TABLE 4 Correlations between MLST and ORFs used for POT

POT no.	ST (CC or allele profile)	No. of isolates	Presence or absence of ORFs for the POT analysis by group:											
			ORFs for identification of bacterial species[a]					ORFs for calculation of POT no.[b]						
			atpA	OXA-51	pittii-6	nosocomialis-3	Asp-1	AB57_2484	ACICU_02042	ACICU_02966	ACICU_01870	AB57_3308	ACICU_03137	AB57_0815
A. baumannii														
122	ST2 (CC2)	27	+	+	−	−	−	+	+	+	+	−	+	−
69	ST1 (CC1)	3	+	+	−	−	−	+	−	−	−	+	−	+
0	ST235 (CC33)	1	+	+	−	−	−	−	−	−	−	−	−	−
8	ST33 (CC33)	18	+	+	−	−	−	−	−	−	+	−	−	−
8	ST148	1	+	+	−	−	−	−	−	−	+	−	−	−
32	CC33 (3-5-7-1-12-1-2)	2	+	+	−	−	−	−	+	−	−	−	−	−
32	ST239 (CC216)	1	+	+	−	−	−	−	+	−	−	−	−	−
44	New (1-4-2-1-42-1-4)	1	+	+	−	−	−	−	+	−	+	+	−	−
44	ST40	1	+	+	−	−	−	−	+	−	+	+	−	−
10	ST52	1	+	+	−	−	−	−	−	−	+	−	+	−
40	CC10 (1-3-2-1-4-1-4)	1	+	+	−	−	−	−	+	−	+	−	−	−
41	ST49	1	+	+	−	−	−	−	+	−	+	−	−	+
56	ST142	1	+	+	−	−	−	−	+	+	+	−	−	−
72	ST152	4	+	+	−	−	−	+	−	−	+	−	−	−
73	ST212	1	+	+	−	−	−	+	−	−	+	−	−	+
92	ST246	1	+	+	−	−	−	+	−	+	+	+	−	−
96	CC216 (3-4-2-2-7-2-2)	1	+	+	−	−	−	+	+	−	−	−	−	−
104	ST34 (CC34)	9	+	+	−	−	−	+	+	−	+	−	−	−
104	New (27-2v-2v-1-9-2-5)	3	+	+	−	−	−	+	+	−	+	−	−	−
104	ST145 (CC216)	1	+	+	−	−	−	+	+	−	+	−	−	−
106	CC109 (3-4-2-2-9-1-5)	1	+	+	−	−	−	+	+	−	+	−	+	−
108	ST133	1	+	+	−	−	−	+	+	−	+	+	−	−
A. pittii														
66	NA[c]	9	+	−	+	−	−	+	−	−	−	−	+	−
70	NA	1	+	−	+	−	−	+	−	−	−	+	+	−
74	NA	2	+	−	+	−	−	+	−	−	+	−	+	−
74	NA	1	+	−	−	−	−	+	−	−	+	−	+	−
76	NA	1	+	−	+	−	−	+	−	−	+	+	−	−
78	NA	6	+	−	+	−	−	+	−	−	+	+	+	−
A. nosocomialis														
97	NA	1	+	−	−	+	−	+	+	−	−	−	−	+
104	NA	26	+	−	−	+	−	+	+	−	+	−	−	−
105	NA	49	+	−	−	+	−	+	+	−	+	−	−	+
105	NA	1	+	−	−	−	−	+	+	−	+	−	−	+
Acinetobacter species close to *A. nosocomialis*														
41	NA	7	+	−	−	−	+	−	+	−	+	−	−	+
105	NA	4	+	−	−	−	+	+	+	−	+	−	−	+
109	NA	2	+	−	−	−	+	+	+	−	+	+	−	+
125	NA	2	+	−	−	−	+	+	+	+	+	+	−	+

[a] Five ORFs, i.e., *atpA*, OXA-51, pittii-6, nosocomialis-3, and Asp-1, were used in the identification of each *Acinetobacter* isolate.

[b] Seven ORFs, i.e., AB57_2484, ACICU_02042, ACICU_02966, ACICU_01870, AB57_3308, ACICU_03137, and AB57_0815, were used for calculation of the POT number of each isolate.

[c] NA, not adopted.

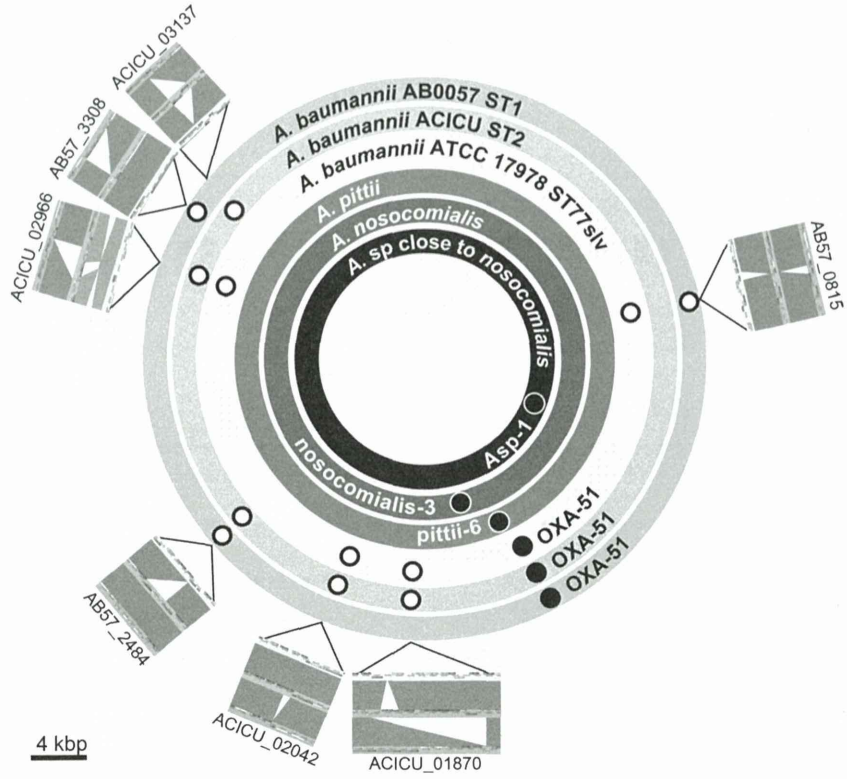

FIG 1 Distributions of small genomic islets and species-specific markers on genomes of representative strains. Open circles indicate SGIs, and closed circles indicate species-specific genetic markers. Locations of *atpA* selected as the universal marker of *Acinetobacter* species are not provided. The genome sequence data of *A. pittii*, *A. nosocomialis*, and *Acinetobacter* species close to *A. nosocomialis* are still draft data at present. The positions of each marker were decided by mapping the contigs containing markers on the basis of the genome of *A. baumannii* ACICU. Genome comparison maps illustrated by Artemis Comparison Tool (http://www.sanger.ac.uk/resources/software/act/) are arranged outside the circles, indicating genomes. The outer, second outer, and third outer rings indicate the genomes of *A. baumannii* AB0057, ACICU, and ATCC 17978, respectively. The dark-gray drawings in genome comparison maps indicate matches between the sequences, and the silver drawing in ACICU_02966 indicates an inversion match. The calibration bar indicates 4 kbp on all seven genome comparison maps. A large color version of this figure with high resolution is available in Fig. S1 in the supplemental material.

other *A. calcoaceticus-A. baumannii* complex strains, although the sequence similarities ranged from 80% to 97%.

Strains belonging to the same CC showed very similar distribution patterns in 16 of the 24 SGI ORFs (Table 1). The CC identities of strains belonging to the major CC were identified based on the distribution patterns of those 16 SGI ORFs. However, the distribution patterns of the 16 SGI ORFs in strains of Pasteur ST145 could not be distinguished from those of a novel ST (allele numbers 27-2v-2v-1-9-2-5).

SGI ORFs adopted for multiplex PCR amplification (Table 1) were selected according to the following principles. First, ORFs that were found exclusively in international clones I or II but were absent among most clones other than the epidemic ones were selected. The ORFs specific to international clone I were AB57_0815 and AB57_3308, and those specific to international clone II were ACICU_02966 and ACICU_03137. Thus, the international clones can be identified and distinguished by detecting the four ORFs AB57_0815, AB57_3308, ACICU_02966, and ACICU_03137. Second, three ORFs (AB57_2484, ACICU_02042, and ACICU_01870) were selected, because their distribution patterns in the epidemic clones were divergent in at least two alleles compared with those found in other nonepidemic lineages. These 3 ORFs were finally adopted to improve the discriminatory power of the test. The distribution patterns of the 7 SGI ORFs among the *A. baumannii* clones and *Acinetobacter* species are shown in Fig. 1 and Table 4; see also Fig. S1 in the supplemental material.

Two ORFs (*atpA* and *sucD*) were selected as the candidates for universal markers of *Acinetobacter* species by comparing whole-genome sequences. These alleles were found among all *Acinetobacter* species in the BLAST databases (whole-genome sequencing [WGS] database as of 8 May 2013) showing higher percent sequence similarities (>80%) than other orthologs. *atpA* was finally chosen as the marker specific to *Acinetobacter* species and the marker for the internal control of PCR amplification.

Genetic markers specific to *A. pittii*, *A. nosocomialis*, and *Acinetobacter* spp. close to *A. nosocomialis* were also searched for their whole-genome data. ORFs designated pittii-6, nosocomialis-3, and Asp-1 (Table 2) were finally chosen as markers specific to *A. pittii*, *A. nosocomialis*, and *Acinetobacter* spp. close to *A. nosocomialis*, respectively (Table 2). The pittii-6 marker was chosen from nine candidate markers, and its specificity and sensitivity were 100% and 95%, respectively (Table 2). As pittii-6 was also found in *A. calcoaceticus* genome sequences, with an 83% sequence similarity in its nucleotide sequence level, primers were designed for the specific detection of *A. pittii*. The nosocomialis-3 marker was chosen from 3 candidates, and its specificity and sensitivity were 100% and 99%, respectively. The Asp-1 marker was chosen from

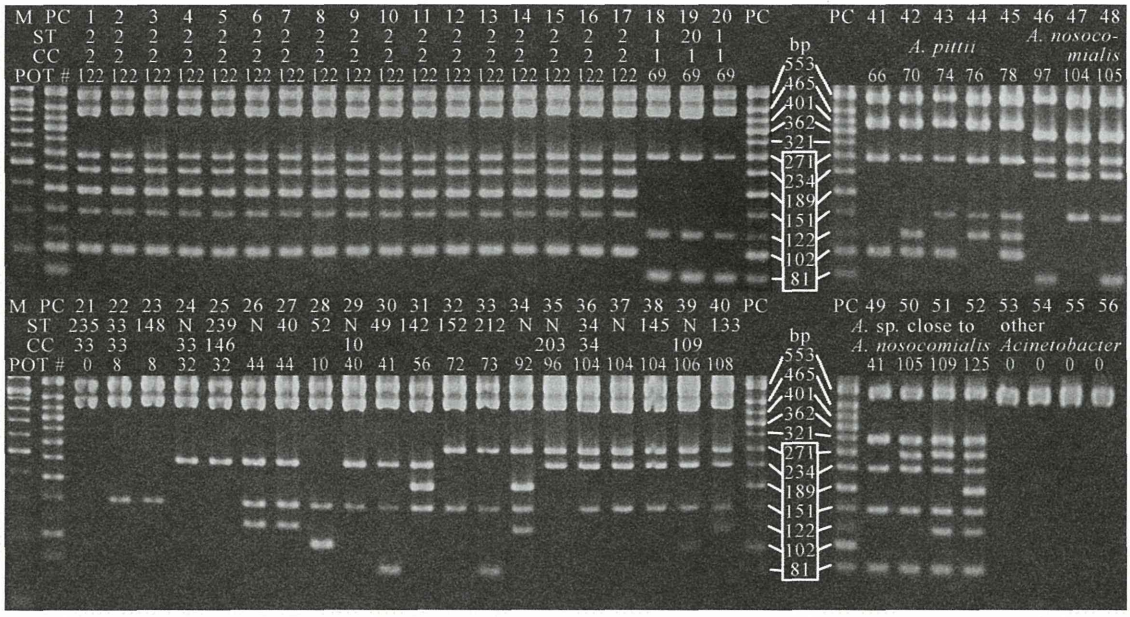

FIG 2 Agarose gel electrophoresis patterns of PCR-based ORF typing using 12-plex PCR. Lane M, 50-bp ladder marker; lane PC, positive control; lanes 1 to 40, *A. baumannii*; lanes 41 to 45, *A. pittii*; lanes 46 to 48, *A. nosocomialis*; lanes 49 to 52, *Acinetobacter* species close to *A. nosocomialis*; lane 53, *A. radioresistens*; lane 54, *A. ursingii*; lane 55, *A. bereziniae*; lane 56, *A. soli*. International clones II (lanes 1 to 17) and I (lanes 18 to 20) showed unique patterns and can be distinguished from other *A. baumannii* clones (lanes 21 to 40) and other *Acinetobacter* species (lanes 41 to 56). The POT numbers of both *A. baumannii* ST49 (lane 30) and an *Acinetobacter* species close to *A. nosocomialis* (lane 49) become 41, but these isolates can apparently be discriminated from each other by the ladder patterns of the upper 5 fragments from bp 321 to bp 553. ST, sequence type; CC, clonal complex. "N" in ST lines indicates a novel ST. The ladder used for the binary digitization of the genotype of each isolate is shown in the white box. The binary numbers corresponding to each band were 1 for 81 bp, 2 for 102 bp, 4 for 122 bp, 8 for 151 bp, 16 for 189 bp, 32 for 234 bp, and 64 for 271 bp, from the bottom of the ladder in the box. The remaining 5 bands from 321 bp to 553 bp were used for the identification of *Acinetobacter* spp.

5 candidates, and its specificity and sensitivity were 100% and 100%, respectively.

The ladder patterns of the PCR amplicons described above were clearly distinguishable by the 12-plex PCR established as the POT in the present study (Fig. 2). The same results were obtained by all four thermal cyclers we evaluated. To substantiate the 12-plex PCR, 44 *A. baumannii* strains used for SGI ORF screening were tested by both monoplex PCR and POT, and complete data agreement was observed between the two methods.

A total of 81 *A. baumannii* strains, which have been classified into 18 CCs by MLST, were analyzed by the POT method. International clones I and II were distinguished from other genetic lineages with more than two differences in the bands of their ladder patterns. According to the ladder patterns of seven ORFs, *A. baumannii* strains were classified into 17 POT types, 11 of which exhibited one-to-one correspondence to the CCs. Moreover, clinically isolated *Acinetobacter* species other than *A. baumannii* can be classified into three to five POT types at present (Table 4).

DISCUSSION

In the present study, we first showed that the newly established POT is capable of rapidly identifying *A. baumannii* international clones in ordinary clinical laboratories without performing nucleotide sequencing analyses of multiple genes as with MLST. To increase the feasibility of the test, the number of SGI ORFs adopted for POT was optimized. International clones I and II were fully distinguished by this method from other clones or lineages of *Acinetobacter* species. Moreover, the CCs of *A. baumannii* can be estimated by POT. The discriminatory power of POT can be controlled by optimizing the number of ORFs and loci selected for analysis. Such a newly developed POT method that compares the distribution patterns of ORFs and/or SGIs in each clinical isolate may well promise to be an easy and rapid genotyping method for identifying bacterial genetic lineages and molecular epidemiology, which is feasible in ordinary clinical microbiology laboratories.

Indeed, several methods to identify international clones using PCR have been reported (10, 11). However, they cannot identify newly emerging multidrug-resistant epidemic clones that might spread in the future. In fact, multidrug-resistant isolates other than the *A. baumannii* international clones have been reported (12–18). The POT method constructed in the present study is applicable to the identification of new CCs of *A. baumannii* or *A. calcoaceticus-A. baumannii* complex species, including *A. nosocomialis* and *A. pittii*, in the future.

Although SGIs were first reported from *Salmonella enterica* in 2001 by whole-genome analysis (6), little attention has been paid to them so far. Using genomic comparisons of *S. aureus* strains, we found that the distribution patterns of SGI ORFs correlate with the clonal complex in *S. aureus* (5). In the present study, it was also proven that the distribution patterns of ORFs in SGIs correlated well with the CCs in *A. baumannii*. This finding indicates that a very similar concept can be applicable even to various bacterial genera, and that close correlations between the distribution patterns of SGIs and CCs may be a general phenomenon in the microbial world. In fact, the CCs of *P. aeruginosa* were successfully predicted with a strategy and protocol similar to those of the POT constructed in the present study (M. Suzuki and Y. Iinuma, unpublished data).

To obtain the genetic information of clinical isolates from antimicrobial resistance surveillance data, digitized numeric data provided by new genotyping methods, like the POT, contribute to easy and feasible genotyping. Since the POT is very simple and requires equipment only for PCR and agarose gel electrophoresis, this can become a routine performance method in many ordinary clinical microbiology laboratories in various countries and regions, including developing countries around the world. If many clinical microbiologists and researchers would employ the POT for genotyping of clinical isolates, they could report the genotype of each clinical isolate as a digitized numeric number, and this would make it very easy to quickly compare the genotypes of clinical isolates with those of other clinical isolates recovered in different continents or areas. Therefore, the POT would enable us to identify newly emerging genetic lineages in the very early stage of their outbreak. Present weak antimicrobial resistance surveillance and monitoring systems depending mainly on the antimicrobial resistance phenotypes of clinical isolates can be reinforced from the genetic viewpoint by using the POT instead of MLST.

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REFERENCES

1. **Sabat AJ, Budimir A, Nashev D, Sá-Leão R, van Dijl Jm Laurent F, Grundmann H, Friedrich AW, ESCMID Study Group of Epidemiological Markers (ESGEM).** 2013. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill. **18:** pii=20380. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20380.
2. **Kempf M, Rolain JM.** 2012. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int. J. Antimicrob. Agents **39:**105–114. http://dx.doi.org/10.1016/j.ijantimicag.2011.10.004.
3. **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. http://dx.doi.org/10.1016/j.ijantimicag.2012.09.008.
4. **Suzuki M, Tawada Y, Kato M, Hori H, Mamiya N, Hayashi Y, Nakano M, Fukushima R, Katai A, Tanaka T, Hata M, Matsumoto M, Takahashi M, Sakae K.** 2006. Development of a rapid strain differentiation method for methicillin-resistant *Staphylococcus aureus* isolated in Japan by detecting phage-derived open-reading frames. J. Appl. Microbiol. **101:** 938–947. http://dx.doi.org/10.1111/j.1365-2672.2006.02932.x.
5. **Suzuki M, Matsumoto M, Takahashi M, Hayakawa Y, Minagawa H.** 2009. Identification of the clonal complexes of *Staphylococcus aureus* strains by determination of the conservation patterns of small genomic islets. J. Appl. Microbiol. **107:**1367–1374. http://dx.doi.org/10.1111/j.1365-2672.2009.04321.x.
6. **Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MT, Sebaihia M, Baker S, Basham D, Brooks K, Chillingworth T, Connerton P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG.** 2001. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature **413:**848–852. http://dx.doi.org/10.1038/35101607.
7. **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. http://dx.doi.org/10.1128/JCM.44.3.827-832.2006.
8. **Carver T, Berriman M, Tivey A, Patel C, Böhme U, Barrell BG, Parkhill J, Rajandream MA.** 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. Bioinformatics **24:** 2672–2676. http://dx.doi.org/10.1093/bioinformatics/btn529.
9. **Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL.** 2006. Identification of *Acinetobacter baumannii* by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species. J. Clin. Microbiol. **44:**2974–2976. http://dx.doi.org/10.1128/JCM.01021-06.
10. **Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL.** 2007. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. Clin. Microbiol. Infect. **13:** 807–815. http://dx.doi.org/10.1111/j.1469-0691.2007.01759.x.
11. **Matsui M, Suzuki S, Suzuki M, Arakawa Y, Shibayama K.** 2013. Rapid discrimination of *Acinetobacter baumannii* international clone II lineage by pyrosequencing SNP analyses of *bla*(OXA-51-like) genes. J. Microbiol. Methods **94:**121–124. http://dx.doi.org/10.1016/j.mimet.2013.05.014.
12. **Decousser JW, Jansen C, Nordmann P, Emirian A, Bonnin RA, Anais L, Merle JC, Poirel L.** 2013. Outbreak of NDM-1-producing *Acinetobacter baumannii* in France, January to May 2013. Euro Surveill. **18:**31. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20547.
13. **Tena D, Martínez NM, Oteo J, Sáez D, Vindel A, Azañedo ML, Sánchez L, Espinosa A, Cobos J, Sánchez R, Otero I, Bisquert J.** 2013. Outbreak of multiresistant OXA-24- and OXA-51-producing *Acinetobacter baumannii* in an internal medicine ward. Jpn. J. Infect. Dis. **66:**323–326. http://dx.doi.org/10.7883/yoken.66.323.
14. **Ahmed-Bentley J, Chandran AU, Joffe AM, French D, Peirano G, Pitout JD.** 2013. Gram-negative bacteria that produce carbapenemases causing death attributed to recent foreign hospitalization. Antimicrob. Agents Chemother. **57:**3085–3091. http://dx.doi.org/10.1128/AAC.00297-13.
15. **Kempf M, Rolain JM, Azza S, Diene S, Joly-Guillou ML, Dubourg G, Colson P, Papazian L, Richet H, Fournier PE, Ribeiro A, Raoult D.** 2013. Investigation of *Acinetobacter baumannii* resistance to carbapenems in Marseille hospitals, south of France: a transition from an epidemic to an endemic situation. APMIS **121:**64–71. http://dx.doi.org/10.1111/j.1600-0463.2012.02935.x.
16. **Schuetz AN, Huard RC, Eshoo MW, Massire C, Della-Latta P, Wu F, Jenkins SG.** 2012. Identification of a novel *Acinetobacter baumannii* clone in a US hospital outbreak by multilocus polymerase chain reaction/electrospray-ionization mass spectrometry. Diagn. Microbiol. Infect. Dis. **72:**14–19. http://dx.doi.org/10.1016/j.diagmicrobio.2011.09.012.
17. **Acosta J, Merino M, Viedma E, Poza M, Sanz F, Otero JR, Chaves F, Bou G.** 2011. Multidrug-resistant *Acinetobacter baumannii* harboring OXA-24 carbapenemase, Spain. Emerg. Infect. Dis. **17:**1064–1067. http://dx.doi.org/10.3201/eid/1706.091866.
18. **Zhong Q, Xu W, Wu Y, Xu H.** 2012. Clonal spread of carbapenem non-susceptible *Acinetobacter baumannii* in an intensive care unit in a teaching hospital in China. Ann. Lab. Med. **32:**413–419. http://dx.doi.org/10.3343/alm.2012.32.6.413.
19. **Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodrìguez-Valera F.** 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J. Clin. Microbiol. **43:**4382–4390. http://dx.doi.org/10.1128/JCM.43.9.4382-4390.2005.

mixed Filipino-White heritage; both had IgG persistently detected beyond 12 months of age and were monitored clinically for retinochoroiditis. Their charts contained no information regarding maternal exposure or risk factors. During the 15-year period, the rate of diagnosed congenital toxoplasmosis was 3.8 (95% CI 1.5–9.2) per million live births. There were no infant deaths for which congenital toxoplasmosis was recorded as a cause. We were unable to study fetal deaths because the corresponding cause-of-death codes were not readily available.

Historically, the lowest prevalence of *T. gondii* infection has been recorded in the western United States (*5*). The rate of clinically apparent congenital toxoplasmosis in this study was lower than that found during the late 1980s through early 1990s in the New England Newborn Screening Program initially after birth (2 per 521,555 live births [3.8 per million] versus 5 per 635,000 live births [7.9 per million], respectively) (*6*). However, the prevalence of *T. gondii* infection has decreased in the United States since the 1990s (*1*).

Our study is subject to several limitations. Our approach would only detect clinically apparent cases, and the results should be considered a minimal estimate of congenital infection. Some cases may not have been recorded in the electronic system, but this omission is not likely for severe illness, repeated hospital or clinic visits, or outside consultation. The small number of cases makes the rate of diagnosed congenital toxoplasmosis somewhat imprecise; a few missed cases would increase the rate considerably. In addition, we were not able to evaluate fetal deaths; however, stillbirth is reportedly a rare complication of congenital toxoplasmosis (*7*). Although we found a low rate of diagnosed congenital toxoplasmosis in northern California, population-based studies to evaluate rates of the disease in other geographic areas would be beneficial.

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References

1. Jones JL, Kruszon-Moran D, Rivera H, Price C, Wilkins PP. *Toxoplasma gondii* Seroprevalence in the United States 2009–2010 and comparison with the past two decades. Am J Trop Med Hyg. 2014;90:1135–39. http://dx.doi.org/10.4269/ajtmh.14-0013
2. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009;39:1385–94. http://dx.doi.org/10.1016/j.ijpara.2009.04.003
3. Remington JS, McLeod R, Wilson CB, Desmonts G. Toxoplasmosis 2011. In: Remington JS, Klein JO, Wilson CB, Nizet V, Maldonado YA, editors. Infectious diseases of the fetus and newborn infant. 7th ed. Philadelphia, PA: Elsevier Saunders; 2011. p. 918–1041.
4. Krieger N. Overcoming the absence of socioeconomic data in medical records: validation and application of a census-based methodology. Am J Public Health. 1992;82:703–10. http://dx.doi.org/10.2105/AJPH.82.5.703
5. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB, et al. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. Am J Epidemiol. 2001; 154:357–65. http://dx.doi.org/10.1093/aje/ 154.4.357
6. Guerina NG, Hsu HW, Meissner HC, Maguire JH, Lynfield R, Stechenberg B, et al.; The New England Regional Toxoplasma Working Group. Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. N Engl J Med. 1994;330:1858–63. http://dx.doi.org/10.1056/NEJM199406303302604
7. Freeman K, Oakley L, Pollak A, Buffolano W, Petersen E, Semprini AE, et al. Association between congenital toxoplasmosis and preterm birth, low birthweight and small for gestational age birth. BJOG. 2005;112:31–7. http://dx.doi.org/10.1111/j.1471-0528.2004.00299.x

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Invasive Infection Caused by Carbapenem-Resistant *Acinetobacter soli*, Japan

To the Editor: Infections caused by *Acinetobacter* spp., especially *A. baumannii*, have been increasingly documented in recent years. Carbapenems tend to be empirically prescribed as first-choice drugs for severe invasive infections caused by *Acinetobacter* spp. other than *A. baumannii* because these microbes are usually susceptible to carbapenems. However, infections with carbapenem-resistant *Acinetobacter* spp. have been increasingly reported during the past 15 years. In *A. baumannii*, carbapenems are usually inactivated by intrinsic oxacillinase (OXA)-51–like, acquired OXA-23–like, or OXA-58–like carbapenemases. Moreover, production of acquired metallo-β-lactamases (MBLs) of the Verona integron (VIM), imipenemase (IMP), or New Delhi (NDM) types has been detected among carbapenem-resistant *Acinetobacter* species, including *A. baumannii*, *A. junii*, *A. bereziniae*, *A. nosocomialis*, and *A. pittii* (*1*). We report a case of infection with carbapenem-resistant *A. soli* producing another

MBL type, Tripoli MBL 2 (TMB-2), in a man in Japan.

A man in his 60s who had mesenteric injury, pelvic fracture, and intestinal perforation from a traffic accident was admitted to Okazaki City Hospital in Aichi, Japan, on May 3, 2013. After surgery, cefmetazole was prescribed on May 6 (1 g 2×/d for 7 d). On May 12, symptoms of infection developed in the patient, and 2 sets of blood samples were drawn from different vessels for bacterial culture. The following day, cefmetazole was discontinued, and ciprofloxacin (0.3 g 2×/d) and piperacillin/tazobactam (4.5 g 2×/d) were started. *Acinetobacter* isolates resistant to piperacillin/tazobactam and carbapenems were then recovered from the blood samples, so piperacillin/tazobactam was discontinued on May 14. After that, ceftriaxone (2 g 2×/d) and gentamicin (0.04 g 2×/d) were successively prescribed, in addition to ciprofloxacin; the symptoms of infection improved, and all antimicrobial drugs were discontinued by May 26. Additional blood cultures performed on May 17, 21, and 28 yielded negative results for *Acinetobacter* spp. However, the patient's condition worsened on June 5. Meropenem (0.5 g 4×/d) was then given, but the patient died of multiorgan failure on June 7.

The bacterial isolates from the initial blood cultures were identified as *A. soli* by nucleotide sequencing of the *rpoB* and *gyrB* genes and assigned identification no. HK001. MICs of β-lactams, measured by the agar dilution method in accordance with the guideline M07-A9 of the Clinical and Laboratory Standards Institute (http://clsi.org), were as follows: sulbactam/ampicillin, >128 mg/L; piperacillin, >128 mg/L; tazobactam/piperacillin, >128 mg/L; cefotaxime, >64 mg/L; ceftazidime, >64 mg/L; aztreonam, 64 mg/L; cefmetazole, >128 mg/L; imipenem, 8 mg/L; meropenem, 32 mg/L; and doripenem, 32 mg/L. However, MICs of gentamicin, amikacin, levofloxacin, ciprofloxacin, colistin, and tigecycline were below the breakpoints of susceptibility as listed in Clinical and Laboratory Standards Institute document M100-S23. Carbapenem resistance was not transferred from *A. soli* HK001 to *Escherichia coli* strain CSH-2 (*metB* F^- NA^r Rif^r) by conjugation. A double-disk synergy test was initially performed by using sodium mercaptoacetic acid (SMA) (*2*) and ceftazidime and meropenem disks (Eiken Chemical Co., Ltd, Tokyo, Japan), and results suggested MBL production. The modified Hodge test was then performed, and ertapenem and meropenem disks gave clear positive results (data not shown). PCR was performed to detect bla_{OXA-23}–like, $bla_{OXA-24/40}$–like, bla_{OXA-51}–like, bla_{OXA-58}–like, bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2}, bla_{NDM-1}, bla_{SMB-1}, and bla_{TMB-1} genes. Nucleotide sequence analyses showed that the *A. soli* isolate harbored bla_{TMB-2} and bla_{OXA-58}. The modified SMA-disk method (*3*) was reevaluated to determine whether it could successfully detect TMB-2 production in *A. soli* HK001. Apparent positive results were obtained when disks containing imipenem, meropenem, or ertapenem were used, particularly when the edge-to-edge distance between 2 disks containing SMA and a carbapenem, respectively, was kept at 5 mm (Figure, top row). However, when the distance between the ertapenem and SMA disks was ≥10 mm, MBL production was more difficult to detect (Figure, lower 2 rows). This finding may be the result of co-production of OXA-58 by the isolate.

More than 30 *Acinetobacter* species had been registered by January 2012 (*4*); *A. soli* was initially isolated from the soil of a mountain forest in South Korea in 2007 (*5*) and has been recovered from blood cultures of 5 neonates in Brazil (*6*). Carbapenem-resistant *A. soli* co-harboring bla_{IMP-1} and bla_{OXA-58}–like genes was identified in April 2011 in Japan and is frequently recovered from bacteremia patients (*7*). TMB-1 was reported in 2012 in an *Achromobacter xylosoxidans* isolate from a hospital in Tripoli, Libya (*8*); TMB-2 was later reported in Japan

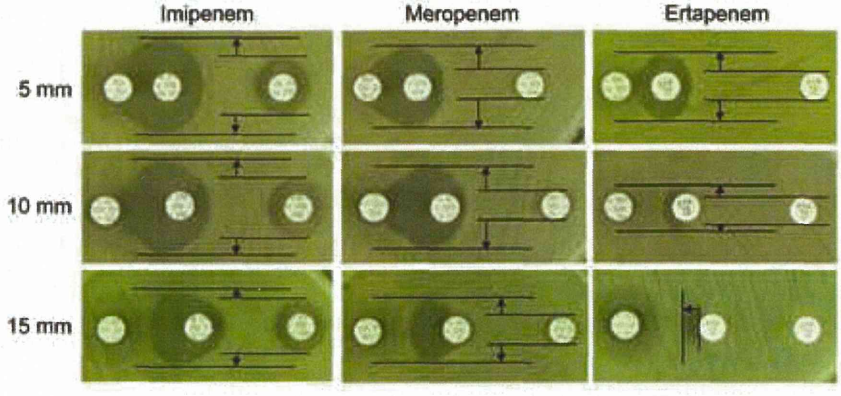

Figure. Results of double-disk synergy testing of the *Acinetobacter soli* isolate HK001 identified in a man in Japan. Testing was performed by using disks containing sodium mercaptoacetic acid (SMA) and the carbapenems imipenem, meropenem, and ertapenem. Apparent expansion of growth inhibition zone around a carbapenem disk placed near a SMA disk compared with that around a disk of carbapenem alone is seen on Mueller-Hinton agar if the isolate produces metallo-β-lactamases (*2,3*). When the edge-to-edge distance between 2 disks containing a carbapenem and SMA, respectively, was kept at 5 mm, expansion of the growth inhibition zone became clearer than for those kept at a distance of 10 mm and 15 mm, regardless of carbapenems used. Vertical expansion of growth inhibition zones by the effect of SMA is indicated by arrows; ertapenem gave the clearest result when the disk distance was kept at 5 mm (top right panel), even though *A. soli* HK001 co-produces oxacillinase 58–like carbapenemase, which is hardly inhibited by SMA.

(*9*). The TMB-2–producing *A. soli* strain that we isolated came from a blood culture, indicating that *A. soli* is a potential cause of bloodstream infections or bacteremia. *A. soli* has also been detected in lice and keds of domestic animals (*10*), indicating that *A. soli* may inhabit natural environments and that injuries and bites by arthropods might present a risk for invasive infections. Isolates of *Acinetobacter* species, particularly those recovered from blood culture, should be identified to species type to enable further evaluation of the clinical significance of carbapenem-resistant *A. soli* strains.

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References

1. Yamamoto M, Nagao M, Matsumura Y, Hotta G, Matsushima A, Ito Y, et al. Regional dissemination of *Acinetobacter* species harboring metallo-β-lactamase genes in Japan. Clin Microbiol Infect. 2013;19:729–36. http://dx.doi.org/10.1111/1469-0691.12013
2. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. J Clin Microbiol. 2000;38:40–3.
3. Hattori T, Kawamura K, Arakawa Y. Comparison of test methods for detecting metallo-β-lactamase-producing Gram-negative bacteria. Jpn J Infect Dis. 2013; 66:512–8.
4. Malhotra J, Anand S, Jindal S, Rajagopal R, Lal R. *Acinetobacter indicus* sp. nov., isolated from a hexachlorocyclohexane dump site. Int J Syst Evol Microbiol. 2012;62:2883–90. http://dx.doi.org/10.1099/ijs.0.037721-0
5. Kim D, Baik KS, Kim MS, Park SC, Kim SS, Rhee MS, et al. *Acinetobacter soli* sp. nov., isolated from forest soil. J Microbiol. 2008;46:396–401. http://dx.doi.org/10.1007/s12275-008-0118-y
6. Pellegrino FL, Vieira VV, Baio PV, dos Santos RM, dos Santos AL, Santos NG, et al. *Acinetobacter soli* as a cause of bloodstream infection in a neonatal intensive care unit. J Clin Microbiol. 2011;49:2283–5. http://dx.doi.org/10.1128/JCM.00326-11
7. Endo S, Yano H, Kanamori H, Inomata S, Aoyagi T, Hatta M, et al. High frequency of *Acinetobacter soli* among *Acinetobacter* isolates causing bacteremia at a Japanese tertiary hospital. J Clin Microbiol. 2014;52:911–5. http://dx.doi.org/10.1128/JCM.03009-13
8. El Salabi A, Borra PS, Toleman MA, Samuelsen Ø, Walsh TR. Genetic and biochemical characterization of a novel metallo-β-lactamase, TMB-1, from an *Achromobacter xylosoxidans* strain isolated in Tripoli, Libya. Antimicrob Agents Chemother. 2012;56:2241–5. http://dx.doi.org/10.1128/AAC.05640-11
9. Suzuki S, Matsui M, Suzuki M, Sugita A, Kosuge Y, Kodama N, et al. Detection of Tripoli metallo-β-lactamase 2 (TMB-2), a variant of bla_{TMB-1}, in clinical isolates of *Acinetobacter* spp. in Japan. J Antimicrob Chemother. 2013;68:1441–2. http://dx.doi.org/10.1093/jac/dkt031
10. Kumsa B, Socolovschi C, Parola P, Rolain JM, Raoult D. Molecular detection of *Acinetobacter* species in lice and keds of domestic animals in Oromia Regional State, Ethiopia. PLoS ONE. 2012;7:e52377. http://dx.doi.org/10.1371/journal.pone.0052377

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Spread of Vaccinia Virus to Cattle Herds, Argentina, 2011

To the Editor: Since 1999, several zoonotic outbreaks of vaccinia virus (VACV) infection have been reported in cattle and humans in rural areas of Brazil. The infections have caused exanthematous lesions on cows and persons who milk them, and thus are detrimental to the milk industry and public health services (*1*,*2*). In Brazil during the last decade, VACV outbreaks have been detected from the north to the extreme south of the country (*1*–*4*). Because Brazil shares extensive boundaries with other South American countries, humans and cattle on dairy and beef-producing farms in those countries may be at risk of exposure to VACV. To determine if VACV has spread from Brazil to Argentina, we investigated the presence of VACV in serum samples from cattle in Argentina.

During 2011, we obtained serum samples from 100 animals (50 dairy and 50 beef cattle) on farms in Córdoba, Corrientes, Entre Ríos, and Santa Fe Provinces in Argentina (online Technical Appendix, panel A, http://wwwnc.cdc.gov/EID/article/20/9/14-0154-Techapp1.pdf). No VACV cases had been reported in humans or cattle in these provinces. However, Corrientes Province borders the Brazilian state of Rio Grande do Sul, where VACVs (Pelotas 1 and Pelotas 2 viruses) were isolated during an outbreak affecting horses in 2008 (*2*).

To determine the presence of neutralizing antibodies in the serum samples, we used an orthopoxvirus 70% plaque-reduction neutralization test as described (*4*). On the basis of previous studies that detected viral DNA in serum samples (*4*–*6*), we used real-time PCR to amplify the highly conserved orthopoxvirus vaccinia growth factor (*vgf*) gene DNA (P.A. Alves, unpub. methods).

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〈報　告〉

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

渡邊　珠代[1)]・丹羽　隆[1,2)]・土屋麻由美[1)]・外海　友規[1,2)]・太田　浩敏[1,3)]・村上　啓雄[1)]

Surveillance of Infection Control Measures among All Hospitals Collecting Infection Prevention Medical Fees in Gifu Prefecture

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(2014年6月6日 受付・2014年11月25日 受理)

要　旨

岐阜県では，2012年4月より県内の全感染防止対策加算算定病院(以下，加算病院)を対象に，感染対策チーム(ICT)活動の質についてのサーベイランスを開始した．今回，このサーベイランス結果について報告する．2012年4月から2014年2月までの23ヶ月間の，ICT活動(会議およびラウンド回数)，薬剤耐性菌等［メチシリン耐性黄色ブドウ球菌(MRSA)，基質特異性拡張型βラクタマーゼ(ESBL)産生菌，*Clostridium difficile*(CD)トキシン)］の検出，血液培養，擦式アルコール製剤および抗菌薬の使用量についての毎月のデータを解析した．その結果，ICT会議の開催回数，血液培養の複数セットの採取率，擦式アルコール製剤の使用量の増加が認められた．一方，MRSAの新規検出率は増加，ESBL産生菌の新規および総検出率は軽度増加傾向にあり，MRSAの総検出率，CDトキシンの検出率には，明確な増加や減少傾向は認めなかった．抗菌薬の使用状況にも大きな変化は認めなかった．本サーベイランスにより，岐阜県内の加算病院の感染対策活動の実態把握が可能となった．また，一部の調査項目，特にICTの努力で比較的改善しやすいと考えられる項目に関しては，有意な改善が認められた．他の項目の動向も含め，引き続き解析を継続したい．

Key words：サーベイランス，感染制御，感染対策チーム，擦式アルコール製剤，抗菌薬

はじめに

平成24年度診療報酬改定により，感染防止対策加算(以下，加算)の算定病院(以下，加算病院)同士の連携と，感染防止対策の強化が求められるようになった[1)]．医療関連感染対策の活動内容について，自施設のレベルを推し量ることや，互いに比較することができるよう，我々は岐阜県において，全加算病院を対象に，毎月のデータを収集し，フィードバックするサーベイランスシステムを構築した．

今回，岐阜県内の全加算算定病院の感染対策活動を評価することを目的として，23ヶ月間のサーベイランスデータを解析したので報告する．

岐阜大学医学部附属病院 [1)]生体支援センター，[2)]薬剤部，[3)]検査部

対象と方法

1. 対象

岐阜県内の全ての加算病院を対象とした．

2. 方法

岐阜大学医学部附属病院生体支援センターが，サーベイランスの事務局となった．データの収集は，電子メー

ルを介して事務局から岐阜県内の加算病院の感染対策担当者宛てに，表計算ソフト(マイクロソフトエクセル2010，マイクロソフト社)を使用したフォーマットファイル(図1)を送付した．各病院の担当者は月次データを入力後にファイルを返信し，事務局でデータを集計した．本研究では，2012年4月から2014年2月までの月次データを解析した．

調査項目は，病院基礎情報，感染対策チーム(infection control team: ICT)活動，薬剤耐性菌等の検出，血液培養検査，擦式アルコール製剤(alcohol-based hand rub: ABHR)および抗菌薬の使用量とした．以下に各項目の収集データと解析方法を示す．

1)　病院基礎情報

算定中の加算，病床数，文部科学省の定める月間在院患者延数(入院日および退院日を含む)，細菌検査室の有無のデータを収集した．

2)　ICT活動

ICT会議およびラウンドの実施回数を収集した．

病院名＿＿＿＿＿＿　加算＿＿＿＿　病床数＿＿＿＿床　細菌検査室＿＿＿＿
のべ在院日数：＿＿＿＿＿＿　(文科省数/入院日・退院日を含める)　2013年4月データ

①ICT活動の状況

ICTミーティング、会議の実施回数	回
ICTラウンドの実施回数	回
その他の活動(　　)	回

②薬剤耐性菌等の検出状況(患者数でお答えください。

		総数	新規
MRSA			
ESBL産生菌			
内訳	ESBL産生*E. coli*		
	ESBL産生*K. pneumoniae*		
	ESBL産生*K. oxytoca*		
	ESBL産生*P. mirabilis*		
CDトキシン			
その他※(　　)			

※その他の項目には、MDRP、VREなどをご記入下さい。枠が足りない場合は、以下にご記入ください。

③感染症患者の発生状況

血液培養提出数		セット
1セットのみの血液培養提出数		セット
血液培養陽性数		セット
汚染検体数		セット

複数セット採取率自動計算 %

④病院感染対策の実施状況

手指消毒用アルコール製剤の使用量

各製剤の1回あたりの至適使用量がお判りでしたら、ご記入下さい。

使用しているアルコール製剤名	使用量	測定している場合の測定方法	1回量	手指消毒用アルコール使用率
①	mL		mL	自動計算　mL/1000入院患者数・日
②	mL		mL	自動計算　mL/1000入院患者数・日
③	mL		mL	自動計算　mL/1000入院患者数・日

⑤抗菌薬の使用状況

系統	薬品名	商品名(代表例)	採用の有無	月間使用量(g)
アミノグリコシド	ストレプトマイシン	ストレプトマイシン	<選択>	
	カナマイシン	カナマイシン	<選択>	
	アミカシン	アミカシン	<選択>	
	ゲンタマイシン	ゲンタシン	<選択>	
	ジベカシン	パニマイシン	<選択>	
	トブラマイシン	トブラシン	<選択>	
	イセパマイシン	エクサシン	<選択>	
	ベカナマイシン	カネンドマイシン	<選択>	
	リボスタマイシン	ビスタマイシン	<選択>	
	スペクチノマイシン	トロビシン	<選択>	
テトラサイクリン	ミノサイクリン	ミノマイシン	<選択>	
リンコマイシン	クリンダマイシン	ダラシンS	<選択>	
	リンコマイシン	リンコシン	<選択>	
マクロライド	エリスロマイシン	エリスロシン	<選択>	
	アジスロマイシン	ジスロマック	<選択>	
ペニシリン	ベンジルペニシリン	ペニシリンG	<選択>	
	アンピシリン	ビクシリン	<選択>	
	アンピシリン	ビクシリンS	<選択>	
	ピペラシリン	ペントシリン	<選択>	
	アスポキシシリン	ドイル	<選択>	
	アンピシリン	ユナシンS	<選択>	
	ピペラシリン	ゾシン	<選択>	

図1　フォーマットファイル(抜粋)

3)　薬剤耐性菌等の検出

メチシリン耐性黄色ブドウ球菌(methicillin-resistant *Staphylococcus aureus*: MRSA)，基質特異性拡張型βラクタマーゼ(extended spectrum beta lactamase: ESBL)産生菌，*Clostridium difficile*(CD)が産生する毒素(以下，CD トキシン)のそれぞれの，新規検出数および総検出数を患者数単位で収集した．各検出患者数を月間在院患者延数で除した後に 1,000 を乗じ，1,000 入院患者・日あたりの検出率を算出した．

4)　血液培養検査

血液培養提出数，1 セットのみの血液培養提出数，血液培養陽性数，汚染検体数を収集した．血液培養検査において，各セットのうち，嫌気ボトル，好気ボトルあるいは小児ボトルの少なくとも 1 本から菌が検出された場合を血液培養陽性と定義した．汚染検体の定義[2]は，2 セット以上の血液培養が採取されていた場合に 1 セットのみが陽性かつ検出菌が *Propionibacterium acnes*, coagulase-negative staphylococci (CNS), *Bacillus* spp., *Corynebacterium* spp. の場合，あるいは，1 セットのみの血液培養採取で陽性かつ，検出菌が *P. acnes*, CNS, *Bacillus* spp., *Corynebacterium* spp. の場合とした．ただし，これらの菌が検出された場合でも，持続して菌が検出されている場合や，明らかに起因菌と考えられる場合は汚染菌から除外し，一方で，感染性心内膜炎以外の場合は，口腔内レンサ球菌は汚染菌とした．血液培養の提出数をそれぞれ病床数，月間在院患者延数で除した後，それぞれ 100 および 1,000 を乗じ，100 病床数あたりの血液培養提出率および，1,000 入院患者・日あたりの血液培養提出率を算出した．血液培養陽性率および汚染率は，それぞれ血液培養陽性数，汚染検体数を血液培養提出数で除して算出した．複数セット採取率は，血液培養提出数から 1 セットのみの血液培養提出数を減じたものを血液培養提出数で除して算出した[3]．

5)　ABHR 使用量

各施設で採用されている製剤が異なるため，各施設での使用量をその製剤の至適使用量で除した推定使用回数を算出した．複数の ABHR が採用されている施設に関しては，それぞれの製剤の推定使用回数の合計を算出した．さらに，ABHR 使用回数を月間在院患者延数で除して，入院患者・日あたりの ABHR 使用率を算出した．

6)　抗菌薬使用量

入院症例に使用した各注射用抗菌薬の実使用量を収集した．抗菌薬の実使用量をもとに 1 日投与量(defined daily dose: DDD)を算出[4]し，DDD を月間在院患者延数で除して，抗菌薬使用密度(antimicrobial use density: AUD)を算出した．なお，ペニシリン G については，100 万単位を 1 g と換算した．

データ解析の結果は，年 2 回全ての加算施設が集まる合同カンファランスで報告するとともに，各加算病院に対してフィードバックデータを送付した．フィードバックデータには，全加算施設の平均値，加算 1 および加算 2 施設のそれぞれの平均値，および各病院のデータを，項目別にグラフで表示した．フィードバック用データの一部を図 2 に例示する．

統計解析は正規性検定を行った上，正規分布に従わないことを確認した上で，Kruskal-Wallis 検定を行った．有意水準は $p<0.05$ とした．なお，統計ソフトは IBM

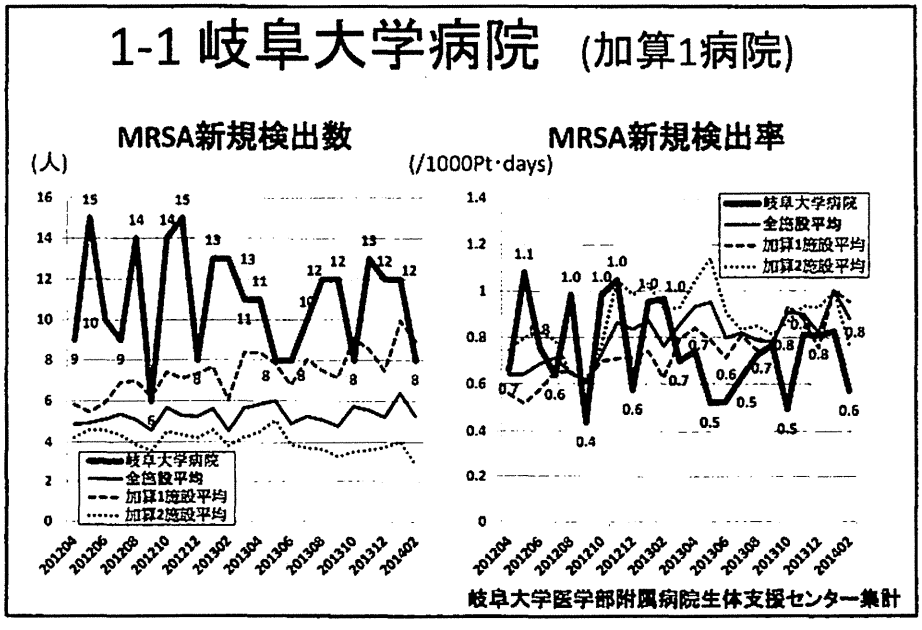

図2　フィードバックデータの一部(例)