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Comprehensive analysis of imipenemase (IMP)-type metallo-Beta-lactamase showing global distribution threating Asia

Pisut Pongchaikul [1,2,3,*] and Paninee Mongkolsuk [1]

[1] Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan 10540 Thailand; pisut.pon@mahidol.edu (P.P.), phaninae@gmail.com (P.M.)

[2] Integrative Computational BioScience Center, Mahidol University, Nakhon Pathom 73170 Thailand (P.P.)

[3] Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom; pisutp@liverpool.ac.uk (P.P.)

* Correspondence: pisut.pon@mahidol.edu

Abstract: Antibiotic resistance, particularly beta-lactam resistance, is a major problem worldwide. Imipenemase or IMP-type metallo-beta-lactamase (MBL) has become a more prominent enzyme, especially in Asia, since it was discovered in the 1990s in Japan. There are currently more than 91 variants of IMP-type enzymes. The most commonly identified variant of IMP-type enzymes is IMP-1 variant. IMP-type MBLs have been identified in more than 10 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 in the region. This work investigated a variety of currently available IMP-type MBLs in both global level and regional level. Out of 88 variants of IMP-type MBLs reported worldwide, only 32 variants were found to have susceptibility profiles. Most of the 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: beta-lactamase; carbapenemase; antimicrobial resistance

1. Introduction

Multidrug resistance organisms, especially β-lactamase-habouring pathogens, is a major global public health problem worldwide resulting in high mortality, high morbidity and rising economic costs [1]. The β-lactamase enzyme, that 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 7,270 enzymes available in the β-lactamase database (www.bldb.eu). β-lactamase can be classified into four classes, based on Ambler classification; class A, C, D are serine protease-derived β-lactamases while class B is the metallo-or zinc dependent β-lactamase (MBL) [3].

Imipenemase (IMP) is encoded by *bla*$_{IMP}$ genes. Along with other enzymes in this group: Verona Integron-encoded Metallo-β-lactamase (VIM), São Paulo metallo-beta-lactamase (SPM) and German imipenemase (GIM), IMP belongs to class B β-lactamase and has carbapenemase activity [4]. Similar to other MBLs, IMP 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 IMP-1 was first reported in Japan in 1988 from P. aeruginosa strain GN17203 [6]. There are currently 88 variants of IMP reported worldwide.

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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 number of available sequences. To understand the comprehensive picture of *bla*$_{IMP}$ 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 literatures

A comprehensive literature search was performed by PM and PP on Pubmed/Medline and EMBASE until 30th 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 *bla*$_{IMP}$ gene was reported. Articles were excluded when the English version was not available.

2.2. *bla*$_{IMP}$ gene sequence retrieval and analysis

A total number of 88 sequences of IMP-type metallo-beta-lactamase genes (*bla*$_{IMP}$) were found and downloaded from both beta-lactamase databases [7] (last accessed November 2021) and GenBank database in November 2021. IMP-36, -50 and -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].

2.3. Phylogenetic tree estimation

Prior to the construction of phylogenetic tree, the model test was conducted to estimate the most appropriate model using built-in functions in MEGA (Kumar, 2018). The maximum likelihood phylogenetic tree with 1,000 bootstraps was constructed using General Time Reversible (GTR) model with gamma distribution for nucleotide sequences using FastTree [10]. The tree was visualised in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and annotated in interactive Tree of Life (iTOL) [11].

3. Results

3.1 Distribution of IMP-type MBLs

A search of NCBI database and EMBASE using "IMP and beta-Lactamases" for gene encoding *bla*$_{IMP}$ 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

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currently deposited on NCBI's GenBank. These 88 variants were identified in 29 species across 32 countries (Table 1). According to the genes submitted to GenBank and literature search, the detection of *bla*$_{IMP}$ 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
IMP-1	*Achromobacter xylosoxidans*	Japan	EF027105.1, KF032823.1, KF032821.1,KF032820.1
	Comamonas thiooxydans	Japan	AP025194.1
	P. aeruginosa	Japan	AB983593.1
		Thailand	[12]
		Malaysia	KX987869.1
		China	AY386702.1, AY912485.1
		Iran	KR703251.1,JX648311.1, JX644173.1, JQ766530.1
		Nepal	LC636409.1
		Singapore	AY168635.1,AY625689.1,AY625688.1, AY625687.1, AY625686.1
		Egypt	KX452681.1
		- (Direct submitted in Brazil)	GU831553.1,GU831552.1,GU831551.1,GU831550.1,GU831549.1,
		- (Submitted UK, unpublished)	MH594579.1
		Turkey	DQ842025.1
		India	KF570107.1
		USA	MK388919.1,MF479262.1
	P.putida	Singapore	AY251052.1

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	Pseudomonas fluorescens	Singapore	AY250709.1
	S.marcescens	Japan	AB162950.1,AB162949.1,AB162948.1,AB162947.1,NG_049172.1
	K.pneumoniae	Iran	LC512050.1, LC512051.1
	K.pneumoniae	Japan	[13]
	Acinetobacter spp.	Korea	[14]
	Acinetobacter bereziniae	Korea	EU014166.1,EU686386.1
	Acinetobacter calcoaceticus	Thailand	HM185482.1
	A. baumannii	Japan	[15]
		- (Submitted Korea, unpublished)	EF375699.1
		Iran	KR080548.1, KF723585.1
		- (Submitted Brazil, unpublished)	KF381490.1,KF381489.1,KF381488.1,KF381487.1
		Thailand	HM036079.1
	Acinetobacter pittii	Korea	GQ288398.1, GQ288393.1
		Taiwan	GU064942.1, GU064941.1,GQ864268.1
		Japan	AB753459.1
	Acinetobacter nosocomialis	Korea	GQ288394.1
		Taiwan	GU064940.1,GU064939.1, GU064938.1
	Citrobacter freundii	Japan	AB754498.1
	Citrobacter youngae	- (Direct submitted in Ireland)	MW847603.1
	Enterobacter aerogenes	Japan	[15]
	Enterobacter cloacae	- (Direct submitted in Japan)	LC508022.1

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		Japan	[15]
		China	MK088089.1
	Enterobacter hormaechei	China	MG287118.1
	E.coli	Japan	[16]
		Iran	LC512049.1
	Proteus mirabilis	Brazil	KY057362.1
	Proteus vulgaris	Japan	[16]
	Providencia rettgeri	Japan	AB754496.1
	Leclercia adecarboxylata	China	KJ531212.1
IMP-2	*A.baumanni*	Italy	AJ243491.1, NG_049183.1
		India	KC588963.1
	Serratia marcescens	Japan	AB182996.1
	P. aeruginosa	India	KC588963.1
IMP-3	*Shigella flexneri*	- (Published in USA)	NG_049194.1
IMP-4	*A.baumanni*	Hong Kong	NG_049203.1,AF445082.1,AF244145.1
		Singapore	DQ532122.1,AY795963.1,AY590475.1
	Acinetobacter calcoaceticus	- (Direct submit Malaysia,unpublished)	DQ307573.1
	Citrobacter freundii	China	EU368857.1, JQ818252.1
	E.coli	China	AB636651.1
		- (Direct submit India)	MF169878.1
	Enterobacter cloacae	China	KF699334.1
		Korea	KY884003.1

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		Japan	LC198842.1
	Enterobacter aero-genes	China	KF184385.1
	K. pneumoniae	China	EU368858.1,JQ808503.1, JN106667.1, KF184388.1,FJ384365.1,KF680003.1
	K. oxytoca	China	JQ820404.1,KY913900.1
	P. aeruginosa	China	DQ297664.1
		Malaysia	GQ221782.1
IMP-5	*A.baumanni*	Portugal	NG_049212.1,JF810083.1
IMP-6	*E. coli*	Japan	AB753460.1
	S.marcescens	Japan	NG_049220.1,AB040994.1
	Providencia rettgeri	Japan	AB754497.1
	P. aeruginosa	Japan	AB188812.1
		Korea	EU117233.1
IMP-7	*P. aeruginosa*	Canada	NG_049221.1,AF318077.1
		Czech	JX982232.1
		Japan	LC091209.2,LC091210.2
		Malaysia	GQ221781.1,AF416736.2,GU213192.1
		India	HM641894.1
		Singapore	AY625685.1
		Slovakia	EF601914.1
IMP-8	*A.baumanni*	Taiwan	EF127959.1
		China	DQ845788.1
	E. coli	Singapore	KF534724.1
	Enterobacter cloa-cae	Taiwan	[17]
		China	JQ820405.1
	K. pneumoniae	China	JQ820406.1, EU368856.1
		Taiwan	NG_049222.1,AF322577.2
		Tunisia	HE605039.1

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	K. oxytoca	China	HQ651093.1
	S.marcescens	Taiwan	EU042136.1
IMP-9	*P. aeruginosa*	China	AY033653,EU176818.1, KF184386.1,KF255597.1, KF255596.1,KF255595.1,
		- (Direct submit China)	HM106459.1
IMP-10	*Achromobacter xylosoxidans*	Japan	AB074435.1,AB195638.1
	P. aeruginosa	Japan	AB074434.1,AB074433.1,NG_049173.1,AB195637.1
		- (Direct submit in Japan,Unpublished)	DQ288156.1
	P. putida	Italy	AJ420864.1
	K. pneumoniae	Tunisia	HE605040.1
IMP-11	*P. aeruginosa*	Japan	AB074437.1
	A.baumanni	Japan	AB074436, NG_049174.1
	Enterobacter cloacae	Japan	LC628821.1
IMP-12	*P. putida*	Italy	NG_049175.1
IMP-13	*P. aeruginosa*	Italy	FJ172676.1,FJ172674.1,AJ512502.1,NG_049176.1
		France	JX131371.1
		Thailand	GU207399.1
	P. monteilii	Italy	JN091097.1
	K. pneumoniae	Tunisia	HE605041.1
IMP-14	*Achromobacter xylosoxidans*	Thailand	KJ406506.2,KJ406505.2
	P. aeruginosa	Thailand	AY553332.1,NG_049177.1
IMP-15	*P. aeruginosa*	Thailand	NG_049178.1,AY553333.1
		Vietnam	LC075716.1
		Spain	KC310496.1
IMP-16	*P. aeruginosa*	Brazil	AJ584652.2,NG_049179.1

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IMP-17	*P. aeruginosa*	Italy	NG_049180.1
IMP-18	*P. aeruginosa*	USA	AY780674.2,NG_049181.1
		Mexico	HM138673.1
		- (Direct submit in Costa Rica,unpublished)	KC907377.2
		- (Direct submit in Japan,unpublished)	AB587676.1
IMP-19	*A.baumanni*	Iran	JQ766528.1
		Japan	AB184977.1
	Achromobacter xylosoxidans	Japan	AB201263.1
	Enterobacter cloacae	Japan	AB201264.1
	Aeromonas caviae	France	NG_049182.1
	K. pneumoniae	- (Direct submit in Japan,unpublished)	LC062960.1
	P. aeruginosa	Japan	AB184976.1
	P. putida	Japan	AB201265.1
	S.marcescens	Poland	MH071810.1,MF678587.1
IMP-20	*P. aeruginosa*	Japan	AB196988, NG_049184.1
IMP-21	*P. aeruginosa*	Japan	AB204557,NG_049185.1
IMP-22	*Providencia rettgeri*	Japan	AB754495.1
	P. aeruginosa	Austria	FM876313.1
	Pseudomonas fluorescens	Italy	DQ361087.2,NG_049186.1
IMP-23	*Citrobacter freundii*	China	NG_049187.1
IMP-24	*Serratia marcescens*	Taiwan	EF192154.1,NG_049188.1
IMP-25	*P. aeruginosa*	China	EU352796
		Korea	EU541448.1, NG_049189.1
		- (Direct submit in China,unpublished)	KY081418.1,KY081417.1,HM175876.1
	Stenotrophomonas maltophilia	- (Direct submit in China)	GU944726.1
IMP-26	*Enterobacter cloacae*	China	HQ685900.1

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	P. aeruginosa	Malaysia	JQ629930.1
	P. aeruginosa	Nepal	LC636067.1
	P. aeruginosa	Singapore	GU045307.1,NG_049190.1
	P. aeruginosa	Vietnam	LC075717.1
IMP-27	*Morganella morganii*	Mexico	KY847875.1,KY847873.1
	Proteus mirabilis	USA	JF894248.1
		- (Direct submit in USA)	NG_049191.1
	Providencia rettgeri	USA	KY847874.1
IMP-28	*K. oxytoca*	Spain	HQ263342.1,NG_049192.1
IMP-29	*P. aeruginosa*	France	HQ438058.1, JQ041634,NG_049193.1
IMP-30	*Escherichia coli*	China	KM589497.1
	P. aeruginosa	Russia	NG_049195.1
IMP-31	*P. aeruginosa*	Germany	KF148593.1,NG_049196.1
IMP-32	*K. pneumoniae*	Thailand	NG_049197.1,JQ002629.1
IMP-33	*P. aeruginosa*	Italy	JN848782,NG_049198.1
IMP-34	*K. oxytoca*	Japan	AB700341.1, NG_049199.1
	Acinetobacter colistiniresistens	Japan	LC276939.1
IMP-35	*P. aeruginosa*	German	JF816544.1,NG_049200.1
IMP-36	Not found in NCBI database and pubmed		
IMP-37	*P. aeruginosa*	Franch	JX131372.1,NG_049201.1
IMP-38	*K. pneumoniae*	China	HQ875573.1, NG_049202.1
IMP-39	*P. aeruginosa*	Franch	MK507818.1, NG_064724.1
IMP-40	*P. aeruginosa*	Japan	AB753457,NG_049204.1
IMP-41	*P. aeruginosa*	Japan	AB753458,NG_049205.1
IMP-42	*Acinetobacter soli*	Japan	AB753456.1,NG_049206.1
IMP-43	*P. aeruginosa*	Japan	NG_049207.1
IMP-44	*P. aeruginosa*	Japan	NG_049208.1
IMP-45	*P. aeruginosa*	China	KJ510410.1,NG_049209.1
		France	KU984333.1

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IMP-46	*P. putida*	France	MK543944.1, MK507819.1, NG_064725.1
IMP-47	*Serratia marcescens*	- (Direct submit USA)	KP050486.1
IMP-48	*P. aeruginosa*	- (Direct submit USA, unpublished)	NG_049210.1,KM087857.1
IMP-49	*P. aeruginosa*	Brazil	NG_049211, KP681694.1
IMP-50	Not found in NCBI database and pubmed		
IMP-51	*P. aeruginosa*	Vietnam	NG_049213.1,LC031883.1
IMP-52	*E.coli*	Japan	NG_049214.1,LC055762.1
IMP-53	*P. aeruginosa*	- (Direct submit USA)	NG_049215.1
IMP-54	*P. aeruginosa*	Thailand	KU052795.1,NG_049216.1
IMP-55	*A.baumanni*	Iran	KU299753.1, NG_049217.1
IMP-56	*P. aeruginosa*	Mexico	KU351745.1
		Guatemala	KU315553.1,NG_049218.1
IMP-57	Not found in NCBI database and pubmed		
IMP-58	*P. putida*	Denmark	KU647281.1,NG_049219.1
IMP-59	*E. coli*	Australia	KX196782.1, NG_055477.1
IMP-60	*Enterobacter cloacae*	Japan	LC159227.1, NG_050945.1
IMP-61	*A. baumannii*	- (Direct submit in Germany,unpublished)	KX462700.1, NG_051166.1
IMP-62	*P. aeruginosa*	Mexico	KX753224.1,NG_051513.1
IMP-63	*P. aeruginosa*	France	KX821663.1, NG_052049.1
IMP-64	*Proteus mirabilis*	USA	NG_054710.1,KX949735.2
IMP-65	*P. aeruginosa*	Thailand	KY315991.1, NG_066508.1
IMP-66	*E. coli*	Japan	LC190726.1, NG_054676.1
IMP-67	*Providencia rettgeri*	- (Direct submit in USA, unpublished)	MF281100.1, NG_055271.1
IMP-68	*K.pneumoniae*	Japan	MF669572.1, NG_055584.1
IMP-69	*Providencia sp.*	China	MF678349.1, NG_055665.1
IMP-70	*P. aeruginosa*	Germany	MG748725.1,NG_056176.1

	Providencia rettgeri	Japan	LC348383.1
IMP-71	*P. aeruginosa*	France	MG818167.1
IMP-72	*P. aeruginosa*	Mexico	MH021847.1
IMP-73	*P. aeruginosa*	Japan	MH021848.1, NG_057463.1
IMP-74	*P. aeruginosa*	Brazil	MH243349.1, NG_057606.1
IMP-75	*P. aeruginosa*	Mexico	MH243350.1, MW692112.1, NG_057607.1
IMP-76	*P. aeruginosa*	Japan	NG_061409.1
IMP-77	*P. aeruginosa*	Japan	NG_061410.1
IMP-78	*P. aeruginosa*	Japan	NG_061411.1
IMP-79	*P. aeruginosa*	France	MG873561.1, NG_061626.1
IMP-80	*P. aeruginosa*	Japan	NG_062274.1
IMP-81	*P. aeruginosa*	Columbia	MN267699.1
IMP-82	*P. aeruginosa*	- (Direct submit in Germany,unpublished)	MN057782.1,
		- (Direct submit in USA, unpublished)	NG_065873.1
IMP-83	*P. aeruginosa*	Mexico	MN104595.1, NG_065874.1
IMP-84	*P. aeruginosa*	- (Direct submit in Switzerland,unpublished)	MN219692.1
	P. aeruginosa	- (Direct submit in USA, unpublished)	NG_065875.1
IMP-85	*P. aeruginosa*	France	MN510335.1, NG_066696.1
IMP-86	*P. aeruginosa*	China	MT241520.1, NG_076650.1
IMP-87	*P. aeruginosa*	China	MT241521.1, NG_076651.1
IMP-88	*P. aeruginosa*	Japan	LC558310.1, NG_070737.1
IMP-89	*P. putida*	China	NG_070738.1
IMP-90	*P. aeruginosa*	- (Direct submit in Germany,unpublished)	MW811441.1
		- (Direct submit in USA,unpublished)	NG_074713.1
IMP-91	*P. aeruginosa*	China	MZ702721.1, NG_076634.1

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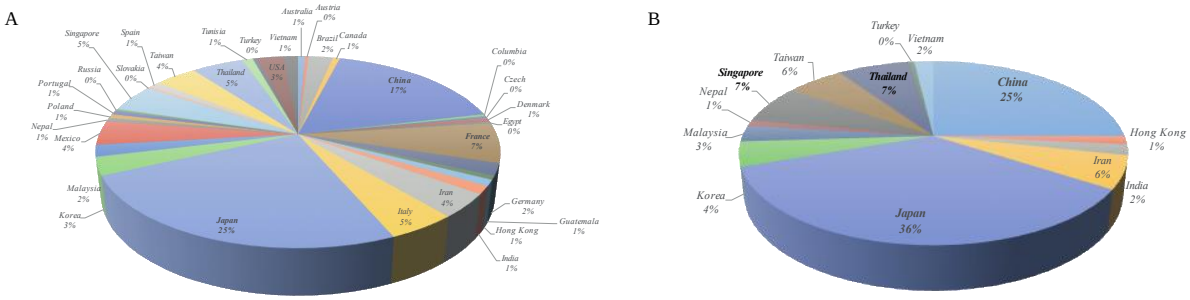

Figure 1. Distribution of IMP-type metallo-beta-lactamase genes (A) worldwide (B) in Asia.

By focusing on Asia in where more than half of the reporting countries (69%) were…, there were only 12 countries (China – including Hong Kong, India, Iran, Japan, Korea, Malaysia, Nepal, Singapore, Thailand, Turkey and Vietnam) reported the presence of *bla*$_{IMP}$ in their countries. Japan and China remained the first (36%) and the second (25%) most frequently *bla*$_{IMP}$ identified countries. Thailand and Singapore were the third most frequently reported countries (Figure 1B). The most frequently reported *bla*$_{IMP}$ carrier was *Pseudomonas aeruginosa*, followed by *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*. By considering the variant of *bla*$_{IMP}$ in countries with high prevalence of *bla*$_{IMP}$ in Asia, IMP-1 was the most frequently reported in Japan (23%) and Singapore (50%). IMP-4 and IMP-14 were the most frequently reported from China (27%) and Thailand (27%), respectively (Figure 2A-D).

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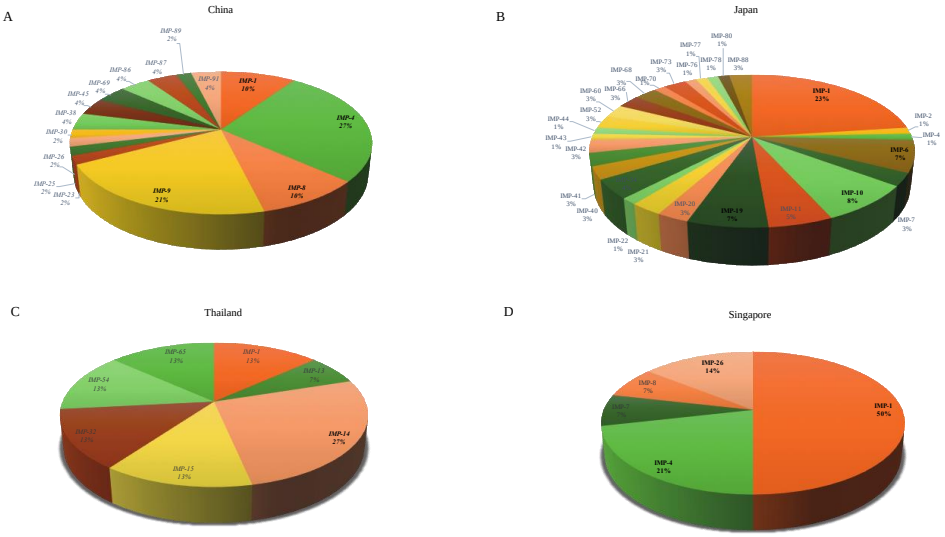

Figure 2. Distribution of *bla*$_{IMP}$ genes in 4 countries in Asia: (A) Japan, (B) China, (C) Thailand and (D) Singapore

3.2. In silico analysis of IMP-type MBLs

In silico analysis of IMP-type MBL genes was conducted to investigate the diversity of enzymes. By 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; Figure S1). These sequences were residues of a lactam ring-catalytic site. The overall analysis showed 79.3% - 96.7% amino acid sequence similarity.

Phylogenetic tree was constructed to visualise the relationship of the enzymes. IMP-MBL enzymes were separated into three main clusters (Figure 3). Group I contains 38 variants, including IMP-2, IMP-8, IMP-12, IMP-13, IMP-14, IMP-17, IMP-18, IMP-19, IMP-20, IMP-23, IMP-24, IMP-27, IMP-31, IMP-32, IMP-33, IMP-35, IMP-37, IMP-39, IMP-46, IMP-47, IMP-48, IMP-49, IMP-54, IMP-56, IMP-63, IMP-64, IMP-65, IMP-67, IMP-69, IMP-71, IMP-72, IMP-75, IMP-83, IMP-84, IMP-86, IMP-87, IMP-90, and IMP-91. Noticeably, IMP-12, IMP-63 and IMP-90, previously identified as group II, 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, including IMP-1, IMP-3, IMP-4, IMP-5, IMP-6, IMP-7, IMP-9, IMP-10, IMP-15, IMP-25, IMP-26, IMP-28, IMP-29, IMP-30, IMP-34, IMP-38, IMP-40, IMP-42, IMP-43, IMP-45, IMP-51, IMP-52, IMP-53, IMP-55, IMP-59, IMP-60, IMP-61, IMP-62, IMP-66, IMP-70, IMP-73, IMP-76, IMP-77, IMP-78, IMP-79, IMP-80, IMP-81, IMP-82, IMP-85, IMP-88, and IMP-89. Lastly, group III contains nine variants, including IMP-11, IMP-16, IMP-21, IMP-22, IMP-41, IMP-44, IMP-58, IMP-68, and IMP-74.

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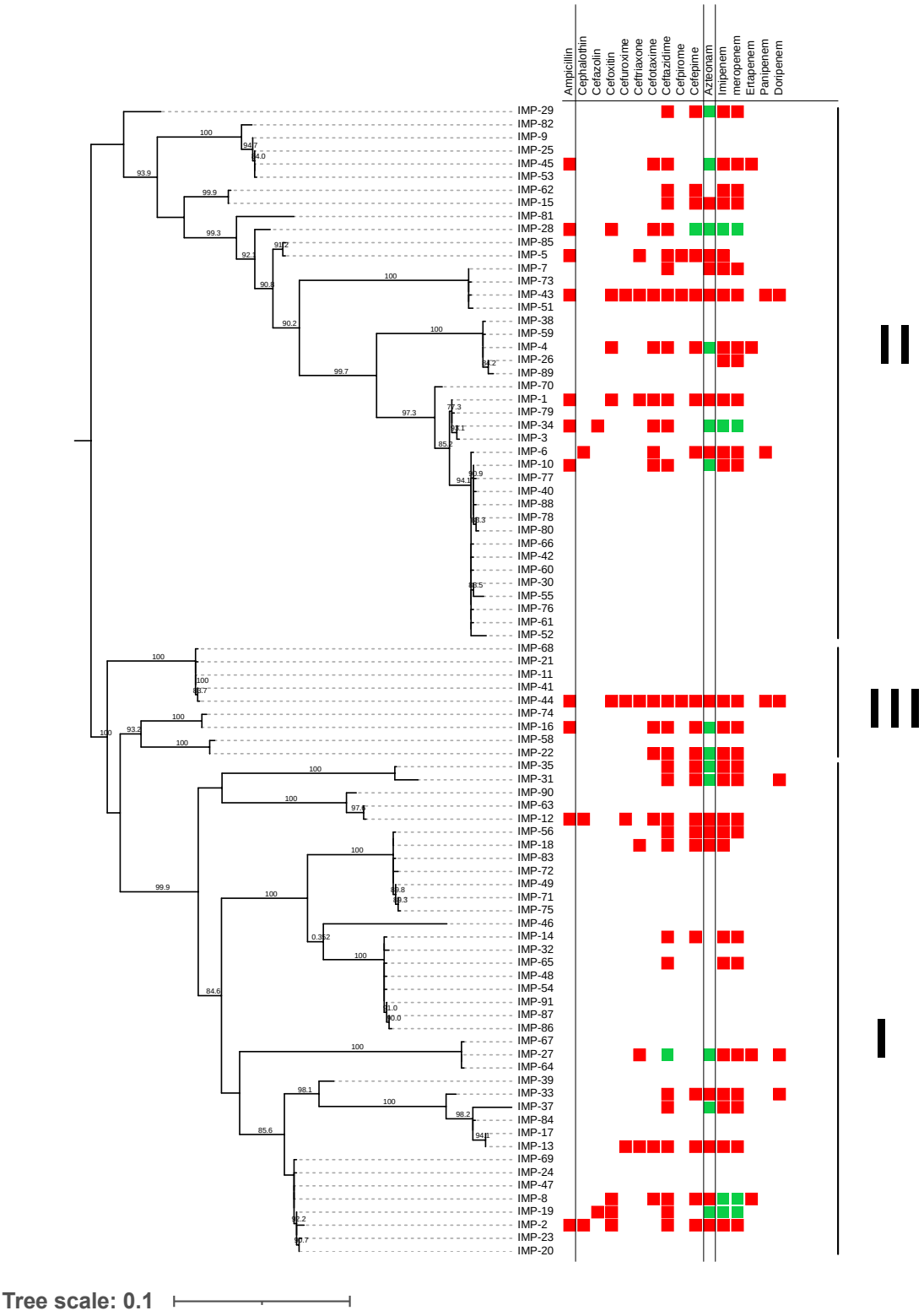

Figure 3. Phylogenetic relationship of *bla*$_{IMP}$ genes. Unrooted maximum likelihood phylogenetic tree constructed using nucleotide sequences of 88 *bla*$_{IMP}$ gene with 1,000 bootstrap supports was visualised together with antibiotic susceptibility profile of 32 variants of *bla*$_{IMP}$ gene.

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3.3. Resistance of IMP MBL variants

The pattern of antibiotic susceptibility of each *bla*$_{IMP}$ variant was obtained from the articles to investigate whether the variation in each variant was associated with susceptibility. By reviewing literature, most of the antibiotic agents tested were in the group of cephalosporin and carbapenem (Figure 3), especially anti-pseudomonal antibiotics, since *P. aeruginosa* was the most abundant species identified to possess *bla*$_{IMP}$ gene. Out of 88 available variants, susceptibility profile was reported only in 32 variants (Figure 3, right panel). Overall, strains with *bla*$_{IMP}$ were resistant to several beta-lactam antibiotics.

For carbapenem, almost all of the isolates with *bla*$_{IMP}$ 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 *bla*$_{IMP}$ – carrying species. Likewise, isolates with *bla*$_{IMP}$ were resistant to cephalosporins. Aztreonam, a monobactam, was also shown to have less effect on *bla*$_{IMP}$ carriers.

By combining antibiotic susceptibility profile with phylogenetic tree to investigate the relationship between clustering and susceptibility, it was found that susceptibility pattern was not associated with phylogenetic tree (Figure 3).

4. Discussion

The importance of clinically important bacteria has been increasing due to the multidrug resistance caused by the production of drug-inactivating enzymes, especially beta-lactamases[18]. More critically, carbapenemase enzyme has been increasingly identified in pathogens that are associated with nosocomial infections [19,20]. This study is the first to comprehensively investigate the epidemiology and diversity of IMP-type MBLs, a class B beta-lactamase with carbapenemase ability.

An IMP-type MBL is encoded by *bla*$_{IMP-N}$ gene (N = no. of variant) which can be located on the chromosome or the plasmid, which facilitates the transfer of *bla*$_{IMP}$ genes via horizontal gene transfer [21,22]. Our study showed that the *bla*$_{IMP}$ gene was detected in clinically relevant species, including *P. aeruginosa* and *A. baumannii*, which are associated with hospital-associated infection and listed in "Priority 1: CRITICAL" list of antibiotic resistant pathogens by WHO (https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed). Interestingly, our analysis revealed that the top 3 countries that *bla*$_{IMP}$ genes were detected were all Asian countries: Japan, China and Thailand. Japan is the first place where IMP-type MBLs (IMP-1) were reported [6]. There were 28, 15, 7, and 5 variants of *bla*$_{IMP}$ gene identified in Japan, China, Thailand, and Singapore, respectively. A recent study revealed that carbapenemases – derived *P. aeruginosa* – are distributed thoroughly in every part of Thailand [23]. However, the epidemiological study of IMP variants in Japan and China has not been conducted. It is, therefore, important to note that *bla*$_{IMP}$ gene is one of the causes of antibiotic resistance in Asia.

Phylogenetic tree is commonly used to investigate the evolutionary relationship of genes or organisms. Our findings revealed that a reconstructed phylogenetic tree using 88

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bla$_{IMP}$ variants clustered the genes into three main groups (Figure 2). In a broad picture, this tree was similar to a previous version [23]. 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 *bla*$_{IMP}$ is nearly well-defined except that some branches remain dynamic depending on the number of genes added to the tree. The change of position on the phylogenetic tree could be caused by the increased number of tested genes in our study.

A search for antibiotic susceptibility profiles revealed that only 32 variants (out of 88) were tested for their susceptibility. The profile showed that 3rd generation cephalosporins and carbapenem were less effective against most strains with *bla*$_{IMP}$. Interestingly, Aztreonam remained active to the strains with some types of *bla*$_{IMP}$. However, the association between susceptibility and phylogenetic tree was absent. This is supported by the finding showing the sequence of the active site (catalytic site) was highly conserved within the members of MBLs [24]. It is of note that nucleotide or amino acid substitutions outside the active site might not affect the beta-lactam-hydrolysing activity of the enzyme. In addition, the susceptibility profile of strains containing each *bla*$_{IMP}$ variant must be performed to ensure the association between substitution/phylogenetic tree and antibiotic resistance pattern. All in all, the finding of this work demonstrated that antibiotic resistance-associated genes 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, has caused public health problems worldwide. This study is the first to comprehensively analyse all currently available variants of IMP-type MBLs and associated susceptibility. Asian countries, especially Japan and China, are presently under a wide spread of *bla*$_{IMP}$-carrying bacteria, listed in the WHO's antibiotic-resistant bacteria. An unrooted phylogenetic backbone of *bla*$_{IMP}$ gene variants demonstrated 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: www.mdpi.com/xxx/s1, Figure S1: Multiple sequence alignment of amino acid sequence of 88 *bla*$_{IMP}$ variants.

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