

Nosocomial Outbreak of VIM-1-Producing *Klebsiella pneumoniae* Isolates of Multilocus Sequence Type 15: Molecular Basis, Clinical Risk Factors, and Outcome

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We study the epidemiology, molecular basis, clinical risk factors, and outcome involved in the clonal dissemination of VIM-1-producing *Klebsiella pneumoniae* isolates in the hospital setting. All patients infected/colonized by carbapenem-nonsusceptible *K. pneumoniae* (CNSKP) in 2009 were included. Molecular epidemiology was studied by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Antibiotic resistance genes were analyzed by PCR and sequencing. Plasmids were studied by PFGE with S1 nuclease digestion and for incompatibility group by a PCR-based replicon typing scheme. Risk factors associated with CNSKP colonization/infection were assessed by an observational case-control study. All 55 patients studied were infected ($n = 28$) or colonized ($n = 27$) by VIM-1-producing *K. pneumoniae*. All but one acquired isolates of a single clone (PFGE cluster 1 [C1], sequence type 15 [ST15]), while another clone (PFGE C2, ST340) was detected in four patients. C1 isolates also produced the new extended-spectrum β -lactamase SHV-134. bla_{VIM-1} was carried in a class 1 integron and an untypeable plasmid of ~ 50 bp. The number of days that the patient received mechanical ventilation, the use of parenteral nutrition, previous treatment with linezolid, and treatment with extended-spectrum cephalosporins for more than 7 days were detected to be independent risk factors for CNSKP acquisition. The VIM-1-producing *K. pneumoniae* ST15 clone has a high capacity to spread among intensive care unit patients with severe underlying conditions. A high rate of associated mortality and great difficulty in controlling the spread of this clone, without permanent behavioral changes in the personnel, were observed.

Carbapenemases hydrolyze all beta-lactam antibiotics, including carbapenems (5, 19, 33, 37), and their high potential for rapid, wide dissemination constitutes a major clinical and public health threat (5, 18, 19, 24, 26–28, 37). Class B carbapenemases include metallo- β -lactamases (MBLs), such as VIM, IMP, and NDM-1 (19, 24). Acquired MBLs are increasingly reported from *Enterobacteriaceae* (5, 9, 14, 10, 24, 35, 37).

Klebsiella pneumoniae is a major cause of nosocomial infections, particularly in intensive care units (ICUs), representing a relevant potential clinical risk. During the last decade, VIM-type MBLs have spread in *K. pneumoniae* (2, 21, 31, 32, 35, 38) and outbreaks of such strains have been reported (2, 21, 32).

In Spain, VIM-1-producing *Enterobacteriaceae* have been described to be associated with single cases, small outbreaks (6, 31, 36), or polyclonal spread affecting different bacterial species (25, 35).

This study describes an extensive nosocomial outbreak caused by a clonal multidrug-resistant strain of *K. pneumoniae* producing VIM-1. Our aims were to assess the epidemiology, molecular basis, clinical risk factors, and outcomes involved in the acquisition and dissemination of VIM-1-producing *K. pneumoniae* in the hospital setting.

MATERIALS AND METHODS

Study design and bacterial isolates. During 2009, an increase in the isolation of carbapenem-nonsusceptible *K. pneumoniae* (CNSKP) isolates was noted in the University Hospital Puerta de Hierro Majadahonda (UHPHM) in Madrid, Spain. This observation prompted the present investigation. UHPHM is a tertiary care hospital with 613 inpatient beds, 52 intensive care beds, and over 17,000 hospital admissions per year.

All the patients infected and/or colonized by CNSKP isolates between January 2009 and December 2009 were included in this study. An infected case caused by CNSKP was defined according to CDC criteria (17); a colonized case was defined as a patient carrying CNSKP without clinical evidence of infection.

Bacterial identification and susceptibility testing. Species identification and antibiotic susceptibility testing were performed by broth microdilution (MicroScan; Siemens Healthcare Diagnostics, Deerfield, IL) and by Etest (AB Biodisk, Solna, Sweden). Susceptibility results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (8). Antibiotic-susceptible *Escherichia coli* ATCC 25922 and carbapenem-resistant *E. coli* MN4 (30) were used as quality control strains. Tigecycline susceptibility was determined by the Etest and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (13).

Carbapenem nonsusceptibility was considered to be resistance or intermediate susceptibility to one of more of the three carbapenem antibiotics tested: imipenem, meropenem, and ertapenem.

The modified Hodge test was carried out using an imipenem disk;

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imipenem susceptibility alone and in combination with EDTA was also determined (Etest; AB Biodisk, Solna, Sweden).

Molecular epidemiology. The genetic relatedness between the *K. pneumoniae* isolates was determined by pulsed-field gel electrophoresis (PFGE) after total chromosomal DNA digestion with XbaI (30).

K. pneumoniae isolates representing the different PFGE clusters were further studied by multilocus sequence typing (MLST) according to the Institut Pasteur scheme (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>); last accessed December 2010).

Antibiotic resistance mechanisms and class 1 integron characterization. Standard PCR conditions were used to amplify genes coding for carbapenemases (*bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}), extended-spectrum β -lactamases (ESBLs; *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}), and plasmid-mediated AmpC (*bla*_{CMY}, *bla*_{FOX}, *bla*_{MOX}, *bla*_{DHA}, *bla*_{EBC}, *bla*_{ACC}). Aminoglycoside acetylase resistance genes [*aac*(3)-IIa, *aac*(6')-Ib] and 16S RNA methylases (*armA*, *rmtA*, *rmtB*) were also screened (15, 30).

Class 1 integron structures, the integrase gene *intI1*, and the variable regions, as well as the linkage of the *bla*_{SHV-134} allele with IS26, were screened by PCR amplification and DNA sequencing (12, 34).

In addition, the entire sequences of the *ompK35* and *ompK36* genes in four isolates representing the two CNSKP clones detected were analyzed by PCR and DNA sequencing (20).

Conjugation assay and plasmid characterization. Conjugation experiments were performed with four donor *K. pneumoniae* clinical isolates, previously selected according to PFGE results, using the kanamycin-azide resistant *E. coli* HB101 as a recipient. Putative transconjugants were selected on Mueller-Hinton agar plates containing kanamycin (100 μ g/ml), azide (160 μ g/ml), and cefotaxime (4 μ g/ml).

Plasmids were classified according to their incompatibility group by a PCR-based replicon-typing scheme (3, 4). PFGE with S1 nuclease digestion of whole genomic DNA (S1-PFGE) was used to detect plasmids as previously described (16).

Case-control clinical study. A retrospective observational case-control study was conducted among patients admitted to the medical ICU (MICU) or surgical ICU (SICU) between January and December 2009.

All the ICU patients were screened by culture of rectal swab specimens at least once a week until hospital discharge. A case was defined as a patient admitted to either of the two ICUs during the study period who presented infection or colonization by VIM-1-producing *K. pneumoniae* after 48 h of admission in the ICU. Control patients were randomly selected from monthly lists of patients admitted to the ICUs during the same period as the case patient with an ICU stay greater than 48 h and with no evidence of CNSKP infection or colonization in any of the clinical or surveillance samples taken.

Death associated with the infection was defined as persistence of signs and symptoms of infection by VIM-1-producing *K. pneumoniae* at the time of death or death occurring within 14 days after the diagnosis of VIM-1-producing *K. pneumoniae* infection without evidence of another obvious cause.

Information about the potential clinical risk factors (underlying medical conditions, as well as surgical procedures, including use and duration of invasive devices and type and duration of antimicrobial treatment) were recorded. In addition, Charlson modified index (1, 7) and Apache II scores were also recorded on admission to the ICU (22).

The periods used for the determination of risk factors were from admission until the date of colonization or infection by VIM-1-producing *K. pneumoniae* (for cases) and from admission to the time of discharge from the ICU (for controls).

Statistical analysis. Comparison of discrete variables was performed by univariate logistic regression. Continuous variables were compared by Student's *t* test or the Wilcoxon rank sum (Mann-Whitney) test, as appropriate. Two multivariate logistic regression models were developed with the purpose of determining the potential independent risk factors associated with VIM-1-producing *K. pneumoniae* infection/colonization, adjusted by Apache II score: a general model and a model that included

only the use of antibiotics as a risk factor. On the basis of a complete model, which included all the risk factors that showed a *P* value of <0.20 in the univariate analysis, the final model was built using the maximum likelihood method using a backward strategy of selecting variables. Adjusted odds ratios (ORs), including their 95% confidence intervals (CIs), were calculated. Two-tailed tests were used for all analyses. *P* values of <0.05 were considered statistically significant. Statistical procedures were performed using the Stata (version 9.0 SE) statistical package.

RESULTS AND DISCUSSION

Patients and infections. During 2009, CNSKP isolates were obtained from clinical samples from 55 patients admitted to either the SICU (*n* = 36, 65.5%) or the MICU (*n* = 19, 34.5%). Of the 55 patients, 28 (50.9%) were infected by CNSKP, while 27 (49.1%) had no clinical evidence of infection and were considered colonized. The incidence of new cases in the SICU was significantly higher than that in the MICU (16.7 versus 6.1 cases/1,000 patient-days) (relative risk, 2.7; 95% CI, 1.6 to 4.7; *P* < 0.001). Thirty-five (63.6%) patients were male. Twenty-one (38.2%) were >65 years old, and 34 (61.8%) were >18 and \leq 65 years old. The median MICU/SICU stay was 36 days (range, 3 to 101 days).

The clinical characteristics, treatments, and outcomes of the patients infected by CNSKP are detailed in Table 1. The most prevalent infections diagnosed were pneumonia and catheter-associated bacteremia, with seven cases (25%) each. Of the 27 patients colonized, CNSKP was recovered from exudates of the rectum (22 cases; 78.6%), trachea (3 cases; 11.1%), urethra (1 case; 3.7%), and bladder catheter (1 case; 3.7%).

Of the 55 patients studied, 45 (81.8%) had previous underlying conditions, and 25 of them (55.6%) had more than one. The most prevalent underlying diseases were neoplasia (13 cases; 25.5%), immunosuppression due to solid organ transplantation (12 cases; 21.8%), and heart diseases (21 cases; 38.2%).

Thirteen (46.4%) infected patients and 11 (40.7%) colonized patients, all of them with severe underlying diseases, died.

Twenty-two of the 28 (78.6%) infected patients were treated with antibiotics active against CNSKP, mainly tigecycline in monotherapy (9 cases; 32.1%) or in combination with colistin (11 cases; 39.3%) (Table 1). Of the remaining 6 infected patients, 2 were treated with meropenem or ertapenem because their isolates' MICs for both antibiotics were 1 μ g/ml and interpreted to be susceptible according to previous CLSI criteria (ertapenem MIC \leq 2 μ g/ml and meropenem MIC \leq 4 μ g/ml; document M100-S20 [7a]), 2 did not receive treatment due to early death, and the clinical condition of 2 patients improved after central venous catheter or bladder catheter removal (Table 1).

Nineteen of the 28 patients infected (67.9%) received adjuvant treatment, mainly change or removal of the central venous catheter (14 patients) (Table 1).

Susceptibility testing of CNSKP isolates. During the study period, 335 CNSKP isolates were recovered from different samples from the 55 patients studied. All of them were resistant to ampicillin, amoxicillin-clavulanic acid, cefoxitin, cefotaxime, cef-tazidime, piperacillin-tazobactam, gentamicin, tobramycin, ciprofloxacin, and co-trimoxazole; 36% were susceptible to amikacin. The MICs for tigecycline ranged from 0.25 μ g/ml to 4 μ g/ml (MIC₅₀ = 0.5 μ g/ml, MIC₉₀ = 1 μ g/ml). All isolates were susceptible to colistin (MICs < 2 μ g/ml).

Imipenem, meropenem, and ertapenem MICs ranged from 1 to >8 μ g/ml (MIC₅₀ and MIC₉₀, >8 μ g/ml in all cases). The

TABLE 1 Clinical characteristics of patients infected with VIM-1-producing *Klebsiella pneumoniae*^a

Gender	Age (yr)	Unit	Charlson index score	Apache II index	Type of infection	Antimicrobial therapy	Total no. of antimicrobial days	Adjunctive therapy	Outcome
M	60	SICU	3	28	Pneumonia	TGC + CST	50	None	Death
F	66	MICU	3	12	UTI	AMK	8	BC change	Cure
M	67	MICU	4	25	Pneumonia	Meropenem	12	CVC change	Cure
M	53	SICU	2	8	Intra-abdominal	TGC	9	None	Cure
M	46	MICU	1	21	CAB	TGC	4	CVC removal	Cure
M	35	SICU	3	17	CAB	TGC	34	CVC change	Cure
F	65	MICU	0	26	Meningitis	TGC + CST	26	CVC change	Cure
F	64	MICU	7	23	UTI	TGC	11	None	Death
F	38	SICU	0	20	CAB	None	0	PVC change	Cure
M	61	MICU	4	15	LRTI	TGC + CST	10	CVC change	Death
F	54	SICU	4	23	Pneumonia, CAB	TGC + CST	10	CVC and ET change	Death
M	68	SICU	6	17	Meningitis	TGC	7	CVC and VS change	Death
M	61	MICU	3	28	Pneumonia	TGC + CST	25	None	Cure
M	43	MICU	2	31	Pneumonia	TGC + CST	43	None	Cure
F	72	SICU	3	23	UTI	None	0	CVC change	Death
M	46	SICU	3	15	Meningitis	None	0	PVC change	Death
M	34	MICU	0	11	UTI	None	0	BC change	Cure
M	43	SICU	3	18	Pneumonia, CAB	TGC + CST	7	CVC and PVC change	Death
M	51	SICU	4	26	LRTI	Ertapenem	12	PVC removal	Cure
M	39	SICU	1	24	LRTI	AMK + TGC + CST	28	None	Cure
M	54	SICU	2	21	LRTI	TGC + CST	28	None	Death
M	80	SICU	2	16	Intra-abdominal	TGC	3	CVC change	Death
F	58	MICU	2	23	UTI	TGC	2	BC change	Cure
M	69	SICU	7	19	CAB	TGC + CST	38	CVC change	Death
M	59	SICU	6	29	Soft tissue	TGC	31	None	Death
F	63	SICU	4	21	LRTI	TGC + CST	21	CVC removal	Cure
M	35	SICU	0	17	CAB	TGC	2	None	Cure
F	66	SICU	3	29	Pneumonia	TGC + CST	38	CVC and PD change	Death

^a M, male; F, female; MICU, medical intensive care unit; SICU, surgical intensive care unit; AMK, amikacin; TGC, tigecycline; CST, colistin; CAB, catheter-associated bacteremia; UTI, urinary tract infection; LRTI, lower respiratory tract infection; CVC, central venous catheter; PVC, peripheral venous catheter; BC, bladder catheter; ET, endotracheal tube; VS, ventricular shunt; PD, percutaneous drainage.

modified Hodge test with imipenem or imipenem-EDTA Etest strips was positive for all CNSKP isolates.

Molecular epidemiology. In total, 99 representative CNSKP isolates were subjected to further molecular epidemiology studies. PFGE results revealed two well-defined clusters. Cluster 1 (C1) was predominant in both infected ($n = 27$) and colonized ($n = 27$) patients, while isolates of cluster 2 (C2) were isolated from only four patients, although only one was infected; the three patients colonized by isolates of C2 were also infected ($n = 1$) or colonized ($n = 2$) by isolates of C1.

According to the minor banding pattern differences found, C1 could be subdivided into four subtypes, C1A to C1D; two subtypes

were also identified in C2, C2A and C2B (Fig. 1). By MLST analysis, C1 and C2 were identified to be sequence type 15 (ST15) (six isolates tested, three of subtype C1A and one each of C1B, C1C, and C1D) and ST340 (three isolates tested, two of subtype C2A and one of C2B), respectively (Fig. 1). This is the first description of VIM-1-producing *K. pneumoniae* ST15, an MLST type previously associated with CTX-M-15-producing epidemic clones in Hungary and Denmark (11, 29). ST340 is a single-locus variant of the widely disseminated KPC-producing ST258 strain (18).

Antibiotic resistance genes and class 1 integron characterization. *bla*_{VIM-1} was identified in both the C1/ST15 and C2/ST340 clones. *bla*_{VIM-1} was carried in a class 1 integron in the following

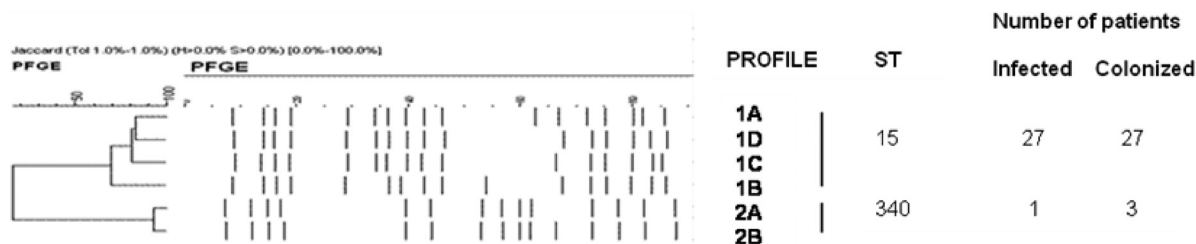


FIG 1 Dendrogram illustrating the PFGE profiles and MLST types of the two different clones of VIM-1-producing *Klebsiella pneumoniae* isolates and the number of patients infected and/or colonized by each. One patient was infected by clone C1/ST15 and colonized by clone C2/ST340. Two patients were colonized by both C1/ST15 and C2/ST340.

TABLE 2 Antibiotic susceptibilities of VIM-1-producing *Klebsiella pneumoniae* isolates K534 of clone C1/ST15 and K535 of clone C2/ST340 and their transconjugants, TC1K534, TC2K534 and TC1K535

Antibiotic ^a	MIC (μg/ml)					
	K534	TC1K534	TC2K534	K535	TCK535	<i>E. coli</i> HB101
Ampicillin	>16	>16	>16	>16	>16	≤4
Amox/clav	>16	>16	8	>16	>16	≤4
Cefazolin	>16	>16	>16	>16	>16	≤4
Cefoxitin	>16	>16	≤4	>16	16	≤4
Cefotaxime	>64	32	4	>64	64	≤1
Ceftazidime	>128	128	32	>128	64	≤1
Cefepime	>16	8	2	>16	4	≤1
Aztreonam	>16	≤1	>16	≤1	≤1	≤1
Imipenem	>32	4	0.12	16	4	0.12
Meropenem	>32	1	0.03	4	1	0.03
Ertapenem	>32	1	0.015	4	1	0.015
Piper/taz	>64	>64	≤8	>64	>64	≤8
Ciprofloxacin	>2	≤0.12	≤0.12	>2	≤0.12	≤0.12
Gentamicin	>8	≤2	>8	4	≤2	≤2
Tobramycin	>8	4	>8	8	4	≤2
Amikacin	32	≤8	16	≤8	≤8	≤8

^a Amox/clav, amoxicillin-clavulanic acid; Piper/taz, piperacillin-tazobactam.

cassette combination: *intI1* (integrase gene)–*bla*_{VIM-1}–*aac(6′)-Ib* (tobramycin resistance gene, also called *aacA4*)–*dhfrII* (trimethoprim resistance gene)–*aadA1* (streptomycin resistance gene)–*catB2* (chloramphenicol resistance gene)–*qacEδ1/sul-1* (quaternary ammonium compound resistance gene/sulfonamide resistance gene).

C1/ST15 isolates also had the *bla*_{SHV-134} gene encoding the new ESBL SHV-134 (<http://www.lahey.org/studies/webt.asp>). *bla*_{SHV-134} has a single nucleotide change at position 448 (C → G), in comparison with the sequence of *bla*_{SHV-12}, which codes for an amino acid substitution at position 154 (Q → E) (GenBank accession number [HM559945](http://www.ncbi.nlm.nih.gov/nuccore/HM559945)). The insertion sequence IS26 was detected linked 73 bp upstream of *bla*_{SHV-134}, as previously described in the *bla*_{SHV-12} gene (12). This fact supports the idea of genetic evolution of *bla*_{SHV-134} from the *bla*_{SHV-12} gene.

The *bla*_{TEM-1} gene and the aminoglycoside resistance gene *aac(3′)-IIa* were also identified in *K. pneumoniae* isolates representative of both clones.

A selected sample of four *K. pneumoniae* isolates representing

the different clones (two C1/ST15 isolates and two C2/ST340 isolates) with MICs of >8 μg/ml to imipenem, meropenem, and ertapenem was studied further to determine *ompK35* and *ompK36* sequences. In the two C1/ST15 isolates, the DNA sequences of *ompK35* and *ompK36* showed point mutations at positions 690 (G → A) and 360 (C → A), generating TGA and TAA premature stop codons, respectively. In the two C2/ST340 isolates, the sequence of *ompK35* had a point mutation at position 520 (C → T), generating a TAG premature stop codon, whereas no changes were detected in the *ompK36* sequence.

Conjugation assay and plasmid characterization. Carbapenem-susceptible *E. coli* transconjugants were obtained from isolates of the C1/ST15 and C2/340 clones (Table 2). All transconjugants carried a plasmid of ~50 kb that was untypeable by PCR and from which positive identification of the *bla*_{VIM-1} gene and a class 1 integron was obtained (Table 3). Untypeable plasmids of a similar size were found in a recent study by Miró et al. (25). Previous European studies had detected *bla*_{VIM-1} in association with plasmids of incompatibility groups N (32), I (35), and HI2 (25, 35).

TABLE 3 Molecular characteristics of VIM-1-producing *Klebsiella pneumoniae* isolates K534 of clone C1/ST15 and K535 of clone C2/ST340 and their transconjugants, TC1K534, TC2K534 and TC1K535

Characteristic	Result ^a					
	K534	TC1K534	TC2K534	K535	TCK535	<i>E. coli</i> HB101
Modified Hodge test	Pos	Pos	Neg	Pos	Pos	Neg
Imipenem-EDTA synergy test	Pos	Pos	Neg	Pos	Pos	Neg
<i>bla</i> _{VIM-1}	Pos	Pos	Neg	Pos	Pos	Neg
<i>bla</i> _{SHV-134}	Pos	Neg	Pos	Neg	Neg	Neg
<i>bla</i> _{TEM-1}	Pos	Neg	Pos	Pos	Neg	Neg
<i>aac(6′)-Ib</i>	Pos	Pos	Neg	Pos	Pos	Pos
<i>aac(3′)-IIa</i>	Pos	Neg	Pos	Pos	Neg	Neg
Class 1 integron	Pos	Pos	Neg	Pos	Pos	Pos
PFGE profile	C1			C2		
MLST	ST15			ST340		
Plasmid Inc group		Untypeable	FIIA		Untypeable	
PFGE S1 fragment size (kb)	~170, ~75, ~50	~50	~75	~250, ~170, ~50	~50	

^a Pos, positive; Neg, negative.

TABLE 4 Univariate analysis of risk factors linked to infection or colonization by VIM-1-producing *Klebsiella pneumoniae*

Characteristic ^a	Case patients (n = 55)	Control patients (n = 55)	OR	95% CI	P
Median (range) no. of days at risk	18 (5–63)	7 (3–110)			0.001 ^b
Mean (SD) Apache II risk index per 10 units of increment	41.0 (22.9)	18.8 (8.3)	1.5	0.9–2.5	0.08
Mean (SD) Charlson index	3.2 (2.2)	3.5 (2.2)	0.9	0.8–1.1	0.5
No. (%) of patients with the following specific risk factors:					
Chronic lung disease	1 (1.8)	5 (9.1)	0.2	0.03–2.2	0.1
Diabetes mellitus	7 (12.8)	11 (20.0)	0.6	0.2–1.6	0.3
Chronic renal insufficiency	6 (10.9)	6 (10.9)	1	0.3–3.3	1
Malignancy	14 (25.5)	13 (23.6)	1.1	0.5–2.6	0.8
Cardiovascular disease	21 (38.2)	24 (43.6)	0.8	0.4–1.7	0.6
Liver disease	3 (5.6)	6 (10.9)	0.5	0.1–2	0.3
Blood disease	3 (5.5)	4 (7.3)	0.7	0.2–3.5	0.7
Neurologic disease	10 (18.2)	5 (9.9)	2.2	0.7–7.0	0.1
Immunosuppression	12 (21.8)	16 (29.1)	0.7	0.3–1.7	0.3
No. (%) of patients with the following health care-associated factors:					
Presence of CVC (days)					
0–7	6 (10.9)	34 (61.8)	1		
8–21	29 (52.7)	16 (29.1)	10.3	3.6–29.7	<0.001
>21	20 (36.4)	5 (9.1)	22.7	6.1–83.9	<0.001
Presence of PVC (days)					
0–7	14 (25.5)	32 (58.2)	1		
>7	41 (74.6)	23 (41.8)	4.1	1.8–9.2	0.001
Presence of mechanical ventilation (days)					
0–7	7 (14.9)	34 (72.3)	1		
8–14	14 (29.8)	8 (17)	6.7	2.4–18.6	<0.001
>14	26 (55.3)	5 (10.6)	5.0	7.0–84.2	<0.001
Presence of nasogastric tube (days)					
0–7	9 (19.2)	25 (74.5)	1		
8–14	12 (25.5)	9 (19.2)	4.4	1.6–12.3	0.004
>14	26 (55.3)	3 (6.4)	5.0	6.5–74.2	<0.001
Tracheostomy	31 (66)	9 (19.2)	7.0	3.0–16.5	<0.001
Parenteral nutrition	33 (70.2)	16 (34)	4.1	2.4–12.6	<0.001
Mean total with invasive devices	5.2 (1)	3.6 (1.4)	2.8	1.9–4.1	<0.001
Median (range) total hospital LOS (days)	36 (3–101)	7 (3–110)			<0.001 ^b
Mean (SD) total no. of antimicrobials	5.0 (2.3)	2.9 (2.4)	1.4	1.2–1.7	<0.001
No. (%) of patients with the following antimicrobial use characteristics:					
Total length of antimicrobial use (days)					
0–14	20 (36.4)	40 (72.7)	1		
15–21	15 (27.3)	8 (14.6)	3.8	1.4–10.3	0.01
>21	20 (36.4)	7 (12.7)	5.7	2.1–15.8	0.001
Extended-spectrum cephalosporin use for >7 days	15 (27.8)	4 (7.3)	4.8	1.5–15.5	0.009
Antimicrobial(s) used					
Quinolones	46 (83.6)	30 (54.6)	4.6	1.8–10.4	0.001
Carbapenems	32 (58.2)	15 (27.3)	3.7	1.7–8.3	0.001
Piperacillin-tazobactam	24 (43.6)	12 (21.8)	2.8	1.2–6.4	0.016
Aminoglycosides	5 (9.1)	6 (10.9)	1.2	0.4–4.3	0.751
Linezolid	38 (69.1)	12 (21.8)	8.0	3.4–18.9	<0.001

^a CVC, central venous catheter; PVC, peripheral venous catheter; LOS, length of stay.

^b Mann-Whitney U test.

In addition, cefotaxime-resistant but carbapenem-susceptible transconjugants were also obtained from isolates of the C1/ST15 clone that were TEM-1 and SHV-134 producers and carried a single IncFIIA plasmid of ~75 kb (Tables 2 and 3). Simultaneous production of VIM-1 and ESBLs of the SHV family has previously

been described in *K. pneumoniae* (2, 32, 35), but this is the first report of the novel ESBL SHV-134 and VIM-1. *bla*_{SHV-134} is located in a conjugative IncFII plasmid other than the plasmid harboring the *bla*_{VIM-1} gene. Previous reports described the association of *bla*_{SHV-12} with IncFII plasmids (12, 29).

TABLE 5 Potential risk factors associated with VIM-1-producing *Klebsiella pneumoniae* colonization or infection as determined by multivariate analysis^a

Model	OR	95% CI	P
General model			
Presence of mechanical ventilation (days)	1.2	1.1–1.3	<0.001
Use of parenteral nutrition	3.6	1.4–9.4	0.009
Model with specific antimicrobials^b			
Extended-spectrum cephalosporins (>7 days)	4.2	1.1–15.3	0.032
Linezolid	7.5	3.1–18.3	<0.001

^a Multivariate analyses were performed by logistic regression adjusted for Apache II index.

^b The remaining risk factors included in the general model are not shown because the ORs, 95% CIs, and *P* values did not change significantly.

Case-control clinical study. All the 55 patients infected and/or colonized by VIM-1-producing *K. pneumoniae* isolates and 55 control patients were included in the study. No differences between case and control patients regarding age, gender, and frequency of surgery could be found (Table 4).

The total hospital stay was longer for cases than for controls (median stay, 36 versus 7 days; *P* < 0.001), as was the Apache II index (21.4 versus 18.8; *P* = 0.08). No significant differences in frequency of underlying diseases were observed between the two groups; however, the case-fatality ratio was significantly higher for case patients than for control patients (45.5% and 30.9%, respectively; *P* = 0.003).

In general, infection or colonization by VIM-1-producing *K. pneumoniae* was significantly associated with total previous exposure to invasive devices and a lengthened stay in the ICU (*P* < 0.001). Previous exposures included, in particular, central and peripheral venous catheters, mechanical ventilation, a nasogastric tube, parenteral nutrition, and a tracheotomy. Cases also received

more antibiotics and received antibiotics for longer periods of time than their control counterparts (Table 4). Furthermore, use of quinolones, carbapenems, piperacillin-tazobactam, linezolid, and extended-spectrum cephalosporins for more than 7 days was associated with a greater risk of acquiring CNCKP (Table 4). The main independent risk factors identified by the general multivariate model were the number of days that the patient received mechanical ventilation and the use of parenteral nutrition; previous treatment with linezolid and extended-spectrum cephalosporins for more than 7 days was identified as a risk factor by the antimicrobial use model (Table 5).

Taking into account the fact that the size of this observational study precludes the identification of several independent factors, which would otherwise be interrelated, the factors that were identified indicated that very sick patients who required intensive care for long periods of time was at greater risk for infection or colonization by VIM-1-producing *K. pneumoniae*. These factors give greater opportunities to acquire bacteria, especially in an environment where intensive antibiotic treatments select those species resistant to many antibiotics. One of the interesting findings in this observational study is the independent association of a previous exposure of cases to linezolid. On the one hand, it could be signaling those patients who developed more severe infections and were empirically treated for infections with Gram-positive microorganisms, but, on the other hand, linezolid could be helping to eradicate patients' Gram-positive bacterial flora, thereby allowing colonization by resistant Gram-negative bacteria.

Epidemic curve and infection control measures. The epidemic curve of patients infected by CNSKP in the two ICU units is displayed in Fig. 2. Almost all the MICU cases appeared in the second half of the year, from week 27 to year end. The dissemination of CNSKP from the SICU to the MICU during the summer period was linked to the fact that the MICU was closed; therefore, MICU patients were allocated to one of the SICU modules and

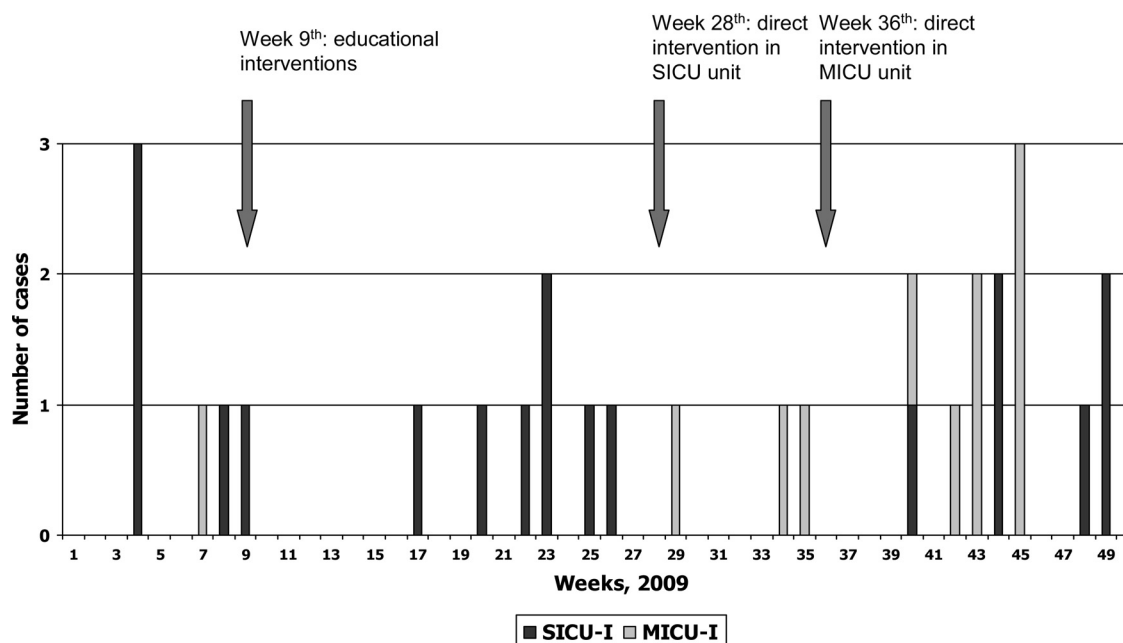


FIG 2 Epidemic curve of cases of infection by VIM-producing *Klebsiella pneumoniae* in two intensive care units. SICU-I, infected patients in surgical intensive care unit; MICU-I, infected patients in medical intensive care unit.

nurses, registered nurses, warden, and cleaning personnel were shared by MICU and SICU patients. This circumstance allowed dissemination of the bacteria between the two populations, indicating that contact transmission is a key factor for the spread of the epidemic clone.

Infection control personnel identified all patients with VIM-1-producing *K. pneumoniae* isolates recovered from any clinical specimen according to the microbiology laboratory reports. In addition, active surveillance of all patients admitted to the ICUs was performed once a week. Patients identified to be harboring VIM-1-producing *K. pneumoniae* were assigned to contact precautions, including a single room, when available, or patient cohorting and the use of gowns and gloves that were discarded after caring for a patient. In addition, standard precautions were reinforced for all patients admitted to the ICUs, including improvement of hand hygiene compliance through the use of alcohol rubs before and after caring for patients. The outbreak described here did not stop during 2010 (data not shown) but remained at a lower incidence toward endemicity (incidence rate, 4.4 cases/1,000 patient-days in 2010). Eradication of these bacteria in this vulnerable population can be very difficult to achieve, in spite of drastic measures, such as an aggressive infection control strategy.

In this outbreak, we observed three periods without cases of infection: between weeks 10 and 16, 31 and 33, and 36 and 39 (Fig. 2). These periods followed both the educational series in the SICU on week 9 and the direct observation of the personnel and the requirement to comply with basic hand hygiene and contact precaution measures on weeks 28 to 29 and 36 to 37 for the SICU and MICU, respectively (Fig. 2). The latter intervention is costly and could not be continued due to economic constraints. Other potentially effective but costly interventions, which include the cohorting of carriers and staff or even closing of the units and the total removal of all environmental as well as patient reservoirs, were not undertaken (23).

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