

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

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12
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14
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25 **Abstract**

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

43 **Introduction**

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

54 More generally, it is apparent that the population structure of *A.*
55 *baumannii* comprises three major international lineages, named European clones
56 I, II, and III [4, 5]. A subgroup of European clone II involving clonal complex (CC)
57 92, has spread globally [6], and is wide spread in China [7] and Korea [8]; it is also
58 recorded in Australia [9].

59 While these strains remain relatively rare in Japan, it is a matter of
60 concern [10]. In the past reports about *Acinetobacter* spp. in Japan, *Acinetobacter*
61 *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.

81

82 *Identification at the level of species/genomic species*

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

94

95 *Antimicrobial susceptibility testing*

96 Minimum inhibitory concentrations were measured by the broth
97 microdilution method of the Clinical and Laboratory Standards Institute [15].
98 Antimicrobial agents tested were as follows: ampicillin, ceftazidime, imipenem,
99 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 ISAb_a*

111 *bla*_{OXA} genes were sought by multiplex PCR (Table 1) [17]. The presence of
112 an ISAb_a-type insertion sequence upstream of *bla*_{OXA} genes was investigated by
113 PCR and nucleotide sequencing, using combinations of the forward primers for
114 ISAb_a 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

119 DNA fragments were separated with a CHEF MAPPER (Bio-Rad) for 18.5 h at
120 14 °C with a 1 to 17s linear ramp of 6V/cm. Restriction patterns were analyzed
121 with Fingerprinting II software (Bio-Rad) and cluster analysis was performed by
122 the unweighted pair-group method with mathematical averaging. Position
123 tolerance and optimization were set at 1.5% and 1.5%, respectively. Only
124 restriction fragments larger than 50 kb were used for analysis. Isolates with
125 >85% similarity were assigned to the same strain subgroup.

126

127 *Multilocus sequence typing (MLST)*

128 Sequence types (STs) of *A. baumannii* isolates were determined according
129 to the MLST scheme (<http://pubmlst.org/abaumannii/>) [18, 19]. Clonal complexes
130 (CCs) were determined by eBURST version 3 (<http://eburst.mlst.net/>) with
131 definition of the groups by sharing alleles at ≥ 6 of 7 loci and bootstrap values of
132 1000 [20].

133

134 *Statistical analysis*

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

138

139 *Nucleotide sequence accession number*

140 The nucleotide sequence of *bla*_{OXA-206} was assigned accession number

141 AB634250.

142 **Results**

143 *Isolates with reduced susceptibilities to carbapenems*

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

148 Among the 598 clinical isolates of *Acinetobacter* spp. (333 from respiratory
149 tract, 45 from urinary tract, 79 from blood, 74 from pus, and 67 from other sites),
150 27 isolates (4.5%) were resistant to either or both imipenem and panipenem (17
151 from respiratory tract, 2 from urinary tract, 2 from blood, 2 from pus, and 4 from
152 other sites).

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

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

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

163

164 *Antimicrobial susceptibility*

165 Antibiogram data are described in Table 2. Compared with
166 non-*baumannii* *Acinetobacter* spp., *A. baumannii* was more frequently
167 non-susceptible to other classes of drug, namely, ampicillin/sulbactam (71% in *A.*
168 *baumannii* vs. 0% in non-*baumannii*, $p<0.01$) and ciprofloxacin (93% vs. 31%,
169 $p<0.01$).

170

171 *MBL-producing isolates*

172 Thirteen of the 27 isolates were determined to have MBLs by phenotypic
173 testing and PCR showed 12 of these to carry *bla*_{IMP-1}. All of them except one were
174 non-*baumannii* *Acinetobacter* isolates. The *bla*_{IMP-2} was detected in one *A.*
175 *nosocomialis* isolate. No MBL genes were found in isolates negative by the
176 phenotypic test.

177

178 *Class D carbapenemases and ISAba*

179 Sequencing of the PCR products revealed that the *bla*_{OXA-51-like} gene harbored by

180 12/14 *A. baumannii* isolates was *bla*_{OXA-66} and one isolate harbored *bla*_{OXA-64},
181 while one isolate harbored *bla*_{OXA-206}, a new variant gene. The isolate with
182 *bla*_{OXA-64} also had IS*Aba3*-like-*bla*_{OXA-58} and the isolate with *bla*_{OXA-206}, a single
183 amino acid variant of OXA-66 carried IS*Aba1*-*bla*_{OXA-23}. Of these 14, six carried
184 *bla*_{OXA-23} and one had *bla*_{OXA-58}. One *A. lwoffii* isolate and the three *A. pittii*
185 isolates harbored *bla*_{OXA-58}. IS*Aba1* was located 34-bp upstream of the *bla*_{OXA-66}
186 and *bla*_{OXA-206} genes, or 8-bp upstream of the *bla*_{OXA-23} gene. IS*Aba3*-like was
187 located 17-bp upstream of *bla*_{OXA-58}. IS*Aba1* was not detected upstream of
188 *bla*_{OXA-66} in TUM10629 and upstream of *bla*_{OXA-64} in TUM 10635, and IS*Aba3*-like
189 was not found upstream of *bla*_{OXA-58} in *A. lwoffii* TUM 10655.

190 *Genetic relatedness of A. baumannii isolates*

191 The *A. baumannii* isolates were classified into 3 subgroups by PFGE
192 (Figure). PFGE subgroups A and B comprised 7 and 6 isolates, respectively,
193 whereas one isolate was unique. PFGE subgroup A consisted of five isolates,
194 TUM10629 to TUM10633 (Table 2), carrying both *bla*_{OXA-66} and *bla*_{OXA-23} from one
195 hospital in the Kanto region, TUM10641 carrying *bla*_{OXA-206} and *bla*_{OXA-23}, and
196 TUM10642 from outside Kanto region. All isolates from Kanto region with only
197 *bla*_{OXA-66} were classified into the PFGE subgroup B. The unique isolate, allocated
198 to subgroup C, was TUM10635, harboring *bla*_{OXA-64}, *bla*_{OXA-58}, and *bla*_{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*_{OXA-66} belonged to CC92 is compatible with the finding that the

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

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

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

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

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

245 In contrast to *A. baumannii*, almost all isolates of non-*baumannii*
246 *Acinetobacter* isolates proved to produce MBLs (Table 2; 92% in non-*baumannii*
247 vs 7.1% in *A. baumannii*, p<0.01). PCR and sequencing revealed *bla*_{IMP-1} in all but
248 one MBL-producing isolates. Previous studies in East Asia have demonstrated a
249 similar, but not identical, trend: in Korea, carbapenem-resistant *A. nosocomialis*
250 and *A. calcoaceticus*, harbored the VIM-2 type MBL gene [25, 29]. In Taiwan,
251 MBL genes of *bla*_{IMP-1} and *bla*_{VIM-11} were detected in *A. pittii* and *A. nosocomialis*
252 [22]. In these reports, MBLs were not detected in *A. baumannii*. The difference
253 of MBL gene might reflect the predominant MBL genes among other pathogens;
254 in Japan, the IMP-1-type MBL is most prevalent among *Pseudomonas aeruginosa*
255 [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*_{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*_{OXA-23} gene. The MBL producing *A.*
265 *baumannii* was rare; only one isolate belonging to CC276 and containing *bla*_{OXA-64}
266 and *bla*_{OXA-58} also harbored *bla*_{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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382

383

384 **FIGURE LEGEND**

385 **Figure.** Pulsed-field gel electrophoresis (PFGE) of 14 carbapenem-non-susceptible
386 *Acinetobacter baumannii* isolates in Japan. *A. baumannii* comprised PFGE
387 subgroup A, B and C at a level 85% (indicated by the broken line). While PFGE
388 subgroup A and B belonged to CC92, PFGE subgroup C with unique band pattern
389 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 AAAAGG GGA TT		
OXA-58 like Fw	AAG TAT TGG GGC TTG TGC TG		
OXA-58 like Rv	CCC CTC TGC GCT CTA CAT AC		
<i>ISAb</i> a-1Fw	CAC GAA TGC AGAAGT TG	<i>ISAb</i> a family	[2]
<i>ISAb</i> a-1Rv	CGA CGA ATA CTA TGA CAC		[3]
<i>ISAb</i> a-3 like	AGC AAT ATC TCG TAT ACC GC		
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-susceptible *Acinetobacter* species in Japan.

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

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

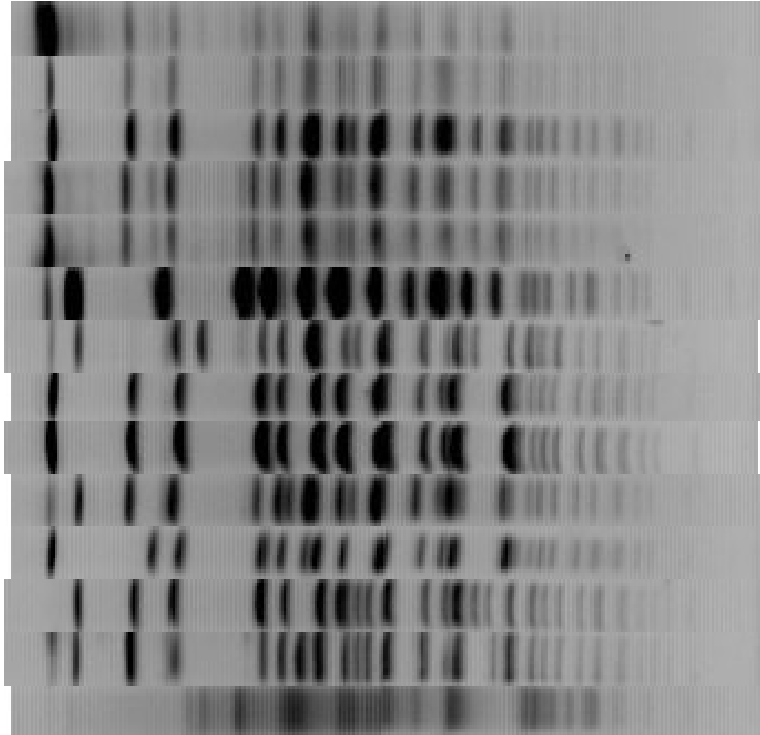
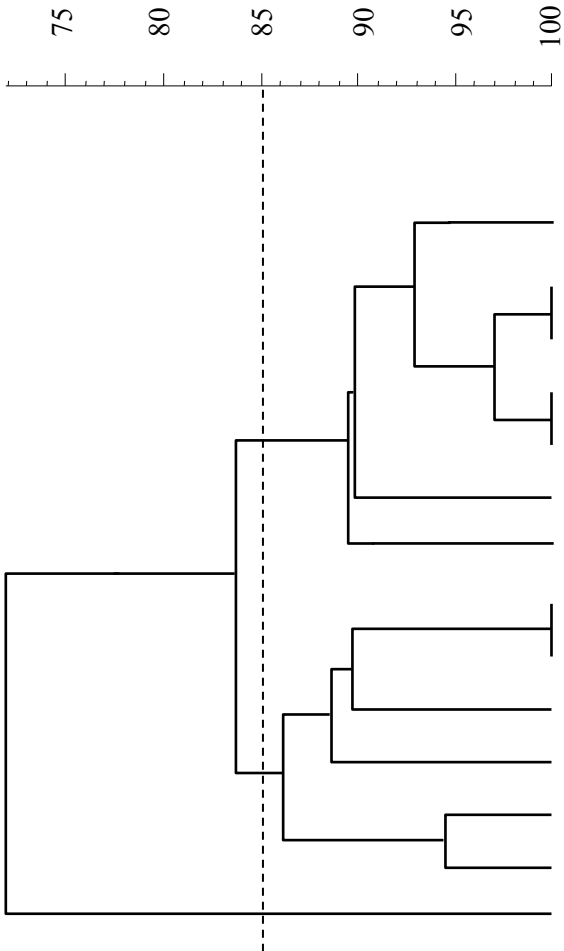
‡ Hp means hospital number.

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

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

†Detection of IS*Aba3*-like at 17-bp upstream from *bla*_{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