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 ± 1 dilution).

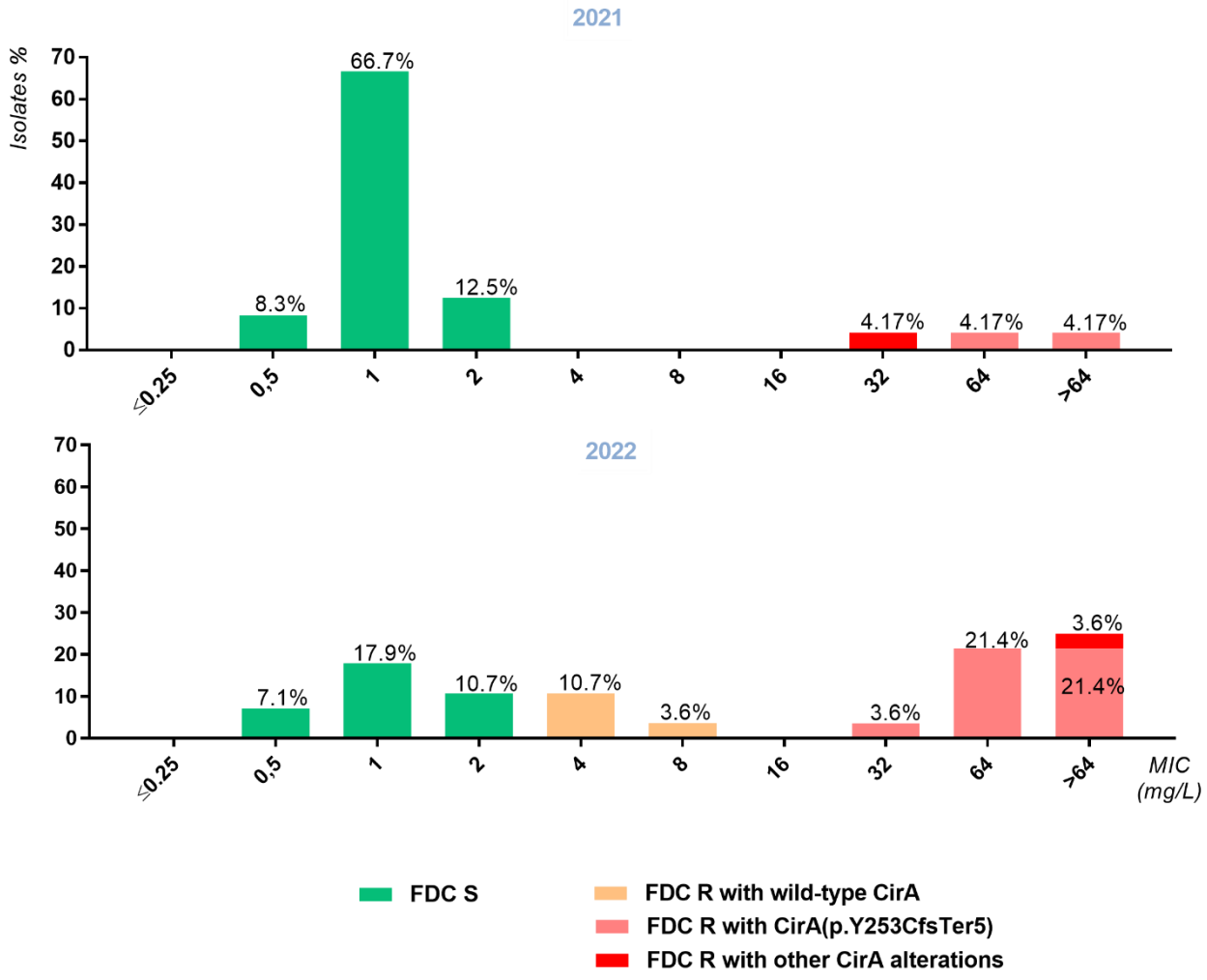

Figure S2. CirA aminoacidic sequences comparison of KP-1PI, as FDC susceptible reference strain, with the high-level FDC resistant isolates 20218 (p.G75W), 22651 (p.D426Ter) and 21074 (p.Y253CfsTer5), drew using *MView*.

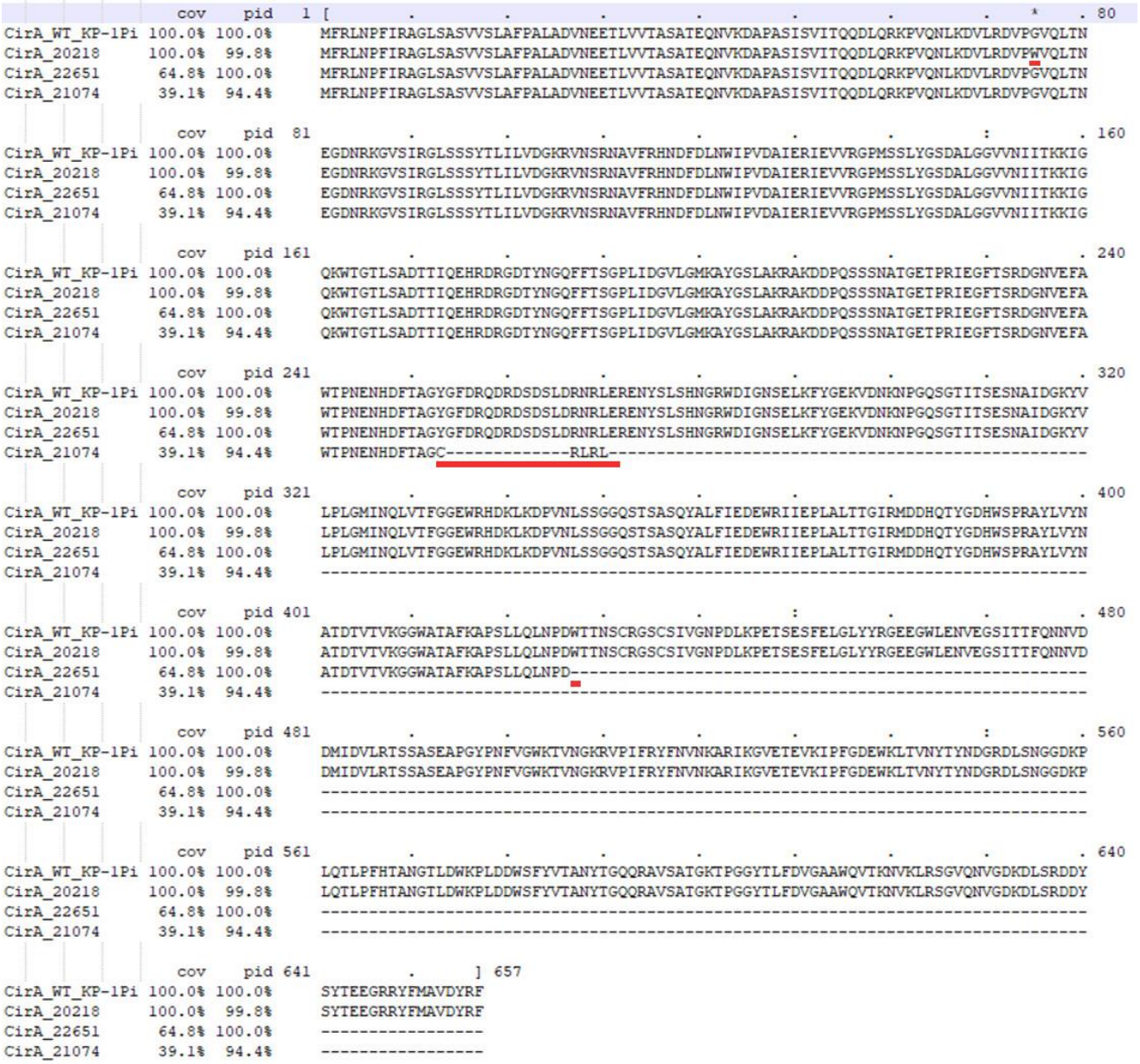

Table S3. Primers, probes and reaction conditions for the molecular detection of the CirA$_{Y253CfsTer5}$ mutants.

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 µL, including 3 µL 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.