Multicenter survey for carbapenemase-producing Enterobacterales in central Japan

Yuki Hara1,2, Mitsutaka Iguchi3, Nobuyuki Tetsuka4, Hiroshi Morioka3, Aki Hirabayashi5, Masato Suzuki5, Yuka Tomita6, Keisuke Oka3 and Tetsuya Yagi1,3

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

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

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BACKGROUND

The rapid spread of carbapenemase-producing Enterobacterales (CPE) is a worldwide public health concern that poses a serious threat to clinical settings. 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. At present, CPE is endemic in Southeast Asia and some parts of Europe, but it is still rare in Japan. Although the prevalence of carbapenem-resistant Enterobacterales (CRE) is low according to the report of the Japan Nosocomial Infections Surveillance (JANIS), 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. 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.
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. 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 applied by each laboratory. Carbapenemase production was screened using the modified carbapenem inactivation method (mCIM) according to the CLSI description.

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 (blaKPC, blaGES, bladaa, blaNDM, blaOXA-48, blaOXA-23, blaOXA-24, and blaOXA-55) were screened in all mCIM-positive, intermediate, and carbapenem-resistant strains, regardless of the results of mCIM. Common extended-spectrum β-lactamases (blaCTX-M-1, blaCTX-M-2, blaCTX-M-9, blaCTX-M-8/25, blaTEM, and blaSHV) and AmpC (blaSOX, blATRA, and blaCIT) genes were screened in all collected strains using previously described primers. 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. 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*.

| Bacterial strains         | 2015 Jan–March | 2016 Jan–March | 2017 Jan–March | 2018 Jan–March | 2019 Jan–March | 5 year average incidence rates |
|---------------------------|----------------|----------------|----------------|----------------|----------------|-------------------------------|
| *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*    | 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%                         |

Table 2 Incidence rates of carbapenemase-producing Enterobacterales
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.

| Bacterial strain | n | 2015 Jan–March | 2016 Jan–March | 2017 Jan–March | 2018 Jan–March | 2019 Jan–March |
|------------------|---|----------------|----------------|----------------|----------------|----------------|
| Escherichia coli | 8 | blαIMP-1 (2)   | blαNDM-5 (1)   | blαIMP-1 (1)   | blαIMP-1 (1)   | blαNDM-5 (2)   |
| Klebsiella pneumoniae | 24 | blαIMP-1 (4) | blαIMP-1 (1) | blαIMP-1 (8) | blαIMP-6 (2) | blαIMP-1 (4) | none |
| Klebsiella oxytoca | 10 | none | blαIMP-1 (2) | blαIMP-1 (2) | blαIMP-1 (4) | blαIMP-1 (2) |
| Klebsiella aerogenes | 0 | none | none | none | none | none |
| Enterobacter cloacae complex | 23 | blαIMP-1 (5) | blαIMP-1 (5) | blαIMP-1 (8) | blαIMP-1 (4) | blαIMP-1 (1) |
| Total | 65 | blαIMP-1 (11) | blαIMP-6 (1) | blαNDM-5 (1) | blαIMP-1 (19) | blαIMP-1 (13) | blαNDM-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.
Molecular characteristics of CPE

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

| Hospital | n  | 2015 Jan–March | 2016 Jan–March | 2017 Jan–March | 2018 Jan–March | 2019 Jan–March |
|----------|----|----------------|----------------|----------------|----------------|----------------|
|          |    | 2015 Jan–March | 2016 Jan–March | 2017 Jan–March | 2018 Jan–March | 2019 Jan–March |
| Hospital A | 14 | ST78 (1)       | ST78 (4)       | ST78 (6)       | ST133 (1)      | none           |
| Hospital B | 5  | ST113 (1)      | 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 E | 1  | none           | ST29 (1)       | none           | none           | none           |
| Hospital F | 1  | ST78 (1)       | ST78 (4)       | ST78 (6)       | ST78 (2)       | ST78 (1)       |
| Hospital G | 2  | none           | ST78 (6)       | ST78 (2)       | ST78 (1)       | none           |
| Hospital H | 1  | none           | none           | none           | none           | ST78 (1)       |
| Hospital I | 1  | none           | ST78 (6)       | ST78 (2)       | ST78 (1)       | none           |
| Total     | 23 | ST78 (2)       | ST78 (6)       | ST78 (2)       | ST78 (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  | 2015 Jan–March | 2016 Jan–March | 2017 Jan–March | 2018 Jan–March | 2019 Jan–March |
|----------|----|----------------|----------------|----------------|----------------|----------------|
|          |    | 2015 Jan–March | 2016 Jan–March | 2017 Jan–March | 2018 Jan–March | 2019 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)       | none           |
| Hospital F | 2  | ST70 (1)       | none           | ST592 (1)      | none           | none           |
| Hospital G | 2  | none           | none           | ST592 (1)      | ST517 (1)      | none           |
| Hospital H | 1  | none           | none           | ST461 (1)      | none           | none           |
| Hospital I | 4  | ST517 (2)      | none           | ST716 (3)      | ST716 (1)      | ST716 (1)      |
| Total     | 23 | ST517 (2)      | ST37 (3)       | ST716 (3)      | ST716 (1)      | ST716 (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. 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. 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. IMP-6, which was found in 2001, was more resistant to meropenem than imipenem and was distributed mainly in the western region, including the Kinki District. 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, 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, continuous monitoring of the trends of this specific clone in our region is necessary. According to a report by Tetsuka et al., 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, were not detected in this study. However, ST37, which is distributed worldwide in relation to carbapenemase production, 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.

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CONFLICT OF INTEREST

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

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Molecular characteristics of CPE

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