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1 **An IncP plasmid carrying the colistin resistance gene *mcr-1***
2 **in *Klebsiella pneumoniae* from hospital sewage**

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11

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13

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15

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19

20 **Abstract**

21 A *Klebsiella pneumoniae* strain of ST313 recovered from hospital sewage was found
22 to carry the plasmid-borne colistin resistance gene *mcr-1*, which was bracketed by two
23 copies of the insertion sequence IS*Apl1* on a 57-kb self-transmissible IncP type
24 plasmid of a new IncP-1 clade. The carriage of *mcr-1* on a self-transmissible
25 broad-host-range plasmid highlights that *mcr-1* has the potential to be spread beyond
26 the *Enterobacteriaceae*.

27 Colistin is the last resort antimicrobial agents to treat the infections caused by many
28 Gram-negative bacteria. Recently, a plasmid-borne colistin resistance gene, *mcr-1*, has
29 been found in *Escherichia coli* and *Klebsiella pneumonia* from human and animals in
30 China (1). A few follow-up studies have found *mcr-1*-carrying *E. coli* in many
31 countries in Africa (2), Europe (3-8), Asia (1-3, 9, 10) and North America (9, 11). In
32 addition, *mcr-1* has also been found in *Kluyvera ascorbata* (12) and several species of
33 *Salmonella* (13-15). All of above findings suggest that *mcr-1* has been widely
34 distributed and imposes an emerging threat for clinical management and public and
35 animal health. During a screening study for the presence of colistin-resistant
36 *Enterobacteriaceae* in hospital sewage, we found a *mcr-1*-carrying *K. pneumonia*,
37 which is reported here.

38
39 *K. pneumoniae* strain WCHKP1511 was recovered from the influx mainstream of
40 hospital sewage at West China Hospital, Chengdu, western China, in November 2015.
41 Strain WCHKP1511 grew on CHROMAgar Orientation agar plates (CHROMAgar,
42 Paris, France) containing 4 µg/ml colistin and 64 µg/ml linezolid. Species
43 identification was established by partially sequencing the *gyrB* gene (16). Strain
44 WCHKP1511 was resistant to colistin (MIC, 8 µg/ml), polymyxin B (MIC, 8 µg/ml),
45 chloramphenicol (MIC, 128 µg/ml) and tetracycline (MIC, 64 µg/ml) but was
46 susceptible to amikacin (MIC, 0.5 µg/ml), ceftazidime (MIC, ≤0.5 µg/ml), cefotaxime
47 (MIC, 0.03 µg/ml), ciprofloxacin (MIC, 0.03 µg/ml), imipenem (MIC, 0.125 µg/ml)

48 and tigecycline (MIC, 1 µg/ml) as determined using the microdilution broth method
49 following recommendations of the Clinical Laboratory Standards Institute (CLSI)
50 (17). WCHKP1511 was susceptible to fosfomycin (MIC, 32 µg/ml) as determined
51 using the agar dilution method following recommendations of CLSI (17). In addition,
52 strain WCHKP1511 was resistant to ampicillin and trimethoprim-sulphamethoxazole,
53 intermediate to amoxicillin-clavuanate, gentamicin, tobramycin and nitrofurantoin,
54 and susceptible to aztreonam, cefazolin, cefepime, cefoxitin, ceftriaxone, ertapenem,
55 levofloxacin and piperacillin-tazobactam as determined using the Vitek II automated
56 system (bioMerieux, Lyon, France). Breakpoints defined by FDA and by EUCAST
57 were used for tigecycline and colistin, respectively; otherwise, those defined by CLSI
58 were applied. Strain WCHKP1511 was found to carry *mcr-1* by PCR and sequencing
59 (1, 12). Although *mcr-1* has been widely found in *E. coli*, *mcr-1*-carrying *K.*
60 *pneumoniae* remains uncommon and had only been found in eastern China (Jiangsu
61 and Zhejiang provinces) before (1, 18).

62

63 Strain WCHKP1511 was subjected to the 150-bp paired-end whole genome
64 sequencing with a ca. 200× coverage using the HiSeq 2500 Sequencer (Illumina, San
65 Diego, CA, USA). A total of 3,784,972 reads and 567,745,800 clean bases were
66 generated, which were assembled into 275 contigs (230 contigs ≥ 1,000 bp) in length
67 (N50, 67,217 bp) with a 57.46% GC content using the Spades program (19).

68

69 WCHKP1511 belonged to ST313, which was determined by using the genomic
70 sequence to query the multi-locus sequence typing database of *K. pneumoniae*
71 (<http://bigsdh.web.pasteur.fr/klebsiella/klebsiella.html>). In the *K. pneumoniae* MLST
72 database, only one ST313 strain, KML 2185, which was recovered from human blood
73 in the Netherlands in 2007, has been deposited. *K. pneumoniae* strains carrying *mcr-1*
74 in Jiangsu province belonged to ST25 (20), while the STs of those in Zhejiang
75 province were unknown. ST313 was not closely related to ST25, as only 2 out of 7
76 alleles were identical between the two STs.

77

78 Antimicrobial resistance genes were predicted using ResFinder from the Center for
79 Genomic Epidemiology (<http://genomicepidemiology.org/>). In addition to *mcr-1*,
80 WCHKP1511 had multiple genes mediating resistance to aminoglycosides
81 (*aac(3)-Iva*, *aadA2*, *aph(3')-Ia* and *aph(4)-I*), β -lactams (*bla*_{TEM-135} and a new *bla*_{SHV}
82 variant), chloramphenicol (*floR*), fosfomycin (*fosA*), quinolones (*oqxA* and *oqxB*),
83 sulphonamides (*sul2*), trimethoprim (*dfrA12*) and tetracycline (*tet(A)*) as predicted by
84 ResFinder. *bla*_{TEM-135} encodes a broad-spectrum β -lactamase (21). Of note, although
85 strain WCHKP1511 carried *fosA*, it was susceptible to fosfomycin, which warrants
86 further investigation. *bla*_{SHV} in strain WCHKP1511 is a new variant, which encodes a
87 SHV enzyme with an amino acid difference (Thr14Asn, the position is based on the
88 ATG start condon) from SHV-111, the closest match. The new SHV has been assigned
89 SHV-195 by the NCBI β -lactamase classification system

90 (www.ncbi.nlm.nih.gov/pathogens/submit_beta_lactamase/). As strain WCHKP1511
91 was susceptible to third generation cephalosporins, *bla*_{SHV-195} is unlikely to encode an
92 extended-spectrum β -lactamase (ESBL). The complete coding sequence of *bla*_{SHV-195}
93 was cloned onto the pBC SK vector (Agilent, Santa Clara, CA, USA), which was
94 electroporated into *E. coli* DH5 α . *E. coli* transformants containing *bla*_{SHV-195} were
95 resistant to ampicillin (MIC, >256 μ g/mL) and cephalothin (32 μ g/mL) but were
96 susceptible to aztreonam, ceftazidime, cefotaxime, ceftiofur and imipenem
97 determined using the broth microdilution method (17). This confirmed that *bla*_{SHV-195}
98 encodes a broad-spectrum rather than an ESBL.

99

100 In addition, there was a predicted bleomycin-resistance gene, designated ORFble here,
101 which was not identified by Resfinder but was identified by the Prokka annotation
102 tool (22) and was confirmed by Protein-BLAST in strain WCHKP1511. The complete
103 coding sequence of ORFble was amplified with primers 1511_ble_BamHI_F
104 (CGCGGATCCTTGGTTCACCATGAAGATG)/1511_ble_EcoRI_R
105 (CCGGAATTCCGGCCGATTGCTGAACAGATTA), was cloned onto pBC SK and
106 was electroporated into *E. coli* DH5 α . ORFble-containing transformants were selected
107 on LB agar plates containing 25 μ g/mL chloramphenicol and the presence of ORFble
108 in transformants was confirmed by PCR and sequencing. However, MIC (0.25 μ g/mL)
109 of zeocin (Thermo Fisher Scientific, Waltham, MA, USA), a bleomycin, against *E.*
110 *coli* DH5 α transformant containing ORFble was the same as that against DH5 α as

111 determined using the broth microdilution method (17). This suggests that ORFble did
112 not mediate resistance to bleomycin and its function remains undetermined.

113

114 Conjugation experiments were carried out in broth using azide-resistant *E. coli* strain
115 J53 as the recipient and 2 µg/ml colistin plus 150 µg/ml sodium azide was used for
116 selecting transconjugants. The presence of *mcr-1* in transconjugants was confirmed
117 using PCR. In strain WCHKP1511, *mcr-1* could be transferred to *E. coli* J53 at a
118 frequency of 10⁻² cells per donor cell by mating, suggesting that *mcr-1* was carried by
119 a self-transmissible plasmid, which was assigned pMCR_1511. The sequence of
120 pMCR_1511 was completely circularised with gaps between contigs closed by Sanger
121 sequencing of amplicons from PCRs using primers designed based on available contig
122 sequences. pMCR_KP1511 was 57,278 bp in size and had no known antimicrobial
123 resistance genes other than *mcr-1*. Unlikely the previously-described *mcr-1*-carrying
124 IncI2 plasmid pHNSHP45 (GenBank accession number KP347127) (1),
125 pMCR_KP1511 belonged to the IncP type, a broad-host-range incompatibility group.
126 Plasmid pKH-457-3-BE carrying *mcr-1* in *E. coli* from Belgium was found to have an
127 IncP backbone (3). However, the sequence of pKH-457-3-BE was not available for
128 further analysis. Nonetheless, it has been suggested that pKH-457-3-BE was 99%
129 similarity and 73% coverage with the IncHI2 plasmid pHXY0908 (GenBank
130 accession number KM877269) in *Salmonella enterica* serotype Typhimurium (8).
131 pMCR_KP1511 had only a 6% coverage with pHXY0908, indicating that

132 pMCR_1511 was very different from pKH-457-3-BE and pKH-457-3-BE may not be
133 a true IncP plasmid but is likely of IncHI2.

134

135 pMCR_1511 has the typical IncP-1 plasmid backbone (23) containing the *trfA*
136 encoding the replication initiation protein, two *par* modules for plasmid partitioning,
137 two conjugative regions *tra* (17.6 kb) and *trb* (12.7 kb), the host-lethal
138 protein-encoding *kil* genes and their regulator *kor* (stands for *kil-override*) genes, and a
139 toxin-antitoxin *higA-B* system (Figure 1). The backbone of pMCR_1511 was highly
140 similar (99% identity) with that on plasmid pHNFP671 (GenBank accession number
141 KP324830), which was an IncP plasmid in *E. coli* isolate FP671 from Guangzhou,
142 China but did not carry *mcr-1*. IncP-1 plasmid has six assigned clades, i.e., α , β , γ , δ , ϵ
143 and ζ , among which β clade has $\beta 1$ and $\beta 2$ two subclades (24). To determine of which
144 clade pMCR_1511 was, the sequence of 30 genes belonging to the IncP-1 backbone
145 was retrieved from pMCR_1511, concatenated and then aligned to the counterparts of
146 one representative plasmid of each clade including $\beta 1$ and $\beta 2$ subclades as described
147 previously (25). Phylogenetic analysis of the IncP-1 plasmid backbone revealed that
148 pMCR_1511 and pHNFP671 belonged to a new IncP-1 clade (Figure S1 in the
149 supplementary file).

150

151 When compared the sequence of pHNFP671, pMCR_1511 has two unique regions
152 (Figure 1). One of the unique region harbored *mcr-1* and the other contained ORFble.

153 The sequence comparison of pMCR_1511 and pHNFP671 allowed us to analyze the
154 genetic context of *mcr-1* in detail. Like most genetic contexts available in the
155 GenBank, *mcr-1* was located downstream of the insertion sequence *ISAp11* on
156 pMCR_1511. However, there was another *ISAp11*, which was interrupted (see below),
157 downstream and therefore *mcr-1* was bracketed by two copies of *ISAp11* on
158 pMCR_1511. It has been known that *ISAp11* is able to generate 2-bp direct target
159 repeats (DR) upon insertion (26)
160 (https://www-is.biotoul.fr/index.html?is_special_name=ISAp11). The 2-bp flanking
161 sequences of the region bracketed by the two copies of *ISAp11* were identical (AC,
162 Figure 2). When the region formed by two copies of *ISAp11* and one of the 2-bp
163 flanking sequences were subtracted artificially, the joined sequence perfectly matched
164 that of an open reading frame (orf) with unknown function on plasmid pHNFP671. It
165 therefore proved that the 2-bp sequence was truly DR generated by *ISAp11* rather than
166 coincidence and the two *ISAp11* formed a composite transposon to mobilize *mcr-1*
167 gene. A very recent analysis revealed that the *ISAp11*-formed composite transposon
168 carrying *mcr-1* has also been seen on either the chromosome or a plasmid (IncH or
169 unknown Inc groups) of seven *E. coli* strains (27). The *ISAp11*-formed composite
170 transposon carrying *mcr-1* is all located at different locations, which are also different
171 from the location on pMCR_1511, in the seven *E. coli* strains and is flanked by 2-bp
172 DR in five strains (27). The previous analysis (27) and the findings in the present

173 study suggest that the IS*ApII*-formed composite transposon is a common vehicle for
174 mediating the spread of *mcr-1*.

175

176 The IS*ApII* downstream of *mcr-1* on pMCR_1511 was interrupted by the insertion of
177 Tn3 with the characteristic 5-bp DR (Figure 2). The Tn3 was also disrupted by IS26
178 and most of the Tn3 was absent, which may be due to the action of IS26. It is well
179 known that the insertion of IS26 can lead to the deletion of the adjacent sequence of
180 the insertion site (28). Alternative explanation for the absence of most part of Tn3 is
181 that the insertion of the second IS26 and the recombination between the two IS26
182 could lead to the loss of the intervening region. Although IS*ApII* downstream of
183 *mcr-1* was interrupted by Tn3, the right-end inverted repeat (IRR) of the IS*ApII*
184 remained intact (Figure 2). The transposase encoded by the IS*ApII* upstream of *mcr-1*
185 had the potential to recognize the IRR of the IS*ApII* downstream of *mcr-1* and then
186 could realize the mobilization of the region bracketed by the two copies of the IS*ApII*.

187

188 In conclusion, the plasmid-borne colistin resistance gene *mcr-1* was found in a *K.*
189 *pneumoniae* of an infrequently encountered ST from hospital sewage. *mcr-1* was
190 carried by a self-transmissible IncP plasmid, which is a broad-host-range type of
191 plasmids and has the potential to mediate the dissemination of *mcr-1* from the
192 *Enterobacteriaceae* to other Gram-negative bacteria such as *Pseudomonas aeruginosa*.
193 *mcr-1* was bracketed by two copies of IS*ApII*, which were able to form a composite

194 transposon and represented a common mechanism for mediating the mobilization of
195 *mcr-1*.

196

197 **Nucleotide sequence accession number.** Reads and the Whole Genome Shotgun
198 Sequencing project of *K. pneumoniae* strain WCHKP1511 have been deposited into
199 DDBJ/EMBL/GenBank under accession number SRR3170679 and LSMF00000000,
200 respectively. The sequence of pMCR_1511 has been deposited into
201 DDBJ/EMBL/GenBank under accession number KX377410.

202

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208

209 **References**

- 210 1. **Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G,**
211 **Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H,**
212 **Liang Z, Liu JH, Shen J.** 2015. Emergence of plasmid-mediated colistin
213 resistance mechanism MCR-1 in animals and human beings in China: a
214 microbiological and molecular biological study. *Lancet Infect Dis* **16**:161-168.
- 215 2. **Olaitan AO, Chabou S, Okdah L, Morand S, Rolain J-M.** 2016.
216 Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis* **16**:147.
- 217 3. **Malhotra-Kumar S, Xavier BB, Das AJ, Lammens C, Butaye P, Goossens**
218 **H.** 2016. Colistin resistance gene *mcr-1* harboured on a multidrug resistant
219 plasmid. *Lancet Infect Dis* **16**:283-284.

- 220 4. **Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agerso**
221 **Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas RS, Cavaco LM,**
222 **Hansen DS, Aarestrup FM, Skov RL.** 2015. Detection of *mcr-1* encoding
223 plasmid-mediated colistin-resistant *Escherichia coli* isolates from human
224 bloodstream infection and imported chicken meat, Denmark 2015. Euro
225 Surveill **20**:doi: 10.2807/1560-7917.ES.2015.2820.2849.30085.
- 226 5. **Haenni M, Poirel L, Kieffer N, Châtre P, Saras E, Métayer V, Dumoulin R,**
227 **Nordmann P, Madec J-Y.** 2016. Co-occurrence of extended spectrum β
228 lactamase and MCR-1 encoding genes on plasmids. Lancet Infect Dis
229 **16**:281-282.
- 230 6. **Falgenhauer L, Waezsada S-E, Yao Y, Imirzalioglu C, Käsbohrer A,**
231 **Roesler U, Michael GB, Schwarz S, Werner G, Kreienbrock L,**
232 **Chakraborty T.** 2016. Colistin resistance gene *mcr-1* in extended-spectrum
233 β -lactamase-producing and carbapenemase-producing Gram-negative bacteria
234 in Germany. Lancet Infect Dis **16**:282-283.
- 235 7. **Arcilla MS, van Hattem JM, Matamoros S, Melles DC, Penders J, de Jong**
236 **MD, Schultz C.** 2016. Dissemination of the *mcr-1* colistin resistance gene.
237 Lancet Infect Dis **16**:147-149.
- 238 8. **Poirel L, Kieffer N, Liassine N, Thanh D, Nordmann P.** 2016.
239 Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of
240 *Escherichia coli*. Lancet Infect Dis **16**:281.
- 241 9. **Stoesser N, Mathers AJ, Moore CE, Day NPJ, Crook DW.** 2016. Colistin
242 resistance gene *mcr-1* and pHNSHP45 plasmid in human isolates of
243 *Escherichia coli* and *Klebsiella pneumoniae*. Lancet Infect Dis **16**:285-286.
- 244 10. **Suzuki S, Ohnishi M, Kawanishi M, Akiba M, Kuroda M.** 2016.
245 Investigation of a plasmid genome database for colistin-resistance gene *mcr-1*.
246 Lancet Infect Dis **16**:284-285.
- 247 11. **Mulvey MR, Mataseje LF, Robertson J, Nash JHE, Boerlin P, Toye B,**
248 **Irwin R, Melano RG.** 2016. Dissemination of the *mcr-1* colistin resistance
249 gene. Lancet Infect Dis **16**:289-290.
- 250 12. **Zhao F, Zong Z.** 26 September 2016. *Kluyvera ascorbata* carrying the *mcr-1*
251 colistin resistance gene from hospital sewage. Antimicrob Agents Chemother
252 10.1128/AAC.01165-16.
- 253 13. **Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC,**
254 **Nightingale KK, Bugarel M, Ison SA, Scott HM, Loneragan GH.** 2016.
255 Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis
256 **16**:144-145.
- 257 14. **Hu Y, Liu F, Lin IYC, Gao GF, Zhu B.** 2016. Dissemination of the *mcr-1*
258 colistin resistance gene. Lancet Infect Dis **16**:146-147.
- 259 15. **Tse H, Yuen K-Y.** 2016. Dissemination of the *mcr-1* colistin resistance gene.
260 Lancet Infect Dis **16**:145-146.
- 261 16. **Yamamoto S, Harayama S.** 1995. PCR amplification and direct sequencing

- 262 of *gyrB* genes with universal primers and their application to the detection and
263 taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol*
264 **61**:1104-1109.
- 265 17. **CLSI**. 2013. Performance Standards for Antimicrobial Susceptibility Testing;
266 Twenty-Third Informational Supplement. M100-S23. Clinical and Laboratory
267 Standards Institute, Wayne, PA, USA.
- 268 18. **Du H, Chen L, Tang Y-W, Kreiswirth BN**. 2016. Emergence of the *mcr-1*
269 colistin resistance gene in carbapenem-resistant *Enterobacteriaceae*. *Lancet*
270 *Infect Dis* **16**:287-288.
- 271 19. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS,**
272 **Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV,**
273 **Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA**. 2012. SPAdes: a new
274 genome assembly algorithm and its applications to single-cell sequencing. *J*
275 *Comput Biol* **19**:455-477.
- 276 20. **Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, Xie X, Feng P, Tang**
277 **YW, Kreiswirth BN, Chen L, Du H**. 2016. Complete sequences of
278 *mcr-1*-harboring plasmids from extended spectrum β -lactamase (ESBL)- and
279 carbapenemase-producing *Enterobacteriaceae* (CPE). *Antimicrob Agents*
280 *Chemother* **60**:4351-4354.
- 281 21. **Pasquali F, Kehrenberg C, Manfreda G, Schwarz S**. 2005. Physical linkage
282 of Tn3 and part of Tn1721 in a tetracycline and ampicillin resistance plasmid
283 from *Salmonella Typhimurium*. *J Antimicrob Chemother* **55**:562-565.
- 284 22. **Seemann T**. 2014. Prokka: rapid prokaryotic genome annotation.
285 *Bioinformatics* **30**:2068-2069.
- 286 23. **Pansegrau W, Lanka E, Barth PT, Figurski DH, Guiney DG, Haas D,**
287 **Helinski DR, Schwab H, Stanisich VA, Thomas CM**. 1994. Complete
288 nucleotide sequence of Birmingham IncP alpha plasmids. Compilation and
289 comparative analysis. *J Mol Biol* **239**:623-663.
- 290 24. **Sen D, Brown CJ, Top EM, Sullivan J**. 2013. Inferring the evolutionary
291 history of IncP-1 plasmids despite incongruence among backbone gene trees.
292 *Mol Biol Evol* **30**:154-166.
- 293 25. **Norberg P, Bergstrom M, Jethava V, Dubhashi D, Hermansson M**. 2011.
294 The IncP-1 plasmid backbone adapts to different host bacterial species and
295 evolves through homologous recombination. *Nat Commun* **2**:268.
- 296 26. **Tegetmeyer HE, Jones SC, Langford PR, Baltes N**. 2008. IS*Apl1*, a novel
297 insertion element of *Actinobacillus pleuropneumoniae*, prevents ApxIV-based
298 serological detection of serotype 7 strain AP76. *Vet Microbiol* **128**:342-353.
- 299 27. **Snesrud E, He S, Chandler M, Dekker JP, Hickman AB, McGann P, Dyda**
300 **F**. 12 September 2016. A Model for Transposition of the Colistin Resistance
301 Gene *mcr-1* by IS*Apl1*. *Antimicrob Agents Chemother* doi:
302 10.1128/AAC.01457-16.
- 303 28. **Mollet B, Iida S, Arber W**. 1985. Gene organization and target specificity of

304 the prokaryotic mobile genetic element IS26. *Mol Gen Genet* **201**:198-203.

305

306

307 **Figure legends**

308 **Figure 1.** Genetic structure of IncP plasmid pMCR_1511 carrying *mcr-1* and the
309 comparison with IncP plasmid pHNFP671. Regions and genes that are indicated are
310 ORFble (a predicted [but actually not] bleomycin resistance gene), *higA-B* (encoding
311 a toxin/antitoxin system), *mcr-1*, *kor-par-kli* (for plasmid maintenance), *tra* and *trb*
312 (the two conjugation-encoding regions), and *trfA* (encoding the plasmid replication
313 initiation protein). Backbones of the two IncP plasmids are almost identical.
314 Compared to pHNFP671, pMCR_1511 carried two additional regions that contained
315 either *mcr-1* or ORFble.

316

317 **Figure 2.** Genetic context of *mcr-1* on pMCR_1511. Genetic context of *mcr-1* on the
318 IncI2 plasmid pHNSHP45 (GenBank accession number KP347127) and the
319 corresponding region on the IncP plasmid pHNFP671 (GenBank accession number
320 KP324830) are shown for comparison. orfs that encode hypothetical proteins with
321 unknown function are indicated in white except that the one disrupted by the
322 IS*AplI*-formed composite transposon on pMCR_1511 is shown in black ($\Delta 1$ and $\Delta 2$).
323 Other genes shown are *nikB* (encoding relaxase of the plasmid), *ydgA* (DNA
324 topoisomerase III), *ydfA* (transcriptional regulator), *parA* (resolvase), *bla*_{TEM} (shown
325 as a white arrow in Tn3) and *traB* (conjugative protein). The 2-bp direct repeat (GA)
326 abutting the IS*AplI*-*mcr-1*-*pho* region on pHNSHP45 and the 2-bp direct repeat (AC)
327 abutting the IS*AplI*-formed composite transposon on pMCR_1511 are shown. On

328 pMCR_1511, the IS*ApII* downstream of *mcr-1* was interrupted by Tn3, which was
329 interrupted by IS26.