Title Page

Plasmidome analysis of carbapenem-resistant Enterobacteriaceae isolated in Vietnam

Running title. Plasmidome analysis of CRE in Vietnam

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Abstract

Carbapenem-resistant Enterobacteriaceae (CRE) represent a serious threat to public health due to limited management of severe infections and high mortality. The rate of resistance of Enterobacteriaceae 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-1}, spread via plasmids among Enterobacteriaceae in Vietnam. In this study, we performed detection and molecular characterization of bla_{NDM-1}-carrying plasmids in CRE isolated in Vietnam, and identified several possible cases of horizontal transfer of plasmids both within and among species of bacteria. Twenty-five carbapenem-resistant isolates from Enterobacteriaceae clinically isolated in a reference medical institution in Hanoi were sequenced on Illumina short-read sequencers, and 12 isolates harboring bla_{NDM-1} were sequenced on an Oxford Nanopore Technologies long-read sequencer to obtain complete plasmid sequences. 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-1}, leading to multidrug resistance of their bacterial hosts. These results provide insight into the genetic basis of CRE in Vietnam, and could help control nosocomial infections.

Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) represent a serious threat to public health due to limited management of severe infections and high mortality. Carbapenem-hydrolyzing β-lactamase (carbapenemase) genes, such as bla_{NDM}, bla_{KPC}, and bla_{OXA-48}, that confer resistance to a broad range of β-lactams including third-generation cephalosporins and carbapenems are predominantly encoded on conjugative plasmids and have been transferred among Enterobacteriaceae around the world [1]. Therefore, it is very important to detect carbapenemase-carrying plasmids and to aggregate information on the plasmids spreading in each country. According to a report from China [2], CRE isolates simultaneously harbored other important antimicrobial resistance (AMR) genes, including those encoding extended-spectrum β-lactamase (ESBL) that hydrolyze third-generation cephalosporins, plasmid-mediated quinolone resistance, and aminoglycoside resistance genes, suggesting that the isolates harbored plasmids associated with multidrug resistance.

Several studies have revealed the co-existence of clinically relevant AMR genes on the same plasmid. The acquired fluoroquinolone resistance gene *qnr*, which encodes an efflux pump that excretes quinolones, was co-carried with carbapenemase genes in the same plasmids in Taiwan [3], and with bla_{KPC} in Israel [4]. Aminoglycoside acetyltransferases such as AAC(6')-Ib and 16S ribosomal RNA (rRNA) methyltransferases such as ArmA and RmtB are involved in well-known mechanisms of high-level resistance to aminoglycosides. In Switzerland, 16S rRNA methyltransferase genes have been reported to be co-carried with bla_{NDM-1}, in the same plasmids [5]. Another acquired fluoroquinolone resistance gene, *qep* [6], which also encodes a

67 quinolone efflux pump, co-existed with aminoglycoside resistance and ESBL genes in
68 the same plasmids in China [7]. The spread of plasmids co-carrying fluoroquinolone,
69 aminoglycoside, and carbapenem resistance genes both within and among species of
70 bacteria poses a threat to public health. In this study, we report the detailed genetic
71 structures of the plasmids that co-carried bla_{NDM-1} with other important AMR genes; this
72 knowledge provides insight into characteristics and transmission of the plasmids among
73 Enterobacteriaceae in Vietnam.

74 The Vietnamese Ministry of Health developed a national action plan on AMR in 2013
75 [8]. The Viet Nam Resistance (VINARES) project implements measures in the following
76 areas: infection control and healthcare-associated infections (HAI), antimicrobial
77 consumption, and microbiological analysis and reporting capacity [9]. Through VINARES,
78 a surveillance network consisting of 16 central and provincial-level medical institutions
79 was established in 2013. Vietnam has implemented AMR surveillance and stewardship
80 according to the national action plan, and research on AMR using whole-genome
81 sequencing has begun. According to VINARES data, the rate of resistance of *Klebsiella*
82 *pneumoniae* to third-generation cephalosporins, carbapenems, aminoglycoside, and
83 fluoroquinolones was 66.4%, 17.1%, 29.5%, and 53.0%, respectively, in Vietnam [10],
84 whereas the rate was 11.4%, 0.6%, 7.9%, and 9.3%, respectively, in UK [11], and 17.2,
85 4.3%, 4.5%, and 16.8%, respectively, in U.S.A [12]. The rate of resistance to major
86 antimicrobials, including carbapenems, is much higher in Vietnam than in Western
87 countries, but the reasons remain unknown due to the lack of genomic epidemiology
88 research. A point prevalence survey with screening for colonization by CRE in 12 medical
89 institutions in Vietnam revealed that 52% of patients were colonized with CRE [13].
90 Previously, we frequently detected bla_{NDM} genes in CRE isolated in Vietnam [14], and

91 suspected that *bla*$_{NDM}$ genes were spreading via plasmids among Enterobacteriaceae in
92 medical institutions and communities. To examine the prevalence of important AMR
93 genes in plasmids, whole-genome sequencing of CRE isolates is required. Long-read
94 sequencing is useful for constructing complete sequences of whole plasmids and
95 tracking horizontal transfer of AMR plasmids during nosocomial infections [15]. Recently,
96 long-read nanopore sequencing using MinION from Oxford Nanopore Technologies
97 (ONT) has been applied to such investigations [16,17].
98 In this study, we performed nanopore sequencing to achieve comprehensive
99 characterization of plasmids carrying *bla*$_{NDM-1}$ among clinical isolates of
100 Enterobacteriaceae from Vietnam.
101

102 Results

103 A total of 122 isolates of ESBL-producing Enterobacteriaceae were collected in daily
104 diagnosis in a reference medical institution in Hanoi, Vietnam between 2013 and 2017.
105 Twenty-five CRE isolates, including 19 *K. pneumoniae*, two *Escherichia coli*, two
106 *Citrobacter freundii*, one *Morganella morganii*, and one *Proteus mirabilis* isolates, were
107 sequenced on Illumina short-read sequencers, and confirmed actually carbapenemase-
108 positive and carrying carbapenemase genes (12 $bla_{\text{NDM-1}}$, five $bla_{\text{NDM-4}}$, eight $bla_{\text{KPC-2}}$, one
109 $bla_{\text{OXA-48}}$, and one $bla_{\text{OXA-181}}$). As shown in Fig. 1, many isolates had the same types of
110 carbapenemase genes and plasmid replicons. For example, *M. morganii* MH16-367M,
111 *P. mirabilis* MH13-009N, *C. freundii* MH17-012N, and *E. coli* MH13-051M had the IncN
112 replicon and $bla_{\text{NDM-1}}$, suggesting that they shared a common plasmid carrying $bla_{\text{NDM-1}}$
113 that had been transferred via conjugation. Multilocus sequence typing (MLST) classified
114 three *K. pneumoniae* isolates (MH15-289M, MH16-398D, and MH15-258M) as
115 sequence type 11 (ST11), and another three *K. pneumoniae* isolates (MH15-208H,
116 MH15-191M, and MH13-055M) as sequence type 395 (ST395). The isolates that
117 belonged to the same STs had the same replicons and $bla_{\text{NDM-1}}$, suggesting that they
118 shared a common plasmid carrying $bla_{\text{NDM-1}}$ and had been transferred via conjugation or
119 suggesting that their common origin clonally disseminated. Enterobacteriaceae
120 harboring $bla_{\text{NDM-1}}$ were first reported in Vietnam in 2013 [18], suggesting that $bla_{\text{NDM-1}}$ is
121 both important and prevalent in this country; hence, there is an urgent need to elucidate
122 the mechanism of plasmid transmission. Twelve isolates harboring $bla_{\text{NDM-1}}$ and one
123 isolate harboring $bla_{\text{NDM-4}}$ were sequenced on a MinION nanopore sequencer, yielding
124 complete sequences of the plasmids carrying the gene. The results of BLAST search
125 revealed that some plasmids had high sequence identities with each other and with

126 plasmids in the GenBank database (Figs. 2, 3, 4, and 5). However, *bla*$_{NDM-4}$-carrying
127 plasmid pMH16-335M_1 was not identical to any other plasmids (data not shown).
128 *P. mirabilis* MH13-009N and *M. morganii* MH16-367M had only one plasmid, pMH13-
129 009N_1 (72.6 kb) and pMH16-367M_1 (73.1 kb), respectively; the sequences of these
130 plasmids were almost identical, including the *bla*$_{NDM-1}$ and IncFII(pSE11)::IncN hybrid
131 replicon regions (Figs. 2A and 2B). Both of the plasmids carried *bla*$_{NDM-1}$ and an integron
132 cassette that contains the class 1 integrase gene (*intI1*) and the AMR genes (*bla*$_{TEM-1b}$,
133 *rmtB*, and *qepA1*). The same integron cassette was observed in previous reports [6],[19].
134 In another case, *E. coli* MH13-051M had five plasmids, and *C. freundii* MH17-012N had
135 four plasmids. *E. coli* MH13-051M harbored an IncFII(pRSB107)::IncN hybrid plasmid
136 carrying *bla*$_{NDM-1}$, pMH13-051M_1 (111.5 kb), which shared common structures with two
137 plasmids, pMH17-012N_1 (39.2 kb) and pMH17-012N_2 (87.6 kb), both found in *C.*
138 *freundii* MH17-012N (Figs. 2C and 2D). The plasmid pMH13-051M_1 and the smaller
139 IncR plasmid pMH17-012N_1 carried AMR genes (*bla*$_{NDM-1}$ and *bla*$_{TEM-1b}$), and the only
140 pMH13-051M_1 carried *rmtB* and ESBL gene *bla*$_{CTX-M-55}$. The larger
141 IncFII(pHN7A8)::IncN hybrid plasmid pMH17-012N_2 carried no known AMR genes, but
142 did have a set of conjugation-associated type IV secretion system (T4SS) genes, which
143 shared common structures with plasmid pMH13-051M_1.
144 BLAST search showed that *P. mirabilis* pMH13-009N_1 was highly identical with
145 *Salmonella enterica* FDAARGOS_70 plasmid unnamed1 from human in U.S.A. (100.0%
146 identity in 61% region of pMH13-009N_1), which carried *bla*$_{TEM-1B}$ (Fig. 3A). Also, *C.*
147 *freundii* pMH17-012N_2 was highly identical with *E. coli* p103-2-4 in China (99.9%
148 identity in 94% region of pMH17-012N_2) and with *Salmonella enterica* serovar
149 Enteritidis p12367A from human in China (99.9% identity in 73% region of pMH17-

150 012N_2) (Fig. 3B). Though the plasmid pMH17-012N_2 had no AMR gene, the plasmids
151 p103-2-4 and p12367A carried several important AMR genes, such as *rmtB*, bla_{TEM-1b},
152 and $bla_{CTX-M-55}$ (Fig. 3B).

153 Furthermore, as shown in Fig. 4, three *K. pneumoniae* isolates belonging to ST11
154 (MH15-289M, MH16-398D, and MH15-258M) shared identical IncFIA(HI1)::IncA/C2
155 hybrid plasmids carrying bla_{NDM-1} (147–149 kb), and all of these plasmids carried other
156 important AMR genes, such as *qnrB9*, *rmtC*, bla_{OXA-1}, and $bla_{CTX-M-15}$. BLAST search
157 showed that these three plasmids were highly identical with *K. pneumoniae* plasmid
158 tig00000169_pilon in U.S.A. (99.8% identity in 82% region of pMH15-289M_1) and with
159 *E. coli* pK71-77-1-NDM from human in Norway (99.9% identity in 62% region of pMH15-
160 289M_1). The plasmids tig00000169_pilon and pK71-77-1-NDM carried *rmtC*, bla_{NDM-1},
161 and bla_{CMY-6}, and the plasmid tig00000169_pilon further carried *qnrB58* and bla_{SHV-11}.
162 *K. pneumoniae* MH16-398D had 235 sequence variants (214 single-nucleotide variants
163 [SNVs], 14 multiple-nucleotide variants [MNVs], four deletions, two insertions, and one
164 replacement), and *K. pneumoniae* MH15-258M had six sequence variants (five SNVs
165 and one replacement) relative to *K. pneumoniae* MH15-289M.

166 As shown in Fig. 5, another three *K. pneumoniae* isolates belonging to ST395 (MH15-
167 208H, MH15-191M, and MH13-055M) shared identical IncFII(Yp) plasmids carrying
168 bla_{NDM-1} (75–76 kb), and all plasmids carried another important AMR gene, *rmtB*. BLAST
169 search showed that these three plasmids were highly identical with *Enterobacter*
170 *hormaechei* pNDM1_045001 from human in China (99.9% identity in 98% region of
171 pMH15-208H_1) and with *K. pneumoniae* pSECR18-2374C from human in South Korea
172 (99.9% identity in 95% region of pMH15-208H_1). The plasmid pNDM1_045001 carried
173 *rmtB*, bla_{TEM-1b}, and bla_{NDM-1}, and the plasmid pSECR18-2374C carried *rmtB*, bla_{TEM-1b},

174 and *bla*$_{NDM-4}$. *K. pneumoniae* MH15-191M had three sequence variants (two SNVs and
175 one MNV) and *K. pneumoniae* MH13-055M had only one SNV relative to *K. pneumoniae*
176 MH15-208H.

177 The *bla*$_{NDM-1}$-carrying plasmids, *P. mirabilis* pMH13-009N_1 and *M. morganii* pMH16-
178 367M_1 were successfully transferred to recipient *E. coli* at a frequency of 10^{-2}–10^{-3} *in*
179 *vitro*, however, *C. freundii* pMH17-012N_1 and pMH17-012N_2, *E. coli* pMH13-051M_1,
180 *K. pneumoniae* pMH15-258M_1, and *K. pneumoniae* pMH15-208H_1 were not
181 transferred to recipient *E. coli* under our experimental conditions (data not shown).
182

183 Discussion

184 In this study, we performed plasmidome analysis (i.e., comprehensive genetic analysis
185 of plasmids) on plasmids carrying carbapenemase genes in CRE isolated from patients
186 in Vietnam. We focused on CRE isolates harboring *bla*$_{NDM}$, one of the most important
187 carbapenemase genes in the world, and completely sequenced 12 plasmids carrying
188 *bla*$_{NDM-1}$ from *K. pneumoniae*, *E. coli*, *C. freundii*, *M. morganii*, and *P. mirabilis* isolates
189 and one plasmid carrying *bla*$_{NDM-4}$ from *K. pneumoniae* isolate. Some plasmids were
190 nearly identical to each other, suggesting that they represent common and important
191 AMR plasmids disseminated in this country. Moreover, the co-existence of clinically
192 relevant AMR genes, such as ESBL, aminoglycoside resistance, and fluoroquinolone
193 resistance genes, with *bla*$_{NDM-1}$ was observed in plasmids pMH13-009N_1, pMH16-
194 367M_1, pMH15-289M_1, pMH16-398D_1, and pMH15-258M_1. Hence, the spread of
195 the plasmids both within and among species of bacteria will pose a threat to public health.

196 Almost identical IncFII(pSE11)::IncN hybrid plasmids, pMH13-009N_1 and pMH16-
197 367M_1, were found in *P. mirabilis* and *M. morganii* isolates, respectively (Figs. 2A and
198 2B). These plasmid shared common structures with the IncN plasmid from *S. enterica*
199 isolate in U.S.A (Fig. 3A). IncN plasmids are prevalent in the microbiota of animals, and
200 disseminate AMR genes such as *bla*$_{CTX-M-1}$ and *qnr* [20], suggesting that their origin
201 plasmid could be disseminated in communities, including humans, animals, and the
202 environment, in Vietnam and other countries.

203 The IncFII(pRSB107)::IncN hybrid plasmid carrying *bla*$_{NDM-1}$, pMH13-051M_1 in *E. coli*
204 MH13-051M, had partial sequence identity with an IncR plasmid carrying *bla*$_{NDM-1}$,
205 pMH17-012N_1, and an IncFII(pHN7A8)::IncN hybrid plasmid carrying conjugation
206 genes but no known AMR gene, pMH17-012N_2, both in *C. freundii* MH17-012N (Figs.

207 2C and 2D). The plasmid pMH17-012N_2 shared common structures with the plasmids
208 p103-2-4 from *E. coli* in China and p12367A from *Salmonella enterica* in China, carrying
209 many AMR genes (Fig. 3B). Rearrangement of conjugation genes plays an important
210 role in the dissemination of AMR genes between bacteria [21]. Because known IncR
211 plasmids are non-transferable [22], an IncR plasmid carrying AMR genes, such as
212 pMH17-012N_1, and another plasmid carrying conjugation genes, such as pMH17-
213 012N_2, could be assumed to have been fused into a single plasmid with both AMR and
214 conjugation genes, such as pMH13-051M_1, in a bacterium, and then been propagated
215 by conjugation to another bacterium. Reports of IncR plasmids carrying various AMR
216 genes have been increasing. The pool of AMR genes on IncR plasmids is thought to
217 spread to transmissible plasmids via recombination, contributing to the high evolutionary
218 plasticity of bacterial genomes [23]. IncFII(pRSB107)::IncN hybrid plasmids such as
219 pMH13-051M_1 are partially homologous to the pHN7A8 plasmid, which carries several
220 AMR genes, such as $bla_{\text{CTX-M-65}}$, *fosA3*, and *rmtB*, and is widespread in *E. coli* from
221 animals in China [24]. The plasmid pMH13-051M_1 also carries $bla_{\text{CTX-M-55}}$, *fosA3*, and
222 *rmtB* in addition to $bla_{\text{NDM-1}}$ and $bla_{\text{TEM-1B}}$.
223 The IncFII(pSE11)::IncN, IncFII(pRSB107)::IncN, and IncFII(pHN7A8)::IncN hybrid
224 plasmids shown in Figs. 2A and 2C consisted of two replicons belonging to different
225 incompatibility groups, IncFII and IncN. Similarly, IncFIA(HI1)::IncA/C2 hybrid plasmids
226 from three *K. pneumoniae* ST11 isolates consisted of two replicons belonging to different
227 incompatibility groups, IncFIA and IncA/C2 (Fig. 4). Hybrids of multiple replicons
228 belonging to different incompatibility groups represent a plasmid strategy for expansion
229 of host range and dissemination of important acquired genes, including AMR genes,
230 among bacteria [25]. The plasmid pMH15-289M_1 was similar in structure to those

231 reported in U.S.A and Europe, and the plasmid pMH15-208H_1 was similar in structure
232 to those reported in Asia (Figs. 4 and 5). These *K. pneumoniae* and plasmids would have
233 also been spreading in medical institutions and community in Vietnam.

234 It is important to estimate whether *K. pneumoniae* isolates belonging to the same STs
235 were originated from the same clones and spread within the medical institution.
236 According to a report [26], SNVs accumulated in *K. pneumoniae* (ST258) at a rate of 3.9
237 SNVs per year. Our analysis of SNVs of *K. pneumoniae* (ST11) revealed 214 SNVs in
238 MH16-398D relative to MH15-289M; based on the rate of accumulation, it could take
239 many years for these many SNVs to arise. This suggests that many ST11 clones would
240 be prevalent in the medical institutions and communities, and that they have intermixed
241 for a long time. In support of this theory, a report [27] showed that *K. pneumoniae* isolates
242 from medical institutions, sewage, canals, and agricultural waste water were intermixed
243 in the phylogenetic classification. Because ST11 is one of major international epidemic
244 clones of *K. pneumoniae* [28], an ST11 clone carrying this AMR plasmid could have
245 disseminated in a medical institution or local community in Hanoi, Vietnam. Another three
246 isolates of *K. pneumoniae* (ST395) are more clonal because only a few SNVs were
247 present in MH15-191M and MH13-055M relative to MH15-208H.
248

249 Conclusion

250 Plasmidome analysis in this study enabled estimation of the origins and diversity of
251 plasmids carrying *bla*$_{NDM-1}$ in Vietnam. Hybrid analysis with both Illumina short-read and
252 ONT long-read sequencing is a promising method for detecting important AMR plasmids
253 in CRE isolates and controlling nosocomial infections.
254

255 Materials and Methods

256 Bacterial isolates

257 A total of 122 ESBL-producing Enterobacteriaceae isolates were collected from blood,
258 sputum, urine, and pus from patients in daily diagnosis in a reference medical institution
259 in Hanoi, Vietnam between 2013 and 2017. Bacterial species identification and
260 antimicrobial susceptibility testing were performed by Vitek 2 (BioMèrieux) and E-test
261 (BioMèrieux). Carbapenemase production was examined by CarbaNP test according to
262 the Clinical and Laboratory Standards Institute (CLSI) guidelines. Major carbapenemase
263 genes (bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{IMP}, and bla_{VIM}) were detected by a multiplex PCR
264 method [29]. Twenty-five carbapenem-resistant and carbapenemase-positive isolates
265 (19 *K. pneumoniae*, two *E. coli*, two *C. freundii*, one *M. morganii*, and one *P. mirabilis*)
266 were subjected to the whole-genome sequencing analysis described below.

267

268 Whole-genome sequencing and bioinformatics analysis

269 Whole genome sequencing using MiniSeq, MiSeq, and/or HiSeq systems (Illumina)
270 was performed for phylogenetic and MLST analysis, and detection of acquired AMR
271 genes and plasmid replicon types. A maximum-likelihood phylogenetic tree was
272 generated by PhyML from a concatenated core-gene alignment consisting of 13,305
273 SNVs constructed using the Roary pipeline (https://sanger-pathogens.github.io/Roary/).
274 Sequence types, carbapenemase genes, and plasmid replicon types were analyzed
275 using MLST v2.0, ResFinder v3.2 with minimum threshold of 90% identity and 60%
276 coverage, and PlasmidFinder v2.1 with minimum threshold of 90% identity and 60%
277 coverage, respectively, on the Center for Genomic Epidemiology (CGE) server at
278 Technical University of Denmark (http://www.genomicepidemiology.org).

279 Twelve Enterobacteriaceae isolates with *bla*$_{NDM-1}$ (seven *K. pneumoniae*, one *E. coli*,
280 two *C. freundii*, one *M. morganii*, and one *P. mirabilis*) and one *K. pneumoniae* isolate
281 with *bla*$_{NDM-4}$ were further sequenced on a MinION nanopore sequencer (Oxford
282 Nanopore Technologies) using the SQK-RAD002 or SQK-RBK001 kits and R9.4
283 flowcells to obtain complete sequences of plasmids carrying carbapenem resistance
284 genes. *De novo* assembly was performed using Canu v1.5 [30] and Miniasm [31], error
285 correction using Illumina reads with CLC Genomics Workbench v12.0 (QIAGEN), and
286 coding sequences (CDS) annotation using the PATRIC server (https://www.patricbrc.org).
287 Sequence variant detection was performed using CLC Genomics Workbench. Linear
288 comparison of sequences was performed using BLAST and visualized by Easyfig
289 (http://mjsull.github.io/Easyfig/). *bla*$_{NDM-1}$, other important AMR genes (ARG), type IV
290 secretion system (T4SS)-associated genes involved in conjugation that were detected
291 by SecReT4 program [32], and mobile gene elements (MGE) were identified manually
292 from CDS annotations.
293 Draft genome and complete plasmid sequences of CRE isolated in Vietnam have been
294 deposited at GenBank/EMBL/DDBJ under BioProject number PRJDB6655.
295
296 Bacterial conjugation
297 Bacterial conjugation was performed according to the following protocol. The same
298 amount of Luria-Bertani (LB) broth cultures of each donor bacteria and the recipient
299 azide–resistant *E. coli* J53 (*F- met pro Azi*^{r}), were mixed and spotted onto Mueller-Hinton
300 agar and then incubated at 37°C overnight. The mixed cells were recovered and
301 suspended into PBS buffer, plated onto LB agar after 10-fold serial dilution, and
302 incubated at 37°C overnight. Transconjugants were selected on LB agar containing 2

303 μg/mL of meropenem and 100 μg/mL of sodium azide.

304

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Conflicts of Interest

None to declare.

320 Figure Legends

321 Figure 1.

322 CRE isolates sequenced on Illumina systems. Red nodes, but not black nodes, show
323 isolates subsequently sequenced with ONT MinION. Bar lengths represent the number
324 of substitutions per site in the core genome. Minimum inhibitory concentrations (MICs)
325 of meropenem (MEM) and imipenem (IPM) of isolates, years in which the bacteria were
326 isolated, plasmid replicon types, and sizes of bla_{NDM}-carrying plasmids are shown.

327

328 Figure 2.

329 bla_{NDM-1}-carrying plasmids in CRE isolates sequenced using ONT MinION and Illumina
330 systems. Sets of plasmids from *P. mirabilis* MH13-009N and *M. morganii* MH16-367M,
331 and from *E. coli* MH13-051M and *C. freundii* MH17-012N, are shown. (A) and (C) Linear
332 comparison of bla_{NDM-1}-carrying plasmid sequences. Sets of plasmids from *P. mirabilis*
333 MH13-009N and *M. morganii* MH16-367M (A and B), and from *E. coli* MH13-051M and
334 *C. freundii* MH17-012N (C and D), are shown. Red, yellow, green, blue, and gray arrows
335 indicate bla_{NDM-1}, other important AMR genes (ARG), type IV secretion system (T4SS)-
336 associated genes involved in conjugation, mobile gene elements (MGE), and other
337 genes, respectively.

338

339 Figure 3.

340 (A) and (B) Linear comparison of plasmid sequences from Vietnam and other countries.
341 *P. mirabilis* pMH13-009N_1 and *Salmonella enterica* FDAARGOS_70 plasmid
342 unnamed1 (accession: CP026053) (A), and *C. freundii* pMH17-012N_2, *E. coli* p103-2-
343 4 (accession: CP034846), and *Salmonella enterica* serovar Enteritidis p12367A

344 (accession: CP041177) (B) are shown.

345

346 Figure 4.

347 Linear comparison of *bla*$_{NDM-1}$-carrying plasmid sequences from Vietnam and other

348 countries. *K. pneumoniae* pMH15-289M_1, *K. pneumoniae* pMH16-398D_1, *K.*

349 *pneumoniae* pMH15-258M_1, *K. pneumoniae* tig00000169_pilon (accession:

350 CP021952), and *E. coli* pK71-77-1-NDM (accession: CP040884) are shown.

351

352 Figure 5.

353 Linear comparison of *bla*$_{NDM}$-carrying plasmid sequences from Vietnam and other

354 countries. *K. pneumoniae* pMH15-208H_1, *K. pneumoniae* pMH15-191M_1, *K.*

355 *pneumoniae* pMH13-055M_1, *E. hormaechei* pNDM1_045001 (accession: CP043383),

356 and *K. pneumoniae* pSECR18-2374C (accession: CP041930) are shown.

357

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464

Fig. 1

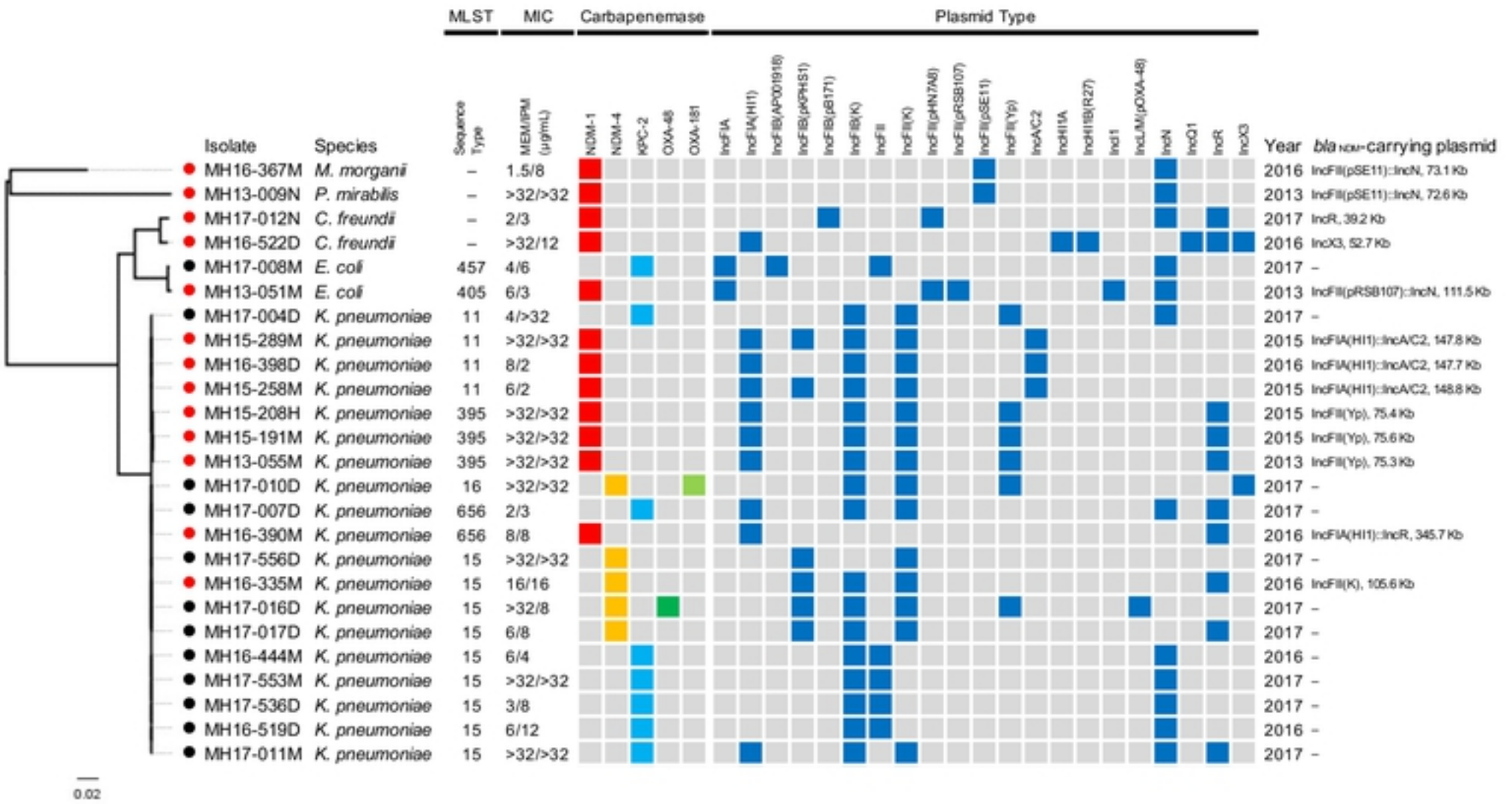

MLST
MIC
Carbapenemase
Plasmid Type
Isolate
Species
Sequence Type
MEM/IPM (μg/mL)
NDM-1
NDM-4
KPC-2
OXA-48
OXA-181
IncFIA
IncFIA(HI1)
IncFIB(AP001918)
IncFIB(pKPHS1)
IncFIB(pB171)
IncFIB(K)
IncFII
IncFII(K)
IncFII(pHN7A8)
IncFII(pRSB107)
IncFII(pSE11)
IncFII(Yp)
IncA/C2
IncHI1A
IncHI1B(R27)
IncI1
IncL/M(pOXA-48)
IncN
IncQ1
IncR
IncX3
Year
bla NDM-carrying plasmid
MH16-367M M. morganii – 1.5/8 2016 IncFII(pSE11)::IncN, 73.1 Kb
MH13-009N P. mirabilis – >32/>32 2013 IncFII(pSE11)::IncN, 72.6 Kb
MH17-012N C. freundii – 2/3 2017 IncR, 39.2 Kb
MH16-522D C. freundii – >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 –
MH15-289M K. pneumoniae 11 >32/>32 2015 IncFIA(HI1)::IncA/C2, 147.8 Kb
MH16-398D K. pneumoniae 11 8/2 2016 IncFIA(HI1)::IncA/C2, 147.7 Kb
MH15-258M K. pneumoniae 11 6/2 2015 IncFIA(HI1)::IncA/C2, 148.8 Kb
MH15-208H K. pneumoniae 395 >32/>32 2015 IncFII(Yp), 75.4 Kb
MH15-191M K. pneumoniae 395 >32/>32 2015 IncFII(Yp), 75.6 Kb
MH13-055M K. pneumoniae 395 >32/>32 2013 IncFII(Yp), 75.3 Kb
MH17-010D K. pneumoniae 16 >32/>32 2017 –
MH17-007D K. pneumoniae 656 2/3 2017 –
MH16-390M K. pneumoniae 656 8/8 2016 IncFIA(HI1)::IncR, 345.7 Kb
MH17-556D K. pneumoniae 15 >32/>32 2017 –
MH16-335M K. pneumoniae 15 16/16 2016 IncFII(K), 105.6 Kb
MH17-016D K. pneumoniae 15 >32/8 2017 –
MH17-017D K. pneumoniae 15 6/8 2017 –
MH16-444M K. pneumoniae 15 6/4 2016 –
MH17-553M K. pneumoniae 15 >32/>32 2017 –
MH17-536D K. pneumoniae 15 3/8 2017 –
MH16-519D K. pneumoniae 15 6/12 2016 –
MH17-011M K. pneumoniae 15 >32/>32 2017 –
0.02

Fig. 2

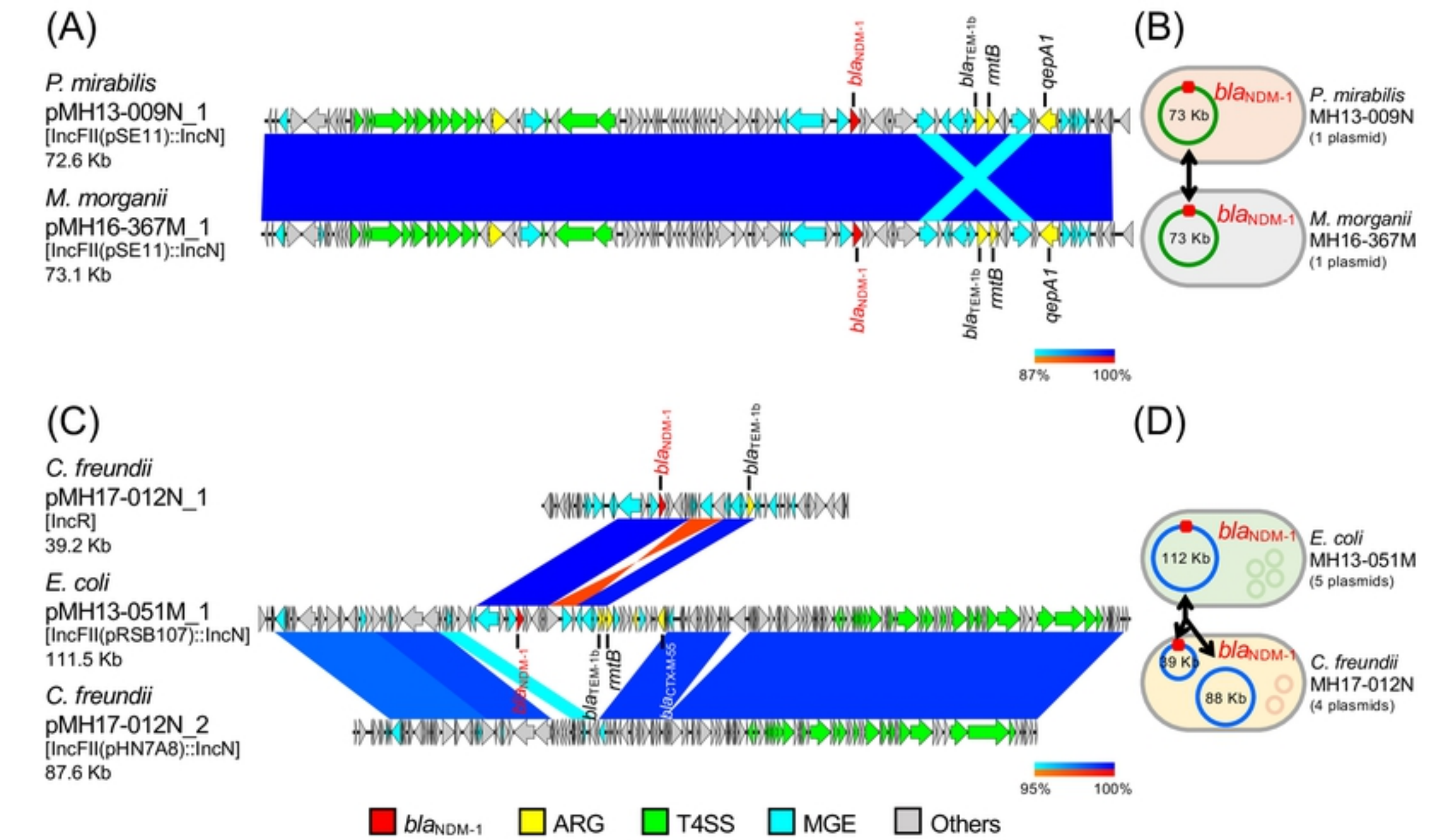

(A)
P. mirabilis
pMH13-009N_1
[IncFII(pSE11)::IncN]
72.6 Kb
M. morganii
pMH16-367M_1
[IncFII(pSE11)::IncN]
73.1 Kb
blaNDM-1
blaTEM-1b
rmtB
qepA1
87%
100%
(B)
73 Kb
P. mirabilis
MH13-009N
(1 plasmid)
M. morganii
MH16-367M
(1 plasmid)
(C)
C. freundii
pMH17-012N_1
[IncR]
39.2 Kb
E. coli
pMH13-051M_1
[IncFII(pRSB107)::IncN]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

Fig. 3

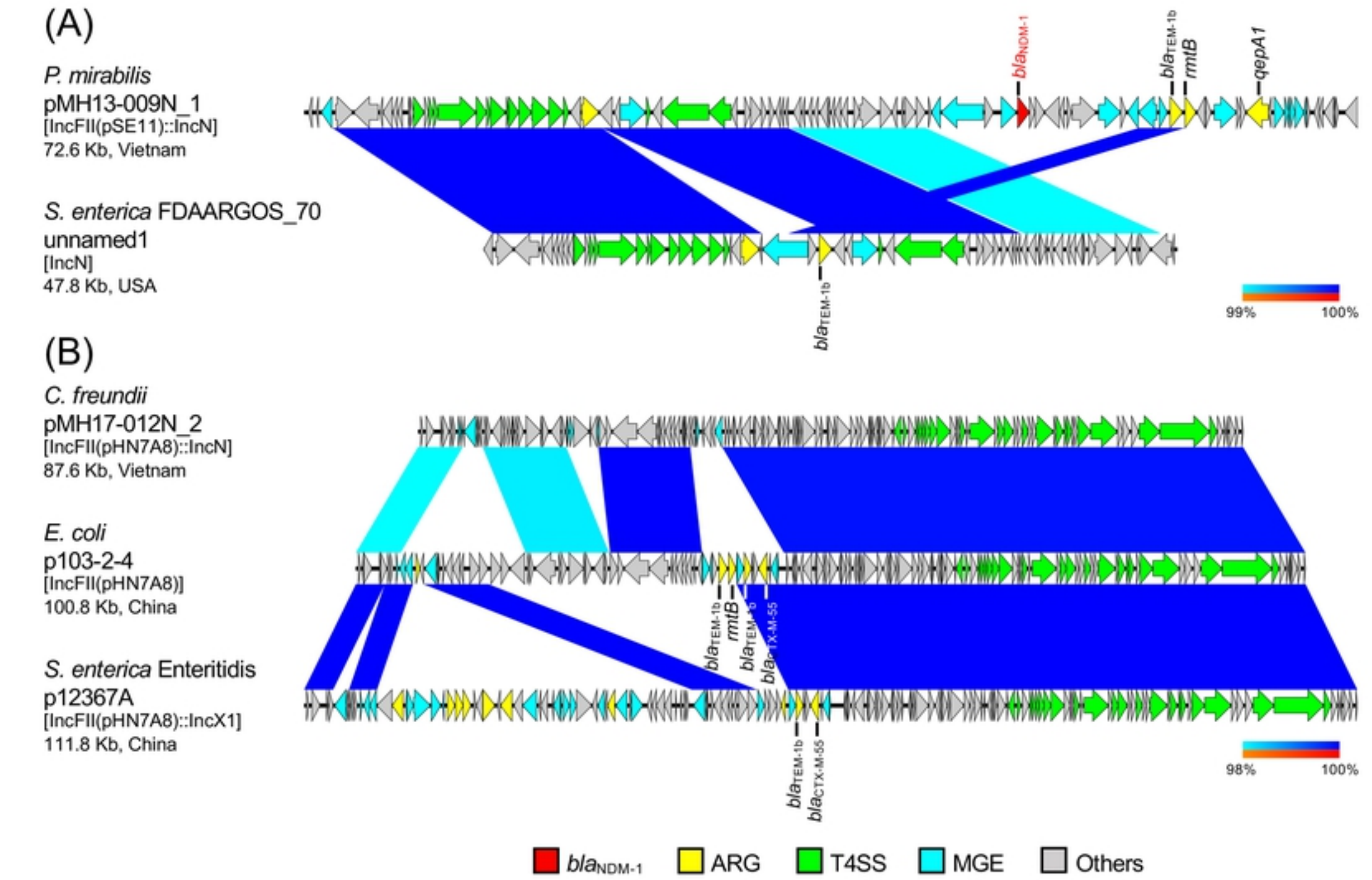

(A)
P. mirabilis
pMH13-009N_1
[IncFII(pSE11)::IncN]
72.6 Kb, Vietnam
S. enterica FDAARGOS_70
unnamed1
[IncN]
47.8 Kb, USA
blaNDM-1
blaTEM-1b
rmtB
qepA1
blaTEM-1b
99%
100%
(B)
C. freundii
pMH17-012N_2
[IncFII(pHN7A8)::IncN]
87.6 Kb, Vietnam
E. coli
p103-2-4
[IncFII(pHN7A8)]
100.8 Kb, China
S. enterica Enteritidis
p12367A
[IncFII(pHN7A8)::IncX1]
111.8 Kb, China
blaTEM-1b
rmtB
blaTEM-1b
blaCTX-M-55
blaTEM-1b
blaCTX-M-55
98%
100%
blaNDM-1
ARG
T4SS
MGE
Others

Fig. 4

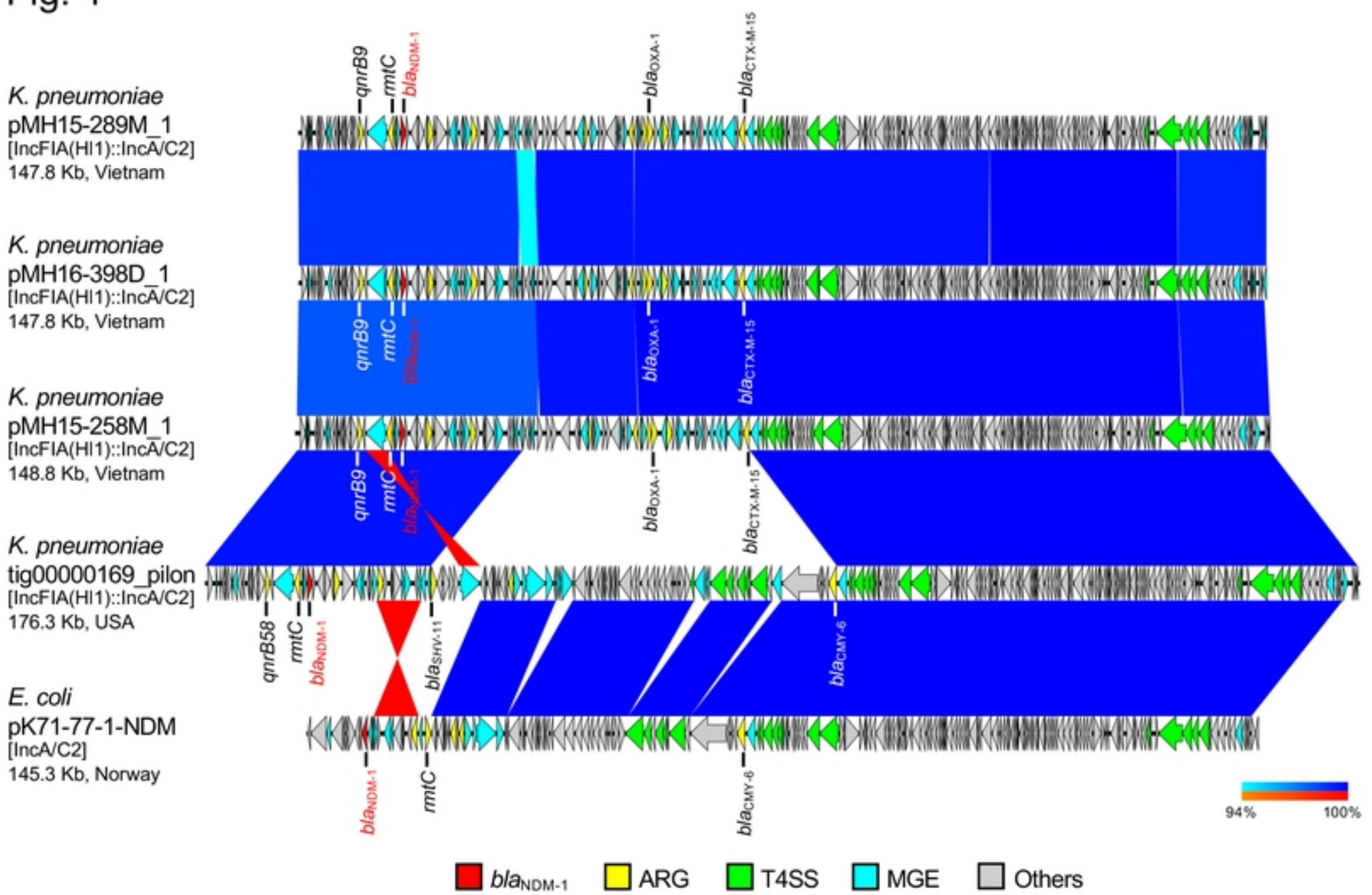
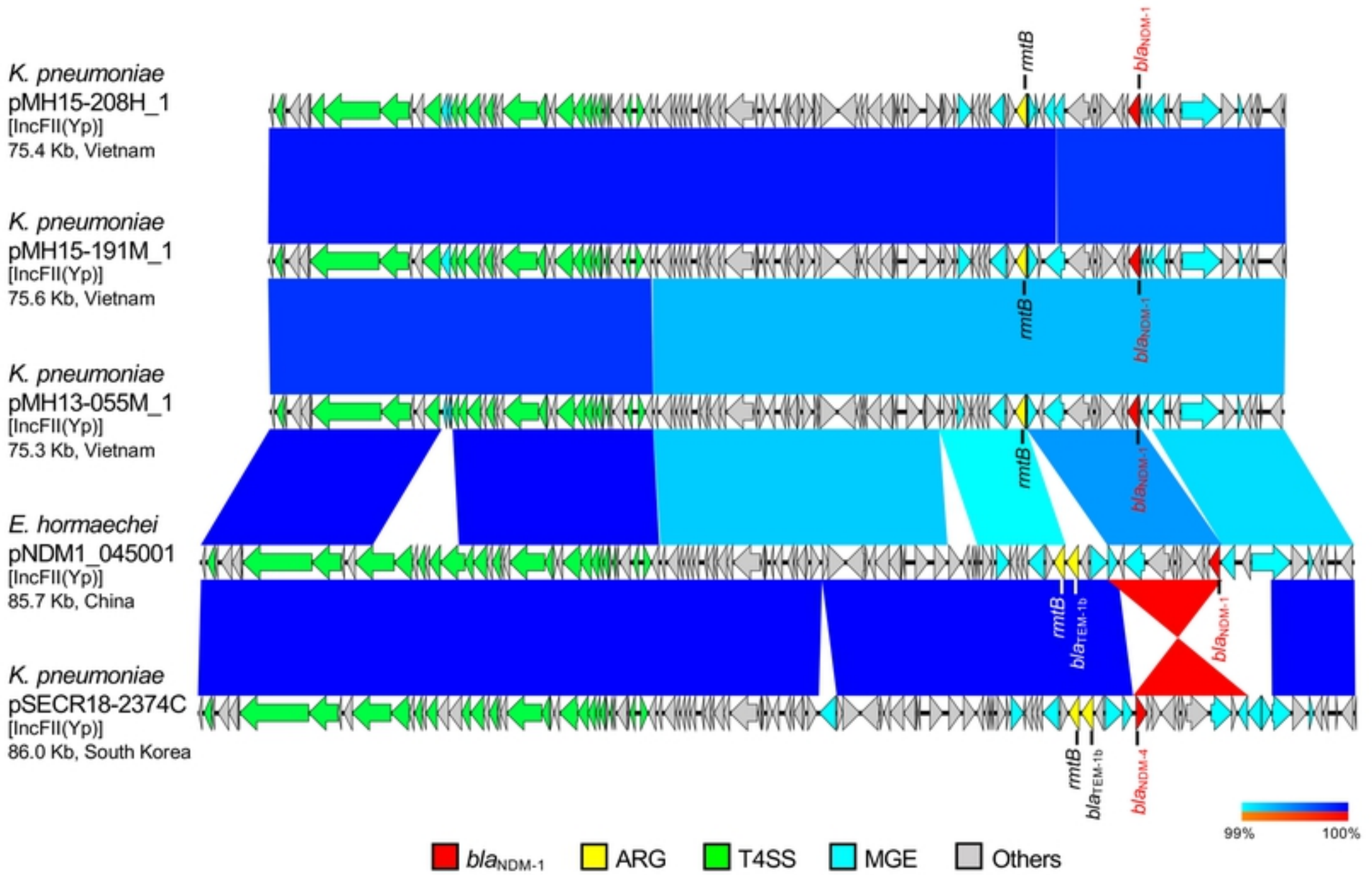

K. pneumoniae
pMH15-289M_1
[IncFIA(HI1)::IncA/C2]
147.8 Kb, Vietnam
K. pneumoniae
pMH16-398D_1
[IncFIA(HI1)::IncA/C2]
147.8 Kb, Vietnam
K. pneumoniae
pMH15-258M_1
[IncFIA(HI1)::IncA/C2]
148.8 Kb, Vietnam
K. pneumoniae
tig00000169_pilon
[IncFIA(HI1)::IncA/C2]
176.3 Kb, USA
E. coli
pK71-77-1-NDM
[IncA/C2]
145.3 Kb, Norway
qnrB9
rmtC
blaNDM-1
blaOXA-1
blaCTX-M-15
qnrB58
blaSHV-11
blaCMY-6
94%
100%
blaNDM-1
ARG
T4SS
MGE
Others

Fig. 5


K. pneumoniae
pMH15-208H_1
[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
rmtB
blaNDM-1
blaTEM-1b
blaNDM-4
99%
100%
blaNDM-1
ARG
T4SS
MGE
Others