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Molecular characterization of carbapenem-non-susceptible *Acinetobacter* spp. in Japan; Predominance of multidrug-resistant *Acinetobacter baumannii* clonal complex 92 and IMP-type metallo-ß-lactamase-producing non-*baumannii* *Acinetobacter* species

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Keywords: *Acinetobacter* spp., metallo-ß-lactamase, carbapenem-hydrolyzing class D ß-lactamase, amplified ribosomal DNA restriction analysis (ARDRA)

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Abstract

We conducted an epidemiological study concerning carbapenem-non-susceptible clinical isolates of *Acinetobacter* spp. in Japan by molecular procedures including carbapenemase gene identification and amplified ribosomal DNA restriction analysis. Among 598 clinically isolated *Acinetobacter* spp. in 2007, 27 (4.5%) were non-susceptible to carbapenems. Most carbapenem-non-susceptible *Acinetobacter baumannii* (13/14) belonged to clonal complex (CC) 92, harbored *bla*$_{\text{OXA-51-like}}$ genes, including novel *bla*$_{\text{OXA-206}}$, downstream of IS*Aba*1, and were recovered mainly from the Kanto region. Carbapenem-non-susceptible *A. baumannii* CC92 isolates were further divided by pulsed-field gel electrophoresis into two groups, one of which was characterized by the presence of *bla*$_{\text{OXA-23}}$. One *A. baumannii* CC276 isolate carried *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{OXA-58}}$. Almost all non-*baumannii* *Acinetobacter* isolates (12/13), including *Acinetobacter pittii* (formerly *Acinetobacter* genomic species 3) and *Acintobacter nosocomialis* (formerly *Acinetobacter* genomic species 13TU), produced IMP-type metallo-ß-lactamases, and were recovered from various regions in Japan. This is the first report describing the nationwide molecular epidemiology of carbapenem-non-susceptible *Acinetobacter* spp. with genomic species level identification in Japan.

Introduction

Among *Acinetobacter* species, *Acinetobacter baumannii* is the most important nosocomial pathogens, frequently resistant to multiple antimicrobials. Carbapenems play an important role for treatment of *Acinetobacter* infections, but carbapenem-resistant *A. baumannii* have spread worldwide rapidly in these two decades with multiple resistant means [1]; OXA-type class D carbapenemases, which are intrinsic or acquired, could be potentiated by promoter sequences located within IS*Aba* insertion sequences [2, 3]. Metallo-ß-lactamases (MBLs) also confer carbapenem resistance on this pathogen. ß-lactamases are shown to work synergistically with other mechanisms including alteration of drug permeability and efflux pumps [1].

More generally, it is apparent that the population structure of *A. baumannii* comprises three major international lineages, named European clones I, II, and III [4, 5]. A subgroup of European clone II involving clonal complex (CC) 92, has spread globally [6], and is wide spread in China [7] and Korea [8]; it is also recorded in Australia [9].

While these strains remain relatively rare in Japan, it is a matter of concern [10]. In the past reports about *Acinetobacter* spp. in Japan, *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex (ACB complex) including

62 *Acinetobacter pittii* (formerly *Acinetobacter* genomic species 3) and *Acinetobacter*
63 *nosocomialis* (formerly *Acinetobacter* genomic species 13 TU) had been mentioned
64 as "*A. baumannii*" and its mechanism of carbapenem resistance was explained by
65 MBLs; epidemiological information including genetic species identification and
66 class D carbapenemase is lacking [11, 12].
67 Here, we characterized the genetic mechanisms of carbapenem-
68 non-susceptibility in clinical *Acinetobacter* spp. from a nationwide surveillance
69 study in Japan, and analyzed the molecular epidemiology of
70 carbapenem-non-susceptible isolates.
71
72 **Materials and methods**
73
74 *Bacterial strains*
75 A total of 598 clinical isolates of *Acinetobacter* spp. were collected from 72
76 hospitals and other healthcare institutions as part of a nationwide survey
77 between January and December 2007 by the Levofloxacin Surveillance Group
78 [13]. The maximum number of isolates collected per institution was 10. Only one
79 isolate was accepted from each patient. Isolates with resistance to imipenem or
80 panipenem (MICs ≥8 mg/L) in the survey [13] were investigated further.

Identification at the level of species/genomic species

Biochemical identification was performed in 27 carbapenems non-susceptible isolates by the BD Phoenix automated system (BD Diagnostic Systems, Sparks, MD, USA). Amplified ribosomal DNA restriction analysis (ARDRA) was performed to identify species and genomic species [14]. Reference of ARDRA patterns were obtained from website (http://users.ugent.be/~mvaneech/ARDRA/Acinetobacter.html). PCR amplification and nucleotide sequencing of intrinsic *bla*$_{\text{OXA-51-like}}$ gene were performed to confirm identification of *A. baumannii* (Table 1). Nucleotide sequences of the amplified products were determined using an ABI 310 genetic analyzer with Big Dye terminator Ver.3.1 cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA).

Antimicrobial susceptibility testing

Minimum inhibitory concentrations were measured by the broth microdilution method of the Clinical and Laboratory Standards Institute [15]. Antimicrobial agents tested were as follows: ampicillin, ceftazidime, imipenem, meropenem and minocycline (Sigma Chemical Co. St Louis, MO, USA),

100 gentamicin and sulbactam (Wako Pure Chemical, Osaka, Japan), ciprofloxacin
101 (MP Biomedicals, Solon, OH, USA), and cefepime (Bristol-Myers Squibb, Tokyo,
102 Japan).
103
104 *Phenotypic and genotypic tests for MBLs*
105 Production of MBLs was screened by the double-disk synergy test using
106 ceftazidime (30 μg) and sodium mercaptoacetate (30 μg) discs (Eiken Chemical,
107 Inc., Tokyo, Japan) [16]. Genes for IMP-1, IMP-2, VIM-1, and VIM-2
108 carbapenemases were sought by PCR (Table 1) [12].
109
110 *Detection of class D carbapenemase genes and associated ISAba*
111 *bla*$_{OXA}$ genes were sought by multiplex PCR (Table 1) [17]. The presence of
112 an IS*Aba*-type insertion sequence upstream of *bla*$_{OXA}$ genes was investigated by
113 PCR and nucleotide sequencing, using combinations of the forward primers for
114 IS*Aba* and the reverse primer for the relevant *bla*$_{OXA}$ gene.
115
116 *Molecular typing by pulsed-field gel electrophoresis (PFGE)*
117 Agarose gel plugs containing *Apa* I-digested genomic DNA were prepared
118 with the CHEF Bacterial Genomic DNA Plug Kit (Bio-Rad, Hercules, CA). The

DNA fragments were separated with a CHEF MAPPER (Bio-Rad) for 18.5 h at 14 °C with a 1 to 17s linear ramp of 6V/cm. Restriction patterns were analyzed with Fingerprinting II software (Bio-Rad) and cluster analysis was performed by the unweighted pair-group method with mathematical averaging. Position tolerance and optimization were set at 1.5% and 1.5%, respectively. Only restriction fragments larger than 50 kb were used for analysis. Isolates with >85% similarity were assigned to the same strain subgroup.

Multilocus sequence typing (MLST)

Sequence types (STs) of *A. baumannii* isolates were determined according to the MLST scheme (http://pubmlst.org/abaumannii/) [18, 19]. Clonal complexes (CCs) were determined by eBURST version 3 (http://eburst.mlst.net/) with definition of the groups by sharing alleles at ≥6 of 7 loci and bootstrap values of 1000 [20].

Statistical analysis

Distribution of drug resistance, as well as resistance determinants, was estimated by Fisher's exact test. A p value <0.05 was considered as a statistically significant difference.

Nucleotide sequence accession number

The nucleotide sequence of *bla*$_{\text{OXA-206}}$ was assigned accession number AB634250.

Results

Isolates with reduced susceptibilities to carbapenems

Acinetobacter spp. were isolated as follows; 182 isolates were from Kanto region, 26 isolates were from Hokkaido, 77 isolates were from Tohoku region, 86 isolates were from Tokai and Hokuriku region, 134 isolates were from Kansai and Chugoku region, and 93 isolates were from Kyusyu region.

Among the 598 clinical isolates of *Acinetobacter* spp. (333 from respiratory tract, 45 from urinary tract, 79 from blood, 74 from pus, and 67 from other sites), 27 isolates (4.5%) were resistant to either or both imipenem and panipenem (17 from respiratory tract, 2 from urinary tract, 2 from blood, 2 from pus, and 4 from other sites).

These 27 carbapenem-non-susceptible isolates were further identified at species/genomic species level by ARDRA identification; 51.9% (14/27) of tested *Acinetobacter* species was identified as *A. baumannii*. In agreement with ARDRA-based idenitification, all 14 *A. baumannii* isolates were detected $bla_{\text{OXA-51 like}}$ gene by PCR, a hallmark of this species.

Carbapenem-non-susceptible *A. baumannii* were isolated in two areas. Of the 14 *A. baumannii* isolates, 13 were from five different hospitals in Kanto region (13/182), and one was from Kyushu (1/93). In contrast, the non-*baumannii*

isolates were from more diverse areas; Kanto, Tokai, Kyushu, Hokkaido and Hokuriku (Table 2).

Antimicrobial susceptibility

Antibiogram data are described in Table 2. Compared with non-*baumannii Acinetobacter* spp., *A. baumannii* was more frequently non-susceptible to other classes of drug, namely, ampicillin/sulbactam (71% in *A. baumannii* vs. 0% in non-*baumannii*, $p<0.01$) and ciprofloxacin (93% vs. 31%, $p<0.01$).

MBL-producing isolates

Thirteen of the 27 isolates were determined to have MBLs by phenotypic testing and PCR showed 12 of these to carry $bla_{\text{IMP-1}}$. All of them except one were non-baumannii *Acitetobacter* isolates. The $bla_{\text{IMP-2}}$ was detected in one *A. nosocomialis* isolate. No MBL genes were found in isolates negative by the phenotypic test.

Class D carbapenemases and ISAba

Sequencing of the PCR products revealed that the $bla_{\text{OXA-51-like}}$ gene harbored by

12/14 *A. baumannii* isolates was *bla*$_{\text{OXA-66}}$ and one isolate harbored *bla*$_{\text{OXA-64}}$, while one isolate harbored *bla*$_{\text{OXA-206}}$, a new variant gene. The isolate with *bla*$_{\text{OXA-64}}$ also had IS*Aba3*-like-*bla*$_{\text{OXA-58}}$ and the isolate with *bla*$_{\text{OXA-206,}}$ a single amino acid variant of OXA-66 carried IS*Aba1*-*bla*$_{\text{OXA-23}}$. Of these 14, six carried *bla*$_{\text{OXA-23}}$ and one had *bla*$_{\text{OXA-58}}$. One *A. lwoffii* isolate and the three *A. pittii* isolates harbored *bla*$_{\text{OXA-58}}$. IS*Aba1* was located 34-bp upstream of the *bla*$_{\text{OXA-66}}$ and *bla*$_{\text{OXA-206}}$ genes, or 8-bp upstream of the *bla*$_{\text{OXA-23}}$ gene. IS*Aba3*-like was located 17-bp upstream of *bla*$_{\text{OXA-58}}$. IS*Aba1* was not detected upstream of *bla*$_{\text{OXA-66}}$ in TUM10629 and upstream of *bla*$_{\text{OXA-64}}$ in TUM 10635, and IS*Aba3*-like was not found upstream of *bla*$_{\text{OXA-58}}$ in *A. lwoffii* TUM 10655.

Genetic relatedness of A. baumannii isolates

The *A. baumannii* isolates were classified into 3 subgroups by PFGE (Figure). PFGE subgroups A and B comprised 7 and 6 isolates, respectively, whereas one isolate was unique. PFGE subgroup A consisted of five isolates, TUM10629 to TUM10633 (Table 2), carrying both *bla*$_{\text{OXA-66}}$ and *bla*$_{\text{OXA-23}}$ from one hospital in the Kanto region, TUM10641 carrying *bla*$_{\text{OXA-206}}$ and *bla*$_{\text{OXA-23}}$, and TUM10642 from outside Kanto region. All isolates from Kanto region with only *bla*$_{\text{OXA-66}}$ were classified into the PFGE subgroup B. The unique isolate, allocated to subgroup C, was TUM10635, harboring *bla*$_{\text{OXA-64}}$, *bla*$_{\text{OXA-58}}$, and *bla*$_{\text{IMP-1}}$.

199 The isolates of PFGE subgroup A and B were determined to belong to CC92
200 (i.e. ST208 and ST219). TUM10635, the *A. baumannii* isolate with IMP-1, had
201 novel sequences in *gdhB* and *gpi* and was assigned to ST276.
202
203 **Discussions**
204 The prevalence of carbapenem-non-susceptibility among *Acinecobacter*
205 spp. in Japan (4.5%) was at lower level than reports in other regions: 26.9% in
206 Korea [21], 49% in Taiwan [22], 50-52.4% in China [23], and 22-26% in Europe
207 [24]. Moreover, as observed in Korea [25], carbapenem-non-susceptible *A.*
208 *baumannii* was more frequently resistant to ampicillin/sulbactam and
209 ciprofloxacin than for non-*baumannii* isolates.
210 Geographically, carbapenem-non-susceptible *A. baumannii* isolates were
211 recovered mainly from the Kanto region, while non-*baumannii* isolates were
212 distributed in various regions.
213 While ST92 has been the global epidemic clone among
214 carbapenem-non-susceptible *A. baumannii*,[26] 13 out of 14
215 carbapenem-non-susceptible *A. baumannii* isolates belonged to ST208 or its
216 single variant ST219, the member of CC92 in our study. The fact that the isolates
217 carrying $bla_{\text{OXA-66}}$ belonged to CC92 is compatible with the finding that the

isolates carrying *bla*$_{\text{OXA-66}}$ often belonged to STs such as ST98 (formerly ST34) included in CC92 demonstrated in a recent report [27] (Table 2). CC92 has increasingly been documented as a globally disseminated linage included in European clone II, often with multidrug resistance [6, 9]. *bla*$_{\text{OXA-51-like}}$ gene can confer carbapenem-non-susceptibility to the bacteria if IS*Aba1* providing promoter sequences for overexpression is located adjacent to *bla*$_{\text{OXA-51-like}}$[2].

PFGE subgroup A was characterized by the presence of *bla*$_{\text{OXA-23}}$, an important determinant for carbapenem-resistance. Five isolates of PFGE subgroup A were recovered from a single hospital in Kanto region, suggesting possible clonal spread due to nosocomial transmission or local endemicity. Nevertheless, one isolate (TUM10642) of the same PFGE subgroup was recovered from Kyushu, a region very distant from Kanto. In contrast to PFGE subgroup A, *bla*$_{\text{OXA-23}}$ was absent among isolates of PFGE subgroup B; these strains seemed to owe their carbapenem-low-susceptibility to *bla*$_{\text{OXA-66}}$ with a promoter within IS*Aba1* [2].

In our results, PFGE revealed that ST208 contained at least two clones, PFGE subgroup A and B, and most of the isolates belonging to subgroup A harbored *bla*$_{\text{OXA-23}}$, while the isolates belonging to subgroup B harbored only *bla*$_{\text{OXA-51 like}}$ gene. These result demonstrated that PFGE has a greater discriminatory power

than MLST. While CC92 carrying *bla*$_{\text{OAX-23}}$ has been documented worldwide [6, 9], CC92 with and without *bla*$_{\text{OXA-23}}$ were observed as carbapenem-non-susceptible strains in this study.

One *A. baumannii*, TUM10635, showed several distinctive aspects: the presence of both the *bla*$_{\text{OXA-58}}$ and *bla*$_{\text{IMP-1}}$ gene, a novel ST 276, the foundation of CC 276, and a unique PFGE band pattern (Figure). Interestingly, its intrinsic *bla*$_{\text{OXA-51-like}}$ gene, OXA-64, was also reported in NDM-1 type metallo-ß-lactamase-producing *A. baumannii* from Germany [28].

In contrast to *A. baumannii*, almost all isolates of non-*baumannii* *Acintetobacter* isolates proved to produce MBLs (Table 2; 92% in non-*baumannii* vs 7.1% in *A. baumannii*, $p<0.01$). PCR and sequencing revealed *bla*$_{\text{IMP-1}}$ in all but one MBL-producing isolates. Previous studies in East Asia have demonstrated a similar, but not identical, trend: in Korea, carbapenem-resistant *A. nosocomialis* and *A. calcoaceticus*, harbored the VIM-2 type MBL gene [25, 29]. In Taiwan, MBL genes of *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{VIM-11}}$ were detected in *A. pittii* and *A. nosocomialis* [22]. In these reports, MBLs were not detected in *A. baumannnii*. The difference of MBL gene might reflect the predominant MBL genes among other pathogens; in Japan, the IMP-1-type MBL is most prevalent among *Pseudomonas aeruginosa* [30]. While previous study in Japan explained carbapenem-resistant *"A.*

256 *baumannii*" due mainly to production of MBLs [11], however their data did not
257 analyze species/ genomic species identification by ARDRA. It will be necessary to
258 identify more detailed species for the antibiotic susceptibility surveillance of
259 *Acinetobacter* species. Meanwhile, *bla*$_{\text{OXA-58}}$ located 17-bp downstream of
260 IS*Aba3*-like elements was detected mainly in isolates of *A. pittii*, similar to the
261 situation in Taiwan [22].

262 Our study revealed that most carbapenem-non-susceptible *A. baumannii*
263 belonged to CC 92 known as a worldwide disseminated clone, and consisted of at
264 least two lineages with or without *bla*$_{\text{OXA-23}}$ gene. The MBL producing *A.*
265 *baumannii* was rare; only one isolate belonging to CC276 and containing *bla*$_{\text{OXA-64}}$
266 and *bla*$_{\text{OXA-58}}$ also harbored *bla*$_{\text{IMP-1}}$. This is the first report showing the
267 differences of β-lactamases among carbapenem-non-susceptible *Acinetobacter* spp.
268 with genomic species level identification in Japan. These observations enhance
269 our understanding of the epidemiology of carbapenem-non-susceptible
270 *Acinetobacter* spp..

271

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FIGURE LEGEND

Figure. Pulsed-field gel electrophoresis (PFGE) of 14 carbapenem-non-susceptible *Acinetobacter baumannii* isolates in Japan. *A. baumannii* comprised PFGE subgroup A, B and C at a level 85% (indicated by the broken line). While PFGE subgroup A and B belonged to CC92, PFGE subgroup C with unique band pattern belonged to CC 276 (Table 2).

390**Table 1.** Primers used in this study

Primers	Sequence(5' to 3')	Aim	Ref
OXA-51 like Fw	TAA TGC TTT GAT CGC CCT TG	multiplex PCR	[17]
OXA-51 like Rv	TGG ATT GCA CTT CAT CTT GG		
OXA-23 like Fw	GAT CGG ATT GGA CCA GA		
OXA-23 like Rv	ATT TCT GAC CGC ATT TCC AT		
OXA-24 like Fw	GGT TAG TTG GCC CCC TTA AA		
OXA-24 like Rv	AGT TGA GCG AAA AGG GGA TT		
OXA-58 like Fw	AAG TAT TGG GGC TTG TGC TG		
OXA-58 like Rv	CCC CTC TGC GCT CTA CAT AC		
IS*Aba*-1Fw	CAC GAA TGC AGA AGT TG	IS*Aba* family	[2]
IS*Aba*-1Rv	CGA CGA ATA CTA TGA CAC		
IS*Aba*-3 like	AGC AAT ATC TCG TAT ACC GC		[3]
OXA-23 W F	GGG CAT ATG AAT AAA TAT TTT ACT TGC TAT GTG G	simplex PCR	This study
OXA-23 W R	GGG GGA TCC TTA AAT AAT ATT CAG CTG TTT TAA TGA TTT C		
OXA-51 W F	GGG GGC ATA TGA ACA TTA AAG CAC TCT TAC		
OXA-51 W R	CCC GGA TCC TGC TAT AAA ATA CCT AAT TG		
OXA-58 W F	GGG CCA TGG GTA TGA AAT TAT TAA AAA TAT TGA GTT TAG TT		
OXA-58 W R	CCG GAT CCT GTT ATA AAT AAT GAA AAA C		
OXA-51 likeS F	AAA GCT TCC GCT ATT CC	Sequence	This study
OXA-51like S R	GGA GTA ATT TTT AGA GGA CC		
IMP-1 F1	ACC GCA GCAGAG TCT TTG	MBLs	[12]
IMP-1 R1	ACA ACC AGT TTT GCC TTA CC		
IMP-2 F2	GTT TTA TGT GTA TGC TTC C		
IMP-2 R2	AGC CTG TTC CCA TGT AC		
VIM-1 F3	AGT GGT GAG TAT CCG ACA G		
VIM-1 R3	ATG AAA GTG CGT GGA GAC		
VIM-2 F4	ATG TTC AAA CTT TTG AGT AAG		
VIM-2 R4	CTA CTC AAC GAC TGA GCG		

Table 2. Characterization of carbapenem-non-susuceptible *Acinetobacter* species in Japan.

isolate No.	Species	ß-lactamase				PFGE	MLST		Hp	Location of hospitals	Sample	MIC (mg/L)							
		OXA-51	OXA-23	OXA-58	MBL		ST	CC				SAM	CAZ	FEP	IPM	MEM	GEN	MIN	CIP
10629	*A.baumannii*	66	23**			A	208	92	1	Kanto	Pus	32/16	>256	128	64	64	>512	8	64
10630	*A.baumannii*	66*	23**			A	208	92	1	Kanto	Sputum	128/64	256	256	64	128	8	16	128
10631	*A.baumannii*	66*	23**			A	208	92	1	Kanto	Blood	64/32	128	128	64	128	4	8	64
10632	*A.baumannii*	66*	23**			A	208	92	1	Kanto	Sputum	32/16	128	128	64	128	2	8	32
10633	*A.baumannii*	66*	23**			A	208	92	1	Kanto	Pus	64/32	128	128	64	128	4	8	32
10634	*A.baumannii*	66*				B	208	92	1	Kanto	Sputum	4/2	256	64	8	16	8	≤0.25	≤0.06
10635	*A.baumannii*	64		58†	IMP-1	C	276	276	1	Kanto	Sputum	4/2	128	16	2	8	128	4	64
10636	*A.baumannii*	66*				B	219	92	2	Kanto	Sputum	8/4	128	64	4	8	>512	2	128
10637	*A.baumannii*	66*				B	219	92	2	Kanto	Sputum	32/16	256	128	4	16	>512	2	>128
10638	*A.baumannii*	66*				B	219	92	2	Kanto	Sputum	32/16	256	128	4	16	>512	4	>128
10639	*A.baumannii*	66*				B	208	92	3	Kanto	Other	32/16	256	64	16	32	>512	2	32
10640	*A.baumannii*	66*				B	208	92	4	Kanto	Sputum	64/32	128	32	2	8	256	2	64
10641	*A.baumannii*	206*	23**			A	208	92	5	Kanto	Other	128/64	256	256	32	128	4	8	128
10642	*A.baumannii*	66*				A	208	92	6	Kyusyu	Urine	4/2	128	16	8	16	256	2	64
10643	*A. pittii*			58†	IMP-1				7	Hokkaido	Sputum	8/4	8	8	16	8	4	≤0.25	1
10644	*A. pittii*			58†					8	Kanto	Sputum	4/2	256	256	32	32	8	≤0.25	16
10645	*A. pittii*			58†	IMP-1				9	Kanto	Urine	4/2	256	128	64	64	1	≤0.25	64
10646	*A.calcoaceticus*				IMP-1				10	Tokai	Blood	2/1	>512	512	32	128	>512	≤0.25	2
10647	*A.calcoaceticus*				IMP-1				10	Tokai	Sputum	4/2	>512	256	32	64	4	≤0.25	0.13
10648	*A.calcoaceticus*				IMP-1				10	Tokai	Sputum	2/1	512	64	16	64	16	≤0.25	0.13
10649	*A.calcoaceticus*				IMP-1				10	Tokai	Sputum	4/2	>512	512	64	128	64	≤0.25	0.25

10650	*A. nosocomialis*		IMP-1	10	Tokai	Sputum	2/1	512	256	64	128	>512	≤0.25	32
10651	*A. nosocomialis*		IMP-2	11	Kyusyu	Sputum	4/2	512	256	16	32	>512	≤0.25	≤0.06
10652	*A. nosocomialis*		IMP-1	12	Kyusyu	Sputum	2/1	512	128	16	32	256	≤0.25	≤0.06
10653	*A. nosocomialis*		IMP-1	12	Kyusyu	Sputum	4/2	512	256	32	16	2	≤0.25	4
10654	*A.lwoffii*		IMP-1	10	Tokai	blood	4/2	512	256	32	64	0.5	≤0.25	0.125
10655	*A.lwoffii*	58	IMP-1	13	Hokuriku	Other	1/0.5	256	32	8	16	1	≤0.25	≤0.06

SAM, ampicillin/sulbactam; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; GEN, gentamicin; MIN, minocyclin; CIP, ciprofloxacin; PFGE, pulsed-fielded gel electrophoresis; MLST, multilocus sequencing type; ST, sequence type; CC, clonal complex.

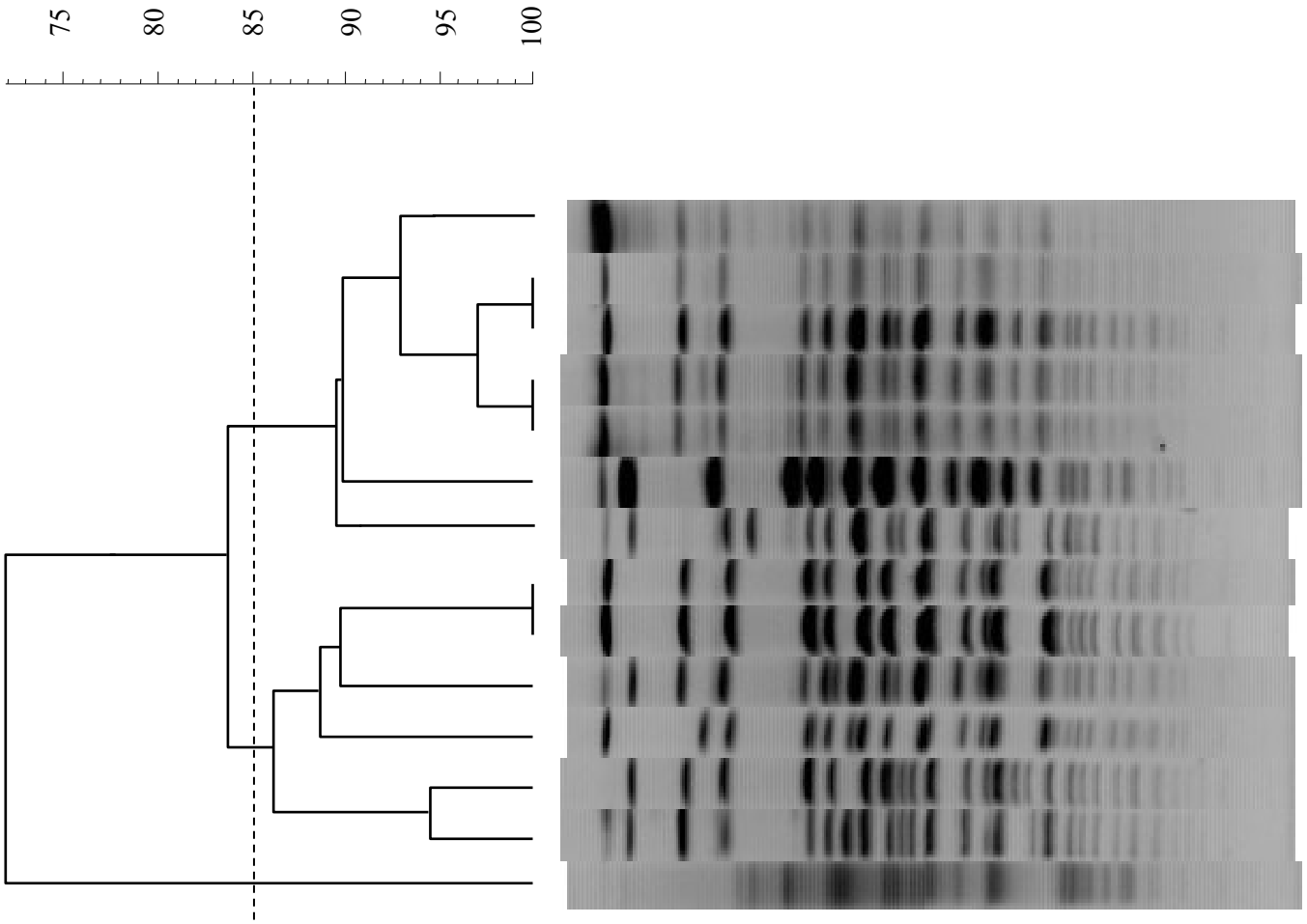
‡ Hp means hospital number.

*Detection of IS*Aba1* at 34-bp upstream from *bla*$_{\text{OXA-51 like}}$ gene.

** Detection of IS*Aba1* at 8-bp upstream from *bla*$_{\text{OXA-23}}$ gene.

†Detection of IS*Aba3*-like at 17-bp upstream from *bla*$_{\text{OXA-58}}$ gene.

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]



Isolate name	PFGE subgroup	Clonal Complex
TUM10642	A	92
TUM10632	A	92
TUM10633	A	92
TUM10630	A	92
TUM10631	A	92
TUM10629	A	92
TUM10641	A	92
TUM10637	B	92
TUM10638	B	92
TUM10634	B	92
TUM10636	B	92
TUM10639	B	92
TUM10640	B	92
TUM10635	C	276