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Plasmid analysis of NDM metallo-β-lactamase-producing Enterobacterales isolated in Vietnam

Running title. Plasmid analysis of NDM-producing Enterobacterales in Vietnam

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Abstract

Carbapenem-resistant Enterobacterales (CRE) represent a serious threat to public health due to the lack of treatment and high mortality. The rate of antimicrobial resistance of Enterobacterales isolates to major antimicrobials, including carbapenems, is much higher in Vietnam than in Western countries, but the reasons remain unknown due to the lack of genomic epidemiology research. A previous study suggested that carbapenem resistance genes, such as the carbapenemase gene *bla*$_{NDM}$, spread via plasmids among Enterobacterales in Vietnam. In this study, we characterized *bla*$_{NDM}$-carrying plasmids in Enterobacterales isolated in Vietnam, and identified several possible cases of horizontal transfer of plasmids both within and among species of bacteria. Twenty-five carbapenem-nonsusceptible isolates from a medical institution in Hanoi were sequenced on Illumina short-read sequencers, and 13 *bla*$_{NDM}$-positive isolates, including isolates of *Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii*, *Morganella morganii*, and *Proteus mirabilis*, were further sequenced on an Oxford Nanopore Technologies long-read sequencer to obtain complete plasmid sequences. Almost identical 73 kb IncFII(pSE11)::IncN hybrid plasmids carrying *bla*$_{NDM-1}$ were found in a *P. mirabilis* isolate and an *M. morganii* isolate. A 112 kb IncFII(pRSB107)::IncN hybrid plasmid carrying *bla*$_{NDM-1}$ in an *E. coli* isolate had partially identical sequences with a 39 kb IncR plasmid carrying *bla*$_{NDM-1}$ and an 88 kb IncFII(pHN7A8)::IncN hybrid plasmid in a *C. freundii* isolate. 148–149 kb IncFIA(HI1)::IncA/C2 plasmids and 75–76 kb IncFII(Yp) plasmids, both carrying *bla*$_{NDM-1}$ were shared among three sequence type 11 (ST11) isolates and three ST395 isolates of *K. pneumoniae*, respectively. Most of the plasmids co-carried genes conferring resistance to clinically relevant antimicrobials, including third-generation cephalosporins, aminoglycosides, and fluoroquinolones, in addition to *bla*$_{NDM-}$

49 $_1$. These results provide insight into the genetic basis of CRE in Vietnam, and could help

50 control nosocomial infections.

51

52 **Introduction**

53 Carbapenems have a broad spectrum of antimicrobial activity and are reserved for the
54 treatment of infections caused by multidrug-resistant Gram-negative bacteria including
55 Enterobacterales. The increase of cases infected by carbapenem-resistant
56 Enterobacterales (CRE) is a serious threat to public health due to limited management
57 of severe infections and risk of increased mortality [1,2]. Carbapenem-hydrolyzing β-
58 lactamase (carbapenemase) genes, such as bla_{NDM}, bla_{KPC}, and bla_{OXA-48}, that confer
59 resistance to a broad range of β-lactams including third-generation cephalosporins and
60 carbapenems are predominantly encoded on conjugative plasmids and have been
61 transferred among Enterobacterales around the world [3]. There was a report that out of
62 27 carbapenem-resistant *Klebsiella pneumoniae* isolated from patients in a medical
63 institution in Hanoi, Vietnam from 2014 to 2015, two isolates belonging to sequence type
64 395 (ST395) harbored bla_{NDM-1}, and five isolates belonging to ST15, ST16, and ST2353
65 harbored bla_{NDM-4} [4]. There was another report that six *K. pneumoniae* isolates with
66 bla_{NDM-1} were isolated from water samples in Hanoi, Vietnam in 2011 and belonging to
67 ST283 [5]. Furthermore, we previously detected bla_{NDM} in 68.1% (47/69) of CRE isolates
68 from 45 patients in a medical institution in Hanoi, Vietnam from 2010 to 2012, and some
69 of isolates could be shown to transfer bla_{NDM} to *Escherichia coli* [6], suggesting that
70 bla_{NDM} had spread via plasmids among Enterobacterales in medical institutions and
71 communities in Vietnam.

72 The common replicon types of bla_{NDM}-carrying plasmids associated with
73 Enterobacterales includes IncA/C, IncFIA, IncFIB, IncFII, IncH, IncL/M, IncN, and IncX
74 [7,8]. The plasmids occasionally co-carried other clinically relevant antimicrobial
75 resistance (AMR) genes, such as extended-spectrum β-lactamase (ESBL) genes

76 conferring resistance to third-generation cephalosporins, such as *bla*$_{CTX-M}$, 16S ribosomal
77 RNA (rRNA) methyltransferase genes conferring high-level resistance to
78 aminoglycosides, such as *armA* and *rmt*, and fluoroquinolone resistance genes, such as
79 *qnr* and *qep* [9]. Metallo-β-lactamase gene *bla*$_{NDM-1}$ is often observed in a Tn*125*
80 transposon in carbapenem-resistant *Acinetobacter* spp. Tn*125* is bracketed by two
81 copies of insertion sequences, IS*Aba125*, belonging to the IS*30* family [10,11]. On
82 plasmids of CRE, *bla*$_{NDM-1}$ is also observed in the Tn*125*-like mobile gene element (MGE)
83 which contains IS*Aba125* [11–14].
84 In this study, we report the detailed structures of *bla*$_{NDM-1}$-carrying plasmids, and co-
85 existing AMR genes and MGEs on the plasmids in Enterobacterales isolated in Vietnam.
86 This knowledge provides insight into genetic characteristics and potential transmissions
87 of the plasmids among Enterobacterales in Vietnam for the first time.
88

Results

Molecular characterization of carbapenem-nonsusceptible Enterobacterales isolated in Vietnam

A total of 122 isolates of ESBL-producing Enterobacterales were collected in daily diagnosis from individual patients in a medical institution in Hanoi, Vietnam between 2013 and 2017. Twenty-five Enterobacterales isolates, including *K. pneumoniae* (19/25, 76.0%), *E. coli* (2/25, 8.0%), *Citrobacter freundii* (2/25, 8.0%), *Morganella morganii* (1/25, 4.0%), and *Proteus mirabilis* (1/25, 4.0%) isolates, from clinical specimens, including blood (12/25, 48.0%), pus (10/25, 40.0%), urine (2/25, 8.0%), and sputum (1/25, 4.0%), were nonsusceptible to meropenem or imipenem and tested positive with CarbaNP test. The minimum inhibitory concentrations (MICs) of meropenem ranged from 1.5 to >32 μg/mL (median at 6 μg/mL) and those of imipenem ranged from 2 to >32 μg/mL (median at 12 μg/mL) (Fig. 1 and Table S1).

Whole-genome sequencing of all 25 carbapenem-nonsusceptible Enterobacterales isolates with short-read sequencers resulted in the draft genome sequences ranged from 4.1 Mb to 5.9 Mb sizes with 132 to 868 contigs covered by at least x60 sequencing data (Table S1). Carbapenemase gene $bla_{\text{NDM-1}}$ was detected in 12 Enterobacterales isolates (12/25, 48.0%), including *K. pneumoniae* (7/25, 28.0%), *C. freundii* (2/25, 8.0%), *E. coli* (1/25, 4.0%), *M. morganii* (1/25, 4.0%), and *P. mirabilis* (1/25, 4.0%) isolates. Carbapenemase gene $bla_{\text{NDM-4}}$ was detected in five *K. pneumoniae* isolates (5/25, 20.0%), in which two isolates co-harbored other carbapenemase gene $bla_{\text{OXA-48-like}}$ ($bla_{\text{OXA-48}}$ and $bla_{\text{OXA-181}}$, respectively) (2/25, 8.0%). Carbapenemase gene $bla_{\text{KPC-2}}$ was detected in eight Enterobacterales isolates (8/25, 32.0%), including *K. pneumoniae* (7/25, 28.0%) and *E. coli* (1/25, 4.0%) isolates (Fig. 1 and Table S1).

113 Multilocus sequence typing (MLST) analysis revealed that the 19 carbapenemase-
114 producing *K. pneumoniae* isolates belonged to five sequence types (STs), including
115 ST15 (9/19, 47.4%), ST11 (4/19, 21.1%), ST395 (3/19, 15.8%), ST656 (2/19, 10.5%),
116 and ST16 (1/19, 5.3%). The two *E. coli* isolates with $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-2}}$ belonged to
117 ST405 and ST457, respectively (Fig. 1). The presence of $bla_{\text{NDM-1}}$ and replicons
118 IncFII(pSE11) and IncN were detected in *M. morganii* MH16-367M and *P. mirabilis*
119 MH13-009N, suggesting that these isolates might harbor $bla_{\text{NDM-1}}$ on the identical
120 IncFII(pSE11), IncN, or IncFII(pSE11)::IncN plasmid (Fig. 1).
121 MLST classified three *K. pneumoniae* isolates (MH15-289M, MH16-398D, and MH15-
122 258M) as ST11 and another three *K. pneumoniae* isolates (MH15-208H, MH15-191M,
123 and MH13-055M) as ST395. Interestingly, the presence of $bla_{\text{NDM-1}}$ and replicons
124 IncFIA(HI1), IncFIB(K), and IncFII(K) were detected in all six isolates (Fig. 1). In other
125 examples, in the nine *K. pneumoniae*-ST15 isolates, $bla_{\text{NDM-4}}$, IncFIB(pKPHS1), and
126 IncFII(K) replicons were detected in four isolates (MH17-556D, MH16-335M, MH17-
127 016D, and MH17-017D), and $bla_{\text{KPC-2}}$, IncFIB(K), and IncN replicons were detected in
128 five isolates (MH16-444M, MH17-553M, MH17-536D, MH16-519D, and MH17-011M)
129 (Fig. 1).
130

131 **Putative transfer of $bla_{\text{NDM-1}}$-carrying plasmids between Enterobacterales isolated**
132 **in Vietnam**

133 To investigate the similarities of the $bla_{\text{NDM-1}}$-carrying plasmids in more detail, long-read
134 sequencing was performed for 12 $bla_{\text{NDM-1}}$-positive Enterobacterales isolates, including
135 seven *K. pneumoniae* belonging to ST11 (three isolates), ST395 (three isolates), and
136 ST656 (one isolate), two *C. freundii*, one *E. coli* belonging to ST405, one *M. morganii*,

137 and one *P. mirabilis* isolates, and one *bla*$_{NDM-4}$-positive *K. pneumoniae* isolate belonging
138 to ST15 were further sequenced with the long-read sequencer. Hybrid analysis using
139 short-read and long-read sequencing data resulted in complete sequences of 13 *bla*$_{NDM}$-
140 carrying plasmids that ranged from 39.2 kb to 345.7 kb sizes (covered by at least x125
141 long-read sequencing data; Table S2). Interestingly, many *bla*$_{NDM}$-carrying plasmids co-
142 carried other clinically relevant AMR genes, such as 16S rRNA methyltransferases genes
143 (9/13, 69.2%) and quinolone resistance genes (6/13, 46.2%) on the same plasmid (Table
144 S2).

145 BLAST search revealed that several *bla*$_{NDM-1}$-carrying plasmids had high sequence
146 identities with each other and with plasmids in the GenBank database (Figs. 2, 3, 4, and
147 5). However, *bla*$_{NDM-1}$-carrying plasmid pMH16-522D_1 (52.7 kb plasmid IncX3 replicon,
148 accession: AP018571) in *C. freundii* MH16-522D, *bla*$_{NDM-1}$-carrying plasmid pMH16-
149 390M_1 [345.7 kb plasmid with IncFIA(HI1)::IncR replicons, accession: AP018583] in *K.*
150 *pneumoniae* MH16-390M, and *bla*$_{NDM-4}$-carrying plasmid pMH16-335M_1 [105.6 kb
151 plasmid with IncFII(K) replicon, accession: AP018584] in *K. pneumoniae* MH16-335M
152 were not homologous to any other plasmids in this study (Table S2). BLAST search
153 revealed that numerous plasmids in Enterobacterales isolated around the world had 98-
154 100% identity in more than 70% of the regions of pMH16-522D_1, pMH16-390M_1, and
155 pMH16-335M_1, respectively. All three plasmids harbored additional β-lactamase
156 and/or *qnr* genes: *C. freundii* pMH16-522D_1 carried *bla*$_{SHV-12}$, *K. pneumoniae* pMH16-
157 390M_1 carried *bla*$_{CTX-M-3}$, *bla*$_{TEM-1b}$, and *qnrB6*, and *K. pneumoniae* pMH16-335M_1
158 carried *bla*$_{OXA-9}$, *bla*$_{SHV-106}$, and *bla*$_{SHV-28}$ (Table S2). Furthermore, all three plasmids
159 contained the Tn*125*-like MGEs around *bla*$_{NDM}$, and the sequences were highly identical
160 to the reference sequence of *Tn*125 (accession: HQ857107) (96.9 to 100% identity with

161 87-89% region) [14].

162 *P. mirabilis* MH13-009N (isolated in 2013) and *M. morganii* MH16-367M (isolated in
163 2016) had only one plasmid, pMH13-009N_1 [72.6 kb plasmid with IncFII(pSE11)::IncN
164 replicons, accession: AP018566] and pMH16-367M_1 [73.1 kb plasmid with
165 IncFII(pSE11)::IncN replicons, accession: AP018565], respectively; the sequences of
166 these plasmids were almost identical (100% identity in 99% regions), including the
167 $bla_{\text{NDM-1}}$ and IncFII(pSE11)::IncN hybrid replicon regions (Figs. 2A and 2B). Both of the
168 plasmids carried $bla_{\text{NDM-1}}$ and an integron cassette that contains the class 1 integrase
169 gene (*intI1*) and the AMR genes, such as $bla_{\text{TEM-1b}}$, *rmtB*, and *qepA1*. The same integron
170 cassette was observed on *E. coli* pHPA (10.5 kb plasmid with IncFII replicon, accession:
171 AB263754) isolated from a human in Japan in 2002 [15]. Carbapenemase gene $bla_{\text{NDM-}}$
172 $_{1}$ was surrounded by several MGEs, including IS*Aba125*, IS*CR21*, and two IS*26*, on *P.*
173 *mirabilis* pMH13-009N_1 and *M. morganii* pMH16-367M_1, and shared identical
174 sequences with Tn*125* (Fig. 2A): 100% identity with 89% region of the reference
175 sequence (accession: HQ857107) [14,16,17]. The nearly identical $bla_{\text{NDM-1}}$-carrying
176 plasmids, *P. mirabilis* pMH13-009N_1 and *M. morganii* pMH16-367M_1 were
177 successfully transferred to recipient *E. coli* at a frequency of 10^{-2}–10^{-3} *in vitro*.

178 *E. coli* MH13-051M (isolated in 2013) harbored $bla_{\text{NDM-1}}$ on plasmid pMH13-051M_1
179 [111.5 kb plasmid with IncFII(pRSB107)::IncN replicons, accession: AP018572], which
180 had partially identical sequences with two plasmids, pMH17-012N_1 (39.2 kb plasmid
181 with IncR replicon, accession: AP018567, 100% identity in 24% region of pMH13-
182 051M_1) and pMH17-012N_2 [87.6 kb plasmid with IncFII(pHN7A8)::IncN replicons,
183 accession: AP018568, 98.8% identity in 78% region of pMH13-051M_1], both found in
184 *C. freundii* MH17-012N (isolated in 2017) (Figs. 2C and 2D). *E. coli* pMH13-051M_1

185 [IncFII(pRSB107)::IncN plasmid] and *C. freundii* pMH17-012N_1 (smaller IncR plasmid)
186 commonly carried *bla*$_{NDM-1}$ and *bla*$_{TEM-1b}$, and pMH13-051M_1 further carried *bla*$_{CTX-M-55}$
187 and *rmtB*. *C. freundii* pMH17-012N_2 [larger IncFII(pHN7A8)::IncN plasmid] carried no
188 known AMR genes, but did have a set of conjugation-associated type IV secretion
189 system (T4SS) genes, which shared identical sequences with *E. coli* pMH13-051M_1.
190 The genetic structures surrounding *bla*$_{NDM-1}$ in *E. coli* pMH13-051M_1 and *C. freundii*
191 pMH17-012N_1 contained MGEs, including IS*Aba125*, IS*CR21*, and two IS*26*, and
192 shared identical sequences with Tn*125* (Fig. 2C): 98.6% and 100% identity with 89%
193 region of the reference sequence (accession: HQ857107) [14].
194

195 **Comparison of *bla*$_{NDM-1}$-carrying plasmids in Vietnam with those in other countries**

196 Next, we compared *bla*$_{NDM-1}$–carrying plasmids identified in Vietnam with those
197 reported in other countries. A set of conjugation-associated T4SS genes in *P. mirabilis*
198 pMH13-009N_1 [72.6 kb plasmid with IncFII(pSE11)::IncN replicons] was partially
199 identical with *Salmonella enterica* FDAARGOS_70 plasmid unnamed1 (47.8 kb plasmid
200 with IncN replicon, accession: CP026053, 100% identity in 61% region of pMH13-
201 009N_1) isolated from a human in the United States in 2013, which carried *bla*$_{TEM-1b}$ (Fig.
202 3A). However, *S. enterica* FDAARGOS_70 plasmid unnamed1 did not carry *bla*$_{NDM-1}$ as
203 *P. mirabilis* pMH13-009N_1 did. *C. freundii* pMH17-012N_2 [87.6 kb plasmid with
204 IncFII(pHN7A8)::IncN replicons and no AMR genes] was highly identical with *E. coli*
205 p103-2-4 [100.8 kb plasmid with IncFII(pHN7A8) replicons, accession: CP034846,
206 99.9% identity in 94% region of pMH17-012N_2] from a goose farm in China in 2018 and
207 with *S. enterica* serovar Enteritidis p12367A [111.8 kb plasmid with
208 IncFII(pHN7A8)::IncX1 replicons, accession: CP041177, 99.9% identity in 73% region of

209 pMH17-012N_2] isolated from a human in China in 2013 (Fig. 3B). *E. coli* p103-2-4 and
210 *S. enterica* serovar Enteritidis p12367A carried several AMR genes, such as $bla_{\text{CTX-M-55}}$
211 and $bla_{\text{TEM-1b}}$.

212 Furthermore, three *K. pneumoniae* isolates belonging to ST11 (MH15-289M, MH16-
213 398D, and MH15-258M isolated in 2015, 2016, and 2015, respectively) shared nearly
214 identical $bla_{\text{NDM-1}}$-carrying plasmids, pMH15-289M_1, pMH16-398D_1, and pMH15-
215 258M_1 [147–149 kb plasmids with IncFIA(HI1)::IncA/C2 replicons, accessions:
216 AP018577, AP018578, and AP018579, respectively, 99.6% identity in 99% regions], and
217 all of these plasmids carried other AMR genes, such as $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, *rmtC*, and
218 *qnrB9* (Fig. 4). $bla_{\text{NDM-1}}$ was surrounded by several MGEs, including IS*3000*, IS*1*, and
219 IS*CR1*. These three plasmids were highly identical with *K. pneumoniae* plasmid
220 tig00000169_pilon [176.3 kb plasmid with IncFIA(HI1)::IncA/C2 replicons, accession:
221 CP021952, 99.8% identity in 82% region of pMH15-289M_1] in the United States and
222 with *E. coli* pK71-77-1-NDM (145.3 kb plasmid with IncA/C2 replicon, accession:
223 CP040884, 99.9% identity in 62% region of pMH15-289M_1) isolated from a human in
224 Norway in 2010. The plasmids tig00000169_pilon and pK71-77-1-NDM had identical
225 sequences in the T4SS region of pMH15-289M_1, and carried $bla_{\text{NDM-1}}$, $bla_{\text{CMY-6}}$, and
226 *rmtC*, and the plasmid tig00000169_pilon further carried $bla_{\text{SHV-11}}$ and *qnrB58*. *K.*
227 *pneumoniae* MH16-398D had 235 sequence variants (214 single-nucleotide variants
228 [SNVs], 14 multiple-nucleotide variants [MNVs], four deletions, two insertions, and one
229 replacement), and *K. pneumoniae* MH15-258M had six sequence variants (five SNVs
230 and one replacement) relative to *K. pneumoniae* MH15-289M.

231 Another three *K. pneumoniae* isolates belonging to ST395 (MH15-208H, MH15-191M,
232 and MH13-055M isolated in 2015, 2015, and 2013, respectively) shared nearly identical

233 *bla*$_{NDM-1}$-carrying plasmids, pMH15-208H_1, pMH15-191M_1, and pMH13-055M_1 [75–
234 76 kb plasmids with IncFII(Yp) replicon, accessions: AP018580, AP018581, and
235 AP018582, respectively, 99.8–99.9% identity in 99% regions], and all plasmids carried
236 another AMR gene, *rmtB* (Fig. 5). *bla*$_{NDM-1}$ in these plasmids was surrounded by several
237 MGEs, including IS*Aba125*, IS*1*, and IS*91*, and shared partially identical sequences with
238 Tn*125* (Fig. 5): 99.5-100% identity with 88-89% region of the reference sequence
239 (accession: HQ857107) [14]. These three plasmids were highly identical with
240 *Enterobacter hormaechei* pNDM1_045001 [85.7 kb plasmid with IncFII(Yp) replicon,
241 accession: CP043383, 99.9% identity in 98% region of pMH15-208H_1] isolated from a
242 human in China in 2018 and with *K. pneumoniae* pSECR18-2374C [86.0 kb plasmid with
243 IncFII(Yp) replicon, accession: CP041930, 99.9% identity in 95% region of pMH15-
244 208H_1] isolated from a human in South Korea in 2018. *E. hormaechei* pNDM1_045001
245 and *K. pneumoniae* pSECR18-2374C had a partially identical sequence of conjugation-
246 associated T4SS genes with pMH15-208H_1. *E. hormaechei* pNDM1_045001 carried
247 *bla*$_{NDM-1}$, *bla*$_{TEM-1b}$, and *rmtB*, and *K. pneumoniae* pSECR18-2374C carried *bla*$_{NDM-4}$,
248 *bla*$_{TEM-1b}$ and *rmtB*. *K. pneumoniae* MH15-191M had three sequence variants (two SNVs
249 and one MNV) and *K. pneumoniae* MH13-055M had only one SNV relative to *K.*
250 *pneumoniae* MH15-208H.

251 In bacterial conjugation experiments with *E. coli*, *C. freundii* pMH17-012N_1 (39.2 kb
252 plasmid with IncR replicon), pMH17-012N_2 [87.6 kb plasmid with IncFII(pHN7A8)::IncN
253 replicons], *E. coli* pMH13-051M_1 [111.5 kb plasmid with IncFII(pRSB107)::IncN
254 replicons], *K. pneumoniae* pMH15-258M_1 [148.8 kb plasmid with IncFIA(HI1)::IncA/C2
255 replicons], and *K. pneumoniae* pMH15-208H_1 [75.4 kb plasmid with IncFII(Yp) replicon]
256 were not transferred to recipient *E. coli* under our experimental conditions though a set

257 of T4SS-associated genes were detected in *C. freundii* pMH17-012N_2, *E. coli* pMH13-

258 051M_1, *K. pneumoniae* pMH15-258M_1, and *K. pneumoniae* pMH15-208H_1.

259

260 **Discussion**

261 The Viet Nam Resistance (VINARES), a nationwide surveillance network consisting of
262 16 central and provincial-level medical institutions was established in Vietnam in 2013
263 [18]. According to VINARES data, the rates of resistance of *K. pneumoniae* to third-
264 generation cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones were
265 66.4%, 17.1%, 29.5%, and 53.0%, respectively, in Vietnam [19], whereas the rates were
266 13.6%, 0.5%, 6.5%, and 8.7%, respectively, in the United Kingdom in 2013 [20], and
267 17.2%, 4.3%, 4.5%, and 16.8%, respectively, in the United States in 2010 [21]. Though
268 the rates of resistance of Enterobacterales to major antimicrobials are much higher in
269 Vietnam than in Western countries, the genetic causes remain unknown.

270 In this study, we performed comprehensive genetic analysis on plasmids carrying
271 bla_{NDM} carbapenemase genes in Enterobacterales isolated from patients in Vietnam. We
272 focused on carbapenem-nonsusceptible Enterobacterales isolates harboring bla_{NDM}, one
273 of the most prevalent carbapenemase genes in the world, and completely sequenced 12
274 plasmids carrying bla_{NDM-1} from *K. pneumoniae* (ST11, ST395, and ST656), *E. coli*
275 (ST405), *C. freundii*, *M. morganii*, and *P. mirabilis* isolates and one plasmid carrying
276 bla_{NDM-4} from *K. pneumoniae* isolate (ST15). Nearly identical plasmids carrying bla_{NDM-1}
277 were detected in different bacterial species of this study, suggesting that they represent
278 common and important AMR plasmids disseminated in Vietnam (Figs. 2, 4, and 5).
279 Moreover, the co-existence of clinically relevant AMR genes, such as ESBL genes (e.g.,
280 bla_{CTX-M}, bla_{OXA}, and bla_{TEM}), aminoglycoside resistance genes (e.g., *armA* and *rmt*), and
281 fluoroquinolone resistance genes (e.g., *qep* and *qnr*), with bla_{NDM-1} was observed in
282 several plasmids, such as *P. mirabilis* pMH13-009N_1 (Fig. 2) and *K. pneumoniae*
283 pMH15-289M_1 (Fig. 4). Hence, the spread of the plasmids both within and among

284 species of bacteria would have caused an increase in multidrug-resistant bacteria and
285 the infected hosts in Vietnam, and will pose a threat to public health.

286 Almost identical IncFII(pSE11)::IncN hybrid plasmids, pMH13-009N_1 and pMH16-
287 367M_1, were found in *P. mirabilis* and *M. morganii* isolates, respectively (Figs. 2A and
288 2B). These plasmids had identical sequences including a set of conjugation-associated
289 T4SS genes with the IncN plasmid from *S. enterica* isolated in the United States (Fig.
290 3A). IncN plasmids are prevalent in the microbiota of animals, and disseminate AMR
291 genes such as $bla_{\text{CTX-M-1}}$ and *qnr* [22], suggesting that IncN plasmids with the same origin
292 of *P. mirabilis* pMH13-009N_1 and *M. morganii* pMH16-367M_1 could be disseminated
293 in communities, including humans, animals, and the environment, in Vietnam and other
294 countries.

295 The IncFII(pRSB107)::IncN hybrid plasmid pMH13-051M_1 in *E. coli*, had partial
296 sequence identity with the IncR plasmid pMH17-012N_1 and the IncFII(pHN7A8)::IncN
297 hybrid plasmid pMH17-012N_2 both in *C. freundii* (Figs. 2C and 2D). Rearrangement of
298 conjugation genes plays an important role in the dissemination of AMR genes between
299 bacteria [23]. Because known IncR plasmids are non-transferable [24], an IncR plasmid
300 carrying AMR genes (e.g. *C. freundii* pMH17-012N_1) and another plasmid carrying
301 conjugation genes (e.g. *C. freundii* pMH17-012N_2) could be assumed to have been
302 fused into a single plasmid with both AMR and conjugation genes (e.g. *E. coli* pMH13-
303 051M_1) in a bacterium. Subsequently the plasmid was propagated by conjugation to
304 another bacterium. There is an increasing number of reports of IncR plasmids carrying
305 various AMR genes and the pool of AMR genes on IncR plasmids is thought to spread
306 to transmissible plasmids via recombination, contributing to the high evolutionary
307 plasticity of bacterial genomes [25]. Further analysis on the possible recombination

308 events are needed to understand the ways of transmission of these plasmids between
309 bacterial isolates and species.

310 The IncFII(pHN7A8)::IncN hybrid plasmid pMH17-012N_2 had highly identical
311 sequences including a set of conjugation-associated T4SS genes with the plasmids *E.*
312 *coli* p103-2-4 and *S. enterica* Enteritidis p12367A, that carry several AMR genes, such
313 as $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-1b}}$, but no bla_{NDM} (Fig. 3B). The IncFII plasmid pHN7A8
314 (accession: JN232517), which also carries several AMR genes, such as $bla_{\text{CTX-M-65}}$, *rmtB*,
315 and *fosA3*, and is widespread in *E. coli* from farm and companion animals in China [26],
316 suggesting that plasmids with IncFII(pHN7A8) replicon are associated with AMR.

317 $bla_{\text{NDM-1}}$ was found on several hybrid plasmids, such as IncFII(pSE11)::IncN,
318 IncFII(pRSB107)::IncN hybrid plasmids (Figs. 2A and 2C), and IncFIA(HI1)::IncA/C2
319 hybrid plasmids from three *K. pneumoniae* ST11 isolates (Fig. 4). Hybrids of multiple
320 replicons belonging to different incompatibility groups represent a plasmid strategy for
321 expansion of host range and dissemination of acquired AMR genes among bacteria [27].
322 The IncFIA(HI1)::IncA/C2 hybrid plasmids or IncFII(Yp) plasmids were reported from
323 other regions, including the United States, Europe, and Asia (Figs. 4 and 5).
324 IncFIA(HI1)::IncA/C2 plasmids and IncFII(Yp) plasmids were detected in *K. pneumoniae*
325 in this study, suggesting that these plasmids and/or bacterial isolates would have also
326 been spreading in medical institutions and community in Vietnam.

327 The $bla_{\text{NDM-1}}$-surrounding regions in plasmids, *P. mirabilis* pMH13-009N_1, *M. morganii*
328 pMH16-367M_1, *C. freundii* pMH17-012N_1, and *E. coli* pMH13-051M_1 (Fig. 2), *K.*
329 *pneumoniae* pMH15-208H_1, pMH15-191M_1, and pMH13-055M_1 (Fig. 5), and *K.*
330 *pneumoniae* pMH16-390M_1, pMH16-335M_1, and *C. freundii* pMH16-522D_1 had
331 almost identical sequence including Tn*125* which was discovered in *A. baumannii*

332 [10,14,16]. Tn*125* includes IS*Aba125* and IS*CR21*, but sequences of the Tn*125*-like
333 region in *P. mirabilis* pMH13-009N_1, *M. morganii* pMH16-367M_1, and *E. coli* pMH13-
334 051M_1 (Fig. 2) include two IS*26* in addition to IS*Aba125* and IS*CR21*. This Tn*125*-like
335 structure has also been detected in other plasmids from Enterobacterales, such as *C.*
336 *freundii* pCF104a-T3 (accession: MN657241) isolated from a human in Germany in 2015
337 [17]. This report showed that IS*26*-mediated transfer of $bla_{\text{NDM-1}}$-containing Tn*125*-like
338 regions had occurred in different Enterobacterales species within one medical institution.
339 The MGEs surrounding $bla_{\text{NDM-1}}$ on plasmids, *K. pneumoniae* pMH15-289M_1,
340 pMH16-398D_1, and pMH15-258M_1 (Fig. 4), were highly identical with the
341 corresponding sequence of *E. coli* pEC2-NDM-3 (accession: KC999035) isolated from a
342 human in Australia in 2010 though pEC2-NDM-3 carried $bla_{\text{NDM-3}}$ with a part of the same
343 set of MGEs, including IS*CR1*. IS*CR1* was suggested to provide a vehicle for $bla_{\text{NDM-1}}$
344 dissemination [8, 28].
345 MLST analysis is helpful to estimate whether bacterial isolates belonging to the same
346 STs were originated from the same clones and spread within the medical institution, and
347 whole-genome SNV-based analysis enables us to estimate more accurate genetic
348 relationship. According to a report [29], SNVs accumulated in *K. pneumoniae* ST258
349 isolates at a rate of 3.9 SNVs per year. Our analysis of SNVs of *K. pneumoniae* ST11
350 isolates (Fig. 4) revealed 214 SNVs in MH16-398D relative to MH15-289M. This
351 suggests that different ST11 clones are present in the medical institutions and
352 communities. In support of this theory, a report [30] showed that *K. pneumoniae* isolates
353 from medical institutions, sewage, canals, and agricultural waste water were intermixed
354 in the phylogenetic classification. Because ST11 is one of major international epidemic
355 clones of *K. pneumoniae* [31], an ST11 clone carrying AMR plasmid could have

356 disseminated in a medical institution or local community in Hanoi, Vietnam. Another three
357 ST395 isolates of *K. pneumoniae* (Fig. 5) are very clonal based on the rate of SNV
358 accumulation [29] and could be disseminated from the same clone within the medical
359 institution because only a few SNVs were present in MH15-191M and MH13-055M
360 relative to MH15-208H.
361

362 **Conclusion**

363 Plasmid analysis in this study shows the complex structures and diversity of plasmids
364 carrying *bla*$_{NDM-1}$ in Vietnam. Hybrid analysis with both Illumina short-read and ONT long-
365 read sequencing is a promising method for detecting important AMR plasmids in CRE
366 isolates and the real-time analysis is useful for controlling nosocomial infections.
367

368 **Materials and Methods**

369 **Bacterial isolates**

370 A total of 122 ESBL-producing Enterobacterales isolates were collected from selected
371 specimens, including blood, sputum, urine, and pus from both in-patients and out-
372 patients with infections in daily diagnosis in a reference medical institution in Hanoi,
373 Vietnam between 2013 and 2017. All bacterial isolates were excluded from duplicates
374 from the same patient. The medical institution has 700 beds, and includes 13 surgical
375 departments and 10 internal medicine departments. The medical services are provided
376 for both military and civil patients. Bacterial species identification, prediction of ESBL
377 activity, and antimicrobial susceptibility testing were performed by Vitek 2 (BioMèrieux)
378 and E-test (BioMèrieux). ESBL gene(s)-harboring isolates could also harbor other
379 important AMR genes, including carbapenemase gene(s). According to the Clinical and
380 Laboratory Standards Institute (CLSI) 2020 guidelines, breakpoints of meropenem and
381 imipenem are ≤1 μg/mL (susceptible), 2 μg/mL (intermediate), ≥4 μg/mL (resistant),
382 respectively. Carbapenemase activity was examined by CarbaNP test according to the
383 CLSI 2020 guidelines. Major carbapenemase genes (bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{IMP}, and
384 bla_{VIM}) were detected by a multiplex PCR method [32]. Twenty-five carbapenem-
385 nonsusceptible and carbapenemase-positive isolates (19 *K. pneumoniae*, two *E. coli*,
386 two *C. freundii*, one *M. morganii*, and one *P. mirabilis*) were subjected to the whole-
387 genome sequencing analysis described below.

388

389 **Preparation of genomic DNA**

390 For short-read sequencing, genomic DNAs of bacterial isolates were extracted with
391 the phenol-chloroform method, and purified with QIAquick PCR Purification Kit

392 (QIAGEN) according to the manufacturer's instructions. For long-read sequencing, high-
393 molecular-weight genomic DNAs of bacterial isolates were extracted with MagAttract
394 HMW DNA Kit (QIAGEN) according to the manufacturer's instructions. The extracted
395 genomic DNAs were quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific).
396

397 **Whole-genome sequencing and bioinformatics analysis**

398 Whole-genome sequencing was performed to examine the prevalence of important
399 AMR genes on plasmids. Long-read sequencing is useful for constructing complete
400 sequences of whole plasmids and tracking horizontal transfer of AMR plasmids during
401 nosocomial infections [33]. Recently, long-read nanopore sequencing using MinION
402 nanopore sequencer from Oxford Nanopore Technologies (ONT) has been applied to
403 such investigations [34,35].

404 Whole genome sequencing using MiniSeq or HiSeq systems (Illumina) was performed
405 for phylogenetic and MLST analysis, and screening of acquired AMR genes and plasmid
406 replicon types. Library for Illumina sequencing (insert size of 500-900 bp) was prepared
407 using Nextera XT DNA Library Prep Kit (Illumina). Paired-end sequencing was performed
408 using MiniSeq (2 x 150 bp) or HiSeq 4000 systems (2 x 150 bp). Trimming and *de novo*
409 assembly of paired-end reads was performed using CLC Genomics Workbench v12.0
410 (QIAGEN) with default parameters. A maximum-likelihood phylogenetic tree was
411 generated by PhyML from a concatenated core-gene alignment consisting of 13,305
412 SNVs constructed using the Roary pipeline (https://sanger-pathogens.github.io/Roary/).
413 For PhyML, we used the following parameters that indicate the GTR +G4 model of DNA
414 substitution with estimation of the shape parameter of the gamma distribution by
415 maximizing the likelihood: -m GTR -c 4 -a e.

416 Twelve Enterobacterales isolates with *bla*$_{NDM-1}$ (seven *K. pneumoniae*, one *E. coli*, two
417 *C. freundii*, one *M. morganii*, and one *P. mirabilis*) and one *K. pneumoniae* isolate with
418 *bla*$_{NDM-4}$ were further sequenced on MinION (ONT) using the SQK-RAD002 or SQK-
419 RBK001 kits and R9.4 flowcells according to the manufacturer's instructions to obtain
420 complete sequences of plasmids carrying carbapenem resistance genes. *De novo*
421 assembly was performed using Canu v1.5 [36] and Miniasm [37] with default parameters.
422 The overlap region in the assembled contig was detected by genome-scale sequence
423 comparison using LAST (http://last.cbrc.jp) and was trimmed manually. Illumina paired-
424 end reads were mapped onto the resulted circular sequences, and error correction was
425 performed by extracting consensus of mapped reads using CLC Genomics Workbench
426 v12.0 with default parameters.
427 Sequence types, carbapenemase genes, and plasmid replicon types were analyzed
428 using MLST v2.0, ResFinder v3.2 with minimum threshold of 90% identity and 60%
429 coverage, and PlasmidFinder v2.1 with minimum threshold of 90% identity and 60%
430 coverage, respectively, on the Center for Genomic Epidemiology (CGE) server at
431 Technical University of Denmark (http://www.genomicepidemiology.org).
432 Coding sequences (CDS) annotation using RASTtk on the PATRIC v3.6.3 server
433 (https://www.patricbrc.org) with default parameters. Sequence variant detection was
434 performed using CLC Genomics Workbench v12.0 with default parameters of Fixed
435 Ploidy Variant Detection (Ploidy=1). Linear comparison of sequences was performed
436 using BLAST with the default settings (the nucleotide collection database and the
437 megablast program), and visualized by Easyfig (http://mjsull.github.io/Easyfig/). *bla*$_{NDM-1}$,
438 other important AMR genes (ARG), type IV secretion system (T4SS)-associated genes
439 involved in conjugation that were detected by SecReT4 program [38], and mobile gene

440 elements (MGEs) were identified manually from CDS annotations and basically analyzed
441 by comparing the sequences analyzed in previous studies.
442 Draft genome and complete plasmid sequences of carbapenem-nonsusceptible
443 Enterobacterales isolated in Vietnam have been deposited at GenBank/EMBL/DDBJ
444 under BioProject number PRJDB6655.
445
446 **Bacterial conjugation**
447 Bacterial conjugation was performed using six *bla*$_{NDM-1}$-carrying plasmid-positive
448 Enterobacterales isolates [*P. mirabilis* MH13-009N, *M. morganii* MH16-367M, *C. freundii*
449 MH17-012N, *E. coli* MH13-051M (ST405), *K. pneumoniae* MH15-258M (ST11), and *K.*
450 *pneumoniae* MH15-208H (ST395)] according to the following protocol. The same amount
451 of Luria-Bertani (LB) broth cultures of each donor bacteria and the recipient azide–
452 resistant *E. coli* J53 (ATCC BAA-2731, *F- met pro Azi*^{r}), were mixed and spotted onto
453 Mueller-Hinton agar and then incubated at 37°C overnight. The mixed cells were
454 recovered and suspended into PBS buffer, plated onto LB agar after 10-fold serial dilution,
455 and incubated at 37°C overnight. Transconjugants were selected on LB agar containing
456 2 μg/mL of meropenem and 100 μg/mL of sodium azide.
457

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Conflicts of Interest

None to declare.

465 **Figure Legends**

466 **Figure 1**

467 Carbapenem-nonsusceptible Enterobacterales isolates sequenced on Illumina
468 systems. Red nodes, but not black nodes, show isolates subsequently sequenced with
469 ONT MinION. Bar lengths represent the number of substitutions per site in the core
470 genome constructed using the Roary pipeline (see Materials and Methods). Sequence
471 types of multilocus sequence typing (MLST) analysis determined from genomes,
472 minimum inhibitory concentrations (MICs) of meropenem (MEM) and imipenem (IPM) of
473 isolates, carbapenemase genes detected by ResFinder and replicon types detected by
474 PlasmidFinder in genomes, years in which the bacteria were isolated, and replicon types
475 and sizes of *bla*$_{NDM}$-carrying plasmids are shown.

476

477 **Figure 2**

478 *bla*$_{NDM-1}$-carrying plasmids and the detailed genetic structures around *bla*$_{NDM-1}$ in
479 carbapenem-nonsusceptible Enterobacterales isolates sequenced using ONT MinION
480 and Illumina systems. Sets of plasmids from *P. mirabilis* MH13-009N (pMH13-009N_1,
481 accession: AP018566) and *M. morganii* MH16-367M (pMH16-367M_1, accession:
482 AP018565), and from *E. coli* MH13-051M (pMH13-051M_1, accession: AP018572) and
483 *C. freundii* MH17-012N (pMH17-012N_1, accession: AP018567 and pMH17-012N_2,
484 accession: AP018568), are shown. (A) and (C) Linear comparison of *bla*$_{NDM-1}$-carrying
485 plasmid sequences. (B) and (D) Schematic representation of possible transfer of
486 plasmids, including *bla*$_{NDM-1}$-carrying plasmids. Circles within bacterial cells show
487 plasmids, and red squares on plasmids show *bla*$_{NDM-1}$. Sets of plasmids from *P. mirabilis*
488 MH13-009N and *M. morganii* MH16-367M (A and B), and from *E. coli* MH13-051M and

489 *C. freundii* MH17-012N (C and D), are shown. Red, yellow, green, blue, and gray arrows
490 indicate *bla*$_{NDM}$, other important AMR genes (ARGs), type IV secretion system (T4SS)-
491 associated genes involved in conjugation, mobile gene elements (MGEs), and other
492 genes, respectively. The colors in comparison of plasmids show percent identity and
493 sequence direction as indicated. Blue for matches in the same direction and red for
494 matches in the inverted direction.
495
496 **Figure 3**
497 (A) and (B) Linear comparison of plasmid sequences from Vietnam and other countries.
498 *P. mirabilis* pMH13-009N_1 (this study, accession: AP018566) and *Salmonella enterica*
499 FDAARGOS_70 plasmid unnamed1 (accession: CP026053) (A), and *E. coli* pMH13-
500 051M_1 (this study, accession: AP018572), *C. freundii* pMH17-012N_2 (this study,
501 accession: AP018568), *E. coli* p103-2-4 (accession: CP034846), and *Salmonella*
502 *enterica* serovar Enteritidis p12367A (accession: CP041177) (B) are shown. Red, yellow,
503 green, blue, and gray arrows indicate *bla*$_{NDM}$, other important AMR genes (ARGs), type
504 IV secretion system (T4SS)-associated genes involved in conjugation, mobile gene
505 elements (MGEs), and other genes, respectively. The colors in comparison of plasmids
506 show percent identity and sequence direction as indicated. Blue for matches in the same
507 direction and red for matches in the inverted direction.
508
509 **Figure 4**
510 Linear comparison of *bla*$_{NDM-1}$-carrying plasmid sequences and the detailed genetic
511 structures around *bla*$_{NDM-1}$ from Vietnam and other countries. *K. pneumoniae* pMH15-
512 289M_1 (this study, accession: AP018577), *K. pneumoniae* pMH16-398D_1 (this study,
513 accession: AP018578), *K. pneumoniae* pMH15-258M_1 (this study, accession:

514 AP018579), *K. pneumoniae* tig00000169_pilon (accession: CP021952), and *E. coli*
515 pK71-77-1-NDM (accession: CP040884) are shown. Red, yellow, green, blue, and gray
516 arrows indicate bla_{NDM}, other important AMR genes (ARGs), type IV secretion system
517 (T4SS)-associated genes involved in conjugation, mobile gene elements (MGEs), and
518 other genes, respectively. The colors in comparison of plasmids show percent identity
519 and sequence direction as indicated. Blue for matches in the same direction and red for
520 matches in the inverted direction.
521
522 **Figure 5**
523 Linear comparison of bla_{NDM}-carrying plasmid sequences and the detailed genetic
524 structures around bla_{NDM-1} from Vietnam and other countries. *K. pneumoniae* pMH15-
525 208H_1 (this study, accession: AP018580), *K. pneumoniae* pMH15-191M_1 (this study,
526 accession: AP018581), *K. pneumoniae* pMH13-055M_1 (this study, accession:
527 AP018582), *E. hormaechei* pNDM1_045001 (accession: CP043383), and *K.*
528 *pneumoniae* pSECR18-2374C (accession: CP041930) are shown. Red, yellow, green,
529 blue, and gray arrows indicate bla_{NDM}, other important AMR genes (ARGs), type IV
530 secretion system (T4SS)-associated genes involved in conjugation, mobile gene
531 elements (MGEs), and other genes, respectively. The colors in comparison of plasmids
532 show percent identity and sequence direction as indicated. Blue for matches in the same
533 direction and red for matches in the inverted direction.
534
535 **Table S1**
536 Bacterial species and isolates, specimen types for the bacterial isolation, years in
537 which the bacteria were isolated, and minimum inhibitory concentrations (MICs) of

538 meropenem (MEM) and imipenem (IPM) of isolates are shown. Also, sequence types of
539 multilocus sequence typing (MLST) analysis determined from genomes, carbapenemase
540 genes detected by ResFinder in genomes, sizes and contigs of genomes, coverages in
541 short-read sequencing, Illumina sequencing platforms, and accession numbers of
542 genomes are shown. According to the CLSI 2020 guidelines, Breakpoints of meropenem
543 and imipenem are as follows: ≤1 μg/mL, susceptible; 2 μg/mL, intermediate; ≥4 μg/mL,
544 resistant (R).
545
546 **Table S2**
547 Bacterial species and isolates, and *bla*$_{NDM}$-carrying plasmids are shown. Also, replicon
548 types detected by PlasmidFinder and antimicrobial resistance genes (ARGs) detected
549 by ResFinder in plasmids, sizes of plasmids, and coverages in long-read sequencing,
550 and accession numbers of plasmids are shown.
551

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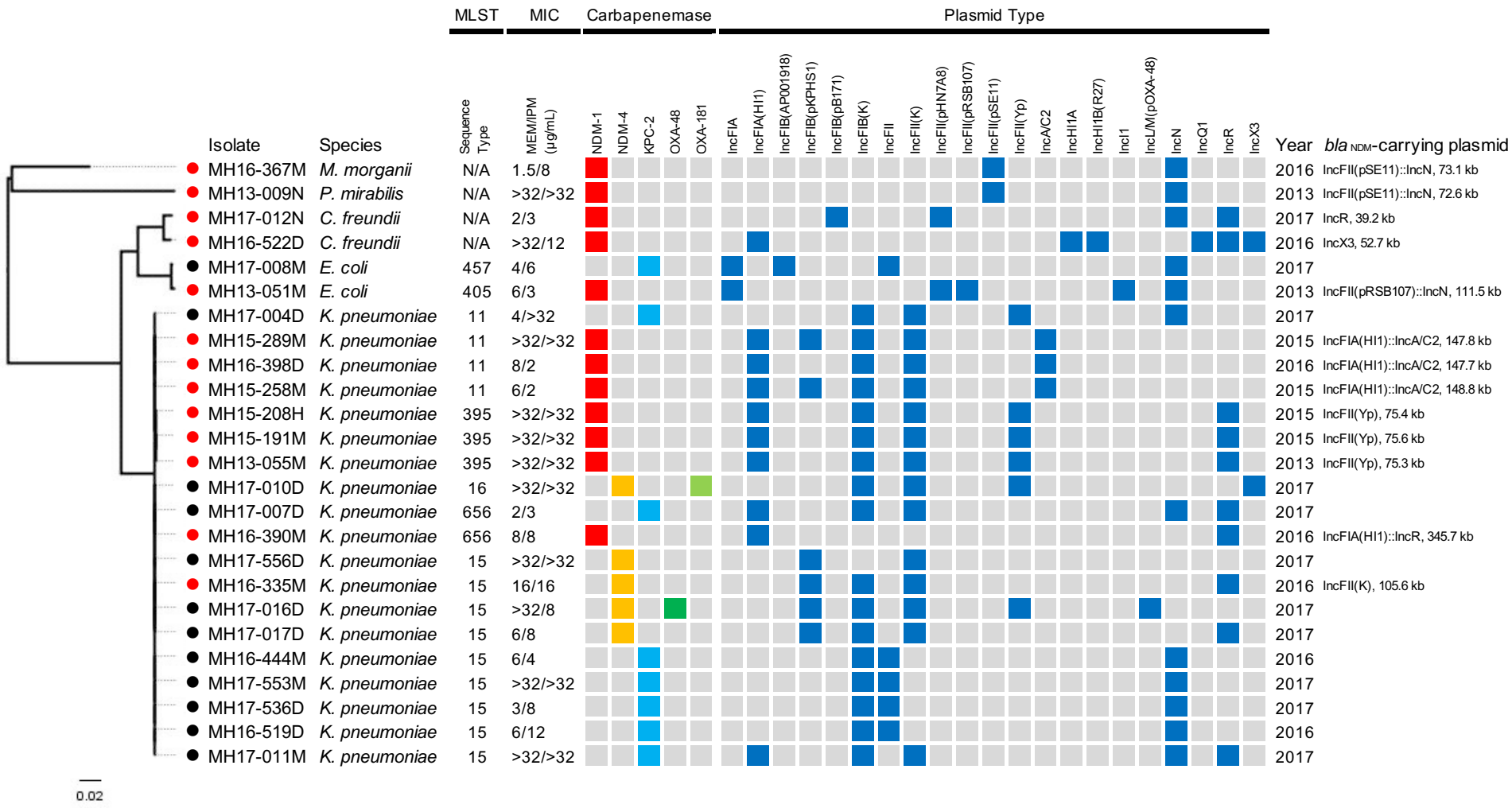
MLST
MIC
Carbapenemase
Plasmid Type
Isolate
Species
Sequence Type
MEM/IPM (μg/mL)
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NDM-4
KPC-2
OXA-48
OXA-181
IncFIA
IncFIA(HI1)
IncFIB(AP001918)
IncFIB(pKPHS1)
IncFIB(pB171)
IncFIB(K)
IncFII
IncFII(K)
IncFII(pHN7A8)
IncFII(pRSB107)
IncFII(pSE11)
IncFII(Yp)
IncA/C2
IncHI1A
IncHI1B(R27)
IncI1
IncL/M(pOXA-48)
IncN
IncQ1
IncR
IncX3
Year
bla NDM-carrying plasmid
MH16-367M M. morganii N/A 1.5/8 2016 IncFII(pSE11)::IncN, 73.1 kb
MH13-009N P. mirabilis N/A >32/>32 2013 IncFII(pSE11)::IncN, 72.6 kb
MH17-012N C. freundii N/A 2/3 2017 IncR, 39.2 kb
MH16-522D C. freundii N/A >32/12 2016 IncX3, 52.7 kb
MH17-008M E. coli 457 4/6 2017
MH13-051M E. coli 405 6/3 2013 IncFII(pRSB107)::IncN, 111.5 kb
MH17-004D K. pneumoniae 11 4/>32 2017
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MH16-519D K. pneumoniae 15 6/12 2016
MH17-011M K. pneumoniae 15 >32/>32 2017
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Fig. 1

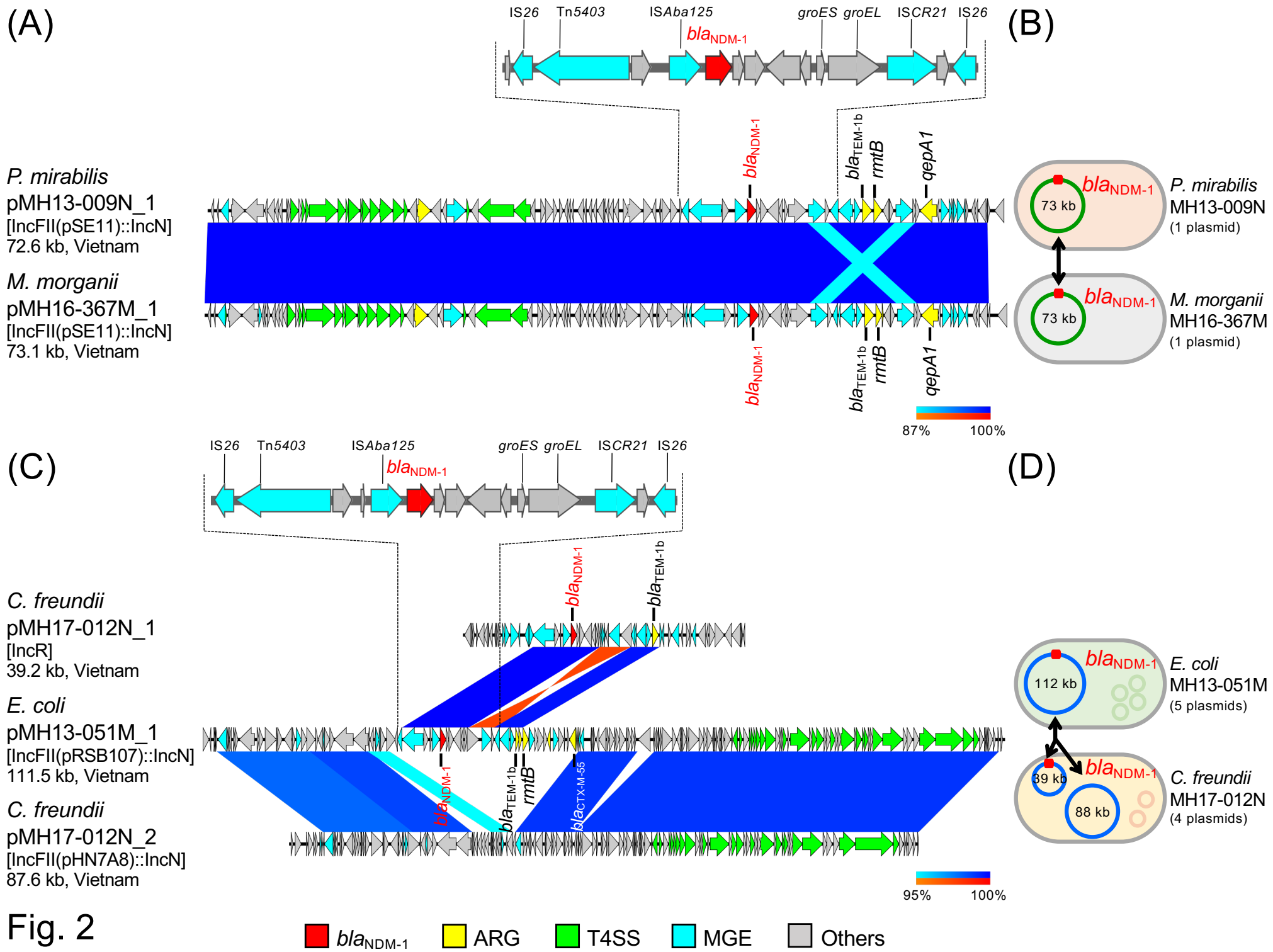
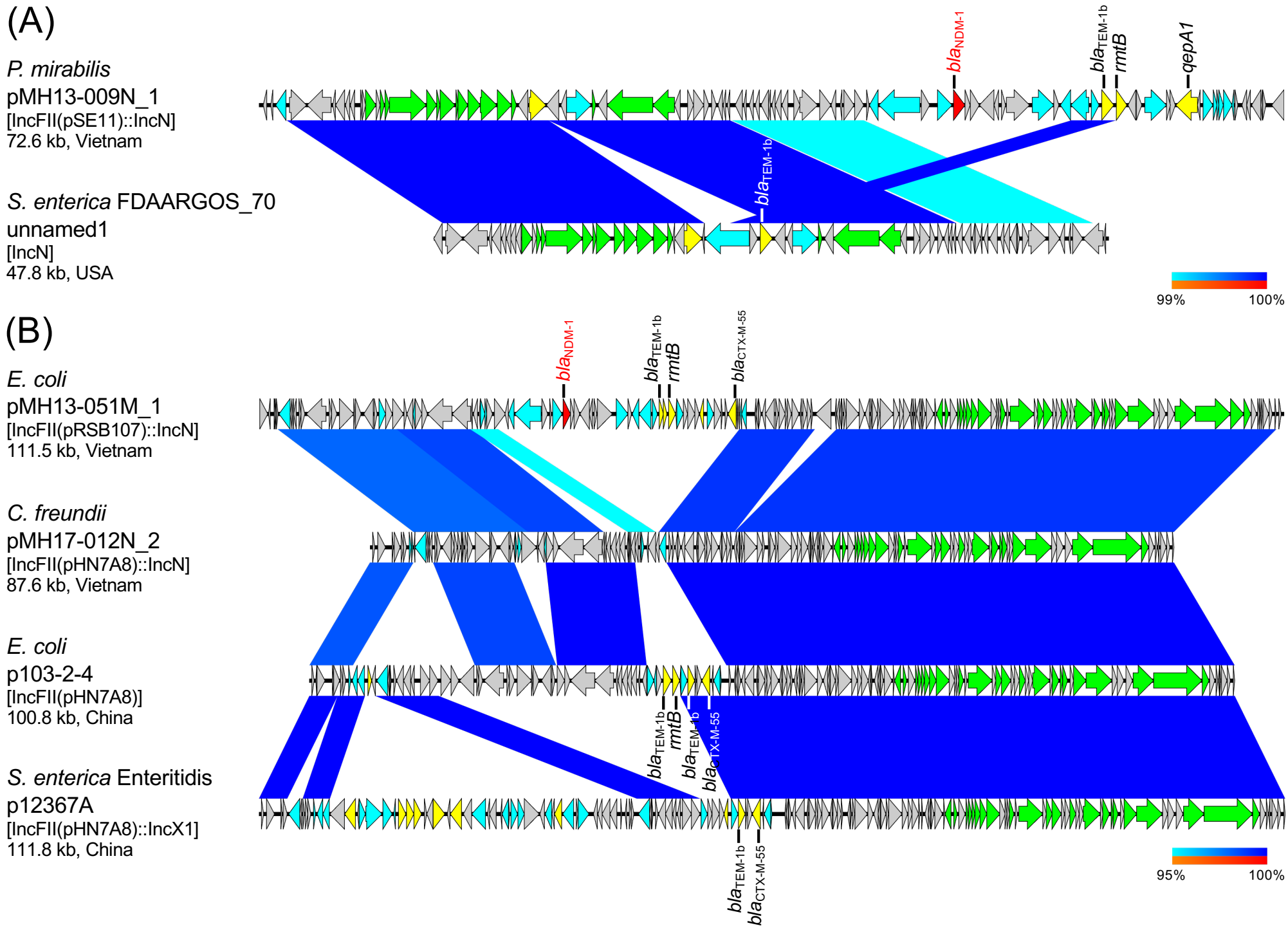

(A)
IS26
Tn5403
ISAba125
blaNDM-1
groES groEL
ISCR21
IS26
(B)
P. mirabilis
pMH13-009N_1
[IncFII(pSE11)::IncN]
72.6 kb, Vietnam
M. morganii
pMH16-367M_1
[IncFII(pSE11)::IncN]
73.1 kb, Vietnam
blaNDM-1
blaTEM-1b
rmtB
qepA1
87%
100%
73 kb
P. mirabilis
MH13-009N
(1 plasmid)
M. morganii
MH16-367M
(1 plasmid)
(C)
(D)
C. freundii
pMH17-012N_1
[IncR]
39.2 kb, Vietnam
E. coli
pMH13-051M_1
[IncFII(pRSB107)::IncN]
111.5 kb, Vietnam
C. freundii
pMH17-012N_2
[IncFII(pHN7A8)::IncN]
87.6 kb, Vietnam
blaCTX-M-55
95%
112 kb
39 kb
88 kb
E. coli
MH13-051M
(5 plasmids)
C. freundii
MH17-012N
(4 plasmids)
ARG
T4SS
MGE
Others

Fig. 2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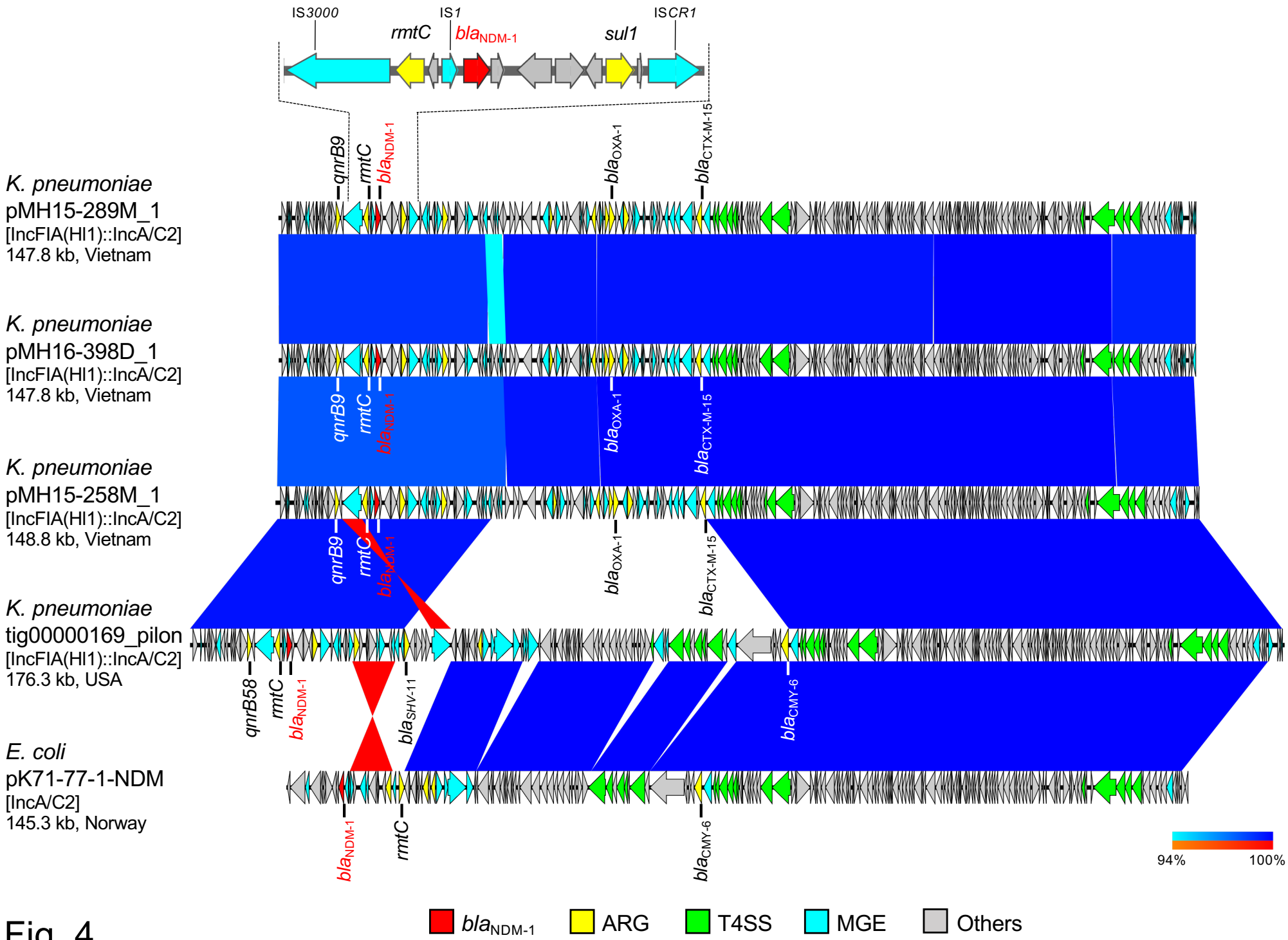
S. enterica FDAARGOS_70
unnamed1
[IncN]
47.8 kb, USA
blaNDM-1
blaTEM-1b
rmtB
qepA1
blaTEM-1b
99%
100%
(B)
E. coli
pMH13-051M_1
[IncFII(pRSB107)::IncN]
111.5 kb, Vietnam
C. freundii
pMH17-012N_2
[IncFII(pHN7A8)::IncN]
87.6 kb, Vietnam
E. coli
p103-2-4
[IncFII(pHN7A8)]
100.8 kb, China
S. enterica Enteritidis
p12367A
[IncFII(pHN7A8)::IncX1]
111.8 kb, China
blaNDM-1
blaTEM-1b
rmtB
blaCTX-M-55
blaTEM-1b
rmtB
blaTEM-1b
blaCTX-M-55
blaTEM-1b
blaCTX-M-55
95%
100%
blaNDM-1
ARG
T4SS
MGE
Others

Fig. 3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IS1
ISCR1
rmtC
bla NDM-1
sul1
K. pneumoniae
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[IncFIA(HI1)::IncA/C2]
147.8 kb, Vietnam
K. pneumoniae
pMH16-398D_1
[IncFIA(HI1)::IncA/C2]
147.8 kb, Vietnam
K. pneumoniae
pMH15-258M_1
[IncFIA(HI1)::IncA/C2]
148.8 kb, Vietnam
K. pneumoniae
tig00000169_pilon
[IncFIA(HI1)::IncA/C2]
176.3 kb, USA
E. coli
pK71-77-1-NDM
[IncA/C2]
145.3 kb, Norway
qnrB9
rmtC
bla NDM-1
bla OXA-1
bla CTX-M-15
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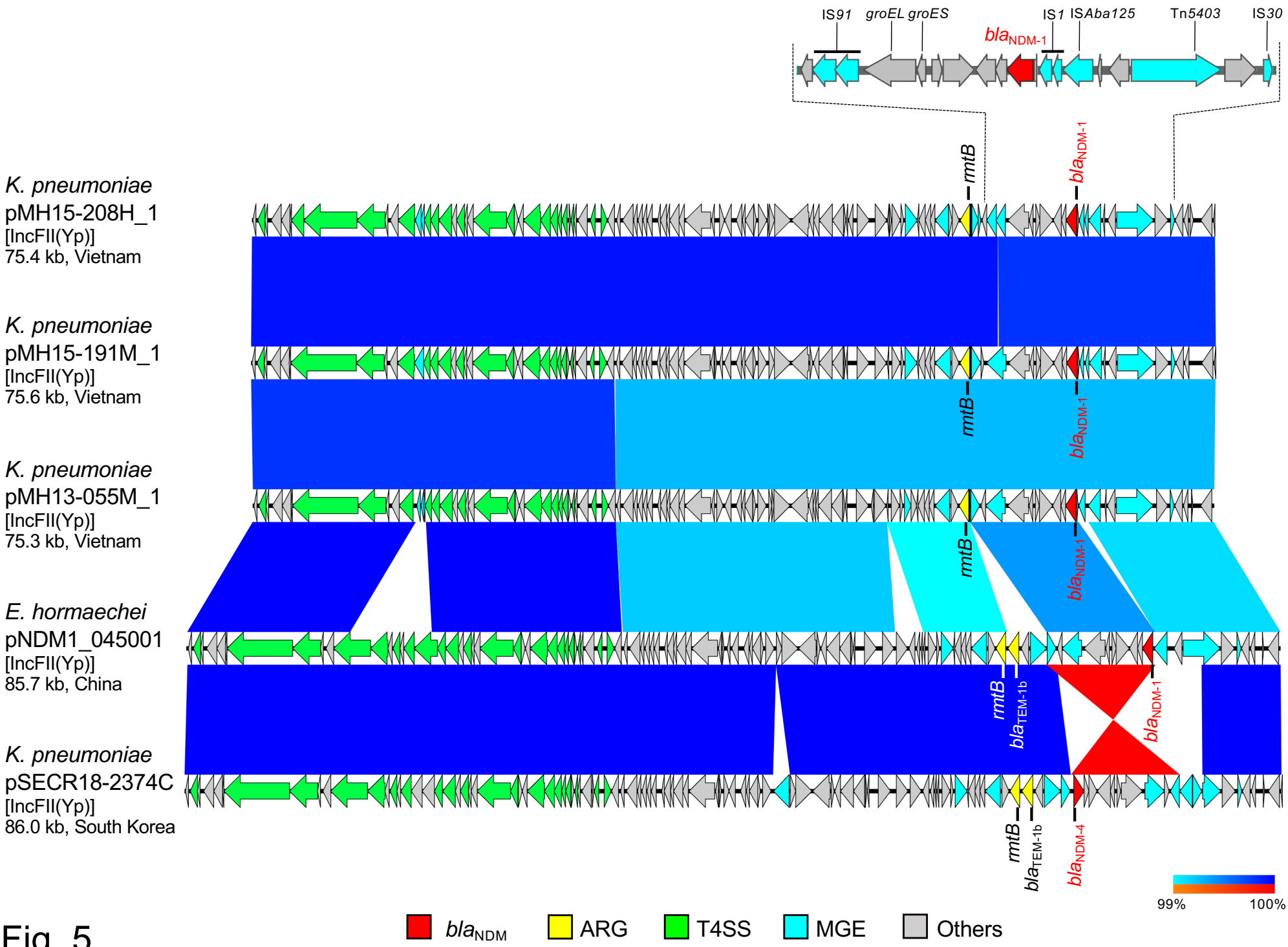
bla SHV-11
bla CMY-6
94%
100%
bla NDM-1
ARG
T4SS
MGE
Others

Fig. 4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groEL groES
IS1
ISAba125
Tn5403
IS30
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rmtB
blaNDM-1
K. pneumoniae
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[IncFII(Yp)]
75.4 kb, Vietnam
K. pneumoniae
pMH15-191M_1
[IncFII(Yp)]
75.6 kb, Vietnam
K. pneumoniae
pMH13-055M_1
[IncFII(Yp)]
75.3 kb, Vietnam
E. hormaechei
pNDM1_045001
[IncFII(Yp)]
85.7 kb, China
K. pneumoniae
pSECR18-2374C
[IncFII(Yp)]
86.0 kb, South Korea
blaTEM-1b
blaNDM-4
99%
100%
blaNDM
ARG
T4SS
MGE
Others

Fig. 5

Species	Isolate	Specimen	Year	MEM MIC	IPM MIC	MLST	Carbapenemase gene	Size	Contig	Coverage	Platform	Accession no.
M. morganii	MH16-367M	blood	2016	1.5	8 (R)	N/A	*bla*$_{NDM-1}$	4,132,245 bp	297	88.2x	MiniSeq	BFCJ00000000
P. mirabilis	MH13-009N	urine	2013	>32 (R)	>32 (R)	N/A	*bla*$_{NDM-1}$	4,116,783 bp	868	296.9x	MiniSeq	BFCK01000000
C. freundii	MH17-012N	urine	2017	2	3	N/A	*bla*$_{NDM-1}$	5,555,817 bp	156	235.1x	HiSeq 4000	BFCL01000000
C. freundii	MH16-522D	pus	2016	>32 (R)	12 (R)	N/A	*bla*$_{NDM-1}$	5,311,537 bp	182	119.4x	MiniSeq	BFCM01000000
E. coli	MH17-008M	blood	2017	4 (R)	6 (R)	457	*bla*$_{KPC-2}$	5,406,936 bp	606	129.6x	HiSeq 4000	BFCN01000000
E. coli	MH13-051M	blood	2013	6 (R)	3	405	*bla*$_{NDM-1}$	5,656,255 bp	259	107.9x	MiniSeq	BFCO00000000
K. pneumoniae	MH17-004D	pus	2017	4 (R)	>32 (R)	11	*bla*$_{KPC-2}$	5,694,352 bp	280	108.3x	HiSeq 4000	BFCP00000000
K. pneumoniae	MH15-289M	blood	2015	>32 (R)	>32 (R)	11	*bla*$_{NDM-1}$	5,771,413 bp	159	158.8x	HiSeq 4000	BFCQ00000000
K. pneumoniae	MH16-398D	pus	2016	8 (R)	2	11	*bla*$_{NDM-1}$	5,640,361 bp	263	87.3x	MiniSeq	BFCR00000000
K. pneumoniae	MH15-258M	blood	2015	6 (R)	2	11	*bla*$_{NDM-1}$	5,868,091 bp	377	444.1x	MiniSeq	BFCS00000000
K. pneumoniae	MH15-208H	sputum	2015	>32 (R)	>32 (R)	395	*bla*$_{NDM-1}$	5,685,569 bp	202	216.5x	HiSeq 4000	BFCT00000000
K. pneumoniae	MH15-191M	blood	2015	>32 (R)	>32 (R)	395	*bla*$_{NDM-1}$	5,787,568 bp	375	350.8x	MiniSeq	BFCU01000000
K. pneumoniae	MH13-055M	blood	2013	>32 (R)	>32 (R)	395	*bla*$_{NDM-1}$	5,718,215 bp	248	255.0x	HiSeq 4000	BFCV00000000
K. pneumoniae	MH17-010D	pus	2017	>32 (R)	>32 (R)	16	*bla*$_{NDM-4}$, *bla*$_{OXA-181}$	5,608,215 bp	160	261.8x	HiSeq 4000	BFCW01000000
K. pneumoniae	MH17-007D	pus	2017	2	3	656	*bla*$_{KPC-2}$	5,637,531 bp	149	131.1x	HiSeq 4000	BFCX00000000
K. pneumoniae	MH16-390M	blood	2016	8 (R)	8 (R)	656	*bla*$_{NDM-1}$	5,638,714 bp	219	97.1x	MiniSeq	BFCY00000000
K. pneumoniae	MH17-556D	pus	2017	>32 (R)	>32 (R)	15	*bla*$_{NDM-4}$	5,533,931 bp	132	126.3x	HiSeq 4000	BFCZ01000000
K. pneumoniae	MH16-335M	blood	2016	16 (R)	16 (R)	15	*bla*$_{NDM-4}$	5,840,408 bp	274	140.2x	MiniSeq	BFDA00000000
K. pneumoniae	MH17-016D	pus	2017	>32 (R)	8 (R)	15	*bla*$_{NDM-4}$, *bla*$_{OXA-48}$	5,871,712 bp	429	64.8x	MiniSeq	BFAI01000000
K. pneumoniae	MH17-017D	pus	2017	6 (R)	8 (R)	15	*bla*$_{NDM-4}$	5,847,449 bp	250	203.0x	HiSeq 4000	BFDB00000000
K. pneumoniae	MH16-444M	blood	2016	6 (R)	4 (R)	15	*bla*$_{KPC-2}$	5,640,233 bp	288	122.7x	MiniSeq	BFDC00000000
K. pneumoniae	MH17-553M	blood	2017	>32 (R)	>32 (R)	15	*bla*$_{KPC-2}$	5,673,369 bp	177	138.2x	HiSeq 4000	BFDD01000000
K. pneumoniae	MH17-536D	pus	2017	3	8 (R)	15	*bla*$_{KPC-2}$	5,652,240 bp	292	74.8x	MiniSeq	BFDE00000000
K. pneumoniae	MH16-519D	pus	2016	6 (R)	12 (R)	15	*bla*$_{KPC-2}$	5,682,307 bp	287	78.7x	MiniSeq	BFDF00000000
K. pneumoniae	MH17-011M	blood	2017	>32 (R)	>32 (R)	15	*bla*$_{KPC-2}$	5,652,498 bp	202	182.1x	HiSeq 4000	BFDG00000000

Table S1

Species	Isolate	Plasmid	Replicon type	ARG (β-lactam)	ARG (aminoglycoside)	ARG (quinolone)	ARG (others)	Size	Coverage	Accession no.
M. morganii	MH16-367M	pMH16-367M_1	IncFII(pSE11) ::IncN	*bla*$_{NDM-1}$, *bla*$_{TEM-1B}$	*rmtB*	*qepA1*	*tet(A)*	73.1 kb	1860.7x	AP018565
P. mirabilis	MH13-009N	pMH13-009N_1	IncFII(pSE11) ::IncN	*bla*$_{NDM-1}$, *bla*$_{TEM-1B}$	*rmtB*	*qepA1*	*tet(A)*	72.6 kb	758.8x	AP018566
C. freundii	MH17-012N	pMH17-012N_1	IncR	*bla*$_{NDM-1}$, *bla*$_{TEM-1B}$	N.D.	N.D.	N.D.	39.2 kb	930.6x	AP018567
C. freundii	MH17-012N	pMH17-012N_2	IncFII(pHN7A8) ::IncN	N.D.	N.D.	N.D.	N.D.	87.6 kb	930.6x	AP018568
C. freundii	MH16-522D	pMH16-522D_1	IncX3	*bla*$_{NDM-1}$, *bla*$_{SHV-12}$	N.D.	N.D.	N.D.	52.7 kb	924.5x	AP018571
E. coli	MH13-051M	pMH13-051M_1	IncFII(pRSB107) ::IncN	*bla*$_{NDM-1}$, *bla*$_{CTX-M-55}$, *bla*$_{TEM-1B}$	*rmtB*	N.D.	*fosA3*	111.5 kb	388.7x	AP018572
K. pneumoniae	MH15-289M	pMH15-289M_1	IncFIA(HI1) ::IncA/C2	*bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*rmtC*, *aac(6')-Ib-cr*, *aac(3)-IIa*, *aac(3)-IId*	*qnrB9*	*catA2*, *catB3*, *sul1*, *dfrA14*	147.8 kb	125.0x	AP018577
K. pneumoniae	MH16-398D	pMH16-398D_1	IncFIA(HI1) ::IncA/C2	*bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*rmtC*, *aac(6')-Ib-cr*, *aac(3)-IIa*, *aac(3)-IId*	*qnrB9*	*catA2*, *catB3*, *sul1*, *dfrA14*	147.7 kb	224.1x	AP018578
K. pneumoniae	MH15-258M	pMH15-258M_1	IncFIA(HI1) ::IncA/C2	*bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*rmtC*, *aac(6')-Ib-cr*, *aac(3)-IIa*, *aac(3)-IId*	*qnrB9*	*catA2*, *catB3*, *sul1*, *dfrA14*	148.8 kb	584.9x	AP018579
K. pneumoniae	MH15-208H	pMH15-208H_1	IncFII(Yp)	*bla*$_{NDM-1}$	*rmtB*	N.D.	N.D.	75.4 kb	252.3x	AP018580
K. pneumoniae	MH15-191M	pMH15-191M_1	IncFII(Yp)	*bla*$_{NDM-1}$	*rmtB*	N.D.	N.D.	75.6 kb	788.4x	AP018581
K. pneumoniae	MH13-055M	pMH13-055M_1	IncFII(Yp)	*bla*$_{NDM-1}$	*rmtB*	N.D.	N.D.	75.3 kb	243.8x	AP018582
K. pneumoniae	MH16-390M	pMH16-390M_1	IncFIA(HI1) ::IncR	*bla*$_{NDM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{TEM-1B}$	*aac(6')-Ib-cr*, *aadA16*	*qnrB6*	*ARR-3*, *sul1*, *dfrA27*	345.7 kb	431.1x	AP018583
K. pneumoniae	MH16-335M	pMH16-335M_1	IncFII(K)	*bla*$_{NDM-4}$, *bla*$_{OXA-9}$, *bla*$_{SHV-106}$, *bla*$_{SHV-28}$	*aac(6')-Ib-cr*, *aadA1*	N.D.	N.D.	105.6 kb	180.6x	AP018584

Table S2