表１　迅速
遺伝子抽出およびLAMP法を用いたノロウイルスの検出：結果

		LAMP G2		
		LAMP GII		
		陽性	陰性	計
IC(イムノキャッチ®)	陽性	9	1*	10
	陰性	2	23**	25
計		11	24	35

*長期入院患者でノロウイルス胃腸炎は臨床的に否定的
**1検体はLAMP GI陽性となった
※陽性検体（遺伝子抽出液）は10^3希釈までLAMP陽性となった

図１　ノロウイルスLAMP法の感度（1～10^5 copies/sample）

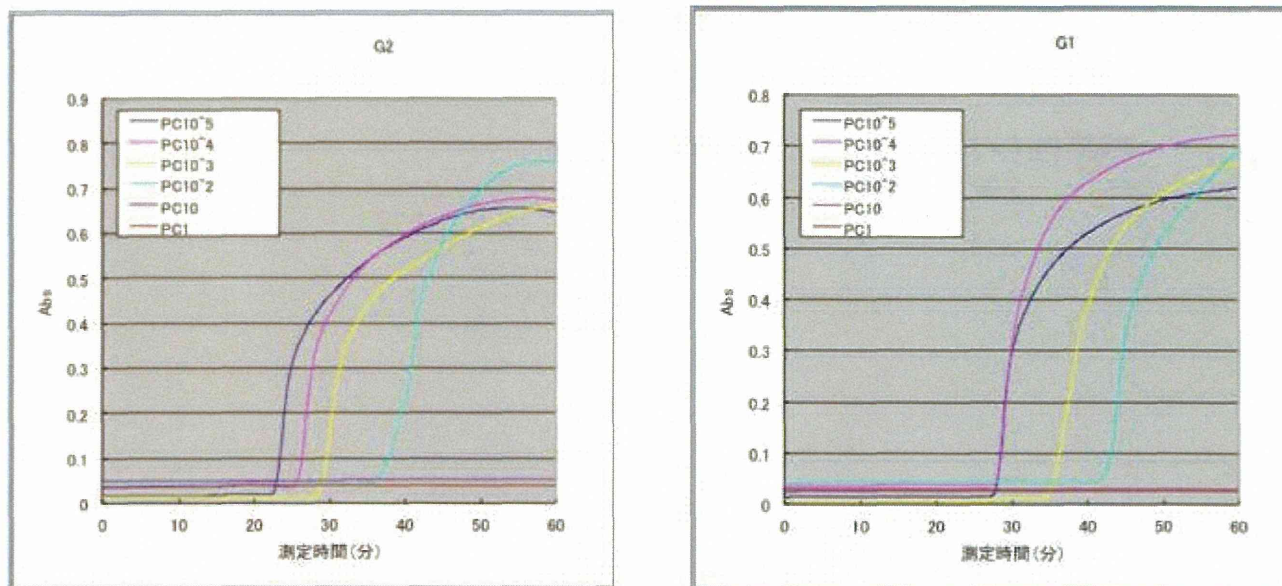
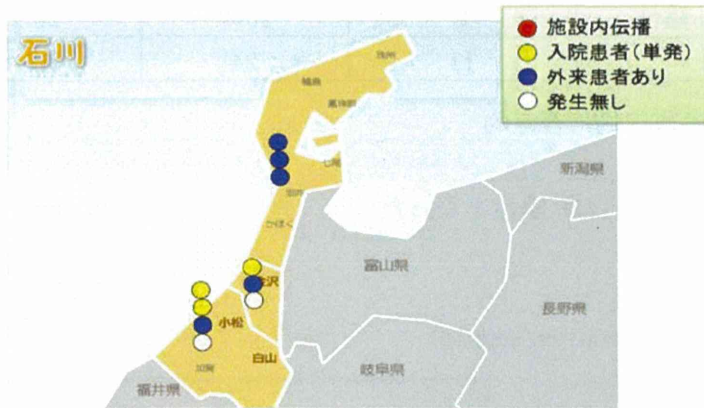
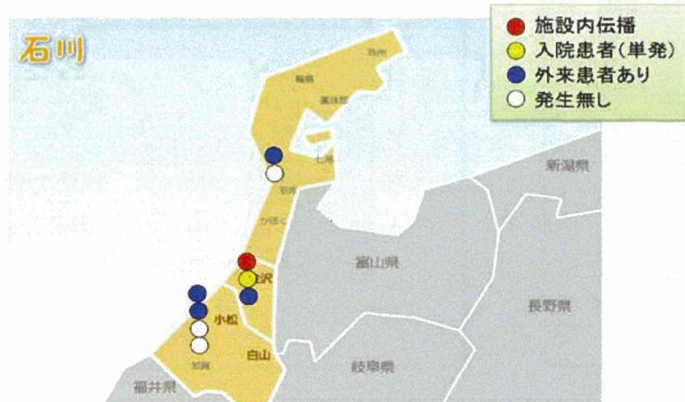


図2　石川県における感染性（ノロウイルス）胃腸炎流行状況

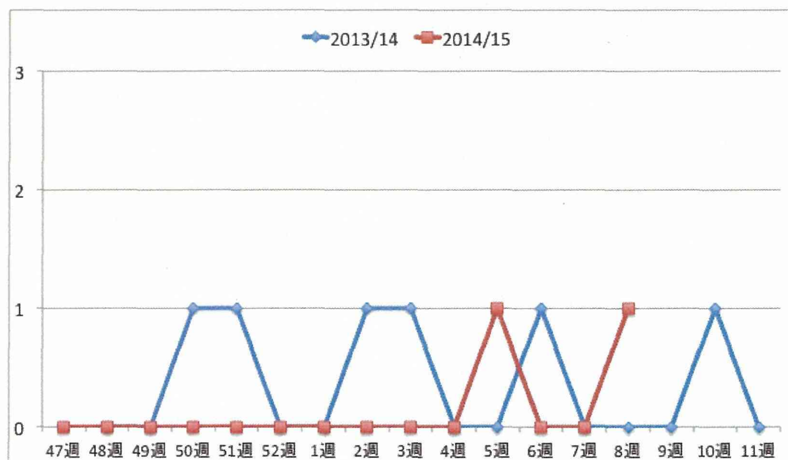
a）感染性（ノロウイルス）胃腸炎流行状況・2015 年第 2 週（2015/1/5～1/11）



b）感染性（ノロウイルス）胃腸炎流行状況・2015 年第 8 週（2015/2/16～2/22）



c）施設内伝播が発生した施設数（2013/14 シーズンおよび 2014/15 シーズン）



Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の 編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Suzuki M, Hosoba E, Matsui M, Arakawa Y.	New PCR-based open reading frame typing method for easy, rapid, and reliable identification of *Acinetobacter baumannii* international epidemic clones without performing multilocus sequence typing	J Clin Microbiol.	52	2925-2932	2014
Kitanaka H, Sasano M, Yokoyama S, Suzuki M, Jin W, Inayoshi M, Hori M, Wachino J, Kimura K, Yamada K, Arakawa Y.	Invasive infection caused by carbapenem-resistant *Acinetobacter soli,* Japan.	Emerg Infect Dis.	20	1574-1576	2014
渡邉珠代、丹羽 隆、土屋麻由美、外海友規、太田浩敏、村上啓雄	岐阜県内感染防止対策加算算定全病院での感染対策活動に関するサーベイランス結果報告	日本環境感染学会雑誌	30	44-55	2015
藤本 修平	感染対策サーベイランスにおける新しい取り組み-耐性菌時代の院内感染対策と 2DCM-web-	化学療法の領域	30	224(1108)-238(1122)	2014
藤本 修平	耐性菌と戦う臨床細菌検査の有効活用法 -電子化による感染対策の高精度化-	日本臨床微生物学会雑誌	25	1-9	2014
D. Minh Nguyen, Deguchi H, Ichikawa M, Saito T, And Fujimoto S	An Analysis on Risk of Influenza-Like Illness Infection in a Hospital Using Agent-Based Simulation.	Public Health Frontier	3	63-74	2014

Ⅳ．研究成果の刊行物・別刷・資料

JCM
Journals.ASM.org

New PCR-Based Open Reading Frame Typing Method for Easy, Rapid, and Reliable Identification of *Acinetobacter baumannii* International Epidemic Clones without Performing Multilocus Sequence Typing

Masahiro Suzuki,[a,b] Eriko Hosoba,[c] Mari Matsui,[d] Yoshichika Arakawa[b]

Laboratory of Bacteriology, Aichi Prefectural Institute of Public Health, Nagoya, Japan[a]; Department of Bacteriology, Nagoya University Graduate School of Medicine, Nagoya, Japan[b]; Department of Infectious Diseases and Immunology, Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan[c]; Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo, Japan[d]

Antimicrobial resistance issues have become a global health concern. The rapid identification of multidrug-resistant microbes, which depends on microbial genomic information, is essential for overcoming growing antimicrobial resistance challenges. However, genotyping methods, such as multilocus sequence typing (MLST), for identifying international epidemic clones of *Acinetobacter baumannii* are not easily performed as routine tests in ordinary clinical laboratories. In this study, we aimed to develop a novel genotyping method that can be performed in ordinary microbiology laboratories. Several open reading frames (ORFs) specific to certain bacterial genetic lineages or species, together with their unique distribution patterns on the chromosomes showing a good correlation with the results of MLST, were selected in *A. baumannii* and other *Acinetobacter* spp. by comparing their genomic data. The distribution patterns of the ORFs were visualized by agarose gel electrophoresis after multiplex PCR amplification and digitized. *A. baumannii* sequence types (STs) corresponding to international clones I and II were successfully discriminated from other STs and *Acinetobacter* species by detecting the distribution patterns of their ORFs using the multiplex PCR developed here. Since bacterial STs can be easily expressed as digitized numeric data with plus (+) expressed as 1 and minus (−) expressed as 0, the results of the method can be easily compared with those obtained by different tests or laboratories. This PCR-based ORF typing (POT) method can easily and rapidly identify international epidemic clones of *A. baumannii* and differentiate this microbe from other *Acinetobacter* spp. Since this POT method is easy enough to be performed even in ordinary clinical laboratories, it would also contribute to daily infection control measures and surveillance.

Antimicrobial resistance has become a global health concern. The World Health Organization has stated that weak or absent antimicrobial resistance surveillance and monitoring systems accelerate the emergence and spread of antimicrobial resistance (see http://www.who.int/drugresistance/documents/surveillancereport/en/). One of the weak points of current antimicrobial resistance surveillance and monitoring systems is the absence of genetic data for the bacterial isolates. Microbial genotyping is indispensable for a precise understanding of the genetic lineages of clinical isolates that cause nosocomial outbreaks (1).

Acinetobacter baumannii is one of the major multidrug-resistant nosocomial pathogens. In particular, *A. baumannii* epidemic clones, the so-called international clones I and II, usually show multidrug resistance, and only limited antimicrobials are efficacious for treating infections caused by them (2). On the other hand, *A. baumannii* clinical isolates other than the epidemic international clones are still susceptible to several antimicrobials. The performance of appropriate precautions that target the epidemic clones is indispensable for blocking their further nosocomial transmission. Therefore, it has become very important to rapidly discriminate the *A. baumannii* epidemic clones from other nonepidemic *A. baumannii* lineages and non-*baumannii Acinetobacter* species, such as *Acinetobacter nosocomialis* and *Acinetobacter pittii*. In this regard, multilocus sequence typing (MLST) is indeed useful for the exact identification of the epidemic clones, which are classified into several sequence types (STs), such as ST1 and ST2, by MLST performed at the Institut Pasteur. ST1 and ST2 are also assigned to clonal complex 109 (CC109) and CC92, respectively, by the MLST of Bartual et al. (19) as reported by Zarrilli et al. (3) However, MLST of *Acinetobacter* clinical isolates can be performed only in limited cases of nosocomial outbreaks even in Japan, and this results in a delay in the ability to alert for the emergence and spread of epidemic clones in hospital settings. Early identification of epidemic clones of *A. baumannii* is very important especially in the areas where they have not been prevalent yet. Therefore, the establishment of easy and rapid genotyping methods has been much awaited.

The construction of new analytical methods that make it easy to obtain genetic information of clinical isolates in ordinary clinical laboratories is desired. We consider that the most convenient way to simplify microbial genotyping would be to display the re-

Received 12 April 2014 **Returned for modification** 6 May 2014
Accepted 28 May 2014
Published ahead of print 4 June 2014
Editor: R. Patel
Address correspondence to Yoshichika Arakawa, yarakawa@med.nagoya-u.ac.jp.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.01064-14.
Copyright © 2014, American Society for Microbiology. All Rights Reserved.
doi:10.1128/JCM.01064-14

TABLE 1 ORF candidates relating to small genomic islets (SGI) and their distributions among the *A. baumannii* genomes[a]

SGI ORF candidate[b]	ORF corresponding to the SGI ORF candidates among the indicated *A. baumannii* strain (Pasteur sequence type, GenBank accession no.)[c]			
	AB0057 (ST1, CP001182)	ACICU (ST2, CP000863)	ATCC17978 (ST77slv[d], CP000521)	SDF (ST17, CU468230)
ACICU_00180*		ACICU_00180 (100)	A1S_0157 (99)	
AB57_0388*	AB57_0388 (100)			
AB57_0454*	AB57_0454 (100)		Between A1S_0376[e] and A1S_0378 (99)	Between ABSDF3133 and ABSDF3134 (98)
AB57_0526	AB57_0526 (100)			Between ABSDF 3067 and ABSDF3068 (99)
ACICU_00563*		ACICU_00563 (100)		ABSDF2963 (100)
AB57_0815*#	AB57_0815 (100)		A1S_0767 (99)	
AB57_1987	AB57_1987 (100)	ACICU_01794 (99)		ABSDF1977 (98)
AB57_2085	AB57_2085 (100)		Between A1S_1754 and A1S_1755 (96)	
ACICU_01870*#		ACICU_01870 (100)	A1S_1782 (95)	ABSDF1960 (96)
ACICU_02042 *#		ACICU_02042 (100)	A1S_1927 (99)	
AB57_2484*#	AB57_2484 (100)	ACICU_02351 (98)		
ACICU_02468		ACICU_02468 (100)	A1S_2266 (99)	ABSDF1260 (97)
ACICU_02520*	Between AB57_2751 and AB57_2752 (99)	ACICU_02520 (100)	Between A1S_2318 and A1S_2319 (99)	
ACICU_02597		ACICU_02597 (100)		
AB57_2930	AB57_2930 (100)	ACICU_02697 (99)	A1S_2485 (98)	
ACICU_02886	AB57_3056 (96)	ACICU_02886 (100)	A1S_2641 (97)	
ACICU_02966*#		ACICU_02966 (100)	Between A1S_2707 and A1S_2708 (98)	ABSDF0764 (98)
AB57_3308*#	AB57_3308 (100)			
ACICU_03137*#		ACICU_03137 (100)		ABSDF0546 (98)
AB57_3624*	AB57_3624 (100)	ACICU_03369 (99)	Between A1S_3168 and A1S_3169 (99)	
ACICU_03379*		ACICU_03379 (100)		ABSDF0314 (95)
ACICU_03418*		ACICU_03418 (100)	Between A1S_3220 and A1S_3221 (99)	ABSDF0260 (100)
A1S_3257			A1S_3257 (100)	ABSDF3356 (98)
ACICU_03581*		ACICU_03581 (100)	A1S_3381 (99)	ABSDF3529 (99)

[a] SGI, small genomic islet.
[b] ORFs showing the same distribution patterns among clonal isolates are indicated by an asterisk (*), and ORFs selected for PCR-based ORF typing are indicated by a hash tag (#).
[c] Numbers in parentheses are the percent sequence similarities over representative SGI ORFs listed in the first column.
[d] slv, single locus variant.
[e] When nucleotide sequences corresponding to an SGI ORF candidate are found in the genomes of some *A. baumannii* strains but they have not been named in the annotated genome data, the ORFs flanking the nucleotide sequence similar to the SGI ORF candidate are provided.

sults as "1" for "+" and "0" for "−", the so-called binary typing, which does not require any further handling of specimens, such as performing nucleotide sequence analyses, counting the allelic repeats, or analyzing complicated restriction enzyme digestion patterns. We previously succeeded in developing a genotyping method for *Staphylococcus aureus* by detecting the distribution patterns of its open reading frames (ORFs) using multiplex PCR that can be replaced with pulsed-field gel electrophoresis (PFGE) (4, 5). In the genotyping of *S. aureus*, the distribution patterns of small genomic islets (SGIs) showed good correlations with the clonal complex (CC) types obtained by MLST. SGIs consist of one to several ORFs (6). Therefore, we hypothesized that the CCs of *A. baumannii* and the *Acinetobacter calcoaceticus-A. baumannii* complex might be also estimated or predicted by detecting the distribution patterns of SGIs specific to each *Acinetobacter* species. The distribution patterns of SGIs can easily be visualized by agarose gel electrophoresis after multiplex PCR; therefore, clone typing of isolates can be performed in many ordinary microbiology laboratories in which equipment for only PCR and agarose gel electrophoresis is available.

In the present study, therefore, we developed a new multiplex PCR-based method for easy, rapid, and reliable discrimination of the clonal complexes of *A. baumannii*, especially the epidemic clones.

MATERIALS AND METHODS

Bacterial isolates. A total of 226 *Acinetobacter* clinical isolates collected from patients in Japan between 2001 and 2012, including 79 *A. baumannii*, 20 *A. pittii*, 77 *A. nosocomialis*, 15 *Acinetobacter* species close to *A. nosocomialis*, 24 *A. radioresistens*, three *A. ursingii*, three *A. bereziniae*, two *A. soli*, one *A. junii*, one *Acinetobacter* genomic species 13BJ, and one *Acinetobacter* genomic species 14BJ, were used. These isolates were identified using their *rpoB* gene sequence (7). Two American Type Culture Collection (ATCC) reference strains available in our laboratory (*A. baumannii* strains ATCC 19606 and ATCC BAA-1605) were also used. The 79 *A. baumannii* clinical isolates and two ATCC reference strains were analyzed by MLST. The isolates were cultured overnight on soy bean casein digest agar plates at 37°C, and chromosomal DNA was extracted with the QuickGene SP kit DNA tissue (SP-DT) (Wako Pure Chemical Industries, Osaka, Japan). MLST analysis was performed according to the protocol of the Institut Pasteur MLST databases (http://www.pasteur.fr/mlst). The clustering of related STs, which was defined as a CC, was determined with the aid of the eBURST program (http://eburst.mlst.net/).

For *Acinetobacter*-specific ORF screening, *Pseudomonas aeruginosa* strain JCM 14847, *Pseudomonas putida* strain JCM 13063, *Pseudomonas fluorescens* strain JCM 5963, *Pseudomonas stutzeri* strain JCM 5965, *Pseu-*

TABLE 2 Species-specific ORF candidates

ORF[a]	Contig no., nucleotide position[b]	No. found/no. tested for *Acinetobacter* organism: *A. baumannii*	*A. pittii*	*A. nosocomialis*	*Acinetobacter* species close to *A. nosocomialis*	Other *Acinetobacter* species
pittii-1	9, 490–1095	0/8	0/4	0/8	0/4	NT[c]
pittii-2	19, 296023–297078	0/8	3/4	0/8	0/4	NT
pittii-3	21, 113290–112808	5/8	2/4	0/8	0/4	NT
pittii-4	23, 336772–337306	5/8	3/4	0/8	0/4	NT
pittii-5	23, 435468–436845	0/8	2/4	1/8	4/4	NT
pittii-6*	25, 270084–271118	0/81	19/20	0/77	0/15	0/35
pittii-7	26, 56553–57056	0/8	2/4	0/8	0/4	NT
pittii-8	31, 96908–97756	0/8	3/4	0/8	1/4	NT
pittii-9	31, 288435–286909	0/8	0/4	0/8	1/4	NT
nosocomialis-1	6, 48398–49115	0/8	0/4	7/8	0/4	NT
nosocomialis-2	12, 64890–63445	8/8	0/4	8/8	1/4	NT
nosocomialis-3*	90, 13009–11208	0/81	0/20	76/77	0/15	0/35
Asp-1*	12, 41330–40363	0/81	0/20	0/77	15/15	0/35
Asp-2	15, 52010–54341	0/8	0/4	0/8	0/4	NT
Asp-3	15, 207828–209480	0/8	0/4	0/8	0/4	NT
Asp-4	16, 155456–156670	0/8	0/4	0/8	1/4	NT
Asp-5	21, 98536–99348	0/8	0/4	0/8	0/4	NT

[a] The ORFs selected for species identification are indicated by an asterisk.
[b] ORFs were selected from *A. pittii* D499 (pittii-1 to -9 [GenBank accession no. AGFH00000000]), *A. nosocomialis* NCTC 8102 (nosocomialis-1 to -3 [GenBank accession no. AIEJ00000000]), and *Acinetobacter* species GG2 (Asp-1 to -5 [GenBank accession no. ALOW00000000]).
[c] NT, not tested.

domonas nitroreducens strain JCM 2782, *Azotobacter vinelandii* strain JCM 21475, and *Brevundimonas diminuta* strain JCM 2788 were used as a negative control. These strains were provided by the Japan Collection of Microorganisms, Riken BioResource Center (BRC), which participates in the National BioResource Project of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT). *Achromobacter xylosoxidans* and *Escherichia coli* clinical isolates were also used as a negative control.

Searching small genomic islets from *A. baumannii* whole-genome sequences. The whole-genome DNA sequences of six *A. baumannii* strains, AB0057 (DDBJ/EMBL/GenBank accession no. CP001182), AB307-0294 (GenBank accession no. CP001172), AYE (GenBank accession no. CU459141), ACICU (GenBank accession no. CP000863), ATCC 17978 (GenBank accession no. CP000521), and SDF (GenBank accession no. CU468230), were obtained from an Internet database (PubMed [http://www.ncbi.nlm.nih.gov/sites/entrez]) and compared to each other using the MBGD website (http://mbgd.genome.ad.jp/) and blast+ (National Center for Biotechnology Information, Bethesda, MD, USA), with a tabular output option, and homologues were visualized by ACT (8). The nonconserved regions among the six strains were identified and selected as potential SGIs. Among the selected SGIs, those containing single to several ORFs without the presence of structures resembling insertion sequences, transposases, or integrases were selected for the determination of CCs. Nonconserved regions with larger structures, such as transposons, prophages, and antimicrobial resistance islands, were excluded. The distribution patterns of the SGI candidates (Table 1) were investigated by PCR using 42 *A. baumannii* representative clinical isolates and two ATCC strains.

Searching species-specific ORFs from whole-genome sequences. The whole-genome DNA sequences of four *A. pittii* (strains D499 [GenBank accession no. AGFH00000000], DSM 9306 [AIEF00000000], DSM 21653 [AIEK00000000], and SH024 [NZ_ADCH00000000]), two *A. nosocomialis* (strains NCTC 8102 [AIEJ00000000] and RUH2624 [NZ_ACQF00000000]), three *Acinetobacter calcoaceticus* (strains PHEA-2 [CP002177], DSM 30006 [NZ_APQI00000000], and RUH2202 [NZ_ACPK00000000]), one *A. bereziniae* (strain LMG 1003 [NZ_AIEI00000000]), one *Acinetobacter haemolyticus* (strain ATCC 19194 [NZ_ADMT00000000]), one *Acinetobacter johnsonii* (strain SH046 [NZ_ACPL00000000]), one *A. junii* (strain SH025 [NZ_ACPM00000000]), three *Acinetobacter lwoffii* (strains NCTC 5866 [AIEL00000000], SH145 [NZ_ACPN00000000], and WJ10621 [NZ_AFQY00000000]), one *Acinetobacter parvus* (strain DSM 16617 [AIEB00000000]), four *A. radioresistens* (strains DSM 6976 [AIDZ00000000], SH164 [NZ_ACPO00000000], SK82 [NZ_ACVR00000000], and WC-A-157 [ALIR00000000]), one *A. ursingii* (strain DSM 16037 [AIEA00000000]), one *Acinetobacter venetianus* (strain RAG-1 [AKIQ00000000]), and four *Acinetobacter* species that have not been given scientific names (GG2 [ALOW00000000], ATCC 27244 [ABYN00000000], HA [NZ_AJXD00000000], and NBRC 100985 [NZ_BAEB00000000]) and the genomic data of six *A. baumannii* isolates mentioned in the section above were obtained from PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez) and compared using blast+. ORFs showing high percent sequence similarities among all *Acinetobacter* species used in the method were selected as candidates of markers specific to *Acinetobacter* species. ORFs found only in *A. pittii*, *A. nosocomialis*, or *Acinetobacter* species close to *A. nosocomialis* (corresponding to strain GG2) genomes were selected as candidates of species-specific ORFs (Table 2). The presence of species-specific ORFs was screened by PCR for representative isolates, including eight *A. baumannii*, four *A. pittii*, eight *A. nosocomialis*, and four *Acinetobacter* species close to *A. nosocomialis*.

Multiplex PCR detection of selected ORFs to identify international clones. To maximize the discriminatory power and reliability of the identification of international clones, seven ORFs in separate SGIs were selected for multiplex PCR detection in order to identify international clones with a minimum difference of two bands in the detected ORF ladder patterns of other CCs among the isolates used in this study (Table 1).

For easy execution, the selected ORFs were detected by multiplex PCR, which we call PCR-based ORF typing (POT). The primer pairs for detecting ORFs in the seven SGIs, *Acinetobacter*-specific ORF, bla_{OXA-51} (9), and three species-specific ORFs (Table 2) were designed for multiplex PCR detection (Table 3). As ORFs in SGIs were found among *A. pittii*, *A. nosocomialis*, and *Acinetobacter* spp. close to *A. nosocomialis*, as well as for *A. baumannii*, the primers were designed to adapt universally to those *Acinetobacter* species.

Template DNAs for multiplex PCR were prepared by suspending bac-

TABLE 3 Primers finally selected for multiplex PCR

Target ORF	Primer direction	Sequence (5′ to 3′)[a]	Final concn (μM)	Amplicon size (bp)
atpA	Forward	CTGAACCTAGAACAGGATTCAGT	0.2	553
	Reverse	TCACGGAAGTATTCACCCAT	0.2	
OXA-51	Forward	GCTTCGACCTTCAAAATGCT	0.2	465
	Reverse	TCCAGTTAACCAGCCTACTTGT	0.2	
pittii-6	Forward	CATGTAGGTAGTCAAATGCCTG	0.2	401
	Reverse	CCGCTGGTGATGCTTTATTC	0.2	
nosocomialis-3	Forward	GTGATCGTGGTGATAGCTGG	0.2	362
	Reverse	GTAAGTTCCTGTTGCAACTCC	0.2	
Asp-1	Forward	GGATCTTTAACTCCATGGCTC	0.2	321
	Reverse	GATTATCrTGTAATAACCACGCAC	0.2	
AB57_2484	Forward	TATGTACAAAGCCAACCGGA	0.2	271
	Reverse	GAATTTGAGCdGAAGCCATTA	0.2	
ACICU_02042	Forward 1	CCGCGTCTTTCATAATAAGCAA	0.1	234
	Forward 2	CCACGTCTCTCATAATAAGCAA	0.1	
	Reverse 1	TGGAGAAATAGATTCTTCAAAAGTTGT	0.1	
	Reverse 2	TGCAGAAATAGATTCTTCAmAAATTGT	0.1	
ACICU_02966	Forward	ACCGTAyCCCTTTTTAATAAGTTCA	0.2	189
	Reverse	GGGCAAACTTATCATAGTTATATCGAC	0.2	
ACICU_01870	Forward	GCTGCAACCCAACCAATwA	0.2	151
	Reverse	AATTGGCTTCGhTGGATATTTATG	0.2	
AB57_3308	Forward	GCAACAGTTTCAAAATTAAATGG	0.2	122
	Reverse 1	ACTGTTTGTATGGGTATTGCAG	0.1	
	Reverse 2	ACTGTTTGTATAGGCATTGCAG	0.1	
ACICU_03137	Forward	CCyGCACTGCTCTACGATAATG	0.2	102
	Reverse	TTGyTCATAATGAAAAGCCGCA	0.2	
AB57_0815	Forward	CTTTAGAmGAGGCACGTTGGTTTG	0.2	81
	Reverse	TTTCACAyGGCTCACCGT	0.2	

[a] Mixed nucleotide residues were described according to a standard code (r, A/G; d, A/G/T; m, A/C; y, C/T; w, A/T; h, A/C/T).

terial cells in 100 μl of Tris-EDTA buffer (pH 8.0) at a turbidity of McFarland standard 0.5 to 2, heating at 100°C for 10 min, and centrifugation at 14,000 rpm (approximately 15,000 × *g*) for 1 min. Next, POT was carried out with the four thermal cyclers, i.e., GeneAmp PCR system 9700 (Life Technologies Japan, Tokyo, Japan), Applied Biosystems 2720 (Life Technologies Japan), GeneAtlas 322 (Astec, Fukuoka, Japan), and the Thermal Cycler Dice Gradient (TaKaRa Bio, Otsu, Japan), to validate their compatibility on the same platforms. The primer mixture was prepared by mixing all primers listed in Table 3 to 100× the final concentration. PCR was carried out in a 20-μl mixture containing 2 μl of the heat extract template DNA, prepared as described above, PCR buffer (3 mM Mg^{2+}), 0.2 mM each deoxynucleoside triphosphate (dNTP), 0.8 units of FastStart *Taq* DNA polymerase (Roche Diagnostics, Mannheim, Germany), and 0.2 μl of the primer mixture. The sequences and final concentrations of the primers are shown in Table 3. All four DNA preparations extracted from clinical *A. baumannii* (POT 122 [ST2] in Table 4), *A. pittii* (POT 78 in Table 4), *A. nosocomialis* (POT 105 in Table 4), and *Acinetobacter* spp. close to *A. nosocomialis* (POT 105 in Table 4) were mixed and used as the DNA template for both the positive control and the ladder marker in PCR. The thermal conditions were as follows: 95°C for 10 min, 30 cycles at 95°C for 30 s, 60°C for 30 s, 72°C for 2 min, and then at 4°C for several hours before agarose gel electrophoresis. The PCR products (2 μl) were electrophoresed on 4% agarose gels (NuSieve 3:1; Lonza, Basel, Switzerland) in 0.5× Tris-borate-EDTA (TBE) at 100 V for 50 min; the bands were then visualized with ethidium bromide.

After PCR, the seven SGI ORFs were scored in the order of their PCR amplicon size, with either "1" for "+"or "0" for "−" (binary code), depending on the presence or absence, respectively, of the band of amplicon DNA. These scores were then converted to decimal numbers, i.e., POT numbers. The results of each SGI binary were multiplied by 2^n ($n = 6 - 0$) and added. For example, the binary code of ST2 (1111010) was converted to 122 as follows: $1 \times 64 + 1 \times 32 + 1 \times 16 + 1 \times 8 + 0 \times 4 + 1 \times 2 + 0 \times 1$. Furthermore, each POT number was represented by a numerical label, ranging from a POT of 0 (0000000) to a POT of 127 (1111111).

RESULTS

A total of 24 SGI candidates (Table 1) were selected by comparing the whole-genome data of the six *A. baumannii* strains (AB0057, AB307-0294, AYE, ACICU, ATCC 17978, and SDF). Highly conserved (95 to 100% sequence similarity) DNA sequences were found in most of the SGIs of the six *A. baumannii* strains checked, and nucleotide sequence identities were also observed among