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Prevalence of carbapenem-resistant Enterobacterales with bla_{IMP-6} predominance in hospitals from 2018 to 2021 in Nara, Japan

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Objectives: Despite the global health risk of carbapenem-resistant Enterobacterales (CRE), especially carbapenemase-producing Enterobacterales (CPE), Japan reports a significantly low frequency of CRE with a predominance of IMP-type carbapenemases. This study aimed to investigate the prevalence and characteristics of CRE isolated from hospitals in the city of Nara, Japan.

Methods: We obtained 171 CRE isolates from 16 791 Enterobacterales isolated at 23 hospitals in Nara between January 2018 and December 2021. Isolates of CPE were characterized through antimicrobial susceptibility testing, the carbapenem inactivation method, PCR and DNA sequencing. Genotypic diversity of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* was determined via MLST and PFGE.

Results: The prevalence of CRE between 2018 and 2021 was 1.02%, gradually decreasing from 1.13% to 0.74%. Ninety-nine isolates were identified as CPE, representing six species. Ninety-seven CPE isolates harboured bla_{IMP-6}, while the remaining two carried either bla_{IMP-1} or bla_{IMP-19}. Genotype analysis identified ST131 as the dominant genotype for *E. coli*, but none for *K. pneumoniae*. PFGE results suggested clonal spread of CPE in Hospital A, where CRE was isolated in high numbers ($n=44$).

Conclusions: In this study, CRE prevalence was marginally higher than previously reported in Japan, but still low in frequency. A predominance of Enterobacterales harbouring bla_{IMP-6} was confirmed in Nara. The spread of CPE at Hospital A suggested the possibility of a nosocomial outbreak due to bla_{IMP-6} transmission via plasmids or clonal spread. Continued monitoring is crucial for effective management of CRE prevalence in the region.

Introduction

Antimicrobial resistance (AMR) is a global threat that contributes to serious adverse consequences such as therapeutic failure, increased morbidity, mortality and healthcare costs. This has prompted the World Health Organization to issue a red alert and create a priority list of antibiotic-resistant bacteria for the research and development of effective drugs. Carbapenem-resistant Enterobacterales (CRE) was included in the 'critical priority pathogens' category owing to their impact on mortality, disease burden and circulation at the human–animal–environment interface.[1] Global dissemination of CRE/carbapenemase-producing Enterobacterales (CPE) currently presents a serious threat from a public health perspective, and CRE/CPE outbreaks have been reported worldwide.[2] The resistance mechanism involves carbapenemase production or porin loss with extended-spectrum β-lactamases and/or AmpC-type β-lactamases.[3] CRE/CPE may spread through clonal bacterial strain expansion or horizontal carbapenemase gene transfer via plasmids.[4] Carbapenemase genes can transfer their resistance by transmission across strains and Enterobacterales species. These could result in a CRE outbreak and should be monitored for trends.[5,6]

The most common carbapenemases among Enterobacterales are KPC, NDM, IMP, VIM and OXA-48-like enzyme.[7] The distributions of these enzymes differ by geographical location.[8] KPC occurs in the USA, NDM is localized to the Indian subcontinent,

and IMP is found in Japan. Furthermore, KPC, OXA-48, NDM and VIM carbapenemases are the most common carbapenemases in Europe, while OXA-48 is the most prevalent in Turkey.[9] CRE/CPE prevalence varies by country and region. A report from Greece revealed that 62.3% of Greek *Klebsiella pneumoniae* were resistant to carbapenems.[8] A surveillance programme in the USA described 14.4% of Enterobacterales carrying KPC-2 or KPC-3.[10] Contrarily, CRE/CPE prevalence is still low in Japan (0.31%),[11] despite a few outbreaks caused by IMP producers at some medical institutions.[12]

The distribution of IMP variants differs regionally; $bla_{IMP\text{-}1}$ is widely distributed in Japan but especially dominant in the Kanto region. Although $bla_{IMP\text{-}6}$ is predominant in the Kinki region,[13,14] $bla_{IMP\text{-}19}$ has been recently reported, especially in Kyoto and Shiga.[15] The IMP-1 group includes important variants such as IMP-1 and IMP-6, whereas IMP-19 belongs to the IMP-2 group.[16,17] Nevertheless, detailed information regarding carbapenemase gene distribution is lacking. The treatment strategy of CRE/CPE infection depends on the carbapenemase genotype because of its substrate specificity.[18–20] For example, IMP-6 producers are susceptible to imipenem[21] and also used for susceptibility testing in Japan. However, they are sometimes overlooked by automated testing in clinical laboratories and treated with carbapenems.[14,22] Thus, proper investigation of molecular epidemiology is crucial for effective management and treatment of CRE/CPE.[23] Furthermore, the low frequency of CRE isolation in Japan has led to nationwide studies, but a detailed regional analysis is needed to determine the factors contributing to the predominant spread of IMP-producing isolates. In this study, we aimed to elucidate the prevalence and characteristics of CRE isolated from city hospitals in Nara, Japan, as well as the potential of a nosocomial outbreak with high CRE dissemination.

Materials and methods

Bacterial isolates

We collected 16 791 clinical isolates of Enterobacterales from 23 hospitals in the city of Nara, Japan, between January 2018 and December 2021. They were obtained either from infection sites or through colonization/screening. Only one isolate per patient was included in this study. Bacterial species were identified using the MicroScan WalkAway system (Siemens Healthineers Diagnostics, USA) and MALDI biotyper (Bruker Daltonics, USA) available in each hospital and verified using VITEK MS (Sysmex bioMérieux, Japan) at Nara Medical University. The MicroScan WalkAway system, VITEK-2 (Sysmex bioMérieux), DPS192iX (Eiken Chemical Co., Ltd., Japan), and IA20MIC mkII (Eiken Chemical Co., Ltd.) were used to screen the isolates, of which 171 were included in this study. CRE is defined in Japan as *Enterobacteriaceae* with: (i) meropenem MIC ≥ 2 mg/L or (ii) imipenem MIC ≥ 2 mg/L and cefmetazole MIC ≥ 64 mg/L.[24] CRE was isolated from urine ($n=98$), sputum ($n=35$), stool ($n=11$), nasal mucosa ($n=6$), blood ($n=5$), intraperitoneal drain ($n=4$), pus ($n=4$), bronchi ($n=2$), bile ($n=1$), vagina ($n=1$) and unknown ($n=4$).

Antimicrobial susceptibility testing and CPE screening

The MIC of the collected isolates was evaluated using the agar dilution method according to the CLSI guidelines,[25] with *Escherichia coli* ATCC 29522 as the quality control. The carbapenem inactivation method (CIM) was performed for the 171 CRE isolates to screen carbapenemase production, as previously described.[26,27]

PCR identification and sequencing of β-lactamase genes

PCR was performed for all CPE isolates to detect the carbapenemase (bla_{IMP}, bla_{VIM}, bla_{KPC}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM}) and other β-lactamase ($bla_{CTX\text{-}M}$ and plasmid-mediated AmpC β-lactamase) genes.[28–31] The genotype was identified using gene-specific PCR and confirmed through DNA sequencing.[32,33] The sequences were analysed with DNASTAR Lasergene (DNASTAR, USA) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

MLST of E. coli *and* K. pneumoniae

To identify the genetic relatedness of the isolates, MLST was performed using Achtman schemes for *E. coli* and Institut Pasteur schemes for *K. pneumoniae*. Housekeeping genes in *E. coli* (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) and *K. pneumoniae* (*rpoB*, *phoE*, *infB*, *gapA*, *mdh*, *pgi* and *tonB*) were sequenced and analysed with DNASTAR Lasergene.[34,35] DNA sequence variations were analysed using an MLST database for *E. coli* (https://pubmlst.org/bigsdb? db=pubmlst_mlst_seqdef) and *K. pneumoniae* (https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl? db=pubmlst_klebsiella_seqdef&page=batchSequenceQuery) to determine sequence types (STs).

PFGE typing of E. coli *and* K. pneumoniae

Similarities between the CPE isolates (9 *E. coli* strains and 30 *K. pneumoniae* strains) from Hospital A were identified through PFGE, using a CHEF-DR III system (Bio-Rad Laboratories, Richmond, CA, USA) and the *Xba*I restriction enzyme (Takara Bio Inc.), as previously described.[36,37] Electrophoresis conditions for *E. coli* involved running at 5.3–49.9 s for 20 h at 14°C with a voltage of 6 V/cm^2. For *K. pneumoniae*, the conditions included 5–20 s for 4 h, followed by 25–50 s for 18 h at 14°C, also with a voltage of 6 V/cm^2. The PFGE patterns were interpreted according to the criteria outlined by Tenover *et al.*[38]: 'indistinguishable, isolates with the same number of bands and appearing identical or differing by only one band from that of the reference strain; 'closely related', isolates with two to three bands different from those of the reference strain; and 'possibly related', isolates with up to six bands different from those of the reference strain.

Conjugation experiments

Transferability of the carbapenemase genes was investigated through conjugation experiments using CPE as the donor and sodium azide-resistant *E. coli* J53 as the recipient, as previously described.[39] Luria–Bertani broth cultures of donor strains and recipient *E. coli* J53 at exponential phase were mixed at a ratio of 1:1 by volume, and these mating mixtures were incubated overnight at 37 °C. Transconjugants on Luria–Bertani agar plates containing cefpodoxime (8 mg/L) and sodium azide (100 mg/L) were selected, and the resistance genes transferred from the donor strains were verified by PCR.

Results

Prevalence and identification of CRE isolates

Among the 16 791 isolates obtained, 171 from 14 hospitals were identified as CRE during the study period (Table 1). The average prevalence of CRE between 2018 and 2021 was 1.02%, with a gradual decline from 1.13% in 2018 to 0.74% in 2021. The CRE isolates included 40 *E. coli*, 59 *K. pneumoniae*, 23 *Enterobacter cloacae* complex, 44 *Klebsiella aerogenes*, 3 *Klebsiella oxytoca* and 2 *Citrobacter freundii* isolates.

Table 1. Prevalence of CRE in hospitals in Nara, Japan, between 2018 and 2021

		CRE		Species of CRE					
Year	No. of isolates	No. of isolates (%)	No. of hospitals	*K. pneumoniae*	*E. coli*	*E. cloacae* complex	*K. aerogenes*	*K. oxytoca*	*C. freundii*
2018	4065	46 (1.13)	9	20	8	6	11	1	0
2019	4519	51 (1.13)	9	16	17	8	7	2	1
2020	4170	44 (1.06)	9	15	11	6	12	0	0
2021	4037	30 (0.74)	7	8	4	3	14	0	1
Total	16 791	171 (1.02)	14	59	40	23	44	3	2

Characteristics and antimicrobial susceptibility of CRE isolates

Ninety-nine CRE isolates were positive for CIM and carried carbapenemase genes. These were categorized as CPE (Table 2). The remaining 72 CIM negative isolates without any carbapenemase genes were categorized as non-CPE. Table 2 displays the antimicrobial susceptibilities of the isolates. Almost CPE isolates were non-susceptible to piperacillin, cefmetazole, cefotaxime and ceftazidime, and all of them were susceptible to imipenem. The MIC of meropenem for 97 CPE isolates harbouring $bla_{\text{IMP-6}}$ was higher than that of imipenem. The MIC of piperacillin decreased more significantly when the agent was combined with tazobactam in CPE than in non-CPE isolates. CPE isolates consisted of mainly *E. coli* (38/99) and *K. pneumoniae* (52/99), and 97 of them harboured $bla_{\text{IMP-6}}$, one carried $bla_{\text{IMP-1}}$, and another harboured $bla_{\text{IMP-19}}$ (Table 3). The isolates carrying $bla_{\text{IMP-1}}$ and $bla_{\text{IMP-19}}$ were isolated in 2021. Ninety-three of the 97 IMP-6 producers also harboured CTX-M β-lactamase genes [$bla_{\text{CTX-M-2}}$ ($n=90$), $bla_{\text{CTX-M-3}}$ ($n=1$), $bla_{\text{CTX-M-15}}$ ($n=1$) or $bla_{\text{CTX-M-27}}$ ($n=1$)]. Non-CPE isolates primarily consisted of the *E. cloacae* complex (17/72) and *K. aerogenes* (42/72) rather than *E. coli* and *K. pneumoniae*. None of the CRE isolates harboured any plasmid-mediated AmpC β-lactamase.

*Genotypes of CPE isolates (*E. coli *and* K. pneumoniae*)*

STs for CPE isolates were identified through MLST analysis, with *E. coli* ($n=37$) spanning seven different STs [ST12 ($n=2$), ST14 ($n=5$), ST38 ($n=2$), ST69 ($n=4$), ST95 ($n=1$), ST131 ($n=21$) and ST1177 ($n=2$); Table 3]. ST131 was the dominant genotype in *E. coli* isolated from 6 of the 14 hospitals. On the other hand, *K. pneumonia* ($n=52$) exhibited 12 different STs [ST37 ($n=5$), ST45 ($n=3$), ST111 ($n=1$), ST200 ($n=1$), ST307 ($n=1$), ST399 ($n=1$), ST466 ($n=4$), ST768 ($n=1$), ST846 ($n=19$), ST1606 ($n=9$), ST3919 ($n=6$) and ST3954 ($n=1$)]. Although isolates with ST846 and ST1606 were prevalent, they were mostly obtained from the same hospital—Hospital A. Other hospitals lacked dominant STs such as *E. coli* ST131.

The distribution of STs of the *E. coli* and *K. pneumoniae* between 2018 and 2021 is shown in Table S1 (available as Supplementary data at *JAC-AMR* Online). Comparing isolates from before the COVID-19 pandemic (2018–19) and during the pandemic (2020–21), we found some STs of *E. coli* (ST131) and *K. pneumoniae* (ST37, ST45, ST466 and ST846) that were isolated across years. Other STs of *E. coli* and *K. pneumoniae* were sporadically isolated before and during the pandemic, respectively.

Clonal relatedness of CPE isolates in Hospital A

Forty-four CPE isolates (43 IMP-6 producers and 1 IMP-1 producer) were isolated from Hospital A (Table 3), which was the highest number among all hospitals (Hospitals A–N). PFGE analysis confirmed the clonal relatedness of the CPE isolates from Hospital A. One PFGE band pattern (including 'indistinguishable') was obtained for each ST (ST12 of *E. coli*, ST466, ST846, ST1606 and ST3919 of *K. pneumoniae*), except for ST131. Isolates belonging to the same ST were considered as clonal strains. On the other hand, ST131 displayed three PFGE band patterns, indicating multiple strain types.

Transferability of carbapenemase-producing genes

Conjugation experiments were used to test all CPE isolates for transferability of carbapenemase gene determinants. Transconjugants containing carbapenemase-encoding plasmids were obtained from 84 CPE isolates (84.8%). The average transfer frequency of all CPE isolates was 1.5×10^{-3} (range 10^{-7}–10^{-2}) (Table 3). All 84 transconjugants harboured $bla_{\text{IMP-6}}$. Eighty-one co-carried $bla_{\text{CTX-M-2}}$, one co-harboured $bla_{\text{CTX-M-3}}$, one co-carried $bla_{\text{CTX-M-15}}$, and one co-harboured $bla_{\text{CTX-M-27}}$.

Discussion

In this study, we revealed the prevalence and molecular characteristics of CRE isolated from 23 hospitals in Nara, Japan. The prevalence of CRE was 1.02% between 2018 and 2021, which was higher than that previously reported (0.31%–0.33%) for the same time period.[11,40] CRE prevalence was low, but it was observed in the majority of hospitals (14/23) during the study period. The 171 CRE isolates included 99 CPE and 72 non-CPE. *K. pneumoniae* ($n=52$) was predominant in all CPE isolates, followed by *E. coli* ($n=37$), whereas *K. aerogenes* ($n=42$) and *E. cloacae* complex ($n=17$) were dominant in the non-CPE isolates. *K. aerogenes* and *E. cloacae* complex, which encoded chromosomal AmpC β-lactamase, are thought to have acquired carbapenem resistance through loss of outer membrane porins or overexpression of efflux pumps.[3,41]

All CPE isolates in this study carried IMP-type carbapenemase genes, consistent with the Japan CPE profile.[14] The globally reported KPC, NDM and OXA-48 carbapenemase genes were not detected. Ninety-seven of the 99 CPE isolates harboured $bla_{\text{IMP-6}}$, confirming the predominance of $bla_{\text{IMP-6}}$ in Nara. Notably, all CPE isolated between 2018 and 2020 only carried $bla_{\text{IMP-6}}$, whereas those harbouring $bla_{\text{IMP-1}}$ or $bla_{\text{IMP-19}}$ emerged

Table 2. Resistance genes and antimicrobial susceptibilities of CPE- and non-CPE-classified species

		MIC range (mg/L)[a]/no. of non-susceptible isolates (%)[b]										
Category	Species (no. of isolates)	PIPC	PIPC /TAZ	CMZ	CTX	CAZ	CFPM	IPM	MEPM	LVFX	AMK	CST
CPE (99)	*K. pneumoniae* (52) *E. coli* (37) *E. cloacae* complex (6) *K. aerogenes* (2) *K. oxytoca* (1) *C. freundii* (1)	4–>256 /95 (96.0)	0.5 –>256 /9 (9.1)	2–>256 /81 (81.8)	4–>256 /99 (100)	0.5–>256 /96 (97.0)	0.5–>256 /31 (31.3)	<0.063–1 /0 (0)	0.125–32 /42 (42.4)	<0.063–128 /38 (38.4)	1–16 /16 (16.2)	0.5–1 /0 (0)
Non-CPE (72)	*K. pneumoniae* (7) *E. coli* (3) *E. cloacae* complex (17) *K. aerogenes* (42) *K. oxytoca* (2) *C. freundii* (1)	0.125–>256 /25 (34.7)	0.25–>256 /22 (30.6)	1–>256 /69 (95.8)	<0.063–>256 /26 (36.1)	0.125–>256 /26 (36.1)	<0.063–>256 /7 (9.7)	<0.063–16 /17 (23.6)	<0.063–16 /9 (12.5)	<0.063–32 /9 (12.5)	1–16 /4 (5.6)	0.5–>8 /1 (1.4)

[a]PIPC, piperacillin; TAZ, tazobactam; CTX, cefotaxime; CAZ, ceftazidime; CMZ, cefmetazole; CFPM, cefepime; IPM, imipenem; MEPM, meropenem; LVFX, levofloxacin; AMK, amikacin; CST, colistin.

[b]Breakpoint for non-susceptible in accordance with the CLSI document M100 (25).

Table 3. Genotypes, resistance genes and transferability of CPE isolates between hospitals (A–N)

Species	ST	No. of CPE isolates	No. of CPE isolates from hospitals A–N[a]														Resistance genes (no. of isolates)	Transferability (average conjugation frequency)
			A[b]	B	C	D	E	F	G	H	I	J	K	L	M	N		
E. coli	12	2	2 (1 pattern)														IMP-6, CTX-M-2 (2)[d]	28/37 (2.3×10^{-3})
	14	5		5													IMP-6, CTX-M-2 (5)	
	38	2					2										IMP-6, CTX-M-2 (2)	
	69	4				3		1									IMP-6, CTX-M-2 (3)[d], IMP-6 (1)	
	95	1	1														IMP-6, CTX-M-2 (1)[d]	
	131	21	7 (3 patterns)	2			5	1		1				5			IMP-6, CTX-Ms (20)[c,d], IMP-6 (1)[d]	
	1177	2			2												IMP-6, CTX-M-2 (1), IMP-6, CTX-M-3 (1)	
K. pneumoniae	37	5					4			1							IMP-6, CTX-M-2 (5)[d]	49/52 (1.7×10^{-3})
	45	3					3										IMP-6, CTX-M-2 (3)	
	111	1					1										IMP-6, CTX-M-2 (1)	
	200	1										1					IMP-6, CTX-M-2 (1)	
	307	1	1														IMP-6, CTX-M-2 (1)	
	399	1													1		IMP-19 (1)[d]	
	466	4	2 (1 pattern)									1				1	IMP-6, CTX-M-2 (4)	
	768	1								1							IMP-6, CTX-M-2 (1)	
	846	19	13 (1 pattern)		6												IMP-6, CTX-M-2 (18), IMP-6 (1)[d]	
	1606	9	9 (1 pattern)														IMP-6, CTX-M-2 (9)	
	3919	6	6 (1 pattern)														IMP-6, CTX-M-2 (6)	
	3954	1		1													IMP-6, CTX-M-2 (1)	
E. cloacae complex	—	6		5						1							IMP-6, CTX-M-2 (5), IMP-6 (1)[d]	5/6 (2.4×10^{-3})
K. aerogenes	—	2	1							1							IMP-6, CTX-M-2 (2)[d]	1/2 (7.8×10^{-5})
K. oxytoca	—	1	1														IMP-6, CTX-M-2 (1)	1/1 (8.2×10^{-4})
C. freundii	—	1	1														IMP-1 (1)[d]	0/1
Total		99	44	13	8	3	15	2	0	5	0	2	0	5	1	1		84/99 (1.5×10^{-3})

[a]Hospitals in which CRE was isolated (A–N).
[b]Numbers in parentheses are the number of PFGE band patterns obtained from each ST isolate.
[c]CTX-Ms include CTX-M-2 (18), CTX-M-15 (1) and CTX-M-27 (1).
[d]Includes strains for which resistance genes were not transferred during conjugation experiments.

for the first time in 2021. Since these genes are dominant in Kanto, and Kyoto and Shiga, respectively,[15,42] the CPE isolates harbouring bla_{IMP-1} or bla_{IMP-19} could have entered Nara from these regions. In East Asia, CRE tends to be highly isolated in China and Korea, where NDM and KPC are frequently detected.[8,43–45] The differences between Japan and other countries may be attributed to the fact that Japan is an island nation, and the low isolation rate of CRE and the dominance of IMP producers in Nara and Japan are noteworthy.

All *E. coli* genotypes, dominated by ST131 (21/37), harboured bla_{IMP-6}, represented seven STs and were isolated from 6 of 14 hospitals. To the best of our knowledge, this is the first report to describe the genotype of IMP-6-producing *E. coli* in Japan. *E. coli* ST131 was the dominant genotype in extended-spectrum β-lactamase-producing *E. coli*,[46,47] corresponding with carbapenemase-producing *E. coli*. *E. coli* ST131 is associated with the rapid global spread of AMR and poses a major threat to public health.[48] Our findings suggest that the predominance of bla_{IMP-6} in the Kinki region is likely due to the dissemination of IMP-6-producing *E. coli* ST131. *K. pneumoniae*, predominantly harbouring bla_{IMP-6} (51/52), exhibited 12 different STs with no dominant type across the hospitals. We have previously reported that carbapenemase-producing *K. pneumoniae* isolated in Japan mostly carry bla_{IMP-6}, while others harbour bla_{IMP-1}.[49] No dominant genotypes were identified, akin to this study.

International high-risk clonal lineages, including clonal complex 258 (ST11, ST258, ST340 and ST512), ST37, ST147 and

ST307, are globally disseminated.[50,51] In this study, five strains of ST37 and one of ST307 were detected. Although these genotypes were not the predominant type and there was no apparent spread between many hospitals in this region, ST37 isolates carrying bla_{IMP-1} were observed in the central region of Japan.[52] Additionally, potential outbreaks have been reported in other areas of the world,[51] which warrants close monitoring.

We also evaluated the distribution of STs of the *E. coli* and *K. pneumoniae* before the pandemic (2018–19) and during the pandemic (2020–21) (Table S1). Some STs of *E. coli* (ST131) and *K. pneumoniae* (ST466 and ST846) were isolated both before and during the pandemic, as well as in several hospitals. These isolates may have spread clonally or colonized. On the other hand, isolates of other STs were sporadically isolated before and during the pandemic. These isolates may have been affected by the pandemic, but due to the small number of isolates, this is speculative and needs to be verified with more data. A report from the Centers for Disease Control and Prevention highlighted a 35% increase in hospital-onset infections due to CRE during the first year of the pandemic.[53,54] A recent report also alerted about an increased detection of CRE worldwide during the pandemic.[44,45,55] Furthermore, the distribution of CRE resistance genes has changed in some countries during the pandemic.[55,56] The prevalence of CRE isolated from 23 hospitals in Nara is still low, and the influence of the pandemic on the emergence of AMR remains unclear. However, Nara is one of the leading tourist cities in Japan, and the number of tourists from abroad has been increasing after the pandemic. CRE monitoring and surveillance should continue to assess the impact of COVID-19 on the emergence of AMR.

Most CPE isolates were isolated from Hospital A, indicating spread via nosocomial infection. To verify this possibility, we conducted PFGE for the *E. coli* and *K. pneumoniae* strains isolated from the hospital. In *E. coli*, the ST131 isolates exhibited three PFGE band patterns, suggesting that these isolates were not spread by a clonal strain. In *K. pneumoniae*, the ST846, ST1606 and ST3919 isolates showed the same PFGE band pattern within each ST, indicating spread by clonal strains. These genotypes were no high-risk clones but considered to have spread via nosocomial infection.[50] In particular, isolates ST466 and ST846 were isolated across years, suggesting that these pathogens may have colonized hospital facilities (Table S1).

Conjugation experiments revealed that the average transfer frequency of resistance genes in all CPE isolates was high (1.5×10^{-3}), with 80 of the 90 isolates harbouring both bla_{IMP-6} and $bla_{CTX-M-2}$. Thus, those transconjugants are assumed to possess highly transferable plasmids such as pKPI-6, a self-transmissible IncN-type plasmid carrying bla_{IMP-6} and $bla_{CTX-M-2}$, already epidemic in Japan.[57] The nosocomial outbreak in Hospital A is similar to a case of long-term nosocomial infection at Osaka National Hospital, in which IncN-type plasmids, including pKPI-6, played a key role in bla_{IMP-6} transmission.[58] This highlights the importance of monitoring and preventing the spread of CPE via plasmids.

This study has some limitations. First, the prevalence of CRE in this study reflects the high number of CRE isolations in a single hospital (Hospital A); therefore, the actual prevalence of CRE in the city of Nara was probably lower than indicated. Second, whole-genome sequencing was not performed for the 72 non-CPE isolates, which limits our understanding of carbapenem resistance through loss of outer membrane porins combined with production of chromosomal AmpC β-lactamase. This is a task for future studies. Finally, a comprehensive evaluation of CRE spread in the region through whole-genome sequencing and plasmid characterization would have augmented the ST and PFGE analyses performed in this study.

Conclusions

Our findings reveal the notable prevalence, yet low frequency (1.02%), of CRE in the hospitals in Nara between 2018 and 2021, even though CRE increased in many regions of the world during the COVID-19 pandemic. All CPE isolates carried IMP-type carbapenemase genes, with *K. pneumoniae* and *E. coli* harbouring bla_{IMP-6} emerging as the principal strains. IMP-6-producing *E. coli* were identified for the first time in Japan, and *E. coli* ST131 was the predominant genotype. The diverse *K. pneumoniae* genotypes, lack of high-risk clones and clonal relatedness in Hospital A highlight the various CRE dissemination patterns.

After the COVID-19 pandemic, the number of foreign tourists visiting Nara has increased, raising concerns about the possibility of AMR import. If carbapenemase-producing bacteria such as NDM and KPC were isolated in Nara, they could be imported AMR strains. Continued surveillance and detailed molecular analyses are crucial for understanding transmission dynamics and detecting changes in the trends and risks of potential spread in the region.

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Transparency declarations

None to declare.

Author contributions

R.K. was involved in data curation, investigation and writing—original draft. R.N. was involved in conceptualization, funding acquisition, investigation and writing—original draft. A.N., T.H., R.T., Y.S., K.Y., S.H. and K.T. were involved in investigation. S.A. was involved in investigation and writing—original draft. D.K. was involved in resources and investigation. R.M. was involved in project administration and supervision. T.K. was involved in conceptualization, investigation, resources and data curation. H.Y. was involved in conceptualization, funding acquisition, project administration and supervision. All authors have approved the final article.

Ethical approval

Not required.

Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online.

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