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Supplementary

Genomics and Virulence of *Klebsiella Pneumoniae* Kpnu95 ST1412 Harboring a Novel Incf Plasmid Encoding *Bla*CTX-M-15 and *Qnrs1* Causing Community Urinary Tract Infection

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1. Supplementary Materials

Table S1. Average generation times of KpnU95, the cured, and the plasmid reconstituted cured strain

***K. pneumoniae* isolate**	**Average generation time ± SD (min) in different media**		
	LB	BM2	Artificial urine
KpnU95 (Clinical)	42.21 ± 0.50	73.45 ± 2.01	95.37 ± 2.09
KpnU95ΔpKpnU95 (Cured)	41.82 ± 0.88	73.64 ± 2.22	103.85 ± 2.68*
KpnU95ΔpKpnU95/pKpnU95 (Cured reconstituted)	40.94 ± 1.69	73.44 ± 1.06	89.99 ± 7.50

* Significantly long generation time of KpnU95ΔpKpnU95 versus KpnU95 clinical strain (p value = 0.0361) using Student's t-test.

Table S2. Average generation times of KpnU95, the cured, and the plasmid reconstituted cured strain in the presence of copper

***K. pneumoniae* isolate**	**Average generation time ± SD (minutes) in different $CuSO_4$ concentration [mM], p value**			
	0	2	4	8
KpnU95 (Clinical)	51.93 ± 0.005	50.53 ± 0.48	54.56 ± 1.35	78.85 ± 3.87
KpnU95ΔpKpnU95 (Cured)	52.14 ± 4.12	57.41 ± 0.35**	63.33 ± 1.18*	86.29 ± 2.77
KpnU95ΔpKpnU95/pKpnU95 (Cured reconstituted)	51.93 ± 0.03	52.46 ± 0.18	62.59 ± 0.002*	76.25 ± 0.34

* $p \leq 0.05$ and ** $p \leq 0.005$ versus KpnU95 with Student's t-test.

Table S3. PCR primers to screen the presence of pKpnU95 accessory unique genes encoded on pU95.

Primer	**Sequence (5′ – 3′)**	**Target**	**Amplicon Size (bp)**	**Reference**
CTX-M1-F	AAAAATCACTGCGCCAGTTC	*bla*$_{\text{CTX-M-group1}}$	415	[1]
CTX-M1-R	AGCTTATTCATCGCCACGTT			
umuD_ pU95_F	ATGTTCTTAATTCCAATGGAAAATCC	*umuD*	423	This study
umuD_ pU95_R	TTACAGATTACCCCGGGCA			
IncF(K)_pU95_F	GATCATTCGCTCGATGTCTG	*IncFIB(K)*	571	This study
IncFIB(K)+partRep_pU95_R	CCCGCTGATGAGTTTGGGAT			
qnrS1_ pU95_F	ATGGAAACCTACAATCATACATAT CG	*qnrS1*	657	This study
qnrS1_ pU95_R	TTAGTCAGGATAAACAACAATACCCA			
HisP_pU95_F	ATGCGTGATTATGCTATTGAG	*hisP*	819	This study
HisP_pU95_R	TCATGCTGACTCCTTCAATGC			
chrA_pU95_F	ATGAACGATACTGCCAGGA	*chrA*	1206	This study
chrA_pU95_R	TCACAGTGCTAAACTCAACAAC			
pcoB_pU95_F	ATGCTGTTGAAAACGTCTCG	*pcoB*	1818	This study
pcoB_pU95_R	TCATTCCTCCACCCGGACTT			

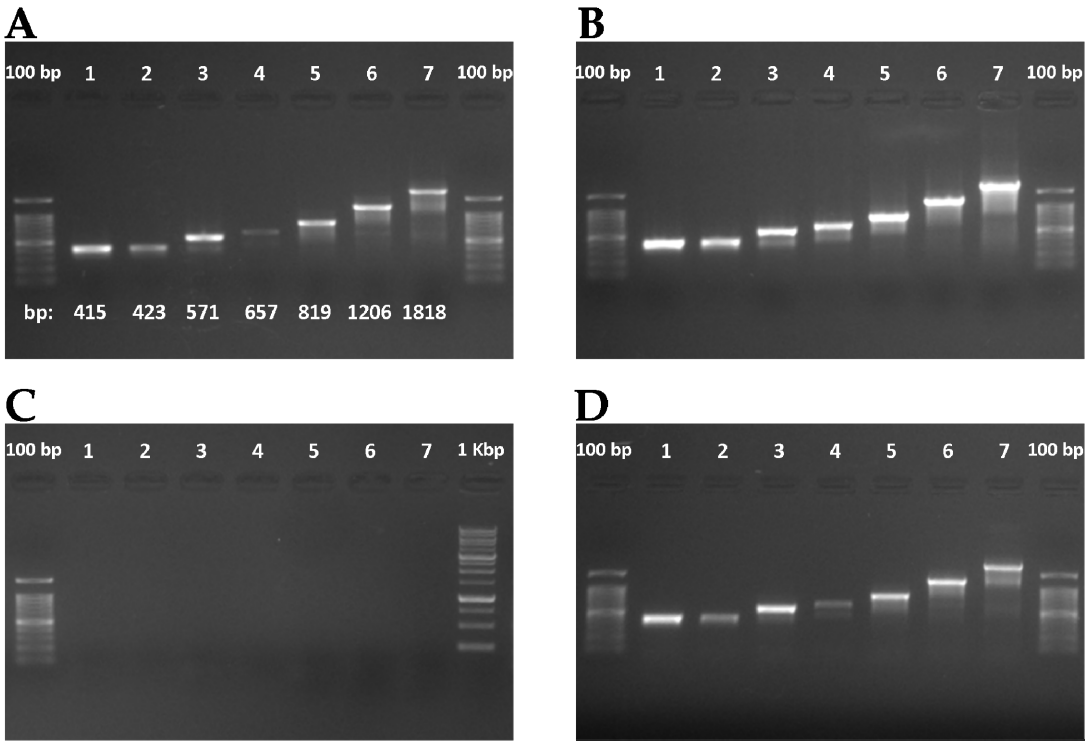

Figure S1. Targeted PCR screening for validating the presence/absence of pKpnU95 plasmid in the different studied strains. The PCR amplified seven genes encoded on pKpnU95. The expected amplicons are presented in the clinical KpnU95 strain (amplicon sizes appear in base pairs, bp) (**A**); PCR performed on the purified plasmid pKpnU95 (**B**); lysate of the cured strain KpnΔpKpnU95 (**C**), and from the reconstituted strain KpnU95-cured/pKpnU95 (**D**). The seven pKpnU95 amplified genes in panels **A-D** are: lane 1 - *bla*$_{\text{CTX-M-group1}}$; lane 2- *umuD*; lane 3 - IncFIB(K); lane 4 - *qnrS1*; lane 5 - *hisP*; lane 6 - *chrA*; lane 7 - *pcoB*. The DNA molecular weight markers are 1kb and 100bp ladders (GeneDirex). Electrophoresis was performed in 1% agarose.

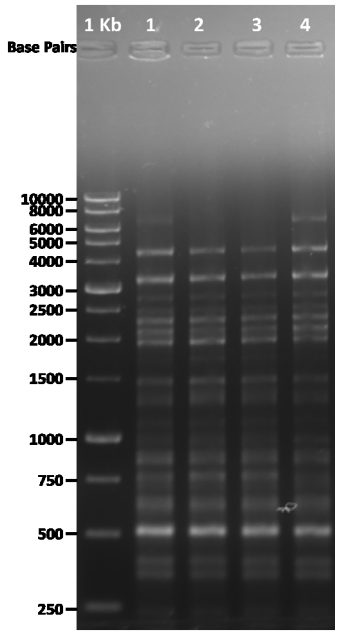

Figure 2. ERIC-PCR analysis of KpnU95 strains. ERIC-PCR pattern of crude DNA lysates of KpnU95, lane 1; KpnU95 after passages at 42ºC as a control, lane 2; KpnΔpKpnU95, lane 3, and KpnΔpKpnU95/pKpnU95, lane 4. The DNA molecular weight marker is a 1kb ladder (Bio-Labs). Electrophoresis was performed in 1% agarose gel.

References

1. Woodford, N.; Fagan, E.J.; Ellington, M.J. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. *J. Antimicrob. Chemother.* **2006**, *57*, 154–155.