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EPIDEMIOLOGY AND SURVEILLANCE

Distribution and Molecular Characterization of *Acinetobacter baumannii* International Clone II Lineage in Japan

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ABSTRACT Multidrug-resistant (MDR) *Acinetobacter* spp. have been globally disseminated in association with the successful clonal lineage *Acinetobacter baumannii* international clone II (IC II). Because the prevalence of MDR *Acinetobacter* spp. in Japan remains very low, we characterized all *Acinetobacter* spp. ($n = 866$) from 76 hospitals between October 2012 and March 2013 to describe the entire molecular epidemiology of *Acinetobacter* spp. The most prevalent species was *A. baumannii* ($n = 645$; 74.5%), with *A. baumannii* IC II ($n = 245$) accounting for 28.3% of the total. Meropenem-resistant isolates accounted for 2.0% ($n = 17$) and carried IS*Aba1*-$bla_{\text{OXA-23-like}}$ ($n = 10$), bla_{IMP} ($n = 4$), or IS*Aba1*-$bla_{\text{OXA-51-like}}$ ($n = 3$). Multilocus sequence typing of 110 representative *A. baumannii* isolates revealed the considerable prevalence of domestic sequence types (STs). *A. baumannii* IC II isolates were divided into the domestic sequence type 469 (ST469) ($n = 18$) and the globally disseminated STs ST208 ($n = 14$) and ST219 ($n = 4$). ST469 isolates were susceptible to more antimicrobial agents, while ST208 and ST219 overproduced the intrinsic AmpC β-lactamase. *A. baumannii* IC II and some *A. baumannii* non-IC II STs (e.g., ST149 and ST246) were associated with fluoroquinolone resistance. This study revealed that carbapenem-susceptible *A. baumannii* IC II was moderately disseminated in Japan. The low prevalence of acquired carbapenemase genes and presence of domestic STs could contribute to the low prevalence of MDR *A. baumannii*. A similar epidemiology might have appeared before the global dissemination of MDR epidemic lineages. In addition, fluoroquinolone resistance associated with *A. baumannii* IC II may provide insight into the significance of *A. baumannii* epidemic clones.

KEYWORDS *Acinetobacter* spp., *Acinetobacter baumannii* international clone II, MLST, bla_{ADC}

The prevalence of multidrug-resistant (MDR) *Acinetobacter* spp. has increased globally during the last 2 decades, and high rates of carbapenem resistance, as high as more than 50% of *Acinetobacter* species isolates, have been reported from Asian countries (1–3). The emergence and dissemination of several clonal lineages contribute to the increasing prevalence of MDR *Acinetobacter* spp. (4). One of the most successful clonal lineages, *Acinetobacter baumannii* international clone II (IC II), corresponds to clonal complex 92 (CC92) according to the multilocus sequence typing (MLST) Oxford scheme developed by Bartual et al. (5) and to sequence type 2 (ST2) of the alternative Pasteur MLST scheme. *A. baumannii* IC II is associated with nosocomial outbreaks and multidrug resistance (6) and has spread globally (7).

In Japan, *A. baumannii* IC II is also reported to be strongly associated with multidrug

Received 26 October 2017 **Returned for modification** 15 November 2017 **Accepted** 28 November 2017

Accepted manuscript posted online 4 December 2017

Citation Matsui M, Suzuki M, Suzuki M, Yatsuyanagi J, Watahiki M, Hiraki Y, Kawano F, Tsutsui A, Shibayama K, Suzuki S. 2018. Distribution and molecular characterization of *Acinetobacter baumannii* international clone II lineage in Japan. Antimicrob Agents Chemother 62:e02190-17. https://doi.org/10.1128/AAC.02190-17.

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TABLE 1 Species identifications and specimen types

Identification	No. of isolates (% of total)	Specimen type						GenBank accession no. of *rpoB* sequence of reference strain[a]
		Respiratory	Urine	Wound	Blood	Stool	Other	
A. baumannii	645 (74.5)	498	57	34	13	8	35	DQ207471
IC II	245 (28.3)	215	21	6	0	1	2	
Non-IC II	400 (46.2)	283	36	28	13	7	33	
A. nosocomialis	83 (9.6)	73	1	4	1	1	3	EU477118
A. pittii	60 (6.9)	38	2	1	3	2	14	EU477114
A. seifertii	18 (2.1)	13	2	2	1	0	0	EU477126
A. bereziniae	13 (1.5)	13	0	0	0	0	0	DQ207475
A. junnii/A. grimontii	12 (1.4)	8	0	1	0	1	2	DQ207483/DQ207486
A. soli	11 (1.3)	10	0	0	0	0	1	HQ148175
A. ursingii	8	7	0	0	0	0	1	DQ231239
Genomic sp. between 1 and 3	5	2	1	0	1	1	0	EU477122
A. radioresistens	2	1	1	0	0	0	0	DQ207489
A. guillouiae	2	0	1	1	0	0	0	DQ207476
A. calcoaceticus	1	1	0	0	0	0	0	DQ207474
A. baylyi	1	1	0	0	0	0	0	EU477155
A. beijerinckii	1	1	0	0	0	0	0	EU477124
A. johnsonii	1	1	0	0	0	0	0	DQ207485
Genomic sp. 15BJ/genomic sp. 16	1	0	0	1	0	0	0	EU477133/EU477135
Acinetobacter spp.[b]	2	2	0	0	0	0	0	
Total (% of total)	866	669 (77.3)	65 (7.5)	44 (5.1)	19 (2.2)	13 (1.5)	56 (6.5)	

[a]The following *Acinetobacter* species (GenBank accession no. of *rpoB* sequence of reference strain) were not isolated in this study: *A. gerneri* (DQ207482), *A. venetianus* (EU477136), *A. towneri* (DQ207493), *A. tandoii* (DQ207491), *A. haemolyticus* (DQ207484), *A. schindleri* (DQ207490), *A. bouvetii* (DQ207473), *A. lwoffii* (DQ207487), *A. tjernbergiae* (EU477153), *A. gyllenbergii* (EU477148), *A. parvus* (DQ207488), *A. brisouii* (KJ124836), *A. albensis* (KR611814), *A. gandensis* (KJ569689), *A. kookii* (KM821031), *A. guangdongensis* (KR611818), *A. indicus* (KJ124838), *A. bohemicus* (KJ124834), *A. harbinensis* (KF803234), *A. rudis* (KJ124837), *A. nectaris* (KJ124840), *A. qingfengensis* (KC631629), *A. puyangensis* (JX499272), genomic sp. 6 (DQ207480), genomic sp. 9 (DQ207481), genomic sp. 13BJ (EU477132), genomic sp. 14BJ (EU477147), genomic sp. 15TU (EU477119), genomic sp. 17 (EU477134).

[b]Isolates were unidentifiable at the species level because the partial *rpoB* sequences had low identity with the closest reference sequences (91.9% and 85.5%), though they were identified as *Acinetobacter* spp. using 16S rRNA sequencing and Vitek2.

or carbapenem resistance (8–10) and has caused nosocomial outbreaks (11–13). However, the prevalence of MDR *Acinetobacter* species isolates (criteria are described in Materials and Methods section) remains low in Japan. According to the annual open report 2015 of the clinical laboratory division of Japan Nosocomial Infections Surveillance (JANIS) (14), a national surveillance system, MDR *Acinetobacter* species isolates accounted for only 0.5% of *Acinetobacter* species isolates, and the rates of imipenem and meropenem resistance were 3.2% and 1.8%, respectively (https://janis.mhlw.go.jp/english/report/open_report/2015/4/1/ken_Open_Report_Eng_201500_clsi2012.pdf). Therefore, previous studies mainly focused on MDR or carbapenem-nonsusceptible isolates cannot elucidate the entire molecular epidemiology of *Acinetobacter* spp. and the reason for the low levels of resistance in Japan. Thus, in this study, we characterized clinical *Acinetobacter* species isolates, including carbapenem-susceptible isolates, collected nationwide in Japan.

RESULTS

Bacterial isolates and species identification. A total of 932 isolates from 78 hospitals were characterized. Of the 932 isolates, 886 isolates from 76 hospitals were identified as *Acinetobacter* spp. and subjected to further investigation. Distributions of species and specimen types are shown in Table 1. *A. baumannii* was the most prevalent (n = 645; 74.5%), followed by *Acinetobacter nosocomialis* and *Acinetobacter pittii*. Among *A. baumannii* isolates, 245 (38.0%) belonged to IC II, accounting for 28.3% of all *Acinetobacter* spp.

Only 19 *Acinetobacter* species isolates were isolated from blood. Interestingly, none of these was *A. baumannii* IC II, while 13 (68.4%) were *A. baumannii* non-IC II. Respiratory specimens were the most frequent specimens from which *Acinetobacter* spp. were isolated. Unlike isolates from blood, *A. baumannii* IC II tended to be isolated from respiratory specimens more often than *A. baumannii* non-IC II, with 87.8% (215/245) of

TABLE 2 Rates of antimicrobial resistance among isolates collected in this study and comparison with JANIS data

Antimicrobial agent(s)	% of isolates collected in this study resistant among:				% of *Acinetobacter* species isolates resistant (no. of isolates tested) in JANIS annual report 2013[b]
	IC II (n = 245[a])	Non-IC II (n = 400)	Non-*baumannii* (n = 221)	All isolates (n = 866)	
Piperacillin-tazobactam	24.5	0.8	0.9	7.5	7.8 (4,953)
Ampicillin-sulbactam	8.2	0.0	0.0	2.3	5.8 (4,498)
Ceftazidime	69.8	2.0	0.9	20.9	10.0 (20,856)
Cefepime	48.2	4.0	0.0	15.5	9.2 (15,394)
Imipenem	3.7	1.3	0.0	1.6	3.6 (16,947)
Meropenem	4.9	1.3	0.0	2.0	3.7 (17,027)
Gentamicin	51.0	6.0	2.7	17.9	9.5 (19,422)
Amikacin	24.5	0.0	0.0	6.9	3.5 (20,863)
Levofloxacin	87.8	13.0	2.7	31.5	8.3 (20,040)
Ciprofloxacin	100	24.8	6.8	41.5	No data
Minocycline	13.5	0.0	0.0	3.8	No data
Colistin	0.0	0.3	1.8	0.6	No data
Polymyxin B	0.0	0.0	0.9	0.2	No data

[a]n = number of isolates.
[b]Rates of resistance in 2013 JANIS data (15) were recalculated based on the breakpoints of CLSI M100-S25 (36).

A. baumannii IC II isolates derived from respiratory specimens and 70.8% (283/400) of *A. baumannii* non-IC II isolates derived from these specimens ($P < 0.001$).

The 866 isolates were obtained from 698 patients, and demographic information was available for 676 patients. There were 407 males (60.2%) and 269 females (39.8%). The age distribution of the 676 patients ranged between 0 and 97 years with a median age of 65 and was similar between males and females. The age and sex distributions were similar across patients who carried *A. baumannii* IC II, *A. baumannii* non-IC II, and non-*baumannii Acinetobacter* species isolates.

Differences in antimicrobial susceptibilities among *A. baumannii* IC II, *A. baumannii* non-IC II, and non-*baumannii Acinetobacter* species isolates. The rates of antimicrobial resistance were compared among isolates categorized as *A. baumannii* IC II (n = 245), *A. baumannii* non-IC II (n = 400), and non-*baumannii Acinetobacter* spp. (n = 221) (Table 2). *A. baumannii* IC II exhibited a higher proportion of resistant isolates than the other two groups for all antimicrobial agents except colistin and polymyxin B. Notably, all *A. baumannii* IC II isolates were resistant to ciprofloxacin, whereas only 18.4% of the isolates in the other two groups were resistant. There were six MDR isolates, of which two were *A. baumannii* IC II and four *A. baumannii* non-IC II. All MDR isolates were susceptible to minocycline, colistin, and polymyxin B. Colistin-resistant isolates were identified as *A. baumannii* non-IC II (n = 1), *Acinetobacter beijerinkii* (n = 1), *Acinetobacter bereziniae* (n = 2), and *Acinetobacter seifertii* (n = 1).

To better characterize the epidemiology of these isolates throughout Japan, the prevalence of antimicrobial-resistant isolates in this study was compared with data from JANIS (Table 2) (https://janis.mhlw.go.jp/english/report/open_report/2013/3/1/ken_Open_Report_Eng_201300.pdf) (15). The proportions of isolates resistant to ceftazidime, cefepime, gentamicin, amikacin, and levofloxacin were higher in this study than in JANIS. *A. baumannii* IC II isolates exhibited significantly higher rates of resistance to these antimicrobial agents than did the other two groups. This suggests a higher proportion of *A. baumannii* IC II isolates in this study than in isolates from the hospitals participating in JANIS.

MIC_{90}s and MIC_{50}s for the three groups described above are shown in Table 3. It is notable that *A. baumannii* IC II isolates had higher MIC_{50}s and MIC_{90}s than the other two groups, even for some antimicrobial agents for which *A. baumannii* IC II isolates exhibited low rates of resistance, such as ampicillin-sulbactam, imipenem, and meropenem.

Distribution of acquired carbapenem resistance genes among *Acinetobacter* spp. The distribution of acquired carbapenem resistance genes is shown in Table 4. None of the isolates were positive for $bla_{\text{OXA-40-like}}$, $bla_{\text{OXA-58-like}}$, bla_{VIM} group, or bla_{TMB} group genes. $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$, encoding naturally occurring oxacillinases,

TABLE 3 Distribution of MIC_{90}s and MIC_{50}s for *A. baumannii* IC II, *A. baumannii* non-IC II, and non-*baumannii Acinetobacter* species isolates

Antimicrobial agent(s)	MIC range (μg/ml)[a]	Value(s) (μg/ml) for isolates in indicated group					
		MIC_{90}			MIC_{50}		
		IC II (*n* = 245[b])	Non-IC II (*n* = 400)	Non-*baumannii* (*n* = 221)	IC II (*n* = 245)	Non-IC II (*n* = 400)	Non-*baumannii* (*n* = 221)
Piperacillin-tazobactam	≤8/4 to ≥512/4	256/4	≤8/4	≤8/4	≤8/4	≤8/4	≤8/4
Ampicillin-sulbactam	≤1/0.5 to 64/32	16/8	2/1	2/1	4/2	≤1/0.5	≤1/0.5
Ceftazidime	≤1 to ≥64	≥64	8	8	≥64	2	4
Cefepime	≤1 to ≥64	≥64	16	8	16	2	2
Imipenem	≤0.125 to 64	2	0.5	0.5	1	0.25	0.25
Meropenem	≤0.125 to 64	2	1	0.5	1	0.25	0.25
Gentamicin	≤1 to ≥128	≥128	8	2	16	≤1	≤1
Amikacin	≤2 to ≥256	≥256	8	4	4	≤2	≤2
Levofloxacin	≤0.25 to ≥64	16	8	0.5	8	≤0.25	≤0.25
Ciprofloxacin	≤0.25 to ≥64	≥64	≥64	1	≥64	≤0.25	≤0.25
Minocycline	≤1 to ≥64	16	≤1	≤1	4	≤1	≤1
Colistin	≤0.5 to ≥8	≤0.5	≤0.5	1	≤0.5	≤0.5	≤0.5
Polymyxin B	≤0.5 to ≥8	≤0.5	≤0.5	1	≤0.5	≤0.5	≤0.5

[a]Distribution of MICs for all isolates.
[b]*n* = number of isolates.

were detected in all 645 *A. baumannii* and both *Acinetobacter radioresistens* isolates, respectively. Among 17 meropenem-resistant isolates (MIC of ≥8 μg/ml), the most prevalent gene was IS*Aba1*-$bla_{OXA-23-like}$ (*n* = 10). Three isolates had IS*Aba1* upstream from $bla_{OXA-51-like}$, all of which were bla_{OXA-80}. In contrast, the other 47 isolates with IS*Aba1*-$bla_{OXA-51-like}$ were not resistant to meropenem, and the $bla_{OXA-51-like}$ sequences were bla_{OXA-66} in all but two isolates that carried bla_{OXA-80} and $bla_{OXA-104}$, respectively. Overall, IS*Aba1*-$bla_{OXA-23-like}$ and IS*Aba1*-$bla_{OXA-51-like}$ were mainly detected in *A. baumannii* IC II and bla_{IMP} was detected in *A. baumannii* non-IC II isolates.

MLST. A total of 110 nonduplicate *A. baumannii* isolates, consisting of 37 *A. baumannii* IC II and 73 *A. baumannii* non-IC II isolates from 70 hospitals, were subjected to MLST (Table 5). All IC II isolates belonged to ST2 according to the Pasteur scheme, and they were further classified into four STs, ST469, ST208, ST219, and ST1353, based on the Oxford scheme. *A. baumannii* non-IC II isolates belonged to diverse STs, with the 73 isolates being assigned to 46 and 50 different STs using the Pasteur and Oxford schemes, respectively. Most of these STs have been previously reported in Japan or represented novel STs identified in this study. Four isolates from three hospitals were identified as ST25, which is a successful lineage other than international clones I to III (4), using the Pasteur scheme.

Differences in antimicrobial susceptibilities among STs. Among *A. baumannii* IC II isolates, isolates belonging to ST208 and its single-locus variant (SLV) ST219 exhibited significantly higher rates of resistance to piperacillin-tazobactam, ceftazidime, and cefepime than those belonging to ST469 (Table 6). Notably, all ST208 and ST219 isolates

TABLE 4 Distribution of acquired carbapenem resistance genes

Gene	No. of isolates with meropenem MIC of:								
	8 μg/ml (*n* = 17[a])				≤4 μg/ml (*n* = 849)				
	A. baumannii IC II	*A. baumannii* non-IC II	Non-*baumannii Acinetobacter* spp.	Subtotal	*A. baumannii* IC II	*A. baumannii* non-IC II	Non-*baumannii Acinetobacter* spp.	Subtotal	Total
bla_{IMP}		4		4					4
IS*Aba1*-$bla_{OXA-23-like}$	9	1		10					10
IS*Aba1*-$bla_{OXA-51-like}$	3			3	46	1		47	50
No tested genes				0	187	394	221	802	802
Total	12	5	0	17	233	395	221	849	866

[a]*n* = number of isolates.

TABLE 5 MLST data and rates of ciprofloxacin resistance among STs

Group (no. of isolates)	Pasteur ST (no. of isolates)	Country(ies) with isolates belonging to ST in database[a]	Oxford ST (no. of isolates)	Country(ies) with isolates belonging to ST in database[a]	No. (%) of ciprofloxacin-resistant isolates[b]
IC II (n = 37)	ST2 (37)	Multiple countries	ST469 (18)	None	18 (100)
			ST208 (14)	Multiple countries	14 (100)
			ST219 (4)	Japan	4 (100)
			ST1353 (1)	Japan[c]	1
Non-IC II (n = 73)	ST149 (5)	Japan	ST862 (5)	None	5 (100)
	ST406 (5)	Japan, USA	ST310 (5)	USA	2 (40)
	ST25 (4)	Multiple countries	ST229 (4)	Brazil, Mexico, USA	2 (50)
	ST34 (4)	Sweden, Czech Republic	ST432 (2)	China, Czech Republic	0
			ST1352 (2)	Japan[c]	0
	ST213 (4)	Japan	ST1351 (4)	Japan[c]	0
	ST151 (3)	Japan	ST1365 (2)	Japan[c]	0
			ST1381 (1)	Japan[c]	0
	ST152 (3)	Japan	ST1373 (3)	Japan[c]	0
	ST246 (3)	Japan	ST1362 (3)	Japan[c]	3 (100)
	ST33 (2)	Japan, USA	ST1364 (1)	Japan[c]	0
			ST1372 (1)	Japan[c]	0
	ST40 (2)	China, Czech Republic, Japan	ST373 (1)	China, Czech Republic, Japan	0
			ST528 (1)	None	0
	ST138 (2)	Taiwan, Germany	ST1354 (2)	Japan[c]	0
	ST448 (2)	Japan[c]	ST1361 (2)	Japan[c]	0
	ST22 (1)	The Netherlands	ST1382 (1)	Japan[c]	1
	ST106 (1)	China	ST605 (1)	None	0
	ST108 (1)	USA	ST105 (1)	Argentina, USA	0
	ST113 (1)	China, USA, Brazil	ST873 (1)	USA	0
	ST120 (1)	Japan	ST1383 (1)	Japan[c]	0
	ST150 (1)	Japan	ST718 (1)	None	0
	ST154 (1)	Spain, Saudi Arabia, USA	ST943 (1)	USA	0
	ST193 (1)	Brazil, Poland	ST1377 (1)	Japan[c]	0
	ST240 (1)	Japan, Switzerland	ST1370 (1)	Japan[c]	0
	ST241 (1)	Japan, Brazil, USA	ST613 (1)	USA	0
	ST267 (1)	Australia, Spain	ST942 (1)	Australia	1
	ST331 (1)	China	ST1366 (1)	Japan[c]	0
	ST412 (1)	Iraq, USA	ST1349 (1)	Japan[c]	0
	ST439 (1)	Japan[c]	ST1380 (1)	Japan[c]	0
	ST441 (1)	Japan[c]	ST1350 (1)	Japan[c]	0
	ST442 (1)	Japan[c]	ST1355 (1)	Japan[c]	0
	ST443 (1)	Japan[c]	ST1356 (1)	Japan[c]	0
	ST444 (1)	Japan[c]	ST1357 (1)	Japan[c]	0
	ST445 (1)	Japan[c]	ST1358 (1)	Japan[c]	0
	ST446 (1)	Japan[c]	ST1359 (1)	Japan[c]	0
	ST447 (1)	Japan[c]	ST1360 (1)	Japan[c]	0
	ST449 (1)	Japan[c]	ST1367 (1)	Japan[c]	0
	ST450 (1)	Japan[c]	ST1368 (1)	Japan[c]	0
	ST451 (1)	Japan[c]	ST1369 (1)	Japan[c]	0
	ST452 (1)	Japan[c]	ST1371 (1)	Japan[c]	0
	ST453 (1)	Japan[c]	ST1374 (1)	Japan[c]	0
	ST454 (1)	Japan[c]	ST1375 (1)	Japan[c]	0
	ST455 (1)	Japan[c]	ST1376 (1)	Japan[c]	0
	ST456 (1)	Japan[c]	ST1384 (1)	Japan[c]	0
	ST874 (1)	Japan[c]	ST1363 (1)	Japan[c]	0
	ST875 (1)	Japan[c]	ST1342 (1)	None	0
	ST876 (1)	Japan[c]	ST1378 (1)	Japan[c]	1
	ST877 (1)	Japan[c]	ST1379 (1)	Japan[c]	0
	ST897 (1)	Japan[c]	ST1116 (1)	None	0

[a]Country(ies) where the isolates corresponding to the STs were submitted to the PubMLST *Acinetobacter baumannii* isolate database (http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_abaumannii_isolates:id1%96id3421).
[b]Percentages are indicated only for STs including more than two resistant isolates.
[c]Novel registration in this study.

were resistant to ceftazidime and all ST469 isolates were susceptible to it. It has been reported that the overproduction of the intrinsic AmpC cephalosporinase ADC results from the insertion of IS*Aba1* sequences upstream from bla_{ADC} and is responsible for cephalosporin resistance in *A. baumannii* (16). To clarify the differences in antimicrobial

TABLE 6 Comparison of rates of antimicrobial resistance among ST208, ST219, and ST469

Antimicrobial agent(s)	No. (%) of resistant isolates belonging to: ST208 and ST219 (n = 18 isolates) (allele profiles 1-3-3-2-2-97-3 and1-3-3-2-2-101-3)	ST469 (n = 18 isolates) (allele profile 1-12-3-2-2-103-3)	P value
Piperacillin-tazobactam	8 (44.4)	0 (0.0)	0.003
Ampicillin-sulbactam	2 (11.1)	1 (5.6)	1
Ceftazidime	18 (100)	0 (0.0)	<0.001
Cefepime	14 (77.8)	1 (5.6)	<0.001
Imipenem	1 (5.6)	0 (0.0)	1
Meropenem	1 (5.6)	0 (0.0)	1
Gentamicin	13 (72.2)	7 (38.9)	0.092
Amikacin	6 (33.3)	0 (0.0)	0.019
Levofloxacin	16 (88.9)	14 (77.8)	0.658
Ciprofloxacin	18 (100)	18 (100)	
Minocycline	3 (16.7)	3 (16.7)	1
Colistin	0 (0.0)	0 (0.0)	
Polymyxin B	0 (0.0)	0 (0.0)	

susceptibilities among STs, β-lactamase genes and upstream ISs were identified (Table 7). The bla_{ADC} from ST208 and ST219 isolates encoded ADC-30 or the single-amino-acid variant ADC-73 (see Fig. S1a in the supplemental material). In all of these isolates, the strong promoter sequence presumed to derive from IS*Aba1* was detected upstream from bla_{ADC}, suggesting the overproduction of the intrinsic AmpC β-lactamase (Fig. S1b). In contrast, the bla_{ADC} gene in all but one ST469 isolate encoded ADC-25 and the original promoter sequence, rather than the promoter derived from the upstream insertion, was detected (Fig. S1a and b). There were no notable differences in other β-lactamase genes among ST208, ST219, and ST469 isolates.

Among the 73 non-IC II isolates, 58 (79.5%) were susceptible to all antimicrobial agents tested. Fifteen (20.5%) were resistant to ciprofloxacin, and among these, only four were also resistant to antimicrobial agents other than fluoroquinolones. Fluoroquinolone resistance appeared to be associated with specific STs, such as ST149 and ST246 in the Pasteur scheme (Table 5). Four isolates that were resistant to several classes of antimicrobial agents belonged to ST149, ST246, ST25, and ST406.

DISCUSSION

As carbapenem resistance among *Acinetobacter* spp. remains below 5% in Japan, we predicted a similar low prevalence of *A. baumannii* IC II. However, in this study, *A. baumannii* IC II isolates accounted for 28.3% of clinically isolated *Acinetobacter* spp. and were distributed among about half of the participating hospitals. This means that *A. baumannii* IC II is already widespread in Japan but is carbapenem susceptible. In our previous study in 2001, which collected 264 *Acinetobacter* species isolates from 88

TABLE 7 β-Lactamase-encoding genes detected in ST208, ST219, and ST469 isolates

ST, allele profile	No. of isolates	$bla_{OXA\text{-}51\text{-}like}$	bla_{ADC}[a]	Other β-lactamase-encoding gene(s)
ST208, 1-3-3-2-2-97-3	6	$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}30}$	
	4	$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}30}$	$bla_{TEM\text{-}1}$
	2	IS*Aba1*-$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}30}$	
	1	$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}30}$	$bla_{PER\text{-}1}$, $bla_{TEM\text{-}1}$
	1	$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}73}$	$bla_{TEM\text{-}1}$
ST219, 1-3-3-2-2-101-3	3	$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}30}$	
	1	$bla_{OXA\text{-}66}$	IS-$bla_{ADC\text{-}30}$	IS*Aba1*-$bla_{OXA\text{-}23}$
ST469, 1-12-3-2-2-103-3	9	$bla_{OXA\text{-}66}$	$bla_{ADC\text{-}25}$	
	8	$bla_{OXA\text{-}66}$	$bla_{ADC\text{-}25}$	$bla_{TEM\text{-}1}$
	1	$bla_{OXA\text{-}66}$	$bla_{ADC\text{-}153}$ (novel)	

[a]For ISs of $bla_{ADC\text{-}30}$ and $bla_{ADC\text{-}73}$, the strong promoter sequence (−35, TTAGAA, and −10, TTATTT) presumed to derive from IS*Aba1* was detected upstream from bla_{ADC} genes. $bla_{ADC\text{-}153}$, a novel variant of bla_{ADC}, was deposited in GenBank under accession number KY997074.

hospitals, *A. baumannii* IC II accounted for only 3% (unpublished data). Although it is difficult to directly compare the data from these studies, as the isolate collection methods were not strictly the same, *A. baumannii* IC II might have gradually become disseminated in Japan without notice.

The isolates in this study appear to include a relatively higher proportion of *A. baumannii* IC II than did the isolates from hospitals participating in JANIS. This difference in the proportion of *A. baumannii* IC II isolates might be related to the characteristics of the participating hospitals. The JANIS annual report in 2013 (15) consisted of data from 745 hospitals, most of which are acute care hospitals (https://janis.mhlw.go.jp/english/report/open_report/2013/3/1/ken_Open_Report_Eng_201300.pdf). In contrast, the participating hospitals in this study included those that have specific beds for long-term care. Thus, our data are consistent with a previous report that long-term-care facilities are potential reservoirs of MDR organisms, including carbapenem-resistant *A. baumannii* (17).

The dissemination of carbapenem-susceptible *A. baumannii* IC II shown in this study suggests that epidemic clonal lineages of *A. baumannii* did not emerge and disseminate as carbapenem-resistant clones. Instead, this lineage may have evolved into a carbapenem-resistant or MDR lineage by acquiring various antimicrobial resistance genes during or after dissemination. This hypothesis is supported by previous reports. During the 1980s and 1990s, *A. baumannii* international clones I, II, and III (previously European clones I, II, and III) spread throughout Europe and caused outbreaks there, but they were usually susceptible to carbapenem (18, 19), like the *A. baumannii* IC II isolates in this study. Then, the emergence and proliferation of carbapenemase-producing European clones I and II with $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$, or $bla_{\text{OXA-58-like}}$ genes resulted in increasing rates of carbapenem resistance throughout Europe in the early 2000s (20). In this study, *A. baumannii* isolates with $bla_{\text{OXA-23-like}}$, which is the major carbapenemase gene in *A. baumannii* worldwide, remained rare, which may be one reason for the low prevalence of carbapenem-resistant *A. baumannii* isolates in Japan. However, *A. baumannii* IC II with IS*Aba1* upstream from $bla_{\text{OXA-51-like}}$ accounted for 5.7% ($n = 50$) of the total isolates. Clones with IS*Aba1* upstream from $bla_{\text{OXA-51-like}}$, such as the SE clone, spread in Europe before the dissemination of the OXA-23-producing clone (21). Therefore, it may be necessary to monitor the prevalence of the carbapenemase gene, not just rates of carbapenem resistance.

MLST using the Oxford scheme revealed that ST469, previously ST76 (10), and ST1353 are putative domestic STs of *A. baumannii* IC II in Japan, as to our knowledge they have been reported only from Japan, with the exception of the first registered ST469 isolate from China. Unlike ST208 and ST219, ST469 isolates were susceptible to most of the antimicrobial agents tested. Interestingly, the intrinsic AmpC cephalosporinase ADC-25 detected in ST469 was also detected in *A. baumannii* strain A320 (GenBank accession number JN247441), an old IC II isolate from the Netherlands in 1982. ADC-30 with IS*Aba1*, detected in ST208 and ST219 isolates, has been reported more recently and frequently, such as in *A. baumannii* strain MDR-ZJ06 (China in 2006) (22), *A. baumannii* strain A91 (Australia in 2005) (23), and *A. baumannii* strain AB78 (United States in 2007) (24). Some research groups have investigated the genetic diversity of *A. baumannii* IC II using whole-genome sequencing and divided isolates into various clades (25, 26). Though detailed investigation and comparison of ST469 and ST208 isolates is a subject for a future study, the domestic IC II, ST469, might be closely related to the carbapenem-susceptible ancestral IC II that was disseminated in Europe in the 1980s and 1990s. That is, the molecular epidemiology of *Acinetobacter* spp. in Japan might be similar to that in Europe in previous decades. This may represent another reason why MDR *A. baumannii* remains rare in Japan.

The discriminatory power of MLST using the Oxford scheme has been considered to be too high in some cases (27, 28); however, our data suggest that the Oxford scheme is useful for classifying *A. baumannii* IC II into meaningful groups.

Among *A. baumannii* non-IC II isolates, most of the other STs were novel in this study or previously identified in Japan (10, 29), suggesting the presence of domestic types of *A. baumannii* in addition to ST469.

Fluoroquinolone resistance was associated with not only *A. baumannii* IC II but also some specific STs in the *A. baumannii* non-IC II isolates, such as ST149 and ST246, according to the Pasteur scheme. Notably, all IC II isolates were resistant to ciprofloxacin. Fluoroquinolone resistance is considered an important factor in the success of the epidemic clone *A. baumannii* IC II. In regard to *A. baumannii* non-IC II, ST149 was first identified by our group as an MDR isolate (10). Later, ST149 was reported as a quinolone-resistant isolate in Japan (29), suggesting the possibility of a novel epidemic clonal lineage, according to Shrestha et al. (30). Also, ST246 was first identified as a quinolone-resistant isolate in Japan (29), and ST246 harboring $bla_{\text{OXA-23-like}}$ caused a nosocomial outbreak in a hospital (31). Further monitoring of the STs will be required, considering the possibility of novel epidemic clones. The presence of these quinolone-resistant isolates and likely quinolone-resistant STs resulted in the discrepancy between the MIC_{50}s and MIC_{90}s of *A. baumannii* non-IC II isolates for fluoroquinolones shown in Table 3.

In conclusion, the low prevalence of acquired carbapenemase genes and the presumptive domestic STs of *A. baumannii* IC II might contribute to the maintenance of low rates of antimicrobial resistance among *A. baumannii*. The contrast in the epidemiological features of these isolates in Japan compared to those in other countries suggests that our control measures against *Acinetobacter* spp. have achieved a measure of success. In the "National Action Plan on Antimicrobial Resistance," we set out to "enhance global multidisciplinary countermeasures against antimicrobial resistance" (http://www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/0000138942.pdf). Japan may therefore provide an international contribution toward antimicrobial resistance control measures around the world. In addition, our results suggest that fluoroquinolones may hold the key to overcoming epidemic clones.

MATERIALS AND METHODS

Collection of bacterial isolates and relevant information. A total of 86 National Hospital Organization hospitals in Japan participated in this study on a voluntary basis. The 86 hospitals are located in 42 prefectures among the 47 prefectures across Japan (see Fig. S2 in the supplemental material) and include both acute care and long-term care hospitals. Between October 2012 and March 2013, all clinical isolates identified as *Acinetobacter* spp. were collected, regardless of antimicrobial susceptibility. Duplicate isolates from multiple specimens from the same patient were included. Patient information (age, gender, day of isolation, specimen type, and history of hospitalization abroad) was listed in an information sheet at each participating hospital, and the information sheets were sent to our laboratory with the isolates.

Species level identification and discrimination of *A. baumannii* IC II. Species level identification was confirmed by partial *rpoB* sequence (nucleotide positions 2955 to 3775), with sequences compared with those of reference strains using the neighbor-joining method (32, 33). When the partial *rpoB* sequence yielded ≥97.5% identity with the closest reference sequence (Table 1), the isolate was identified as the same species as the reference strain. In this regard, the interspecies identities of the partial *rpoB* sequences of genomic sp. 15BJ (GenBank accession number EU477133) and genomic sp. 16 (GenBank accession number EU477135) and those of *Acinetobacter junnii* (GenBank accession number DQ207483) and *Acinetobacter grimontii* (GenBank accession number DQ207486) are 99.0% and 98.4%, respectively, and *A. grimontii* has been reported as a later heterotypic synonym of *A. junnii* (34). Therefore, we did not differentiate between these species. Isolates unidentifiable by *rpoB* sequencing were confirmed as species other than *Acinetobacter* spp. by 16S rRNA sequencing and the Vitek2 system (bioMérieux, Marcy l'Etoile, France) and excluded from further investigation. Discrimination of *A. baumannii* IC II was performed by pyrosequencing single-nucleotide polymorphism (SNP) analyses of $bla_{\text{OXA-51-like}}$ genes (35).

Antimicrobial susceptibility testing. MICs for 13 antimicrobial agents, including piperacillin-tazobactam, ampicillin-sulbactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin, levofloxacin, ciprofloxacin, minocycline, colistin, and polymyxin B, were determined by the broth microdilution method using dry plate Eiken (Eiken Chemical Co., Tokyo, Japan). Resistance was determined based on the recommended breakpoints of CLSI document M100-S25 (36). Multidrug resistance was based on the criteria of the Infectious Diseases Control Law in Japan and defined as satisfying all three of the following MIC criteria: (i) imipenem and/or meropenem MIC of ≥16 μg/ml for carbapenems, (ii) ciprofloxacin MIC of ≥4 μg/ml and/or levofloxacin MIC of ≥8 μg/ml for fluoroquinolones, and (iii) amikacin MIC of ≥32 μg/ml.

Detection of carbapenem resistance genes. Carbapenemase genes and the upstream insertion sequence (IS) were detected by PCR using primers specific for bla_{IMP}, bla_{TMB}, bla_{VIM}, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, and IS*Aba1*F (37–39). The primers for bla_{TMB} were designed in this study (forward, 5′-GTCATTTCGCTTTTGCCAACGAAG-3′, and reverse, 5′-CAGCGGTCGCCGTGATTGGCCTTG-3′). $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ were reported as naturally occurring oxacillinase genes in *A. baumannii* and *A. radioresistens*, respectively (40, 41). Therefore, in this study, the isolates in which IS*Aba1* was present upstream from $bla_{\text{OXA-51-like}}$ or $bla_{\text{OXA-23-like}}$ were regarded as isolates with carbapenem

resistance genes. The entire nucleotide sequences of $bla_{OXA-51-like}$ genes were determined by PCR and sequencing using OXA-69A and OXA-69B primers (40).

MLST and identification of β-lactamase genes from whole-genome sequencing data. A total of 110 nonduplicate *A. baumannii* isolates were analyzed using MLST. The 110 isolates were selected as follows. One representative isolate was selected from each hospital (70 isolates from 70 hospitals). In addition, isolates with antimicrobial susceptibility patterns or $bla_{OXA-51-like}$ sequences that were different from the first representative isolate were also selected from each hospital (40 isolates from 32 hospitals). Genomic DNA was subjected to whole-genome sequencing using the Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). *De novo* assembly of derived paired-end sequence reads was performed with CLC Genomics Workbench version 8.5.1 (CLC bio, Aarhus, Denmark). Sequence types (STs) in both the Oxford and Pasteur schemes were identified based on assembled contigs using MLST1.8 provided by the Center for Genomic Epidemiology (42). Novel allele sequences were reconfirmed by Sanger sequencing. Novel alleles and novel ST profiles were submitted to the MLST database (http://pubmlst.org/abaumannii/) for assignment.

β-Lactamase-encoding genes were detected based on assembled contigs using ResFinder 2.1 with a threshold of 90% identity (43). The nucleotide sequences of β-lactamase-encoding genes were confirmed by comparison with the reference sequence listed on the Lahey β-lactamase site (http://www.lahey.org/Studies/) and in NCBI's β-Lactamase Data Resources (https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/). The promoter sequences of bla_{ADC} were identified by reference to a previous report (16).

This study was reviewed and approved by the Ethical Review Board of the National Institute of Infectious Diseases (Tokyo, Japan).

Statistical analysis. All calculations were computed with SAS software.

Accession number(s). A novel variant of bla_{ADC} has been deposited in NCBI's β-Lactamase Data Resources under GenBank accession number KY997074 as $bla_{ADC-153}$.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02190-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We are grateful to National Hospital Organization and all of the participating hospitals for providing bacterial isolates, to Kumiko Kai and Yoshie Taki for technical assistance, and to Paul Higgins and curators of the *Acinetobacter baumannii* MLST database for their assistance in submitting our MLST data.

This work was supported by the Ministry of Health, Labor and Welfare of Japan (grant number H24-Shinkou-Ippan-010), the Research Program on Emerging and Re-emerging Infectious Diseases of the Japan Agency for Medical Research and Development (AMED), and a grant-in-aid for young scientists (B) from the Japan Society for the Promotion of Science (grant number 16K19208).

REFERENCES

1. Kamolvit W, Sidjabat HE, Paterson DL. 2015. Molecular epidemiology and mechanisms of carbapenem resistance of *Acinetobacter* spp. in Asia and Oceania. Microb Drug Resist 21:424–434. https://doi.org/10.1089/mdr.2014.0234.
2. Yong D, Shin HB, Kim YK, Cho J, Lee WG, Ha GY, Choi TY, Jeong SH, Lee K, Chong Y, KONSAR group. 2014. Increase in the prevalence of carbapenem-resistant *Acinetobacter* isolates and ampicillin-resistant non-typhoidal *Salmonella* species in Korea: a KONSAR study conducted in 2011. Infect Chemother 46:84–93. https://doi.org/10.3947/ic.2014.46.2.84.
3. Ruan Z, Chen U, Jiang Y, Zhou H, Zhou Z, Fu Y, Wang H, Wang Y, Yu Y. 2013. Wide distribution of CC92 carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* in multiple provinces of China. Int J Antimicrob Agents 42:322–328. https://doi.org/10.1016/j.ijantimicag.2013.06.019.
4. Zarrilli R, Pournaras S, Giannouli M, Tsakris A. 2013. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. Int J Antimicrob Agents 41:11–19. https://doi.org/10.1016/j.ijantimicag.2012.09.008.
5. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriquez-Valera F. 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 43:4382–4390. https://doi.org/10.1128/JCM.43.9.4382-4390.2005.
6. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 5:e10034. https://doi.org/10.1371/journal.pone.0010034.
7. Karah N, Sundsfjord A, Towner K, Samuelsen Ø. 2012. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. Drug Resist Updat 15:237–247. https://doi.org/10.1016/j.drup.2012.06.001.
8. Endo S, Yano H, Hirakata Y, Arai K, Kanamori H, Ogawa M, Shimojima M, Ishibashi N, Aoyagi T, Hatta M, Yamada M, Tokuda K, Kitagawa M, Kunisima H, Kaku M. 2012. Molecular epidemiology of carbapenem-non-susceptible *Acinetobacter baumannii* in Japan. J Antimicrob Chemother 67:1623–1626. https://doi.org/10.1093/jac/dks094.
9. Kouyama Y, Harada S, Ishii Y, Saga T, Yoshizumi A, Tateda K, Yamaguchi K. 2012. Molecular characterization of carbapenem-non-susceptible *Acinetobacter* spp. in Japan: predominance of multidrug-resistant *Acinetobacter baumannii* clonal complex 92 and IMP-type metallo-β-lactamase-producing non-*baumannii Acinetobacter* species. J Infect Chemother 18:522–528. https://doi.org/10.1007/s10156-012-0374-y.
10. Matsui M, Suzuki S, Yamane K, Suzuki M, Konda T, Arakawa Y, Shibayama K. 2014. Distribution of carbapenem resistance determinants among epidemic and non-epidemic types of *Acinetobacter* species in Japan. J Med Microbiol 63:870–877. https://doi.org/10.1099/jmm.0.069138-0.
11. Asai S, Umezawa K, Iwashita H, Ohshima T, Ohashi M, Sasaki M, Hayashi H, Matsui M, Shibayama K, Inokuchi S, Miyachi H. 2014. An outbreak of

bla$_{OXA-51-like}$- and *bla*$_{OXA-66}$-positive *Acinetobacter baumannii* ST208 in the emergency intensive care unit. J Med Microbiol 63:1517–1523. https://doi.org/10.1099/jmm.0.077503-0.
12. Alshahni MM, Asahara M, Kawakami S, Fujisaki R, Matsunaga N, Furukawa T, Teramoto T, Makimura K. 2015. Genotyping of *Acinetobacter baumannii* strains isolated at a Japanese hospital over five years using targeted next-generation sequencing. J Infect Chemother 21:512–515. https://doi.org/10.1016/j.jiac.2015.03.009.
13. Ushizawa H, Yahata Y, Endo T, Iwashita T, Misawa M, Sonobe M, Yamagishi T, Kamiya H, Nakashima K, Matsui T, Matsui M, Suzuki S, Shibayama K, Doi M, Irie F, Yamato S, Otomo Y, Oishi K. 2016. An epidemiological investigation of a nosocomial outbreak of multidrug-resistant *Acinetobacter baumannii* in a critical care center in Japan, 2011-2012. Jpn J Infect Dis 69:143–148. https://doi.org/10.7883/yoken.JJID.2015.049.
14. Ministry of Health, Labour and Welfare of Japan. 2017. Annual open report 2015 (all facilities), Japan Nosocomial Infections Surveillance (JANIS) [CLSI2012 version], Clinical Laboratory Division. https://janis.mhlw.go.jp/english/report/open_report/2015/4/1/ken_Open_Report_Eng_201500_clsi2012.pdf.
15. Ministry of Health, Labour and Welfare of Japan. 2016. Annual open report 2013 (all facilities), Japan Nosocomial Infections Surveillance (JANIS), Clinical Laboratory Division. https://janis.mhlw.go.jp/english/report/open_report/2013/3/1/ken_Open_Report_Eng_201300.pdf.
16. Héritier C, Poirel L, Nordmann P. 2006. Cephalosporinase overexpression resulting from insertion of IS*Aba1* in *Acinetobacter baumannii*. Clin Microbiol Infect 12:123–130. https://doi.org/10.1111/j.1469-0691.2005.01320.x.
17. Lim CJ, Cheng AC, Kennon J, Spelman D, Hale D, Melican G, Sidjabat HE, Peterson DL, Kong DC, Peleg AY. 2014. Prevalence of multidrug-resistant organisms and risk factors for carriage in long-term care facilities: a nested case-control study. J Antimicrob Chemother 69:1972–1980. https://doi.org/10.1093/jac/dku077.
18. Dijkshoorn L, Aucken H, Gerner-Smidt P, Janssen P, Kaufmann ME, Garaizar J, Ursing J, Pitt TL. 1996. Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. J Clin Microbiol 34:1519–1525.
19. van Dessel H, Dijkshoorn L, van der Reijden T, Bakker N, Paauw A, van den Broek P, Verhoef J, Brisse S. 2004. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. Res Microbiol 155:105–112. https://doi.org/10.1016/j.resmic.2003.10.003.
20. Towner KJ, Levi K, Vlassiadi M, ARPAC Steering Group. 2008. Genetic diversity of carbapenem-resistant isolates of *Acinetobacter baumannii* in Europe. Clin Microbiol Infect 14:161–167. https://doi.org/10.1111/j.1469-0691.2007.01911.x.
21. Livermore DM. 2012. Fourteen years in resistance. Int J Antimicrob Agents 39:283–294. https://doi.org/10.1016/j.ijantimicag.2011.12.012.
22. Zhou H, Zhang T, Yu D, Pi B, Yang Q, Zhou J, Hu S, Yu Y. 2011. Genomic analysis of the multidrug-resistant *Acinetobacter baumannii* strain MDR-ZJ06 widely spread in China. Antimicrob Agents Chemother 55:4506–4512. https://doi.org/10.1128/AAC.01134-10.
23. Hamidian M, Hall RM. 2013. IS*Aba1* targets a specific position upstream of the intrinsic *ampC* gene of *Acinetobacter baumannii* leading to cephalosporin resistance. J Antimicrob Chemother 68:2682–2683. https://doi.org/10.1093/jac/dkt233.
24. Tian GB, Adams-Haduch JM, Taracila M, Bonomo RA, Wang HN, Doi Y. 2011. Extended-spectrum AmpC cephalosporinase in *Acinetobacter baumannii*: ADC-56 confers resistance to cefepime. Antimicrob Agents Chemother 55:4922–4925. https://doi.org/10.1128/AAC.00704-11.
25. Snitkin ES, Zelazny AM, Montero CI, Stock F, Mijares L, NISC Comparative Sequence Program, Murray PR, Segre JA. 2011. Genome-wide recombination drives diversification of epidemic strains of *Acinetobacter baumannii*. Proc Natl Acad Sci U S A 108:13758–13763. https://doi.org/10.1073/pnas.1104404108.
26. Feng Y, Ruan Z, Shu J, Chen CL, Chiu CH. 2016. A glimpse into evolution and dissemination of multidrug-resistant *Acinetobacter baumannii* isolates in East Asia: a comparative genomics study. Sci Rep 6:24342. https://doi.org/10.1038/srep24342.
27. Hamouda A, Evans BA, Towner KJ, Amyes SG. 2010. Characterization of epidemiologically unrelated *Acinetobacter baumannii* isolates from four continents by use of multilocus sequence typing, pulsed-field gel electrophoresis, and sequence-based typing of *bla*$_{OXA-51-like}$ genes. J Clin Microbiol 48:2476–2483. https://doi.org/10.1128/JCM.02431-09.
28. Tomaschek F, Higgins PG, Stefanik D, Wisplinghoff H, Seifert H. 2016. Head-to-head comparison of two multi-locus sequence typing (MLST) schemes for characterization of *Acinetobacter baumannii* outbreak and sporadic isolates. PLoS One 11:e0153014. https://doi.org/10.1371/journal.pone.0153014.
29. Higuchi S, Shikata M, Chiba M, Hoshino K, Gotoh N. 2014. Characteristics of antibiotic resistance and sequence type of *Acinetobacter baumannii* clinical isolates in Japan and the antibacterial activity of DS-8587. J Infect Chemother 20:256–261. https://doi.org/10.1016/j.jiac.2013.12.001.
30. Shrestha S, Tada T, Miyoshi-Akiyama T, Ohara H, Shimada K, Satou K, Teruya K, Nakano K, Shiroma A, Sherchand JB, Rijal BP, Hirano T, Kirikae T, Pokhrel BM. 2015. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* isolates in a university hospital in Nepal reveals the emergence of a novel epidemic clonal lineage. Int J Antimicrob Agents 46:526–531. https://doi.org/10.1016/j.ijantimicag.2015.07.012.
31. Kubo Y, Komatsu M, Tanimoto E, Sugimoto K, Tanaka S, Migita S, Kondo Y, Yano S, Maehara K, Murata R, Nakamura A, Fujita T, Kawata Y. 2015. Spread of OXA-23-producing *Acinetobacter baumannii* ST2 and ST246 in a hospital in Japan. J Med Microbiol 64:739–744. https://doi.org/10.1099/jmm.0.000077.
32. La Scola B, Gundi VA, Khamis A, Raoult D. 2006. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. J Clin Microbiol 44:827–832. https://doi.org/10.1128/JCM.44.3.827-832.2006.
33. Nemec A, Krizova L, Maixnerova M, van der Reijden TJK, Deschaght P, Passet V, Vaneechoutte M, Brisse S, Dijkshoorn L. 2011. Genotypic and phenotypic characterization of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). Res Microbiol 162:393–404. https://doi.org/10.1016/j.resmic.2011.02.006.
34. Vaneechoutte M, De Baere T, Nemec A, Musílek M, van der Reijden TJ, Dijkshoorn L. 2008. Reclassification of *Acinetobacter grimontii* Carr *et al.* 2003 as a later synonym of *Acinetobacter junii* Bouvet and Grimont 1986. Int J Syst Evol Microbiol 58:937–940. https://doi.org/10.1099/ijs.0.65129-0.
35. Matsui M, Suzuki S, Suzuki M, Arakawa Y, Shibayama K. 2013. Rapid discrimination of *Acinetobacter baumannii* international clone II lineage by pyrosequencing SNP analyses by *bla*$_{OXA-51-like}$ genes. J Microbiol Methods 94:121–124. https://doi.org/10.1016/j.mimet.2013.05.014.
36. Clinical and Laboratory Standards Institute. 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25. CLSI, Wayne, PA.
37. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, Pignatari AC, Tufik S. 2007. Rapid detection and identification of metallo-β-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J Clin Microbiol 45:544–547. https://doi.org/10.1128/JCM.01728-06.
38. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM. 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 27:351–353. https://doi.org/10.1016/j.ijantimicag.2006.01.004.
39. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL. 2006. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett 258:72–77. https://doi.org/10.1111/j.1574-6968.2006.00195.x.
40. Héritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P. 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother 49:4174–4179. https://doi.org/10.1128/AAC.49.10.4174-4179.2005.
41. Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. 2008. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. Antimicrob Agents Chemother 52:1252–1256. https://doi.org/10.1128/AAC.01304-07.
42. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hansman H, Marvig RL, Jelsbak L, Sicheritzs-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11.
43. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.