

# KPC-Like Carbapenemase-Producing *Enterobacteriaceae* Colonizing Patients in Europe and Israel

A. Baraniak,<sup>a</sup> R. Izdebski,<sup>a</sup> J. Fiett,<sup>a</sup> M. Herda,<sup>a</sup> L. P. G. Derde,<sup>b</sup> M. J. M. Bonten,<sup>b</sup> A. Adler,<sup>c</sup> Y. Carmeli,<sup>c</sup> H. Goossens,<sup>d</sup> W. Hryniewicz,<sup>a</sup> C. Brun-Buisson,<sup>e</sup> M. Gniadkowski,<sup>a</sup> on behalf of the MOSAR WP2, WP3, and WP5 Study Groups

National Medicines Institute, Warsaw, Poland<sup>a</sup>; University Medical Center Utrecht, Utrecht, The Netherlands<sup>b</sup>; Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel<sup>c</sup>; University of Antwerp, Antwerp, Belgium<sup>d</sup>; INSERM U957 and Université Paris-Est, Créteil, France<sup>e</sup>

**In a 2008–2011 survey, 17,945 patients in 18 hospital units in Europe and Israel were screened for carriage of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae*, resulting in identification of 124 positive patients. The isolates were dominated by *Klebsiella pneumoniae* sequence type 258 (ST258) KPC-2 and ST512 KPC-3, mainly from Greece and Italy, respectively, whereas Israeli isolates were of diverse species, clones, and KPC variants. Various *bla*<sub>KPC</sub> platforms were observed, among which IncFII<sub>K</sub>-FIB<sub>K</sub> plasmids with *bla*<sub>KPC-2/3</sub> genes in the Tn4401a transposon prevailed.**

Carbapenemase-producing *Enterobacteriaceae* (CPE) constitute an urgent epidemiological issue (1). One of their major, globally spread mechanisms is *Klebsiella pneumoniae* carbapenemases (KPCs), which hydrolyze most  $\beta$ -lactams (1, 2). KPC-2 and -3 are the most prevalent variants, while *K. pneumoniae* is their predominant host species (3, 4). KPCs have occurred in many *K. pneumoniae* clones (sequence types [STs]) (5–8), but ST258 and its close relative ST512 are key players in the pandemic spread (2–4, 6, 9–11). *bla*<sub>KPC</sub> genes are located in Tn4401 transposon variants (12–15) and inserted into plasmids of various replicon types and transmission potentials (5, 7, 16–20). One type of these, pKpQIL, found first in KPC-3-producing *K. pneumoniae* ST258 in Israel, has two specific replicons, FII<sub>K</sub> and FIB<sub>K</sub>, and low conjugation efficiency (21–23). Later, KPC-2- or -3-encoding pKpQIL-like molecules were observed in other countries, usually in *K. pneumoniae* ST258 (2, 3, 10, 24, 25).

During the European Union project MOSAR (Mastering hOSPital Antimicrobial Resistance in Europe), patients in intensive care units (ICUs) and rehabilitation units (RUs) in Europe and Israel were screened for *Enterobacteriaceae* resistant to expanded-spectrum cephalosporins (ESCs) (26). Since KPCs and metallo- $\beta$ -lactamases (MBLs) confer resistance to ESCs (1), the project allowed performance of a large-scale comparative study of the KPC and MBL CPE carriage. A previous report concerned MBL CPE (27), while here we present the KPC data.

Between mid-2008 and mid-2011, all patients in 13 ICUs and five RUs in nine countries ( $n = 17,945$ ) were screened for ESC-resistant (ESC-R) *Enterobacteriaceae* (Table 1). Rectal swabbing was performed regularly from admission until discharge. Swabs were plated onto Brilliance ESB agar (Oxoid, Basingstoke, United Kingdom); enterobacterial colonies were stored for definite analysis. Species were identified with Vitek 2 (bioMérieux, Marcy l'Etoile, France). All isolates were tested for extended-spectrum  $\beta$ -lactamases (ESBLs) and AmpC-type cephalosporinases by the ESB double-disk synergy test (DDST) without and with 250  $\mu$ g/ml cloxacillin (28) and for susceptibility to ertapenem, imipenem, and meropenem. Carbapenemase screening breakpoints were from EUCAST (<http://euca.org>). All suspected CPE isolates were subjected to KPC, MBL, and OXA-48 phenotypic detection, using the combined disk test with phenylboronic acid (PBA CDT) (29), the DDST with EDTA (30), and the temocillin

disk (31), respectively. All nonduplicate PBA CDT-positive organisms were tested by PCR for *bla*<sub>KPC</sub> genes (32), and this test was performed also for putative MBL producers (27).

A total of 124 patients carrying 127 unique KPC CPE organisms were identified in 6 of 18 clinical sites, located in Greece (centers AT,  $n = 44$ , and LA,  $n = 35$ ), France (center RP,  $n = 1$ ), Israel (center LH,  $n = 6$ , and TA,  $n = 16$ ), and Italy (center FS,  $n = 22$ ) (Table 1). They were 59.0% of all patients with CPE. Four Greek patients had *K. pneumoniae* strains coproducing KPC and MBL (VIM-1) and were reported previously too (27). The results for individual countries concurred with those of other reports. Since 2008, after the nationwide outbreak in 2006 and 2007, KPCs in Israel have been endemic at a lower level (2, 33, 34). Consistently, the KPC cases in the Israeli RUs were scattered during the study, being  $\sim 1\%$  of all patients screened and  $\sim 2\%$  of those with ESC-R organisms (Table 1). The KPC spread in Greece commenced in 2007 and was much advanced by mid-2008 (2, 34–36). Both Greek ICUs recorded KPC cases from the survey start, and their contribution to all patients screened and to ESC-R *Enterobacteriaceae* carriers was  $\sim 6\%$  and  $\sim 35\%$ , respectively. Italy reported the first KPC case in 2008, followed by an outbreak progressing rapidly from 2010 (2, 34, 37). The RU FS, screening patients from February 2009 to February 2011, had its first 2 cases late in 2009 and then 12 in 2010 and 8 in the first 2 months of 2011, being  $\sim 3\%$  of all patients and  $\sim 6\%$  of those with ESC-R organisms.

The *bla*<sub>KPC</sub> amplicons were digested by RsaI (Fermentas, Vilnius, Lithuania), which distinguishes *bla*<sub>KPC-2</sub> and *bla*<sub>KPC-3</sub> (38),

Received 12 November 2015 Returned for modification 9 December 2015

Accepted 18 December 2015

Accepted manuscript posted online 28 December 2015

Citation Baraniak A, Izdebski R, Fiett J, Herda M, Derde LPG, Bonten MJM, Adler A, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M, on behalf of the MOSAR WP2, WP3, and WP5 Study Groups. 2016. KPC-like carbapenemase-producing *Enterobacteriaceae* colonizing patients across Europe and Israel. *Antimicrob Agents Chemother* 60:1912–1917. doi:10.1128/AAC.02756-15.

Address correspondence to M. Gniadkowski, [gniadkow@cls.edu.pl](mailto:gniadkow@cls.edu.pl).

A.B. and R.I. contributed equally to this work.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

TABLE 1 Occurrence of patients colonized by KPC CPE in study centers

Country	Center	Unit type	No. of patients enrolled in the study <sup>a</sup>	No. (%) of patients colonized by <i>Enterobacteriaceae</i> producing acquired ESC-hydrolyzing $\beta$ -lactamases <sup>b,c,d</sup>	No. (%) of patients colonized by CPE <sup>b,d,e</sup>	No. (%) of patients colonized by KPC CPE <sup>d</sup>
France	HM	ICU	2,373	256 (10.8)	1 (0.04) <sup>e</sup>	
France	RP	ICU	1,328	85 (6.4)	1 (0.08) <sup>f</sup>	1 (0.08)
France	SJ	ICU	1,049	51 (4.9)	4 (0.4)	
Greece	AT	ICU	796	117 (14.7)	53 (6.7) <sup>g</sup>	44 (5.5)
Greece	LA	ICU	558	99 (17.7)	83 (14.9) <sup>h</sup>	35 (6.3)
Italy	CA	ICU	788	49 (6.2)	2 (0.3)	
Latvia	RI	ICU	1,464	526 (35.9)	10 (0.7)	
Luxemburg	LU	ICU	1,823	54 (3.0)		
Portugal	PO	ICU	910	18 (2.0)		
Portugal	VR	ICU	628	24 (3.8)	1 (0.2)	
Slovenia	GO	ICU	919	32 (3.5)		
Slovenia	LJ	ICU	685	115 (16.8)		
Spain	BA	ICU	1,069	41 (3.8)		
France	BM	RU	410	76 (18.5)		
Israel	LH	RU	564	177 (31.4)	6 (1.1) <sup>i</sup>	6 (1.1)
Israel	TA	RU	1,650	870 (52.7)	16 (1.0) <sup>j</sup>	16 (1.0)
Italy	FS	RU	704	340 (48.3)	28 (4.0)	22 (2.8)
Spain	GI	RU	227	104 (45.8)	5 (2.2)	
Total			17,945	3,034 (16.9)	210 (1.2)	124 (0.7)

<sup>a</sup> All patients that were swabbed at least once at a clinical center, regardless of the length of hospitalization.

<sup>b</sup> These numbers were shown also in the report on colonization by MBL CPE in MOSAR centers (27).

<sup>c</sup> Acquired ESC-hydrolyzing  $\beta$ -lactamases include ESBLs, AmpC-type cephalosporinases, MBLs, and KPCs.

<sup>d</sup> Patients in this column include both those who were colonized at admission and those who were colonized due to in-hospital transmission.

<sup>e</sup> Carbapenemases include KPCs and MBLs except in one patient in the French ICU HM who was colonized with *E. coli* coproducing OXA-48 carbapenemase and ESBL.

<sup>f</sup> This patient was colonized by KPC-producing *K. pneumoniae* and MBL-producing *E. coli*.

<sup>g</sup> One patient was colonized by KPC-producing *E. coli* and MBL-producing *K. pneumoniae*.

<sup>h</sup> Four patients were colonized by *K. pneumoniae* coproducing KPC and MBL.

<sup>i</sup> One patient was colonized by KPC-producing *E. coli* and *K. pneumoniae*.

<sup>j</sup> Two patients were colonized by two different KPC producers: either *C. freundii* and *E. coli* or *E. coli* and *K. pneumoniae*.

followed by sequencing for representative isolates. KPC-producing isolates were typed by pulsed-field gel electrophoresis (PFGE) as described previously (39). PFGE types and subtypes were distinguished visually according to the method of Tenover et al. (40). Selected isolates were analyzed also by multilocus sequence typing (MLST) (41–44); databases available at <http://pubmlst.org/cfreundii/> (*Citrobacter freundii*), <http://pubmlst.org/ecloacae/> (*Enterobacter cloacae*), <http://mlst.warwick.ac.uk/mlst/dbs/Ecoli> (*Escherichia coli*), and <http://bigsdw.web.pasteur.fr/klebsiella/klebsiella.html> (*K. pneumoniae*) were used for assigning STs. *E. cloacae* STs and  $\beta$ -lactamases were shown previously (45).

*K. pneumoniae* isolates, being the predominant species ( $n = 110$ ; 86.6%), were classified into 10 STs (Table 2). ST258 prevailed ( $n = 76$ ; 69.1%) and was observed in all but one of the sites (FS, Italy), dominating in Greece with  $bla_{KPC-2}$  ( $n = 73$ ; 93.6%). The next-most-prevalent clone, ST512 ( $n = 21$ ; 19.1%), was originally identified in this study in an Israeli isolate from 2008 (<http://bigsdw.web.pasteur.fr/klebsiella/klebsiella.html>). This single-locus variant (SLV) of ST258 carried  $bla_{KPC-3}$  and dominated in the Italian RU FS ( $n = 19$ ; 86.4%) but was sporadic in Israel. Four KPC-2- and VIM-1-positive Greek isolates belonged to ST147, the major VIM producer in Greece (27), while the remaining STs represented single isolates with KPC-2 or -3 in individual sites. *C. freundii*, *E. cloacae*, and *E. coli*, usually producing KPC-2s, were identified vastly in Israel and were clonally diverse, except for *E.*

*coli* ST131, of which there were three KPC-2 or -3 isolates. Most of the *E. cloacae*, *E. coli*, and *K. pneumoniae* isolates represented international clones (45, 46). For *C. freundii*, the clonality data are scarce (27, 41, 47), but KPC-producing *C. freundii* ST14, originally identified in this study, was found in 2015 in Malaysia [<http://pubmlst.org/cfreundii/>]. In general, the clonality plus KPC type data were congruent with data in national reports. The high KPC CPE diversity in the Israeli centers corresponds to the endemicity situation following the polyclonal outbreak of KPC-2 and the clonal spread of *K. pneumoniae* ST258 KPC-3 (7, 19, 22, 48, 49), even if other studies still indicate the importance of *K. pneumoniae* ST258/ST512 (50). In contrast, the high prevalence of ST258 KPC-2 in Greece and ST512 KPC-3 in Italy reflected their clonal dissemination in real time (35–37). This study is also yet another report on KPC-producing *E. coli* ST131, which has been repeatedly identified in Israel (4, 49, 51, 52).

The location of  $bla_{KPC}$  genes within Tn4401-like transposons and polymorphism of these was analyzed by PCR mapping (12). For the Tn4401g variant (15), an additional primer was designed (5'-GTTCCACTGAGCGTCAGAC-3') for use with primer 3781L (12) (expected product size, 370 bp). All  $bla_{KPC}$  genes were located in Tn4401 variants (12). The main type was Tn4401a (12), observed in all isolates from Greece, Italy, and France and in 9/22 Israeli isolates, including most *K. pneumoniae* isolates with  $bla_{KPC-2}$  or  $bla_{KPC-3}$  (Table 2). Tn4401c (14) and Tn4401g (15)

**TABLE 2** Geographic distribution, species, clones, pulsotypes, S1 plasmid profiles, plasmids and Tn4401 transposons with *bla*<sub>KPC</sub> genes, and other acquired β-lactamases of KPC CPE isolates<sup>a</sup>

Center	Species	ST (CC or CG) <sup>b</sup>	No. of isolates	No. of pulsotypes (no. of subtypes)	S1 profile <sup>c</sup>	Size of plasmid (kb); replicon(s) of plasmid with <i>bla</i> <sub>KPC</sub> gene(s) <sup>d</sup>	<i>bla</i> <sub>KPC</sub> <sup>e</sup>	Tn4401 variant <sup>f</sup>	MBL, ESBL, and/or AmpC (no. of isolates) <sup>g</sup>
AT (Greece)	<i>E. coli</i>	ST10 (CC10)	1	1	Eco1	~130; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	TEM-1
	<i>K. pneumoniae</i>	ST258 (CG258)	43	2 (18)	Kpn1	~120; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	SHV-12 + TEM-1
					Kpn2	~115; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	SHV-12 + TEM-1
LA (Greece)	<i>K. pneumoniae</i>	ST17 (CG17)	1	1	Kpn4	~115; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	SHV-5 + TEM-1
	<i>K. pneumoniae</i>	ST147 (CC147) <sup>h</sup>	4	1 (2)	Kpn6	~115; <i>FII<sub>K</sub> + FIB<sub>K</sub></i>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	VIM-1 + TEM-1
					Kpn9	~100; <i>FII<sub>K</sub> + FIB<sub>K</sub></i>			
	<i>K. pneumoniae</i>	ST258 (CG258)	30	1 (12)	Kpn2	~115; <i>FII<sub>K</sub> + FIB<sub>K</sub></i>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	SHV-12 + TEM-1
				Kpn10	~70; NT	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	SHV-12	
FS (Italy)	<i>K. pneumoniae</i>	ST16 (CG17)	1	1	Kpn7	~90; NT	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	CTX-M-15
	<i>K. pneumoniae</i>	ST45 (CG485)	1	1	Kpn3	~115; <i>FII<sub>K</sub> + FIB<sub>K</sub></i>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1
	<i>K. pneumoniae</i>	ST383 (CC42)	1	1	Kpn8	~100; <b>FII<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	CMY-4 + TEM-1
	<i>K. pneumoniae</i>	ST512 (CG258)	19	1 (9)	Kpn2/6	~115; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1 (19); SHV-12 + CMY-2 (1); OXA-1 (1) <sup>i</sup>
RP (France)	<i>K. pneumoniae</i>	ST258 (CG258)	1	1	ND	ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	CTX-M-15 + SHV + TEM-1
LH (Israel)	<i>E. cloacae</i>	ST78 (CC74) <sup>j</sup>	1	1	Ecl1	ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	SHV-12 + TEM-1 <sup>k</sup>
	<i>E. coli</i>	ST131 (CC131)	2	1 (2)	Eco2	~75; N	<i>bla</i> <sub>KPC-2</sub>	Tn4401g	TEM-1
	<i>E. coli</i>	ST167 (CC10)	1	1	Eco4	~90; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	SHV-12 + TEM-1
	<i>E. coli</i>	ST1571	1	1	Eco3	ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	SHV-12 + TEM-1
	<i>K. pneumoniae</i>	ST258 (CG258)	1	1	Kpn5	~115; <i>FII<sub>K</sub> + FIB<sub>K</sub></i>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1
	<i>K. pneumoniae</i>	ST512 (CG258)	1	1	Kpn11	~150; N	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	-
TA (Israel)	<i>C. freundii</i>	<b>ST14</b>	1	1	Cfr1	~80; N	<i>bla</i> <sub>KPC-2</sub>	Tn4401g	TEM-1 + OXA-1
	<i>C. freundii</i>	<b>ST12</b>	1	1	Cfr2	~80; N	<i>bla</i> <sub>KPC-2</sub>	Tn4401g	CTX-M-15 + TEM-1
	<i>C. freundii</i>	ND	1	1	Cfr3	~80; N	<i>bla</i> <sub>KPC-2</sub>	Tn4401g	SHV-12 + TEM-1 + OXA-1
	<i>C. freundii</i>	<b>ST10</b>	1	1	Cfr4	~300; ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	TEM-1 + OXA-1
	<i>C. freundii</i>	<b>ST15</b>	1	1	Cfr4	~300; ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	TEM-1 + OXA-1
	<i>E. cloacae</i>	<b>ST118</b> <sup>l</sup>	1	1	Ecl3	~320; ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	CTX-M-27 + SHV-12 + TEM-1 <sup>k</sup>
	<i>E. cloacae</i>	<b>ST146</b> <sup>l</sup>	1	1	Ecl2	~300; ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	TEM-1 <sup>k</sup>
	<i>E. coli</i>	ST69 (CC69)	1	1	Eco8	~70; N	<i>bla</i> <sub>KPC-2</sub>	NT	TEM-1
	<i>E. coli</i>	ST131 (CC131)	1	1	Eco5	~115; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1
	<i>E. coli</i>	ST216	1	1	Eco6	~60; N	<i>bla</i> <sub>KPC-2</sub>	Tn4401g	TEM-1 + OXA-1
	<i>E. coli</i>	<b>ST3541</b>	1	1	Eco7	ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	CTX-M-15 + SHV-12 + CMY-2 + TEM-1 + OXA-1
	<i>K. pneumoniae</i>	ST17 (CG17)	1	1	Kpn12	~140; N	<i>bla</i> <sub>KPC-2</sub>	Tn4401g	TEM-1 + OXA-1
	<i>K. pneumoniae</i>	ST34 (CC34)	1	1	Kpn13	ND	<i>bla</i> <sub>KPC-2</sub>	Tn4401c	CTX-M-15 + SHV-12 + TEM-1 + OXA-1
	<i>K. pneumoniae</i>	ST36 (CG485)	1	1	Kpn14	~115; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1
<i>K. pneumoniae</i>	ST258 (CG258)	1	1	Kpn2	~115; <i>FII<sub>K</sub> + FIB<sub>K</sub></i>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1	
<i>K. pneumoniae</i>	ST383 (CC42)	1	1	Kpn15	~115; <b>FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	CTX-M-15 + CMY-4 + TEM-1	
<i>K. pneumoniae</i>	<b>ST512</b> (CG258)	1	1	Kpn16	~140; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-3</sub>	Tn4401a	TEM-1 + OXA-1	
<i>K. pneumoniae</i>	ST833 (CG258)	1	1	Kpn17	~100; <b>FII<sub>K</sub> + FIB<sub>K</sub></b>	<i>bla</i> <sub>KPC-2</sub>	Tn4401a	SHV-12	

<sup>a</sup> Other acquired β-lactamases include MBLs, ESBLs, AmpCs, and broad-spectrum β-lactamases. CC, clonal complex; CG, clonal group; ND, not determined; NT, nontypeable; FII<sub>K</sub>, FIB<sub>K</sub>, and N are plasmid replicon types.

<sup>b</sup> New STs are indicated in bold. Numerous reports on *K. pneumoniae* ST512 have been published since 2012 (2, 11, 34, 37); however, this ST was identified originally in this study (isolate identifier 578 in the *K. pneumoniae* MLST database [http://bigsdw.web.pasteur.fr]). In groups of four or more isolates, MLST was performed for representative isolates, based on the PFGE data.

<sup>c</sup> In large groups of isolates of the same ST/pulsotype (*K. pneumoniae* ST258 and ST512), the S1 analysis was performed for representative isolates. S1 plasmid profiles are numbered within species groups of isolates; profiles differed from each other by number and/or size of plasmids.

<sup>d</sup> Plasmids found in transformants are shown in bold. Replicons shown in italics represent the probable types of *bla*<sub>KPC</sub> plasmids (PBRT and pKpQIL PCR mapping was performed on DNA of clinical isolates).

<sup>e</sup> In groups of four or more isolates of the same ST/pulsotype, *bla*<sub>KPC</sub> sequencing was performed for representative isolates; for the remaining isolates, RsaI PCR-restriction fragment length polymorphism analysis distinguishing *bla*<sub>KPC-2</sub> and *bla*<sub>KPC-3</sub> sequences (38) was carried out.

<sup>f</sup> In groups of four or more isolates of the same ST/pulsotype, PCR mapping of Tn4401-like elements was performed for representative isolates.

<sup>g</sup> In groups of four or more isolates with the same ST/pulsotype and *bla* genes, PCR profile sequencing was performed for representative isolates.

<sup>h</sup> These isolates were also included in the study of MBL CPE isolates identified during the MOSAR project (27).

<sup>i</sup> All isolates of this group produced TEM-1; one isolate produced additionally SHV-12 and CMY-2, and another one produced OXA-1.

<sup>j</sup> STs and β-lactamases of the *E. cloacae* isolates from LH and TA were reported previously (45).

were found only in Israel in various species and clones, always containing *bla*<sub>KPC-2</sub>. Tn4401a has been the main type of Tn4401, strongly associated with *K. pneumoniae* ST258 worldwide (6, 10, 18, 21, 36), while Tn4401c has been observed in diverse KPC-2-producing organisms in Israel (15, 49). Interestingly, Tn4401c-derived Tn4401g was identified only recently in a single *K. pneumoniae* KPC-2 isolate recovered in Israel in 2008 (15), whereas in this study, it occurred frequently in *C. freundii*, *E. coli*, and *K. pneumoniae*.

Plasmid profiling and identification of *bla*<sub>KPC</sub>-carrying plasmids was done with nuclease S1 (New England BioLabs, Beverly, MA) analysis (53) and hybridization with the *bla*<sub>KPC</sub> probe, using the enhanced-chemiluminescence (ECL) Random-Prime labeling and detection system (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). The analysis comprised 44 isolates of all species, STs, and pulsotypes (15 *K. pneumoniae* ST258/ST512 isolates), revealing highly varied plasmid profiles, with *bla*<sub>KPC</sub>-carrying plasmids ranging in size from ~60 to ~320 kb (Table 2). Plasmid DNA of 27 isolates of various species, STs, and S1 profiles was purified with the Qiagen plasmid midi kit (Qiagen, Hilden, Germany) and electroporated into *E. coli* DH5 $\alpha$ , with transformant selection with 0.5  $\mu$ g/ml imipenem or 1  $\mu$ g/ml cefotaxime. Subsequently, plasmids of the transformants were purified and subjected to PCR-based replicon typing (PBRT) (54–57). KPC-positive transformants were obtained for 22 isolates (Table 2). PBRT revealed that 12 of these had plasmids with FII<sub>K</sub> and FIB<sub>K</sub> replicons (alternating in two cases) of ~90 to ~140 kb. PCR mapping, performed as proposed by Baraniak et al. (10), showed that all these were of the pKpQIL type (21), and molecules positive in that assay were identified also in selected isolates for which no transformants were available (Table 2). The pKpQIL-like plasmids carried *bla*<sub>KPC-2</sub> or *bla*<sub>KPC-3</sub> (Tn4401a) and were hosted mainly in *K. pneumoniae* ST258 and ST512 isolates; however, these occurred also in other organisms (10, 22, 23). The other group was IncN plasmids of ~60 to ~150 kb, identified in various *C. freundii*, *E. coli*, and *K. pneumoniae* Israeli strains, usually carrying *bla*<sub>KPC-2</sub> (Tn4401g). These plasmids have been observed among diverse KPC-2-producing *E. coli* and non-CG258 *K. pneumoniae* isolates in Israel (15, 49). However, some of our isolates fell beyond this pattern, like *K. pneumoniae* ST833 (SLV of ST258), with *bla*<sub>KPC-2</sub> on a pKpQIL-like plasmid or *K. pneumoniae* ST512 with *bla*<sub>KPC-3</sub> on an IncN molecule. Finally, the *bla*<sub>KPC-2</sub> gene in the Tn4401c variant was observed in *C. freundii* and *E. cloacae* in large plasmids (~300 to ~320 kb) that could not be separated by transfer despite repeated attempts; their replicon types thus remained undetermined.

The KPC CPE isolates were analyzed for other acquired  $\beta$ -lactamase genes, namely, *bla*<sub>SHV-5/SHV-12</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>CMY-2</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>OXA-1</sub> types, by PCR and sequencing (32, 58–60). The isolates had various  $\beta$ -lactamase combinations, including SHV- and CTX-M-like ESBLs, AmpCs of the CMY-2 type, and broad-spectrum TEM-1 and OXA-1 enzymes (Table 2).

We assessed the KPC CPE carriage among ICU and RU patients on a large international scale, using the same time frame and methodology. Not surprisingly, KPC producers were found mainly in the countries which reported their wide spread, i.e., Greece, Italy, and Israel (2, 33–37). Considering the study period, 2008 to 2011, the rhythm of occurrence of cases in individual centers and characteristics of the organisms reflected the situation in the countries, i.e., the onset and advanced stage of nationwide

outbreaks in Italy and Greece, respectively, and the postoutbreak endemicity in Israel (33, 35–37). The analysis provided a comparative snapshot of the geographic and quantitative distribution of species/clones, Tn4401 transposon variants, and *bla*<sub>KPC</sub>-carrying plasmids, often observed in national reports. Also, this has been one of the first studies of *C. freundii* and *E. cloacae* that included MLST data.

## ACKNOWLEDGMENTS

The MOSAR WP2, WP3, and WP5 Study Groups also included the following: M. J. Dautzenberg, University Medical Center Utrecht, Utrecht, The Netherlands; M. Kazma and S. Navon-Venezia, Division of Epidemiology and Preventive Medicine, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel; S. Malhotra-Kumar and C. Lammens, University of Antwerp, Antwerp, Belgium; P. Legrand, Hôpital Henri Mondor, Créteil, France; D. Annane, Hôpital Raymond Poincaré, Garches, France; A. Chalfine, Groupe Hospitalier Paris, Saint Joseph, Paris, France; H. Giamarellou, Attikon General Hospital, Athens, Greece; G. L. Petrikos, Laikon General Hospital, University of Athens, Athens, Greece; G. Nardi, Azienda Ospedaliera S. Camillo Forlanini, Rome, Italy; A. Balode and U. Dumpis, Paul Stradins University Hospital, Riga, Latvia; P. Stammel, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg; I. Aragão, Central Hospital of Porto, Porto, Portugal; F. Esteves, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal; I. Muzlovic, University Medical Centre Ljubljana, Ljubljana, Slovenia; V. Tomic, University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia; A. Torres Martí, Hospital Clínic, University of Barcelona, Barcelona, Spain; C. Lawrence, Hôpital Maritime de Berck, Berck, France, and Garches, Paris, France; J. Salomon, INSERM, Institut Pasteur, Cnam, Paris, France; M. Paul, Loewenstein Hospital, Ra'anana, Israel; Y. Lerman, Geriatric Division, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; A. Rossini and A. Salvia, Fondazione Santa Lucia IRCCS, Rome, Italy; and J. Vidal Samsó and J. Fierro, Institute Guttmann, Barcelona, Spain.

We thank curator teams of the Medical University in Białystok, Poland, the National Center for Global Health and Medicine in Tokyo, Japan, the University of Warwick in Warwick, United Kingdom, and the Institut Pasteur in Paris, France, for curating the MLST data of *C. freundii*, *E. cloacae*, *E. coli*, and *K. pneumoniae*, respectively, and making them publicly available.

## FUNDING INFORMATION

National Science Centre, Poland provided funding to Anna Baraniak, Radosław Izdebski, Janusz Fiett, Małgorzata Herda, Waleria Hryniewicz, and Marek Gniadkowski under grant number UMO-2012/07/B/NZ6/03528. European Commission (EC) provided funding to Anna Baraniak, Radosław Izdebski, Janusz Fiett, Małgorzata Herda, Lennie Derde, Marc J Bonten, Amos Adler, Yehuda Carmeli, Herman Goossens, Waleria Hryniewicz, Christian Brun-Buisson, and Marek Gniadkowski under grant number LSHP-CT-2007-037941.

## REFERENCES

1. Nordmann P, Poirel L. 2014. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect* 20:821–830. <http://dx.doi.org/10.1111/1469-0691.12719>.
2. Muñoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP. 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 13:785–796. [http://dx.doi.org/10.1016/S1473-3099\(13\)70190-7](http://dx.doi.org/10.1016/S1473-3099(13)70190-7).
3. Pitout JD, Nordmann P, Poirel L. 2015. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother* 59:5873–5884. <http://dx.doi.org/10.1128/AAC.01019-15>.
4. Mathers AJ, Peirano G, Pitout JD. 2015. The role of epidemic resistance



- plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clin Microbiol Rev* 28:565–591. <http://dx.doi.org/10.1128/CMR.00116-14>.
5. Cuzon G, Naas T, Truong H, Villegas MV, Wisell KT, Carmeli Y, Gales AC, Venezia SN, Quinn JP, Nordmann P. 2010. Worldwide diversity of *Klebsiella pneumoniae* that produce  $\beta$ -lactamase *bla*<sub>KPC-2</sub> gene. *Emerg Infect Dis* 16:1349–1356. <http://dx.doi.org/10.3201/eid1609.091389>.
  6. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, Brolund A, Giske CG. 2009. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 53:3365–3370. <http://dx.doi.org/10.1128/AAC.00126-09>.
  7. Leavitt A, Carmeli Y, Chmelnitsky I, Goren MG, Ofek I, Navon-Venezia S. 2010. Molecular epidemiology, sequence types, and plasmid analyses of KPC-producing *Klebsiella pneumoniae* strains in Israel. *Antimicrob Agents Chemother* 54:3002–3006. <http://dx.doi.org/10.1128/AAC.01818-09>.
  8. Curiao T, Morosini MI, Ruiz-Garbajosa P, Robustillo A, Baquero F, Coque TM, Canton R. 2010. Emergence of *bla*<sub>KPC-3</sub>-Tn4401a associated with a pKPN3/4-like plasmid within ST384 and ST388 *Klebsiella pneumoniae* clones in Spain. *J Antimicrob Chemother* 65:1608–1614. <http://dx.doi.org/10.1093/jac/dkq174>.
  9. Navon-Venezia S, Leavitt A, Schwaber MJ, Rasheed JK, Srinivasan A, Patel JB, Carmeli Y. 2009. First report on a hyperepidemic clone of KPC-3-producing *Klebsiella pneumoniae* in Israel genetically related to a strain causing outbreaks in the United States. *Antimicrob Agents Chemother* 53:818–820. <http://dx.doi.org/10.1128/AAC.00987-08>.
  10. Baraniak A, Grabowska A, Izdebski R, Fiett J, Herda M, Bojarska K, Zabicka D, Kania-Pudlo M, Mlynarczyk G, Zak-Pulawska Z, Hryniewicz W, Gniadkowski M. 2011. Molecular characteristics of KPC-producing *Enterobacteriaceae* at the early stage of their dissemination in Poland, 2008–2009. *Antimicrob Agents Chemother* 55:5493–5499. <http://dx.doi.org/10.1128/AAC.05118-11>.
  11. Warburg G, Hidalgo-Grass C, Partridge SR, Tolmasky ME, Temper V, Moses AE, Block C, Strahilevitz J. 2012. A carbapenem-resistant *Klebsiella pneumoniae* epidemic clone in Jerusalem: sequence type 512 carrying a plasmid encoding *aac(6′)-Ib*. *J Antimicrob Chemother* 67:898–901. <http://dx.doi.org/10.1093/jac/dkr552>.
  12. Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. 2008. Genetic structures at the origin of acquisition of the  $\beta$ -lactamase *bla*<sub>KPC</sub> gene. *Antimicrob Agents Chemother* 52:1257–1263. <http://dx.doi.org/10.1128/AAC.01451-07>.
  13. Kitchel B, Rasheed JK, Endimiani A, Hujer AM, Anderson KF, Bonomo RA, Patel JB. 2010. Genetic factors associated with elevated carbapenem resistance in KPC-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 54:4201–4207. <http://dx.doi.org/10.1128/AAC.00008-10>.
  14. Naas T, Cuzon G, Truong HV, Nordmann P. 2012. Role of ISKpn7 and deletions in *bla*<sub>KPC</sub> gene expression. *Antimicrob Agents Chemother* 56:4753–4759. <http://dx.doi.org/10.1128/AAC.00334-12>.
  15. Chmelnitsky I, Shklyar M, Leavitt A, Sadovsky E, Navon-Venezia S, Ben Dalak M, Edgar R, Carmeli Y. 2014. Mix and match of KPC-2 encoding plasmids in *Enterobacteriaceae*-comparative genomics. *Diagn Microbiol Infect Dis* 79:255–260. <http://dx.doi.org/10.1016/j.diagmicrobio.2014.03.008>.
  16. Andrade LN, Curiao T, Ferreira JC, Longo JM, Climaco EC, Martinez R, Bellissimo-Rodrigues F, Basile-Filho A, Evaristo MA, Del Peloso PF, Ribeiro VB, Barth AL, Paula MC, Baquero F, Canton R, Darini AL, Coque TM. 2011. Dissemination of *bla*<sub>KPC-2</sub> by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* species in Brazil. *Antimicrob Agents Chemother* 55:3579–3583. <http://dx.doi.org/10.1128/AAC.01783-10>.
  17. Gootz TD, Lescoe MK, Dib-Hajj F, Dougherty BA, He W, Della-Latta P, Huard RC. 2009. Genetic organization of transposase regions surrounding *bla*<sub>KPC</sub> carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. *Antimicrob Agents Chemother* 53:1998–2004. <http://dx.doi.org/10.1128/AAC.01355-08>.
  18. Gomez SA, Pasteran FG, Faccione D, Tijet N, Rapoport M, Lucero C, Lastovetska O, Albornoz E, Galas M, Group KPC, Melano RG, Corso A, Petroni A. 2011. Clonal dissemination of *Klebsiella pneumoniae* ST258 harbouring KPC-2 in Argentina. *Clin Microbiol Infect* 17:1520–1524. <http://dx.doi.org/10.1111/j.1469-0691.2011.03600.x>.
  19. Goren MG, Navon-Venezia S, Chmelnitsky I, Carmeli Y. 2010. Carbapenem-resistant KPC-2-producing *Escherichia coli* in a Tel Aviv Medical Center, 2005 to 2008. *Antimicrob Agents Chemother* 54:2687–2691. <http://dx.doi.org/10.1128/AAC.01359-09>.
  20. Mataseje LF, Boyd DA, Willey BM, Prayitno N, Kreiswirth N, Gelosia A, Poutanen SM, Low DE, Jenkins SG, Katz K, Mulvey MR. 2011. Plasmid comparison and molecular analysis of *Klebsiella pneumoniae* harbouring *bla*<sub>KPC</sub> from New York City and Toronto. *J Antimicrob Chemother* 66:1273–1277. <http://dx.doi.org/10.1093/jac/dkr092>.
  21. Leavitt A, Chmelnitsky I, Carmeli Y, Navon-Venezia S. 2010. Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. *Antimicrob Agents Chemother* 54:4493–4496. <http://dx.doi.org/10.1128/AAC.00175-10>.
  22. Leavitt A, Chmelnitsky I, Ofek I, Carmeli Y, Navon-Venezia S. 2010. Plasmid pKpQIL encoding KPC-3 and TEM-1 confers carbapenem resistance in an extremely drug-resistant epidemic *Klebsiella pneumoniae* strain. *J Antimicrob Chemother* 65:243–248. <http://dx.doi.org/10.1093/jac/dkp417>.
  23. Adler A, Shklyar M, Schwaber MJ, Navon-Venezia S, Dhaher Y, Edgar R, Solter E, Benenson S, Masarwa S, Carmeli Y. 2011. Introduction of OXA-48-producing *Enterobacteriaceae* to Israeli hospitals by medical tourism. *J Antimicrob Chemother* 66:2763–2766. <http://dx.doi.org/10.1093/jac/dkr382>.
  24. Garcia-Fernandez A, Villa L, Carta C, Venditti C, Giordano A, Venditti M, Mancini C, Carattoli A. 2012. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob Agents Chemother* 56:2143–2145. <http://dx.doi.org/10.1128/AAC.05308-11>.
  25. Chen L, Chavda KD, Melano RG, Jacobs MR, Koll B, Hong T, Rojzman AD, Levi MH, Bonomo RA, Kreiswirth BN. 2014. Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York Hospitals. *Antimicrob Agents Chemother* 58:2871–2877. <http://dx.doi.org/10.1128/AAC.00120-14>.
  26. Derde LP, Cooper BS, Goossens H, Malhotra-Kumar S, Willems RJ, Gniadkowski M, Hryniewicz W, Empel J, Dautzenberg MJ, Annane D, Aragao I, Chalfine A, Dumpis U, Esteves F, Giamarellou H, Muzlovic I, Nardi G, Petrikos GL, Tomic V, Marti AT, Stammet P, Brun-Buisson C, Bonten MJ. 2014. Interventions to reduce colonisation and transmission of antimicrobial-resistant bacteria in intensive care units: an interrupted time series study and cluster randomised trial. *Lancet Infect Dis* 14:31–39. [http://dx.doi.org/10.1016/S1473-3099\(13\)70295-0](http://dx.doi.org/10.1016/S1473-3099(13)70295-0).
  27. Papagiannitsis CC, Izdebski R, Baraniak A, Fiett J, Herda M, Hrabak J, Derde LP, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M. 2015. Survey of metallo- $\beta$ -lactamase-producing *Enterobacteriaceae* colonizing patients in European ICUs and rehabilitation units, 2008–11. *J Antimicrob Chemother* 70:1981–1988. <http://dx.doi.org/10.1093/jac/dkv055>.
  28. Drieux L, Brossier F, Sougakoff W, Jarlier V. 2008. Phenotypic detection of extended-spectrum  $\beta$ -lactamase production in *Enterobacteriaceae*: review and bench guide. *Clin Microbiol Infect* 14(Suppl 1):S90–S103.
  29. Doi Y, Potoski BA, Adams-Haduch JM, Sidjabat HE, Pasculle AW, Paterson DL. 2008. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type  $\beta$ -lactamase by use of a boronic acid compound. *J Clin Microbiol* 46:4083–4086. <http://dx.doi.org/10.1128/JCM.01408-08>.
  30. Lee K, Lim YS, Yong D, Yum JH, Chong Y. 2003. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo- $\beta$ -lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 41:4623–4629. <http://dx.doi.org/10.1128/JCM.41.10.4623-4629.2003>.
  31. Glupczynski Y, Huang TD, Bouchahrouf W, Rezende de Castro R, Bauraing C, Gerard M, Verbruggen AM, Deplano A, Denis O, Bogaerts P. 2012. Rapid emergence and spread of OXA-48-producing carbapenem-resistant *Enterobacteriaceae* isolates in Belgian hospitals. *Int J Antimicrob Agents* 39:168–172. <http://dx.doi.org/10.1016/j.ijantimicag.2011.10.005>.
  32. Baraniak A, Izdebski R, Herda M, Fiett J, Hryniewicz W, Gniadkowski M, Kern-Zdanowicz I, Filczak K, Lopaciuk U. 2009. Emergence of *Klebsiella pneumoniae* ST258 with KPC-2 in Poland. *Antimicrob Agents Chemother* 53:4565–4567. <http://dx.doi.org/10.1128/AAC.00436-09>.
  33. Schwaber MJ, Lev B, Israeli A, Solter E, Smollan G, Rubinovitch B, Shalit I, Carmeli Y. 2011. Containment of a country-wide outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals via a nationally implemented intervention. *Clin Infect Dis* 52:848–855. <http://dx.doi.org/10.1093/cid/cir025>.

34. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P. 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 18:413–431. <http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x>.
35. Pournaras S, Protonotariou E, Voulgari E, Kristo I, Dimitroulia E, Vitti D, Tsalidou M, Maniatis AN, Tsakris A, Sofianou D. 2009. Clonal spread of KPC-2 carbapenemase-producing *Klebsiella pneumoniae* strains in Greece. *J Antimicrob Chemother* 64:348–352. <http://dx.doi.org/10.1093/jac/dkp207>.
36. Giakkoupi P, Papagiannitsis CC, Miriagou V, Pappa O, Polemis M, Tryfinopoulou K, Tzouveleki LS, Vatopoulos AC. 2011. An update of the evolving epidemic of bla<sub>KPC-2</sub>-carrying *Klebsiella pneumoniae* in Greece (2009–10). *J Antimicrob Chemother* 66:1510–1513. <http://dx.doi.org/10.1093/jac/dkr166>.
37. Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, AMCLICRE Survey Participants, Pantosti A, Pagani L, Luzzaro F, Rossolini GM. 2013. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill* 18:20489. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20489>.
38. Lopez JA, Correa A, Navon-Venezia S, Correa AL, Torres JA, Briceno DF, Montealegre MC, Quinn JP, Carmeli Y, Villegas MV. 2011. Intercontinental spread from Israel to Colombia of a KPC-3-producing *Klebsiella pneumoniae* strain. *Clin Microbiol Infect* 17:52–56. <http://dx.doi.org/10.1111/j.1469-0691.2010.03209.x>.
39. Seifert H, Dolzani L, Bressan R, van der Reijden T, van Strijen B, Stefanik D, Heersma H, Dijkshoorn L. 2005. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 43:4328–4335. <http://dx.doi.org/10.1128/JCM.43.9.4328-4335.2005>.
40. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33:2233–2239.
41. Bai L, Xia S, Lan R, Liu L, Ye C, Wang Y, Jin D, Cui Z, Jing H, Xiong Y, Bai X, Sun H, Zhang J, Wang L, Xu J. 2012. Isolation and characterization of cytotoxic, aggregative *Citrobacter freundii*. *PLoS One* 7:e33054. <http://dx.doi.org/10.1371/journal.pone.0033054>.
42. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 43:4178–4182. <http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005>.
43. Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, Shimojima M, Kirikae T. 2013. Multilocus sequence typing (MLST) for characterization of *Enterobacter cloacae*. *PLoS One* 8:e66358. <http://dx.doi.org/10.1371/journal.pone.0066358>.
44. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 60:1136–1151. <http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x>.
45. Izdebski R, Baraniak A, Herda M, Fielt J, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M. 2015. MLST reveals potentially high-risk international clones of *Enterobacter cloacae*. *J Antimicrob Chemother* 70:48–56. <http://dx.doi.org/10.1093/jac/dku359>.
46. Woodford N, Turton JF, Livermore DM. 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev* 35:736–755. <http://dx.doi.org/10.1111/j.1574-6976.2011.00268.x>.
47. Gaibani P, Ambretti S, Farruggia P, Bua G, Berlinger A, Tamburini MV, Cordovana M, Guerra L, Mazzetti M, Roncarati G, Tenace C, Moro ML, Gagliotti C, Landini MP, Sambri V. 2013. Outbreak of *Citrobacter freundii* carrying VIM-1 in an Italian hospital, identified during the carbapenemases screening actions, June 2012. *Int J Infect Dis* 17:e714–e717. <http://dx.doi.org/10.1016/j.ijid.2013.02.007>.
48. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y. 2008. Isolation of imipenem-resistant *Enterobacter* species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. *Antimicrob Agents Chemother* 52:1413–1418. <http://dx.doi.org/10.1128/AAC.01103-07>.
49. Adler A, Miller-Roll T, Assous MV, Geffen Y, Paikin S, Schwartz D, Weiner-Well Y, Hussein K, Cohen R, Carmeli Y. 2015. A multicenter study of the clonal structure and resistance mechanism of KPC-producing *Escherichia coli* isolates in Israel. *Clin Microbiol Infect* 21:230–235. <http://dx.doi.org/10.1016/j.cmi.2014.10.008>.
50. Adler A, Hussein O, Ben-David D, Masarwa S, Navon-Venezia S, Schwaber MJ, Carmeli Y, Post-Acute-Care Hospital Carbapenem-Resistant Enterobacteriaceae Working Group. 2015. Persistence of *Klebsiella pneumoniae* ST258 as the predominant clone of carbapenemase-producing Enterobacteriaceae in post-acute-care hospitals in Israel, 2008–13. *J Antimicrob Chemother* 70:89–92. <http://dx.doi.org/10.1093/jac/dku333>.
51. Naas T, Cuzon G, Gaillot O, Courcol R, Nordmann P. 2011. When carbapenem-hydrolyzing  $\beta$ -lactamase KPC meets *Escherichia coli* ST131 in France. *Antimicrob Agents Chemother* 55:4933–4934. <http://dx.doi.org/10.1128/AAC.00719-11>.
52. O'Hara JA, Hu F, Ahn C, Nelson J, Rivera JJ, Pasculle AW, Doi Y. 2014. Molecular epidemiology of KPC-producing *Escherichia coli*: occurrence of ST131-*fimH30* subclone harboring pKpQIL-like IncFIIk plasmid. *Antimicrob Agents Chemother* 58:4234–4237. <http://dx.doi.org/10.1128/AAC.02182-13>.
53. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. *Anal Biochem* 226:235–240. <http://dx.doi.org/10.1006/abio.1995.1220>.
54. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 63:219–228. <http://dx.doi.org/10.1016/j.mimet.2005.03.018>.
55. Villa L, Garcia-Fernandez A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. *J Antimicrob Chemother* 65:2518–2529. <http://dx.doi.org/10.1093/jac/dkq347>.
56. Garcia-Fernandez A, Fortini D, Veldman K, Mevius D, Carattoli A. 2009. Characterization of plasmids harbouring *qnrS1*, *qnrB2* and *qnrB19* genes in *Salmonella*. *J Antimicrob Chemother* 63:274–281. <http://dx.doi.org/10.1093/jac/dkn470>.
57. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A. 2012. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. *Plasmid* 68:43–50. <http://dx.doi.org/10.1016/j.plasmid.2012.03.001>.
58. Woodford N, Fagan EJ, Ellington MJ. 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum  $\beta$ -lactamases. *J Antimicrob Chemother* 57:154–155.
59. Empel J, Baraniak A, Literacka E, Mrowka A, Fielt J, Sadowy E, Hryniewicz W, Gniadkowski M. 2008. Molecular survey of  $\beta$ -lactamases conferring resistance to newer  $\beta$ -lactams in Enterobacteriaceae isolates from Polish hospitals. *Antimicrob Agents Chemother* 52:2449–2454. <http://dx.doi.org/10.1128/AAC.00043-08>.
60. Izdebski R, Baraniak A, Fielt J, Adler A, Kazma M, Salomon J, Lawrence C, Rossini A, Salvia A, Vidal Samsó J, Fierro J, Paul M, Lerman Y, Malhotra-Kumar S, Lammens C, Goossens H, Hryniewicz W, Brun-Buisson C, Carmeli Y, Gniadkowski M. 2013. Clonal structure, extended-spectrum  $\beta$ -lactamases, and acquired AmpC-type cephalosporinases of *Escherichia coli* populations colonizing patients in rehabilitation centers in four countries. *Antimicrob Agents Chemother* 57:309–316. <http://dx.doi.org/10.1128/AAC.01656-12>.