

Additional File 1 to:

Wastewater-based surveillance in Italy leading to the first detection of *mcr-10*-positive *Klebsiella quasipneumoniae*

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Supplementary Table 1. Primers and multiplex PCR protocol for the detection of mobile colistin resistance (*mcr*) genes from 1 to 10 in bacteria isolated from wastewater samples.

Target	Primer sequence (5'-3')	PCR mix		Thermal profile			
<i>mcr-1</i>	F: AGTCCGTTTGTCTTGTGGC R: AGATCCTTGGTCTCGGCTTG	DNA	2 µL	95°C	5 min	x1	
<i>mcr-2</i>	F: CAAGTGTGTTGGTCGCAGTT R: TCTAGCCCGACAAGCATACC						
<i>mcr-3</i>	F: AAATAAAAATTGTTCCGCTTATG R: AATGGAGATCCCCGTTTTT	Dnase-free water	6.75 µL	95°C	30 s	x30	
<i>mcr-4</i>	F: TCACTTTCATCACTGCGTTG R: TTGGTCCATGACTACCAATG						
<i>mcr-5</i>	F: ATGCGGTTGTCTGCATTTATC R: TCATTGTGGTTGTCCTTTTCTG	QIAGEN Multiplex PCR Plus Kit Master Mix (2x)	12.5 µL	60°C	90 s		
<i>mcr-6</i>	F: AGCTATGTCAATCCCGTGAT R: ATTGGCTAGGTTGTCAATC						
<i>mcr-7</i>	F: GCCCTTCTTTTCGTTGTT R: GGTTGGTCTCTTTCTCGT	Primers premix (10 µM)	1.25 µL	72°C	60 s		
<i>mcr-8</i>	F: TCAACAATTCTACAAAGCGTG R: AATGCTGCGGAATGAAG						
<i>mcr-9</i>	F: TTCCTTTGTTCTGGTTG R: GCAGGTAATAAGTCGGTC	Coral Dye	2.5 µL	72°C	10 min		x1
<i>mcr-10</i>	F: TGTTTGATCAGTCCATGATC R: CGTGCCGTTTCGCCAATTA						

Supplementary Table 2. Allele profile of R55, R58 and R73 strains of *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* isolated from wastewater samples.

Gene	<i>gapA</i>	<i>infB</i>	<i>mdh</i>	<i>pgi</i>	<i>phoE</i>	<i>rpoB</i>	<i>tonB</i>
Allele	18	15	novel	16	11	13	51