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Bird's-eye MApping of plasmids (BeMAp) for visualization and comparison of genomic structures of different plasmids by mapping antimicrobial resistance genes on spreadsheets

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ABSTRACT

Effective classification and visualization of multiple antimicrobial resistance plasmids can be challenging, and few tools to analyze similarities among plasmids depending on the location of genes are available. We created a new plasmid mapping program called Bird's-eye MApping of plasmids (BeMAp) to map antimicrobial resistance genes across multiple plasmids onto a spreadsheet and visualize their similarities based on gene types, locations, alignments, and organization. We analyzed plasmids containing various antimicrobial resistance genes, together with genes coding for IMP-type metallo-β-lactamases. Moreover, the mapping of plasmids with antimicrobial resistance genes and Incompatibility (Inc) groups showed that clustered plasmids with a similar organization of antimicrobial resistance genes were not always classified into the same Inc groups, indicating that the program displays multiple plasmids regardless of the Inc group classification. Our results showed that this calculation protocol and mapping strategy could provide a valuable tool for the practical and convenient visualization and comparison of the genomic structure of multiple plasmids in parallel.

1. Introduction

The threat of antimicrobial resistance to modern medicine and the sustainability of global health is increasing (World Health Organization, 2015). In 2015, the World Health Assembly adopted the Global Action Plan on Antimicrobial Resistance and, subsequently, the General Assembly of the United Nations called for globally coordinated operations via the One Health approach (Laxminarayan et al., 2020). Although authorities have been tackling the problem according to the global plan and approach, multidrug-resistant microbes continue to proliferate (van Duin et al., 2020; Jernigan et al., 2020).

Bacterial plasmids are usually double-stranded circular DNA molecules that autonomously replicate in synchrony with bacterial cell division. Plasmids carrying multiple antimicrobial resistance genes confer a variety of genotypes and antimicrobial resistance phenotypes through transfer across bacteria (Nikaido, 2009; Partridge et al., 2018). Antimicrobial resistance genes accumulate onto various plasmids via integrons and are transferred via mobile genetic elements, such as insertion sequences and transposons (Partridge, 2011). In addition, these plasmids can be fused or split, yielding a variety of combinations of antimicrobial resistance genes on plasmids (Kawamura et al., 2017). Moreover, the translocation of antimicrobial resistance genes across plasmids and chromosomes accelerates the spread of these genes. It is necessary to classify plasmids that exhibit multiple, complicated combinations of antimicrobial resistance genes to explore their origin and transmission and elucidate and prevent their spread among antimicrobial resistant bacteria in the context of medicine and public health.

Incompatibility (Inc) grouping is one of the major classifications of plasmids (Carattoli et al., 2005). Through conjugation experiments and PCR analyses, plasmids in the order Enterobacterales can be classified into at least 28 Inc groups, and PlasmidFinder enables the classification of plasmids sequenced using next-generation sequencing (NGS) into Inc groups (Rozwandowicz et al., 2018; Carattoli et al., 2014). Reportedly, each Inc group plasmid tends to be linked to some of the specific antimicrobial resistance genes, as well as hosts and regions of their isolation (Rozwandowicz et al., 2018). Currently, multiple tools can be utilized to

Abbreviations: BeMAp, Bird's-eye MApping of plasmids; Inc, Incompatibility; MBL, metallo-β-lactamase; CDS, coding sequence.

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visualize the similarity of plasmids and analyze their phylogeny. Several tools provide insights into nucleotide sequence similarity and the order of genetic components on some plasmids and enable the visualization of morphogenic and network trees of plasmid relatedness (Sullivan et al., 2011; Tymensen et al., 2019; Ankenbrand et al., 2017; Lanza et al., 2017). However, they fail to display the similarities of genetic structure among multiple plasmids interactively and miss visualization of the genetic structures in the network analysis. Moreover, it is difficult to determine plasmids' relatedness due to the scarcity of contig sequences among plasmids, frequent homologous recombination and genomic rearrangement, together with horizontal transfer (Orlek et al., 2017; Suzuki et al., 2020). Suzuki et al. reported a method to phylogenetically analyze IncI plasmids based on similarities in combinations of open reading frames (ORFs) on plasmids (Suzuki et al., 2020). PCR-based comparison of ORFs on plasmids would enable us to overcome the obstacle that time-consuming analyses based on nucleotide sequencing presents. Nevertheless, in contrast to phylogenetic analysis of chromosomes using Multi Locus Sequence Typing or Pulsed-Field Gel Eelectrophoresis, comparing plasmids of different Inc groups remains difficult because each Inc group plasmid usually has a unique organization of genes (Orlek et al., 2017).

Metallo-β-lactamases (MBLs) are one of the major groups of carbapenemases found in Enterobacterales. They are an IMP type MBL that confers resistance to carbapenems and have been detected predominantly in Japan (Yamagishi et al., 2020). From 2010 to 2014, a prolonged outbreak of IMP-6 carbapenemase-producing multiple bacterial species belonging to the Enterobacterales was reported in a national hospital in Japan (Yamagishi et al., 2020). Because bacteria carrying $bla_{\text{IMP-6}}$, which encodes IMP-6 MBL, are susceptible to imipenem in vitro and tend to be overlooked in clinical laboratories, it was difficult to identify $bla_{\text{IMP-6}}$ spreading across different bacterial genera, species, and different Inc group plasmids, which hindered the quick and comprehensive analyses of multiple plasmids with different Inc types across different bacterial species (Yamagishi et al., 2020; Abe et al., 2020). Therefore, an effective tool for the rapid analysis and visualization of multiple plasmids extensively, needs to be developed.

The purpose of this study was to create a new tool, Bird's-eye MApping of plasmids (BeMAp), to visualize and compare the genomic structure of multiple plasmids. Specifically, we investigated and mapped plasmids carrying $bla_{\text{IMP-6}}$ that were deposited in a public database. Using BeMAp, effective recognition and visualization of similarities in antimicrobial resistance genes around the objective gene ($bla_{\text{IMP-6}}$ or bla_{IMPs}) of multiple plasmids were realized.

2. Materials and methods

2.1. Identification of datasets carrying bla_{IMP-6} or bla_{IMPs} and re-annotation by Prokka

Before performing BeMAp, datasets containing bla_{IMPs} were extracted. Nucleotide sequences of plasmids submitted to the National Center for Biotechnology Information (NCBI) genome database were downloaded in FASTA or GenBank formats. The nucleotide sequence of $bla_{\text{IMP-6}}$ was obtained from accession number AP018758.1 (Protein accession BBE80843.1) (Kanehisa and Goto, 2000). In August 2021, using its sequence, nucleotide data were queried via BLASTn with an *E*-value of 0.05. All datasets in the FASTA format were locally annotated using Prokka (https://github.com/tseemann/prokka/) (Seemann, 2014).

2.2. Preparation for performing BeMAp

The flowchart outlining BeMAp is shown in Fig. 1a. As extracted datasets contained chromosomal sequences or those annotated as "genomic DNA," plasmid datasets were obtained by confirming that they were registered as "plasmid" in the GenBank format, and other types were excluded. To perform a local BLASTn search and avoid duplicate identification of antimicrobial resistance genes, each coding sequence (CDS) in each Prokka-annotated GenBank file was divided into a FASTA file, which was then stored locally.

2.3. Identification of antimicrobial resistance genes on plasmids

As the Clinical and Laboratory Standards Institute (CLSI) provides the performance standard for antimicrobial susceptibility testing against Enterobacterales, *Pseudomonas* spp., and *Acinetobacter* spp. using penicillins, β-lactam combination agents, cephems, monobactams, carbapenems, polymyxins, aminoglycosides, macrolides, tetracyclines, quinolones, folate pathway antagonists, phenicols, fosfomycin, and nitrofurans, resistance genes to these antimicrobial agents were investigated (CLSI, 2020). To identify the antimicrobial resistance genes in plasmids extensively, ResFinder database (last update 2021-08-16) was used (Bortolaia et al., 2020), in which we found no information about resistance genes to nitrofurans. Using the Comprehensive Antibiotic Resistance Database (CARD) (Date 2020-8-6), a new database of resistance genes to nitrofurans was constructed (protein accession numbers AAC73679.1, AAC73938.1, AAP43109.1, and AAP43110.2) (Alcock et al., 2020).

All genes in each plasmid were queried using locally installed BLASTn with these databases to identify antimicrobial resistance genes, as ResFinder identifies genes by BLASTn. For an *E*-value threshold of <0.0001, non-resistance genes, such as integrases and transposases, were identified as antimicrobial resistance genes. Therefore, antimicrobial resistance genes were queried using the condition of *E*-value $= 0$. A CDS was defined as an antimicrobial resistance gene if more than one gene providing resistance to the antimicrobial agent was detected by a query at the CDS.

2.4. Classification by Inc groups and analyzing origins of plasmids carrying the objective gene

During the mapping process, BeMAp classified plasmids according to the Inc group and obtained the features of all plasmids. Using the PlasmidFinder database (database version 2021-07-12), Inc groups of each plasmid were classified by querying FASTA files of plasmids using the BLASTn database with an *E*-value of 0.001 and $>$ 90% nucleotide sequence identity (Carattoli et al., 2014). To distinguish the colors of Inc groups in the mapping, we reindexed Inc groups registered in the database into 18 groups: IncA/C, B/O/K/Z, D, F, G/U, HI, I, L/M, N, P, Q, R, S, T, U, W, X, and Y. By querying descriptions in the GenBank data of each plasmid, such as isolation country and organism, features of each plasmid were obtained.

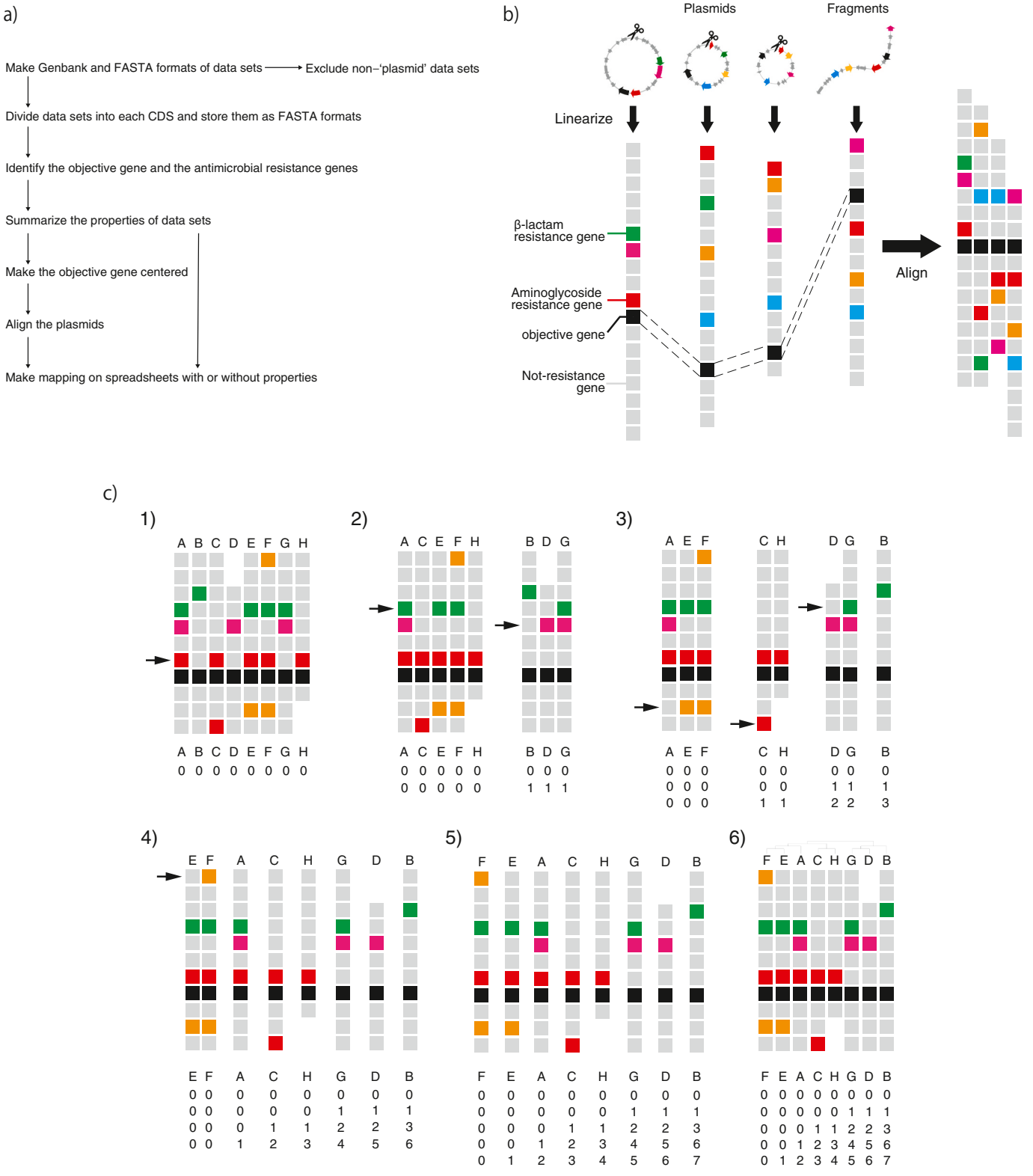
As an output, BeMAp provided a csv file with a summary of the plasmids and features of each plasmid carrying the objective gene ($bla_{\text{IMP-6}}$ or bla_{IMPs}). Additional information is summarized in Supplementary Data S1.

2.5. Mapping antimicrobial resistance genes in plasmids carrying the objective gene (bla_{IMP-6} or bla_{IMPs}) onto a spreadsheet

To visualize the location of the antimicrobial resistance genes on plasmids, genes carried by each plasmid were mapped onto a spreadsheet (Fig. 1b). One cell was filled with one Prokka-annotated gene or protein name, and all genes of one linearized plasmid were arrayed in one column. The complete sequence was determined when the name of a GenBank file contained "complete," and the alignment of genes in the column was arranged such that the objective gene was at the center of the column. Linearized plasmids were aligned side-by-side in the columns on the spreadsheet. As identified plasmids carrying the objective gene contained at least one of the genes, plasmids were arranged to be in the middle of the mapping. Antimicrobial resistance genes were assigned their respective colors. Cells in the upper row of the spreadsheet were filled with the accession numbers for each plasmid. A few

antimicrobial resistance genes, such as *aac(6′)-Ic-br* and *oqxAB*, confer resistance to different types of antimicrobial agents. However, it is difficult to display and distinguish which antimicrobial agents these genes conferred resistance to in the speadsheet (Robicsek et al., 2006; Li et al., 2019). Therefore, we prioritized the following antimicrobial agents to show in the mapping: aminoglycosides, β-lactams, quinolones, macrolides, tetracyclines, folate pathway antagonists, phenicols, fosfomycin, polymyxins, and nitrofurans.

To compare the organization of antimicrobial resistance genes flanking the objective gene, the alignment of genes on plasmids was



(caption on next page)

Fig. 1. Flowchart outlining BeMAp workflow and schematic diagrams for the mapping and alignment of antimicrobial resistance plasmids.
a) Flowchart outlining BeMAp is shown. BeMAp works when GenBank files containing the objective gene and nucleotide sequence data of the objective gene are input.
b) All genes of a linearized plasmid are arrayed in one column. Cells colored with gray represented not-resistance genes and those in other colors represented the objective gene and other antimicrobial resistance genes. All plasmids or fragments carrying an objective gene were aligned side-by-side. As plasmids contained at least one objective gene, they were arranged for the gene to be in the middle of mapping. When the sequences in the dataset were complete, the alignment of genes in the column was arranged such that the objective gene was situated at the center of the columns.
c) Eight columns from A to H are shown. The numbers below the columns indicate the current number of clusters in each column. Arrows indicate the position of R_{max} at each set of plasmids. 1) At row 7, the count of red cells was five, and the red cells and the row were identified as e_{max} and R_{max}, respectively. 2) Columns A, C, E, F, and H contained red cells in row 7 which were absent in columns B, D, and G. Thus, division of the set yielded a set of columns A, C, E, F, and H and a set of columns B, D, and G. As there were two clusters, the former was numbered as 0 and the latter was numbered as 1. In the former and latter clusters, rows 4 and 5 were identified as R_{max}, respectively. 3) and 4) The same dividing procedure was performed. 5) Columns could not be divided, and the procedure was stopped. 6) Synthesis of the divided columns. According to the number of each column below, the dendrogram was constructed as shown above. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

automatically arranged such that the direction of transcription of the objective gene was the same.

The properties of each plasmid, such as the Inc group and the country and organism of isolation, were mapped onto other spreadsheets. Columns for each plasmid, except for cells representing the objective gene and antimicrobial resistance genes, are displayed with the respective colors of the properties. If plasmids were classified into more than two different Inc groups, due to the presence of fused plasmids, they were"Not-identified." The regions to which the countries belong were classified according to the WHO region classification (https://www.who.int/countries). The properties of each plasmid are shown in the upper row of the spreadsheet.

2.6. Alignment of plasmids by an algorithm and formation of dendrogram

To compare plasmids easily and automatically, we aligned them based on the following algorithm (Fig. 1c):

Consider an m × n matrix with each cell containing any value. Some values are common among the columns.

1) Count the numbers of each element in each row
2) Find the largest number of elements (e_{max}) counted at 1) and identify the row that contains e_{max} (R_{max})
3) Divide the matrix into two smaller matrices: one has an e_{max} at R_{max} and the other does not
4) Repeat procedures 1)–3) in the new matrices except for R_{max}
5) Quit repeating procedures when new matrices fail to divide into smaller matrices
6) Synthesize divided matrices

Following this algorithm, elements represent each type of antimicrobial resistance gene and columns represent each plasmid, and then the calculation was done.

When dividing the sets of plasmids into two groups using this algorithm, individual groups were numbered at every dividing step. Plasmids were subsequently clustered based on a unique set of numbers for each plasmid, and a dendrogram was displayed based on the clusters.

2.7. Program availability

The program is implemented in Python and freely available from GitHub as a command line program (https://github.com/yusuketsuda/BeMAp.git).

3. Results

3.1. Collection of plasmids carrying bla_{IMP-6}

In August 2021, 815 datasets were queried via BLASTn using the nucleotide sequence of bla_{IMP-6} with an *E*-value threshold ≤0.05. As these datasets included genes of other IMP-type class B MBLs, these were regarded as datasets containing bla_{IMPs}. Exclusion of non-plasmid datasets and those lacking bla_{IMPs} yielded 303 datasets. By performing strict queries with an E-value of 0 and a complete nucleotide sequence identity, 54 datasets of plasmids containing bla_{IMP-6} were identified. The accession numbers of the datasets containing bla_{IMPs} are listed in Supplementary Data S1.

3.2. Bird's-eye mapping and alignment of plasmids carrying bla_{IMP-6} by comparing features for antimicrobial resistance genes

Using BeMAp, antimicrobial resistance genes in the 54 plasmids carrying bla_{IMP-6} were mapped and aligned such that bla_{IMP-6} was placed in the middle of the map. The map displayed similarities in types, locations, alignments, and organizations of the antimicrobial resistance genes and has been represented with a dendrogram on a spreadsheet (Fig. 2, Supplementary Fig. S1a, and Supplementary Data S2).

When the set of 54 plasmids carrying bla_{IMP-6} was divided 12 times, the number of clusters reached 37, and no new nodes appeared in subsequent divisions.

In plasmids carrying bla_{IMP-6}, antimicrobial resistance genes to aminoglycosides, β-lactams, quinolones, macrolides, tetracyclines, and folate pathway antagonists were identified. However, no antimicrobial resistance genes to phenicols, fosfomycin, polymyxins, or nitrofurans were identified.

3.3. Mapping on the spreadsheet with features of plasmids carrying bla_{IMP-6}

Classified by a query using the PlasmidFinder database, each plasmid carrying bla_{IMP-6} was mapped using the respective colors of the Inc groups on the spreadsheet (Fig. 3, Supplementary Fig. S1b, and Supplementary Data S2). Forty-four plasmids from the left side exhibited similar antimicrobial resistance gene organization, in which bla_{IMP-6} was flanked by two aminoglycoside resistance genes on both sides, followed by a sulfonamide resistance gene (*aacA4-*bla_{IMP-6}*-aadA2-sul1* region; black bar represented in Fig. 3). Among plasmids with a similar organization of antimicrobial resistance genes, 20 and 8 were classified as IncN and IncF, respectively. Eight plasmids were classified as both IncN and IncF. Plasmids with accession numbers AP018741.1 and AP022352.1 were classified as IncN and IncR and as IncF and IncR, respectively. Twenty-one plasmids classified as IncN contained genes for β-lactamase resistance and tetracycline resistance. Four plasmids classified as IncF contained the same cassette, which was composed of four genes encoding for macrolide, trimethoprim, aminoglycoside, and sulfonamide resistance (accession numbers AP022361.1, AP022365.1, AP022366.1, and AP022368.1). The plasmid AP022363.1, classified as IncF, had an inverted orientation of the cassette described above.

The country, city, and organism of origin for each plasmid carrying bla_{IMP-6} were mapped onto spreadsheets (Supplementary Data S2). The plasmids were isolated from Japan and South Korea, and those carrying the *aacA4-*bla_{IMP-6}*-aadA2-sul1* region were isolated from Japan.

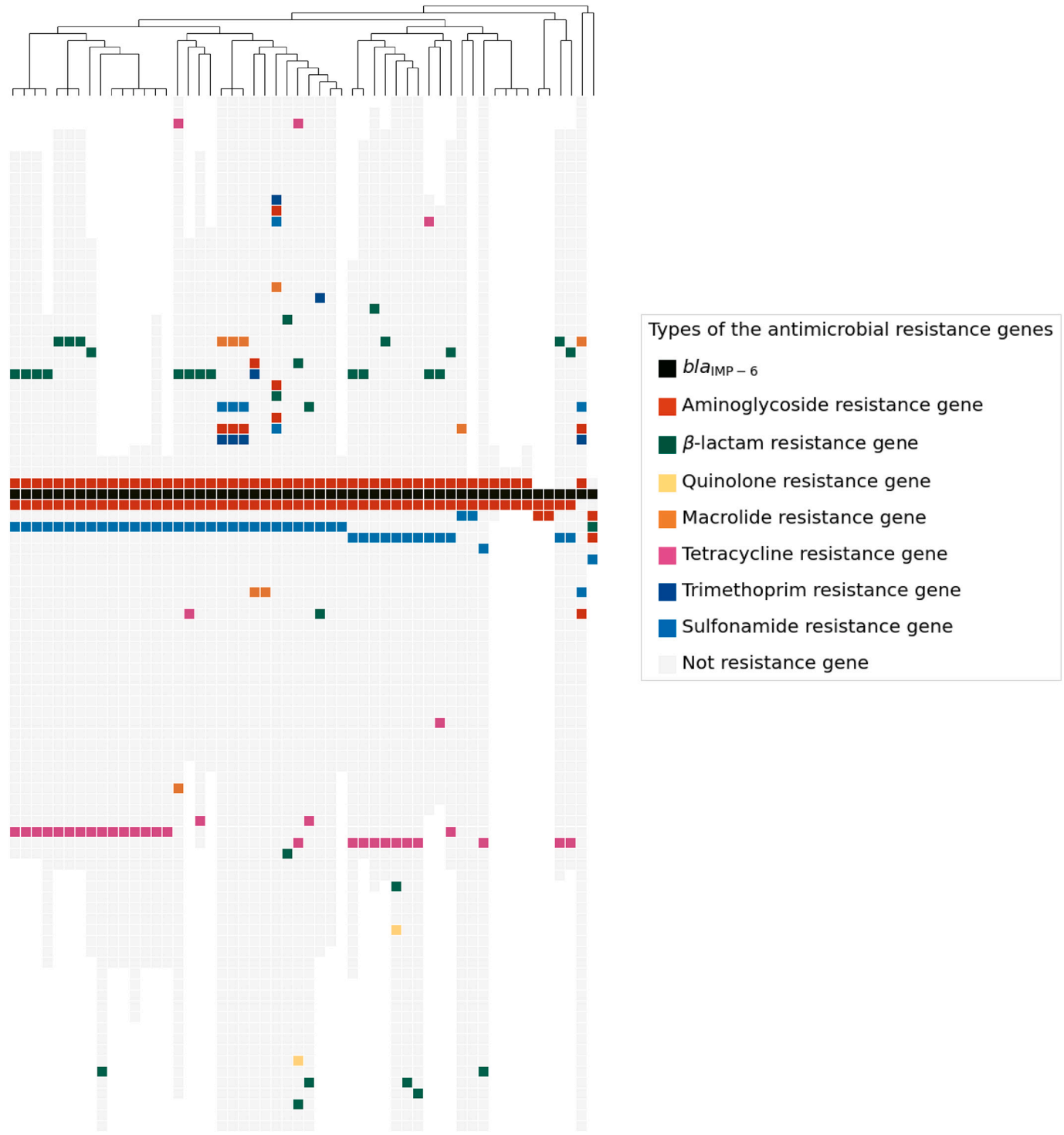


Fig. 2. Enlarged mapping and dendrogram of the antimicrobial resistance genes in plasmids carrying bla_{IMP-6}.
Fifty-four plasmids carrying bla_{IMP-6} were aligned using BeMAp and antimicrobial resistance genes were mapped onto the spreadsheet. Due to its massive complete size, the map is zoomed to show the central clusters of plasmids. At the top of the figure, the dendrogram constructed based on the clustering of these plasmids was displayed. The antimicrobial agents indicated in the legend represent the type of antimicrobial resistance genes present.

Plasmids carrying bla_{IMP-6} were isolated from Enterobacterales and *Pseudomonas aeruginosa*, whereas those carrying the *aacA4*-bla_{IMP-6}-*aadA2*-*sul1* complex were isolated from Enterobacterales alone.

Forty-nine plasmids were isolated from *Homo sapiens*; however, information regarding the hosts of five plasmids was missing.

3.4. Bird's-eye mapping and alignment of plasmids carrying bla_{IMPs}

Three-hundred and three datasets containing bla_{IMPs}, including 54 containing bla_{IMP-6}, were mapped onto a spreadsheet (Fig. 4, Supplementary Fig. S2, and Supplementary Data S3). Nine types of antimicrobial resistance genes other than nitrofurans were identified.

One hundred and seventy-two plasmids were classified into 12 Inc

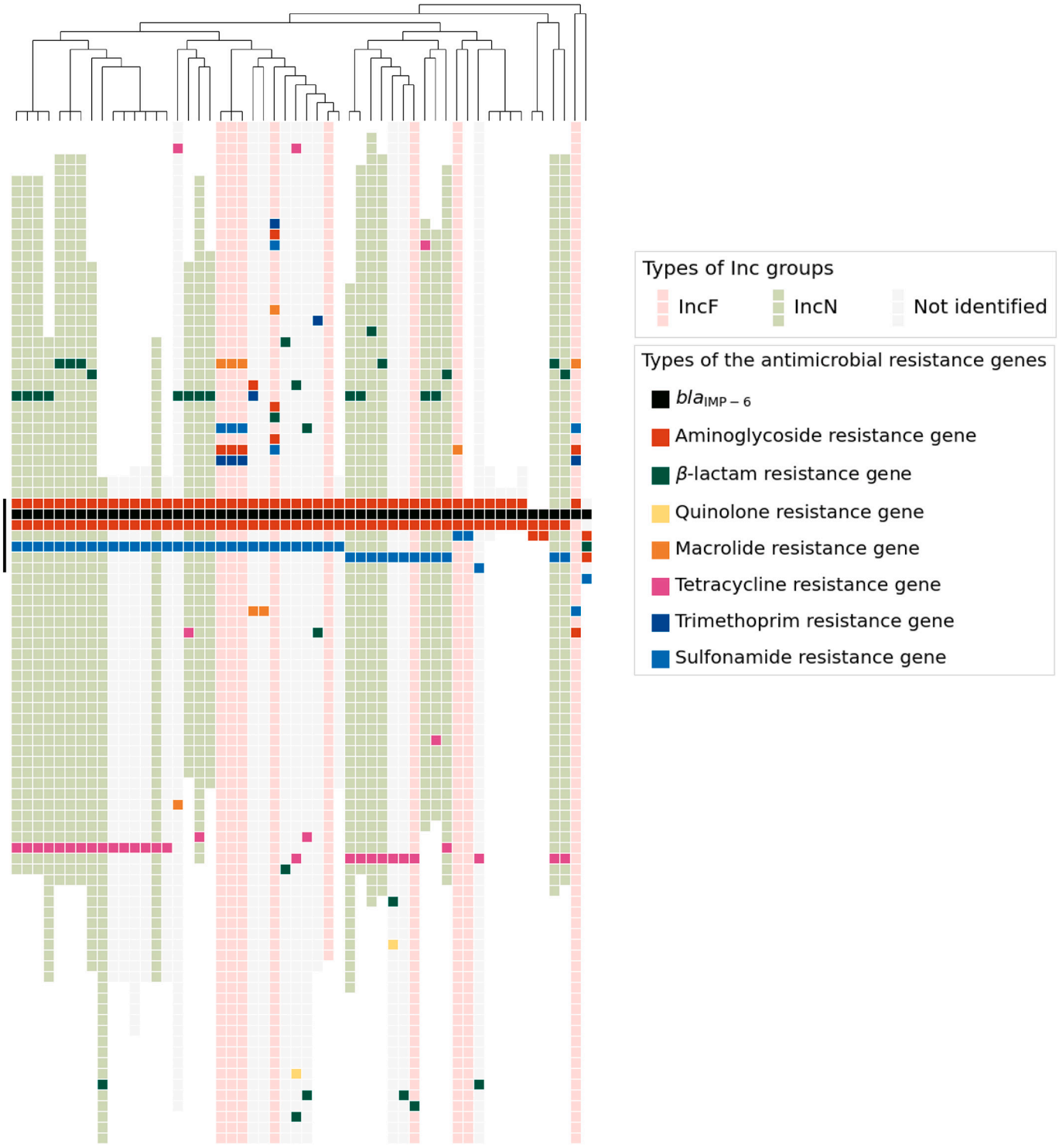


Fig. 3. Enlarged mapping of Inc groups of plasmids carrying bla_{IMP-6}.
In the mapping spreadsheet, the column for each plasmid carrying bla_{IMP-6} is colored according to the identified Inc group. Due to its massive complete size, the map is zoomed to show the central clusters of plasmids. The antimicrobial agents indicated in the legend represent the type of antimicrobial resistance genes present. If a plasmid was identified as belonging to none or more than two Inc groups, it was regarded as "Not-identified." The black bars on the left side of the map indicate the *aacA4*-bla_{IMP-6}-*aadA2*-*sul1* region.

groups, and 105 plasmids remained unclassified. Eighteen plasmids were classified into more than two Inc groups. Of the 172 plasmids classified, 67 were classified into the IncN group.

Eleven plasmids carrying bla_{IMP-4} (accession numbers CP042496.1, CP042514.1, CP042522.1, CP042526.1, CP042532.1, CP042537.1, CP042542.1, CP042548.1, CP042568.1, CP02574.1, and JX101693.1) had similar organization of the antimicrobial resistance genes around bla_{IMP-4} (Fig. 5 and Supplementary Data S3). Of these 11 plasmids, two, with accession numbers CP042496.1 and CP042548.1, were classified as IncF and IncN, respectively, whereas others were classified as IncL/M.

Plasmids carrying bla_{IMPs} were isolated from Asia, Europe, Oceania, and South America. Of the 231 plasmids that had the country of isolation

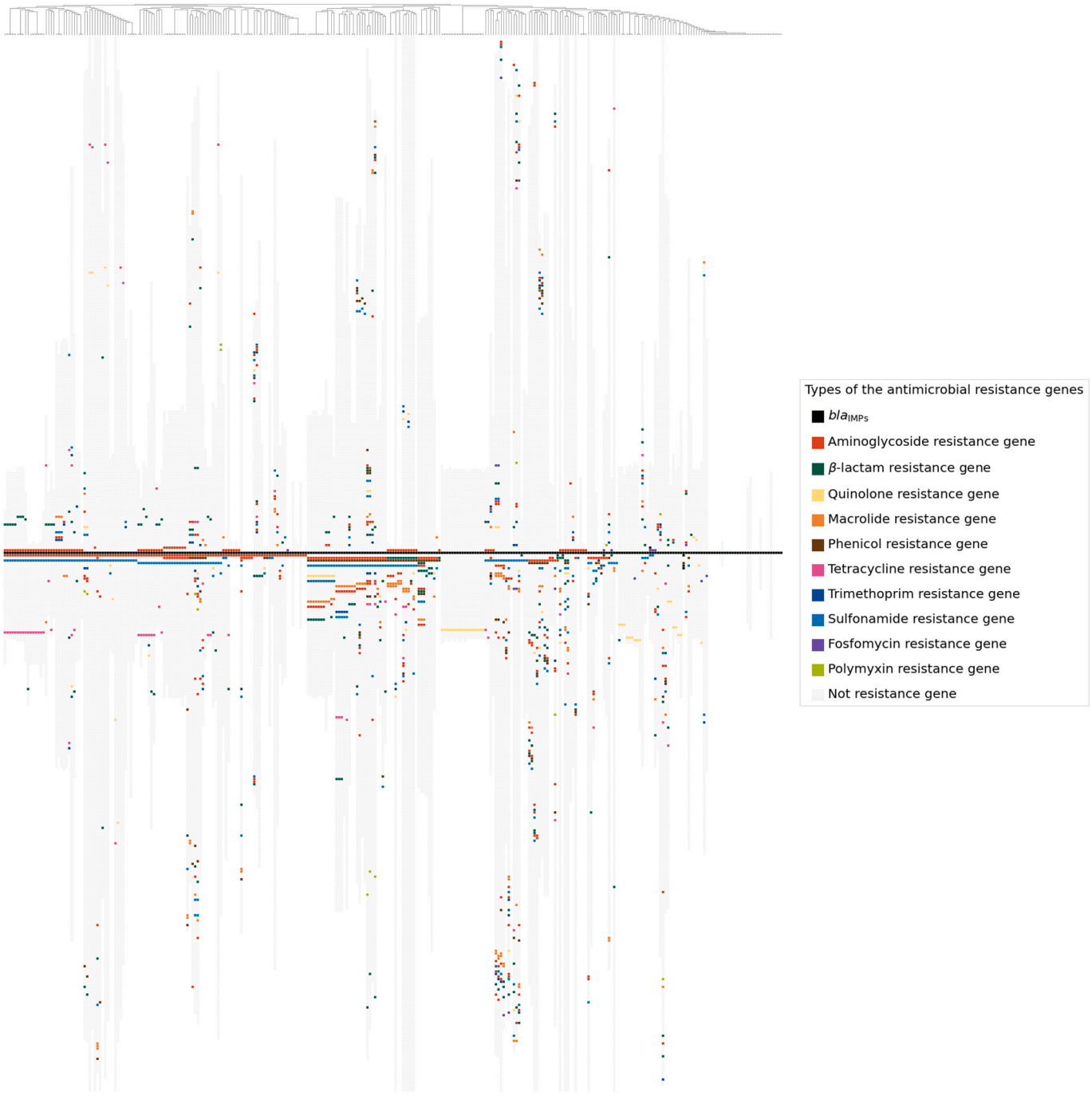


Fig. 4. Enlarged mapping of antimicrobial resistance genes in plasmids carrying *bla*$_{IMPs}$.
Three hundred and three plasmids carrying *bla*$_{IMPs}$ are shown with the dendrogram. Due to its massive complete size, the map is enlarged to show the central clusters of plasmids.

registered, 93 (40.3%) were isolated from Japan. Analysis of plasmids carrying *bla*$_{IMPs}$, based on where these were isolated, exhibited a regional bias in their distribution.

Of the 303 plasmids carrying *bla*$_{IMPs}$, 95 were most frequently isolated from *Klebsiella* spp. Of the 149 plasmids whose hosts were registered, 145 (97.3%) were most frequently isolated from *H. sapiens*.

AP018742.1, AP019247.1, AP019402.1, AP022349.1, and AP022356.1 were in the same cluster and the identities and coverage of nucleotide sequences for these plasmids were above 99% and 100%, respectively (Supplementary Table S1). Although the plasmids shared the same organization of types of antimicrobial resistance observed, AP022349.1 carried *bla*$_{IMP-1}$, whereas other plasmids carried *bla*$_{IMP-6}$ (Supplementary Fig. S3a). KY887594.1, KY887590.1, KY887591.1, and KY887595.1 had the same node (Supplementary Fig. S3b). KY887594.1 carried *bla*$_{IMP-26}$, whereas others carried *bla*$_{IMP-4}$, and KY887594.1, KY887591.1, and KY887595.1 were classified as IncA/C. KY887590.1 was classified as a fusion of IncA/C and IncY. Although the plasmids had different properties, the same organization of the antimicrobial resistance genes was observed in the mapping, and the identities and coverage of their nucleotide sequences were above 99.8% and 84%, respectively (Supplementary Tables S2 and S3).

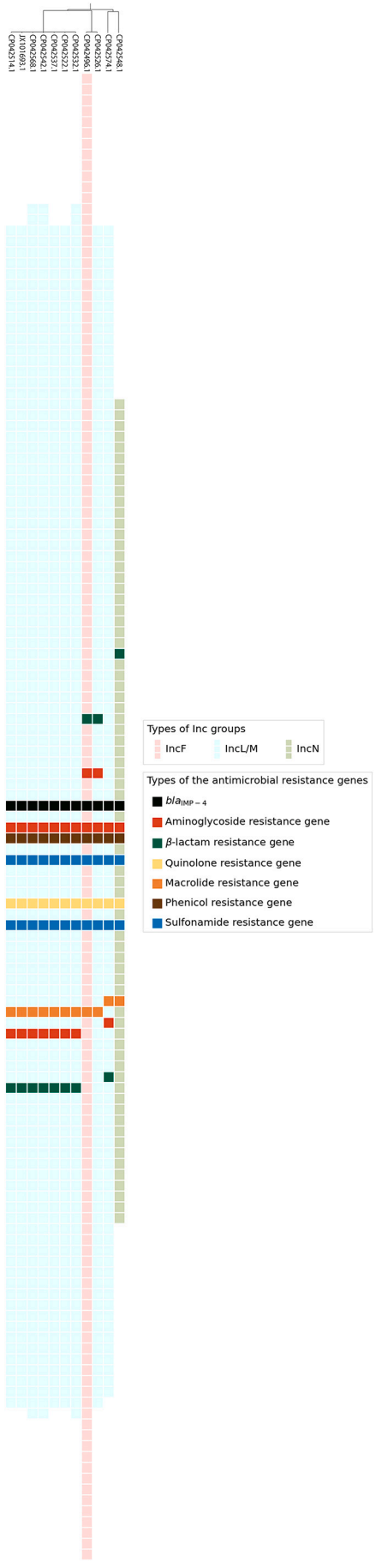


Fig. 5. Inconsistent classification of Inc groups for plasmids carrying bla_{IMP-4}, which have a similar organization of antimicrobial resistance genes
Eleven plasmids carrying bla_{IMP-4} are shown in the same cluster. Nine plasmids were classified as IncL/M. CP042496.1 and CP042548.1 were classified as IncF and IncN, respectively. Although CP042496.1 and CP042526.1 had the same organization of antimicrobial resistance genes, they were classified as IncF and IncL/M, respectively.

4. Discussion

In this study, we mapped and aligned antimicrobial resistance plasmids carrying bla_{IMP-6} or bla_{IMPs} using the novel platform BeMAp and demonstrated its utility in visualizing the genomic similarity of multiple plasmids. Although previously established procedures and algorithms for the alignment depending on the nucleotide or amino acid sequences enabled analyses of plasmids easily, they did not necessarily provide an effective display of the similarities in the organization of antimicrobial resistance genes surrounding a specific gene in multiple plasmids in different Inc groups (Lipman et al., 1989; Larkin et al., 2007). Our new protocol for the alignment of plasmids, that is based on a dividing method using a new algorithm, overcomes obstacles associated with the comparative analysis of plasmids which are caused by mutations, insertion sequences, and homologous recombination, enabling the easy recognition of similarities across many plasmids.

In Enterobacterales, the IMP-type class B MBL, a carbapenemase, has been predominantly isolated in Japan. Forty-one of the 54 plasmids carrying bla_{IMP-6} originated from two investigations in Osaka, Japan (Yamagishi et al., 2020; Abe et al., 2020). As these studies reported that bla_{IMP-6} was spread across different genera, species, and Inc groups, the gene was suitable for the mapping of multiple plasmids with different properties. Plasmids carrying bla_{IMP-6} were mainly classified into the IncN or IncF groups, which exhibit a unique organization of the antimicrobial resistance genes on the map. Thus, our mapping protocol can effectively and rapidly visualize similarities and differences in the organization of antimicrobial resistance genes between plasmids of different Inc types.

There are tools that can analyze plasmids based on Inc grouping and other methods of classification and are suitable for frequent use. Each Inc group correlates with the types, locations, alignments, and organizations of the antimicrobial resistance genes, and the correlation between types and organizations of the antimicrobial resistance genes is clearly shown in the spreadsheet (Rozwandowicz et al., 2018). However, a few plasmids that exhibited variations in antimicrobial resistance gene organization were classified into the same Inc group. For 11 plasmids carrying bla_{IMP-4}, plasmids with an organization similar to those with antimicrobial resistance genes were not always classified into the same Inc groups, suggesting that our method can compare and analyze plasmids regardless of their Inc group classification. Additionally, mapping of Inc groups and the organisms, countries, or regions from where the plasmids were isolated were shown with their respective colors on the spreadsheet. This enabled the recognition of the relationship between the antimicrobial resistance genes and properties of the plasmids.

BeMAp aligns plasmids according to the position and order of antimicrobial resistance genes without a precise identification of these genes. Although BeMAp will offer an option to identify antimicrobial resistance genes precisely, in some cases, identification of types of antimicrobial resistance genes may cause the program to ignore mutations, yielding clusters of similar plasmids. Moreover, the nucleotide sequences of plasmids in the same cluster or node were similar, even when clustered based on the organization of the antimicrobial resistance genes. Therefore, alignment by the types of antimicrobial resistance genes in plasmids using BeMAp may serve as a new approach to classifying plasmids.

However, our study has some limitations. The genetic distance among plasmids could not be calculated using the algorithm and was not

(caption on next column)

reflected in the dendrogram. If one plasmid carried a specific or unique organization of antimicrobial resistance genes only a few cells away from other plasmids in the spreadsheet, these were not included in the same cluster. To address these problems, the alignment must be improved. In the mapping, the length and direction of gene transcription and non-coding regions were not displayed; therefore, new software or web tools to effectively display these factors will be developed in a future study. Although BeMAp can satisfactorily map and analyze plasmids and chromosomes based on the types, locations, alignments, and organizations of antimicrobial resistance genes, owing to large numbers of genes on the chromosome and the increasing burden on the calculation process, the use of a computing system with high performance is recommended. Otherwise, it may take a long time to identify genes and display them on a spreadsheet.

The strengths of BeMAp include that it can identify antimicrobial resistance genes that transfer across different bacterial genera and species via plasmids, and across different types of Inc group plasmids (Yamagishi et al., 2020; Peleg et al., 2005). When antimicrobial resistance genes transfer into a scarce Inc group plasmid in clinical settings, analyzing similarities among these genes becomes difficult because of their unique organization in each Inc group plasmid. This results in the delayed recognition of the nosocomial spread of plasmids and outbreak of antimicrobial resistance genes. Our program can analyze multiple plasmids found in different bacterial genera and species and classify these into different Inc groups, overcoming the difficulties associated with the analysis of plasmids mentioned above. In this study, although we focused on antimicrobial resistance genes in plasmids, the application of our method to databases for other types of genes, such as transposases, integrases, recombinases, or virulence genes, can help us visualize the organization of these genes comprehensively.

5. Conclusions

BeMAp is a novel algorithm that allows for the mapping of multiple plasmids and enables the effective recognition of the types, locations, alignments, and organizations of antimicrobial resistance genes in plasmids via extensive analysis of plasmids carrying $bla_{\text{IMP-6}}$ and bla_{IMPs}. This method may serve as a promising technique for the feasible and practical visualization and comparison of the genomic structure of multiple plasmids.

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CRediT authorship contribution statement

Yusuke Tsuda: Conceptualization, Methodology, Software, Investigation, Data curation, Writing – original draft. **Masahiro Suzuki:** Conceptualization, Software, Supervision. **Jun-ichi Wachino:** Supervision. **Kouji Kimura:** Supervision. **Yoshichika Arakawa:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

None to declare.

Data availability

Data will be made available on request.

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