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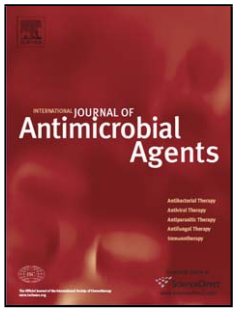

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Characterisation of the CTX-M-15-encoding gene in *Klebsiella pneumoniae* strains from the Barcelona metropolitan area: plasmid diversity and chromosomal integration

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ABSTRACT

The localisation and genetic organisation of *bla*$_{CTX-M-15}$ were studied in 37 CTX-M-15-producing *Klebsiella pneumoniae* isolates collected from 2005 to 2008 within the Barcelona metropolitan area. Polymerase chain reaction (PCR)-based replicon typing and Southern hybridisations were used to identify the *bla*$_{CTX-M-15}$ location. The genetic environment was analysed by PCR mapping and sequencing, and transferability of *bla*$_{CTX-M-15}$ was evaluated by conjugation and transformation assays. The majority of the 37 isolates carried *bla*$_{CTX-M-15}$ in a plasmid location, frequently associated with the ***aac(6')-Ib-cr*** gene. Plasmids encoding *bla*$_{CTX-M-15}$ carried three distinct replicons, i.e. IncFII, IncR and IncFIIk, the latter two not having been described previously in association with *bla*$_{CTX-M-15}$. Several of these plasmids were not self-transferable. Furthermore, in all isolates belonging to sequence type ST-1, *bla*$_{CTX-M-15}$ was found integrated into the *K. pneumoniae* chromosome. In all the studied isolates, the mobile element IS*Ecp1* was found upstream of *bla*$_{CTX-M-15}$, whereas IS*26* was found inserted within IS*Ecp1* in several isolates, in previously unreported positions. In conclusion, these findings indicate that among *K. pneumoniae* strains isolated in the Barcelona metropolitan area, *bla*$_{CTX-M-15}$ is associated with diverse genetic elements, including the IncR and IncFIIk replicons, as reported for the first time here, and the chromosome.

1. Introduction

The spread of antimicrobial-resistant pathogens is a serious clinical and public health problem worldwide. Recombination processes, including homologous recombination and transposition, constitute the major pathways for the capture and expression of resistance genes within mobile genetic elements that thereby intensively contribute to their dissemination. Regarding the high prevalence of *bla*$_{\text{CTX-M-15}}$ in Enterobacteriaceae, several reports have emphasised the importance of the insertion sequences IS*Ecp1* and IS*26* in its mobilisation from the *Kluyvera* chromosome [1,2]. Furthermore, the rapid and international spread of *bla*$_{\text{CTX-M-15}}$ has been mainly associated with the global dissemination of *Escherichia coli* clonal strains (i.e. sequence types ST-131 and ST-405) carrying *bla*$_{\text{CTX-M-15,}}$ mostly within IncFII conjugative plasmids [3]. Detection of CTX-M-15-producing *Klebsiella pneumoniae* is also increasingly documented worldwide and some authors have proposed horizontal transmission of *bla*$_{\text{CTX-M-15}}$ between *E. coli* and *K. pneumoniae* strains by conjugal transfer of IncFII plasmids [4].

In a recent study [5] investigating the presence of CTX-M-15-producing *K. pneumoniae* clonal strains in the Barcelona metropolitan area from 2005 to 2008, we screened for *bla*$_{\text{CTX-M-15}}$ among 111 non-duplicate extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* isolates. We reported 37 CTX-M-15-producing *K. pneumoniae* isolates that, based on pulsed-field gel electrophoresis (PFGE), were classified in eight clonal clusters, corresponding with seven sequence types, as determined by multilocus sequence typing (MLST) [5]. Pulsotypes VII and VIII, which exhibited 79% similarity, belonged to the same sequence type, i.e. ST-1 [5]. In the present report, we describe the location and genetic organisation of *bla*$_{\text{CTX-M-15}}$ in these 37 *K. pneumoniae* isolates.

2. Materials and methods

2.1. Bacterial isolates

The 37 studied *K. pneumoniae* isolates represented all of the CTX-M-15-producing isolates found among 111 ESBL-producing *K. pneumoniae* collected from three hospitals within metropolitan Barcelona between 2005 and 2008, as previously reported [5]. According to 7-gene MLST, these isolates belonged to sequence types ST-1 (*n* = 12), ST-14 (*n* = 1), ST-37 (*n* = 1), ST-147 (*n* = 1), ST-321 (*n* = 1), ST-326 (*n* = 16) and ST-327 (*n* = 5) [5].

2.2. Characterisation of the genetic environment of bla$_{CTX-M-15}$

The genetic environment surrounding *bla*$_{CTX-M-15}$ was investigated in the 37 *K. pneumoniae* isolates by polymerase chain reaction (PCR) mapping and direct sequencing of both strands of the amplicons. To detect nucleotide sequences previously reported to be associated with *bla*$_{CTX-M-15}$, specific primers for the insertion sequences IS*26* and IS*Ecp1* (located upstream of *bla*$_{CTX-M-15}$) and for the open reading frame *orf*477 (located downstream) were used in combination with *bla*$_{CTX-M-15}$-specific primers [1,2,4].

2.3. Determination of plasmid replicon type

Plasmid replicons were identified using the PCR-based replicon typing method as described previously [6], including screening for the IncR replicon that has been recently documented in *Salmonella* strains [7]. In addition, the *repA* gene associated with the replicon from the pKPN4 plasmid was amplified using primers RepFIIk-F (5'-GGTTAAAAACCCGAATCCGG-3') and RepFIIk-R (5'-CAGTCGCGTGTAGTTGTTCC-3'). All positive results were confirmed by sequencing both strands of the amplicons.

2.4. Identification and characterisation of the location of bla$_{CTX-M-15}$

Plasmid number and sizing was performed on all *K. pneumoniae* isolates as well as all associated transconjugants or transformants by PFGE of total DNA digested with *S*1-nuclease as previously described [8]. Restriction fragments from PFGE gels were then transferred onto positively charged nylon membranes and hybridised with specific probes for *bla*$_{CTX-M-15}$, ***aac(6')-Ib-cr*** and each previously identified replicon (i.e. FII, FIIk, R, N and L/M). A chromosomal location of *bla*$_{CTX-M-15}$ in the *K. pneumoniae* isolates was also screened for by the same method using PFGE and Southern hybridisations following I-*Ceu*I nuclease digestion as previously described [9]. Specific probes for the 16S rRNA gene, *bla*$_{SHV-1}$ and seven housekeeping genes (*infB*, *gapA*, *rpoB*, *mdh*, *phoE*, *tonB* and *pgi*) were used for chromosomal detection [10].

2.5. Plasmid mobilisation

Based on the *S*1-nuclease experiment results, several representative isolates from each clonal cluster were selected for conjugation assays. Conjugation was carried out at 37 °C and 30 °C using a rifampicin-resistant derivative of *E. coli* HB101 as recipient, selecting on Luria–Bertani (LB) agar plates supplemented with 100 μg/mL rifampicin and 10 μg/mL cefotaxime. For strains for which no transconjugant could be obtained, plasmids were extracted by the hot alkaline method [11] and were transformed by electroporation into *E. coli* HB101. Transformants were selected on LB agar plates containing 10 μg/mL cefotaxime.

3. Results and discussion

3.1. Genetic environment of bla$_{CTX-M-15}$

Four distinct genetic environments surrounding *bla*$_{CTX-M-15}$ were found among the studied isolates. In isolates belonging to ST-1, ST-326, ST-37 and ST-321, an entire insertion sequence IS*Ecp1* was found 48 bp upstream of *bla*$_{CTX-M-15}$, as already described in several reports [2]. In isolates belonging to ST-327, ST-14 and ST-147, IS*26* was found inserted at, respectively, 209, 497 and 594 nucleotides from the IS*Ecp1* terminus (submitted under GenBank accession nos. GQ845084, GQ845085 and GQ845086, respectively). Although the presence of IS*26* in the genetic environment surrounding *bla*$_{CTX-M-15}$ is well known [2,4], the particular insertion positions observed here have not been previously described, suggesting a different origin or a new reorganisation from those already described. In all isolates studied, open reading frame *orf*477 was detected downstream of *bla*$_{CTX-M-15}$, as previously described [1,12].

3.2. Localisation of bla$_{CTX-M-15}$

PCR-based replicon typing and sequencing showed that replicons IncR, IncFIIA and IncN were present in ST-1 isolates. IncR is a replicon that was recently documented in a *qnrS1*-carrying plasmid from *Salmonella enterica* isolates and corresponds to a replicon of the *K. pneumoniae* plasmid pK245*,* itself described as deriving from the β-replicon of the *K. pneumoniae* plasmid pGSH500 (96% similarity between the protein sequences of the replicases from pK245 and pGSH500) [7,13]. In this study, sequences of the IncR PCR products obtained from the present *Klebsiella* isolates showed 100% identity with the replicase from pK245. Moreover, sequences of the IncFIIA PCR products obtained from ST-1 isolates correspond to replicase RepA from the replicon IncFIIk, as previously

identified in *K. pneumoniae* plasmid pKPN4 (based on GenBank sequence NC_009650) (Table 1) [14].

Specific probe hybridisations of *S*1-digested DNA from ST-1 isolates demonstrated that isolates kp-57, kp-62 and kp-64 carried $bla_{\text{CTX-M-15}}$ and ***aac(6')-Ib-cr*** on plasmids that exhibited replicon IncR (pST1-2,3) (Table 1; Fig. 1). The IncFIIk replicon was identified by the same method in ST-1 isolate 19sp on a 190-kb $bla_{\text{CTX-M-15}}$-carrying plasmid (pST1-1) (Table 1; Fig. 1). No IncN plasmid was found in association with $bla_{\text{CTX-M-15}}$ (Table 1).

The remaining eight ST-1 isolates did not exhibit any plasmid carrying $bla_{\text{CTX-M-15}}$. However, following restriction of their total DNA with the I-*Ceu*I nuclease (i.e. chromosomal restriction), all ST-1 isolates, including the four carrying a plasmidic $bla_{\text{CTX-M-15}}$, specifically hybridised with the $bla_{\text{CTX-M-15}}$ probe on a very large (>2000 kb) I-*Ceu*I-digested fragment. Although this fragment yielded only weak hybridisation with the 16S rRNA probe, it hybridised strongly with the *gapA* (Fig. 2) and $bla_{\text{SHV-1}}$ probes (data not shown), thereby definitively confirming the chromosomal localisation of $bla_{\text{CTX-M-15}}$ in these isolates. Although $bla_{\text{SHV-1}}$ can be found not only in a chromosomal location but also in plasmids, this gene was used for the hybridisation experiment because within the chromosome of *K. pneumoniae* (based on GenBank sequence NC_009648), *gapA* and $bla_{\text{SHV-1}}$ are separated by only 437 kb.

In isolates with the chromosomal $bla_{\text{CTX-M-15}}$, the ***aac(6')-Ib-cr*** gene was found only on plasmids of molecular weight from 35 kb to 60 kb (paST1-1,2,3) (Table 1). These plasmids did not correspond with any of the incompatibility groups tested by replicon typing.

Regarding the remaining 25 *K. pneumoniae* isolates, PCR-based replicon typing and sequencing as well as Southern analysis of *S*1-digested DNA demonstrated that all 16 ST-326 isolates carried *bla*$_{\text{CTX-M-15}}$ plus ***aac(6')-Ib*** and/or ***aac(6')-Ib-cr*** (since these isolates carried both wild-type and bifunctional acetyltransferase-encoding genes [5]), co-localised on IncR plasmids of ca. 75, 85 and 90 kb (pST326-1,2,3) (Table 1). All five ST-327 isolates carried *bla*$_{\text{CTX-M-15}}$ on IncFII plasmids, which were of ca. 60 kb in four isolates (pST327-1) (Table 1; Fig. 1) and 30 kb in the fifth isolate (pST327-2) (Table 1). In the ST-37, ST-147, and ST-321 isolates, both *bla*$_{\text{CTX-M-15}}$ and ***aac(6')-Ib-cr*** were found on IncFII plasmids that ranged in size from 75 kb to 80 kb (pST37-1, pST147 and pST321) (Table 1; Fig. 1). Moreover, ST-37 isolate 23.1sp carried a second copy of these genes on a 290-kb plasmid that was positive for both the FII and FIIk replicons (pST37-2) (Table 1). Notably, this isolate contained a third plasmid of ca. 190 kb that was positive for the IncFIIk replicon (not shown in Table 1). These results are seemingly incompatible since in principle two plasmids belonging to the same Inc group cannot occur in the same host cell. Therefore, it is probable that another unknown replicon is present and expressed in at least one of these plasmids. Finally, isolate kp-71, representing ST-14, carried *bla*$_{\text{CTX-M-15}}$ and ***aac(6')-Ib*** and/or ***aac(6')-Ib-cr*** [5] on a 340-kb plasmid that was negative for all the incompatibility groups tested (pST14) (Table 1; Fig. 1).

Of note, neither IncR plasmids, previously reported in *S. enterica* and *K. pneumoniae*, or IncFIIk plasmids, previously reported only in *K. pneumoniae*, have been described in association with *bla*$_{\text{CTX-M-15}}$. Moreover, none of the *K. pneumoniae* isolates not belonging to ST-1 exhibited a chromosomal copy of *bla*$_{\text{CTX-M-15}}$ (Table 1).

In this study, the IncR and IncFIIk replicons occurred in the majority of isolates (i.e. IncR in 33 isolates and IncFIIk in 26 isolates) (Table 1), with or without being associated with *bla*$_{CTX-M-15}$ or other studied resistance genes. By contrast, the IncFII replicon occurred in only a few isolates (*n* = 8), always associated with *bla*$_{CTX-M-15}$. Although additional studies are needed to confirm these relationships, the present data would suggest that IncR and IncFIIk plasmids may benefit from good stability in *K. pneumoniae*, whereas IncFII plasmids perhaps are maintained in this host by means of selective advantage provided by *bla*$_{CTX-M-15}$. This is consistent with the observation that IncFIIk and IncR plasmids appear to be associated with strains with a high capacity to diffuse and to persist in time, whereas IncFII plasmids were found in strains that have disseminated less extensively [5]. Notably, the genetic environment of *bla*$_{CTX-M-15}$ in all the IncR and IncFIIk plasmids revealed an entire IS*Ecp1* upstream of the gene, which may facilitate gene mobilisation, whereas in some IncFII plasmids the IS*26* insertion within the *tnpA* of IS*Ecp1* may inhibit IS*Ecp1*-mediated mobilisation, trapping the gene in the plasmid, as previously described [15].

3.3. Transferability of bla$_{CTX-M-15}$

To determine whether plasmids carrying *bla*$_{CTX-M-15}$ were self-transferable, conjugation experiments were performed with several isolates (Table 1). Transconjugants were obtained from isolates kp-57 (ST-1), 19sp (ST-1), kp-8 (ST-326), kp-71 (ST-14), 23.1sp (ST-37), kp-28 (ST-147) and 25sp (ST-321). In contrast, *bla*$_{CTX-M-15}$-carrying plasmids could not be similarly self-transferred from isolates kp-62 (ST-1), kp-64 (ST-1), 8H (ST-326), kp-36 (ST-327) and kp-73 (ST-327). However, they could be mobilised by transformation. Isolate kp-30 (ST-1), for which only a chromosomal location of *bla*$_{CTX-M-15}$ was detected, yielded neither transconjugants nor transformants, as expected.

In transconjugants obtained from isolates kp-57, 19sp, kp-71, 23.1sp and 25sp, the plasmids carrying *bla*$_{CTX-M-15}$ were of the same size and carried the same replicon as the plasmids from the donor strains. However, in the remaining two transconjugants (i.e. TC$_{kp-8}$ and TC$_{kp-28}$), the plasmids carrying *bla*$_{CTX-M-15}$ were larger than the corresponding donor strain's *bla*$_{CTX-M-15}$-containing plasmids (Table 1; Fig. 1).

In the case of TC$_{kp-8}$, a 330-kb plasmid was detected that was positive for *bla*$_{CTX-M-15}$ and for both replicons IncR and IncFIIk, whereas in donor strain kp-8 (from ST-326), the *bla*$_{CTX-M-15}$-carrying plasmid had a size of 90 kb and was positive only for IncR. Interestingly, the donor strain carried an additional plasmid of 205 kb, positive for IncFIIk (data not shown). Therefore, we hypothesise that the plasmid detected in the transconjugant was the result of a co-integration event involving both parental plasmids, which carried IncR (90 kb) and IncFIIk (205 kb), respectively.

Furthermore, the deduced FIIk replicon amino acid sequences obtained for all ST-326 isolates (including kp-8), using primers RepFIIk, exhibited 97.6% identity (six amino acid changes) with the reference RepA sequence from pKPN4 as well as 97.6% identity with the replicase associated with the α-replicon carried by plasmid pGSH500, which in addition carried the first-described IncR replicon (based on GenBank sequence AJ009980.1) [15]. This FIIk variant sequence, designated FIIk-α, was also obtained from the TC$_{kp-8}$ *E. coli*, a result that may confirm the hypothesised co-integration event. Transformation experiments involving kp-8 yielded a transformant carrying only the 90-kb IncR plasmid (TF$_{kp-8}$) (Table 1; Fig. 1).

Regarding TC_{kp-28}, a similar recombination event may have occurred involving the IncFII *bla*$_{CTX-M-15}$-carrying plasmid (80 kb) and an IncN plasmid (30 kb) also present in the donor strain kp-28 (from ST-147) (not shown). In contrast, with isolate 23.1sp (from ST-37), although two *bla*$_{CTX-M-15}$-carrying plasmids were detected in the donor strain, in all the conjugation assays only the smaller of these, i.e. the 80-kb IncFII plasmid, was transferred to the recipient (Table 1; Fig. 1).

4. Conclusion

The present findings elucidate a series of genetic elements that likely contribute to the acquisition and maintenance of *bla*$_{CTX-M-15}$ in *K. pneumoniae*, a species of particular clinical significance because of its current association with major hospital outbreaks. In particular, we provide evidence of *bla*$_{CTX-M-15}$ chromosomal integration in *K. pneumoniae* and document the first reported association of *bla*$_{CTX-M-15}$ with the two recently described replicons IncR and IncFIIk. These findings reflect differences in the genetic vectors involved in the carriage of *bla*$_{CTX-M-15}$ in *K. pneumoniae* versus those described in *E. coli.* These differences may contribute to the diffusion of *bla*$_{CTX-M-15}$ within *K. pneumoniae*. Future epidemiological surveillance for *bla*$_{CTX-M-15}$ in *K. pneumoniae* should be carried out to test this hypothesis.

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Competing interests

None declared.

Ethical approval

Not required.

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Fig. 1. (a) Pulsed-field gel electrophoresis (PFGE) analysis of *S*1-digested DNA from transconjugants or transformants obtained from the studied CTX-M-15-producing *Klebsiella pneumoniae* isolates. (b) Southern hybridisation of *S*1-digested DNA with probe for *bla*$_{\text{CTX-M-15}}$. Similar Southern hybridisations using probes for ***aac(6)'-Ib*** and for each replicon were done with *S*1-digested fragments from all study *K. pneumoniae*. The corresponding results are detailed in Table 1. MK, phage λ DNA marker.

Fig. 2. (a) Pulsed-field gel electrophoresis (PFGE) analysis of I-*Ceu*I-digested genomic DNA from CTX-M-15-producing *Klebsiella pneumoniae* isolates representing sequence type ST-1 (kp-44 to kp-52), ST-327 (kp-66 and kp-36), ST-14 (kp-71) and ST-321 (25sp). (b–d) Southern hybridisation of I-*Ceu*I-digested DNA with probes for *bla*$_{\text{CTX-M-15}}$ (b), 16S rRNA (c) and *gapA* (d). Horizontal arrows to the left of blots indicate genomic bands hybridising with the *bla*$_{\text{CTX-M-15}}$ probe. The down-pointing arrow in blot (b) corresponds to the 340-kb *bla*$_{\text{CTX-M-15}}$-carrying plasmid of isolate kp-71. MK, phage λ DNA marker.

Table 1

Identification and characterisation of the location of *bla*$_{CTX-M-15}$ among 37 CTX-M-15-producing *Klebsiella pneumoniae* isolates

ST	Isolate	Year	Replicons detected [a]	*bla*$_{CTX-M-15}$						*aac-6'-Ib*		
				Location				TF/TC		Location [c]	Inc [d]	Plasmid size (kb)
				cr [b]	Plasmid [c]	Inc [d]	Plasmid size (kb)	Recipient/plasmid size (kb)	Inc [d]			
1	kp-57	2007	R	+	pST1-3	R	60	TC$_{kp-57}$/60	R	pST1-3	R	60
1	kp-62	2007	FIIk, R	+	pST1-2	R	55	TF$_{kp-62}$/55	R	pST1-2	R	55
1	kp-64	2007	FIIk, R	+	pST1-2	R	55	TF$_{kp-64}$/55	R	pST1-2	R	55
1	19sp	2005	FIIk	+	pST1-1	FIIk	190	TC$_{19sp}$/190	FIIk	neg.	–	–
1	11.1sp	2005	FIIk, R	+	neg.	–	–	N/D	N/D	paST1-1	neg.	<48
1	17sp	2005	FIIk, R	+	neg.	–	–	N/D	N/D	paST1-1	neg.	<48
1	21sp	2005	FIIk, R	+	neg.	–	–	N/D	N/D	paST1-1,2	neg.	48 and 35
1	kp-30	2007	FIIk, R	+	neg.	–	–	neg.	–	paST1-3	neg.	60
1	kp-44	2007	FIIk, R, N	+	neg.	–	–	N/D	N/D	neg.	–	–
1	15sp	2005	FIIk, R	+	neg.	–	–	N/D	N/D	paST1-1	neg.	48
1	kp-34	2007	R	+	neg.	–	–	N/D	N/D	paST1-1	neg.	48
1	kp-52	2007	R	+	neg.	–	–	N/D	N/D	neg.	–	–
326	1H–13H [e]	2008	FIIk-α [f], R	neg.	pST326-1	R	85	TF$_{8H}$/70	R	pST326	R	85

326	14H	2008	FIIk-α [f], R	neg.	pST326-2	R	75	N/D	N/D	pST326	R	75
326	kp-8	2008	FIIk-α [f], R	neg.	pST326-3	R	90	TF_{kp-8}/90	R	pST326	R	90
								TC_{kp-8}/330	R+FIIk-α [f]	pST326	R	90
326	kp-68	2007	FIIk-α [f], R	neg.	pST326	R	90	N/D	N/D	pST326	R	90
327	kp-36	2007	FII, R	neg.	pST327-1	FII	60	TF_{kp-36}/60	FII	neg.	–	–
327	kp-66	2007	FII, R	neg.	pST327-1	FII	60	N/D	N/D	neg.	–	–
327	kp-42	2007	FII, R	neg.	pST327-1	FII	60	N/D	N/D	neg.	–	–
327	kp-33	2007	FII, R	neg.	pST327-1	FII	60	N/D	N/D	neg.	–	–
327	kp-73	2008	FII, R	neg.	pST327-2	FII	30	TF_{kp-73}/30	FII	neg.	–	–
14	kp-71	2007	R	neg.	pST14	neg.	340	TC_{kp-71}/340	neg.	pST14	neg.	340
37	23.1sp	2005	FII, FIIk	neg.	pST37-1	FII	80	TC_{23sp}/80	FII	pST37-1	FII	80
					pST37-2	FII+FIIk	290	neg. [g]	–	pST37-2	FII+FIIk	290
147	kp-28	2007	FII, N	neg.	pST147	FII	80	TC_{kp-28}/125	FII+N	pST147	FII	80
321	25sp	2005	FII, FIIk	neg.	pST321	FII	75	TC_{25sp}/75	FII	pST321	FII	75

p(ST number), plasmid location; cr, chromosomal location, TF, transformant, TC, transconjugant; Inc, identified replicon; N/D, not done; neg., negative result; PCR, polymerase chain reaction.

[a] Replicon identifications are based on positive amplifications from the PCR-based replicon typing method and sequencing.

[b] Results from the screening of *bla*$_{\text{CTX-M-15}}$ chromosomal location: +, presence of *bla*$_{\text{CTX-M-15}}$ within the chromosome; neg., absence of *bla*$_{\text{CTX-M15}}$ chromosomal location.

[c] Plasmids were arbitrarily named based on the source strain sequence type and plasmid size.

[d] In all *K. pneumoniae* isolates and all transconjugants or transformants, replicons from plasmids containing *bla*$_{\text{CTX-M-15}}$ were identified by PCR-positive amplification and by Southern hybridisation of the corresponding *S*1-digested fragments, as described in Section 2.3.

[e] This group included 13 isolates (1H–13H).

[f] The deduced FIIk amino acid sequences obtained from all isolates belonging to ST-326 showed 97.6% similarity with RepA from pKPN4 and was referred to as FIIk-α, as explained in the Section 3.3.

[g] Conjugation and transformation assays allowed isolation of the 80-kb plasmid; the 290-kb plasmid could not be isolated.

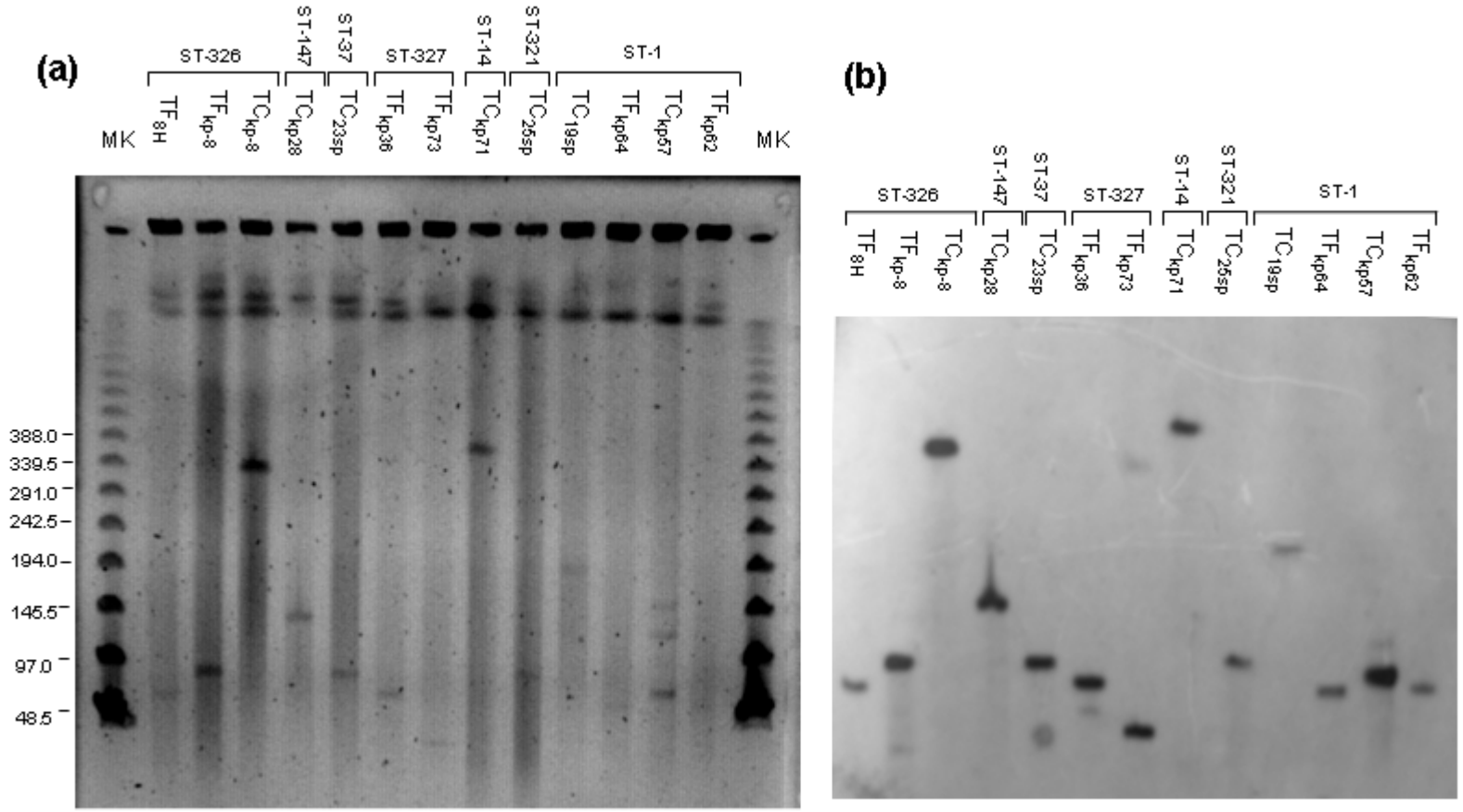

Figure 1: (a) PFGE analysis of *S*1-digested DNA from transconjugants or transformants obtained from the studied CTX-M-15-producing *Klebsiella pneumoniae* isolates. (b) Southern hybridization of *S*1-digested DNA with probe for $\underline{bla_{\text{CTX-M-15}}}$. Similar Southern hybridizations using probes for *aac(6)'-Ib*, and for each replicon, were done with *S*1-digested fragments from all study *K. pneumoniae*. The corresponding results are detailed in Table 1.

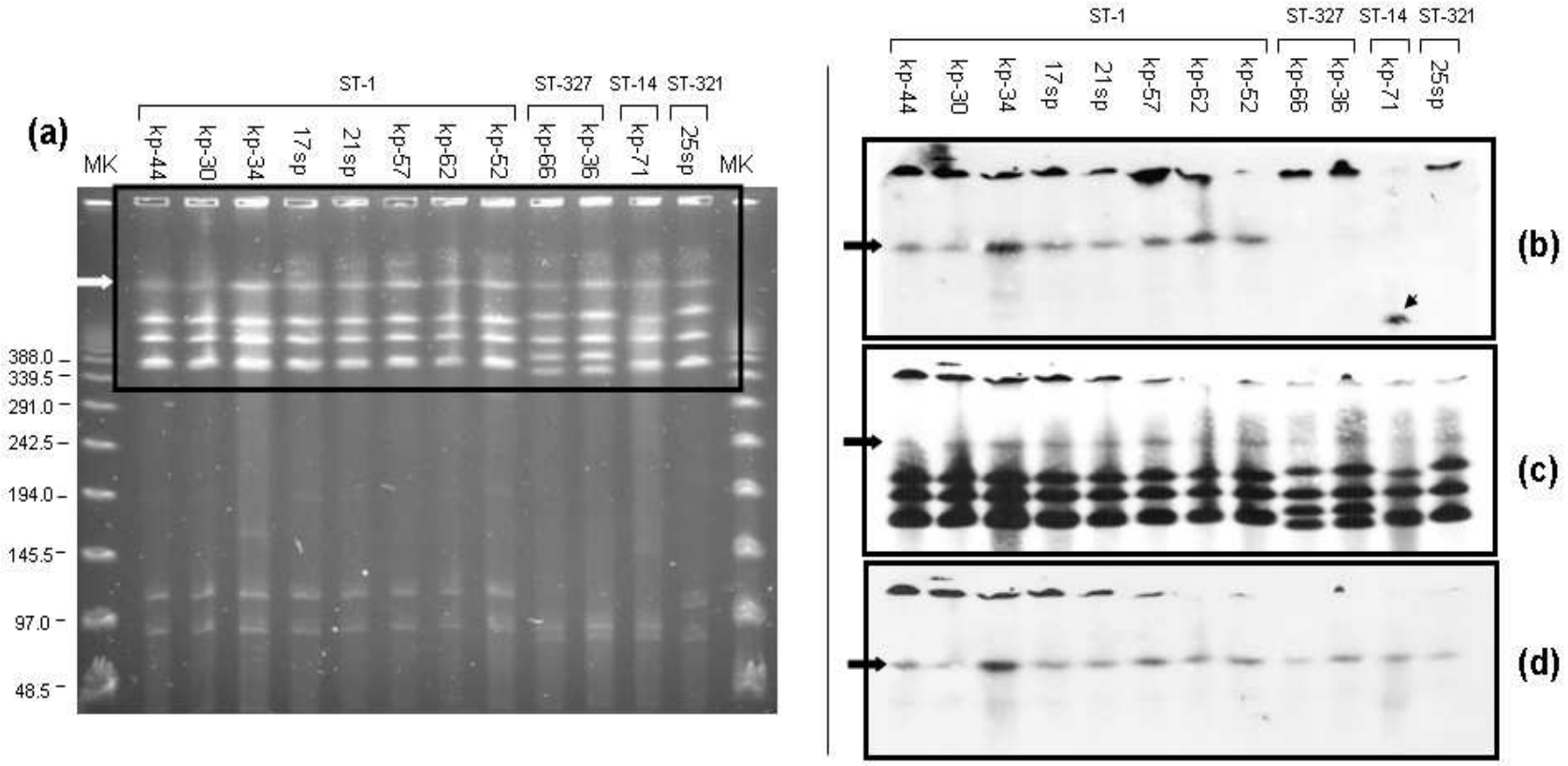

Figure 2: (a) PFGE analysis of I-*Ceu*I-digested genomic DNA from CTX-M-15-producing *Klebsiella pneumoniae* isolates representing ST-1 (kp-44 to kp-52), ST-327 (kp-66 and kp-36), ST-14 (kp-71), and ST-321 (25sp). (b-d) Southern hybridization of I-*Ceu*I-digested DNA with probes for (b) $\underline{bla}_{\text{CTX-M-15}}$, (c) 16S rRNA, and (d) *gapA*. Horizontal arrows at left of blots indicate genomic bands hybridizing with the $\underline{bla}_{\text{CTX-M-15}}$ probe. The down-pointing arrow in blot (b) corresponds with the 340-kb $\underline{bla}_{\text{CTX-M-15}}$-carrying plasmid of isolate kp-71.

Abbreviations: MK, phage λ DNA marker.