Editors' Choice

ORIGINAL PAPER

Nagoya J. Med. Sci. **84**. 630–639, 2022
doi:10.18999/nagjms.84.3.630

Multicenter survey for carbapenemase-producing Enterobacterales in central Japan

Yuki Hara[1,2], Mitsutaka Iguchi[3], Nobuyuki Tetsuka[4], Hiroshi Morioka[3], Aki Hirabayashi[5], Masato Suzuki[5], Yuka Tomita[6], Keisuke Oka[3] and Tetsuya Yagi[1,3]

[1]*Department of Infectious Diseases, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[2]*Department of Clinical Laboratory, Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, Nagoya, Japan*
[3]*Department of Infectious diseases, Nagoya University Hospital, Nagoya, Japan*
[4]*Department of Infection control, Gifu University Graduate School of Medicine, Gifu, Japan*
[5]*Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan*
[6]*Department of Infection control, Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, Nagoya, Japan*

ABSTRACT

Carbapenemase-producing Enterobacterales (CPE) raise concerns about the treatment options for infectious diseases and infection control. We conducted a multicenter study to clarify the molecular epidemiology of CPE in the Aichi Prefecture during the first 3-month period from 2015 to 2019. Carbapenemase production was screened using a modified carbapenem inactivation method, and the genotypes of the carbapenemase genes were determined by polymerase chain reaction sequencing. Genetic relatedness was analyzed using multilocus sequence typing (MLST). Twenty-four hospitals participated in this study. Of the 56,494 Enterobacterales strains detected during the study period, 341 (0.6%) that met the susceptibility criteria were analyzed. Sixty-five of the 341 strains were determined to be CPE, with an incidence rate of 0.12% (65/56,494). The bacterial species responsible for CPE were *Klebsiella pneumoniae* (n = 24), *Enterobacter cloacae* complex (n = 23), *Klebsiella oxytoca* (n = 10), and *Escherichia coli* (n = 8). Most of the carbapenemase genotypes were IMP-1 (58/65), and only three were IMP-6 types. Three *E. coli* strains that produced NDM-5 were detected. MLST analysis showed that Sequence type (ST) 78 was predominant in *E. cloacae* complex CPE (14/23, 60.9%). Meanwhile, various STs were detected in carbapenemase-producing (CP) *K. pneumoniae*, of which ST37 and ST517 were the most common. The incidence rate of CPE in this region was comparable to national data. This 3-month surveillance revealed the spread of ST78 of CP *E. cloacae* complex and ST517 and ST592 of CP *K. pneumoniae* across hospitals, indicating the need to strengthen regional infection control programs.

Keywords: carbapenemase-producing Enterobacterales (CPE), carbapenem-resistant Enterobacterales (CRE), molecular epidemiology, multicenter survey

Abbreviations:
CPE: carbapenemase-producing Enterobacterales
CRE: carbapenem-resistant Enterobacterales
JANIS: Japan Nosocomial Infections Surveillance

Received: October 26, 2021; accepted: December 2, 2021

Corresponding Author: Tetsuya Yagi, MD, PhD
Department of Infectious Diseases, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
Tel: +81-52-744-2955, Fax: +81-52-744-2801, E-mail: tyagi@med.nagoya-u.ac.jp

MLST: multilocus sequence typing
ST: sequence type
CP: carbapenemase-producing

BACKGROUND

The rapid spread of carbapenemase-producing Enterobacterales (CPE) is a worldwide public health concern that poses a serious threat to clinical settings.[1] This is because there are limited antimicrobial agents that can be used for infectious diseases caused by CPE, which is associated with high mortality and expensive medical costs.[2,3] At present, CPE is endemic in Southeast Asia and some parts of Europe, but it is still rare in Japan.[4] Although the prevalence of carbapenem-resistant Enterobacterales (CRE) is low according to the report of the Japan Nosocomial Infections Surveillance (JANIS),[5] which is a nosocomial infection surveillance program conducted by the Ministry of Health, Labour and Welfare, the prevalence of CPE is not fully understood. Moreover, the molecular epidemiology of CPE in a specific region of Japan has not been elucidated, which has made it difficult to design and implement coordinated regional infection control measures.[6] In 2014, we initiated the present regional surveillance for CPE among major acute care hospitals in the Aichi Prefecture in the central region of Japan. This study aimed to analyze the molecular epidemiological characteristics of CPE collected during surveillance from 2015 to 2019.

METHODS

Study setting and study populations

A total of 24 major acute care hospitals in the Aichi Prefecture participated in the study, which comprised three university hospitals, six municipal hospitals, three national hospitals, and 12 private hospitals. The participating hospitals belong to the regional infection control network (Prefectural Infection Control Kasan-1 Network Inter–Conference). The institutional review boards at the Nagoya University Hospital have approved the study (approval number 2017-0396). Informed consent was obtained from all individual participants included in the study.

Bacterial isolates

The clinical microbiology laboratories in the participating hospitals collected all non-duplicate *Escherichia coli*, *Klebsiella* spp. (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella aerogenes*), and *Enterobacter cloacae* complex isolates that met the susceptibility criteria, as shown in Table 1, during the 3-month period from January to March of each year from 2015 to 2019. All isolates were sent to the laboratory for molecular analysis. Isolates of the same species detected from the same patient in different years were analyzed as separate isolates. Information about the sources of specimens, the results of bacterial identification and antimicrobial susceptibility testing, and the number of isolates of each Enterobacterales species for a 3-month period each year were collected.

Table 1 Bacterial isolates inclusion criteria

Strains	Including criteria
Escherichia coli, Klebsiella pneumoniae and *Klebsiella oxytoca*	Ceftriaxone or Ceftazidime resistant and Cefmetazole or Flomoxef resistant Ceftriaxone or Ceftazidime resistant and Imipenem or Meropenem MIC >= 2μg/mL
Enterobacter cloacae complex and *Klebsiella aerogenes*	Ceftriaxone or Ceftazidime resistant and Cefepime or Cefozopran or Cefpirome resistant Ceftriaxone or Ceftazidime resistant and Imipenem or Meropenem MIC >= 2μg/mL

MIC: minimum inhibitory concentration

Bacterial identification, antimicrobial susceptibility profile, and screening of carbapenemase production

All isolates collected in this study were re-identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry using the VITEK MS system (bioMérieux), as previously described.[7] If the results of re-identification were not consistent with the original one, the result of re-identification was adapted to the corresponding susceptibility criteria for the re-identified species to determine whether the isolate was subjected to further analyses. Minimum inhibitory concentrations of cefotaxime, ceftazidime, cefmetazole, flomoxef, imipenem, meropenem, cefepime, cefozopran, and cefpirome were determined according to the Clinical Laboratory Standards Institute (CLSI) M100 document[8] applied by each laboratory. Carbapenemase production was screened using the modified carbapenem inactivation method (mCIM) according to the CLSI description.[8]

Polymerase chain reaction and DNA sequence analysis of bla genes

The DNAs of all isolates were obtained using Cica Geneus® DNA extraction reagent (Kanto Chemical Holdings, Tokyo, Japan) and used as polymerase chain reaction (PCR) templates. Carbapenemase genes (bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-48}, bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58}) were screened in all mCIM-positive, intermediate, and carbapenem-resistant strains, regardless of the results of mCIM. Common extended-spectrum β-lactamases ($bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$, $bla_{CTX-M-8/25}$, bla_{TEM}, and bla_{SHV}) and AmpC (bla_{MOX}, bla_{DHA}, and bla_{CIT}) genes were screened in all collected strains using previously described primers.[9-11] The resultant PCR products were subjected to sequencing analysis for carbapenemase genes, which were consigned to Eurofins Genomics Inc. (Ohta-ku, Tokyo, Japan). Nucleotide sequences were compared and analyzed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Multilocus sequence typing

Multilocus sequence typing (MLST) analysis was performed with all carbapenemase-producing (CP) *K. pneumoniae* and *E. cloacae* complex isolates. Genomic DNA was isolated with Cica Geneus® DNA Extraction Reagent (Kanto Chemical, Tokyo, Japan) and used for the amplification of seven housekeeping genes using primer sets according to the previously reported method.[12,13] DNA sequencing was performed at a commercial laboratory (FASMAC, Kanagawa, Japan), and the consensus regarding the sequence type (ST) was determined using the *Enterobacter cloacae* locus/sequence definitions database (https://pubmlst.org/bigsdb?db=pubmlst_ecloacae_seqdef) and *K. pneumoniae* database (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

Whole-genome sequencing

Additional analysis was performed on strains for which MLST was not performed using the aforementioned method. The isolates were subjected to whole-genome sequencing analysis on a NovaSeq 6000 system (Illumina, San Diego, CA, USA) using the Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA). De novo assembly was performed using CLC Genomics Workbench version 12.0 with default parameters. MLST analysis was performed using MLST version 2.0.4 on the Center for Genomic Epidemiology server at the Technical University of Denmark (http://www.genomicepidemiology.org).

RESULTS

Surveillance data

During the study period, 56,494 Enterobacterales strains were detected in 24 participating hospitals, and 360 strains that met the susceptibility criteria were sent to our laboratory. Nineteen strains were excluded from the study because 10 isolates did not match the antimicrobial susceptibility criteria, seven isolates were misidentified, and two isolates did not grow from the stock. More importantly, 341 strains met the inclusion criteria, the number of which was predominant in the *E. cloacae* complex (n = 134), followed by *E. coli* (n = 96), *K. pneumoniae* (n = 62), *K. aerogenes* (n = 38), and *K. oxytoca* (n = 11). A total of 65 out of 341 (19.1%) strains were identified as CPE and were isolated from 14 hospitals. CPE was detected in five hospitals in 2015 and 2016, which increased to seven hospitals in 2017 and 2018. However, this number decreased to five in 2019. The distribution of CPE among Enterobacterales was *K. pneumoniae* (n = 24), followed by *E. cloacae* complex (n = 23), *K. oxytoca* (n = 10), and *E. coli* (n = 8), with no CPE found in *K. aerogenes*. The annual trend of the CPE detection rates is shown in Table 2, which indicates that CP *K. pneumoniae* and *E. cloacae* complex were increasingly detected from 2015 to 2017 and began to decrease thereafter. No significant changes were observed in CP *E. coli*.

Table 2 Incidence rates of carbapenemase-producing Enterobacterales

Bacterial strains	Incidence rates of carbapenemase-producing Enterobacterales					5 year average incidence rates
	2015 Jan–March	2016 Jan–March	2017 Jan–March	2018 Jan–March	2019 Jan–March	
Escherichia coli	2/5,559 (0.04%)	1/6,475 (0.02%)	1/6,756 (0.02%)	1/7,129 (0.01%)	3/7,269 (0.04%)	0.03%
Klebsiella pneumoniae	6/2,583 (0.23%)	4/2,453 (0.16%)	10/2,817 (0.35%)	4/2,712 (0.14%)	0/2,689 (0%)	0.18%
Klebsiella oxytoca	0/717 (0%)	2/712 (0.28%)	2/874 (0.23%)	4/752 (0.5%)	2/786 (0.25%)	0.25%
Klebsiella aerogenes	0/408 (0%)	0/396 (0%)	0/469 (0%)	0/532 (0%)	0/463 (0%)	0%
Enterobacter cloacae complex	5/907 (0.55%)	5/698 (0.72%)	8/808 (0.99%)	4/836 (0.48%)	1/694 (0.14%)	0.58%
Total	13/10,174 (0.13%)	12/10,734 (0.11%)	21/11,724 (0.18%)	13/11,961 (0.11%)	6/11,901 (0.05%)	0.11%

Carbapenemase genes

The results for carbapenemase gene of CPE have shown in Table 3. IMP-type carbapenemase genes were detected in 62 out of 65 CPE isolates, most of which were IMP-1 producers (n = 58), and three were IMP-6 producers that were detected in *K. pneumoniae* isolates. Three NDM-5 CP *E. coli* strains were identified in 2016 and 2019.

Table 3 Carbapenemase genes of carbapenemase-producing Enterobacterales isolates

Bacterial strain	n	Year				
		2015 Jan–March	**2016 Jan–March**	**2017 Jan–March**	**2018 Jan–March**	**2019 Jan–March**
Escherichia coli	8	$bla_{\text{IMP-1}}$ (2)	$bla_{\text{NDM-5}}$ (1)	$bla_{\text{IMP-1}}$ (1)	$bla_{\text{IMP-1}}$ (1)	$bla_{\text{NDM-5}}$ (2) $bla_{\text{IMP-1}}$ (1)
Klebsiella pneumoniae	24	$bla_{\text{IMP-1}}$ (4) $bla_{\text{IMP-6}}$ (1) bla_{IMP} (1)	$bla_{\text{IMP-1}}$ (4)	$bla_{\text{IMP-1}}$ (8) $bla_{\text{IMP-6}}$ (2)	$bla_{\text{IMP-1}}$ (4)	none
Klebsiella oxytoca	10	none	$bla_{\text{IMP-1}}$ (2)	$bla_{\text{IMP-1}}$ (2)	bla$_{\text{IMP-1}}$ (4)	$bla_{\text{IMP-1}}$ (2)
Klebsiella aerogenes	0	none	none	none	none	none
Enterobacter cloacae complex	23	$bla_{\text{IMP-1}}$ (5)	$bla_{\text{IMP-1}}$ (5)	$bla_{\text{IMP-1}}$ (8)	bla$_{\text{IMP-1}}$ (4)	$bla_{\text{IMP-1}}$ (1)
Total	65	$bla_{\text{IMP-1}}$ (11) $bla_{\text{IMP-6}}$ (1) bla_{IMP} *(1)	$bla_{\text{IMP-1}}$ (11) $bla_{\text{NDM-5}}$ (1)	$bla_{\text{IMP-1}}$ (19) $bla_{\text{IMP-6}}$ (2)	$bla_{\text{IMP-1}}$ (13)	$bla_{\text{IMP-1}}$ (4) $bla_{\text{NDM-5}}$ (2)

*; Because we lost the strains, sequencing and multi locus sequence typing analysis could not be performed.

Multilocus sequence typing of carbapenemase-producing Klebsiella pneumoniae and Enterobacter cloacae complex

K. pneumoniae and *E. cloacae* complex were included in the MLST analysis because they were the most commonly detected species and were detected in several hospitals in the region. MLST analysis of *E. cloacae* complex identified seven unique STs, as shown in Table 4. The most predominant ST was ST78, accounting for 15 (65.2%) of the 23 isolates, followed by ST513 (2/23, 8.7%) and ST113 (2/23, 8.7%). ST25, ST29, ST53, and ST133 were found in a single isolate. Although most ST78 *E. cloacae* complex isolates were detected in Hospital A during the study period, they were also found in Hospital D in 2015, Hospital J in 2018, and Hospital H in 2019.

Table 4 Sequence-typing distribution of carbapenemase-producing *Enterobacter cloacae* complex

Hospital	n	Year				
		2015 Jan–March	2016 Jan–March	2017 Jan–March	2018 Jan–March	2019 Jan–March
Hospital A	14	ST78 (1)	ST78 (4)	ST78 (6) ST25(1)	ST133(1) ST78(1)	none
Hospital B	5	ST113(1) ST513(2)	none	ST53(1)	ST113(1)	none
Hospital C	1	none	ST29(1)	none	none	none
Hospital D	1	ST78(1)	none	none	none	none
Hospital H	1	none	none	none	none	ST78 (1)
Hospital J	1	none	none	none	ST78 (1)	none
Total	23	ST78 (2) ST513(2) ST113(1)	ST78 (4) ST29(1)	ST78 (6) ST25(1) ST53(1)	ST78 (2) ST113(1) ST133(1)	ST78(1)

The numbers in parentheses indicate the number of isolates.
ST: sequence typing

K. pneumoniae CPE strains were divided into eight different STs using MLST, of which the most frequent types were ST517 (6/23, 26.1%) and ST37 (6/23, 26.1%), followed by ST716 (4/23, 17.4%), ST3012, and ST592 (both 2/23, 8.7%) (Table 5). Accordingly, ST70, ST2158, and ST461 were identified in a single isolate. Although each ST was found in a specific hospital, ST592 isolates were detected in Hospitals F and G in 2017. ST517 was predominant in Hospital A; however, it was also detected in Hospital G in 2019.

Table 5 Sequence-typing distribution of carbapenemase-producing *Klebsiella pneumoniae*

Hospital	n	Year			
		2015 Jan–March	2016 Jan–March	2017 Jan–March	2018 Jan–March
Hospital A	7	ST517 (2)	ST517 (1)	ST3012 (2) ST517 (1)	ST517 (1)
Hospital E	7	ST37(2)	ST37 (3)	ST2158 (1)	ST37 (1)
Hospital F	2	ST70 (1)	none	ST592 (1)	none
Hospital G	2	none	none	ST592 (1)	ST517 (1)
Hospital H	1	none	none	ST461 (1)	none
Hospital I	4	none	none	ST716 (3)	ST716 (1)
Total	23	ST517 (2) ST37 (2) ST70 (1)	ST37 (3) ST517 (1)	ST716 (3) ST3012 (2) ST592 (2) ST461 (1) ST517 (1) ST2158 (1)	ST517 (2) ST716 (1) ST37 (1)

The numbers in parentheses indicate the number of isolates.
ST: sequence typing

DISCUSSION

We showed the results of a multicenter 3-month surveillance for CPE from 2015 to 2019 in the Aichi Prefecture, located in the central district of Japan. The results of this study provide important information. First, the incidence rate of CPE in this region was 0.05–0.18% throughout the study period, which was comparable with the national presumptive rate of CPE (approximately 0.1%). As the national surveillance, JANIS, has been collecting data on CRE, which includes both CPE and non-CP CRE, it is difficult to determine the precise trend of laboratory-based detection for CPE in Japan. Second, the distribution of CPE among Enterobacterales and the types of carbapenemases in this region were determined. Third, according to MLST analysis, several specific STs of the CP *E. cloacae* complex and *K. pneumoniae* are spreading across hospitals, indicating the necessity of a coordinated regional infection control program.

In the present study, CPE was mostly detected, in the actual number of isolates, in *K. pneumoniae*, followed by *E. cloacae* complex, *K. oxytoca*, and *E. coli*, which illustrates a similar detection pattern to National Institute of Infectious Diseases reports.[14,15] However, in terms of the 5-year incidence rate, *E. cloacae* complex had the highest incidence rate, followed by *K. oxytoca*, *K. pneumoniae*, and *E. coli*. The detection rate of CP *E. cloacae* complex was high in 2016 and 2017. This may be due to the detection of multiple CP *E. cloacae* complex in some hospitals, and the detection rate of CP *E. cloacae* complex decreased in 2018 and 2019. The detection rate of CP *K. pneumoniae* also showed a significant increase in 2017 when 10 CP *K. pneumoniae* were isolated from six hospitals. However, the detection rate has decreased since 2018. Thus, the increase in CP *E. cloacae* complex and CP *K. pneumoniae* was considered temporary. Monitoring the annual detection rate of CPE would be more desirable for their precise epidemiology. The relatively high incidence rate of CPE in *K. oxytoca* appeared to be characteristic, and further investigation is necessary.

For the most part, the genotype of carbapenemase was IMP-1 type, with a few exceptional isolates of IMP-6-producing *K. pneumoniae* and NDM-5-producing *E. coli*. Another study from a primary care hospital in the Kinki District showed that all CPE isolates produced IMP-6, a variant of IMP-1, which was first detected in a *Serratia marcescens* isolate in Japan.[16] Recently, the wide spread of IMP-type carbapenemases has been reported mainly in Taiwan and eastern China, whereas there are also sporadic reports from other countries.[17] IMP-6, which was found in 2001,[18] was more resistant to meropenem than imipenem and was distributed mainly in the western region, including the Kinki District.[19] These showed the regional differences in the epidemiology of CPE and the type of carbapenemases in Japan. Therefore, regional molecular epidemiology and institutional surveillance are essential for designing an infection control program in each hospital. For example, it is necessary to conduct region-wide surveillance that includes representative post-acute care hospitals, rehabilitation hospitals, long-term care facilities, and nursing homes. This will enable a more precise understanding of the epidemiology of resistant bacteria in the region. Furthermore, collaboration and information sharing with regional public health centers, public health research institutes, and medical and welfare departments of the government are critically important.

MLST analysis showed that ST78 was the most prevalent in *E. cloacae* complex CPE and spread to four different hospitals. A similar observation was reported in other regions of Japan,[20] which showed that the number of hospitals where ST78 was detected has increased since 2018. Since *E. cloacae* complex ST78 is considered to be one of the high-risk clones,[21] continuous monitoring of the trends of this specific clone in our region is necessary. According to a report by Tetsuka et al,[22] CP *E. cloacae* complex was likely to be transmitted horizontally in a hospital setting. In contrast to carbapenemase-non-producing carbapenem-resistant *E. cloacae* complex,

strict contact precautions should be implemented to prevent the transmission of this clone in hospitals.

As for CP *K. pneumoniae*, specific STs, such as ST258, ST147, or ST11, which were recognized as high-risk clones worldwide,[23-25] were not detected in this study. However, ST37, which is distributed worldwide in relation to carbapenemase production,[26,27] was one of the most dominant clones identified in this study. Caution should be paid to in-hospital transmission of CP *K. pneumoniae* ST37, although current epidemiological studies in this region showed that ST37 had been detected in only one hospital, with no apparent spread between hospitals.

This study has some limitations. First, we did not measure antimicrobial susceptibility of the isolates. Therefore, some strains might have been excluded according to the susceptibility criteria for inclusion, and the CPE detection rate might have been overestimated. Second, we collected isolates in only a 3-month period in a year, which possibly caused the CPE clones to spread further. Third, because some of the hospitals with in-house clinical microbiology laboratories in this region participated in this study, we were not able to cover the full situation of CPE in this region.

In conclusion, we revealed the molecular epidemiology and resistance genes of CPE in the Aichi Prefecture in Central Japan. In our region, the incidence rate of CPE was comparable with the national data, and the inter-hospital spread of ST78 of CP *E. cloacae* complex was detected even with a limited, 3-month annual survey. Continuing the regional molecular epidemiological surveillance and monitoring the prevalence of high-risk clones of CPE are essential for strengthening both in-hospital and regional infection control programs to prevent CPE transmission.

ACKNOWLEDGMENTS

We would like to thank the participating members of the Prefectural Infection Control Kasan-1 Network Inter-Conference, a regional infection control network in the Aichi Prefecture. for providing the strains used in the present study: Atsushi Izumi (Higashinagoya National Hospital), Atsushi Kawabata (Toyota Memorial Hospital), Hiroshige Mikamo, Yuka Yamagishi (Aichi Medical University), Junko Fukuhara (Chubu Rosai Hospital), Kenji Inagaki (JCHO Chukyo Hospital), Kiyohito Ishikawa (Fujita Health University), Masami Asai (Daido Hospital), Masao Katayama (Nagoya Medical Center), Masahiro Hirose (Fujita Health University Bantane Hospital), Mitsuru Nishio (Komaki City Hospital), Norihiro Seko (Asahi Rosai Hospital), Satoshi Kayukawa (Nagoya Memorial Hospital), Susumu Iwata (Kasugai Municipal Hospital), Takahumi Niwa (Handa City Hospital), Takayuki Ohe (Gamagori City Hospital), Takeshi Tsuji (Okazaki City Hospital), Toshihiko Yokoyama (Japanese Red Cross Aichi Medical Center Nagoya Daiichi Hospital), Yuichi Kitagawa (National Center for Geriatrics and Gerontology), Yoshikazu Muto (Tosei General Hospital), Yuki Nagata (Toyota Kosei Hospital), and Yasuyuki Sugiura (Anjyo Kosei Hospital). We would like to thank Editage (www.editage.com) for English language editing.

FUNDING

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan (grant no.: H28-Shinkogyousei-ippan-004, 19HA1004) and grant support from MSD K.K. and SHIONOGI & Co., Ltd.

CONFLICT OF INTEREST

The authors declare they hold no conflict of interest in relation to this project.

REFERENCES

1 van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence*. 2017;8(4):460–469. doi:10.1080/21505594.2016.1222343.

2 Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. *Infect Control Hosp Epidemiol*. 2008;29(12):1099–1106. doi:10.1086/592412.

3 Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci Transl Med*. 2012;4(148):148ra116. doi:10.1126/scitranslmed.3004129.

4 Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis*. 2011;11(5):355–362. doi:10.1016/S1473-3099(11)70059-7.

5 Ministry of Health Labour and Welfare, Japan Nosocomial Infections Surveillance Ministry of Health, Labour and Welfare. https://janis.mhlw.go.jp/english/index.asp. Accessed November 1, 2018.

6 Friedman ND, Carmeli Y, Walton AL, Schwaber MJ. Carbapenem-resistant enterobacteriaceae: A strategic roadmap for infection control. *Infect Control Hosp Epidemiol*. 2017;38(5):580–594. doi:10.1017/ice.2017.42.

7 Richter SS, Sercia L, Branda JA, et al. Identification of Enterobacteriaceae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system. *Eur J Clin Microbiol Infect Dis*. 2013;32(12):1571–1578. doi:10.1007/s10096-013-1912-y.

8 Melvin PW, Jean BP, Shelly C, et al, eds. *M100 Performance Standards for Antimicrobial Susceptibility Testing, 28th edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

9 Bogaerts P, Rezende de Castro R, de Mendonça R, Huang TD, Denis O, Glupczynski Y. Validation of carbapenemase and extended-spectrum -lactamase multiplex endpoint PCR assays according to ISO 15189. *J Antimicrob Chemother*. 2013;68(7):1576–1582. doi:10.1093/jac/dkt065.

10 Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. *J Antimicrob Chemother*. 2010;65(3):490–495. doi:10.1093/jac/dkp498.

11 Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol*. 2002;40(6):2153–2162. doi:10.1128/JCM.40.6.2153-2162.2002.

12 Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, Shimojima M, Kirikae T. Multilocus sequence typing (MLST) for characterization of *Enterobacter cloacae*. *PLoS One*. 2013;8(6):e66358. doi:10.1371/journal.pone.0066358.

13 Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol*. 2005;43(8):4178–4182. doi:10.1128/JCM.43.8.4178-4182.2005.

14 National Institute of Infectious Diseases. Surveillance of carbapenemresistant Enterobacteriaceae 2018. *I A S R*. 2019;40:157-158.

15 National Institute of Infectious Diseases. Surveillance of carbapenemresistant Enterobacteriaceae 2019. *I A S R*. 2021;42:123–124.

16 Ito H, Arakawa Y, Ohsuka S, Wacharotayankun R, Kato N, Ohta M. Plasmid-mediated dissemination of the metallo-beta-lactamase gene bla_{IMP} among clinically isolated strains of *Serratia marcescens*. *Antimicrob Agents Chemother*. 1995;39(4):824–829. doi:10.1128/AAC.39.4.824.

17 Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect*. 2014;20(9):821–830. doi:10.1111/1469-0691.12719.

18 Yano H, Kuga A, Okamoto R, Kitasato H, Kobayashi T, Inoue M. Plasmid-encoded metallo-β-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. *Antimicrob Agents Chemother*. 2001;45(5):1343–1348. doi:10.1128/AAC.45.5.1343-1348.2001.

19 National Institute of Infectious Diseases. Surveillance of carbapenemresistant Enterobacteriaceae 2017. *I A S R*. 2018;39:162–163.

20 Aoki K, Harada S, Yahara K, et al. Molecular characterization of IMP-1-producing *Enterobacter cloacae* complex isolates in Tokyo. *Antimicrob Agents Chemother*. 2018;62(3):e02091–e02117. doi:10.1128/AAC.02091-17.

21 Gomez-Simmonds A, Annavajhala MK, Wang Z, et al. Genomic and geographic context for the evolution of high-risk carbapenem-resistant *Enterobacter cloacae* complex clones ST171 and ST78. *mBio*. 2018;9(3):e00542–e00618. doi:10.1128/mBio.00542-18.
22 Tetsuka N, Hirabayashi A, Matsumoto A, et al. Molecular epidemiological analysis and risk factors for acquisition of carbapenemase-producing *Enterobacter cloacae* complex in a Japanese university hospital. *Antimicrob Resist Infect Control*. 2019;8:126. doi:10.1186/s13756-019-0578-3.
23 Pitout JDD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother*. 2015;59(10):5873–5884. doi:10.1128/AAC.01019-15.
24 Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol*. 2014;22(12):686–696. doi:10.1016/j.tim.2014.09.003.
25 Gu D, Dong N, Zheng Z, et al. A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. *Lancet Infect Dis*. 2018;18(1):37–46. doi:10.1016/S1473-3099(17)30489-9.
26 Yang J, Ye L, Guo L, et al. A nosocomial outbreak of KPC-2-producing *Klebsiella pneumoniae* in a Chinese hospital: dissemination of ST11 and emergence of ST37, ST392 and ST395. *Clin Microbiol Infect*. 2013;19(11):E509–E515. doi:10.1111/1469-0691.12275.
27 Zhu J, Sun L, Ding B, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* ST76 and ST37 isolates in neonates. *Eur J Clin Microbiol Infect Dis*. 2016;35(4):611–618. doi:10.1007/s1009.