

# Detection of *mcr-1* gene in undefined *Vibrio* species isolated from clams

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## Supplementary Materials

**Table S1.** The five house-keeping genes (*recA*, *rpoB*, *groEl*, *16S* and *dnaJ*) and respective primers that were used to identify the isolates. The primers that were used to test antimicrobial resistances genes presence belongs to three classes. The first class of antibiotics searched was polymyxins (*mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, *mcr-8* and *mcr-9*). The second class was  $\beta$ -lactams (*bla<sub>IMP</sub>*, *bla<sub>VIM</sub>*, *bla<sub>KPC</sub>*, *bla<sub>NDM</sub>*, *bla<sub>OXA</sub>*, *bla<sub>CTX-M</sub>*, *bla<sub>SHV</sub>* and *bla<sub>TEM</sub>*). The third antimicrobial class searched was quinolone (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qepA*). The virulence genes searched in the three isolates colistin resistant were *chiA*, *vhpA*, *luxR*, *flaC*, *hlyA*, *tlh*, *tdh*, *trh*, *ctxA*, *ompU*, *zot*, *tcpI*, *tcpA*, *sno/sto*, *vvh*, *vpi*, *yrpI*, *ompK* and *vhh*.

Genes	FW	REV	TA (°C)	Reference
<i>recA</i>	5'-GTCTACCAATGGGTCGTATC-3'	5'-GCCATTGTAGCTGTACCAAG-3'	60	[95]
<i>rpoB</i>	5'-GAGTCTTCGAAGTTGTAACC-3'	5'-GGCGAAATGGCWGAGAACCA-3'	51.5	[96]
<i>groEl</i>	5'-TCCARAACATGGGGCGCACAA-3'	5'-ACGTTTGTGCTTCTCGTGTGTCRC-3'	68	[97]
<i>16S rRNA</i>	5'-AGAGTTTGATCCTGGCTCAG-3'	5'-GGTACCTTGTACGACTT-3'	56	[98]
<i>dnaJ</i>	5'-GATYTRCGHTAYAACATGGA-3'	5'-TTCACRCCRTYDAAGAARC-3'	50	[99]
<i>mcr-1</i>	5'-AGTCCGTTTGTCTTGTGGC-3'	5'-AGATCCCTTGGTCTCGGCTTG-3'	60	[100]
<i>mcr-2</i>	5'-CAAGTGTGTTGGTCGCAGTT-3'	5'-TCTAGCCCGACAAGCATACC-3'	58	[100]
<i>mcr-3</i>	5'-AAATAAAAATTGTTCCGCTTATG-3'	5'-AATGGAGATCCCCGTTTTT-3'	58	[100]
<i>mcr-4</i>	5'-TCACTTTCATCACTGCGTTG-3'	5'-TTGGTCCATGACTACCAATG-3'	58	[100]
<i>mcr-5</i>	5'-ATGCGGTGTCTGCATTATC-3'	5'-TCATGTGGTTGTCTTTTCTG-3'	58	[100]
<i>mcr-6</i>	5'-GTCCGGTCAATCCCTATCTGT-3'	5'-ATCACGGGATTGACATAGCTAC-3'	58	[100]
<i>mcr-7</i>	5'-TGCTCAAGCCCTTCTTTTCGT-3'	5'-TTCATCTGCGCCACCTCGT-3'	55	[101]
<i>mcr-8</i>	5'-AACCGCCAGAGCACAGAATT-3'	5'-TTCCCCAGCGATTCTCCAT-3'	60	[101]
<i>mcr-9</i>	5'-CGGTACCGCTACCGCAATAT-3'	5'-ATAACAGCGAGACACCGGTT-3'	58	[101]
<i>bla<sub>IMP</sub></i>	5'-GGAATAGAGTGGCTTAAYTCTC-3'	5'-GGTTTAAAYAAAACAACCACC-3'	50	[102]
<i>bla<sub>VIM</sub></i>	5'-GATGGTGTGTCGCATA-3'	5'-CGAATGCGCAGCACCAG-3'	55	[102]

<i>bla<sub>KPC</sub></i>	5'-CGTCTAGTCTCTGCTGTCTTG-3'	5'-CTTGTCATCCTTGTTAGGCG-3'	52	[102]
<i>bla<sub>NDM</sub></i>	5'-GGTTTGGCGATCTGGTTTC-3'	5'-CGGAATGGCTCATCACGATC-3'	52	[102]
<i>bla<sub>oxa</sub></i>	5'-GCGTGGTTAAGGATGAACAC-3'	5'-CATCAAGTTCAACCCAACCG-3'	52	[103]
<i>bla<sub>CTX-M</sub></i>	5'-ATGTGCAGYACCACTAARGTKATGGC-3'	5'-TGGGTRAARTARGTSAC- CAGAAYCAGCGG-3'	60	[102]
<i>bla<sub>SHV</sub></i>	5'-ATGCGTTATATTCGCCTGTG-3'	5'-TGCTTTGTTATTCGGGCCAA-3'	55	[102]
<i>bla<sub>TEM</sub></i>	5'-TCGCCGCATACACTATTCTCAGAATGA- 3'	5'- ACGCTCACCGGCTCCAGATTAT-3'	50	[102]
<i>qnrA</i>	5'-AGAGGATTTCTCACGCCAGG-3'	5'-TGCCAGGCACAGATCTTGAC-3'	54	[104]
<i>qnrB</i>	5'-GGMATHGAAATTCGCCACTG-3'	5'-TTTGCYGYCGCCAGTCGAA-3'	54	[104]
<i>qnrC</i>	5'-GGGTGTACATTTATTGAATC-3'	5'-TCCACTTACGAGGTTCT-3'	50	[105]
<i>qnrD</i>	5'-CGAGATCAATTTACGGGGAATA-3'	5'-AACAAAGCTGAAGCGCCTG-3'	50	[105]
<i>qnrS</i>	5'-GCAAGTTCATTGAACAGGGT-3'	5'-TCTAAACCGTCGAGTTCGGCG-3'	54	[104]
<i>qepA</i>	5'-AACTGCTTGAGCCCGTAGAT-3'	5'-GTCTACGCCATGGACCTCAC-3'	50	[106]
<i>chiA</i>	5'-GGAAGATGGCGTGATTGACT-3'	5'-GGCATCAATTTCCCAAGAGA-3'	50	[107]
<i>vhpA</i>	5'-CTGAACGACGCCCATTTT-3'	5'-CGCTGACACATCAAGGCTAA-3'	50	[107]
<i>luxR</i>	5'-ATGGACTCAATTGCAAAGAG-3'	5'-TTAGTGATGTTACGGTTGT-3'	50	[107]
<i>flaC</i>	5'-AAATCATTCCAAATCGGTGC-3'	5'-TCTTTGATTCGGCTCTTA-3'	58	[108]
<i>hlyA</i>	5'-GGCAAACAGCGAAACAAATACC-3'	5'-CTCAGCGGGCTAATACGGTTTA-3'	60	[109]
<i>tlh</i>	5'-AAAGCGGATTATGCAGAAGCACTG-3'	5'-GCTACTTTCTAGCATTCTCTGC-3'	54	[110]
<i>tdh</i>	5'-GTAAAGGTCTCTGACTTTTGAC-3'	5'-TGGAATAGAACCTTCATCTTACC-3'	58	[110]
<i>trh</i>	5'-TTGGCTTCGATATTTTCACTATCT-3'	5'-CATAACAAACATATGCCCATTTCCG-3'	58	[110]
<i>ctxA</i>	5'-CGGGCAGATTCTAGACCTCCTG-3'	5'-CGATGATCTTGGAGCATTCCCAC-3'	62	[111]
<i>ompU</i>	5'-ACGCTGACGGAATCAACCAAAG-3'	5'-GCGGAAGTTGGCTTGAAGTAG-3'	61	[111]
<i>zot</i>	5'-TCGCTTAACGATGGC GCGTTTT-3'	5'-AACCCCGTTTCACTTCTA CCA-3'	61	[112]
<i>tcpI</i>	5'-TAGCCTTAGTTCTCA GCAGGCA-3'	5'-GGCAATAGTGTGAGCTC GTTA-3'	60	[112]
<i>tcpA</i>	5'-CCTTCGATCCCCTAA GCAATAC-3'	5'-AGGGTTAGCAACGATGCG TAAG-3'	60	[112]
<i>sno/sto</i>	5'-TCGCATTTAGCCAAA CAGTAGAAA-3'	5'-GCTGGATTGCAACATATT TCGC-3'	55	[112]
<i>vvh</i>	5'-GCTATTTACCGCCGCTCAC-3'	5'-CCGCAGAGCCGTAAACCGAA-3'	55	[113]
<i>vpi</i>	5'-GCAATTTAGGGGCGCGACGT-3'	5'-CCGCTCTTCTTGATCTGGTAG-3'	52	[114]
<i>yrpl</i>	5'-TATTCAACTGAAAGTGTA-3'	5'-ATAGCTCATAATACTGA-3'	60	[115]
<i>ompK</i>	5'-GGCGGTCGCTCTGGTATT-3'	5'-TTGCCATCGTAAGTGCTGTA-3'	55	[116]
<i>vhh</i>	5'-ATCATGAATAAACTATTACGTTACT-3'	5'-GAAAGGATGGTTTGACAAT-3'	55	[117]
<i>toxR</i>	5'-GATTAGGAAGCAACGAAAG-3'	5'-GCAATCACTTCCACTGGTAAC-3'	54	[118]

**Table S2.** Accession numbers from NCBI of the species used to performed MLSA on MEGA X software.

Species strain	Accession number
<i>Vibrio alginolyticus</i> VIO5	AP022861.1
<i>Vibrio anguillarum</i> NB10	LK021130.1
<i>Vibrio atlanticus</i> NC_011753	NC_011753.2
<i>Vibrio barjaei</i> 3062	NR_152641.1
<i>Vibrio bathopelagicus</i> Sal10	NZ_CP062500.1
<i>Vibrio campbellii</i> LA16-V1	NZ_CP021145.1
<i>Vibrio comitans</i> NBRC 102076	NZ_BJLH01000038.1
<i>Vibrio coralliilyticus</i> RE98	CP009617.1
<i>Vibrio crassostreae</i> 00-69-1	NZ_VTXY01000034.1
<i>Vibrio cholerae</i> PS4	NZ_CP077197.1
<i>Vibrio cyclitrophicus</i> ECSMB14105	NZ_CP039700.1
<i>Vibrio diabolicus</i> FDAARGOS_105	NZ_CP014036.1
<i>Vibrio fluvialis</i> A8	NZ_CP053664.1
<i>Vibrio galathea</i> S2757	JXXV01000001.1
<i>Vibrio gazogenes</i> ATCC 43942	NZ_CP018835.1
<i>Vibrio hangzhouensis</i> CGMCC 1.7062	NZ_FNVG01000060.1
<i>Vibrio harveyi</i> ATCC 33843 (392 [MAV])	NZ_CP009467.1
<i>Vibrio ichthyenteri</i> ATCC 700023	AFWF01000003.1
<i>Vibrio jasicida</i> TCFB 0772	NZ_BAOG01000278.1
<i>Vibrio mediterranei</i> QT6D1	NZ_CP018308.1
<i>Vibrio mediterranei</i> 117-T6	NZ_CP033579.1
<i>Vibrio mediterranei</i> Vic-OC-097	NZ_JABXGK01000008.1
<i>Vibrio mediterranei</i> V7A	NZ_PYVE01000089.1
<i>Vibrio navarrensis</i> 20-VB00237	CP065217.1
<i>Vibrio nigripulchritudo</i> SFn1	FO203527.1
<i>Vibrio owensii</i> 20160513VC2W	CP030798.1
<i>Vibrio parahaemolyticus</i> MAVP-R	CP022552.2
<i>Vibrio plantisponsor</i> LMG 24470	NZ_JABEQC00000000.1
<i>Vibrio scopthalmi</i> VS-05	NZ_CP016414.1
<i>Vibrio splendidus</i> BST398	NZ_CP031055.1
<i>Vibrio thalassae</i> CECT 8203	NR_134228.1
<i>Vibrio tubiashii</i> ATCC 19109	NZ_CP009354.1
<i>Vibrio variabilis</i> T01	KP329555.1
<i>Vibrio vulnificus</i> CMCP6	NC_004459.3
<i>Vibrio xuii</i> DSM 17185	MH315871.1
<i>Grimontia hollisae</i> FDAARGOS_111	NZ_CP014056.2
<i>Photobacterium damsela</i> 9046-81	NZ_CP046752.1

**Table S3.** Antimicrobial susceptibility test using colistin, imipenem, cefotaxime, meropenem and ciprofloxacin disks. The halo measurements displayed in mm.

Isolate	Colistin (mm)	Imipenem (mm)	Cefotaxime (mm)	Meropenem (mm)	Ciprofloxacin (mm)
NB04V	13	32	37	30	27
NB08V	20	35	37	39	32
NB09V	22	25	32	32	34
NB10V	35	40	37	36	35
NB11V	22	32	35	36	26
NB12V	25	33	30	35	35
NB13V	40	39	40	37	36
NB14V	39	40	36	38	36
NB15V	18	30	36	34	27
NB16B	22	30	36	33	28
NB17V	22	31	35	34	29
NB18V	22	32	37	35	27
NB19V	13	30	34	32	28
NB20V	14	34	37	40	29
NB21V	20	34	40	36	30
NJ01V	19	30	36	33	28
NJ02V	18	34	38	32	30
NJ04V	19	35	40	33	29
NJ06V	13	32	37	32	26
NJ07V	15	27	35	32	27
NJ10V	40	37	36	40	34
NJ12V	20	34	40	35	30
NJ13V	20	34	34	35	28
NJ14V	22	30	36	35	35
NJ15V	20	30	34	36	27
NJ18V	10	25	30	36	29
NJ21V	0	35	35	32	35
NJ22V	0	34	37	33	32
NB01E	14	35	25	30	40
NB02E	13	32	17	26	30
NB03E	12	32	35	35	34
NB04E	12	30	32	33	33
NB05E	12	29	33	33	37
NJ01E	14	32	20	22	32
NJ02E	12	29	32	32	35
NJ03E	13	32	35	35	33
NJ04E	12	30	33	30	32
NJ05E	13	29	32	32	30
NJ08E	13	30	32	33	27
NJ09E	12	27	32	33	33
NJ10E	13	30	36	36	35
NJ11E	15	32	20	27	35
NJ12E	13	31	33	34	30
EB01V	20	34	36	36	32
EB02V	15	34	40	36	27
EB03V	15	35	40	32	28
EB04V	15	34	38	35	26
EB05V	16	35	32	30	37
EB06V	21	30	34	32	36
EB07V	0	28	34	30	36
EB08V	16	28	32	34	25
EB09V	20	40	36	36	35

<b>EB10V</b>	16	32	35	33	24
<b>EB11V</b>	26	38	40	37	32
<b>EB12V</b>	16	33	35	35	29
<b>EB13V</b>	20	39	40	40	32
<b>EB14V</b>	15	35	40	38	25
<b>EB15V</b>	15	30	36	35	27
<b>EB01E</b>	10	28	33	35	36
<b>EB02E</b>	12	35	32	37	37

**Table S4.** Antimicrobial resistances genes search on all the isolates. The first class of antibiotics searched was polymyxin (*mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, *mcr-8* and *mcr-9*). – indicates non detected gene and + indicates detected gene.

[illegible]

NJ12E	-	-	-	-	-	-	-	-	-
EB01V	-	-	-	-	-	-	-	-	-
EB02V	-	-	-	-	-	-	-	-	-
EB03V	-	-	-	-	-	-	-	-	-
EB04V	-	-	-	-	-	-	-	-	-
EB05V	-	-	-	-	-	-	-	-	-
EB06V	-	-	-	-	-	-	-	-	-
EB07V	+	-	-	-	-	-	-	-	-
EB08V	-	-	-	-	-	-	-	-	-
EB09V	-	-	-	-	-	-	-	-	-
EB10V	-	-	-	-	-	-	-	-	-
EB11V	-	-	-	-	-	-	-	-	-
EB12V	-	-	-	-	-	-	-	-	-
EB13V	-	-	-	-	-	-	-	-	-
EB14V	-	-	-	-	-	-	-	-	-
EB15V	-	-	-	-	-	-	-	-	-
EB01E	-	-	-	-	-	-	-	-	-
EB02E	-	-	-	-	-	-	-	-	-

**Table S5.** Antimicrobial resistances genes search on all the isolates. The second class was  $\beta$ -lactams (*bla<sub>IMP</sub>*, *bla<sub>VIM</sub>*, *bla<sub>KPC</sub>*, *bla<sub>NDM</sub>*, *bla<sub>OXa</sub>*, *bla<sub>CTX-M</sub>*, *bla<sub>SHV</sub>* and *bla<sub>TEM</sub>*). – indicates non detected gene and + indicates detected gene.

Isolate	<i>bla<sub>IMP</sub></i>	<i>bla<sub>VIM</sub></i>	<i>bla<sub>KPC</sub></i>	<i>bla<sub>NDM</sub></i>	<i>bla<sub>OXa</sub></i>	<i>bla<sub>CTX-M</sub></i>	<i>bla<sub>SHV</sub></i>	<i>bla<sub>TEM</sub></i>
NB04V	-	-	-	-	-	-	-	-
NB08V	-	-	-	-	-	-	-	-
NB09V	-	-	-	-	-	-	-	-
NB10V	-	-	-	-	-	-	-	-
NB11V	-	-	-	-	-	-	-	-
NB12V	-	-	-	-	-	-	-	-
NB13V	-	-	-	-	-	-	-	-
NB14V	-	-	-	-	-	-	-	-
NB15V	-	-	-	-	-	-	-	-
NB16B	-	-	-	-	-	-	-	-
NB17V	-	-	-	-	-	-	-	-
NB18V	-	-	-	-	-	-	-	-
NB19V	-	-	-	-	-	-	-	-
NB20V	-	-	-	-	-	-	-	-
NB21V	-	-	-	-	-	-	-	-
NJ01V	-	-	-	-	-	-	-	-
NJ02V	-	-	-	-	-	-	-	-
NJ04V	-	-	-	-	-	-	-	-
NJ06V	-	-	-	-	-	-	-	-
NJ07V	-	-	-	-	-	-	-	-
NJ10V	-	-	-	-	-	-	-	-
NJ12V	-	-	-	-	-	-	-	-
NJ13V	-	-	-	-	-	-	-	-
NJ14V	-	-	-	-	-	-	-	-
NJ15V	-	-	-	-	-	-	-	-
NJ18V	-	-	-	-	-	-	-	-
NJ21V	-	-	-	-	-	-	-	-
NJ22V	-	-	-	-	-	-	-	-
NB01E	-	-	-	-	-	-	-	-
NB02E	-	-	-	-	-	-	-	-
NB03E	-	-	-	-	-	-	-	-
NB04E	-	-	-	-	-	-	-	-

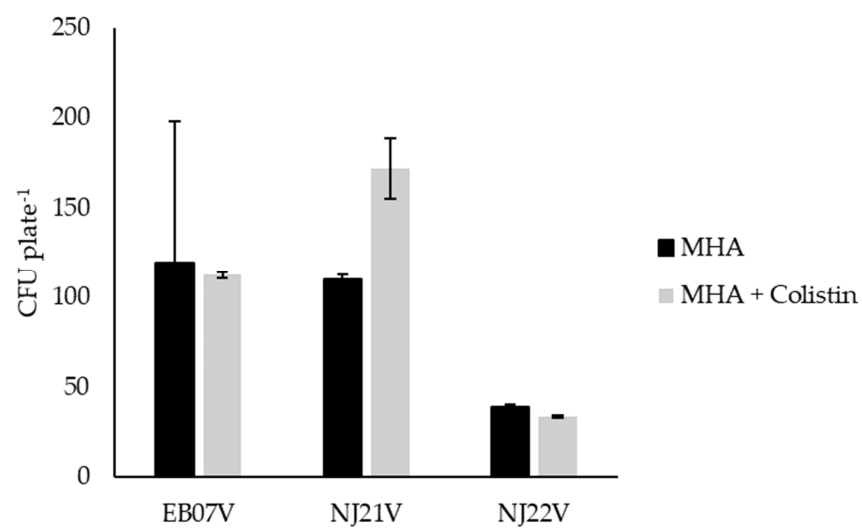
NB05E	-	-	-	-	-	-	-	-
NJ01E	-	-	-	-	-	-	-	-
NJ02E	-	-	-	-	-	-	-	-
NJ03E	-	-	-	-	-	-	-	-
NJ04E	-	-	-	-	-	-	-	-
NJ05E	-	-	-	-	-	-	-	-
NJ08E	-	-	-	-	-	-	-	-
NJ09E	-	-	-	-	-	-	-	-
NJ10E	-	-	-	-	-	-	-	-
NJ11E	-	-	-	-	-	-	-	-
NJ12E	-	-	-	-	-	-	-	-
EB01V	-	-	-	-	-	-	-	-
EB02V	-	-	-	-	-	-	-	-
EB03V	-	-	-	-	-	-	-	-
EB04V	-	-	-	-	-	-	-	-
EB05V	-	-	-	-	-	-	-	-
EB06V	-	-	-	-	-	-	-	-
EB07V	-	-	-	-	-	-	-	-
EB08V	-	-	-	-	-	-	-	-
EB09V	-	-	-	-	-	-	-	-
EB10V	-	-	-	-	-	-	-	-
EB11V	-	-	-	-	-	-	-	-
EB12V	-	-	-	-	-	-	-	-
EB13V	-	-	-	-	-	-	-	-
EB14V	-	-	-	-	-	-	-	-
EB15V	-	-	-	-	-	-	-	-
EB01E	-	-	-	-	-	-	-	-
EB02E	-	-	-	-	-	-	-	-

**Table S6.** Antimicrobial resistances genes search on all the isolates. The third antimicrobial class searched was quinolone (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qepA*). – indicates non detected gene and + indicates detected gene.

Isolate	<i>qnrA</i>	<i>qnrB</i>	<i>qnrC</i>	<i>qnrD</i>	<i>qnrS</i>	<i>qepA</i>
NB04V	-	-	-	-	-	-
NB08V	-	-	-	-	-	-
NB09V	-	-	-	-	-	-
NB10V	-	-	-	-	-	-
NB11V	-	-	-	-	-	-
NB12V	-	-	-	-	-	-
NB13V	-	-	-	-	-	-
NB14V	-	-	-	-	-	-
NB15V	-	-	-	-	-	-
NB16B	-	-	-	-	-	-
NB17V	-	-	-	-	-	-
NB18V	-	-	-	-	-	-
NB19V	-	-	-	-	-	-
NB20V	-	-	-	-	-	-
NB21V	-	-	-	-	-	-
NJ01V	-	-	-	-	-	-
NJ02V	-	-	-	-	-	-
NJ04V	-	-	-	-	-	-
NJ06V	-	-	-	-	-	-
NJ07V	-	-	-	-	-	-

NJ10V	-	-	-	-	-	-
NJ12V	-	-	-	-	-	-
NJ13V	-	-	-	-	-	-
NJ14V	-	-	-	-	-	-
NJ15V	-	-	-	-	-	-
NJ18V	-	-	-	-	-	-
NJ21V	-	-	-	-	-	-
NJ22V	-	-	-	-	-	-
NB01E	-	-	-	-	-	-
NB02E	-	-	-	-	-	-
NB03E	-	-	-	-	-	-
NB04E	-	-	-	-	-	-
NB05E	-	-	-	-	-	-
NJ01E	-	-	-	-	-	-
NJ02E	-	-	-	-	-	-
NJ03E	-	-	-	-	-	-
NJ04E	-	-	-	-	-	-
NJ05E	-	-	-	-	-	-
NJ08E	-	-	-	-	-	-
NJ09E	-	-	-	-	-	-
NJ10E	-	-	-	-	-	-
NJ11E	-	-	-	-	-	-
NJ12E	-	-	-	-	-	-
EB01V	-	-	-	-	-	-
EB02V	-	-	-	-	-	-
EB03V	-	-	-	-	-	-
EB04V	-	-	-	-	-	-
EB05V	-	-	-	-	-	-
EB06V	-	-	-	-	-	-
EB07V	-	-	-	-	-	-
EB08V	-	-	-	-	-	-
EB09V	-	-	-	-	-	-
EB10V	-	-	-	-	-	-
EB11V	-	-	-	-	-	-
EB12V	-	-	-	-	-	-
EB13V	-	-	-	-	-	-
EB14V	-	-	-	-	-	-
EB15V	-	-	-	-	-	-
EB01E	-	-	-	-	-	-
EB02E	-	-	-	-	-	-





**Figure S1.** Plasmid curing of the isolates EB07V, NJ21V and NJ22V. The concentrations of ethidium bromide were: 0.05 g L<sup>-1</sup> for EB07V, 0.025 g L<sup>-1</sup> for NJ21V and NJ22V. The number of CFU was determined in MHA plates with and without colistin.