SUPPLEMENTARY APPENDIX 1

This supplementary material is hosted by *Eurosurveillance* as supporting information alongside the article "Nosocomial outbreak by NDM carbapenemase-producing *Klebsiella pneumoniae* clone with inactivated *cirA* siderophore receptor gene, highly resistant to cefiderocol", on behalf of the authors, who remain responsible for the accuracy and appropriateness of the content. The same standards for ethics, copyright, attributions and permissions as for the article apply. Supplements are not edited by *Eurosurveillance* and the journal is not responsible for the maintenance of any links or email addresses provided therein.

Figure S1. In vitro activity of cefiderocol against NDM-producing Klebsiella pneumoniae clinical isolates, collected from Florence University Hospital in the period January 2021 (n=24) to June 2022 (n=28). When available, replicate isolates from the same patient always exhibited uniform FDC MICs (within \pm 1 dilution).

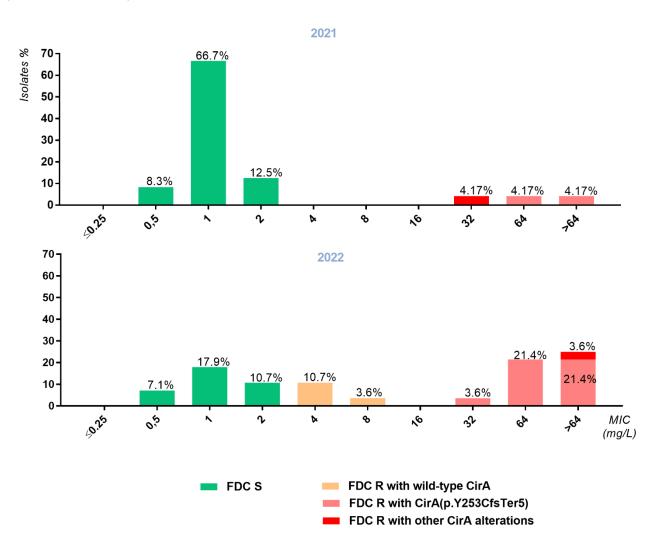


Figure S2. CirA aminoacidic sequences comparison of KP-1PI, as FDC susceptible reference strain, with the high-level FDC resistant isolates 20218 (p.G75W), 22651 (p.D426Ter) and 21074 (p.Y253CfsTer5), drew using *MView*.

					0.0
Cinh UT UD 1Di	cov		1	[80
CirA_WT_KP-1Pi CirA 20218		99.8%		MFRLNPFIRAGLSASVVSLAFPALADVNEETLVVTASATEQNVKDAPASISVITQQDLQKRPVQNLKDVPGVQLIN MFRLNPFIRAGLSASVVSLAFPALADVNEETLVVTASATEQNVKDAPASISVITQQDLQKKPVQNLKDVLKDVPWVQLTN	
		100.0%		MFRLNPFIRAGLSASVVSLAFPALADVNEETLVVTASATEONVKDAPASISVITOODLORKPVONLKDVLRDVPGVOLTN	
CirA 21074		94.4%		MFRLNPFIRAGLSASVVSLAFPALADVNEETLVVTASATEQNVKDAPASISVITQQDLQKRPVQNLKDVPGVQLIN MFRLNPFIRAGLSASVVSLAFPALADVNEETLVVTASATEQNVKDAPASISVITQQDLQKKPVQNLKDVLRDVPGVQLIN	
CIFA_21074	39.18	34.48		NITELNET IRAGEORGY VOLAF FALREVINEET EVV TASATEQUIVED FROTO TOTAL VOLAFO VENERAL VENERAL VIDEO VENERA VIDEO VENERA VIDEO VENERA VIDEO VENERAL VIDEO VENERAL	
	cov	pid	81		160
CirA WT KP-1Pi				EGDNRKGVSIRGLSSSYTLILVDGKRVNSRNAVFRHNDFDLNWIPVDAIERIEVVRGPMSSLYGSDALGGVVNIITKKIG	
THE RESERVE THE PARTY OF THE PA		99.8%		EGDNRKGVSIRGLSSSYTLILVDGKRVNSRNAVFRHNDFDLNWIPVDAIERIEVVRGPMSSLYGSDALGGVVNIITKKIG	
_		100.0%		EGDNRKGVSIRGLSSSYTLILVDGKRVNSRNAVFRHNDFDLNWIPVDAIERIEVVRGPMSSLYGSDALGGVVNIITKKIG	
CirA 21074		94.4%		EGDNRKGVSIRGLSSSYTLILVDGKRVNSRNAVFRHNDFDLNWIPVDAIERIEVVRGPMSSLYGSDALGGVVNIITKKIG	
7					
	cov	pid	161		240
CirA_WT_KP-1Pi	100.0%	100.0%		QKWTGTLSADTTIQEHRDRGDTYNGQFFTSGPLIDGVLGMKAYGSLAKRAKDDPQSSSNATGETPRIEGFTSRDGNVEFA	
CirA_20218	100.0%	99.8%		QKWTGTLSADTTIQEHRDRGDTYNGQFFTSGPLIDGVLGMKAYGSLAKRAKDDPQSSSNATGETPRIEGFTSRDGNVEFA	
CirA_22651	64.8%	100.0%		QKWTGTLSADTTIQEHRDRGDTYNGQFFTSGPLIDGVLGMKAYGSLAKRAKDDPQSSSNATGETPRIEGFTSRDGNVEFA	
CirA_21074	39.1%	94.4%		$\tt QKWTGTLSADTTIQEHRDRGDTYNGQFFTSGPLIDGVLGMKAYGSLAKRAKDDPQSSSNATGETPRIEGFTSRDGNVEFA$	
	cov		241		320
CirA_WT_KP-1Pi				WTPNENHDFTAGYGFDRQDRDSDSLDRNRLERENYSLSHNGRWDIGNSELKFYGEKVDNKNPGQSGTITSESNAIDGKYV	
		99.8%		WTPNENHDFTAGYGFDRQDRDSDSLDRNRLERENYSLSHNGRWDIGNSELKFYGEKVDNKNPGQSGTITSESNAIDGKYV	
-		100.0%		${\tt WTPNENHDFTAGYGFDRQDRDSDSLDRNRLERENYSLSHNGRWDIGNSELKFYGEKVDNKNPGQSGTITSESNAIDGKYV}$	
CirA_21074	39.1%	94.4%		WTPNENHDFTAGCRLRL-	
	cov		321		400
CirA_WT_KP-1Pi				LPLGMINQLVTFGGEWRHDKLKDPVNLSSGGQSTSASQYALFIEDEWRIIEPLALTTGIRMDDHQTYGDHWSPRAYLVYN	
		99.8%		LPLGMINQLVTFGGEWRHDKLKDPVNLSSGGQSTSASQYALFIEDEWRIIEPLALTTGIRMDDHQTYGDHWSPRAYLVYN	
CirA_22651 CirA_21074		100.0%		LPLGMINQLVTFGGEWRHDKLKDPVNLSSGGQSTSASQYALFIEDEWRIIEPLALTTGIRMDDHQTYGDHWSPRAYLVYN	
CIFA_210/4	39.1%	34.48			
	cov	nid	401		480
CirA_WT_KP-1Pi		The second second		ATDTVTVKGGWATAFKAPSLLQLNPDWTTNSCRGSCSIVGNPDLKPETSESFELGLYYRGEEGWLENVEGSITTFQNNVD	-
		99.8%		ATDTVTVKGGWATAFKAPSLLOLNPDWTTNSCRGSCSIVGNPDLKPETSESFELGLYYRGEEGWLENVEGSITTFONNVD	
-		100.0%		ATDTVTVKGGWATAFKAPSLLQLNPD	
CirA 21074		94.4%			
	COV	pid	481		560
CirA_WT_KP-1Pi	100.0%	100.0%		DMIDVLRISSASEAPGYPNFVGWKTVNGKRVPIFRYFNVNKARIKGVETEVKIPFGDEWKLTVNYTYNDGRDLSNGGDKP	
CirA_20218	100.0%	99.8%		DMIDVLRISSASEAPGYPNFVGWKTVNGKRVPIFRYFNVNKARIKGVETEVKIPFGDEWKLTVNYTYNDGRDLSNGGDKP	
CirA_22651	64.8%	100.0%			
CirA_21074	39.1%	94.4%			
			10000		
	COV	•	561		640
CirA_WT_KP-1Pi				LQTLPFHTANGTLDWKPLDDWSFYVTANYTGQQRAVSATGKTPGGYTLFDVGAAWQVTKNVKLRSGVQNVGDKDLSRDDY	
		99.8%		LQTLPFHTANGTLDWKPLDDWSFYVTANYTGQQRAVSATGKTPGGYTLFDVGAAWQVTKNVKLRSGVQNVGDKDLSRDDY	
		100.0%			
CirA_21074	39.1%	94.4%			
	cov	nid	641	. 1 657	
CirA WT KP-1Pi				SYTEEGRRYFMAVDYRF	
		99.8%		SYTEEGRRYFMAVDYRF	
		100.0%			
		94.4%			
	05.18	24.45		4.000 (0.000)	

Table S3. Primers, probes and reaction conditions for the molecular detection of the CirA_{Y253CfsTer5} mutants.

Oligonucleotide name	Sequence (5'-3')	Final concentration in reaction mix (nM)
cirA-rt-fwd	AAGGACGATCCGCAGTCATC	500
cirA-rt-rev	GGCTCAGAGAGTAGTTCTCC	500
cirA-AOUC-rt-p	FAM-GCAGGCTGCAGGCTACGG-MGBNFQ	100

The reaction volume is $16~\mu$ L, including $3~\mu$ L of DNA (obtained with thermal lysis of a water bacterial suspension) approaches. The amplification program consisted of 35 two-step cycles of 15s at 95°C and 60s at 66 °C.

Figure S3. PCR amplification curve for the molecular detection of the CirA_{Y253CfsTer5} mutants. A sample positive for the c.761_767 duplication in *cirA* gene is characterized by a higher intensity of fluorescent emission than samples with the wild type *cirA*, which are revealed with lower efficiency by probes.

