Comprehensive Analysis of Imipenemase (IMP)-Type Metallo-β-Lactamase: A Global Distribution Threatening Asia

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Abstract: Antibiotic resistance, particularly beta-lactam resistance, is a major problem worldwide. Imipenemase or IMP-type metallo-β-lactamase (MBL) has become a more prominent enzyme, especially in Asia, since it was discovered in the 1990s in Japan. There are currently 88 variants of IMP-type enzymes. The most commonly identified variant of IMP-type enzymes is IMP–1 variant. IMP-type MBLs have been detected in more than ten species in Enterobacterales. Pseudomonas aeruginosa is the most frequent carrier of IMP-type enzymes worldwide. In Asia, IMP-type MBLs have been distributed in many countries. This work investigated a variety of currently available IMP-type MBLs at both a global level and a regional level. Out of 88 variants of IMP-type MBLs reported worldwide, only 32 variants were found to have susceptibility profiles. Most of the bacterial isolates carrying IMP-type MBLs were resistant to Carbapenems, especially Imipenem and Meropenem, followed by the 3rd-generation cephalosporins, and interestingly, monobactams. Our results comprehensively indicated the distribution of IMP-type MBLs in Asia and raised the awareness of the situation of antimicrobial resistance in the region.

Keywords: β-lactamase; carbapenemase; antimicrobial resistance

1. Introduction

Multidrug resistance organisms, especially β-lactamase-harbouring pathogens, are a major global public health problem worldwide resulting in high mortality, high morbidity and rising economic costs [1]. The β-lactamase enzyme, which can be produced by both gram-positive bacteria and gram-negative bacteria, inactivates β-lactam antibiotics (i.e., penicillin, cephalosporin, carbapenem and monobactam) by hydrolysing the amide bond of β-lactam ring [2]. Currently, there are more than 7270 enzymes available in the β-lactamase database (Beta-Lactamase database. Available online: www.bldb.eu, accessed on 30 November 2021). β-lactamase can be classified into four classes based on Ambler classification. Class A, C, D include serine protease-derived β-lactamases while class B includes the metallo-or zinc dependent β-lactamase (MBL) [3].

Imipenemase (IMP) is encoded by blaIMP genes. Along with other enzymes in this group: Verona Integron-encoded Metallo-β-lactamase (VIM), São Paulo metallo-β-lactamase (SPM) and German imipenemase (GIM). IMP belongs to class B β-lactamase and has carbapenemase activity [4]. Similar to other MBLs, IMP MBL breaks β-lactam ring with zinc as a catalyst and the enzyme can be inhibited by EDTA. IMP is commonly transferred between organisms, especially Gram-negative bacteria, via class 1 or class 3 of integron [5]. The discovery of blaIMP–1 was first reported in Japan in 1988 from P. aeruginosa strain GN17203 [6]. There are currently 88 variants of IMP reported worldwide.

Even though IMP-type MBLs are important and widely distributed around the world, a comprehensive review of this enzyme has not been conducted. Moreover, a previous phylogenetic construction was restricted due to the limited number of available sequences.
To understand the comprehensive picture of the \textit{bla}\textsubscript{IMP} gene, a review of relevant literature and a phylogenetic tree reconstruction was performed to investigate the distribution of IMP-type MBLs, phylogenetic relationship of the genes, and the association between phylogenetic cluster and antibiotic susceptibility.

2. Materials and Methods

2.1. Review of Literature

A comprehensive literature search was performed by PM and PP on Pubmed/Medline and EMBASE until 30 November 2021 to obtain relevant articles. The search terms used were “IMP and \(\beta\)-Lactamases”. A list of references was stored and the duplicates were removed using Endnote. PM and PP separately screened and selected the titles and the abstracts mentioning IMP metallo-\(\beta\)-lactamase. Articles were included when the prevalence of \(\textit{bla}\textsubscript{IMP}\) gene was reported. Articles were excluded when the English version was not available.

2.2. \(\textit{bla}\textsubscript{IMP}\) Gene Sequence Retrieval and Analysis

A total number of 88 sequences of IMP-type metallo-\(\beta\)-lactamase genes (\(\textit{bla}\textsubscript{IMP}\)) were found and downloaded from both \(\beta\)-lactamase databases [7] (last accessed, November 2021) and GenBank database in November 2021. IMP−36, IMP−50 and IMP−57 could not be found and retrieved from both databases. Multiple sequence alignment of both nucleotide sequences and amino acid sequences was processed using an iterative refinement algorithm in MUSCLE with default parameters [8] and manually edited in MEGA software version 11 [9]. The analysis of overall domain family of the BlaIMP was conducted in Pfam [10].

2.3. Phylogenetic Tree Estimation

Prior to the construction of the phylogenetic tree, the model test was conducted to estimate the most appropriate model using built-in functions in MEGA [9]. The maximum likelihood phylogenetic tree with 1000 bootstraps was constructed using General Time Reversible (GTR) model with gamma distribution for nucleotide sequences using FastTree [11]. The tree was visualised in FigTree (FigTree. Available online: http://tree.bio.ed.ac.uk/software/figtree/, accessed on 30 November 2021) and annotated in the interactive Tree of Life (iTOL) [12].

3. Results

3.1. Distribution of IMP-Type MBLs

A search of the NCBI database and EMBASE using “IMP and \(\beta\)-Lactamases” for gene encoding \(\textit{bla}\textsubscript{IMP}\) demonstrated a variety of variants of IMP-type MBL genes as well as species of IMP-carrying organisms. There were 88 variants of IMP-type MBL genes currently deposited on NCBI’s GenBank. These 88 variants were identified in 29 species across 32 countries (Table 1). Interestingly, most of the \(\textit{bla}\textsubscript{IMP}\) genes identified were from hospital isolates (Table 1). According to the genes submitted to GenBank and the literature search, the detection of \(\textit{bla}\textsubscript{IMP}\) was frequently reported from Japan (25%), followed by China (17%) and France (7%) (Figure 1A).

Table 1. List of currently available IMP-type metallo-\(\beta\)-lactamase genes.

| IMP Type | Host | Country of Isolation | Reference or Accession | Source of Isolates |
|----------|------|----------------------|------------------------|-------------------|
| IMP−1    | \textit{Achromobacter xylosoxidans} | Japan | EF027105.1, KF032823.1, KF032821.1, KF032820.1 | Hospital |
|          | \textit{Comamonas thiooxydans}     | Japan | AP025194.1             | Hospital |
Table 1. Cont.

| IMP Type | Host | Country of Isolation | Reference or Accession | Source of Isolates |
|----------|------|----------------------|------------------------|--------------------|
| IMP−1    |      |                      |                        |                    |

**Pseudomonas aeruginosa**

- **IMP−1**
  - Japan
    - Pseudomonas aeruginosa
      - Reference: AB983593.1
      - Accession: [13]
      - Source: Hospital
    - Thailand
      - Reference: KX987869.1
      - Accession: [13]
      - Source: Hospital
    - Malaysia
      - Reference: AY386702.1
      - Accession: AY912485.1
      - Source: Hospital
    - China
      - Reference: KR703251.1
      - Accession: JX648311, JX644173, JQ766530
      - Source: Hospital
    - Iran
      - Reference: LC636409.1
      - Accession: [13]
      - Source: Hospital
    - Nepal
      - Reference: LC636409.1
      - Accession: [13]
      - Source: N/A
    - Singapore
      - Reference: AY168635.1
      - Accession: AY625689.1, AY625687.1, AY625686.1
      - Source: Hospital
    - Egypt
      - Reference: KX453681.1
      - Accession: [13]
      - Source: Hospital
    - (Direct submission from Brazil)
      - Reference: GU831553.1
      - Accession: GU831552.1, GU831551.1, GU831550.1, GU831549.1, GU831548.1, GU831547.1, GU831546.1
      - Source: N/A
    - (Submitted from the UK, unpublished)
      - Reference: MH594579.1
      - Accession: [13]
      - Source: Hospital
    - Turkey
      - Reference: DQ842025.1
      - Accession: [13]
      - Source: Hospital
    - India
      - Reference: KF570107.1
      - Accession: [13]
      - Source: Hospital
    - USA
      - Reference: MK388919.1
      - Accession: MF479262.1
      - Source: N/A

**Pseudomonas putida**

- Singapore
  - Reference: AY251052.1
  - Accession: [13]
  - Source: Hospital

**Pseudomonas fluorescens**

- Singapore
  - Reference: AY250709.1
  - Accession: [13]
  - Source: Hospital

**Serratia marcescens**

- Japan
  - Reference: AB162950.1
  - Accession: AB162949.1, AB162948.1, AB162947.1, NG_049172.1
  - Source: Hospital

**Klebsiella pneumoniae**

- Iran
  - Reference: LC512050.1
  - Accession: LC512051.1
  - Source: Hospital

**Klebsiella pneumoniae**

- Japan
  - Reference: LC512050.1
  - Accession: LC512051.1
  - Source: Hospital

**Acinetobacter spp.**

- Korea
  - Reference: EU014166.1
  - Accession: EU686386.1
  - Source: Hospital

**Acinetobacter calcoaceticus**

- Thailand
  - Reference: HM185482.1
  - Accession: [13]
  - Source: Hospital

**Acinetobacter baumannii**

- Japan
  - Reference: EF375699.1
  - Accession: [13]
  - Source: Hospital

- Iran
  - Reference: KR080548.1
  - Accession: KF723585.1
  - Source: Hospital

- Thailand
  - Reference: HM036079.1
  - Accession: [13]
  - Source: Hospital

**Acinetobacter pittii**

- Taiwan
  - Reference: GU064942.1
  - Accession: GU064941.1
  - Source: N/A

**Acinetobacter nosocomialis**

- Taiwan
  - Reference: GU064940.1
  - Accession: GU064939.1
  - Source: N/A
Table 1. Cont.

| IMP Type | Host                | Country of Isolation | Reference or Accession | Source of Isolates |
|----------|---------------------|----------------------|------------------------|--------------------|
| IMP−1    | Citrobacter freundii| Japan                | AB754498.1             | N/A                |
|          | Citrobacter youngae | (Direct submission from Ireland) | MW847603.1 | Hospital          |
|          | Enterobacter aerogenes | Japan            | [15]                    | Hospital           |
|          | Enterobacter cloacae | (Direct submission from Japan) | LC508022.1 | Hospital          |
|          | China               | MK088089.1           | Hospital                |                    |
|          | Enterobacter hormaechei | China              | MG287118.1             | N/A                |
|          | Escherichia coli    | Iran                 | LC512049.1             | Hospital           |
|          | Proteus mirabilis   | Brazil               | KY057362.1             | Hospital           |
|          | Proteus vulgaris    | Japan                | [16]                    | Hospital           |
|          | Providencia rettgeri | Japan               | AB754496.1             | N/A                |
|          | Leclercia adecarboxylata | China          | KJ531212.1             | Hospital           |
| IMP−2    | Acinetobacter baumannii | Italy             | AJ243491.1, NG_049183.1 | Hospital          |
|          | Serratia marcescens | Japan                | AB182996.1             | N/A                |
|          | Pseudomonas aeruginosa | India            | KC588963.1             | Hospital           |
| IMP−3    | Shigella flexneri   | (Published in USA)  | NG_049194.1            | N/A                |
|          | Acinetobacter baumannii | Hong Kong         | NG_049203.1, AF445082.1, AF244145.1 | Hospital          |
|          |                      | Singapore           | DQ532122.1, AY795963.1, AY590475.1 | Hospital          |
|          | Acinetobacter calcoaceticus | (Direct submission from Malaysia, unpublished) | DQ307573.1 | N/A                |
| IMP−4    | Citrobacter freundii | China               | JQ818252.1             | N/A                |
|          | Escherichia coli    | China                | AB636651.1             | N/A                |
|          | (Direct submission from India) | China | MF169878.1 | N/A                |
|          | Enterobacter cloacae | China               | KF699334.1             | N/A                |
|          | Korea               | KY884003.1           | Hospital                |                    |
|          | Japan               | LC198842.1           | Hospital                |                    |
|          | Enterobacter aerogenes | China            | KF184385.1             | Hospital           |
|          | Klebsiella pneumoniae | China          | EU368858.1, KF184388.1, FJ384365.1 | Hospital          |
|          |                      | JQ808503.1, JN106667.1, KF680003.1 | N/A                |
|          | Klebsiella oxytoca  | China               | JQ820404.1             | N/A                |
|          |                      | KY913900.1           | Animal                  |                    |
Table 1. Cont.

| IMP Type | Host                      | Country of Isolation | Reference or Accession                     | Source of Isolates |
|----------|---------------------------|----------------------|--------------------------------------------|--------------------|
| IMP−4    | *Pseudomonas aeruginosa*  | China                | DQ297664.1                                 | N/A                |
|          |                           | Malaysia             | GQ221782.1                                 | Hospital           |
| IMP−5    | *Acinetobacter baumannii* | Portugal             | NG_049212.1, JF810083.1                    | Hospital           |
| IMP−6    | *Escherichia coli*        | Japan                | AB753460.1                                 | N/A                |
|          | *Serratia marcescens*     | Japan                | NG_049220.1, AB040994.1                    | Hospital           |
|          | *Providencia rettgeri*    | Japan                | AB754497.1                                 | N/A                |
|          | *Pseudomonas aeruginosa*  | Korea                | EU117233.1                                 | Hospital           |
| IMP−7    | *Pseudomonas aeruginosa*  | Canada               | NG_049221.1, AF318077.1                    | Hospital           |
|          |                           | Czech                | JX982232.1                                 | Hospital           |
|          |                           | Japan                | LC091209.2, LC091210.2                     | Hospital           |
|          |                           | Malaysia             | GQ221781.1, AF416736.2, GU213192.1         | Hospital           |
|          |                           | India                | HM641894.1                                 | Hospital           |
|          |                           | Singapore            | AY625685.1                                 | Hospital           |
|          |                           | Slovakia             | EF601914.1                                 | Hospital           |
|          | *Acinetobacter baumannii* | Taiwan              | EF127959.1                                 | Hospital           |
|          |                           | China                | DQ845788.1                                 | Hospital           |
|          | *Escherichia coli*        | Singapore            | KF534724.1                                 | Hospital           |
| IMP−8    | *Enterobacter cloacae*    | Taiwan               | [16]                                       | Hospital           |
|          |                           | China                | JQ820405.1                                 | N/A                |
|          | *Klebsiella pneumoniae*   | China                | JQ820406.1, EU368856.1                     | Hospital           |
|          |                           | Taiwan               | NG_049222.1, AF322577.2                    | Hospital           |
|          | *Klebsiella oxytoca*      | Tunisia              | HE605039.1                                 | Non-hospital        |
|          | *Serratia marcescens*     | Taiwan               | EU042136.1                                 | N/A                |
| IMP−9    | *Pseudomonas aeruginosa*  | China                | AY033653, EU176818.1                       | Hospital           |
|          |                           | (Direct submission  | KF184386.1, KF255597.1, KF255596.1, KF255595.1 | N/A                |
|          |                           | from China)          | HM106459.1                                 |                    |
|          | *Achromobacter xylosoxidans* | Japan               | AB074435.1, AB195638.1                    | Hospital           |
| IMP−10   | *Pseudomonas aeruginosa*  | Japan                | AB074434.1, AB074433.1, NG_049173.1, AB195637.1 | Hospital           |
|          |                           | (Direct submission  | DQ288156.1                                 | Hospital           |
|          |                           | from Japan, Unpublished) |                                      |                    |
|          | *Pseudomonas putida*      | Italy                | AJ420864.1                                 | Hospital           |
|          | *Klebsiella pneumoniae*   | Tunisia              | HE605040.1                                 | Non-hospital        |
| IMP−11   | *Acinetobacter baumannii* | Japan                | AB074436, NG_049174.1                     | Hospital           |
|          | *Enterobacter cloacae*    | Japan                | LC628821.1                                 | N/A                |
| IMP Type | Host Type | Host | Country of Isolation | Reference or Accession | Source of Isolates |
|----------|-----------|------|----------------------|------------------------|-------------------|
| IMP−12   | *Pseudomonas putida* | Italy | Italy | FJ172676.1, FJ172674.1, AJ512502.1, NG_049176.1 | Hospital |
| IMP−13   | *Pseudomonas aeruginosa* | Italy | France | JX131371.1 | Hospital |
| IMP−14   | *Achromobacter xylosoxidans* | Thailand | Thailand | KJ406506.2, KJ406505.2 | Hospital |
| IMP−15   | *Pseudomonas aeruginosa* | Thailand | Vietnam | LC075716.1 | N/A |
| IMP−16   | *Pseudomonas aeruginosa* | Brazil | Spain | KC310496.1 | Hospital |
| IMP−17   | *Pseudomonas aeruginosa* | Italy | USA | AM780674.2, NG_049181.1 | Hospital |
| IMP−18   | *Pseudomonas aeruginosa* | Japan | Mexico | HM138673.1 | N/A |
| IMP−19   | *Achromobacter baumannii* | Iran | Japan | JQ766528.1 | N/A |
| IMP−20   | *Pseudomonas aeruginosa* | Japan | Japan | AB184977.1 | Hospital |
| IMP−21   | *Pseudomonas aeruginosa* | Japan | Japan | AB201263.1 | N/A |
| IMP−22   | *Providencia rettgeri* | Japan | Japan | AB754495.1 | N/A |
| IMP−23   | *Pseudomonas aeruginosa* | Austria | France | NG_049182.1 | Hospital |
| IMP−24   | *Providencia rettgeri* | Italy | Taiwan | EF192154.1, NG_049188.1 | Hospital |
Table 1. Cont.

| IMP Type | Host                  | Country of Isolation | Reference or Accession                           | Source of Isolates |
|----------|-----------------------|----------------------|--------------------------------------------------|--------------------|
| IMP−25   | **Pseudomonas aeruginosa** | China                | EU352796                                         | Hospital           |
|          |                       | Korea                | EU541448.1, NG_049189.1                          | Hospital           |
|          |                       | (Direct submission from China, unpublished) | KY081418.1, KY081417.1, HM175876.1               | N/A                |
| IMP−26   | **Stenotrophomonas maltophilia** | China                | HQ685900.1                                       | Hospital           |
|          | **Enterobacter cloacae** | Malaysia             | JQ629930.1                                       | Hospital           |
|          | **Pseudomonas aeruginosa** | Nepal                | LC636067.1                                       | Hospital           |
|          | **Pseudomonas aeruginosa** | Singapore           | GU045307.1, NG_049190.1                           | Hospital           |
|          | **Pseudomonas aeruginosa** | Vietnam              | LC075717.1                                       | N/A                |
| IMP−27   | **Proteus mirabilis**  | USA                  | JF894248.1                                       | Hospital           |
|          | **Providencia rettgeri** | USA                  | KY847874.1                                       | N/A                |
| IMP−28   | **Klebsiella oxytoca** | Spain                | HQ263342.1, NG_049192.1                          | Hospital           |
| IMP−29   | **Pseudomonas aeruginosa** | France              | HQ438058.1, JQ9461634, NG_049193.1               | Hospital           |
| IMP−30   | **Escherichia coli**   | China                | KM589497.1                                       | Hospital           |
|          | **Pseudomonas aeruginosa** | Russia              | NG_049195.1                                       | N/A                |
| IMP−31   | **Pseudomonas aeruginosa** | Germany             | KF148593.1, NG_049196.1                          | Hospital           |
| IMP−32   | **Klebsiella pneumoniae** | Thailand            | NG_049197.1, JQ902629.1                          | Hospital           |
| IMP−33   | **Pseudomonas aeruginosa** | Italy               | JN848782, NG_049198.1                            | Hospital           |
| IMP−34   | **Klebsiella oxytoca** | Japan                | AB700341.1, NG_049199.1                           | Hospital           |
|          | **Acinetobacter calcoaceticus** | Japan              | AB700341.1, NG_049199.1                           | Hospital           |
| IMP−35   | **Pseudomonas aeruginosa** | Germany             | JF816544.1, NG_049200.1                          | Hospital           |
| IMP−36   | Not found in NCBI database and pubmed |                     |                                                  |                    |
| IMP−37   | **Pseudomonas aeruginosa** | France              | JX131372.1, NG_049201.1                          | Hospital           |
| IMP−38   | **Klebsiella pneumoniae** | China                | HQ875573.1, NG_049202.1                          | N/A                |
| IMP−39   | **Pseudomonas aeruginosa** | France              | MK50818.1, NG_064724.1                           | Hospital           |
| IMP−40   | **Pseudomonas aeruginosa** | Japan                | AB753457, NG_049204.1                            | N/A                |
| IMP−41   | **Pseudomonas aeruginosa** | Japan                | AB753458, NG_049205.1                            | N/A                |
| IMP−42   | **Acinetobacter soli**  | Japan                | AB753456.1, NG_049206.1                          | N/A                |
| IMP−43   | **Pseudomonas aeruginosa** | Japan                | NG_049207.1                                      | Hospital           |
| IMP−44   | **Pseudomonas aeruginosa** | Japan                | NG_049208.1                                      | Hospital           |
| IMP−45   | **Pseudomonas aeruginosa** | China                | KJ510410.1, NG_049209.1                          | Animal             |
| IMP−46   | **Pseudomonas putida**   | France               | MK543944, MK507819.1, NG_064725.1                 | Hospital           |
| IMP−47   | **Serratia marcescens**  | (Direct submit USA)  | KP050486.1                                       | N/A                |
Table 1. Cont.

| IMP Type | Host                     | Country of Isolation | Reference or Accession | Source of Isolates |
|----------|--------------------------|----------------------|------------------------|--------------------|
| IMP−48   | *Pseudomonas aeruginosa* | (Direct submit USA, unpublished) | NG_049210.1, KM087857.1 | N/A                |
| IMP−49   | *Pseudomonas aeruginosa* | Brazil               | NG_049211, KP681694.1  | N/A                |
| IMP−50   | Not found in NCBI database and pubmed |                      |                        |                    |
| IMP−51   | *Pseudomonas aeruginosa* | Vietnam              | NG_049213.1, LC031883.1 | Hospital           |
| IMP−52   | *Escherichia coli*       | Japan                | NG_049214.1, LC055762.1 | N/A                |
| IMP−53   | *Pseudomonas aeruginosa* | (Direct submit USA)  | NG_049215.1            | N/A                |
| IMP−54   | *Pseudomonas aeruginosa* | Thailand             | KU052795.1, NG_049216.1 | N/A                |
| IMP−55   | *Acinetobacter baumannii*| Iran                 | KU299753.1, NG_049217.1 | Hospital           |
| IMP−56   | *Pseudomonas aeruginosa* | Mexico               | KU351745.1             | Hospital           |
| IMP−57   | Not found in NCBI database and pubmed |                      |                        |                    |
| IMP−58   | *Pseudomonas putida*     | Denmark              | KU647281.1, NG_049219.1 | N/A                |
| IMP−59   | *Escherichia coli*       | Australia            | KX196782.1, NG_055477.1 | N/A                |
| IMP−60   | *Enterobacter cloacae*   | Japan                | LC159227.1, NG_050945.1 | Hospital           |
| IMP−61   | *Acinetobacter baumannii*| (Direct submission from Germany, unpublished) | KX462700.1, NG_051166.1 | Hospital           |
| IMP−62   | *Pseudomonas aeruginosa* | Mexico               | KX753224.1, NG_051513.1 | Hospital           |
| IMP−63   | *Pseudomonas aeruginosa* | France               | KX821663.1, NG_052049.1 | Hospital           |
| IMP−64   | *Proteus mirabilis*      | USA                  | NG_054710.1, KX949735.2 | N/A                |
| IMP−65   | *Pseudomonas aeruginosa* | Thailand             | KY315991.1, NG_065080.1 | Hospital           |
| IMP−66   | *Escherichia coli*       | Japan                | LC190726.1, NG_054676.1 | N/A                |
| IMP−67   | *Providencia rettgeri*   | (Direct submission from USA, unpublished) | MF281100.1, NG_055271.1 | N/A                |
| IMP−68   | *Klebsiella pneumoniae*  | Japan                | MF669572.1, NG_055584.1 | N/A                |
| IMP−69   | *Providencia rettgeri*   | China                | MF678349.1, NG_055665.1 | N/A                |
| IMP−70   | *Providencia rettgeri*   | Germany              | MG748725.1, NG_056176.1 | Hospital           |
| IMP−71   | *Providencia rettgeri*   | Japan                | LC348383.1             | N/A                |
| IMP−72   | *Pseudomonas aeruginosa* | France               | MG818167.1             | Hospital           |
| IMP−73   | *Pseudomonas aeruginosa* | Mexico               | MH021847.1             | N/A                |
| IMP−74   | *Pseudomonas aeruginosa* | Japan                | MH021848.1, NG_057463.1 | N/A                |
| IMP−75   | *Pseudomonas aeruginosa* | Brazil               | MH243349.1, NG_057606.1 | N/A                |
| IMP−76   | *Pseudomonas aeruginosa* | Mexico               | MH243350.1, MW692112.1, NG_057607.1 | N/A |
| IMP−77   | *Pseudomonas aeruginosa* | Japan                | NG_061409.1           | Hospital           |
| IMP−78   | *Pseudomonas aeruginosa* | Japan                | NG_061410.1           | Hospital           |
| IMP−79   | *Pseudomonas aeruginosa* | France               | MG873561.1, NG_061626.1 | Hospital           |
| IMP−80   | *Pseudomonas aeruginosa* | Japan                | NG_062274.1           | Hospital           |
| IMP−81   | *Pseudomonas aeruginosa* | Columbia             | MN267699.1             | N/A                |
According to Figure 1A, Asia accounted for 69% of the reporting countries. The presence of the \textit{bla}_{IMP} gene was reported in 12 countries, namely, China (including Hong Kong), India, Iran, Japan, Korea, Malaysia, Nepal, Singapore, Thailand, Turkey and Vietnam. Focusing on Asia, Japan and China were the first (36%) and the second (25%) most frequently \textit{bla}_{IMP} identified countries. Thailand and Singapore were the third most frequently reported countries (7%) (Figure 1B). The most frequently reported \textit{bla}_{IMP} carriers were \textit{Pseudomonas aeruginosa}, followed by \textit{Acinetobacter baumannii}, \textit{Klebsiella pneumoniae} and \textit{Enterobacter cloacae}. By considering the variant of \textit{bla}_{IMP} in countries with high prevalence of \textit{bla}_{IMP} in Asia, \textit{bla}_{IMP}−1 was the most frequently reported in Japan (23%) and Singapore (50%). \textit{bla}_{IMP}−4 and \textit{bla}_{IMP}−14 were the most frequently reported in China (27%) and Thailand (27%), respectively (Figure 2A–D).

**Table 1. Cont.**

| IMP Type | Host               | Country of Isolation | Reference or Accession          | Source of Isolates |
|----------|--------------------|----------------------|---------------------------------|--------------------|
| IMP−82   | \textit{Pseudomonas aeruginosa} | (Direct submission from Germany, unpublished) | MN057782.1            | Hospital           |
|          |                    | (Direct submission from USA, unpublished) | NG_065873.1            | Hospital           |
| IMP−83   | \textit{Pseudomonas aeruginosa} | Mexico               | MN104595.1, NG_065874.1        | N/A                |
| IMP−84   | \textit{Pseudomonas aeruginosa} | (Direct submission from Switzerland, unpublished) | MN219692.1            | N/A                |
|          |                    | (Direct submission from USA, unpublished) | NG_065875.1            | N/A                |
| IMP−85   | \textit{Pseudomonas aeruginosa} | France               | MN510335.1, NG_066696.1        | Hospital           |
| IMP−86   | \textit{Pseudomonas aeruginosa} | China                | MT241520.1, NG_076650.1        | N/A                |
| IMP−87   | \textit{Pseudomonas aeruginosa} | China                | MT241521.1, NG_076651.1        | N/A                |
| IMP−88   | \textit{Pseudomonas aeruginosa} | Japan                | LC558310.1, NG_070737.1        | Hospital           |
| IMP−89   | \textit{Pseudomonas putida}   | China                | NG_070738.1                    | N/A                |
| IMP−90   | \textit{Pseudomonas aeruginosa} | (Direct submission from Germany, unpublished) | MW811441.1            | Hospital           |
|          |                    | (Direct submission from USA, unpublished) | NG_074713.1            | Hospital           |
| IMP−91   | \textit{Pseudomonas aeruginosa} | China                | MZ702721.1, NG_076634.1        | N/A                |
Figure 1. Distribution of IMP-type metallo-β-lactamase annotated genes (A) worldwide (B) in Asia.

Figure 2. Distribution of \textit{blaIMP} annotated genes in four countries in Asia: (A) Japan, (B) China, (C) Thailand and (D) Singapore. IMP-N is used to represent \textit{blaIMP-N}. 
3.2. In Silico Analysis of IMP-Type MBLs

In silico analysis of IMP-type MBL genes was conducted to investigate the diversity of the genes. Using multiple sequence alignment of 88 variants of IMP-type MBLs, the conserved sequences of active sites were identified as follows: His95, Phe96, His97, Asp99, Ser100, His157, Cys176 and His215 (numbered according to IMP−1; Supplementary Materials Figure S1) [17]. These sequences were residues of a lactam ring-catalytic site. The overall analysis showed 79.3%–96.7% amino acid sequence similarity. To investigate other functions of the protein, we performed protein domain prediction in Pfam. The result showed that this protein contained only one domain, namely ‘Metallo-hydrolase-like-MBL-fold superfamily’, covering from amino acid position 23 to position 234 (result not shown).

A phylogenetic tree was constructed to visualise the relationship of the genes. blaIMP genes were separated into three main clusters (Figure 3). Group I contains 38 variants. Noticeably, blaIMP−12, blaIMP−63 and blaIMP−90, previously identified as group II [18], were currently in a subgroup of group I, called group Ia, with 95.1% bootstrap support. These three variants were isolated from strains with European origin. Group II contains 41 variants. Lastly, group III contains nine variants (Figure 3).

3.3. Resistance of IMP MBL Variants-Carrying Strains

The pattern of antibiotic susceptibility of the isolate carrying each blaIMP variant was obtained from the articles to investigate whether the variation in each variant was associated with susceptibility. The susceptibility profiles were taken from bacterial isolates carrying those blaIMP genes. By reviewing the literature, most of the antibiotic agents tested were in the group of cephalosporin and carbapenem (Figure 3), especially antipseudomonal antibiotics, since P. aeruginosa was the most abundant species identified to possess the blaIMP gene. Out of 88 available variants, susceptibility profile was reported only in 32 variants (Figure 3, right panel). Overall, strains with blaIMP were resistant to several β-lactam antibiotics.

For carbapenem, almost all of the isolates with blaIMP variants were resistant to both meropenem and imipenem. IMP−19, −28, and −34 enzymes were unable to inactivate the carbapenems. Similarly, Cephalosporin was shown to be less active against blaIMP−carrying species. Likewise, isolates with blaIMP were resistant to cephalosporins. Aztreonam, a monobactam, was also shown to have an anti-bacterial effect on most of blaIMP carriers.

By combining the antibiotic susceptibility profile with the phylogenetic tree to investigate the relationship between clustering and susceptibility, it was found that susceptibility pattern was not associated with the phylogenetic tree (Figure 3).
Figure 3. Phylogenetic relationship of bla\textsubscript{IMP} genes. An unrooted maximum likelihood phylogenetic tree constructed using nucleotide sequences of 88 bla\textsubscript{IMP} genes with 1000 bootstrap supports was visualised together with antibiotic susceptibility profile of 32 variants of the bla\textsubscript{IMP} gene. Red squares indicated “resistant” while green squares indicated “susceptible”. IMP-N is used to represent blalMP-N.
4. Discussion

The attention to clinically important bacteria has been rising due to the multidrug resistance caused by the production of drug-inactivating enzymes, especially β-lactamases [19]. More critically, the carbapenemase enzyme has been increasingly detected in pathogens that are associated with nosocomial infections [20,21]. This study is the first to comprehensively investigate the epidemiology and the diversity of IMP-type MBLs, class B β-lactamase with carbapenemase ability.

An IMP-type MBL is encoded by the \( \text{bla}^{\text{IMP-N}} \) gene (\( N = \) an order of variant discovered) which can be located on the chromosome or the plasmid, which facilitates the transfer of the \( \text{bla}^{\text{IMP}} \) gene via horizontal gene transfer [22,23]. Our study showed that the \( \text{bla}^{\text{IMP}} \) gene was detected in clinically relevant species, including \( P. \) aeruginosa and \( A. \) baumannii, which are associated with nosocomial infection and listed in “Priority 1: CRITICAL” list of antibiotic resistant pathogens by WHO (WHO publishes list of bacteria for which new antibiotics are urgently needed. Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed, accessed on 30 December 2021). Interestingly, our analysis revealed that the top two countries where \( \text{bla}^{\text{IMP}} \) genes were detected were both Asian countries: Japan and China. Japan is the first place where IMP-type MBLs (IMP−1) were reported [6]. In Asia, there were 28, 15, 7 and 5 variants of the \( \text{bla}^{\text{IMP}} \) gene identified in Japan, China, Thailand and Singapore, respectively. A recent study revealed that carbapenemases (derived from \( P. \) aeruginosa) are distributed throughout Thailand [18]. However, the epidemiological study of IMP variants in Japan and China has not yet been conducted. It is, therefore, important to note that the \( \text{bla}^{\text{IMP}} \) gene is one of the causes of antibiotic resistance in Asia.

The phylogenetic tree is commonly used to investigate the evolutionary relationship of genes or organisms. Our findings revealed that a reconstructed phylogenetic tree using 88 \( \text{bla}^{\text{IMP}} \) variants clustered the genes into three main groups (Figure 2). In a broad picture, this tree was similar to the previous version [18]. Nevertheless, group Ia, which was previously clustered in group II, was currently identified in group I with high bootstraps. It is important to note that the structure of phylogeny of \( \text{bla}^{\text{IMP}} \) is nearly well-defined although some branches remain dynamic depending on the number of genes added to the tree. The change of position in the phylogenetic tree could be caused by the increased number of tested genes in our study.

A search for antibiotic susceptibility profiles revealed that strains containing 32 variants (out of 88) were tested for their susceptibility. The profile showed that the 3rd-generation cephalosporins and carbapenem were less effective against most strains with the \( \text{bla}^{\text{IMP}} \) gene. Interestingly, Aztreonam is the only agent that is active to the strains with most types of \( \text{bla}^{\text{IMP}} \) (Figure 3). However, the association between susceptibility and the phylogenetic tree was absent. This is supported by the findings showing that the sequence of the active site (catalytic site) was highly conserved within the members of MBLs [17]. It is of note that nucleotide or amino acid substitutions outside the active site might not affect the β-lactam-hydrolysing activity of the enzyme. In addition, the susceptibility profile of the strains containing each \( \text{bla}^{\text{IMP}} \) variant must be performed to ensure the association between the substitution/phylogenetic tree and the antibiotic resistance pattern. It is important to note that the susceptibility profile was taken from bacterial isolates, so the susceptibility can be affected by another mechanism, such as other β-lactamases or efflux pumps [24]. All in all, the findings of this work demonstrated that antibiotic resistance-associated genes were distributed to several regions around the world. This emphasised that the need of discovering or inventing novel antibiotic agents and enforcing antibiotic stewardship is urgent.

5. Conclusions

Carbapenemase, especially IMP-type MBLs, causes public health problems worldwide. This study is the first to comprehensively analyse all currently available variants of IMP-type MBLs and their associated susceptibility. Asian countries, especially Japan and
China, are presently under a wide spread of blaIMP -carrying bacteria which are antibiotic-resistant organisms listed by WHO. An unrooted phylogenetic backbone of blaIMP gene variants illustrated two separate groups without susceptibility or geographical association. This strengthens antibiotic stewardship policy on a global level to control antibiotic resistance problems.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antibiotics11020236/s1, Figure S1: Multiple sequence alignment of amino acid sequence of 88 blaIMP variants.

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