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ARTICLE

# Correlation between the spread of IMP-producing bacteria and the promoter strength of *bla*$_{IMP}$ genes

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## Abstract

The first report of transmissible carbapenem resistance encoded by *bla*$_{IMP-1}$ was discovered in *Pseudomonas aeruginosa* GN17203 in 1988, and *bla*$_{IMP-1}$ has since been detected in other bacteria, including Enterobacterales. Currently, many variants of *bla*$_{IMPs}$ exist, and point mutations in the *bla*$_{IMP}$ promoter have been shown to alter promoter strength. For example, the promoter (Pc) of *bla*$_{IMP-1}$, first reported in *P. aeruginosa* GN17203, was a weak promoter (PcW) with low-level expression intensity. This study investigates whether point mutations in the promoter region have helped to create strong promoters under antimicrobial selection pressure. Using bioinformatic approaches, we retrieved 115 *bla*$_{IMPs}$ from 14,529 genome data of *Pseudomonadota* and performed multiple alignment analyses. The results of promoter analysis of the 115 retrieved *bla*$_{IMPs}$ showed that most of them used the Pc located in class 1 integrons ($n = 112$, 97.4%). The promoter analysis by year revealed that the *bla*$_{IMP}$ population with the strong promoter, PcS, was transient. In contrast, the PcW-TG population, which had acquired a TGn-extended −10 motif in PcW and had an intermediate promoter strength, gradually spread throughout the world. An inverse correlation between Pc promoter strength and Intl1 integrase excision efficiency has been reported previously [1]. Because of this trade-off, it is unlikely that *bla*$_{IMPs}$ with strong promoters will increase rapidly, but the possibility that promoter strength will increase with the use of other integrons cannot be ruled out. Monitoring of the *bla*$_{IMP}$ genes, including promoter analysis, is necessary for global surveillance of carbapenem-resistant bacteria.

## Introduction

The first report of transmissible carbapenem resistance was made in 1988 in *Pseudomonas aeruginosa* GN17203 isolated from a hospital in Toyama Prefecture, Japan [2]. Three years later, Osano et al. reported a novel metallo-beta-lactamase (IMP-1), which is encoded by *bla*$_{IMP-1}$, from *Serratia marcescens* Tn9106 isolated from a patient with a urinary tract infection at a hospital in Aichi Prefecture, Japan in 1991 [3]. Subsequent analysis revealed that *P. aeruginosa* GN17203 harbored the same *bla*$_{IMP-1}$ [4]. Furthermore, Iyobe et al. found that the *bla*$_{IMP-1}$ genes of *P. aeruginosa* GN17203 and *S. marcescens* Tn9106 were located on the gene cassette of a class 1 integron [5]. On the other hand, *bla*$_{IMP-1}$ of *S. marcescens* isolated from another Aichi Prefecture hospital was transmitted by a novel integron [6], which was later shown to be a class 3 integron [7]. In 2016, *bla*$_{IMP-27}$ was reported from *Proteus mirabilis* on the gene cassette of a class 2 integron [8]. Thus, *bla*$_{IMPs}$ have been reported to be transmitted by the class 1, 2 and 3 integrons. Here we report that class 1 integrons are primarily involved in the *bla*$_{IMP}$ propagation.

Class 1 integrons consist of an integron integrase gene (*intI1*) encoding tyrosine recombinase (IntI1), an integron recombination site (*attI*), and a cassette array consisting of 1–200 contiguous gene cassettes [9, 10]. Intl1 catalyzes the insertion and excision of the gene cassette. All gene cassettes have a cassette recombination site (*attC*) and generally consist of a single open reading frame (ORF). Multiresistant integrons (MRIs), which contain multiple gene cassettes encoding drug resistance, have been implicated in multidrug resistance in bacteria [11]. Gene cassettes are generally promoter-less, and their genes are transcribed from the promoter Pc within the coding sequence of Intl1 (Fig. 1a), with transcription levels dependent on

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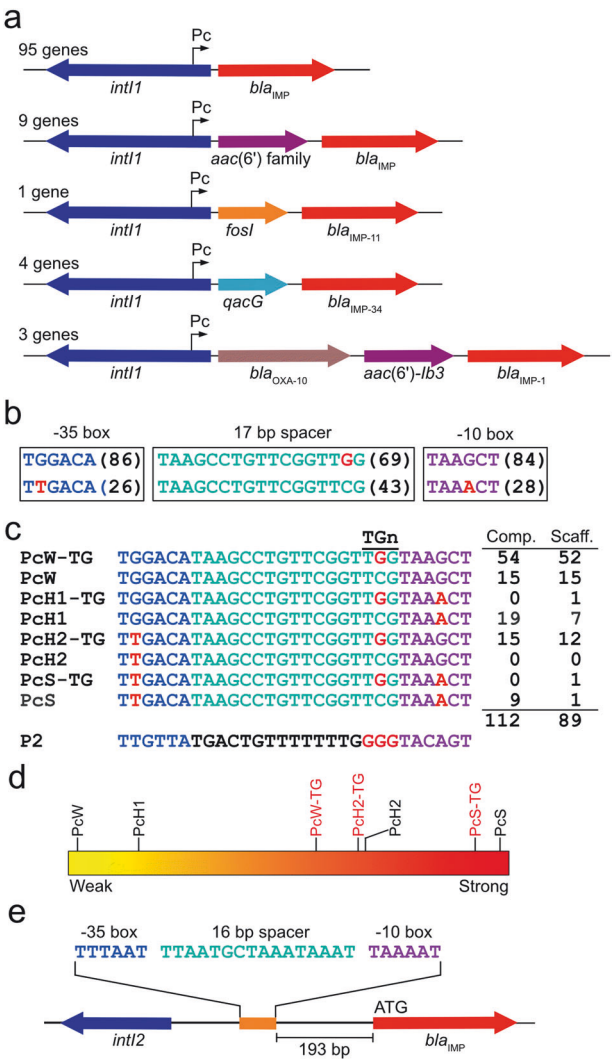


**Fig. 1** Characterization of the $bla_{IMP}$ promoters derived from class 1 (**a**–**d**) and class 2 (**e**) integrons. **a** Distribution of $bla_{IMPs}$ transcribed by Pc on the gene cassette of a class 1 integron. *aac*(6'), aminoglycoside 6'-*N*-acetyltransferase gene; *fosI*, fosfomycin resistance gene; *qacG*, quaternary ammonium compound resistance gene; and *aac*(6')-*Ib3*, aminoglycoside *N*-acetyltransferase gene. **b** Nucleotide sequences of promoter Pc. One point mutation is observed in each sequence of the −35 hexamer, −10 hexamer, and 17 bp spacer. **c** Classification of promoter Pc. Pcs were retrieved from the complete level (Comp.) genomic data ($n = 112$) and from the scaffold level (Scaff.) genomic data ($n = 89$) and the right column indicates the number of promoters detected. **d** Color gradient representing the expression intensity of Pcs obtained by the β-galactosidase assay [1]. **e** Promoter located on a class 2 integron

polymorphisms in the promoter sequence [12–18]. Specifically, several Pc variants are derived by point mutations in the −35 and −10 hexamer sequences. Among them, the four major Pc variants are classified as PcW (weak promoter), PcS (strong promoter), PcH1 (intermediate promoter), and PcH2 (intermediate promoter), according to promoter strength. PcH1 and PcH2 are hybrid-type promoters consisting of −35 and −10 hexamers of PcW and PcS in opposite combinations. In addition to mutations in the −10 and −35 hexamers, Nešvera et al. reported a C-to-G mutation 2 bp upstream of the −10 hexamer of PcW, which forms a TGn-extended −10 motif, in a streptomycin/spectinomycin resistance gene, *aadA2a* [19], and this mutation increased transcription efficiency [19–22]. Jové et al. investigated the promoter expression intensities of four major combinations of Pc and Pc with TGn-extended −10 motifs using a *lacZ* reporter system [1]; their findings are summarized in Fig. 1d. In addition, in some cases there is a second promoter, P2, downstream of Pc, with the spacing between the −35 and −10 hexamers optimized to 17 bp by insertion of three G residues [23], and the sequence of P2 is shown in the bottom row of Fig. 1c. Although the diversity of Pc variants and promoter intensities has been analyzed in detail as mentioned above, how such diversity is involved in the spread of IMP-producing bacteria has not been comprehensively analyzed.

Here, we retrieved 115 $bla_{IMP}$ genes from 14,529 complete-level genome data of *Pseudomonadota* deposited in the GenBank database and performed promoter analysis. We found that the $bla_{IMP}$ population transcribed by the strong promoter PcS is transient and regionally localized, whereas the $bla_{IMP}$ population with the intermediate promoter PcW-TG has increased since the late 2000s. Our findings clearly demonstrate that IMP-producing bacteria with intermediate promoter strength of the $bla_{IMP}$ are gradually spreading throughout the world.

## Materials and methods

### Collection of the $bla_{IMP}$ family from the GenBank database

The $bla_{IMP}$ family was retrieved from 14,529 complete-level genome data (including chromosomes and plasmids) from *Pseudomonadota* (synonym *Proteobacteria*) deposited in the GenBank database using Biopython [24] annotated to metallo-beta-lactamase IMP-X on the annotations already made (data retrieval was done on October 7, 2022). As a result, 115 $bla_{IMP}$ genes were retrieved and their upstream regions were used for promoter analysis. Additional $bla_{IMP4}$ searches were performed using BLAST on 36,214 scaffold-level genome data from *Pseudomonadota* in the GenBank database. This search yielded 200 genes for $bla_{IMP4}$ and its variants (showing >90% homology and 100% query coverage with the IMP-4 protein) and their upstream 290 bp region containing the *intI1*-$bla_{IMP}$ gene order was used for further promoter analysis. This additional search was completed on February 28, 2023.

### Promoter analysis of *bla*$_{IMP}$ and phylogenetic analysis of the *bla*$_{IMP}$ coding region

The promoter region of *bla*$_{IMPs}$ was subjected to multiple alignment analysis using MAFFT (https://mafft.cbrc.jp/alignment/server/) to detect point mutations in the −35 and −10 hexamers, and in the 17 bp spacer sequence. For illustration of the phylogenetic tree, the coding region of *bla*$_{IMP}$ was analyzed by MAFFT and the resulting data were rendered by Phylo.io [25] using the MAFFT sequence alignment server [26]. For the Pc distribution, the world map was rendered by using the rworldmap [27] package in R.

## Results

### Characterization of the *bla*$_{NDM-1}$ promoters

To investigate the *bla*$_{IMP}$ promoters, we retrieved promoter and coding regions of *bla*$_{IMPs}$ from 14,529 full-length *Pseudomonadota* genome data deposited in the GenBank database. A total of 115 *bla*$_{IMPs}$ were retrieved; they were located in plasmids (91 genes) and chromosomes (24 genes) (Fig. 2a). *bla*$_{IMP}$ was first detected in *P. aeruginosa* GN17203, but it is now evident that it has spread to various strains, including Enterobacterales (Fig. 2a). In this study, IMP-4 was the most abundant IMP-type, followed by IMP-1 (Fig. 2b). Promoter analysis of the 115 retrieved *bla*$_{IMPs}$ showed that they were broadly classified into two groups: those derived from class 1 integrons ($n = 112$, 97.4%) and those from class 2 integrons ($n = 3$, 2.6%).

### Analysis of promoter Pc within the class 1 integrons

In the 115 *bla*$_{IMP}$ analyses, 112 Pcs derived from the class 1 integrons were detected. 95 of the 112 *bla*$_{IMPs}$ belonged to the *intl1*-*bla*$_{IMP}$ gene order, while the other 17 genes had other resistance genes arranged between the *intl1* and *bla*$_{IMP}$ in a cassette-like arrangement (Fig. 1a). Multiple sequence alignment analysis using MAFFT (See the Materials and Methods) demonstrated that one point mutation was detected in each of the −35 and −10 hexamers of Pc, which were in coding sequence of the Intl1 (Fig. 1a). For example, among the 112 Pc promoters retrieved, 86 of the −35 hexamers utilized TGGACA and 26 utilized TTGACA. Similarly, there was a frequency bias in the −10 hexamer, with 84 sequences utilizing TAAGCT and 28 sequences

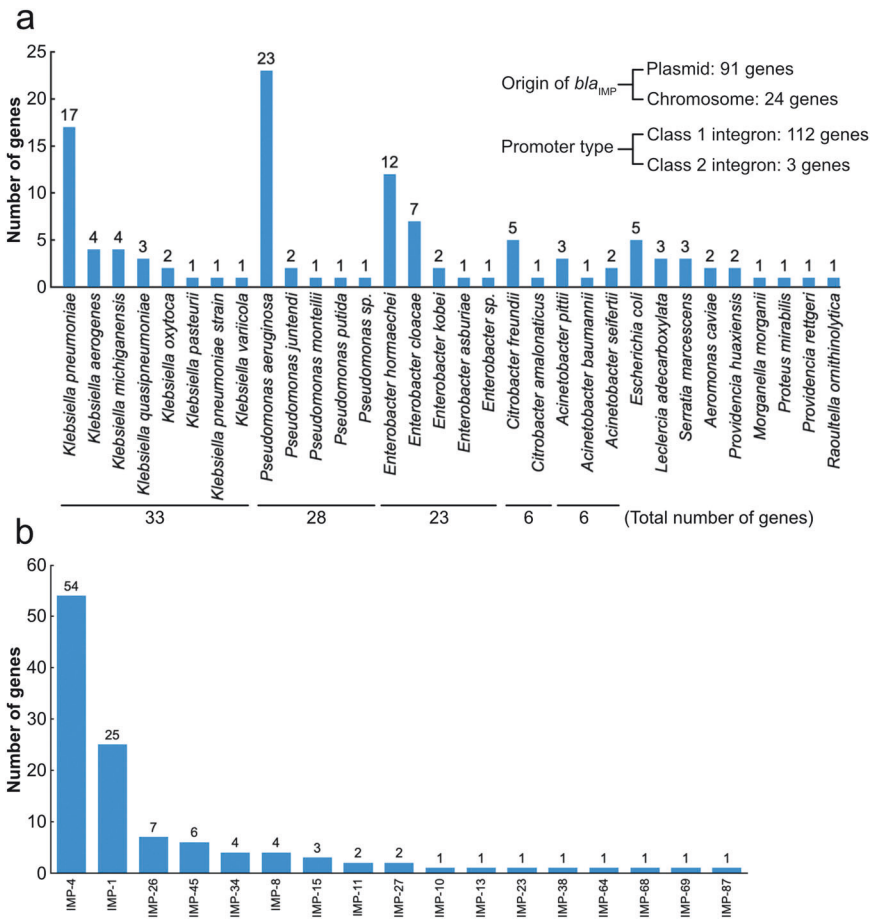


**Fig. 2** Distribution of the *bla*$_{IMP}$ family members. 115 *bla*$_{IMPs}$ were retrieved from the *Pseudomonadota* complete database ($n = 14{,}529$) and classified by bacterial species (**a**) and IMP type (**b**). The vertical axis indicates the number of *bla*$_{IMP}$ genes

utilizing TAAACT (Fig. 1b). Thus, the combination of −35 and −10 hexamers generates PcW, PcH1, PcH2, and PcS as described in the Introduction (Fig. 1c). For the 17 bp spacer sequence between −35 and −10 hexamers, a C-to-G point mutation from two bases upstream of the −10 hexamer was observed and formed the typical TGn-extended −10 motif [22]. For convenience, the PcX promoter with a TGn-extended −10 motif is denoted here as PcX-TG. Including the TGn-mutation, eight promoters could logically be expected to be generated. In this study, we have detected seven Pc derivatives, not including PcH2 (Fig. 1c). In contrast, the second promoter downstream from the Pc region, i.e., P2 [23], was not detected. Thus, the multiple alignment analysis revealed a bias in the Pc region of $bla_{IMP}$. Furthermore, the reason for limiting point mutations to three sites is that the Pc is located on the coding sequence of Intl1, and mutations that destroy the Intl1 coding region are not allowed.

## Yearly transition of the $bla_{IMP}$ Pc promoters

In 1988, IMP-1-producing *P. aeruginosa* GN17203 was discovered for the first time, and it was shown that $bla_{IMP-1}$ is transcribed by the weak promoter, PcW [5]. This leads us to ask, Has the $bla_{IMPs}$ family shifted to $bla_{IMP}$ with a strong promoter under the selection pressure of antimicrobial agents? To answer this question, we chronologically sorted Pc promoters ($n = 109$) from IMP-producing bacteria with known isolation years as shown in Fig. 2, and categorized their promoter types (Fig. 3). As a result, we found that PcW-TG with intermediate promoter strength began to appear around 2007, and promoters with PcW-TG accounted for 47% ($n = 51$) of the IMP-producing bacteria ($n = 109$). The $bla_{IMPs}$ utilizing promoters PcH1 and PcH2-TG were detected sporadically and were a minor population. Interestingly, $bla_{IMPs}$ with the strong promoter, PcS, were only detected between 2012 and 2017, and were restricted to Taiwan [28, 29], China [30, 31], and Australia [32] (Table 1). Thus, the emergence of IMP-producing bacteria with $bla_{IMPs}$ with the strong promoter was transient, but $bla_{IMPs}$ transcribed PcW-TG with the intermediate promoter were found to be dominant.

## Promoter analysis within the class 2 integrons

In 115 $bla_{IMP}$ analyses, we detected three promoters (herein referred to as $P_{intI2}$ for convenience) on the gene cassette of the class 2 integrons as depicted in Fig. 1e. Previously, $bla_{IMP-27}$ utilizing $P_{intI2}$ was detected in IMP-27-producing *Proteus mirabilis* isolated from two patients in the Upper Plains region of the United States in 2009 and 2015, respectively [8]. In the present analysis, $bla_{IMP-27}$ utilizing $P_{intI2}$ was detected in *Morganella morganii* and *P. mirabilis*

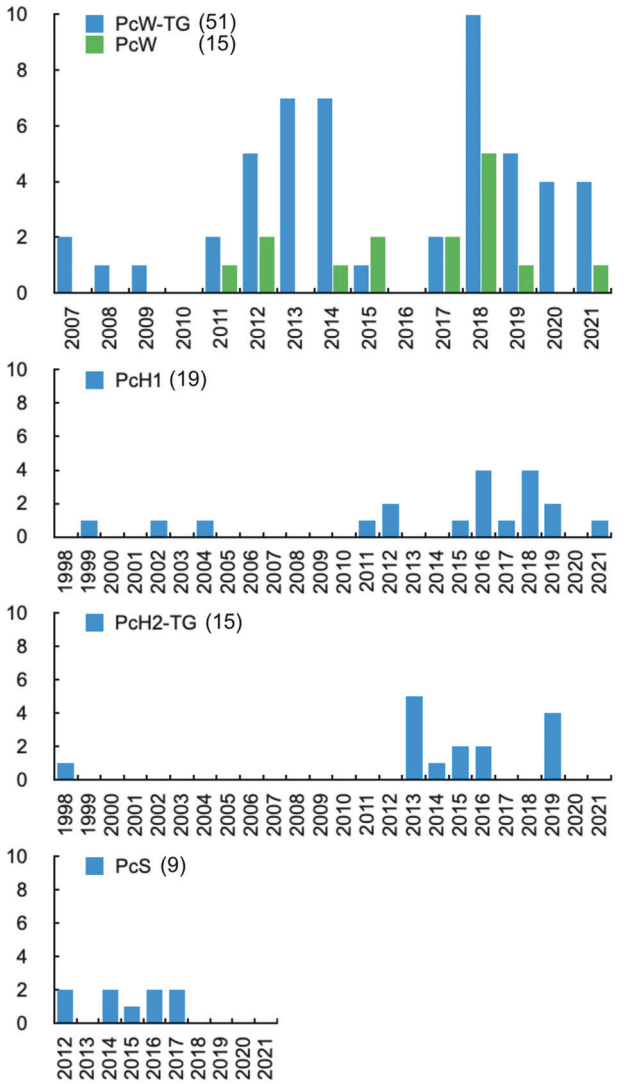


**Fig. 3** Distribution of Pc promoters by years. Pc promoters ($n = 109$) from IMP-producing bacteria with known isolation years were categorized by promoter type and sorted by year

isolated in Winnipeg, Canada [33]. In addition, $bla_{IMP-64}$ utilizing $P_{intI2}$ was detected in *Providencia rettgeri* in Quebec, Canada (Table 2). IMP-64 is a variant of IMP-27 with one amino acid substitution. Collectively, $bla_{IMPs}$ with $P_{intI2}$ are involved in the spread of IMP-27 and its variant, IMP-64, and these are geographically restricted.

## The promoter type of $bla_{IMP-1}$ and $bla_{IMP-4}$ is shifting to PcW-TG

Our results suggest that Pc is being shifted to PcW-TG in the $bla_{IMP}$ family (Fig. 3). To further validate the utilization frequency of Pc, we retrieved the $bla_{IMP-4}$ and its variants (>90% homology with the $bla_{IMP-1}$ product), which are mostly represented in Fig. 2, from 36,214 scaffold-level genome data from *Pseudomonadota* deposited in the

**Table 1** $bla_{IMP}$ genes under the control of the strong promoter, PcS

| Bacterial strain | Origin | IMP type | Promoter | Location | Host | Collection date | Accession number | Reference |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* 1585m1[a] | Plasmid_A | IMP-4 | PcS | Australia | Silver gull | 2012 | CP086389 | [32] |
| *Escherichia coli* 1585m1[a] | Plasmid_B | IMP-4 | PcS | Australia | Silver gull | 2012 | CP086388 | [32] |
| *Acinetobacter pittii* 2014S07-126 | Plasmid | IMP-1 | PcS | Taiwan | Homo sapiens | 2014 | CP033531 | [28] |
| *Acinetobacter pittii* 2014N21-145 | Plasmid | IMP-1 | PcS | Taiwan | Homo sapiens | 2014 | CP033569 | [28] |
| *Acinetobacter seifertii* AS70 | Plasmid | IMP-1 | PcS | Taiwan | Homo sapiens | 2010-2017 | CP061572 | [28] |
| *Acinetobacter seifertii* AS23 | Plasmid | IMP-1 | PcS | Taiwan | Homo sapiens | 2010-2017 | CP061673 | [28] |
| *Enterobacter asburiae* EN3600 | Plasmid | IMP-8 | PcS | China | Homo sapiens | 2015 | CP035637 | [31] |
| *Enterobacter cloacae* ECL42 | Plasmid | IMP-4 | PcS | China | Homo sapiens | 2016 | CP082149 | None |
| *Aeromonas caviae* 710029 | Chromosome | IMP-8 | PcS | China | Homo sapiens | 2016 | CP047981 | [30] |

[a]*E. coli* strain 1585m1 had PcS-type $bla_{IMP-4}$ genes on two different plasmids

Silver gull: Chroicocephalus novaehollandiae

**Table 2** Characterization of $bla_{IMP}$ genes with the promoter $P_{intI2}$

| Bacterial strain | Origin | IMP type | Promoter | Location | Host / source | Collection date | Accession number | Reference |
|---|---|---|---|---|---|---|---|---|
| *Morganella morganii* N18-00103 | Chromosome | IMP-27 | $P_{intI2}$ | Winnipeg, Canada, | Homo sapiens | 2018 | CP048275 | [33] |
| *Proteus mirabilis* N18-00201 | Chromosome | IMP-27 | $P_{intI2}$ | Winnipeg, Canada, | Homo sapiens | 2018 | CP048404 | [33] |
| *Providencia rettgeri* Res13-Sevr-LER2-35 | Plasmid | IMP-64 | $P_{intI2}$ | Quebec, Canada | Manure | 2017 | CP062822 | None |

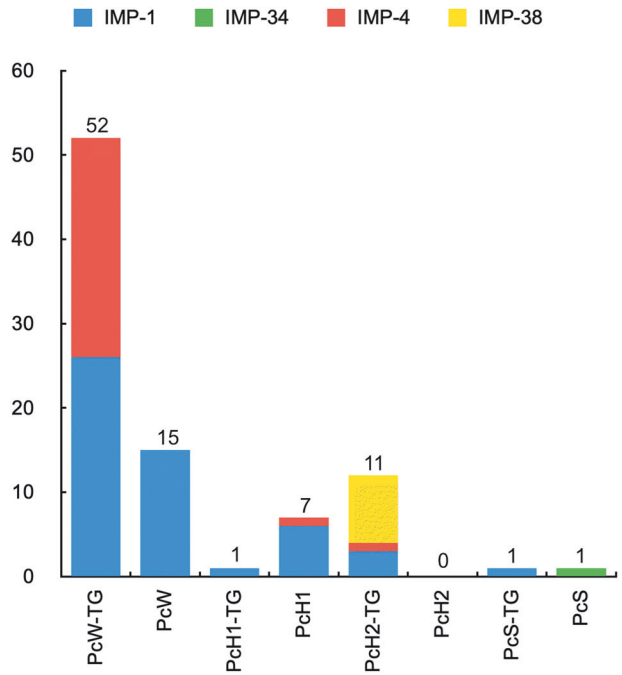


**Fig. 4** Recent distribution of Pcs in $bla_{IMP-1}$, $bla_{IMP-4}$ and their variants. $bla_{IMPs}$ ($n = 89$) were retrieved from 36,214 scaffold-level genome data of *Pseudomonadota* deposited in the GenBank database, and the Pc-type was classified

GenBank database. As a result, 89 $bla_{IMPs}$, which belonged to the *intI1*-$bla_{IMP}$ gene order, encoding IMP-4 and its one amino acid variant, IMP-38, and IMP-1 and its one amino acid variant, IMP-34, were retrieved. These data were collected after 2007, and by using these data, we can speculate how the Pc of $bla_{IMPs}$ has recently shifted and dominated. Consequently, the Pcs of $bla_{IMP-1}$ and $bla_{IMP-4}$ collected after 2007 are also dominated by PcW-TGs ($n = 52$, 58.4%) (Fig. 4). Furthermore, we also confirmed that $bla_{IMPs}$ with a strong promoter, PcS and PcS-TG, were restricted.

## Geographic spread of IMP-1- and IMP-4-producing bacteria and biased promoter utilization

Promoter analysis of $bla_{IMP-1}$ and $bla_{IMP-4}$ demonstrated that $bla_{IMP}$ with PcW-TG is currently spreading (Fig. 4). On the other hand, the correlation between promoter types and countries has not been fully elucidated. Therefore, we retrieved the Pcs of $bla_{IMP-1}$ ($n = 74$) and $bla_{IMP-4}$ ($n = 73$) from the full-length and scaffold-level genomic data and examined their spread and promoter types by country (Fig. 5). Our data demonstrated that $bla_{IMP-1}$ with PcW-TG has been detected mainly in Asian countries, although $bla_{IMP-1}$ genes were also detected in the UK, Brazil, and multiple African countries (Fig. 5b). Thus, the emergence of IMP-1-type-producing bacteria with different Pc promoters, which are geographically distant from Japan, appears to be sporadically distributed, and we further investigated this using a phylogenetic tree analysis (Fig. 6). On the other

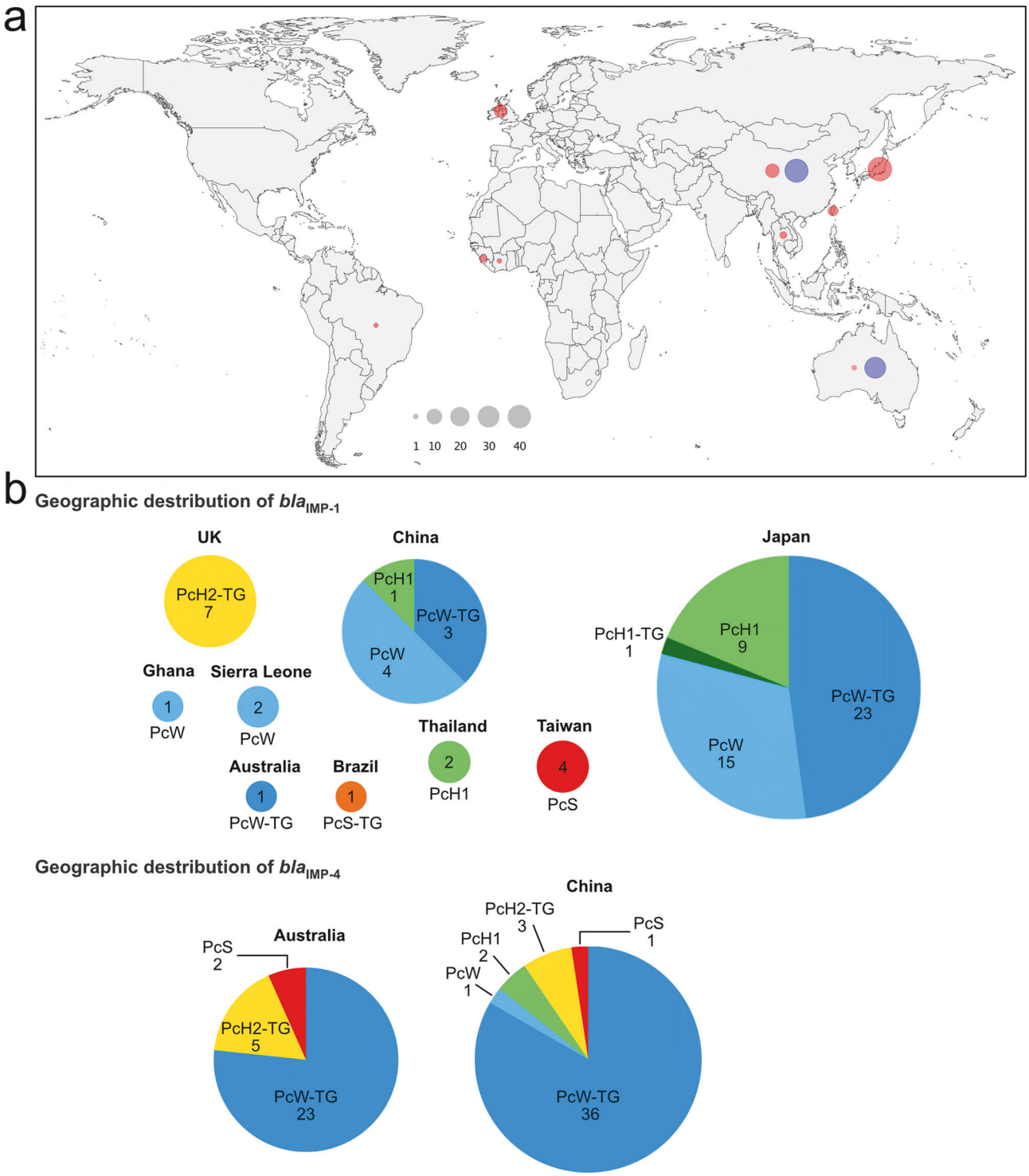


**Fig. 5** Geographic distribution of $bla_{\text{IMP-1}}$ and $bla_{\text{IMP-4}}$ (**a**) and promoter diversity (**b**). Pc promoters of $bla_{\text{IMP-1}}$ ($n = 74$) and blaIMP-4 ($n = 73$) from the full-length and scaffold-level genomic data were collected, and their spread and promoter types were investigated by country. Red and blue circles indicate the number of $bla_{\text{IMP-1}}$ and $bla_{\text{IMP-4}}$ detected, respectively. The world map was rendered by rworldmap as described in the Materials and Methods

hand, detection of $bla_{\text{IMP-4}}$ genes was limited to China and Australia, and again $bla_{\text{IMP-4}}$ with PcW-TG were dominant (Fig. 5c).

The amino acid sequences translated from $bla_{\text{IMP-1}}$ verified above are identical and form the IMP-1 family, but the coding sequence may contain several silent mutations.

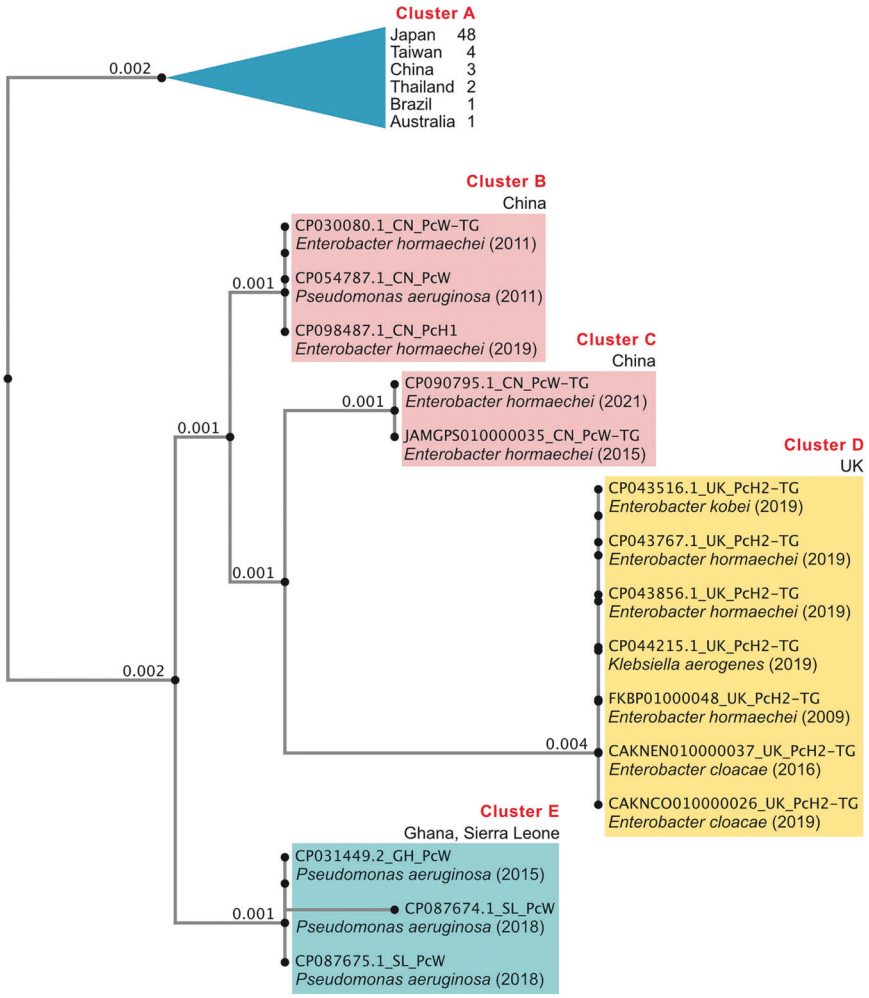


**Fig. 6** Phylogenetic tree of the $bla_{IMP-1}$ coding region. The coding region of $bla_{IMP-1}$ was analyzed by MAFFT and the phylogenetic tree was rendered by Phylo.io as described in the Materials and Methods. For convenience, the $bla_{IMP-1}$ coding regions are grouped into clusters A through E

Therefore, coding sequences of $bla_{IMP-1}$ and $bla_{IMP-4}$ used in this analysis (Fig. 5) were subjected to multiple alignment analysis and the resulting data were used for phylogenetic tree analysis as described in the Materials and Methods. Several silent mutations were observed in the $bla_{IMP-1}$ coding region, resulting in the phylogenetic tree shown in Fig. 6. Of the $bla_{IMP-1}$ coding regions ($n = 74$) used in the analysis, 74.7% (n = 59) shared the same coding sequence, grouped in cluster A, while the remaining 15 genes were grouped into four clusters B ~ E (Fig. 6). As described elsewhere for $bla_{IMP-1}$, it was concluded that IMP-1-producing bacteria from Asia, including Japan and China, grouped in cluster A, and IMP-1-producing bacteria from Europe are geographically independent [34]. In particular, seven strains isolated in the UK, grouped in cluster D, including $bla_{IMP-1}$ of *Enterobacter* complex and $bla_{IMP-1}$ of *Klebsiella aerogenes* [35] had the same silent mutation and the same promoter, PcH2-TG, suggesting that they were not derived from Asia but sporadically distributed in the UK (Table 3). Similarly, three strains from the IMP-1-producing *P. aeruginosa* found in Africa, grouped in cluster E, had silent mutations in their coding sequences. In contrast, the coding sequences of the four $bla_{IMP-1}$ with PcS isolated in Taiwan and the one $bla_{IMP-1}$ with PcS-TG isolated in Brazil (see Fig. 5b) were the same as that of the $bla_{IMP-1}$ isolated in Japan as grouped in cluster A. Interestingly, no silent mutations were observed in the coding sequence of $bla_{IMP-4}$ that spread to China and Australia. Between 1994 and 1998, imipenem-resistant *Acinetobacter* strains were detected in Hong Kong, and this is the first report of IMP-producing bacteria with $bla_{IMP-1}$ [36]. Taken together, these results suggest that the $bla_{IMP-1}$ detected in the UK and continental Africa may not have been of Asian origin and was most likely derived from a geographically isolated source and that IMP-4-producing bacteria spread from Hong Kong and mainland China to Australia.

## Discussion

Promoter analysis was performed on 115 $bla_{IMP}$ genes retrieved from 14,529 complete genomic data of

**Table 3** Characterization of $bla_{IMP-1}$ genes with the promoter PcH2-TG

| Bacterial strain | Origin | IMP type | Promoter | Location | Host | Collection date | Accession number | Reference |
|---|---|---|---|---|---|---|---|---|
| *Enterobacter kobei* EB_P8_L5_01.19 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2019 | CP043516 | [35] |
| *Enterobacter hormaechei* EB_P9_L5_03.19 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2019 | CP043767 | [35] |
| *Enterobacter hormaechei* EB_P6_L3_02.19 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2019 | CP043856 | [35] |
| *Klebsiella aerogenes* KA_P10_L5_03.19 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2019 | CP044215 | [35] |
| *Enterobacter hormaechei* e2071 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2009 | FKBP01000048 | None |
| *Enterobacter cloacae* cpe002 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2016 | CAKNEN010000037 | None |
| *Enterobacter cloacae* cpe090 | Plasmid | IMP-1 | PcH2-TG | UK | Homo sapiens | 2019 | CAKNCO010000026 | None |

*Pseudomonadota*. The results showed that the $bla_{IMPs}$ were divided into those derived from class 1 integrons ($n = 112$, 97.4%) and those from class 2 integrons ($n = 3$, 2.6%), respectively, and no promoters from class 3 integrons were detected. The Pc promoter is characterized by the presence of −35 and −10 hexamers within the coding sequence of Intl1; therefore, promoter mutations disrupting the translation of the coding sequence are not acceptable. The purpose of this study was to analyze alterations in and the spread of the $bla_{IMP}$ promoter under the Intl1 functional constraints.

It is known that there is an inverse correlation between the strength of Pc and the integrase efficiency for the integration and excision of gene cassettes [1], and in the present study, we demonstrated that the $bla_{IMP}$ with PcW-TG, the promoter of intermediate strength, diffuses in this trade-off relationship. Multiple alignment analysis revealed no silent mutations in the $bla_{IMP-4}$ coding sequence, but polymorphisms were observed in the Pc promoter. Thus, point mutations in the Pc region occur independently of mutations in the $bla_{IMP}$ coding sequence. Interestingly, IMP-4-producing *E. coli* 1585m1 has been isolated from the silver gull [32] (Table 1). This strain harbors two plasmids and each plasmid contains $bla_{IMP-4}$ with the strong promoter PcS. Thus, silver gulls living near urban areas may be potential reservoirs of carbapenem resistance.

Due to the fact that most studies are related to IMP typing or enzyme functions of metallo-beta-lactamase, and these data do not cover upstream promoter regions, there is a paucity of information on $bla_{IMP}$ promoter regions. In addition, our analysis method is based on the retrieval of $bla_{IMP}$ according to known annotations using Biopython. For example, the first report of a $bla_{IMP}$ was from *P. aeruginosa* strain GN17203 isolated in 1988 [2], but we were unable to search for the $bla_{IMP}$ because it was not annotated at that time. We expect that more accurate genomic information will be obtained by alteration of the retrieval conditions of the $bla_{IMP}$, which is an issue for the future.

In this study, we modeled the spread of $bla_{IMP}$ with strong promoters as a means for resistant bacteria to survive in the environment, but in reality, we found that $bla_{IMPs}$ with promoters of intermediate strength were widely distributed. In the future, a more detailed analysis of $bla_{IMP}$ diffusion will be possible by including the plasmid replicon type.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

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