The Implementation of a Hospital-wide Practice for the Selective Use of Carbapenems Based on the Monitoring of Susceptibility of *Pseudomonas aeruginosa* Isolates

Toshio OHSHIMA[*1, 2], Satomi ASAI[*3], Miki MIYAZAWA[*1], Yukari YAMAMOTO[*1], Akifumi HISADA[*1], Chie KUMAZAWA[*1], Masayoshi HASHIMOTO[*4], Katsuji FUKAWA[*4], Hideo IWASHITA[*3], Kazuo UMEZAWA[*5], Sanetoshi YAMADA[*6], Yoshiro YAMAMOTO[*7] and Hayato MIYACHI[*3]

[*1]*Department of Medical Laboratory in Tokai University Hospital*
[*2]*Department of Medical Risk and Crisis Management in Chiba Institute of Science*
[*3]*Department of Laboratory Medicine, Tokai University School of Medicine*
[*4]*Department of Pharmacy in Tokai University Hospital*
[*5]*Department of Critical Care and Emergency Medicine, Tokai University School of Medicine*
[*6]*Course of Science and Technology, Tokai University Graduate School of Science and Technology*
[*7]*Department of Mathematics, Tokai University School of Science*

(Received May 9, 2017; Accepted October 5, 2017)

Objectives: To control carbapenem-resistant *Pseudomonas aeruginosa*, we implemented a hospital-wide policy concerning the selective use of carbapenems based on the monitoring of *P. aeruginosa* isolates for susceptibility to five carbapenems using a customized dry plate method. In this study, we retrospectively investigated the outcome of our measures to control carbapenem-resistant *P. aeruginosa*.
Methods: To select effective carbapenems, 100 clinical isolates were collected, and the minimum inhibitory concentration (MIC) to 5 carbapenems (IPM/CS, MEPM, DRPM, BIPM and PAPM/BP) was monitored using a customized dry plate method from 2006 to 2013. Carbapenems, which were associated with a high rate of drug resistance in *P. aeruginosa*, were restricted from use during our intervention study. The antimicrobial use density per 100 bed-days (AUD^{100}) of carbapenems and the detection rates of carbapenem (IPM/CS and MEPM)-resistant *P. aeruginosa* were determined during the period of the intervention.
Results: The isolates consistently showed higher rates of drug-resistant *P. aeruginosa* in IPM/CS and PAPM/BP. Thus, DRPM, MEPM and BIPM were adopted for hospital-wide use. The detection rates of all IPM/CS- and MEPM-resistant *P. aeruginosa* significantly decreased. Meanwhile, the consumption of carbapenems showed an increasing trend.
Conclusions: The outcome of the hospital-wide implementation of the selective use of carbapenems based on periodic monitoring of the susceptibility of *P. aeruginosa* isolates was retrospectively studied. Implementation of this measure might have contributed in part to the control of carbapenem-resistant *P. aeruginosa* in our hospital.

Key words: Drug-resistant *Pseudomonas aeruginosa*, carbapenem, usage restrictions, infection control, customized dry plate

INTRODUCTION

Carbapenems display strong and effective antibacterial activity against a wide spectrum of bacterial infections. Five kinds of carbapenems have been developed: imipenem/cilastatin (IPM/CS), meropenem (MEPM), doripenem (DRPM), biapenem (BIPM) and panipenem/betamipron (PAPM/BP). Carbapenem-resistant *Enterobacteriae* (CRE), multidrug-resistant *Pseudomonas aeruginosa* (MDRP) and multidrug-resistant *Acinetobacter baumannii* are serious issues in the treatment and care of patients in many hospitals in Japan and other countries [1–7].

In tertiary hospitals such as our institute, there are growing numbers of immunocompromised and patients with severe infection being treated using carbapenems. Such situations are well-known risk factors for the rise of carbapenem-resistant bacterial infections. To prevent their emergence and spread, various approaches to drug usage have been implemented, such as restricting the use of antimicrobial agents, using cycling therapy and individual tailoring in therapy [8–10]. However, despite these efforts, no universally effective methods have yet been established.

In our hospital, the detection of carbapenem-resistant *P. aeruginosa* increased continuously from the early 2000s in parallel with the increased consumption of carbapenems, despite the implementation of control measures against hospital-acquired infection, such as the reinforcement of hand hygiene, highlighting the importance of environmental hygiene and the education of medical staff members [11, 12]. Increased carbapenem consumption may have caused environmental antimicrobial pressure and the dominant

Satomi ASAI, Department of Laboratory Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan
Tel: +81-463-93-1121 Fax: +81-463-93-8607 E-mail: sa@is.icc.u-tokai.ac.jp

Table 1 The specimen types of the clinical isolates of *Pseudomonas aeruginosa* that were used in susceptibility monitoring (using the dry plate method) to five carbapenems.

Year/ Type of Specimen (number)	Sputum (or Throat swab)	Urine	Blood	Wound (or pus)	Others	Total
2006	58	19	3	10	10	100
2007	63	14	4	9	10	100
2008	64	18	3	8	7	100
2009	58	18	2	10	12	100
2010	42	26	4	13	15	100
2011	58	17	3	7	15	100
2012	46	26	2	11	15	100
2013	53	22	2	11	12	100

proliferation of carbapenem-resistant *P. aeruginosa* [13, 14].

We suspected that the selective use of carbapenems might be beneficial not only for increasing the individual treatment effect and reducing the total carbapenem use per patient but also for reducing the rates of carbapenem-resistant *P. aeruginosa* by decreasing the environmental antimicrobial pressure with the suspension of certain carbapenems. Furthermore, we expected that the resistance of *P. aeruginosa* isolates to the suspended carbapenems would recover after a period of non-use.

In our hospital, the hospital-wide selective use of three carbapenems based on monitoring the susceptibility of *P. aeruginosa* isolates to five carbapenems was implemented. To ascertain the implementation effect, we investigated the trend in the detection rate of carbapenem-resistant *P. aeruginosa* during the period of implementation of the hospital-wide practice for the selective use of carbapenems.

MATERIALS AND METHODS

There are 803 beds in 14 wards and 36 clinical departments in Tokai University Hospital, including 3 beds in the severe burn unit, 19 beds in the emergency intensive-care unit (EICU), 36 beds in the emergency high-care unit and 32 beds in the ICU.

Assessing the antimicrobial susceptibility of *P. aeruginosa* isolates to five carbapenems using a customized dry plate for the hospital-wide selective use of carbapenems

The collection of *P. aeruginosa* specimens started in September 2006 and was continued until 100 specimens were obtained annually from 2006 to 2013 (800 isolates in total). Regardless of the type of specimen, the initial isolate of *P. aeruginosa* was examined for each patient. Clinical isolates of *P. aeruginosa* were obtained from the sputum (or throat swab), urine, blood, wound pus and other specimens (Table 1). Isolates detected during outbreaks of nosocomial infection were excluded.

Susceptibility for carbapenems was assessed using our customized dry plate method (Eiken Co., Ltd., Tokyo, Japan). The dry plate panel for carbapenems included IPM/CS, MEPM, DRPM, BIPM and PAPM/BP. The drug concentrations were adjusted to 0.25, 1, 2, 4, 8, 16, 32 and 64 μg/mL. The concentration of the bacterial suspension cultured in cation-adjusted Mueller Hinton Broth (CAMHB; Eiken Co., Ltd.) was adjusted to McFarland 0.5 and incubated for 20–24 h at 35 ± 2℃ according to the Clinical and Laboratory Standards Institute (CLSI) guideline [15]. Carbapenems, which were associated with a high rate of drug resistance in *P. aeruginosa* (MIC 8, 16, 32 and 64 μg/mL), were not permitted to use during this study.

Antimicrobial susceptibility testing of all clinical isolates of *P. aeruginosa* in routine laboratory work

The antimicrobial susceptibility in all clinical isolates of *P. aeruginosa* was examined as part of the routine laboratory work by the microdilution broth method based on the CLSI guidelines [15] using a MicroScan WalkAway 96 Plus (Beckman Coulter, Inc., Brea, CA, USA). Carbapenem resistance was defined by IPM/CS and/or MEPM resistance (MIC > 16 μg/mL) and intermediate susceptibility [I] (MIC > 8 μg/mL) according to the 'Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases' established by the Ministry of Health, Labour and Welfare in Japan.

Consumption of carbapenems

The consumption of each of the carbapenems was calculated based on the antimicrobial use density per 100 bed-days (AUD^{100}) using the defined daily dose (DDD) [16].

Statistical analyses

A regression analysis was used to evaluate trends in the rate of carbapenem-resistant ([R] and [I]) isolates and the AUD^{100} from 2007 to 2013.

RESULTS

Antimicrobial susceptibility testing in *P. aeruginosa* isolates against 5 carbapenems using the original dry plate for the hospital-wide selective use of carbapenems

The MICs in approximately 100 isolates of *P. aeruginosa* tested for carbapenem susceptibility during the 8-year period from 2006 to 2013 are shown in Fig. 1. The percentages of isolates with an MIC > 8 μg/mL were 25% for IPM/CS, 36% for PAPM/BP, 19% for MEPM, 19% for BIPM and 8% for DRPM. This pattern of high rates of carbapenem-resistance was similar

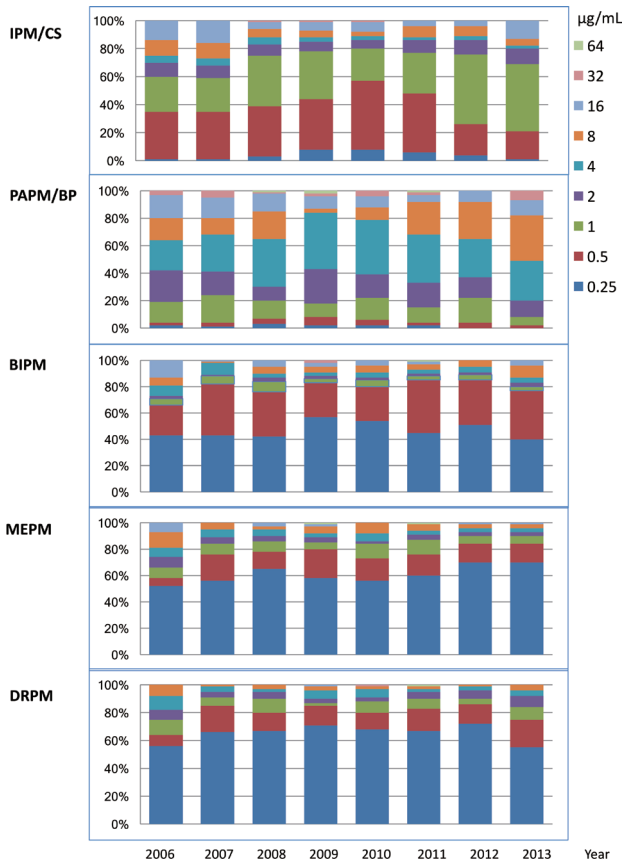

Fig. 1 The measurement of the MIC using the customized dry plate method. To select the more effective carbapenems among IPM/CS, PAPM/BP, MEPM, BIPM and DRPM, the MICs were measured in 100 *Pseudomonas aeruginosa* isolates each year from 2006 to 2013 using our customized dry plate method.

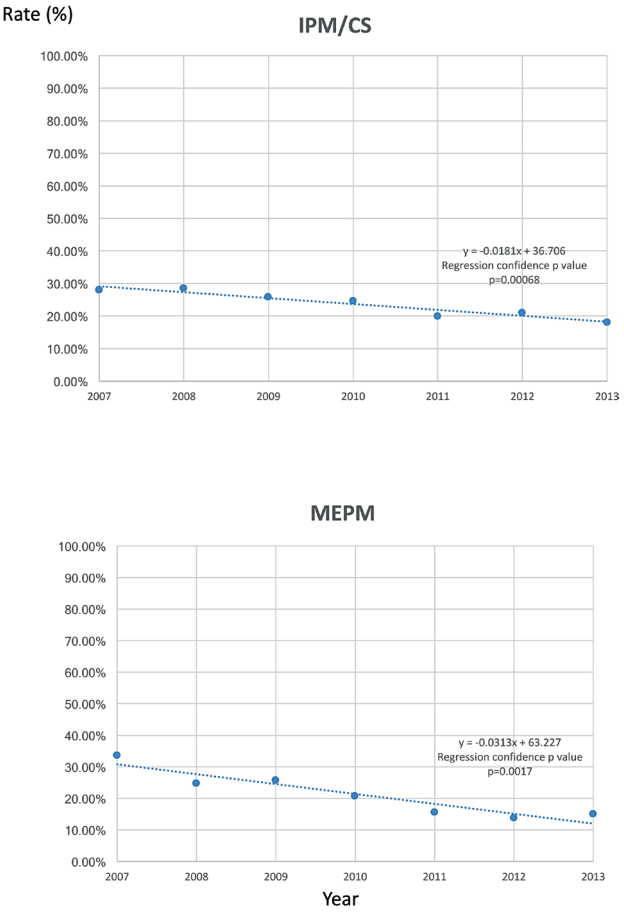

Fig. 2 The detection rate of carbapenem-resistant *Pseudomonas aeruginosa* in the hospital from 2007 to 2013.
The detection rates of IPM/CS-resistant and MEPM-resistant *P. aeruginosa* in Tokai University Hospital are shown in the upper and lower panels, respectively. The rates of both IPM/CS-resistant and MEPM-resistant *P. aeruginosa* decreased significantly during the seven-year study period.

throughout the study period.

Based on the antimicrobial susceptibility testing results in 2006, the three carbapenems MEPM, BIPM and DRPM were permitted to use in the hospital from 2007, and these three carbapenems continued being used for seven years.

Antimicrobial susceptibility testing of all clinical isolates of *P. aeruginosa* in routine laboratory work

The isolates of *P. aeruginosa* that were detected each year in Tokai University Hospital are shown in Table 2. From 2007 to 2013, the rate of IPM/CS-resistant *P. aeruginosa* isolates decreased significantly from 27.96% to 18.05% ($p = 0.00068$), while the rate of MEPM-resistant *P. aeruginosa* decreased from 33.69% to 15.12% ($p = 0.0017$) (Fig. 2).

Consumption of carbapenems

The AUD^{100} values of carbapenems are shown in Fig. 3. The AUD^{100} of total carbapenems showed an increasing trend from 2007 to 2013 ($y = 1.8631x - 3715.6$, $R^2 = 0.5128$, $p = 0.07$). The AUD^{100} of MEPM also showed an increasing trend ($y = 1.3101x - 2613.8$, $R^2 = 0.4733$, $p = 0.0875$).

DISCUSSION

The periodic monitoring of the MICs (obtained using the dry plate method) of 100 clinical isolates of *P. aeruginosa* was performed to optimize the hospital-wide use of carbapenems in order to reduce the risk of the emergence of carbapenem-resistant *P. aeruginosa* and to promote more effective carbapenem treatment. Based on the monitoring during the seven-year period from 2007 to 2013, the three carbapenems DRPM, MEPM and BIPM were selected for use in the hospital. However, the AUD^{100} of MEPM, DRPM and total carbapenems, showed an increasing trend. Furthermore, despite IPM/CS and PAPM/BP not being used since 2007, their MICs were not restored after the suspension. This might have been caused by cross-resistance that developed due to a chemical structure shared among different carbapenems. Essentially, the antibacterial activity of PAPM/BP against *P. aeruginosa* is inferior to that of IMP/CS [17]. DRPM is the most active carbapenem against *P. aeruginosa*. According to reports from other institutions in Japan, MEPM- and DRPM-resistant strains have a high rate of cross-resistance to IPM/CS and PAPM/BP [18, 19]. Contrary to

Table 2 The trends in the detection of *Pseudomonas aeruginosa* in Tokai University Hospital

Year	Total specimens	Total *P. aeruginosa*	IPM/CS-resistant *P. aeruginosa*	MDRP
2006	25,020	4.0% (1,013/25,020)	37.2% (377/1,013)	2.1%
2007	28,462	3.9% (1,099/28,462)	28.0% (307/1,098)	1.9%
2008	25,919	3.4% (870/25,919)	28.5% (248/869)	3.2%
2009	33,848	3.1% (1,053/33,848)	25.8% (272/1,053)	1.9%
2010	36,411	2.1% (761/36,411)	24.6% (187/761)	1.4%
2011	36,007	2.4% (862/36,007)	20.0% (172/862)	1.4%
2012	32,141	2.4% (764/32,141)	20.9% (160/764)	1.2%
2013	38,760	2.1% (820/38,760)	18.0% (148/820)	1.1 %

IPM/CS: imipenem/cilastatin, MDRP: multidrug-resistant *Pseudomonas aeruginosa*.

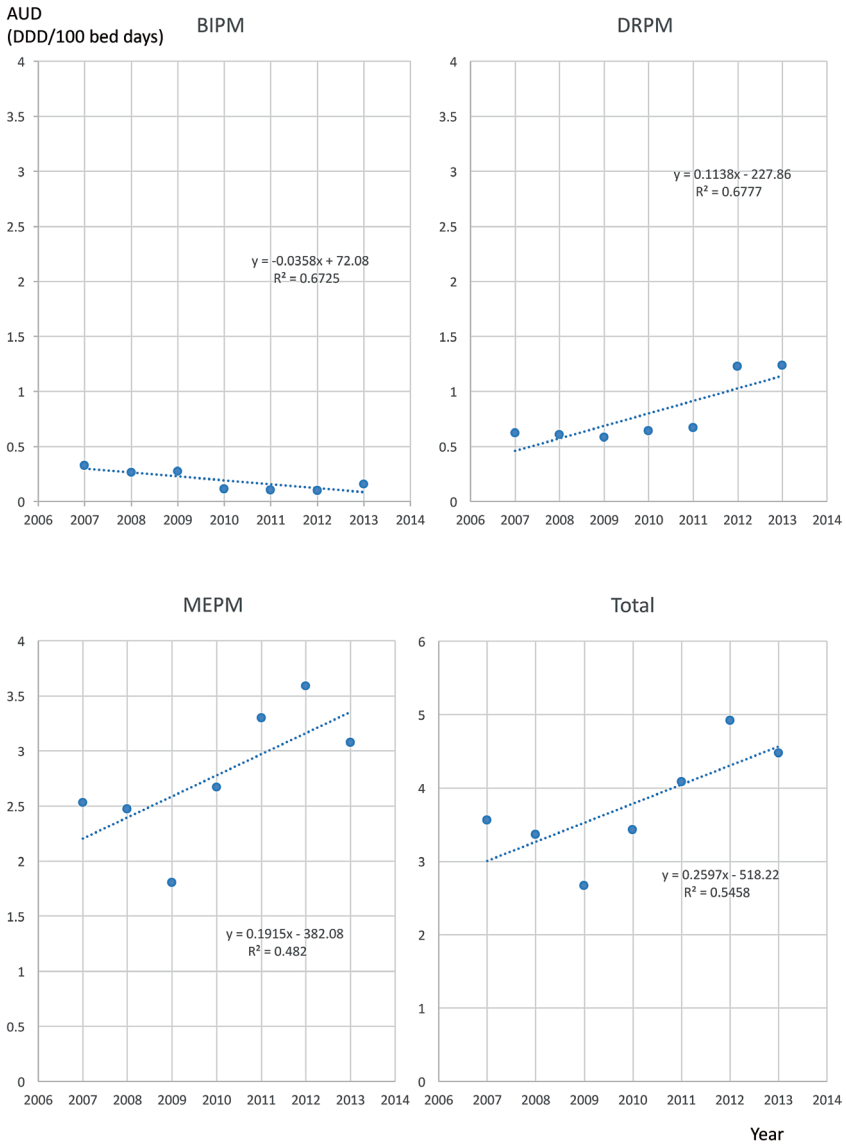

Fig. 3 The antimicrobial use density per 100 bed-days (AUD^{100}) of carbapenems.
The AUD^{100} of MEPM, BIPM, DRPM and total carbapenems is shown. The AUD^{100} of MEPM and total carbapenems showed an increasing trend during the seven-year study period.

the assumption that carbapenem susceptibility would recover after the cessation of the hospital-wide use of carbapenems, the susceptibility to these carbapenems did not change to a significant extent during the study period. This may be because carbapenems, regardless of category, share a chemical structure and thus resistance mechanisms, resulting in cross-resistance of the bacteria to the unused agents [20].

Various mechanisms underlying the acquisition of carbapenem resistance in *P. aeruginosa* have been reported, including OprD porin deficiency, the production of AmpC-β-lactamases and metallo-β-lactamase, and the overexpression of efflux pumps of the resistance nodulation and cell division (RND) efflux systems such as the MexAB-OprM [1, 21–27]. In addition to underlying the development of carbapenem resistance, these mechanisms are considered to be a gateway to the development of MDRP. Thus, the monitoring and control of carbapenem-resistant *P. aeruginosa* is important in the control of MDRP.

The use of a large amount of carbapenems is considered a major risk factor for the development of carbapenem-resistant *P. aeruginosa* [8, 22]. In this study, the detection rate of carbapenem (IPM/CS and/or MEPM)-resistant *P. aeruginosa* in the hospital significantly decreased in the 7-year period after the implementation of the selective use of carbapenems, despite the fact that the AUD^{100} of MEPM and total carbapenems showed an increasing trend. Meanwhile, other measures to control carbapenem-resistant *P. aeruginosa* were intensively performed, including other antimicrobial stewardship, the reinforcement of hand hygiene and environmental hygiene and the education of medical staff members. In combination with the hospital-wide selective use of carbapenems, this multi-modal approach might have contributed to the suppression of carbapenem-resistant *P. aeruginosa*.

The validity of the effects of the intervention in our quasi-experimental design must be discussed [28]. First, the observed results might merely be due to regression to the mean, although this was considered unlikely, since a sufficient number of points were observed during the seven-year study period. Second, the results might be explained by the maturation effect. However, this effect alone cannot explain the observed results in the present study, as a substantial number of new doctors and nurses annually joined the staff. Third, it is difficult to control for major confounding variables in a quasi-experimental design, which is a limitation of our study design. Ideally, confounders such as the severity of illness, the quality of the medical and nursing care (including hand hygiene and environmental hygiene), and antimicrobial prescription practices during the observation period should be measured and adjusted in order to verify the relationship between preventive strategies and any reduction in the rate of carbapenem-resistant *P. aeruginosa*.

In summary, the periodic monitoring of carbapenem susceptibility of clinical isolates of *P. aeruginosa* using the customized dry plate method was considered to be an indicator for the hospital-wide selective use of carbapenems, which might have contributed to the control of carbapenem-resistant *P. aeruginosa* as one aspect of multi-modal infection control measures.

FUNDING

This work was supported by Grant-in-Aid for scientific Research (c) Grant Numbers 26460657 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

This study was approved by the Review board of Tokai University (13R273).

REFERENCES

1) Asai S, Ohshima T, Yoshihara E, Jin G, Umezawa K, Inokuchi S, Miyachi H. Differential co-expression of Mex efflux pumps in a clinical strain of metallo-β-lactamase producing-*Pseudomonas aeruginosa* during the stepwise evolution of resistance to aminoglycosides. *Infect Dis Clin Pract*. 2011; 19: 38–42.
2) Suzuki M, Matsui M, Suzuki S, Rimbara E, Asai S, Miyachi H, *et al*. Genome sequences of multidrug-resistant *Acinetobacter baumannii* strains from nosocomial outbreaks in Japan. *Genome Announc*. 2013; 18: 1. e00476-13. doi: 10.1128/genom A. 00476–13.
3) Asai S, Umezawa K, Iwashita H, Matsui M, Shibayama K, Inokuchi S, *et al*. An outbreak of $bla_{\text{OXA-51-like}}$- and $bla_{\text{OXA-66}}$- positive *Acinetobacter baumannii* ST208 in the emergency intensive care unit. *J Med Microbiol*. 2014; 63: 1517–1523.
4) World Health Organization. Antimicrobial resistance. Global report on surveillance. 2014 Summary. Geneva [Switzerland]: WHO Press; 2014.
5) Royer S, Faria AL, Seki L, Chagas TP, Campos PA, Batistão DW, *et al*. Spread of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clones in patients with ventilator-associated pneumonia in an adult intensive care unit at a university hospital. *Braz J Infect Dis*. 2015; 19: 350–357.
6) Umezawa K, Asai S, Ohshima T, Iwashita H, Ohashi M, Sasaki, *et al*. Outbreak of drug-resistant *Acinetobacter baumannii* ST219 caused by oral care using tap water from contaminated hand hygiene sinks as a reservoir. *Am J Infect Control*. 2015; 43: 1249–1251.
7) Umezawa K, Asai S, Iwashita H, Minakawa Y, Ohashi M, Ohshima T, *et al*. Molecular characteristics of a series of clinical isolates of drug-resistant *Acinetobacter baumannii* ST219 strain: the implications of a sequence analysis of the $bla_{\text{OXA-51-like}}$. *J Emerg Med Trauma Surg Care*. 2016; 3: 017, 1–4.
8) Merz LR, Warren DK, Kollef MH, Fraser VJ. Effects of an antibiotic cycling program on antibiotic prescribing practices in an intensive care unit. *Antimicrob Agents Chemother*. 2004; 48: 2861–2865.
9) Cumpston A, Craig M, Hamadani M, Abraham J, Hobbs GR, Sarwari AR. Extended follow-up of an antibiotic cycling program for the management of febrile neutropenia in a hematologic malignancy and hematopoietic cell transplantation unit. *Transpl Infect Dis*. 2013; 15: 142–149.
10) Yanagihara K, Morinaga Y, Yamada K, Nakamura S, Kurihara S, Yamamoto K, *et al*. Efficacy of switch therapy in community-acquired pneumonia in Japan. *Jpn J Chemother*. 2009; 57: 423–429.
11) Miyachi H, Furuya H, Umezawa K, Itoh Y, Ohshima T, Miyamoto M, Asai S. Controlling methicillin-resistant *Staphylococcus aureus* by stepwise implementation of preventive strategies in a university hospital impact of a link-nurse system on the basis of multidisciplinary approaches. *Am J Infect Control*. 2007; 35: 115–121.
12) Iwashita H, Asai, S, Umezawa K, Ohashi M, Sasaki M, Ohshima T, *et al*. A study on effective disinfection of clinical contact surfaces in dental practice based on bacteriological cultures. (Jpn.) *Jpn J Environ Infect*. 2013; 28: 273–279.
13) Davis MF, Price LB, Liu CM, Silbergeld EK. An ecological perspective on U.S. industrial poultry production: the role of

anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments. *Curr Opin Microbiol*. 2011; 14: 244–250.
14) Joakim Larsson DGJ. Antibiotics in the environment. *Ups J Med Sci*. 2014; 119: 108–112.
15) Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*, 16th Informational supplement M100-S16. CLSI, Wayne, PA. 2006.
16) Yamaguchi K. Nationwide surveillance of parenteral antibiotics containing meropenem: activities against clinically isolated strains in 2004. *Jpn J Antibiot*. 2005; 58: 655–689. (Jpn)
17) Doi Y, Chambers HF: Other Lactam Antibiotics. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. PA: ELSEVIER; 2014: 293–297.
18) Takahashi M, Kaneko T, Taira H. Examination of risk factors influencing the detection of carbapenem-resistant *Pseudomonas aeruginosa*. *Jpn J Environment Infect*. 2010; 25: 104–110. (Jpn)
19) Kakeya H, Yamada K, Nakaie K, Takizawa E, Okada Y, Fujita A, *et al*. A comparison of susceptibility of *Pseudomonas aeruginosa* clinical isolates to carbapenem antibiotics in our hospital. *Jpn J Antibiot*. 2014; 67: 241–248. (Jpn)
20) El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A. Recent updates of carbapenem antibiotics. *Eur J Med Chem*. 2017; 131: 185e195.
21) Masuda N, Ohya S. Cross-resistance to meropenem, cephems, and quinolones in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 1992; 36: 1847–1851.
22) Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. *J Hosp Infect*. 2006; 64: 7–15.
23) Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev*. 2009; 22: 582–610.
24) Rodriguez-Martinez JM, Poirel L, Nordmann P. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicro Agenta Chemother*. 2009; 53: 4783–4788.
25) Poole K. *Pseudomonase aeruginosa* resistance to the max. *Front Microbiol*. 2011; 65: 1–13.
26) Van Limburg M, Shinha B, Lo-Ten-Fore JR, Van Gemert-Pijnen JE. Evaluation of early implementations of antibiotic stewardship program initiatives in nine Dutch hospitals. *Antimicrob Resist Infect Control*. 2014; 3: 33.
27) Dreier J, Ruggerone P. Interaction of antibacterial compounds with RND efflux pumps in *Pseudomonas aeruginosa*. *Front Microbiol*. 2015; 6: 660.
28) Harris AD, Bradham DD, Baumgarten M, Zuckerman IH, Fink JC, Perencevich EN. The use and interpretation of quasi-experimental studies in infectious diseases. *Clin Infect Dis*. 2004; 38: 1586–91.