous situation, a very diverse picture was observed for the $bla_{\text{CTX-M}}$-carrying plasmids. We analyzed 36 plasmids: 22 carried $bla_{\text{CTX-M-15}}$, 5 carried $bla_{\text{CTX-M-9}}$, 4 carried $bla_{\text{CTX-M-14}}$, 2 carried $bla_{\text{CTX-M-2}}$, 2 carried $bla_{\text{CTX-M-3}}$, and 1 carried $bla_{\text{CTX-M-40}}$. All plasmids were from epidemiologically unrelated isolates: 33 were from human cases (6 of these had reported traveling abroad), and 3 were from animals (Table 2). CTX-M-15 was the most common CTX-M subtype identified, and the previously analyzed PFGE patterns for the *E. coli* producers indicated that the spread of CTX-M-15 was not due to a single clone expansion (10). This result is in agreement with other previous studies performed on CTX-

TABLE 2. Characteristics of the CTX-M-carrying plasmids analyzed in this study

Extended-spectrum β-lactamase	Strains	Yr of isolation	Species	Source	Origin	Extra resistance in plasmid[b]	Self transfer[c]	Replicon typing
CTX-M-9	C8-VLA	1999	*S. enterica* serovar Virchow	Human	Spain	SUL, TMP	Yes	HI2
	C16-VLA	2003	*S. enterica* serovar Virchow	Human	United Kingdom	SUL, TMP	Yes	HI2
	C11-VLA	2001	*S. enterica* serovar Virchow	Human	Balearics	SUL, TET, TMP	No	HI2
	C4-VLA	1997	*S. enterica* serovar Virchow	Human	United Kingdom	SUL, TET, TMP	Yes	HI2
	C6-VLA	1997	*S. enterica* serovar Virchow	Human	Spain	NET, SUL, TMP	Yes	HI2
CTX-M-15	E162799-HPA	2001	*E. coli*	Human	United Kingdom	None	Yes	I1
	E177258-HPA	2003	*E. coli*	Human	United Kingdom	None	Yes	I1
	E158740-HPA	2001	*E. coli*	Human	United Kingdom	None	Yes	I1
	E172182-HPA	2003	*E. coli*	Human	United Kingdom	SUL	Yes	I1
	C1-VLA	2001	*S. enterica* serovar Anatum	Human	Pakistan	None	Yes	I1
	UCM267-VLA	2002	*S. enterica* serovar Infantis	Human	Honduras	None	Yes	I1
	ESBL3-VLA	2000	*S. enterica* serovar Ohio	Human	United Kingdom	GEN, SUL, TMP	Yes	I1
	C12-VLA	2002	*S. enterica* serovar Typhimurium	Human	United Kingdom	None	Yes	I1
	C13-VLA	2003	*S. enterica* serovar Typhimurium	Human	United Kingdom	None	Yes	I1
	C15-VLA	2003	*S.* Enteritidis	Human	United Kingdom	GEN	No	FII(1)
	E170743-HPA	2003	*E. coli*	Human	United Kingdom	GEN	No	FII(1)
	E172176-HPA*[a]	2003	*E. coli*	Human	United Kingdom	AMK, GEN, KAN, SUL, TET, TMP	Yes	FII(1), FIA[d]
	E177268-HPA	2003	*E. coli*	Human	United Kingdom	GEN, KAN, STR	No	FII(1), FIA[d]
	E172798-HPA	2003	*E. coli*	Human	United Kingdom	GEN, KAN, TET	No	FII(1), FIA[d]
	E177243-HPA	2003	*E. coli*	Human	United Kingdom	GEN, TET	Yes	FII(1), FIA[d]
	E172172-HPA*	2003	*E. coli*	Human	United Kingdom	AMK, GEN, KAN, SUL, TET, TMP	Yes	FII(1), FIA, FIB[d]
	E169967-HPA**	2002	*E. coli*	Human	United Kingdom	None	Yes	FII(1), FIA, FIB[d]
	E170681-HPA**	2003	*E. coli*	Human	United Kingdom	GEN, SUL, TET, TMP	Yes	FII(2), FIA[d]
	E171897-HPA**	2003	*E. coli*	Human	United Kingdom	GEN, SUL, TET, TMP	No	FII(2), FIA, FIB[d]
	E162237-HPA	2001	*E. coli*	Human	United Kingdom	GEN, SUL, TET, TMP	No	FII(2), FIA, FIB[d]
	E177273-HPA	2003	*E. coli*	Human	United Kingdom	GEN, TET, TMP	Yes	FII(2), FIA, FIB[d]
	E177265-HPA	2003	*E. coli*	Human	United Kingdom	TMP	Yes	Negative
CTX-M-2	E136374-HPA	1998	*E. coli*	Human	United Kingdom	AMK, GEN, KAN, SUL	No	A/C
	C14-VLA	Unknown	*S. enterica* serovar Virchow	Poultry	Ireland	SUL, TET, TMP	No	P
CTX-M-14	C10-VLA	2001	*S. enterica* serovar Enteritidis	Human	United Kingdom	None	Yes	I1
	C159/11-VLA	2004	*E. coli*	Cattle	United Kingdom	None	Yes	K
	Cow 47-VLA	2005	*E. coli*	Cattle	United Kingdom	None	No	Negative
	C3-VLA	2002	*S. enterica* serovar Stanley	Human	Thailand	None	Yes	Negative
CTX-M-3	ESBL7-VLA	2002	*S. enterica* serovar Virchow	Human	United Kingdom	SUL, TMP	Yes	N
	ESBL10-VLA	2002	*S. enterica* serovar Virchow	Human	United Kingdom	SUL, TMP	Yes	N
CTX-M-40	E134200 HPA	1998	*E. coli*	Human	United Kingdom	GEN	No	N

[a] Two clusters of strains (indicated with single and double asterisks) showed a genetic similarity index of 90% by PFGE (10).
[b] Antimicrobial drugs used were the following: amikacin (AMK), chloramphenicol (CHL), gentamicin (GEN), kanamycin (KAN), netilmicin (NET), sulphonamides (SUL), tetracycline (TET), and trimethoprim (TMP).
[c] For the strains where transfer was not achieved by conjugation, transformation experiments were conducted in ElectroMAX streptomycin resistant DH10B cells (Invitrogen, Paisley, United Kingdom) selecting on cefotaxime (10 μg/ml) MacConkey agar plates.
[d] Multireplicon plasmid; each single replicon was used as a probe in Southern blot hybridization experiments performed on purified plasmids, demonstrating their colocalization on the same plasmid.

M-15 *E. coli* producers from the United Kingdom, which demonstrated that this extended-spectrum β-lactamase is prevalent and carried by different *E. coli* lineages (14).

In this study, two different replicons were associated with the $bla_{CTX\text{-}M\text{-}15}$-positive plasmids. Nine of 22 plasmids were self conjugative and positive for the repI1 replicon; the majority was associated with no additional resistance. Twelve of 22 plasmids were positive for repFII, in some strains associated with FIA and/or FIB replicons. In the latter cases the FII, FIA, and FIB replicons were always colocated on the same plasmid by Southern blot hybridization experiments, indicating the presence of multireplicon plasmids carrying the $bla_{CTX\text{-}M\text{-}15}$ gene. To further discern these IncF plasmids, HpaI restriction patterns and the nucleotide sequence of the regulatory antisense CopA RNA were analyzed (12). Nucleotide substitutions in the CopA sequence were considered in the classification of FII plasmids, since the antisense RNA has been demonstrated to participate in the control of replication as well as in the incompatibility behavior of the FII plasmids by sequence divergence (4, 12, 13). The sequence of the 270-bp repF amplicon (6) was determined and aligned to the region between nucleotides 392 to 661 in the IncFII NR1 reference plasmid sequence (EMBL accession no. X02302), corresponding to the CopA antisense RNA. These analyses further discriminated the CTX-M-15-carrying plasmids into two FII subgroups (Fig. S1 in the supplemental material). One group showed a CopA sequence 100% identical to that of the FII reference plasmid (indicated as FII-1) NR1 and also identical to that of the pC15-1a plasmid (EMBL accession no. NC_005327), identified in *E. coli* associated with an outbreak occurring in Canada (5). The pC15-1a plasmid is described as containing a 28.4-kb multidrug resistance region with the $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$,

bla$_{TEM-1}$, *tetA*, *aac*(*6′*)*-Ib*, and *aac*(*3*)*-II* genes inserted into the IncFII plasmid backbone (5). The presence of the same gene variant (*bla*$_{CTX-M-15}$) associated with the same IncFII type of plasmid in the United Kingdom strongly supports the evidence of a wide distribution of this plasmid in different countries. Restriction pattern analysis also confirmed the presence of related but divergent FII plasmids carrying the *bla*$_{CTX-M-15}$ gene (Fig. S2 in the supplemental material). The second subgroup of plasmids, also composed of the two *bla*$_{DHA-1}$-carrying plasmids (Table 1; Fig. S1 in the supplemental material), showed a different CopA sequence [indicated as FII (2) in Table 2]. These data demonstrated that *bla*$_{CTX-M-15}$ can be located on a largely diffused plasmid (IncI1) or can spread associated with a family of related IncFII plasmids also reported from other continents.

The other *bla*$_{CTX-M}$ variants were associated with plasmids belonging to different Inc groups. Interestingly, the plasmids carrying the *bla*$_{CTX-M-9}$ gene were from United Kingdom strains or were associated with travel to Spain, where this gene variant is largely prevalent (9), and were invariably associated with repHI2. These plasmids were all from *S. enterica* serovar Virchow, thus suggesting a recurrent association of HI2 plasmids with this *Salmonella* serotype. Interestingly, the *bla*$_{CTX-M-14}$ variant, although closely related to the *bla*$_{CTX-M-9}$ variant, was associated with different plasmid types, while *bla*$_{CTX-M-3}$ from two *S. enterica* serovar Virchow strains and *bla*$_{CTX-M-40}$ from an *E. coli* strain were both identified on IncN plasmids, despite these genes being more distantly related. These results indicate a great heterogeneity among the CTX-M plasmids with respect to the CMY plasmids that probably correlates with the higher variability of the *bla*$_{CTX-M}$ gene family.

The general overview of the results obtained by PBRT indicate the high prevalence of some replicons, such as repI1, repA/C, and repFII, in association with relevant ESC resistance genes, such as the *bla*$_{CMY-2}$ and *bla*$_{CTX-M-15}$ genes, suggesting a large diffusion of particular plasmids prevailing in the United Kingdom but also identified in other studies in bacterial populations from other continents. These epidemic plasmids seem to prevail in different environments and might spread across different bacterial species in humans as well in animals.

PBRT was demonstrated to be a good method for detecting the replicons in large collections of plasmids. The origin of replication can be considered an additional marker for the constant backbone of the plasmid. The association of replicons with specific plasmid-borne resistance genes opens the possibility of easily detecting and tracing the diffusion of successful plasmids as well as detecting the mobilization capability of a resistance gene among different plasmids.

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REFERENCES

1. **Batchelor, M., F. A. Clifton-Hadley, A. D. Stallwood, G. A. Paiba, R. H. Davies, and E. Liebana.** 2005. Detection of multiple cephalosporin-resistant *Escherichia coli* from a cattle fecal sample in Great Britain. Microb. Drug Resist. **11:**58–61.
2. **Batchelor, M., K. Hopkins, E. J. Threlfall, F. A. Clifton-Hadley, A. D. Stallwood, R. H. Davies, and E. Liebana.** 2005. *bla*$_{CTX-M}$ genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrob. Agents Chemother. **49:**1319–1322.
3. **Batchelor, M., K. Hopkins, E. J. Threlfall, F. A. Clifton-Hadley, A. D. Stallwood, R. H. Davies, and E. Liebana.** 2005. Characterization of AmpC-mediated resistance in clinical *Salmonella* isolates recovered from humans during the period 1992 to 2003 in England and Wales. J. Clin. Microbiol. **43:**2261–2265.
4. **Blomberg, P., K. Nordstrom, and E. G. H. Wagner.** 1992. Replication control of plasmid R1: RepA synthesis is regulated by CopA RNA through inhibition of leader peptide translation. EMBO J. **1:**2675–2683.
5. **Boyd, D. A., S. Tyler, S. Christianson, A. McGeer, M. P. Muller, B. M. Willey, E. Bryce, M. Gardam, P. Nordmann, and M. R. Mulvey.** 2004. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob. Agents Chemother **48:**3758–3764.
6. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228.
7. **Carattoli, A., F. Tosini, W. P. Giles, M. E. Rupp, S. H. Hinrichs, F. J. Angulo, T. J. Barrett, and P. D. Fey.** 2002. Characterization of plasmids carrying CMY-2 from expanded-spectrum cephalosporin-resistant *Salmonella* isolated in the United States between 1996 and 1998. Antimicrob. Agents Chemother. **46:**1269–1272.
8. **Couturier, M., F. Bex, P. L. Bergquist, and W. K. Maas.** 1988. Identification and classification of bacterial plasmids. Microbiol. Rev. **52:**375–395.
9. **Hernandez, J. R., L. Martinez-Martinez, R. Canton, T. M. Coque, A. Pascual, and the Spanish Group for Nosocomial Infections (GEIH).** 2005. Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases in Spain. Antimicrob. Agents Chemother. **49:**2122–2125.
10. **Hopkins, K. L., M. J. Batchelor, E. Liebana, A. P. Deheer-Graham, and E. J. Threlfall.** Characterisation of CTX-M and AmpC genes in human isolates of *Escherichia coli* identified between 1995 and 2003 in England and Wales. Int. J. Antimicrob. Agents, in press.
11. **Liebana, E., M. Gibbs, C. Clouting, L. Barker, F. A. Clifton-Hadley, E. Pleydell, B. Abdalhamid, N. D. Hanson, L. Martin, C. Poppe, and R. H. Davies.** 2004. Characterization of β-lactamases responsible for resistance to extended-spectrum cephalosporins in *Escherichia coli* and *Salmonella enterica* strains from food-producing animals in the United Kingdom. Microb. Drug Resist. **10:**1–9.
12. **Osborn, A. M., F. M. da Silva Tatleyqq, L. M. Steyn, R. W. Pickup, and J. R. Saunders.** 2000. Mosaic plasmids and mosaic replicons: evolutionary lessons from the analysis of genetic diversity in IncFII-related replicons. Microbiology **146:**2267–2275.
13. **Persson, C., E. Gerhart, H. Wagner, and K. Nordstrom.** 1990. Control of replication of plasmid Rl: structures and sequences of the antisense RNA, CopA, required for its binding to the target RNA, CopT. EMBO J. **9:**3767–3775.
14. **Woodford, N., M. E. Ward, M. E. Kaufmann, et al.** 2004. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum b-lactamases in the UK. J. Antimicrob. Chemother. **54:**735–743.