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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Nosocomial Outbreak of VIM-1–producing *Klebsiella pneumoniae* of multilocus sequence type 15: Molecular basis, clinical risk factors, and outcome

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Abstract

We study the epidemiology, molecular basis, clinical risk factors and outcome involved in the clonal dissemination of VIM-1-producing *Klebsiella pneumoniae* in the hospital setting.

All patients infected/colonized by carbapenem non-susceptible *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 by the 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 C1, ST15), while another clone (PFGE C2, ST340) was detected in four patients. C1 isolates also produced the new ESBL SHV-134. $bla_{\text{VIM-1}}$ was carried into a class 1 integron and an untypeable plasmid of ~ 50 bp. The number of days receiving mechanical ventilation, the use of parenteral nutrition, previous treatment with linezolid, and treatment with third generation cephalosporins for more than 7 days were detected as independent risk factors for CNSKP acquisition.

VIM-1 producing *K. pneumoniae* ST15 clone has a high capacity to spread among ICU patients with severe underlying conditions. A high rate of associated mortality and great difficulty in controlling the spread of this clone, without permanent behavioural changes in the personnel, were observed.

Introduction

Carbapenemases hydrolyse 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 associated to single cases, to small outbreaks (6,31,36), or to 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.

Material and Methods

Study design and bacterial isolates

During 2009, an increase in the isolation of carbapenems-non-susceptible *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 E-test (AB-Biodisk, Solna, Sweden). Susceptibility results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (8). Antibiotic-susceptible *E. coli* ATCC 25922 and carbapenems-resistant *E. coli* MN4 (30) were used as quality control strains. Tigecycline susceptibility was determined by the E-test and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (13).

Carbapenems-non-susceptibility was considered as resistance or intermediate susceptibility to one of more of the three carbapenem antibiotics tested: imipenem, meropenem and ertapenem.

Modified Hodge test using an imipenem disk was carried out; imipenem susceptibility alone and in combination with EDTA were also determined (E-test, AB Biodisk, 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 *Xba*I (30).

K. pneumoniae isolates representing the different PFGE clusters were further studied by the 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 (ESBL) (*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 (*arm*A, *rmt*A, *rmt*B) were also screened (15,30).

Class 1 integron structures, the integrase gene *intI*1 and the variable regions, as well as the linkage of *bla*$_{SHV-134}$ allele with IS*26* were screened by PCR amplification and DNA sequencing (12,34).

In addition, the entire sequences of the *ompK*35 and *ompK*36 genes were analyzed by PCR and DNA sequencing in four isolates representing the two CNSKP clones detected (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 μg/ml), azide (160 μg/ml), and cefotaxime (4 μg/ml).

Plasmids were classified according to their incompatibility group by a PCR-based replicon-typing scheme (3). 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 (MICU) or surgical ICU (SICU) between January and December 2009.

All the ICUs patients were screened by rectal cultures 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 hours' admission in the ICU. Control patients were randomly selected from monthly lists of patients admitted to the ICUs during the same period as the patient case, with an ICU stay greater than 48 hours, and with no evidence of CNSKP infection or colonization in any of the clinical or surveillance samples taken.

Death associated to 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 including, as well as surgical procedures, 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 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 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 only included the use of antibiotics as risk factors. On the basis of a complete model, which included all the risk factors that showed a value $p<0.20$ 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) were calculated including their 95% confidence intervals. Two-tailed test were used for all analyses. p values <0.05 were considered statistically significant. Statistical procedures were performed using the Stata 9.0 SE statistical package.

Results and discussion

Patients and infections

During 2009, CNSKP isolates were obtained from clinical samples of 55 patients, admitted in 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 in the MICU (16.7 *vs*. 6.1 cases/1,000 patient-days) (RR: 2.7, 95% CI: 1.6-4.7; $p<0.001$). Thirty-five (63.6%) patients were male. Twenty-one (38.2%) were >65 years old, and 34 (61.8%) were >18 and ≤65 years old. The median number of days of MICU/SICU stay was 36 days (range: 3-101 days).

Clinical characteristics, treatment and outcome 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 one. 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 died, all of them with severe underlying diseases.

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 combined 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 μg/ml and interpreted as susceptible according to previous CLSI

criteria (ertapenem ≤ 2 μg/ml and meropenem ≤ 4 μg/ml, document M100-S20), 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 central venous catheter (14 patients) (Table 1).

Susceptibility testing of CNSKP isolates

During the study period, 335 CNSKP isolates were recovered from different samples of the 55 patients studied. All of them were resistant to ampicillin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime, ceftazidime, piperacillin/tazobactam, gentamicin, tobramycin, ciprofloxacin and cotrimoxazole; 36% were susceptible to amikacin. The MICs for tigecycline ranged from 0.25 μg/ml to 4 μg/ml (MIC_{50}=0.5 μg/ml, MIC_{90}=1 μg/ml). All isolates were susceptible to colistin (MICs < 2 μg/ml).

Imipenem, meropenem and ertapenem MICs ranged from 1 to > 8 μg/ml (MIC_{50} and MIC_{90} >8 μg/ml in all cases). The modified Hodge the imipenem/imipenem-EDTA E-test strips were positive in all CNSKP *K. pneumoniae* 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 cluster 2 (C2) was only isolated from four patients, although only one was infected; the three patients colonized by isolates of C2 cluster were also infected (n=1) or colonized (n=2) by isolates of C1 cluster.

According to the minor banding patterns differences found, C1 cluster could be subdivided into four subtypes, C1A to C1D; also in the C2 cluster, two subtypes were identified, C2A and C2B (Figure 2). By MLST analysis, clusters C1 and C2 were identified as ST15 (six isolates tested; three of subtype C1A, and one of each C1B, C1C and C1D) and ST340 (three isolates tested; two of subtype C2A and one of C2B), respectively (Figure 1). This is the first description of VIM-1-producing *K. pneumoniae* ST15, MLST type previously associated to CTX-M-15-producing epidemic clones in Hungary and Denmark (11,29). The ST340 is a single-locus variant of the widely disseminated KPC-producing ST258 strain (18).

Antibiotic resistance genes and class 1 integron characterization

bla$_{\text{VIM-1}}$ was identified in both C1/ST15 and C2/ST340 clones. *bla*$_{VIM-1}$ was carried into a class 1 integron in the following cassette combination: *intI*1 (integrase gene) - *bla*$_{\text{VIM-1}}$ - *aac(6')-Ib* (tobramycin-resistance gene, also called *aacA4*) - *dhfrII* (thrimethoprim-resistance gene) - *aadA1* (streptomycin-resistance gene) - *cat*B2 (chloramphenicol-resistance) - *qacEδ1/sul-1* (quaternary ammonium compounds-resistance gene/sulphonamides-resistance gene).

C1/ST15 isolates also had the new *bla*$_{\text{SHV-134}}$ gene codifying the ESBL SHV-134, (http://www.lahey.org/studies/webt.asp). *bla*$_{\text{SHV-134}}$ has a single nucleotide change at position 448 (C→G) in comparison with *bla*$_{\text{SHV-12}}$, that codes for an amino acid substitution at position 154 (Q→E) (GeneBank accession number HM559945). The insertion sequence IS*26* was detected linked 73 bp upstream of *bla*$_{\text{SHV-134}}$, as previously described in the *bla*$_{\text{SHV-12}}$ gene (12). This fact supports the idea of genetic evolution of *bla*$_{\text{SHV-134}}$ from the *bla*$_{\text{SHV-12}}$ gene.

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

A selected sample of four *K. pneumoniae* isolates representing the different clones (two C1/ST15 and two C2/ST340 isolates) with MICs > 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 a 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-non-susceptible *E. coli* transconjugants were obtained from isolates of the C1/ST15 and C2/340 clones. All transconjugants carried a plasmid of ~ 50 kb that was untypeable by PCR from which positive identification of *bla*$_{VIM-1}$ gene and class 1 integron were obtained (Table 2). Untypeable plasmids of a similar size were found in a recent study by Miro et al. (25). Previous European studies had detected *bla*$_{VIM-1}$ associated to plasmids of the incompatibility groups N (32), I (35) and HI2 (25,35).

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 (Table 2). Simultaneous production of VIM-1 and ESBLs of the SHV family has been previously described in *K. pneumoniae* (2,32,35), but this is the first report of the novel ESBL SHV-134 and VIM-1. *bla*$_{SHV-134}$ being located in a conjugative IncFII plasmid other than the plasmid

harbouring the *bla*$_{VIM-1}$ gene. Previous reports described the association of *bla*$_{SHV-12}$ with InFII plasmids (12,29).

Case-control clinical study

All the 55 patients infected and/or colonized by VIM-1-producing *K. pneumoniae* and 55 control patients were included in the study. No differences could be found between case and control patients regarding age, gender and frequency of surgery (Table 3).

Total hospital stay was higher for cases than for controls (median stay 36 vs.7 days; $p<0.001$), as well as the Apache II index (21.4 *vs.* 18.8; $p=0.08$). No significant differences in frequency of underlying diseases were observed between both groups, however, the case-fatality ratio was significantly higher for case patients than for control patients (45.5% *vs.* 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 lengthened stay in the ICU ($p<0.001$); in particular exposure to central and peripheral venous catheter, to mechanical ventilation, to nasogastric tube, to parenteral nutrition and to tracheotomy. Cases also received more antibiotics and for longer periods of time than their control counterparts (Table 3). Furthermore, use of quinolones, carbapenems, piperacillin-tazobactan, linezolid and third generation cephalosporins for more than 7 days were associated to a greater risk of acquiring CNCKP (Table 3). The main independents risk factors identified by the general multivariate model were the number of days receiving mechanical ventilation and the use of parenteral nutrition; previous treatment with linezolid and third generation cephalosporins for more than 7 days were identified as risk factors by the antimicrobial use model (Table 4).

Taking in account 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 a group of very sick patients who required intensive care for long periods of time. These factors give greater opportunities to acquire the 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 one hand it could be signalling those patients who developed more severe infection and were empirically treated for gram-positive microorganisms but, on the other hand, lizenolid could be helping to eradicate patients' gram positive 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 ICUs units is displayed in Figure 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 auxiliary personnel were shared with SICU patients. This circumstance allowed dissemination of the bacteria between the two populations indicating that contact transmission is a key factor for the growth 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 as harbouring VIM-1-

producing *K. pneumoniae* were assigned to contact precautions, including 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 lesser incidence towards 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 10-16, 31-33 and 36-39 (Figure 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 28-29 and 36-37 for the SICU and MICU, respectively (Figure 2). This latter intervention is costly and could not be continued due to economic constraints. Other potentially effective, but costly interventions that include the cohorting of carriers and staff, or even closing the units and the total removal of all environmental as well as patient reservoirs, were not undertaken (23).

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543 **Table 1**. Clinical characteristics of patients infected with VIM-1-producing *Klebsiella*
544 *pneumoniae*.
545

Gender	Age (Years)	Unit	Charlson Index Score	Apache II Index Score	Localization of infection	Antimicrobial therapy	Total 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	Intraabdominal	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	Intraabdominal	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

NOTE. 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.

Table 2. Antibiotic susceptibility and molecular characteristics for the VIM-1-producing *Klebsiella pneumoniae* isolates K534 of clone C1/ST15 and K535 of clone C2/ST340, and their transconjugants TC1K534, TC2K534 and TC1K535.

Antibiotics	**K534**	**TC1K534**	**TC2K534**	**K535**	**TCK535**	***E. coli* 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
Tobramicin	>8	4	>8	8	4	≤2
Amikacin	32	≤ 8	16	≤ 8	≤8	≤8
Modified Hodge Test	pos	pos	neg	pos	pos	neg
Imipenem-EDTA synergy test	pos	pos	neg	pos	pos	neg
bla_{VIM-1}	pos	pos	neg	pos	pos	-
$bla_{SHV-134}$	pos	neg	pos	neg	neg	-
bla_{TEM-1}	pos	neg	pos	pos	neg	-
aac (6')-Ib	pos	pos	neg	pos	pos	-
aac(3')-IIa	pos	neg	pos	pos	neg	-
Class 1 integron	pos	pos	neg	pos	pos	-
PFGE profile	C1	-	-	C2	-	-
MLST	ST15	-	-	ST340	-	-
Plasmids Inc groups	-	untypeable	FIIA	-	untypeable	-
PFGE S1	~170kb, ~75kb, ~50kb	~50kb	~75kb	~250kb, ~170kb, ~50kb	~50kb	-

NOTE. Minimal inhibitory concentrations expressed in μg/ml
Amox/Clav, Amoxicillin/Clavulanic acid. Piper/Taz, Piperacillin/Tazobactam

562 **Table 3**. Univariate analysis of risk factors linked to infection or colonization by VIM-
563 1-producing *Klebsiella pneumoniae*
564

Characteristics	Case patients (n=55)	Control patients (n=55)	OR	95% IC	p
Days at risk, median (range)	18(5-63)	7(3-110)			0.001[a]
Apache II risk index, mean (SD) per 10 units of increment	41.0(22.9)	18.8(8.3)	1.5	0.9-2.5	0.08
Charlson Index, mean (SD)	3.2(2.2)	3.5(2.2)	0.9	0.8-1.1	0.5
Patient-specific risk factor					
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
Healthcare-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

Total invasive devices, mean	5.2(1)	3.6(1.4)	2.8	1.9-4.1	<0.001
Total hospital LOS, days, median (range)	36(3-101)	7(3-110)			<0.001 [a]
Total antimicrobial, mean (SD)	5.0(2.3)	2.9(2.4)	1.4	1.2-1.7	<0.001
Total antimicrobial, 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
>7 days third-generation cephalosporins	15(27.8)	4(7.3)	4.8	1.5-15.5	0.009
Use of antimicrobial					
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

NOTE: Data are number (%) of patients, unless otherwise indicated. CI, confidence interval; OR, odds ratio; SD, standard deviation; CVC, central venous catheter; PCV, peripheral venous catheter; LOS, length of stay.

[a] U Mann-Whitney

Table 4. Potential risk factors associated with VIM-1-producing *Klebsiella pneumoniae* colonization or infection as determined by multivariate analysis.

Model	OR	95% IC	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 [a]			
Third-generation cephalosporins (>7 days)	4.2	1.1-15.3	0.032
Linezolid	7.5	3.1-18.3	<0.001

NOTE. CI, confidence interval; OR, odds ratio. Multivariable analyses were performed by logistic regression adjusted for Apache II Index.

[a] The remaining risk factors included in the general model are not shown because the odds ratios [ORs; 95% confidence intervals (CIs)] and *p* values did not change significantly.

Figure 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.

NOTE. One patient was infected by C1/ST15 and colonized by clone C2/ST340. Two patients were colonized by both C1/ST15 and C2/ST340.

Figure 2. Epidemic curve of cases of infection by VIM-producing *Klebsiella pneumoniae* in two intensive care units.

NOTE. SICU-C: Colonized patients in surgical intensive care unit. SICU-I: Infected patients in surgical intensive care unit. MICU-C: Colonized patients in medical intensive care unit. MICU-I: Infected patients in medical intensive care unit.