International Journal of Infectious Diseases 98 (2020) 281–286

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Molecular characteristics of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in Japan: Predominance of CTX-M-15 and emergence of hypervirulent clones

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ARTICLE INFO

Article history:
Received 17 May 2020
Received in revised form 22 June 2020
Accepted 24 June 2020

Keywords:
Klebsiella pneumoniae
Extended-spectrum β-lactamase
CTX-M-15
Hypervirulent

ABSTRACT

Objective: To provide data on the molecular characteristics of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* clinical isolates in Japan.
Methods: A total of 100 clinical isolates of ESBL-producing *K. pneumoniae* collected throughout Japan between June and July 2018 were studied. ESBL genes were analyzed using PCR and DNA sequencing. Transferability of ESBL genes was investigated by conjugation experiments. Plasmid replicon types, virulence genes (*rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344*) associated with hypervirulent *K. pneumoniae* (hvKp), and capsule types were detected using PCR. Genotyping was performed using multilocus sequence typing.
Results: All ESBL-producing isolates carried bla_{CTX-M} genes. The most predominant CTX-M-type identified was CTX-M-15 ($n = 55$). We identified 24 sequence types (STs) among the CTX-M-15 producers, with ST25 ($n = 8$) being the most common. Most of the transconjugants carrying $bla_{CTX-M-15}$ contained the FIIk replicon. Of the 100 ESBL-producing isolates, 31 were hvKp defined by the presence of the virulence genes. These ESBL-producing hvKp isolates belonged to eight STs (STs 23, 25, 36, 65, 86, 268, 412, and 4492), with five capsule types (K1, K2, K20, K57, and undefined).
Conclusions: CTX-M-15 was the predominant ESBL among *K. pneumoniae* isolates from Japan. This study shows that ESBL-producing hvKp strains comprising various clones are emerging in Japan.

Introduction

β-lactam antibiotics are currently the major antimicrobials utilized worldwide for the treatment of serious infections due to *Enterobacteriaceae* including *Klebsiella pneumoniae*. However, increasing use of β-lactam antibiotics has led to the emergence of extended-spectrum β-lactamase (ESBL)-producing strains. These enzymes have been increasingly identified worldwide in *K. pneumoniae* (Calbo and Garau, 2015). It is notable that infections caused by ESBL-producing strains are associated with increased morbidity, mortality, and healthcare-associated costs (Maragakis et al., 2008).

ESBLs can hydrolyze almost all β-lactam antibiotics (except for carbapenems and cephamycins) but are inhibited by clavulanic acid (Paterson and Bonomo, 2005). ESBLs can be classified into three main types, namely TEM, SHV, and CTX-M, among which the CTX-M type, including CTX-M-1, -2, -8, -9, and -25 groups, has emerged as the dominant ESBL type in *K. pneumoniae* strains (Calbo and Garau, 2015; D'Andrea et al., 2013). CTX-M-15, in the CTX-M-1 group, is the most common ESBL in many parts of the world (Calbo and Garau, 2015). The prevalence of ESBLs in *K. pneumoniae* was reported to be around 10% in Japan and more than 20% in the USA and many European and Asian

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https://doi.org/10.1016/j.ijid.2020.06.083

countries (Center for Disease Dynamics, Economics & Policy, 2015; Doi et al., 2017).

K. pneumoniae is primarily responsible for hospital-acquired urinary tract infections, pneumonia, and bacteremia (Podschun and Ullmann, 1998). However, since the mid-1980s, *K. pneumoniae* has also been recognized as the cause of life-threatening, community-acquired infections often involving multiple sites, such as liver abscess with endophthalmitis (Shon et al., 2013; Russo and Marr, 2019). The *K. pneumoniae* strains that cause such infections are termed hypervirulent (Shon et al., 2013) and frequently exhibit the hypermucoviscous phenotype that is detectable as a positive result on the string test, a widely used marker of hypervirulence in *K. pneumoniae* (Fang et al., 2004). Of the >130 capsule types described (Wyres et al., 2016), hypervirulent *K. pneumoniae* (hvKp) strains often belong to the capsule types K1, K2, K5, K20, K54, or K57 (Shon et al., 2013), with K1 and K2 accounting for approximately 70% of hvKp strains (Russo and Marr, 2019). The major virulence genes associated with hvKp include *rmpA*, *rmpA2* (regulators of the mucoid phenotype via increased capsule production), *iucABCD* (biosynthetic genes for the siderophore aerobactin), and *iroBCDN* (biosynthetic genes for the siderophore salmochelin) (Chen et al., 2004; Russo and Marr, 2019; Struve et al., 2015). These genes have been identified in a large virulence plasmid (Chen et al., 2004; Struve et al., 2015). A recent study demonstrated that *rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344* (a metabolic transporter of unknown function) serve as more accurate markers of hypervirulence in *K. pneumoniae* than the string test and the particular capsule types (K1, K2, K5, K20, K54, and K57) (Russo et al., 2018). These hvKp strains have usually been sensitive to a variety of antibiotics except for an intrinsic resistance to ampicillin (Shon et al., 2013). However, more recently, the emergence of ESBL-producing hvKp strains has been described in several countries (Surgers et al., 2016; Zhang et al., 2016). The combination of ESBL with hypervirulence in *K. pneumoniae* may further exacerbate infections caused by this pathogen and prevent successful treatment (Shon et al., 2013).

Although characterization of ESBL-producing *K. pneumoniae* has been undertaken in many countries, limited data are available from Japan. The aims of this study were to characterize the ESBL genes and their transferability, to profile virulence genes (*rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344*) associated with hypervirulence, and to determine the capsule types and sequence types (STs) among ESBL-producing *K. pneumoniae* clinical isolates collected throughout Japan.

Materials and methods

Bacterial isolates and antimicrobial susceptibility testing

A total of 100 consecutive and non-duplicate clinical isolates of ESBL-producing *K. pneumoniae* were collected from 100 hospitals throughout Japan between June and July 2018. Only one isolate was collected from each hospital. These were isolated from urine ($n = 50$), sputum ($n = 32$), blood ($n = 13$), catheter tip ($n = 3$), vagina ($n = 1$), and pharynx ($n = 1$). *K. pneumoniae* isolates were identified with matrix-assisted laser desorption ionization–time of flight mass spectrometry using a Vitek MS system (bioMérieux, Marcy-l'Étoile, France). Initial screening for ESBL was accomplished using the VITEK 2 Advanced Expert System (bioMérieux) according to the manufacturer's instructions. The antimicrobial susceptibility of various antimicrobial agents was determined using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015a), and quality control was performed with *Escherichia coli* ATCC 25922. MICs were interpreted according to breakpoints defined by the CLSI (CLSI, 2015b).

Detection of ESBL genes and DNA sequencing

PCR was performed to detect the presence of $bla_{CTX\text{-}M}$ genes (Dallenne et al., 2010). The CTX-M group was determined by PCR using CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 group-specific primers (Dropa et al., 2015; Kojima et al., 2005; Mena et al., 2006). Amplified PCR products of CTX-M genes were sequenced on an Applied Biosystems 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Conjugation experiments and plasmid replicon typing

Transferability of the ESBL genes was studied by conjugation experiments with ESBL-producing strains as the donor and sodium azide-resistant *E. coli* J53 as the recipient. The experiments were conducted with the broth mating method as previously described (Suzuki et al., 2020). The ESBL genes successfully transferred from the donor strains were verified by PCR. The plasmid content of transconjugants was studied by PCR-based replicon typing (Carattoli et al., 2005; Villa et al., 2010).

Detection of virulence genes and string test for hypermucoviscous phenotype

PCR for *rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344* was conducted using previously described PCR primers and conditions (Russo et al., 2018). In this study, *K. pneumoniae* strains carrying two or more of the five virulence genes (*rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344*) were defined as hvKp, whereas the remainder were defined as non-hvKp (Russo et al., 2018).

Hypermucoviscous phenotype was identified using the string test as described previously (Fang et al., 2004). The formation of a viscous string >5 mm long was defined as a positive result (Fang et al., 2004).

Capsule typing and multilocus sequence typing

PCR detection of capsule types K1, K2, K5, K20, K54, and K57 was performed as described previously (Turton et al., 2010).

Multilocus sequence typing (MLST) was performed using seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB,* and *tonB*) according to the method of Diancourt et al. (Diancourt et al., 2005). DNA sequence variations were analyzed by using an MLST database for *K. pneumoniae* (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Novel alleles and STs were submitted to the curator and assigned as new designations (www.pasteur.fr/mlst).

Results

Capsule type and MLST

MLST analysis identified 44 different STs among the 100 ESBL-producing *K. pneumoniae* isolates, including 5 novel STs (ST4489–4492, ST4494) (Table 1 and Supplementary Table 1). ST25 ($n = 11$) was the most frequently represented ST, followed by ST268 and ST412 ($n = 9$ each), ST23 ($n = 6$), ST37 ($n = 5$), and ST45 and ST307 ($n = 4$ each). The remaining 37 STs were each represented by one to three isolates.

Of the 100 ESBL-producing *K. pneumoniae* isolates, 44 exhibited the K1, K2, K5, K20, K54, or K57 capsule types. The remaining 56 isolates did not exhibit any of the K1, K2, K5, K20, K54, or K57 capsule types. There was an association between specific ST and particular capsule type as shown in Table 2. Isolates with ST23, ST1049, ST268, and ST412 exhibited capsule types K1, K5, K20, and K57, respectively. Isolates with ST25, ST65, ST86, ST101, and

Table 1
Distribution of ESBL types and STs in ESBL-producing *Klebsiella pneumoniae* isolates.

ESBL type	No. of isolates	ST[a]													
		15	17	23	25	36	37	45	65	86	268	307	412	4492	Others (31 STs)
CTX-M-1 group															
CTX-M-15	55	2		3	8		2	3		2	4	4	7		20[b]
CTX-M-3	5			1	1					1					2[c]
CTX-M-2 group															
CTX-M-2	14				1	1	1		1		5		1	1	3[d]
CTX-M-9 group															
CTX-M-14	23		3	2	1		2						1		14[e]
CTX-M-27	3	1						1							1[f]

Abbreviations: ESBL, extended-spectrum β-lactamase; STs, sequence types.
[a] Underlined numbers represent ESBL-producing hypervirulent *K. pneumoniae* isolates.
[b] Others included 15 STs (STs; 4, 54, 101, 219, 273, 280, 290, 323, 336, 610, 1307, 2623, 3176, 3368 and 4490) which were represented by one or two isolates.
[c] Others included ST540 and ST1086.
[d] Others included ST35, ST2286 and ST4489.
[e] Others included 14 STs (STs; 4, 7, 29, 35, 107, 280, 290, 357, 584, 881, 896, 1049, 4491 and 4494).
[f] Others included ST355.

ST4492 exhibited capsule type K2. In addition, isolates with ST7 and ST29 exhibited capsule type K54.

Characterization of ESBLs and MLST

Among the 100 ESBL-producing isolates, all were positive for CTX-M. Among these isolates, 60 were from the CTX-M-1 group, 26 were from the CTX-M-9 group, and 14 were from the CTX-M-2 group. DNA sequencing revealed that the predominant type was CTX-M-15 (55 isolates), followed by CTX-M-14 (23 isolates) (Table 1). The CTX-M-15 producers were divided into 24 different STs. Most of these STs were represented by one to three isolates, except for ST25 ($n = 8$), ST412 ($n = 7$), and ST268 and ST307 ($n = 4$ each).

Antimicrobial susceptibility profile

The results of antimicrobial susceptibility testing are shown in Table 3. All the isolates showed resistance to cefotaxime and were significantly inhibited by clavulanate. Moreover, all the isolates were susceptible to imipenem and meropenem. In addition, 16 and 28 of all isolates were resistant to levofloxacin and gentamicin, respectively.

Table 2
Characteristics of ESBL-producing hypervirulent and non-hypervirulent *Klebsiella pneumoniae* isolates.

Strain group (n)	Capsule type	ST	No. of isolates	Virut-type	Virulence genes					HV (n)	CTX-M type (n)
					rmpA	*rmpA2*	*iucA*	*iroB*	*peg-344*		
hvKp (31)	K1	23	5	VP1	+	+	+	+	+	4	CTX-M-15 (3), CTX-M-14 (2)
	K1	23	1	VP2	–	+	+	+	+	0	CTX-M-3 (1)
	K2	25	1	VP1	+	+	+	+	+	1	CTX-M-14 (1)
	K2	65	1	VP1	+	+	+	+	+	1	CTX-M-2 (1)
	K2	86	2	VP1	+	+	+	+	+	2	CTX-M-3 (1), CTX-M-15 (1)
	K2	86	1	VP2	–	+	+	+	+	0	CTX-M-15 (1)
	K2	4492	1	VP1	+	+	+	+	+	0	CTX-M-2 (1)
	K20	268	7	VP1	+	+	+	+	+	4	CTX-M-2 (5), CTX-M-15 (2)
	K20	268	1	VP3	+	–	–	+	+	0	CTX-M-15 (1)
	K20	268	1	VP4	–	+	+	–	–	0	CTX-M-15 (1)
	K57	412	4	VP1	+	+	+	+	+	3	CTX-M-15 (3), CTX-M-2 (1)
	K57	412	3	VP4	–	+	+	–	–	1	CTX-M-15 (3)
	K57	412	1	VP2	–	+	+	+	+	0	CTX-M-15 (1)
	K57	412	1	VP5	–	+	+	+	–	0	CTX-M-14 (1)
	ND	36	1	VP1	+	+	+	+	+	1	CTX-M-2 (1)
non-hvKp (69)	K2	25	10	NH1	–	–	–	–	–	1	CTX-M-15 (8), CTX-M-3 (1), CTX-M-2 (1)
	K2	101	1	NH1	–	–	–	–	–	0	CTX-M-15 (1)
	K5	1049	1	NH1	–	–	–	–	–	0	CTX-M-14 (1)
	K54	7	1	NH1	–	–	–	–	–	0	CTX-M-14 (1)
	K54	29	1	NH2	–	–	–	+	–	0	CTX-M-14 (1)
	ND	35	1	NH2	–	–	–	+	–	0	CTX-M-14 (1)
	ND	35	1	NH1	–	–	–	–	–	0	CTX-M-2 (1)
	ND	290	1	NH1	–	–	–	–	–	0	CTX-M-14 (1)
	ND	290	1	NH3	–	–	+	–	–	0	CTX-M-15 (1)
	ND	610	2	NH3	–	–	+	–	–	0	CTX-M-15 (2)
	ND	others[a]	49	NH1	–	–	–	–	–	9	CTX-M-15 (27), CTX-M-14 (14), CTX-M-2 (3), CTX-M-27 (3), CTX-M-3 (2)

Abbreviations: ESBL, extended-spectrum β-lactamase; hvKp, hypervirulent *K. pneumoniae*; HV, hypermucoviscous phenotype; ND, not K1, K2, K5, K20, K54, or K57 capsule type; NH, non-hvKp; ST, sequence type; VP, virulence gene profile.
[a] Others included 29 STs (STs; 4, 15, 17, 37, 45, 54, 107, 219, 273, 280, 307, 323, 336, 355, 357, 540, 584, 881, 896, 1086, 1307, 2286, 2623, 3176, 3368, 4489, 4490, 4491, 4494).

Table 3
Susceptibility profile of ESBL-producing *Klebsiella pneumoniae* isolates.

Antimicrobial agents	Total (n = 100)		
	R (%)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
Ampicilin	100	>256	>256
Piperacillin	97	>256	>256
Piperacillin-tazobactam	5	4/4	32/4
Cefmetazole	1	1	4
Cefotaxime	100	128	>256
Cefotaxime-clavulanate	–	≤0.063	0.5
Ceftazidime	59	16	128
Cefepime	52	16	32
Imipenem	0	0.125	0.125
Meropenem	0	≤0.063	≤0.063
Aztreonam	61	16	128
Levofloxacin	16	1	8
Gentamicin	28	0.25	32

Abbreviations: ESBL, extended-spectrum β-lactamase; R, resistant.

Ceftazidime and cefepime resistance rates were higher in isolates with CTX-M-15 than in isolates with other CTX-M enzymes (94.5% vs. 15.5% for ceftazidime and 72.7% vs. 26.6% for cefepime). Of the 100 ESBL-producing *K. pneumoniae* isolates, 8 were resistant to levofloxacin and gentamicin and 7 of these were CTX-M-15 producers. These 7 isolates belonged to 5 STs: ST307 (n = 3), and ST15, ST280, ST1307, and ST3368 (n = 1 each).

Transferability of CTX-M genes and plasmid replicon typing

Conjugation experiments showed that 63 of 100 CTX-M type ESBL-producing *K. pneumoniae* isolates were able to transfer their CTX-M-encoded plasmids to *E. coli* J53. Eight of 63 transconjugants were devoid of replicons (nontypeable) for the incompatibility groups sought by the PCR-based replicon typing method; seven of these carried CTX-M-15 and one carried CTX-M-3. The most common plasmid identified in transconjugants carrying CTX-M-15 or CTX-M-14 was IncFIIk (Table 4). The CTX-M-15-producing or CTX-M-14 producing *K. pneumoniae* isolates harboring IncFIIk showed diverse STs (Supplementary Table 1).

Virulence gene profile and hypermucoviscous phenotype

Of the 100 ESBL-producing *K. pneumoniae* isolates, 31 harbored two or more of the five virulence genes and were identified as hvKp (Table 2). These ESBL-producing hvKp strains were isolated from sputum (n = 16), urine (n = 8), blood (n = 6), and vagina (n = 1). There were 5 virulence gene profiles (VPs) among ESBL-producing hvKp isolates. The most common was VP1 (*rmpA*$^+$, *rmpA2*$^+$, *iucA*$^+$, *iroB*$^+$, and *peg-344*$^+$) (n = 22). The string test was positive for 27 isolates, comprising 17 ESBL-producing hvKp isolates and 10 ESBL-producing non-hvKp isolates. The 31 ESBL-producing hvKp isolates belonged to 8 STs (STs 23, 25, 36, 65, 86, 268, 412, and 4492). ST23, ST268, and ST412 were strongly associated with hvKp, while ST25 was more common among the non-hvKp isolates.

Discussion

This study investigated the molecular characteristics of ESBL-producing *K. pneumoniae* clinical isolates in Japan. $bla_{CTX\text{-}M\text{-}15}$ was the main ESBL gene and was detected in isolates belonging to 24 different STs. The isolates carrying $bla_{CTX\text{-}M\text{-}15}$ were distributed throughout Japan (Supplementary Table 1). Plasmid replicon typing of transconjugants carrying $bla_{CTX\text{-}M\text{-}15}$ showed that most strains belonged to the IncFIIk. We identified 31 of a total of 100 ESBL-producing *K. pneumoniae* isolates as hvKp using virulence markers (*rmpA*, *rmpA2*, *iucA*, *iroB*, and *peg-344*). These ESBL-producing hvKp strains were isolated in hospitals throughout Japan (Supplementary Table 1) and were composed of a variety of clones.

The dominance of the CTX-M family among ESBL-producing *K. pneumoniae* strains was consistent with the global trend. A previous Japanese study in a single hospital, which did not determine the detailed subtypes of CTX-M, showed that the CTX-M-1 group was the predominant ESBL type among ESBL-producing *K. pneumoniae* (Chong et al., 2011). In this study, five different ESBLs were identified with CTX-M-15 of the CTX-M-1 group being the most prevalent. The high frequency of CTX-M-15 was consistent with results of recent studies worldwide (Elhani et al., 2010; Robin et al., 2017; Rodrigues et al., 2014). Our previous study and others showed that CTX-M-14 or CTX-M-27 of the CTX-M-9 group is the most prevalent ESBL type in ESBL-producing *E. coli* from Japan (Komatsu et al., 2018; Yano et al., 2013). The distribution of ESBL genes was, therefore, considered to be different in *K. pneumoniae* and *E. coli* from Japan. However, CTX-M-15-producing *E. coli* has increasingly been detected in Japan (Komatsu et al., 2018; Matsumura et al., 2015; Yano et al., 2013). It is possible that plasmids encoding CTX-M-15 were acquired from *K. pneumoniae*. In fact, our conjugation experiment showed that most (61%, 34/55) of the $bla_{CTX\text{-}M\text{-}15}$ -carrying plasmid can be transferred to recipient *E. coli*. We observed an association between $bla_{CTX\text{-}M\text{-}15}$ and FIIk plasmids, in agreement with previous studies (Robin et al., 2017; Rodrigues et al., 2014). These findings may suggest that the diffusion of CTX-M-15 among *K. pneumoniae* in Japan is linked to FIIk plasmids. An association between $bla_{CTX\text{-}M\text{-}14}$ and FIIk plasmids was also observed. FIIk plasmids were distributed widely across multiple STs, indicating that they are readily transmitted between different clonal lineages of *K. pneumoniae* (Supplementary Table 1).

ST11 and ST147 are international high-risk multiresistant *K. pneumoniae* clones, producing CTX-M-15 or CTX-M-14 (D'Andrea et al., 2013). In addition, ST15 has been detected among CTX-M-15 producers in several countries including Japan (Higashino et al., 2017; Lee et al., 2011; Robin et al., 2017; Rodrigues et al., 2014;

Table 4
Distribution of plasmid replicon types according to CTX-M type identified in *Escherichia coli*-recipient strains.

CTX-M type	No. of strains	Replicon types									
		FIIk	N	F	A/C	I1Iγ	FIIk, N	FIIk, I1Iγ	N, F	F, FIA, FIB	Nontypeable
All	63	35	10	4	1	1	1	1	1	1	8
CTX-M-1 group											
CTX-M-15	34	25		1				1			7
CTX-M-3	3	1		1							1
CTX-M-2 group											
CTX-M-2	10		10								
CTX-M-9 group											
CTX-M-14	14	9		2	1	1	1				
CTX-M-27	2								1	1	

Wang et al., 2013). ST15 ($n = 3$) was found in our study, but ST11 and ST147 were not. The most common ST identified among CTX-M-15 producers in the present study was ST25 ($n = 8$), followed by ST412 ($n = 7$), and ST268 and ST307 ($n = 4$ each). This result differed from that of a previous study in which ST25, ST268, ST307, and ST412 were not found among 55 isolates of CTX-M-15-producing *K. pneumoniae* detected in nine Asian countries excluding Japan (Lee et al., 2011). ST25, ST268, and ST412 were also not reported among CTX-M-15 producers in France, Portugal, Tunisia, the USA, and Canada (Elhani et al., 2010; Peirano et al., 2012; Robin et al., 2017; Rodrigues et al., 2014; Wang et al., 2013). ST307 was reported among CTX-M-15 producers in France (Robin et al., 2017). A recent study revealed that ST307 is associated with a conserved plasmid harboring the $bla_{CTX\text{-}M\text{-}15}$ ESBL gene and several other antimicrobial resistance determinants (Wyres et al., 2019). In the present study, 4 CTX-M-15-producing ST307 isolates were resistant to levofloxacin and 3 of these were also resistant to gentamicin.

Among the 100 ESBL-producing *K. pneumoniae* isolates in the present study, 31 (31%) hypervirulent strains were identified. This rate was higher than that found in a previous study of 230 clinical *K. pneumoniae* strains in China in 2013, of which 72 were ESBL producers, and 11/72 (15%) were predicted to be hvKp based on the presence of *rmpA* (Zhang et al., 2016). A French multicenter study investigated 37 ESBL-producing *K. pneumoniae* strains and showed that none of them harbored *rmpA*, *rmpA2*, or siderophore-encoding genes (Robin et al., 2017). There has been only one previous report of an hvKp strain carrying an ESBL gene from Japan (Harada et al., 2019). However, the present study demonstrated that these strains may be frequently isolated in Japanese hospitals.

We identified various ESBL-producing hvKp clones, of which all except for K2-ST4492 have been identified as hypervirulent clones in previous studies (Harada et al., 2019; Lin et al., 2014; Struve et al., 2015). ST4492 was a novel ST identified in this study and was a single locus variant of ST86, which is one of the typical STs associated with hvKp strains (Russo and Marr, 2019). Previous studies in Japan and Asian countries found that K1-ST23 strains were most common among hvKp strains (Harada et al., 2019; Siu et al., 2011). Although K1-ST23 strains were identified in the present study, K20-ST268 and K57-ST412 strains were the most common among the ESBL-producing hvKp strains identified. Only 17 (54%) of the 31 hvKp strains identified exhibited the hypermucoviscous phenotype. These results support the notion that the absence of this phenotype does not preclude hypervirulence (Catalan-Najera et al., 2017; Russo et al., 2018).

In conclusion, the molecular characterization of Japanese ESBL-producing *K. pneumoniae* isolates in this study revealed that CTX-M-15 is the main ESBL in *K. pneumoniae* from Japan. The diffusion of CTX-M-15 among *K. pneumoniae* from Japan may be associated with FIIk plasmids. Our results also show that ESBL-producing hvKp strains, which include a variety of clones, are emerging and potentially spreading in Japan. Further studies are required to assess the potential threat that ESBL-producing hvKp strains may pose for public health in Japan.

Conflicts of interest

All authors declare that they have no conflict of interest relevant to the study.

Funding

This study was supported by JSPS KAKENHI (grant number:17K10027 and16K09940). The funders had no role in study design, data collection and interpretation, the decision to publish, or preparation of this manuscript.

Ethical approval

Not required.

Acknowledgments

We thank the curatorial team of the Institut Pasteur MLST system (Paris, France) for importing novel alleles and profiles at http://bigsdb.pasteur.fr.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.06.083.

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