Institutional outbreak involving multiple clades of IMP-producing *Enterobacter cloacae* complex sequence type 78 at a cancer center in Tokyo, Japan

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Abstract

Background: Information about the clinical and microbiological characteristics of IMP-producing Enterobacterales has been limited. Here, we describe an institutional outbreak of IMP-producing Enterobacter cloacae complex (ECC) involving multiple clades of ECC sequence type (ST) 78 strains.

Methods: Antimicrobial susceptibility testing, whole-genome sequencing, and conjugation experiments of 18 IMP-producing ECC strains isolated during four-year study period were performed. Species and subspecies were determined by average nucleotide identity analysis and clonal relatedness of the isolates was analyzed with multilocus sequence typing and core-genome single nucleotide polymorphism (SNP) analysis. Relevant clinical information was extracted from medical records.

Results: Fourteen of 18 IMP-producing ECC isolates were determined as Enterobacter hormaechei ST78. Twelve isolates, including 10 isolates belonging to ST78, carried blaIMP-1 in In316-like class 1 integron located on IncHI2 plasmids. Conjugation experiments were successful for 12 isolates carrying blaIMP-1 on IncHI2 plasmids and for an isolate carrying blaIMP-11 on an IncL/M plasmid. Although isolation of ST78 strains was clustered in a 16-months period suggesting nosocomial transmission, these strains were subdivided into three clades by SNP analysis: clade A (n = 10), clade B (n = 1), clade C (n = 3). A part of clonal relatedness was unexpected by the epidemiological information at the time of isolation of the strains. Most of the IMP-producing ECC strains were susceptible to non-β-lactam antibiotics and had relatively low minimum inhibitory concentrations to carbapenems (≤4 μg/mL). Four of five infections caused by IMP-producing ECC were treated successfully.

Conclusions: Whole-genome sequencing analysis revealed the outbreak was caused by three different clades of ST78 strains, where patients had favorable treatment outcome of the infections compared with that caused by Enterobacterales producing other carbapenemases, possibly due to their non-multidrug-resistant phenotype.

Background

Carbapenem-resistant *Enterobacterales* (CRE) has been spreading globally during the last decade and acknowledged as an imminent risk for public health due to the limited treatment options for the infections caused by the organisms [1]. Among the various mechanisms of carbapenem resistance in *Enterobacterales*, production of carbapenemases is clinically the most important. Carbapenemase-producing *Enterobacterales* (CPE) may have a higher risk of detrimental outcomes from invasive infections and of spreading resistance genes in healthcare facilities by clonal expansion or conjugative transfer of resistance plasmids compared with non-carbapenemase-producing CRE [2, 3]. KPC enzymes belonging to Ambler class A, IMP, VIM, NDM enzymes (metallo-b-lactamases: MBLs) belonging to Ambler class B, and OXA-48-group enzymes belonging to Ambler class D are the major carbapenemases produced by *Enterobacterales*. Although KPC enzymes are the most common globally, epidemiology of carbapenemases produced by *Enterobacterales* differs in each region and country of the world [4].

Carbapenem resistance among *Enterobacterales* clinical strains has been relatively infrequent in Japan. A national surveillance conducted by National Institute of Infectious Diseases reported that resistance rates to meropenem of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* complex (ECC) in 2018 were 0.1%, 0.5%, and 1.1%, respectively (https://janis.mhlw.go.jp/english/report/open_report/2018/3/1/ken_Open_Report_Eng_201800_clsi2012.pdf). Although IMP enzymes have been overwhelmingly dominant among carbapenemases produced by *Enterobacterales* in Japan, major species of CPE is different in each geographic area in Japan. While IMP-producing ECC is most common in Tokyo, IMP-producing *K. pneumoniae* and *E. coli* are more common around Osaka [5, 6].

While transmission dynamics of KPC-producing *Enterobacterales* in healthcare facilities have been reported abundantly in the literature, information about transmission of IMP-producing *Enterobacterales* is scarce [7-9]. In addition, characteristics and prognosis of infections caused by IMP-producing *Enterobacterales* remain to be clarified. Although recent global clinical studies have addressed treatment of infections caused by CPE, few cases of infections caused by IMP-producing *Enterobacterales* were included due to their rarity [10].

Starting in July 2014, an outbreak of IMP-producing ECC occurred at a cancer center in Tokyo, Japan over 16-month period. Here, we report the clinical characteristics of infections caused by IMP-producing *Enterobacterales* and the results of microbiological and molecular analysis to infer the route of transmission.

Methods

Setting and design

This is a descriptive study of an institutional outbreak of CPE at a 700-bed cancer center in Tokyo, Japan. It provides care for patients with all type of malignancies. Annually, 17,500 patients are hospitalized with a mean of 12 days. All the CPE isolates between January 2014 and December 2017 were analyzed. In addition, clinical and epidemiological investigation was performed for all the patients who had CPE isolated.

Clinical data collection

Clinical information of the patients with CPE was extracted from the medical records retrospectively and included age, sex, type of malignancy and other comorbidities, date of the first isolation of CPE, type of sample from which CPE was isolated, hospitalized ward, department caring the patient, clinical

significance of the CPE isolates (infection or colonization), use of antimicrobial agents within 30 days prior to the isolation of CPE, type and date of surgery within 90 days prior to the isolation, type of endoscopy within 90 days prior to the isolation, cancer chemotherapy within 90 days prior to the isolation, admission to the hospital during the study period and to other hospitals within a year prior to the isolation, and death within 30 and 90 days after the isolation of CPE. Additionally, the type of infection, antimicrobial treatment, necessity and achievement of source control, and prognosis were reviewed for the cases with infections caused by CPE. Infection and colonization were determined according to the CDC definition [11].

Identification of carbapenemase-producing *Enterobacterales* at the hospital

Routine bacterial identification and antimicrobial susceptibility testing were performed with MicroScan WalkAway (Beckman Coulter, Brea, CA, USA) at the hospital. The results of antimicrobial susceptibility testing were interpreted with CLSI M100-S17 guidelines in 2014, and with CLSI M100-S22 guidelines, in which lower breakpoints of cephalosporins and carbapenems for *Enterobacterales* were adopted, from 2015 through 2017 [12, 13]. Testing for carbapenemase production was performed on the isolates showing non-susceptibility against cefepime or any carbapenems. Carbapenemase production (focusing on MBLs) was confirmed with ceftazidime 30-mg disks (Eiken Chemical, Tokyo, Japan) and sodium mercaptoacetate (SMA) 3-mg disks (Eiken Chemical, Tokyo, Japan). ECC isolates showing enlargement of inhibitory zone diameters around the ceftazidime disk by >5 mm when it was located adjacent to an SMA disk were determined to be carbapenemase-producing *Enterobacterales* [14]. Additionally, modified carbapenem inactivation method (mCIM) according to CLSI M100-S27 guidelines was performed after April 2017 [15].

Active surveillance culture for CPE

A ward-wide active surveillance for multidrug-resistant *Enterobacterales* was conducted for the selected wards intermittently during the study period. All patients except those hospitalized less than three days were included. Stool or rectal swab samples were obtained. If patients had urinary catheters placed, urine was collected. Because epidemiological investigation suggested possible transmission associated with respiratory procedures among post-surgical patients in Surgery-B department, throat swab was also collected from those patients. Culture was performed using CHROMagar ESBL (Kanto Chemical, Tokyo, Japan). Antimicrobial susceptibility testing was performed on the isolates grown on the agar and CPE was identified with the same protocol as clinical culture.

Microbiological and molecular analysis

All CPE isolates detected first from each patient at the hospital were collected and analyzed at a research laboratory. Antimicrobial susceptibility testing was conducted with BD Phoenix NMIC/ID-208 panel (BD Diagnostics, Sparks, Maryland, USA) and results were interpreted according to CLSI M100-S27 guidelines [15]. Carriage of $bla_{\text{IMP-1-group}}$ genes was screened by PCR as described previously [16]. Whole genome sequencing was performed with Illumina Miseq (Illumina, San Diego, California, USA). Genomic DNA libraries were prepared with Nextera XT DNA library preparation kit (Illumina) and were sequenced for 600 cycles (300-bp paired-end reads). Raw reads generated by Miseq were quality trimmed with Trimmomatic tool (version 0.38) and assembled using SPAdes (version 3.12.0). Since all CPE isolates were identified as ECC or related species by MicroScan WalkAway at the hospital, species and subspecies were determined by comparing the average nucleotide identity (ANI) of the genomes of study isolates with those of type strains of ECC using a threshold of >96.5% at ANI Calculator of EZ BioCloud website (https://www.ezbiocloud.net/tools/ani) [17]. Multilocus sequence typing (MLST), plasmid replicon typing, plasmid MLST, and screening of acquired resistance genes were conducted with MLST 1.8, PlasmidFinder 1.3, pMLST 1.4, and ResFinder 3.0, respectively, at Centers for Genomic Epidemiology website (https://cge.cbs.dtu.dk/services/). Structures of integrons were analyzed with Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using nucleotide sequences of contigs containing $bla_{\text{IMP-1-group}}$. Core-genome single nucleotide polymorphism (SNP)-based phylogenetic analysis of ST78 isolates of ECC was performed as described previously using genomic sequence of *E. cloacae* ECNIH3 ST93 (GenBank accession number CP008897) as the reference [6]. Conjugation experiments were carried out with filter mating methods using non-lactose-fermenting *E. coli* ML4909 strain (a rifampin-resistant mutant derived from *E. coli* K-12) as a recipient. Transconjugants were selected on Drigalski lactose agar supplied with moxalactam (16 mg/mL) and rifampin (100 mg/ml). The conjugation experiment was attempted three times for each donor isolate. Carriage of $bla_{\text{IMP-1-group}}$ were confirmed with PCR and PCR-based replicon typing of the plasmids were performed for transconjugants [18].

GenBank accession number

All nucleotide sequences of draft genomes have been deposited in the NCBI database under BioProject accession number PRJDB9939.

Results

Outbreak description

In July 2014, CPE was detected from a hospitalized patient at the cancer center for the very first time. In total, 18 hospitalized patients were found to have CPE during the study period confirmed by clinical culture (n = 13) or surveillance culture (n = 5) (Table 1). Seventeen and one isolates were identified as *E. claocae* and *Cronobacter sakazakii*, respectively, with MicroScan WalkAway. All isolates were positive for production of MBLs by the SMA disk testing. Date of first isolation of CPE from all but one (Patient-18) patients was clustered in a 16-months period (between July 2014 and Oct 2015). The median age of 18 patients was 70 years. Sixteen patients (89%) were male. Seventeen patients (94%) had gastrointestinal malignancy. Six (33%), fourteen (78%), and sixteen (89%) patients had received chemotherapy within 90 days, surgery within 90 days, and antimicrobial therapy within 30 days of the first isolation of CPE, respectively. Although the patients were under the care of 7 different departments, Surgery-A (n = 7), Surgery-B (n = 4), and Medical Oncology-E (n = 3) departments were predominant. At the time of CPE isolation, the patients were located at one of five different wards or one intensive care unit (ICU). Six, five, and three patients were at ICU, Ward-V, and Ward-X, respectively. Thirteen (76%) of seventeen case patients between July 2014 and Oct 2015 had history of hospitalization at multiple wards before the isolation of CPE (Figure 1).

A ward-wide active surveillance was performed twice at Ward-V (November 2014 and February 2015), three times at Ward-X (May, June, and July 2015), and once at Ward Y (November 2014). A total of 191 patients were screened and two patients were positive for carriage of CPE (Patient-7 and -12). Additionally, a patient with a history of admission in Ward-V was screened upon a later admission at Ward-X and was positive for the growth of CPE (Patient-9). Surveillance cultures performed at the discretion of primary physicians immediately after surgery without using selective media turned positive for carriage of CPE in two patients (Patient-2 and -6).

Six patients had infections due to CPE (**Table 2**). Although one patient died of an intraabdominal infection following intestinal perforation on the 11th day of the onset of the infection, infections in other patients were cured without relapse. Two other patients died within 90 days due to reasons unrelated to the carriage of CPE (**Table 1**).

Outbreak management

The patients with a history of isolation of CPE were cared for in a private room under contact precautions according to the infection prevention protocol of the hospital. After July 2014 when the isolation of CPE from patients hospitalized in different wards was documented, occurrence of an institutional outbreak of CPE was notified to the all hospital staffs and strict compliance to the infection prevention protocol was enforced. In addition, direct observation of hand hygiene compliance was initiated by infection preventionists and the data were fed back to each hospital department. Compliance to the infection prevention protocol was thoroughly checked especially at the wards where the patients with CPE was hospitalized. Sampling from hospital environment was not performed. Compliance to the cleaning and disinfection protocol of endoscopes was confirmed and bacterial cultures of the relevant endoscopes, including bronchoscopes for the operating rooms and ICU and duodenoscope, were negative for the growth of CPE.

Microbiological and molecular analysis of CPE

All CPE isolates were non-susceptible to cefotaxime, ceftazidime, and cefepime and all but one isolates were non-susceptible to piperacillin-tazobactam with BD Phoenix NMIC/ID-208 panel. Three isolates were susceptible to aztreonam. Although most of the isolates were non-susceptible to carbapenems, MIC of >4 mg/mL for imipenem and meropenem was observed only in two isolates and one isolate, respectively. Most of the isolates was susceptible to non-b-lactam antibiotics tested (**Table 3**).

Fourteen of eighteen CPE isolates were identified as *E. hormaechei* by ANI and other isolates were *E. hormaechei* subsp. *steigerwaltii* (n = 2), *E. asburiae* (n = 1), and *E. xiangfangensis* (n = 1) (**Table 3**). All *E. hormaechei* isolates were ST78 and isolated between July 2014 and August 2015. While one isolate (TUM17942) carried $bla_{\text{IMP-11}}$, all other isolates carried $bla_{\text{IMP-1}}$. As expected, all isolates carried chromosomal *ampC* genes, but acquired genes for extended-spectrum b-lactamases (ESBL), AmpC, and carbapenemases other than bla_{IMP} were not identified in any isolates. Plasmid replicon for IncHI2A/IncHI2 was documented in 16 of 18 isolates and all 6 isolates in which full nucleotide sequences required for pMLST of IncHI2 plasmids were obtained were pMLST-ST1 (*smr0119*:1-*smr0018*:1). Remaining ten isolates also had allele profile of '1' for *smr00119* (*smr00118* of these isolates had 0.3% difference from allele 1 (n = 4), were not fully sequenced (n = 2), or were non-typable (n = 4)).

All isolates carrying $bla_{\text{IMP-1}}$ had In316 (*intI1*-$bla_{\text{IMP-1}}$-*aac(6')-Ilc*-*sul1*)-like structure. In 7 isolates (TUM17941, TUM17943, TUM17945, TUM17946, TUM17947, TUM17948, and TUM17949), nucleotide sequences of In316 were completely preserved and other isolates had single nucleotide difference (n = 5) or had fragmentation of the structure into multiple contigs (n = 5).

ST78 isolates were divided into three clades by core-genome (4,062,250bp, 87.7% of the genomic sequence of the reference strain) based SNP analysis (**Figure 2**). All patients from whom isolates of clade A were identified had a history of admission at ICU, Ward-V, or Ward-Y. On the other hand, all patients from whom isolates of clade C were identified had a history of multiple admission at Ward-X (**Figure 1**).

Conjugation experiments

In 13 isolates, *bla*$_{IMP}$ was successfully transferred into recipient *E. coli* cell by conjugation experiment (**Table 3**). All transconjugant were positive for PCR using primers for *bla*$_{IMP-1-group}$. TUM17942 yielded transconjugants positive for IncL/M by PCR-based replicon typing. Transconjugants of the remaining isolates were positive for IncHI2 by PCR-based replicon typing.

Discussion

In this study, we described an institutional outbreak of IMP-producing ECC which occurred in 17 patients over 16-months across multiple wards and departments. Core genome-based SNP analysis unexpectedly revealed that the outbreak involved three clades of ST78 isolates suggesting multiple introductions and routes of spread.

ST78 has been recognized as one of the global resistant clones of ECC [19]. ST78 isolates carrying *bla*$_{KPC}$, *bla*$_{VIM}$, and *bla*$_{OXA-48-group}$ have been reported, together with the isolates with *bla*$_{IMP}$ [19-21]. We previously reported that multiple clades of ST78 isolates carrying *bla*$_{IMP-1}$ on IncHI2 plasmids had spread in Tokyo [6]. In this study, we found that multiple clades of ST78 were indeed involved during 16-months outbreak within a single institution. Epidemiological investigation in combination with conventional microbiological analysis was insufficient to elucidate the details of the outbreak, and use of whole-genome sequencing-based analysis, with its resolution to differentiate similar clones, was crucial as described in other outbreaks [7-9].

Two ancestral isolates of clade A (isolates from Patient-1 and -2) had two SNPs differences but no common ancestor of these isolates was identified (**Figure 2**). Nevertheless, the time of introduction of the original isolate of clade A was expected to be within a few months prior to the first identification of IMP-producing ECC at this hospital (July 2014), considering that average substitution rate of IMP-producing ST78 isolates in Tokyo was 4.53 SNPs/genome/year in the previous study [6]. Clade A isolates were detected from 10 patients during one-year period, all of whom had a history of hospitalization at ICU, Ward-V, or Ward-Y (**Figure 1**). We hypothesized that these isolates were transmitted through medical care either via medical device, environment, or healthcare workers during hospitalization in these wards. While Patient-4 had history of hospitalization only at Ward-V, Patient-2, -3, -5 had no history of hospitalization at Ward-V prior to the isolation of clade A isolates. Therefore, involvement of multiple wards in the transmission of clade A isolates was suggested.

An isolate of clade B was isolated only from Patient-11 and carried *bla*$_{IMP-11}$ on an IncL/M plasmid. IMP-11-producing ECC isolates harboring IncL/M plasmids have been reported from Japan [22]. In addition, this patient had a history of recent long-term hospitalization at another hospital. Therefore, acquisition of the clade B isolate likely occurred outside the cancer center.

Clade C isolates were detected from three patients who had been all hospitalized in Ward-X during the same period. IMP-producing isolates were identified from the sputum of Patient-14 and -15 on the next day of the surgery performed by the same department (Surgery-B) at ICU, thus acquisition through respiratory procedure at operating rooms or ICU was suspected based on epidemiological information. However, the whole-genome sequencing analysis revealed that Patient-12 was the index patient and transmission to Patient-14 and 15 most likely occurred during the hospitalization at Ward-X several months prior to the surgery. Cancer patients often require multiple hospitalizations at different wards and are managed by different departments including surgery, radiology, and medical oncology during a long course of treatment. This complexity of care makes it very challenging to infer route of transmission of resistant organisms based on epidemiological investigation alone. Real-time performance of whole-genome sequencing analysis would be more useful in the investigation of these outbreaks involving patients requiring complex medical care.

Patient-10, -16, -17, and -18 carried IMP-producing non-ST78 ECC isolates, which suggests these patients incidentally acquired the isolates unrelated to the outbreak. Notably, two non-ST78 isolates (TUM17947 and TUM17948) carried *bla*$_{IMP-1}$ on IncHI2 plasmids. Carriage of *bla*$_{IMP-1}$ on IncHI2 plasmids by ECC isolates of multiple STs (e.g., ST53, 78, 113, 513, 1047) has been documented in another area (Nagoya) in Japan [23]. Although ST78 isolates appears to predominate among IMP-producing ECC isolates in Tokyo according to our previous study, the nationwide epidemiology remains to be elucidated [6]. Although there is the possibility of conjugative transfer of IncHI2 plasmids carrying *bla*$_{IMP-1}$ between ST78 isolates and non-ST78 isolates in the hospital environment or in the flora of the patients, it is beyond the scope of our analysis.

In this study, most of the IMP-producing ECC isolates were susceptible to non-b-lactam antibiotics and had relatively low MICs (£4 mg/mL) to carbapenems. Furthermore, several isolates were susceptible to non-carbapenem b-lactams such as piperacillin-tazobactam and aztreonam. These patterns of antimicrobial susceptibilities were similar to the IMP-producing *Enterobacterales* in Japan in previous reports [6, 22]. Infections caused by IMP-producing ECC were treated successfully mainly with non-b-lactam antibiotics retaining activity to the organisms except a case of intraabdominal infection without adequate source control (**Table 2**). Better prognosis of infections caused by MBL-producing *Enterobacterales* associated with better antimicrobial susceptibilities compared with those caused by KPC-producing *Enterobacterales*, which was consistent with our observation, was reported in an observational study [24].

Our study has several limitations. First, there could have been missed patients carrying CPE in the outbreak for several reasons. Active surveillance cultures were performed for a limited number of times for selected wards only. In addition, selective media for ESBL, not specific for CPE, was used for surveillance culture. Although previous studies showed that selective media for ESBL had >90% sensitivity for the isolation of CPE as a whole, the ability to identify IMP-producing isolates was unclear [25]. Screening criteria for the routine culture testing for the performance of confirmation testing for carbapenemase production were not strict enough to identify all CPE isolates. However, the lack of isolation of IMP-producing ECC clonal isolates for more than two years after August 2015 suggests successful containment of major transmissions. Second, we have not identified the direct route of introduction and transmission of IMP-producing ECC isolates. Apparent common sources were not identified by clinical epidemiological analysis and surveillance cultures of medical devices such as endoscopes were negative. Third, number of the cases of infections caused by IMP-producing ECC was too limited to analyze the association between the treatment regimen and prognosis.

Conclusions

Involvement of multiple clades of ST78 isolates was documented by whole-genome sequencing analysis of IMP-producing ECC isolates identified in a hospital-wide outbreak. Genetic relatedness of the isolates unexpected by the clinical analysis was uncovered. Real-time performance of whole-genome sequencing of relevant bacterial isolates in complicated epidemiological situations could facilitate identification transmission route, which is vital in containing outbreaks of antimicrobial-resistant organisms.

Abbreviations

CRE: Carbapenem-resistant *Enterobacterales*; CPE: Carbapenemase-producing *Enterobacterales*; MBL: metallo-b-lactamase; ECC: *Enterobacter cloacae* complex; SMA: sodium mercaptoacetate; mCIM: modified carbapenem inactivation method; ANI: average nucleotide identity; MLST: Multilocus sequence typing; BLAST: basic local alignment search tool; SNP: single nucleotide polymorphism; ICU: intensive care unit; ESBL; extended-spectrum b-lactamases

Declarations

Ethics approval and consent to participate

Institutional review board approval was obtained prior to initiating the study (approval number: 2018-1013). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Specific informed consent for this retrospective and non-invasive study was not required by local ethics committee.

Consent for publication

Not applicable since there are no details on individuals reported within the manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

SH reports personal fees from BD, Meiji, Shionogi, Sumitomo Dainippon Pharma, MSD, Astellas, Beckman Coulter Diagnostics, and FUJIFILM Toyama Chemical, and grants from MSD, outside the submitted work. YD reports personal fees from Pfizer, MSD, Shionogi, Janssen, VenatoRx, Entasis, BD, bioMerieux, Gilead, and AstraZeneca, and grants from Pfizer, MSD, Astellas, Shionogi, Kanto Chemical, and Janssen, outside the submitted work. All other authors declare no conflict of interest relevant to the study.

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Authors' contributions

SH designed the study, contributed to the analysis and interpretation of data, and drafted the manuscript. KA contributed to the acquisition, analysis, and interpretation of data. DO, KT, TA, KI, and DS contributed to the acquisition of data. KO, YI, YD, and KM revised the manuscript critically for important intellectual content. BH provided administrative support of the research. All authors read and approved the final manuscript.

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None.

Tables

Table 1. Clinical characteristics of patients from whom carbapenemase-producing *Enterobacterales* strains were isolated.

Patient No.	Time of first isolation	Status[a]	Bacterial species of CPE (MicroScan WalkAway)[b]	Sample	Age[c]	Sex	Ward[c]	Department[c]	Type of malignancy	Other comorbidities	30-/90-day mortality after isolation of CPE
1	Jul. 2014	INF	*Enterobacter cloacae*	Blood	75	M	V	A (Surgery)	GI	Hypertension Diabetes	N/N
2	Jul. 2014	SUR	*Enterobacter cloacae*	Sputum	65	M	ICU	B (Surgery)	GI	Complete situs inversus Hypertension Healed pulmonary tuberculosis	N/N
3	Aug. 2014	INF	*Enterobacter cloacae*	Abscess	69	M	W	A (Surgery) C (Surgery)	GI	Hypertension Cerebral infarction COPD	N/N
4	Sep. 2014	INF	*Enterobacter cloacae*	Intraabdominal fluid	73	M	V	D (Medical oncology)	GI	None	Y/Y
5	Sep. 2014	COL	*Enterobacter cloacae*	Sputum	70	M	ICU	B (Surgery)	GI	None	N/N
6	Sep. 2014	SUR	*Enterobacter cloacae*	Pancreatic fluid	73	M	V	A (Surgery)	GI	Drug-induced cardiomyopathy	N/N
7	Nov. 2014	SUR	*Enterobacter cloacae*	Stool	76	M	V	A (Surgery)	GI	Diabetes Chronic hepatitis C	N/N
8	Feb. 2015	INF	*Enterobacter cloacae*	Intraabdominal fluid	70	M	V	A (Surgery)	GI	Hypertension Hyperlipidemia Diabetes Healed pulmonary tuberculosis	N/N
9	Apr. 2015	SUR	*Enterobacter cloacae*	Stool	65	M	X	E (Medical oncology)	GI	Hyperthyroidism	N/N
10	Apr. 2015	COL	*Cronobacter sakazakii*	Bile	66	M	ICU	A (Surgery)	GI	Hypertension	N/N
11	Apr. 2015	COL	*Enterobacter cloacae*	Urine	65	F	X	E (Medical oncology)	GI	Encephalopathy	N/N
12	May 2015	SUR	*Enterobacter cloacae*	Stool	64	M	X	E (Medical oncology)	GI	None	N/N
13	Jul. 2015	COL	*Enterobacter cloacae*	Sputum	83	M	W	A (Surgery)	GI	Hypertension Diabetes	N/N
14	Jul. 2015	COL	*Enterobacter cloacae*	Sputum	48	M	ICU	B (Surgery)	GI	None	N/Y
15	Aug. 2015	INF	*Enterobacter cloacae*	Sputum	72	M	ICU	B (Surgery)	GI	Hypertension Diabetes Cerebral infarction	N/N
16	Sep. 2015	INF	*Enterobacter cloacae*	Bile	54	F	ICU	A (Surgery)	GI	None	N/N
17	Oct. 2015	COL	*Enterobacter cloacae*	Bile	73	M	Y	F (Surgery)	GI	None	N/Y
18	Apr. 2017	COL	*Enterobacter cloacae*	Sputum	78	M	Z	G (Surgery)	TH	COPD	N/N

[a] Status of the carriage of CPE was abbreviated as INF (infection), COL (colonization detected by clinical cultures), or SUR (colonization detected by surveillance cultures).

[b] Bacterial species identified by MicroScan WalkAway at the hospital were presented.

[c] Age of the patient, ward of hospitalization, and department caring the patient at the time of first isolation of CPE were presented.

[d] Date of surgery was described as the number of days between the precedent surgery and the isolation of CPE.

CPE, carbapenemase-producing *Enterobacterales*; M, male; F, female; Y, Yes; N, No; GI, gastrointestinal; TH, thoracic; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; LS, laryngoscopy; EGS, esophagogastroscopy; CS, colonoscopy; BS, bronchoscopy; DS, Duodenoscopy with endoscopic retrograde cholangiopancreatography; OR, operating room

Table 2. Clinical characteristics and outcomes of infections caused by carbapenemase-producing *Enterobacterales* strains.

Patient No.	Diagnosis of the infectious diseases	Samples positive for CPE	Adequate source control	Antimicrobial treatment[a]	Prognosis	Comment
1	Cholangitis Liver abscess	Blood (Day 1, Day 28) Drained abscess (Day 29)	Established (percutaneous abscess drainage)	(Day 1-3) FEP (IV) (Day 3-8) MEM (IV) + GEN (IV) (Day 9-28) MEM (IV) (Day 29-30) MEM + GEN (IV) + LVX (IV) (Day 31-43) MEM (HD-EX, IV) + GEN (IV)+ LVX (IV) (Day 44-74) TZP (HD-EX, IV) + GEN (IV)+ LVX (IV)	Cure without relapse	Cholangitis with bacteremia due to CPE was treated with MEM (Day 3-28) according to the susceptible result at the hospital. Although fever and bacteremia were once resolved, they recurred on Day 28. CT scan of the abdomen revealed the presence of liver abscess. Percutaneous abscess drainage and antimicrobial retreatment were performed. TZP (Day 44-74) was selected according to the susceptible result at the hospital.
3	Surgical site infection (deep incisional)	Abscess (Day 1)	Established (incision and drainage)	(Day 1-9) TZP (IV) (Day 9-17) MEM (IV) (Day 17-25) LVX (PO)	Cure without relapse	MEM (Day 9-17) was selected according to the susceptible result at the hospital.
4	Intraabdominal infection	Intraabdominal fluid (Day 1)	Unestablished	(Day 1-6) MEM (IV) (Day 6-11) IPM (IV)	Death (Day 11)	Although intestinal perforation was suspected by imaging studies, surgical intervention was not performed because the patient was on do-not-resuscitate order due to his advanced cancer. IPM (Day 6-11) was selected according to the susceptible result at the hospital.
8	Intraabdominal infection	Intraabdominal fluid (Day 1)	Established (percutaneous abscess drainage)	(Day 1-7) AMC (PO)	Cure without relapse	
15	Pneumonia	Sputum (Day 1)	Unnecessary	(Day 1-3) SAM (IV) (Day 3-14) MEM (IV) + LVX (IV)	Cure without relapse	
16	Surgical site infection (deep incisional and intraabdominal space)	Blood (Day 1) Abscess (Day 1) Intraabdominal fluid (Day 3)	Established (incision and drainage of the wound surface, and percutaneous peritoneal drainage)	(Day 1-3) MEM (IV) + GM (IV) (Day 3-21) MEM (HD-EX, IV) + GEN (IV) (Day 22-24) SXT (PO) (Day 24-38) LVX (PO)	Cure without relapse	CPE was first identified from surveillance bile culture during the surgery prior to the onset of infection. SXT was changed to LVX (Day 24) due to the possible side effect of nausea.

[a] Only antimicrobial agents with activity gram-negative organisms were presented. Antimicrobial agents against which the causative organisms were susceptible by antimicrobial susceptibility testing with BD Phoenix NMIC/ID-208 panel interpreted with CLSI M100-S27 guidelines were underlined.

FEP, cefepime; MEM, meropenem; GEN, gentamicin; LVX, levofloxacin; TZP, piperacillin-tazobactam; IPM, imipenem; AMC, amoxicillin-clavulanic acid; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; HD-EX, high-dose and extended infusion; IV, intravenous; PO, oral.

Table 3. Molecular and microbiological characteristics of carbapenemase-producing *Enterobacterales* isolates.

Patient No.	Isolate name	Bacterial species of CPE (ANI)[a]	ST	Clade[b]	Minimum inhibitory concentrations (mg/mL)[c]											Antimicro resistanc genes
					TZP	CTX	CAZ	FEP	ATM	IPM	MEM	LVX	SXT	GEN	AMK	
1	TUM 14647	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	4	4	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
2	TUM 14648	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	2	4	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
3	TUM 14652	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	4	4	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
4	TUM 14654	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	4	2	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
5	TUM 14658	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	4	2	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
6	TUM 14792	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	4	2	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
7	TUM 14797	*E. hormaechei*	78	A	>64/4	>4	>8	>16	>8	4	4	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
8	TUM 17939	*E. hormaechei*	78	A	>64/4	>4	>8	16	>8	4	2	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
9	TUM 17940	*E. hormaechei*	78	A	64/4	>4	>8	>16	8	>4	>4	4	>4/76	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il qnrB6, fo sul1, tet*(I
10	TUM 17941	*E. asburiae*	24	NA	>64/4	>4	>8	>16	£1	4	4	£1	£1/19	4	£8	bla_{IMP-1}, bla_{ACT-1}, *aac(6')-Il fosA, sul*
11	TUM 17942	*E. hormaechei*	78	B	>64/4	>4	>8	8	£1	4	£1	>4	>4/76	£2	£8	bla_{IMP-11}, bla_{TEM-1B} bla_{ACT-5}, *aac(6')-Ia qnrS1, fo sul1*
12	TUM 17943	*E. hormaechei*	78	C	>64/4	>4	>8	>16	>8	>4	4	£1	£1/19	£2	£8	bla_{IMP-1}, bla_{ACT-5}, *aac(6')-Il aph(3")-1 aph(6)-1 qnrB6, fo sul1, sul2 tet*(B)
13	TUM 17944	*E. hormaechei*	78	A	>64/4	>4	>8	16	>8	4	2	£1	£1/19	£2	£8	bla_{IMP-1},

																bla$_{ACT-5}$, *aac(6')-Il qnrB6, fo sul1, tet*(I
14	TUM 17945	*E. hormaechei*	78	C	>64/4	>4	>8	>16	>8	4	4	£1	£1/19	£2	£8	*bla*$_{IMP-1}$, *bla*$_{ACT-5}$, *aac(6')-Il aph(3")-1 aph(6)-1 qnrB6, fo sul1, sul2 tet*(B)
15	TUM 17946	*E. hormaechei*	78	C	>64/4	>4	>8	>16	>8	4	4	£1	£1/19	£2	£8	*bla*$_{IMP-1}$, *bla*$_{ACT-5}$, *aac(6')-Il aph(3")-1 aph(6)-1 qnrB6, fo sul1, sul2 tet*(B)
16	TUM 17947	*E. xiangfangensis*	1331	NA	>64/4	>4	>8	>16	>8	4	4	£1	£1/19	£2	£8	*bla*$_{IMP-1}$, *bla*$_{ACT-16}$, *aac(6')-Il strA, aph(6)-1 qnrS1, fo sul1, tet*(I
17	TUM 17948	*E. hormaechei subsp. steigerwaltii*	113	NA	8/4	>4	>8	16	£1	4	2	£1	£1/19	£2	£8	*bla*$_{IMP-1}$, *bla*$_{ACT-15}$, *aadA1, aac(6')-Il qnrB6, fo sul1*
18	TUM 17949	*E. hormaechei subsp. steigerwaltii*	133	NA	64/4	>4	>8	16	£1	2	£1	4	£1/19	£2	£8	*bla*$_{IMP-1}$, *bla*$_{ACT-7}$, *aac(6')-Il qnrB6, fo sul1, tet*(I

[a] Bacterial species and subspecies confirmed by the comparison of average nucleotide identity of study isolates with those of type strains were presented.

[b] Clades of ST78 isolates determined by core-genome single nucleotide polymorphism-based analysis were presented.

[c] Minimum inhibitory concentrations determined with BD Phoenix NMIC/ID-208 panel were presented.

[d] Plasmid replicon was underlined if a transconjugant of the isolates was positive for the replicon by PCR-based replicon typing

ANI, average nucleotide identity; NA, Not applicable; ST, sequence type; TZP, piperacillin-tazobactam; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; AMK, amikacin.

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Figures

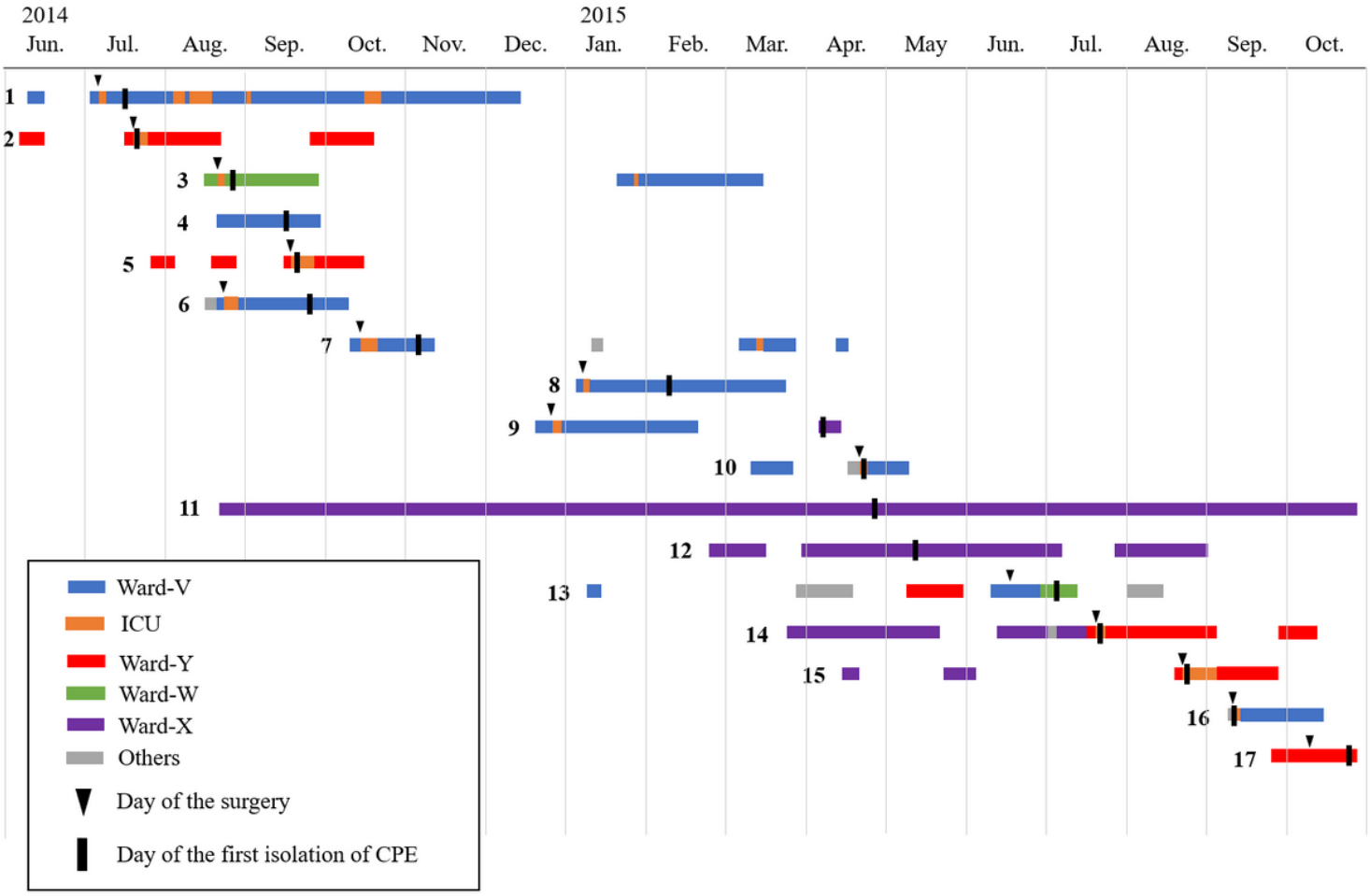

Figure 1

Timeline of hospitalization, surgery, and first isolation of carbapenemase-producing Enterobacterales of the patients. The numbers located at the left end of the bars represent Patient ID.

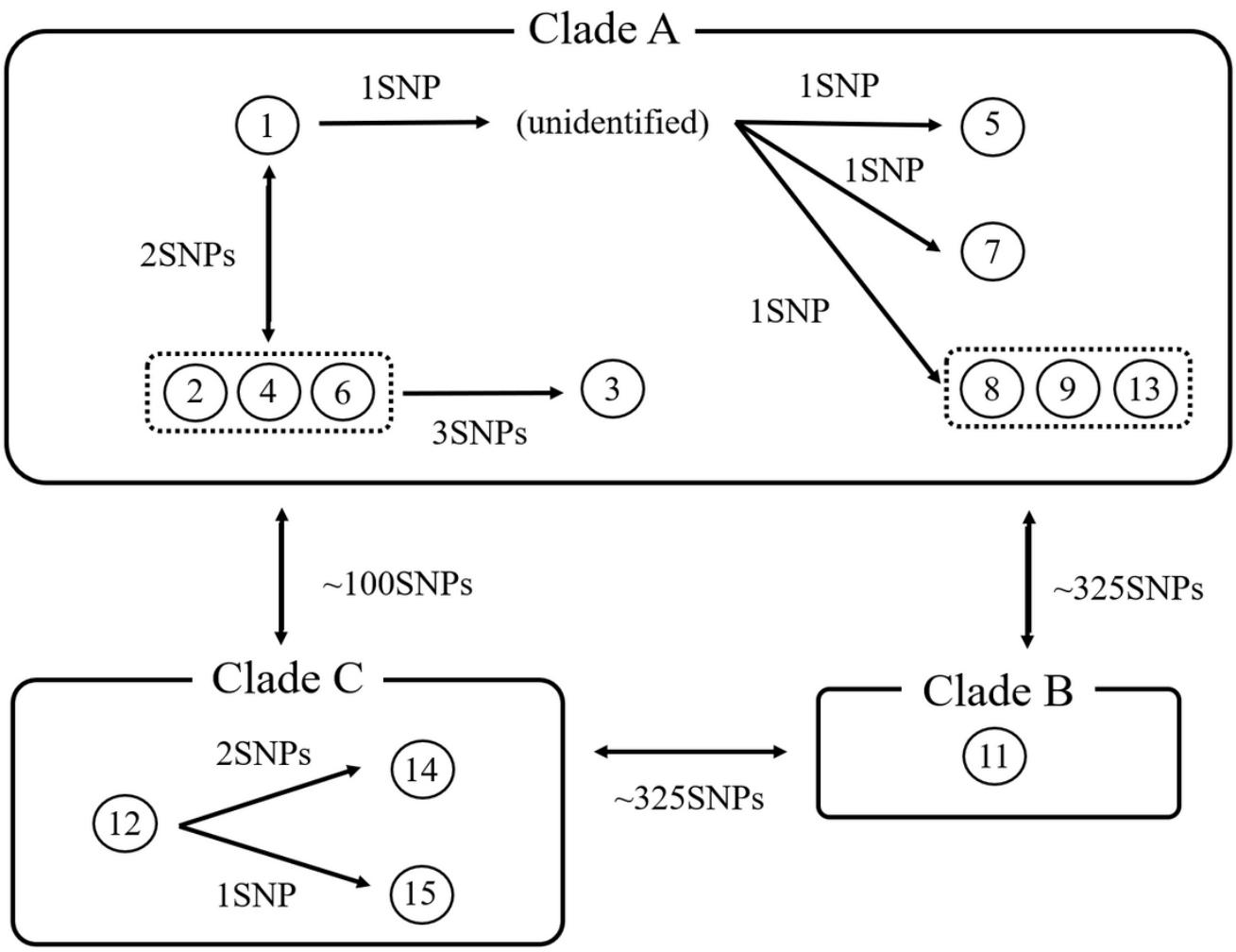

Figure 2

Molecular relatedness of the ST78 isolates from the patients. Isolates were indicated as circles with the patient designation numbers. SNP deference of the isolates from the patients were presented and isolates without SNP difference were enclosed in dotted lines. Isolates belonging to the same clade were enclosed in solid lines.