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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

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

The Vietnamese Ministry of Health developed a national action plan on AMR in 2013 [8]. The Viet Nam Resistance (VINARES) project implements measures in the following areas: infection control and healthcare-associated infections (HAI), antimicrobial consumption, and microbiological analysis and reporting capacity [9]. Through VINARES, a surveillance network consisting of 16 central and provincial-level medical institutions was established in 2013. Vietnam has implemented AMR surveillance and stewardship according to the national action plan, and research on AMR using whole-genome sequencing has begun. According to VINARES data, the rate of resistance of *Klebsiella pneumoniae* to third-generation cephalosporins, carbapenems, aminoglycoside, and fluoroquinolones was 66.4%, 17.1%, 29.5%, and 53.0%, respectively, in Vietnam [10], whereas the rate was 11.4%, 0.6%, 7.9%, and 9.3%, respectively, in UK [11], and 17.2, 4.3%, 4.5%, and 16.8%, respectively, in U.S.A [12]. The rate of resistance 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 point prevalence survey with screening for colonization by CRE in 12 medical institutions in Vietnam revealed that 52% of patients were colonized with CRE [13]. Previously, we frequently detected *bla*$_{NDM}$ genes in CRE isolated in Vietnam [14], and

suspected that *bla*$_{NDM}$ genes were spreading via plasmids among Enterobacteriaceae in medical institutions and communities. To examine the prevalence of important AMR genes in plasmids, whole-genome sequencing of CRE isolates is required. Long-read sequencing is useful for constructing complete sequences of whole plasmids and tracking horizontal transfer of AMR plasmids during nosocomial infections [15]. Recently, long-read nanopore sequencing using MinION from Oxford Nanopore Technologies (ONT) has been applied to such investigations [16,17].

In this study, we performed nanopore sequencing to achieve comprehensive characterization of plasmids carrying *bla*$_{NDM-1}$ among clinical isolates of Enterobacteriaceae from Vietnam.

Results

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

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

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

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

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

Discussion

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

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

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

2C and 2D). The plasmid pMH17-012N_2 shared common structures with the plasmids p103-2-4 from *E. coli* in China and p12367A from *Salmonella enterica* in China, carrying many AMR genes (Fig. 3B). Rearrangement of conjugation genes plays an important role in the dissemination of AMR genes between bacteria [21]. Because known IncR plasmids are non-transferable [22], an IncR plasmid carrying AMR genes, such as pMH17-012N_1, and another plasmid carrying conjugation genes, such as pMH17-012N_2, could be assumed to have been fused into a single plasmid with both AMR and conjugation genes, such as pMH13-051M_1, in a bacterium, and then been propagated by conjugation to another bacterium. Reports of IncR plasmids carrying various AMR genes have been increasing. The pool of AMR genes on IncR plasmids is thought to spread to transmissible plasmids via recombination, contributing to the high evolutionary plasticity of bacterial genomes [23]. IncFII(pRSB107)::IncN hybrid plasmids such as pMH13-051M_1 are partially homologous to the pHN7A8 plasmid, which carries several AMR genes, such as $bla_{\text{CTX-M-65}}$, *fosA3*, and *rmtB*, and is widespread in *E. coli* from animals in China [24]. The plasmid pMH13-051M_1 also carries $bla_{\text{CTX-M-55}}$, *fosA3*, and *rmtB* in addition to $bla_{\text{NDM-1}}$ and $bla_{\text{TEM-1B}}$.

The IncFII(pSE11)::IncN, IncFII(pRSB107)::IncN, and IncFII(pHN7A8)::IncN hybrid plasmids shown in Figs. 2A and 2C consisted of two replicons belonging to different incompatibility groups, IncFII and IncN. Similarly, IncFIA(HI1)::IncA/C2 hybrid plasmids from three *K. pneumoniae* ST11 isolates consisted of two replicons belonging to different incompatibility groups, IncFIA and IncA/C2 (Fig. 4). Hybrids of multiple replicons belonging to different incompatibility groups represent a plasmid strategy for expansion of host range and dissemination of important acquired genes, including AMR genes, 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

Conclusion

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

Materials and Methods

Bacterial isolates

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

Whole-genome sequencing and bioinformatics analysis

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

Twelve Enterobacteriaceae isolates with *bla*$_{NDM-1}$ (seven *K. pneumoniae*, one *E. coli*, two *C. freundii*, one *M. morganii*, and one *P. mirabilis*) and one *K. pneumoniae* isolate with *bla*$_{NDM-4}$ were further sequenced on a MinION nanopore sequencer (Oxford Nanopore Technologies) using the SQK-RAD002 or SQK-RBK001 kits and R9.4 flowcells to obtain complete sequences of plasmids carrying carbapenem resistance genes. *De novo* assembly was performed using Canu v1.5 [30] and Miniasm [31], error correction using Illumina reads with CLC Genomics Workbench v12.0 (QIAGEN), and coding sequences (CDS) annotation using the PATRIC server (https://www.patricbrc.org). Sequence variant detection was performed using CLC Genomics Workbench. Linear comparison of sequences was performed using BLAST and visualized by Easyfig (http://mjsull.github.io/Easyfig/). *bla*$_{NDM-1}$, other important AMR genes (ARG), type IV secretion system (T4SS)-associated genes involved in conjugation that were detected by SecReT4 program [32], and mobile gene elements (MGE) were identified manually from CDS annotations.

Draft genome and complete plasmid sequences of CRE isolated in Vietnam have been deposited at GenBank/EMBL/DDBJ under BioProject number PRJDB6655.

Bacterial conjugation

Bacterial conjugation was performed according to the following protocol. The same amount of Luria-Bertani (LB) broth cultures of each donor bacteria and the recipient azide–resistant *E. coli* J53 (*F- met pro Azi*^{r}), were mixed and spotted onto Mueller-Hinton agar and then incubated at 37°C overnight. The mixed cells were recovered and suspended into PBS buffer, plated onto LB agar after 10-fold serial dilution, and 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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316

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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Fig. 1

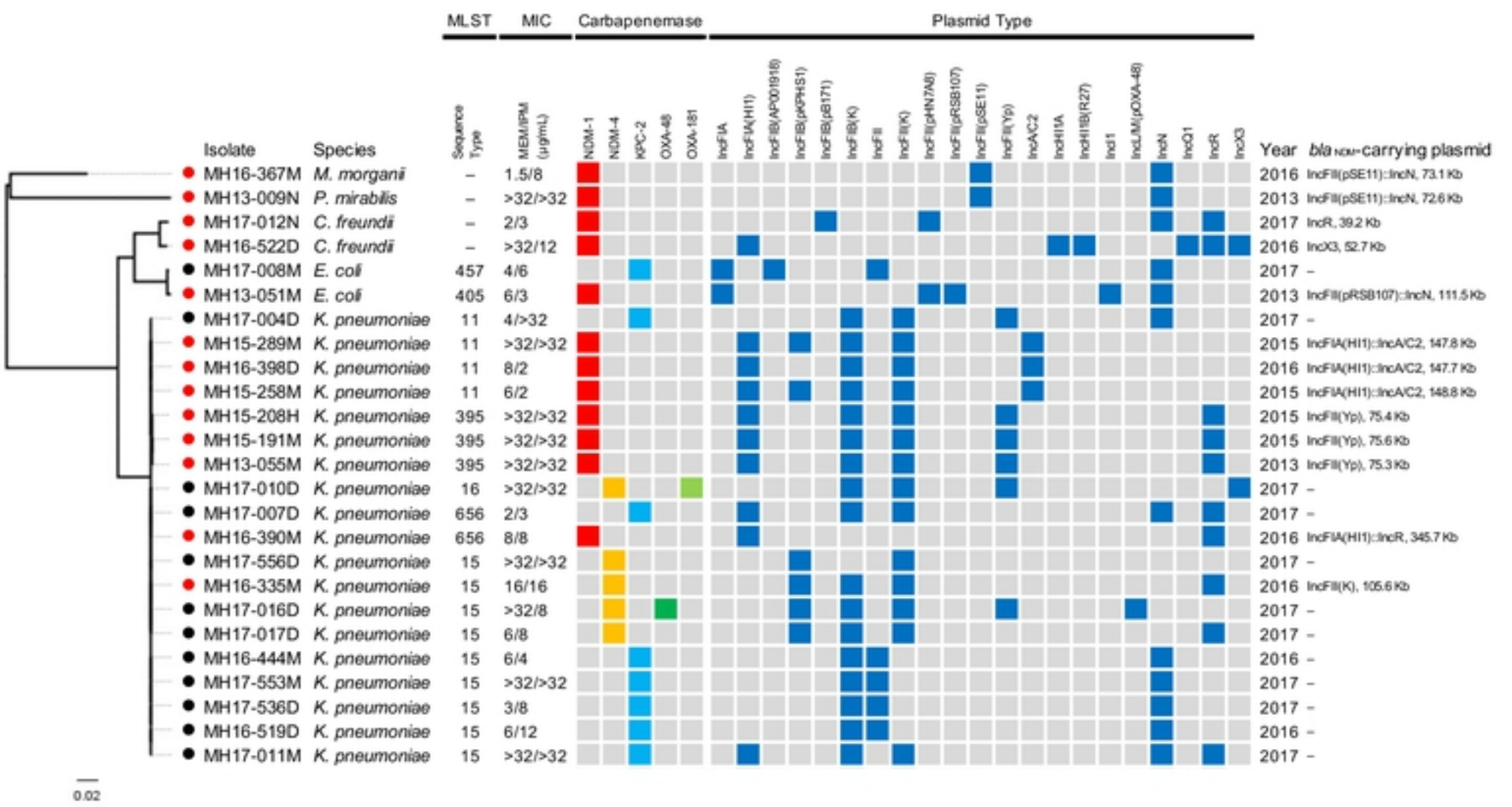

Fig. 2

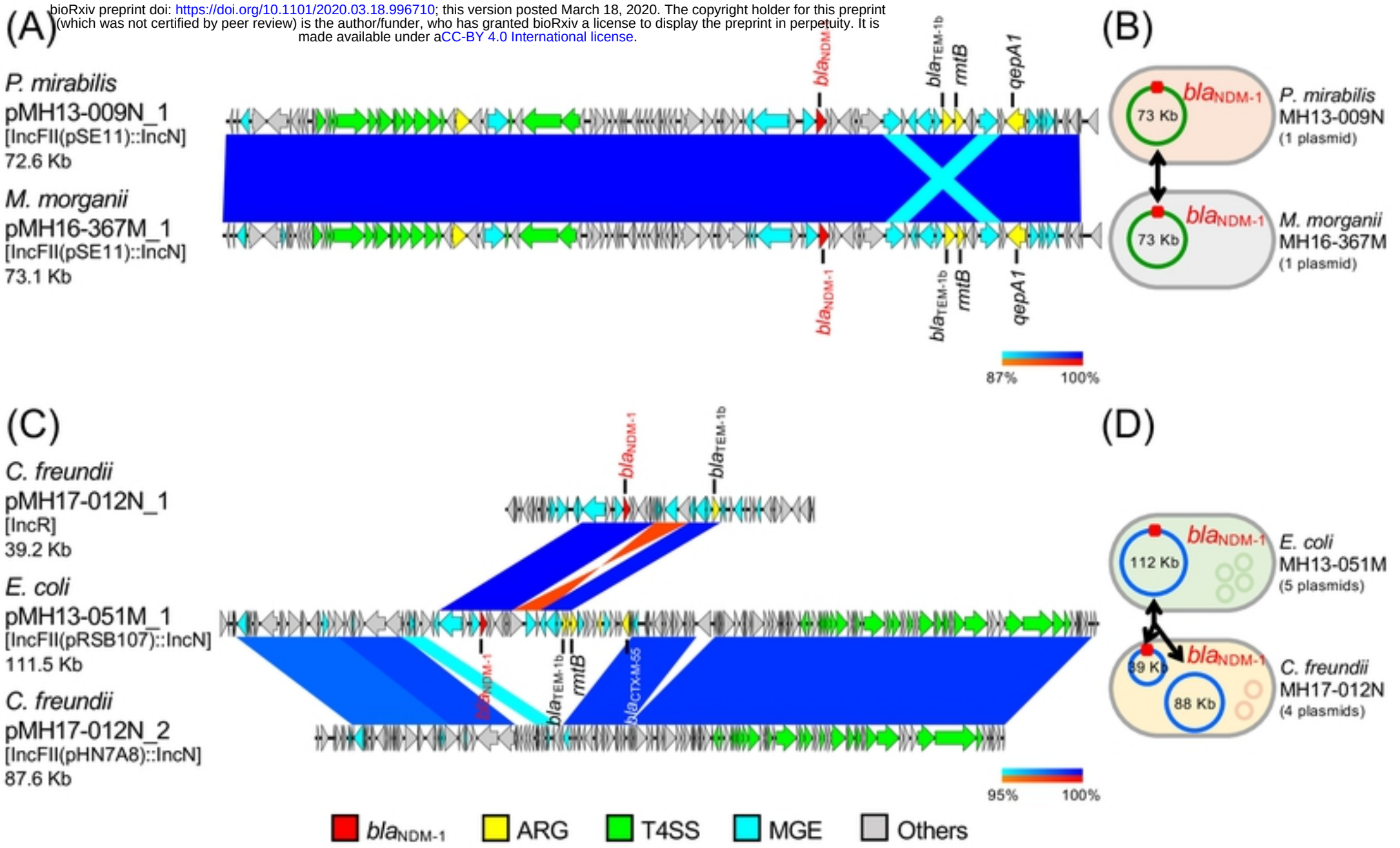

Fig. 3

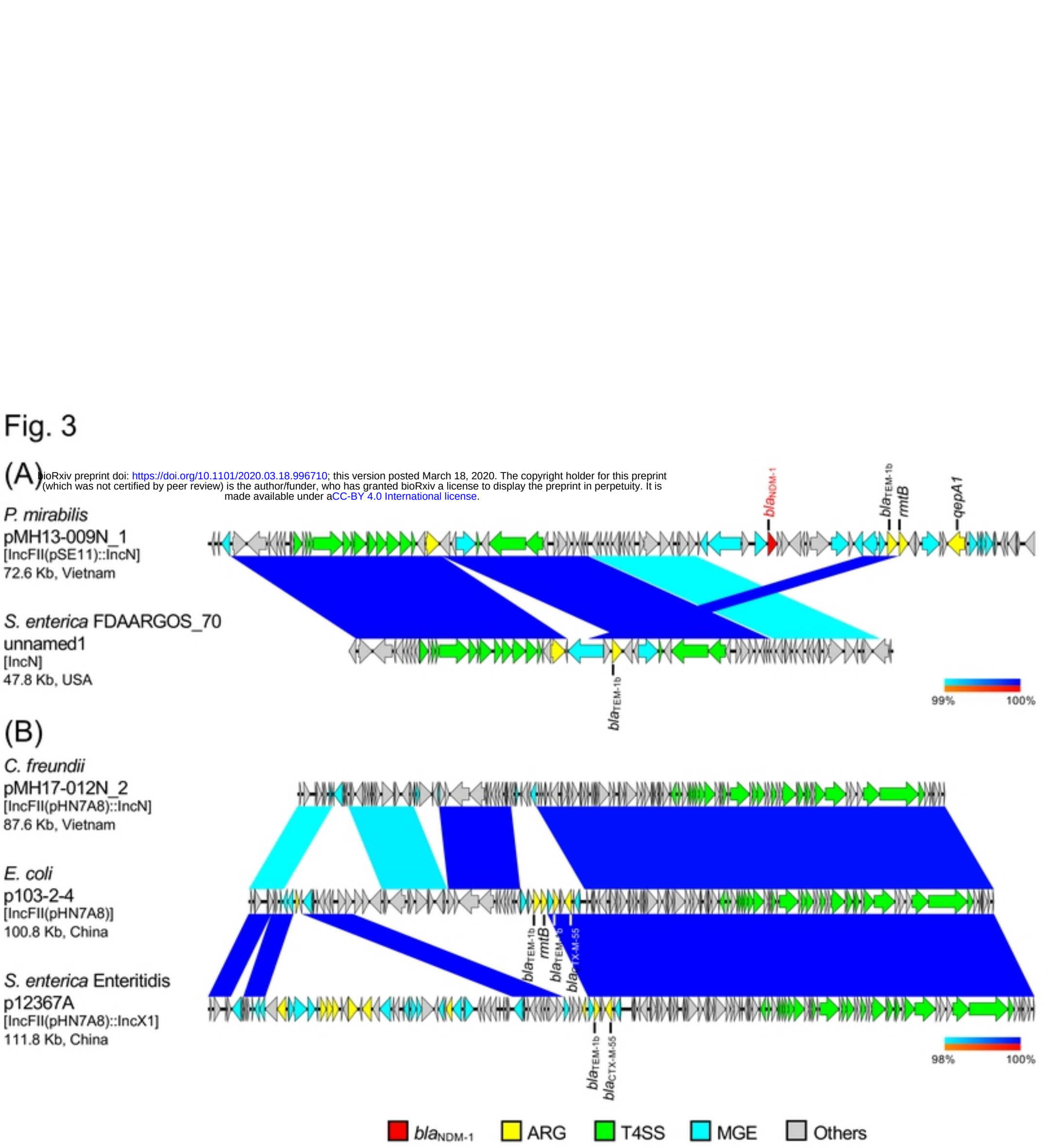

Fig. 4

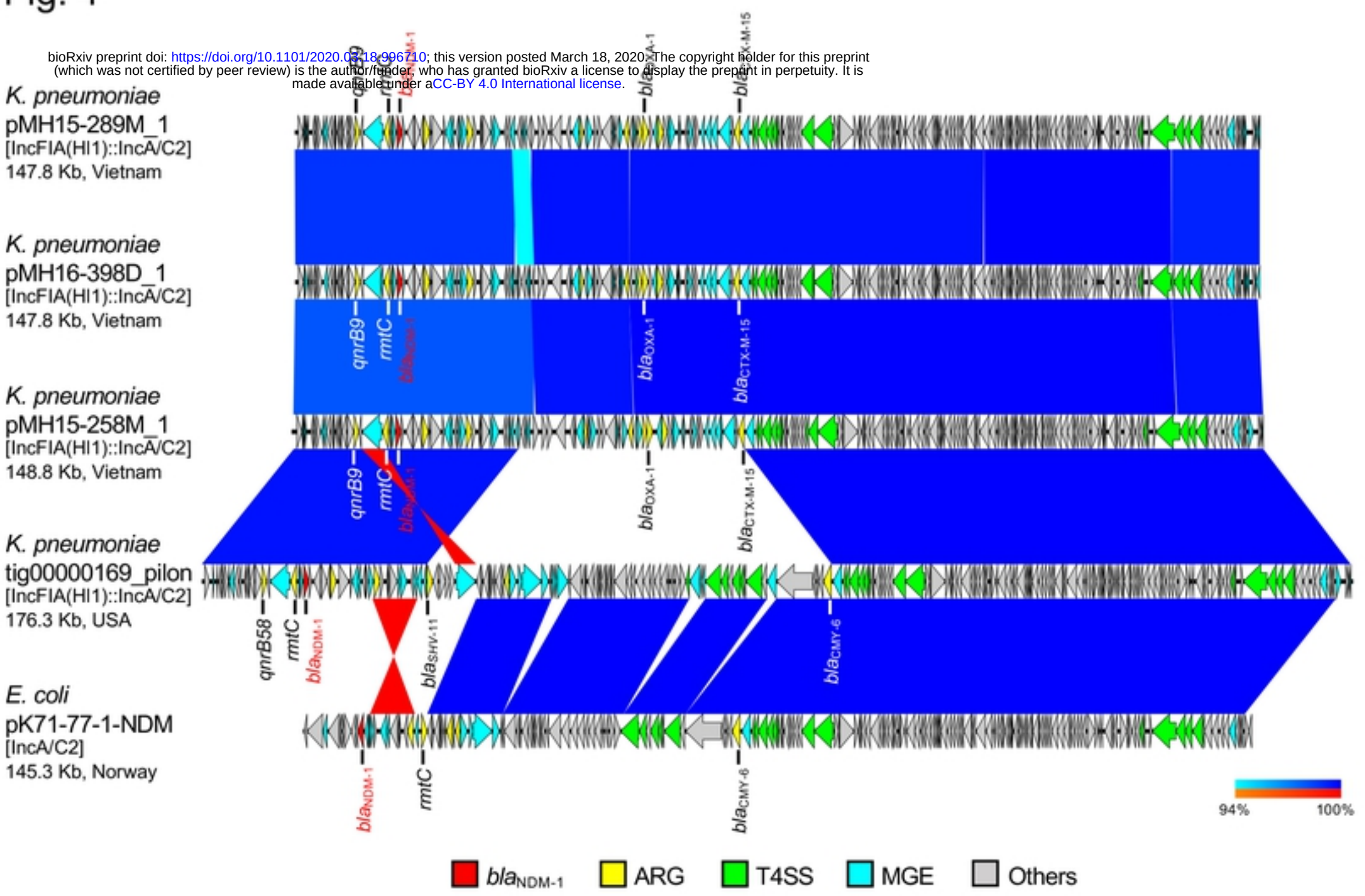

Fig. 5