

# An Outbreak of Infection due to $\beta$ -Lactamase *Klebsiella pneumoniae* Carbapenemase 2–Producing *K. pneumoniae* in a Greek University Hospital: Molecular Characterization, Epidemiology, and Outcomes

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**Background.** We describe the emergence and spread of *Klebsiella pneumoniae* carbapenemase 2 (KPC-2)–producing *K. pneumoniae* at a Greek University hospital.

**Methods.** Isolates with a carbapenem minimum inhibitory concentration  $>1$   $\mu\text{g}/\text{mL}$  and a negative EDTA–imipenem disk synergy test result were submitted to boronic acid disk test and to polymerase chain reaction (PCR) for KPC gene and sequencing. Records from patients who had KPC-2–producing *K. pneumoniae* isolated were retrospectively reviewed. Clinical isolates were submitted to molecular typing using pulsed-field gel electrophoresis, and the  $\beta$ -lactamase content was studied using isoelectric focusing and PCR.

**Results.** From January 2007 through December 2008, 50 patients (34 in the intensive care unit [ICU]) were colonized ( $n = 32$ ) or infected ( $n = 18$ ) by KPC-2–producing *K. pneumoniae*. Increasing prevalence of KPC-2–producing *K. pneumoniae* coincided with decreasing prevalence of metallo- $\beta$  lactamase–producing isolates in our ICU. Multidrug resistance characterized the studied isolates, with colistin, gentamicin, and fosfomycin being the most active agents. Besides KPC-2, clinical isolates encoded TEM-1-like, SHV-11, SHV-12, CTX-M-15, and LEN-19 enzymes. Four different clonal types were detected; the predominant one comprised 41 single patient isolates (82%). Sporadic multiclonal cases of KPC-2–producing *K. pneumoniae* infection were identified from September 2007 through May 2008. The outbreak strain was introduced in February 2008 and disseminated rapidly by cross-transmission; 38 patients (76%) were identified after August 2008. Fourteen cases of bacteremia, 2 surgical site infections, 2 lower respiratory tract infections (1 bacteremic), and 1 urinary tract infection were identified. Most patients received a colistin-containing combination treatment. Crude mortality was 58.8% among ICU patients and 37.5% among non-ICU patients, but attributable mortality was 22.2% and 33.3%, respectively.

**Conclusions.** The emergence of KPC-2–producing *K. pneumoniae* in Greek hospitals creates an important challenge for clinicians and hospital epidemiologists, because it is added to the already high burden of antimicrobial resistance.

During the past decade, clinicians have witnessed a steep increase in the rates of carbapenem resistance

among *Klebsiella pneumoniae* isolates from hospitalized patients in Greece. Data from the Greek System for the Surveillance of Antimicrobial Resistance [1] show that among *K. pneumoniae* blood isolates, carbapenem resistance increased from  $<1\%$  in 2001 to 30% in hospital wards and to 60% in intensive care units (ICUs) in 2008. Until the year 2006, carbapenem resistance in

Received 15 July 2009; accepted 20 September 2009; electronically published 30 December 2009.

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**Clinical Infectious Diseases** 2010;50:364–73

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1058-4838/2010/5003-0010\$15.00  
DOI: 10.1086/649865

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that species was mainly the result of dissemination of the *bla*<sub>VIM-1</sub> gene [2–6].

In late 2007, 2 publications reported the emergence of *bla*<sub>KPC-2</sub> in *K. pneumoniae* isolates in Europe. Both of the involved patients were first hospitalized in Crete, Greece [7, 8]. During 2007–2008, outbreaks of infection due to *K. pneumoniae* carbapenemase 2 (KPC-2)–producing *K. pneumoniae* were identified at 2 hospitals (1 in Crete [9] and 1 in Thessaloniki [10]). A surveillance study organized from February through December 2008 at 21 hospitals in Greece identified the presence of KPC-2–producing *K. pneumoniae* at 18 hospitals in Athens, Crete, and Thessaloniki. Among the 171 isolates studied, 97.1% belonged to the same pulse type, which was found at 17 hospitals [11], suggesting a nationwide dissemination of a hyper-epidemic clone.

These findings were in accordance with an important observation that a dominant KPC-2–producing *K. pneumoniae* strain belonging to the ST 258 lineage has disseminated throughout the United States [12] and was later identified in Israel and Greece [13]. There is evidence that the Greek major clone may have originated in Israel [11], suggesting the possibility of global dissemination of 1 strain type of KPC-2–producing *K. pneumoniae*. The aim of the present study was to describe the emergence of KPC-2–producing *K. pneumoniae* at University General Hospital ATTIKON, in Athens, Greece, focusing on the epidemiological, microbiological, and clinical characteristics of the outbreak and comparing our experience with that of the previously described epidemic of metallo- $\beta$ -lactamase (MBL)–producing *K. pneumoniae* infection in the same setting [4].

## MATERIALS AND METHODS

**Setting.** During the study period (January 2007–December 2008), there were 635 beds in use in our hospital with ~30,000 admissions annually. From January 2007 through October 2008, the hospital's general (surgical and medical) intensive care unit (ICU) had 18 beds in use. Three more beds were added in October 2008. The ICU is separated into 3 compartments, each including an isolation room. Nursing and medical staff are dedicated in each compartment. Antibiotic policies and infection control measures are described elsewhere [4].

**Study design.** This was a retrospective observational cohort study. The computerized databases of the microbiology laboratories were retrospectively searched. All *K. pneumoniae* isolates obtained during the study period from any clinical specimen that exhibited an imipenem or meropenem minimum inhibitory concentration (MIC) >1  $\mu\text{g}/\text{mL}$  and a negative imipenem-EDTA disk synergy test result were further studied for the presence of KPC. Medical records of all patients colonized or infected with KPC–producing *K. pneumoniae* were retrospectively reviewed by an independent physician. Follow-up

was possible until discharge from the hospital or death. The duration of colonization with a KPC–producing isolate before infection was also available, because ICU patients were routinely screened biweekly with use of surveillance cultures [14].

Written consent to use data from patients' files was given by each patient or by a first-degree relative at the time of hospital admission. With regard to the route of acquisition of KPC-2–producing *K. pneumoniae*, horizontal transmission during the current hospitalization was hypothesized for patients who had a negative result of first screening, whereas isolates that were identified on the day of admission or during the first 72 h were characterized as imported from another hospital. The route of acquisition remained undetermined for patients who were screened for the first time >72 h after admission and were positive for KPC-2–producing *K. pneumoniae*. For the clinical and microbiological diagnosis of infections, previously published criteria were used [15–19].

Treatment outcome was evaluated on day 7. Cure was defined as no clinical or laboratory evidence of infection. Improvement was defined as partial resolution of signs, symptoms, and laboratory parameters of infection. Cure and improvement were characterized as a successful outcome; all other outcomes were characterized as failures.

**Microbiological studies.** One isolate per patient (either the first colonizing or the first pathogenic isolate identified) was submitted for further study. Species identification of isolated bacteria and MIC determinations were performed using an automated system (BD Phoenix automated microbiology system; BD Diagnostic Systems). MICs of imipenem, meropenem, ertapenem, gentamicin, and fosfomycin were also evaluated with agar dilution, in accordance with the Clinical Laboratory Standards Institute (CLSI) [20], whereas MICs of colistin and tigecycline were determined using Etest (AB Biodisk), in accordance with the manufacturer's instructions. Results were interpreted in accordance with CLSI criteria [20], with the exception of those for fosfomycin, colistin, and tigecycline. For these agents, the break points proposed by European Committee on Antimicrobial Susceptibility Testing [21] were used (susceptibility,  $\leq 32$  for fosfomycin,  $\leq 2$   $\mu\text{g}/\text{mL}$  for colistin, and  $\leq 1$   $\mu\text{g}/\text{mL}$  for tigecycline), because relevant break points were not available from CLSI. All isolates were screened for MBL production with use of the EDTA–imipenem disk synergy test [22], and isolates for which results were negative were submitted to imipenem–boronic acid disk synergy test as a screening for KPC production [23]. Isoelectric focusing of sonic extracts was performed on precast polyacrylamide gels with a pH 3–10 gradient (Phast Gel IEF 3–9; Amersham Biosciences) for  $\beta$ -lactamase detection. The presence of *bla*<sub>KPC</sub> was confirmed by polymerase chain reaction (PCR) with specific primers [24]. PCR for *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> was performed as described elsewhere [25, 26]. Sequencing of PCR products was performed

**Table 1. Susceptibility Profile of the 50 *Klebsiella pneumoniae* Carbapenemase 2-Producing *K. pneumoniae* Clinical Isolates**

Antimicrobial agent	MIC range, $\mu\text{g}/\text{mL}$	MIC <sub>50</sub> , $\mu\text{g}/\text{mL}$	MIC <sub>90</sub> , $\mu\text{g}/\text{mL}$	Percentage of isolates that were susceptible
Imipenem <sup>a</sup>	16 to >256	64	>256	0
Meropenem <sup>a</sup>	4 to >256	64	>256	2
Ertapenem <sup>a</sup>	32 to >256	256	>256	0
Amikacin	<8 to >32	32	>32	14
Tobramycin	<2 to >8	>8	>8	4
Gentamicin <sup>a</sup>	2 to >256	4	16	70
Colistin <sup>b</sup>	0.125–48	0.5	8	86
Minocycline	1 to >16	4	>16	55.2
Tigecycline <sup>b</sup>	0.5–8	2	3	15.4
Fosfomycin <sup>c</sup>	8 to >256	32	256	54

**NOTE.** MIC, minimum inhibitory concentration; MIC<sub>50</sub>, MIC required to inhibit the growth of 50% of organisms; MIC<sub>90</sub>, required to inhibit the growth of 90% of organisms.

<sup>a</sup> MICs were determined by agar dilution method and were interpreted in accordance with the Clinical Laboratory Standards Institute [20]

<sup>b</sup> MICs were determined by Etest and were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing [21]

<sup>c</sup> MICs were determined by agar dilution method and were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing [21]

by MWG (Eurofins MWG Operon). For sequence analysis, the BLAST program from the National Center for Biotechnology Information Web site was used (<http://www.ncbi.nlm.nih.gov/BLAST>).

The genetic relatedness of all KPC-2-producing *K. pneumoniae* isolates was evaluated using pulsed-field gel electrophoresis (PFGE) analysis. Pulse types were compared with those of MBL-producing *K. pneumoniae* isolated in our institution. DNA was prepared in accordance with standard PFGE methods, and chromosomal restriction fragments obtained after *SpeI* cleavage were visually compared [27].

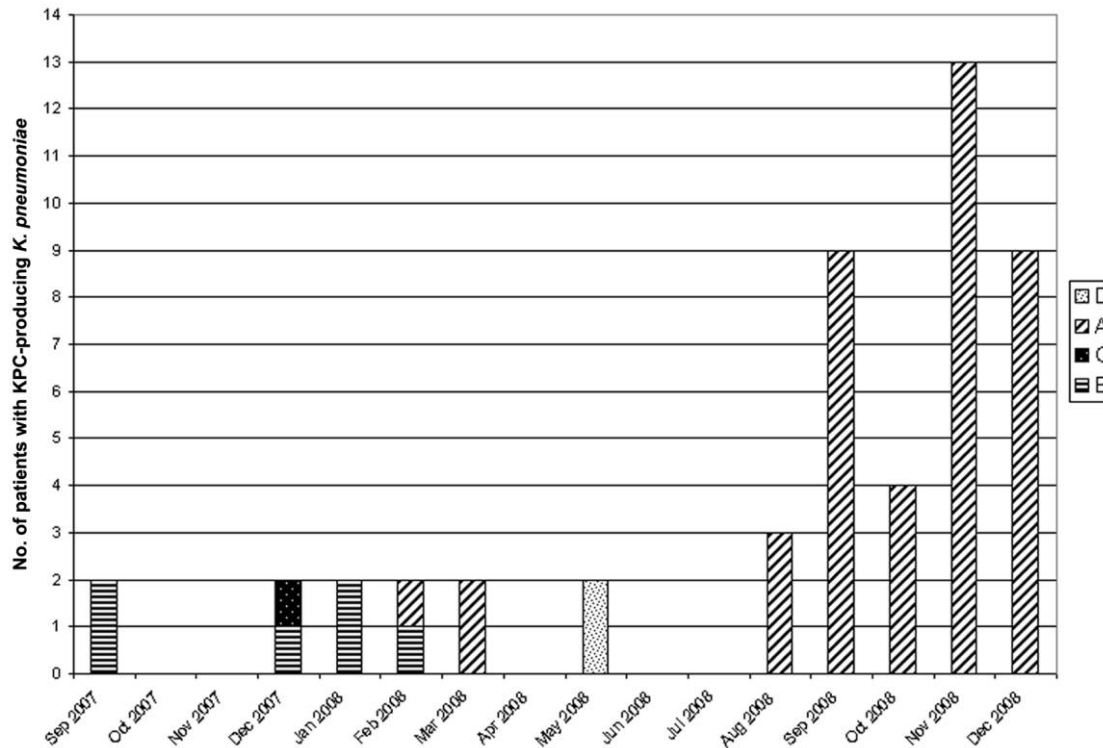
**Environmental cultures.** During November 2008, a point prevalence survey of environmental colonization of KPC-producing *K. pneumoniae* was conducted in our ICU. Samples were obtained for culture by rubbing premoistened swabs repeatedly over designated sites in the immediate vicinity of the patient, over equipment used in patient care, and in the general areas in all the compartments of the ICU. Swab samples were inoculated on MacConkey agar (Becton Dickinson) plates containing imipenem. Procedures of identification and phenotypic and susceptibility testing of isolated gram-negative bacteria were the same as described above.

**Statistical analysis.** Comparative analyses were performed using the  $\chi^2$  test or the Fischer's exact test for assessment of differences in proportions and Student's *t* test for the continuous variables, as appropriate. All tests were 2-tailed, and  $P < .05$  was considered to indicate statistical significance.

## RESULTS

In March 2008, 2 *K. pneumoniae* isolates exhibiting nonsusceptibility to carbapenems but a phenotypic test negative for MBL production were identified. This prompted a database search for similar cases (see above for case definition) that extended retrospectively through January 2007 and prospectively through December 2008. During the study period, a total of 50 patients were either infected (18 [36%]) or colonized (32 [64%]) with a *K. pneumoniae* strain producing a non-MBL carbapenemase, which was identified by PCR and sequencing to be KPC-2. Susceptibilities of the 50 *K. pneumoniae* isolates to various antimicrobials are shown in Table 1. All isolates shared a common multidrug resistance phenotype, although initial MIC testing by the automated Phoenix system showed that 4% and 10% were susceptible to imipenem and meropenem, respectively.

PFGE analysis of the 50 *K. pneumoniae* isolates identified 4 different clonal types, designated A–D (data not shown). The predominant clonal type was A, which comprised 41 single patient isolates (82%) and included subtypes A1 ( $n = 19$ ), A2 ( $n = 19$ ), A3 ( $n = 1$ ), A4 ( $n = 1$ ), and A5 ( $n = 1$ ), differing by 2–4 bands from A1. Six isolates (12%) were clonal type B, including subtypes B1 ( $n = 5$ ) and B2 ( $n = 1$ ), differing by 4–6 bands from each other, whereas 1 (2%) and 2 (4%) isolates were clonal types C and D, respectively. The temporal distribution of KPC producers and their respective clonal type are shown in Figure 1. Pulse types were unrelated to those of MBL-



**Figure 1.** Monthly prevalence of new colonization or infection with *Klebsiella pneumoniae* carbapenemase 2 (KPC-2)-producing *K. pneumoniae* during the study period. Clonal types (A–D) are shown.

producing *K. pneumoniae* isolated during the same period at our institution (data not shown).

In all isolates, molecular studies revealed, in addition to KPC-2 (pI 6.8), a TEM-1-like enzyme and a  $\beta$ -lactamase (pI 7.6), which was identified as the intrinsic SHV-11 in representative isolates. However, the single isolate belonging to clonal type C harbored the chromosomally encoded *bla*<sub>LEN-19</sub> gene. SHV-12 (pI 8.2) was identified in all clonal type A isolates and in 2 clonal type D isolates, and the isolates of B2 and C clonal types also produced CTX-M-15 (pI 8.9).

Sporadic cases of KPC-2-producing *K. pneumoniae* infection were identified from September 2007 through May 2008, representing a multiclonal cluster. Clonal type B was imported by a patient who was hospitalized at 3 other tertiary care hospitals in the Athens area before admission to the ATTIKON ICU, and it was responsible for a limited outbreak because of dissemination to at least 3 more patients in the same ICU compartment. The first isolate belonging to the epidemic clone (A) was introduced in the general ICU in February 2008 by a patient who was transferred from another hospital and was already colonized at admission. This strain was horizontally transmitted to at least 1 additional patient in the same compartment of the ICU and then disappeared. A strain belonging to the same clonal group was reintroduced in the ICU in August 2008 by

another patient already colonized after prolonged hospitalization at another hospital. Thirty-eight patients (76%) with KPC-2-producing *K. pneumoniae* colonization or infection were identified from August through December 2008. Only isolates belonging to clonal group A were responsible for clinical infections in our cohort.

On the basis of criteria described in Materials and Methods, among ICU patients, KPC-2-producing *K. pneumoniae* was acquired by cross-transmission in the ICU in 20 patients (58.8%), it was imported in 10 (29.4%), and the route of acquisition remained undetermined for 4 (11.8%). Among non-ICU patients, KPC-2-producing *K. pneumoniae* was acquired by cross-transmission during the current hospitalization in 4 patients (25%), it was imported in 2 (12.5%), and the route of acquisition remained undefined in 10 (62.5%), because active surveillance was not routinely performed outside the ICU. Among 114 environmental samples from the ICU area that were cultured, results were positive for only 1, which was recovered from the connector of the endotracheal tube of a patient with known colonization by KPC-2-producing *K. pneumoniae*.

Various clinical characteristics of patients from whom KPC-2-producing *K. pneumoniae* was isolated are presented in Table 2. Most of the patients (34 [68%]) were hospitalized in the

**Table 2. Clinical Characteristics of 50 Patients Colonized or Infected with *Klebsiella pneumoniae* Carbapenemase 2 (KPC-2)–Producing *K. pneumoniae* during the Study Period**

Characteristic	ICU patients with KPC-2 <i>K. pneumoniae</i> (n = 34)	Non-ICU patients with KPC-2 <i>K. pneumoniae</i> (n = 16)	P
Male sex	19 (55.9)	7 (43.8)	.65
Age, mean years ± SD	66 ± 16	70 ± 17	.42
Ward			
ICU	34 (100)	0	
Haematology-Oncology	0	2 (12.5)	
Medicine	0	10 (62.5)	
Surgery	0	2 (12.5)	
Cardiac surgery	0	2 (12.5)	
Transferred from another hospital	22 (64.7)	3 (18.8)	.09
Transferred from an ICU	11 (32.4)	4 (25) <sup>a</sup>	.76
Length of stay in current ward before isolation of KPC-2 <i>K. pneumoniae</i> , mean days ± SD	15 ± 18	31 ± 31	.03
Total length of stay in any hospital before isolation of <i>K. pneumoniae</i> , mean days ± SD	32 ± 22	34 ± 30	.79
Total length of stay until death or discharge, mean days ± SD	89 ± 73	62 ± 48	.19
Route of acquisition of KPC-2 <i>K. pneumoniae</i>			
Cross-transmission	20 (58.8)	4 (25)	.26
Imported	10 (29.4)	2 (12.5)	.48
Undetermined	4 (11.8)	10 (62.5)	.01
Source of first isolation of KPC-2 <i>K. pneumoniae</i>			
Feces	22 (64.7)	1 (6.3)	<.01
Blood	5 (14.7)	4 (25)	.47
Bronchial secretions	4 (11.8)	5 (31.3)	.26
Pus	2 (5.9)	3 (18.8)	.33
Central venous catheter	1 (2.9)	1 (6.3)	>.99
Urine	0	2 (12.5)	
Infection	9 (26.5)	9 (56.3)	.17
Receiving mechanical ventilation	30 (93.8)	0	
Receiving renal replacement therapy	6 (18.2)	0	
Immunosuppression	5 (15.2)	5 (31.3)	.29
Median APACHE II score (range)	19 (8–37)	15 (8–30)	.32
Antibiotic therapy during the last month before KPC-2 <i>K. pneumoniae</i> isolation			
Third-generation cephalosporins	3 (9.1)	2 (13.3)	.65
Piperacillin-tazobactam	20 (60.6)	5 (33.3)	.31
Carbapenems	20 (60.6)	8 (53.3)	.81
Quinolones	10 (30.3)	3 (20)	.74
Aminoglycosides	4 (12.1)	2 (13.3)	.99
Colistin	14 (42.4)	2 (13.3)	.19
Tigecycline	4 (12.1)	2 (13.3)	.65
Crude mortality	20 (58.8)	6 (37.5)	.42
Attributable mortality	2 (22.2) <sup>b</sup>	3 (33.3) <sup>b</sup>	>.99

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. APACHE II, Acute Physiology and Chronic Health Evaluation II score [28]; ICU, intensive care unit; SD, standard deviation.

<sup>a</sup> Three patients were transferred from ATTIKON Hospital general ICU, and 1 patient was transferred from ATTIKON cardiac ICU

<sup>b</sup> The denominator was the number of patients with clinical infection (9).

ICU when the KPC-producing isolate was detected. The overall prevalence rate of KPC-2-producing *K. pneumoniae* in our ICU was 1.8 single patient isolates per 100 admissions in 2007 and increased to 9.9 single patient isolates per 100 admissions in 2008. Among the 16 non-ICU patients, 10 (62.5%) were hospitalized in any of the 5 medical wards, and most of them were hospitalized in the medical ward to which 2 colonized ICU patients were transferred before hospital discharge. Overall, the gastrointestinal tract was the most common site of first isolation of KPC-2-producing *K. pneumoniae* (23 [46%] of 50 patients).

The clinical characteristics and outcomes of the 18 patients who received diagnoses of infection are shown in Table 3. Fifty percent of these patients were in the ICU (8 patients in the general ICU and 1 in the cardiac ICU) at the time of diagnosis of KPC-producing *K. pneumoniae* infection. The mean age was 67 years (range, 42–82 years), and 10 patients (55.6%) were male. The median Acute Physiology and Chronic Health Evaluation II (APACHE II) score was 17 (range, 8–37). The mean length of stay in our hospital before infection was 23 days (range, 1–100 days); however, 10 patients were transferred from another hospital, and the total mean length of hospital stay before infection was 27 days (range, 1–100 days; data not shown). All of the patients in this cohort had at least 1 recent hospitalization during the previous 3 months. Among 9 patients for whom surveillance cultures were performed at admission and weekly thereafter, colonization with KPC-producing *K. pneumoniae* preceded clinical infection in 5 (55.6%) for a mean duration of 10 days (range, 2–18 days). Previous rectal colonization was identified in only 2 (22.2%) of the patients with clinical infection.

Bacteremia was diagnosed in 14 patients (77.8%; 8 primary, 2 secondary, and 4 catheter-related cases), surgical site infection was diagnosed in 2 (11.1%), lower respiratory tract infection was diagnosed in 2 (1 with secondary bacteremia), and urinary tract infection was diagnosed in 1. Three (17.6%) of the infected patients were receiving a carbapenem-containing antimicrobial regimen when the infection was diagnosed, and 4 (23.5%) were receiving piperacillin-tazobactam (Table 3). Infection was successfully treated in 12 patients (66.7%) with an antimicrobial regimen containing colistin either as the only active antimicrobial (6 patients) or with an active aminoglycoside (1), tigecycline (3), or a carbapenem and catheter removal (1). Treatment was considered to be unsuccessful in 6 patients (33.3%). Patient 4 received colistin as the active antimicrobial on day 8 and had 2 recurrences of bacteremia after 10 and 28 days of the first diagnosis. Patients 10, 12, 13, and 14 were unsuccessfully treated with a colistin combination regimen; the latter 3 patients were profoundly immunosuppressed. Patient 18 developed a surgical site infection and recurrent bacteremia 11 days after the first infection and a second recurrence 3 days later with a phenotypically and genotypically identical isolate.

Among the 18 patients, infection due to KPC-producing *K. pneumoniae* was considered to have contributed to death in 5 (27.8%), whereas the crude mortality among the cohort of infected patients was 61.1%. The total mean length of hospital stay for infected patients was 62 days (range, 8–161 days).

## DISCUSSION

We described an outbreak of KPC-2-producing *K. pneumoniae* infection at an institution where VIM-1-producing *K. pneumoniae* had reached levels of endemicity. In the hospital's ICU in particular, the prevalence rate of *K. pneumoniae* with an MBL phenotype was 26.5 single patient isolates per 100 admissions in 2006 and 32.9 single patient isolates per 100 admissions in 2007 and decreased to 13.5 single patient isolates per 100 admissions in 2008 (our unpublished data). Of interest, the lowest rate of MBL-producing *K. pneumoniae* coincided with the highest rate of KPC-2-producing *K. pneumoniae* (9.9 single patient isolates per 100 admissions), suggesting a possible replacement of VIM-1 *K. pneumoniae* by the KPC-2-producing *K. pneumoniae*.

In a comparison with our previous experience with MBL-producing Enterobacteriaceae causing clinical infections in the same setting during a 3-year period (2003–2006) [4], we noticed that patients with KPC-2-producing *K. pneumoniae* infection were younger (mean age, 66.5 vs 68.7 years;  $P = .65$ ) and less seriously ill (median APACHE II score, 17 vs 22;  $P = .14$ ). The infection occurred after prolonged hospitalization (mean duration, 23 days) in both cohorts; however, previous colonization was identified in 82% of patients with infection due to MBL-producing Enterobacteriaceae, compared with 55.6% of patients with KPC-2-producing *K. pneumoniae* infection ( $P = .59$ ). The mean duration of colonization before infection was shorter for patients with KPC-2-producing *K. pneumoniae* (9.8 days vs 19 days;  $P = .35$ ). Prior rectal colonization was identified in 72.7% of patients with infection due to MBL-producing Enterobacteriaceae, compared with 22.2% of patients with KPC-2-producing *K. pneumoniae* infection ( $P = .25$ ). Bacteremia was the most common site of infection in both cohorts. Forty-seven percent of patients with infection due to KPC-2-producing *K. pneumoniae* had received carbapenem therapy 3 months before infection, compared with 62.5% of patients with infection due to MBL-producing Enterobacteriaceae ( $P = .63$ ). Crude mortality among patients infected with MBL-producing Enterobacteriaceae was 68.8%, compared with 61.1% among patients infected with KPC-2-producing *K. pneumoniae* ( $P = .83$ ); however, attributable mortality was 18.8% and 27.8%, respectively ( $P = .71$ ). Although the methods of MIC determination differed between these studies, a higher percentage of MBL-producing Enterobacteriaceae isolates exhibited susceptibility to imipenem (47%) or meropenem (58.8%). Finally, molecular epidemiology studies con-

**Table 3. Demographic and Clinical Characteristics and Outcome of 18 Patients with Clinical Infection with *Klebsiella pneumoniae* Carbapenemase 2 (KPC-2)–Producing *K. pneumoniae* during the Study Period**

Patient	Age, years	Date of infection	Ward	Comorbidities	APACHE II score	Site of infection	LOS before infection, days	LOS, days	Total LOS, days	Previous colonization with a KPC-2 <i>K. pneumoniae</i> , source (duration, days)	Treatment outcome (final outcome)	Antimicrobial therapy before isolation of KPC-2 <i>K. pneumoniae</i>	Antimicrobial therapy for the present infection	Comments	Susceptibility phenotype of KPC-2 <i>K. pneumoniae</i> <sup>a</sup>
1	74	26 February 2008	ICU	...	13	VAP and secondary BSI	12	67	67	Rectal (8)	Successful (death)	MOX, MER, COL	MER, COL	<i>Pseudomonas aeruginosa</i> was also isolated in BAL samples; follow-up: 34 days	GEN, COL, FOS, MIN
2	42	7 September 2008	ICU	Cardiomyopathy, heart failure, CAD, DM, sleep apnea, acute renal failure (CVVH)	37	CRBI, sepsis	5	57	57	CVC (7), bronchial (5)	Successful (death)	COL	MER added, GEN added on day 3	Follow-up: 45 days	GEN, COL, FOS
3	46	15 September 2008	ICU, medicine	Sleep apnea, COPD, hypothyroidism	14	CRBI	23	37	37	No	Successful (discharge)	MER	MER, catheter removal	Infection diagnosed 7 days after ICU discharge; discharged from hospital with GEN and MIN on day 11; follow-up: 11 days	GEN, COL, FOS
4	65	20 September 2008, 1 October 2008, 18 October 2008	ICU	Chronic renal failure, DM	22	BSI, CRBI, septic shock, CRBI	9	42	42	No	Recurrence (death)	TZP	CIP added on day 6, COL added on day 8, GEN added (for recurrence) and catheter removal, TIG + AMK and catheter removal (for second recurrence)	Two recurrences (on days 10 and 28); KPC-2 <i>K. pneumoniae</i> bacteremia contributed to death; follow-up: 30 days	GEN, COL, FOS
5	55	27 September 2008	ICU	Alcoholism, epilepsy	11	BSI, sepsis	8	14	14	Unknown	Successful (discharge)	TZP	MER, COL	Follow-up: 5 days	GEN, COL, FOS, MIN
6	76	29 September 2008	ICU	COPD, chronic renal failure	17	CRBI	9	62	62	No	Successful (discharge)	TZP	TIG started on day 5, COL added on day 8	Follow-up: 40 days	GEN, COL, FOS, MIN
7	69	23 October 2008	ICU	COPD	17	BSI	27	147	147	Rectal (18)	Successful (discharge)	CIP	COL and MER added on day 6	Follow-up: 110 days	GEN, COL, FOS, MIN
8	66	23 October 2008	ICU, surgery	Rectal adenocarcinoma postoperative surgical resection—secondary peritonitis	20	SSI	24	161	161	No	Successful (discharge)	TIG, COL	TIG, COL	Infection diagnosed 8 days after ICU discharge; follow-up: 135 days	GEN, COL
9	72	3–5 November 2008	Haematology-oncology	CLL, chemotherapy, thyroid carcinoma, AH	11	BSI	50	77	77	Unknown	Successful (death)	NA	NA	Follow-up: 18 days	GEN, FOS, MIN, TIG
10	58	4 November 2008	Cardiac ICU	Aortic valve stenosis, acute pulmonary edema, DM, AH, end-stage renal failure (CVVH)	11	BSI	55	62	62	Unknown	Failure (death)	COL, MER	COL, MER	Follow-up: 3 days	GEN, COL, FOS, MIN

11	73	11 November 2008	Medicine	Resected bladder carcinoma, bladder reconstruction, chronic renal failure, Parkinson's disease, alcoholism	17	UTI	1	8	Unknown	Successful (discharge)	...	MER, COL	Follow-up: 7 days	GEN, COL, FOS, MIN, TIG
12	55	18-24 November 2008	Haematology-oncology	Refractory AML, persistent neutropenia, portable catheter	NA	BSI	100	113	Unknown	Failure (death)	MER, AMK	COL added on day 2, MER and TIG added on day 4	Persistent bacteremia for 7 days; portable catheter removal on day 10; follow-up: 11 days	GEN, COL
13	80	26 November 2008	Medicine	HCL, chemotherapy, DVT, AH	30	HAP	34	35	Pus (2)	Failure (death)	TIG	MER, COL	Follow-up: 11 days	COL
14	82	27 November 2008	Medicine	NHL, chemotherapy, AH, hip arthroplasty, hypothyroidism	24	BSI, severe sepsis	17	17	Unknown	Failure (death)	TZP, AMK	MER and COL on day 7	Follow-up: 6 days	GEN, COL
15	46	3 December 2008	Cardiac surgery, ICU	CAD, recent CABG, DM, chronic renal failure (CVWH), PVD	23	CRBI	23	60	Unknown	Successful (death)	COL, TIG	AMK added on day 2, TZP added on day 3	Another CRBSI due to KPC-2 <i>K. pneumoniae</i> diagnosed on day 20 and a recurrence with KPC-2 <i>K. pneumoniae</i> and <i>Paeruginosa</i> on day 28; follow-up: 68 days	GEN, FOS, MIN, TIG
16	82	13 December 2008	Medicine	Biliary adenocarcinoma, biliary stent, DM, chronic renal failure	8	Cholangitis, secondary BSI	3	44	Unknown	Successful (death)	...	TZP and AMK, then TIG on day 5	Follow-up: 42 days	GEN, COL, FOS, MIN
17	82	24 December 2008	Surgery	Colon adenocarcinoma postoperative colectomy	21	SSI	10	46	Unknown	Successful (discharge)	...	TZP, then TIG on day 12	Surgical debridement was performed on day 12; follow-up: 39 days	AMK, GEN, FOS, MIN, TIG
18	74	29 December 2008	ICU	Ulcerative colitis while receiving steroids, ischemic colitis, abdominal aorta aneurysm, HT, COPD	17	BSI	1	71	Pus (14)	Reurrence(death)	CIP	MER and COL, TIG added on day 8	Deep SSI and secondary bacteremia due to KPC-2 <i>K. pneumoniae</i> was diagnosed on day 11; <i>Acinetobacter baumannii</i> also isolated in pus; recurrent secondary BSI diagnosed on day 14; follow-up: 42 days	GEN, COL, FOS, MIN

**NOTE.** AH, arterial hypertension; AMK, amikacin; AML, acute myeloid leukemia; APACHE II, Acute Physiology and Chronic Health Evaluation II [28]; BSI, bloodstream infection; CAD, coronary artery disease; CIP, ciprofloxacin; CLL, chronic lymphoid leukemia; COL, colistin; COPD, chronic obstructive pulmonary disease; CRBI, catheter-related BSI; CVC, central venous catheter; CVWH, continuous veno-venous haemofiltration; DM, diabetes mellitus; DVT, deep vein thrombosis; FOS, fosfomicin; GEN, gentamicin; HCL, hairy cell leukemia; ICU, intensive care unit; LOS, length of stay; MER, meropenem; MOX, moxifloxacin; NA, not available; NHL, non-Hodgkin lymphoma; PVD, peripheral vascular disease; SSI, surgical site infection; TIG, tigecycline; TZP, piperacillin-tazobactam; VAP, ventilator-associated pneumonia.

<sup>a</sup> Minimum inhibitory concentration break points applied for susceptibility were imipenem and MER,  $\leq 4 \mu\text{g/mL}$ ; ertapenem,  $\leq 2 \mu\text{g/mL}$ ; GEN and tobramycin,  $\leq 4 \mu\text{g/mL}$ ; AMK,  $\leq 16 \mu\text{g/mL}$ ; netilmicin,  $\leq 8 \mu\text{g/mL}$ ; minocycline,  $\leq 4 \mu\text{g/mL}$ ; CIP,  $\leq 1 \mu\text{g/mL}$ ; trimethoprim-sulfamethoxazole,  $\leq 2 \mu\text{g/mL}/38 \mu\text{g/mL}$ ; FOS,  $\leq 32 \mu\text{g/mL}$ ; COL,  $\leq 2 \mu\text{g/mL}$ , and TIG,  $\leq 1 \mu\text{g/mL}$  [20, 21].

firmed that the outbreak of MBL-producing *K. pneumoniae* infection was polyclonal, whereas that of KPC-2-producing *K. pneumoniae* infection was monoclonal. These differences suggest that the hyperendemic strain of KPC-2-producing *K. pneumoniae*, in addition to dissemination advantage, may even be more virulent than the MBL-producing strains of *K. pneumoniae* circulating in our hospital, although a matched case-control study is required for direct comparison of outcome between infections by *K. pneumoniae* strains with different resistance determinants.

Studies from Israel and the United States have identified poor functional status, ICU stay, transplantation, mechanical ventilation, prolonged hospitalization, and receipt of antibiotics [29, 30] as risk factors for acquisition of KPC-2-producing *K. pneumoniae*. The observational design of our study did not allow for any conclusions regarding potential risk factors for KPC-2-producing *K. pneumoniae* acquisition at our institution.

The epidemiological profile of the present outbreak differs from that of the multiclonal outbreak of KPC-2-producing *K. pneumoniae* that was observed in Israel [31]; however, it is reminiscent of the primarily clonal outbreak described in US hospitals [25, 32, 33]. In the present study, the monoclonal type of the outbreak and the failure to identify a common source or an environmental reservoir implicate patient-to-patient transmission as the main mechanism of spread of KPC-2-producing *K. pneumoniae*. On the basis of the published literature, it appears that this outbreak was part of a nationwide outbreak caused by 1 predominant KPC-2-producing *K. pneumoniae* strain [11], but the strains isolated in our institution were found to also accumulate other  $\beta$ -lactam resistance enzymes (TEM-1-like, SHV-11, SHV-12, LEN-19, and CTX-M-15). In particular, the coexistence of *bla*<sub>KPC-2</sub> with *bla*<sub>CTX-M</sub> type genes in *K. pneumoniae* was previously reported in Brazil [34], China [35], and Israel [31] but not in Greece.

To achieve the containment of the outbreak, infection control measures were intensified throughout the hospital (beginning November 2008). Dedicated infection control personnel were responsible for ensuring compliance with hand hygiene and contact isolation measures and adherence of personnel to meticulous environmental cleaning. Information on the number of new isolates was given daily by the laboratory, and cohorting of colonized or infected patients was practiced when possible. Restriction of the use of carbapenems was also enforced. The efficacy of this multidisciplinary effort cannot be assessed, because the outbreak was still ongoing at the end of the study period.

The susceptibility profile of the isolates recovered during the present outbreak (Tables 1 and 3) underscores the extremely limited therapeutic choices available for the treatment of infected patients. Colistin-containing combinations were most often used (Table 3) in our cohort. Surprisingly, fosfomycin

was active in vitro against 54% of the isolates; however currently, in vivo efficacy of this bacteriostatic agent against KPC-2-producing *K. pneumoniae* has not been evaluated.

We described the emergence of KPC-2-producing *K. pneumoniae* in an institution where MBL-producing *K. pneumoniae* was endemic. Nevertheless, the outbreak strain rapidly disseminated from patient to patient, colonizing the gastrointestinal tract in some of them and causing severe infections shortly thereafter, contributing to death mainly among immunocompromised patients. These observations provide some insight into the epidemiology and the clinical importance of this new threat: namely, KPC-2-producing *K. pneumoniae*.

## Acknowledgments

We thank Zoi Chryssouli and PhD student Natalie Mitchell for technical support.

**Potential conflicts of interest.** All authors: no conflicts.

## References

1. Greek System for the Surveillance of Antimicrobial Resistance. Available at: <http://www.mednet.gr/whonet>. Accessed 30 June 2009.
2. Giakkoupi P, Xanthaki A, Kanelopoulou M, et al. VIM-1 metallo- $\beta$ -lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. *J Clin Microbiol* **2003**; 41:3893–3896.
3. Ikonomidis A, Tokatlidou D, Kristo I, et al. Outbreaks in distinct regions due to a single *Klebsiella pneumoniae* clone carrying a *bla*<sub>VIM-1</sub> metallo- $\beta$ -lactamase gene. *J Clin Microbiol* **2005**; 43:5344–5347.
4. Souli M, Kontopidou FV, Papadomichelakis E, Galani I, Armaganidis A, Giamarellou H. Clinical experience of serious infections caused by Enterobacteriaceae producing VIM-1 metallo- $\beta$ -lactamase in a Greek university hospital. *Clin Infect Dis* **2008**; 46:847–854.
5. Psychogiou M, Tassios PT, Avlami A, et al. Ongoing epidemic of *bla*<sub>VIM-1</sub>-positive *Klebsiella pneumoniae* in Athens, Greece: a prospective survey. *J Antimicrob Chemother* **2008**; 61:59–63.
6. Vatopoulos A. High rates of metallo- $\beta$ -lactamase-producing *Klebsiella pneumoniae* in Greece: a review of the current evidence. *Euro Surveill* **2008**; 13:1–6.
7. Cuzon G, Naas T, Demachy MC, Nordmann P. Plasmid-mediated carbapenem-hydrolyzing  $\beta$ -lactamase KPC-2 in *Klebsiella pneumoniae* isolate from Greece. *Antimicrob Agents Chemother* **2008**; 52:796–797.
8. Tegmark Wisell K, Haeggman S, Gezelius L, et al. Identification of *Klebsiella pneumoniae* carbapenemase in Sweden. *Euro Surveill* **2007**; 12(12):E071220.3.
9. Maltezou HC, Giakkoupi P, Maragos A, et al. Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece). *J Infect* **2009**; 58:213–219.
10. Pournaras S, Protonotariou E, Voulgari E, et al. Clonal spread of KPC-2 carbapenemase-producing *Klebsiella pneumoniae* strains in Greece. *J Antimicrob Chemother* **2009**; 64:348–352.
11. Giakoupi P, Maltezou H, Polemis M, Pappa O, Saroglou G, Vatopoulos A; Greek System for the Surveillance of Antimicrobial Resistance. KPC-2-producing *Klebsiella pneumoniae* infections in Greek hospitals are mainly due to a hyperepidemic clone. *Euro Surveill* **2009**; 14:19218.
12. Kitchel B, Rasheed JK, Patel JB, et al. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* in the United States: clonal expansion of MLST sequence type 258. *Antimicrob Agents Chemother* **2009**; 53:3365–3370.
13. Samuelsen O, Naseer U, Tofteland S, et al. Emergence of clonally related *Klebsiella pneumoniae* isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. *J Antimicrob Chemother* **2009**; 63:654–658.

14. Papadomichelakis E, Kontopidou F, Antoniadou A, et al. Screening for resistant gram-negative microorganisms to guide empiric therapy of subsequent infection. *Intensive Care Med* **2008**; 34:2169–2175.
15. American Thoracic Society Documents. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and health-care-associated pneumonia. *Am J Respir Crit Care Med* **2005**; 171:388–416.
16. Calandra T, Cohen J; International Sepsis Forum. Definition of infection in the ICU consensus conference. The International Sepsis Forum Consensus Conference on Definitions of Infection in the ICU. *Crit Care Med* **2005**; 33:1538–1548.
17. Mermel LA, Farr BM, Sherertz RJ, et al. Guidelines for the management of intravascular catheter-related infections. *Clin Infect Dis* **2001**; 32: 1249–1272.
18. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* **2008**; 36: 309–332.
19. Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* **1992**; 20:864–874.
20. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI document M100-S19. Wayne, PA: Clinical Laboratory Standards Institute, **2009**.
21. European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints. Available at: <http://www.srga.org/eucastwt/MICTAB/index.html>. Accessed 10 June 2009.
22. Lee K, Chong Y, Shin HB, et al. Modified Hodge and EDTA-disk synergy tests to screen metallo- $\beta$ -lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* **2001**; 7:88–91.
23. Tsakris A, Kristo I, Poulou A, et al. Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* in the clinical laboratory. *J Clin Microbiol* **2009**; 47:362–367.
24. Bradford PA, Bratu S, Urban C, et al. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30  $\beta$ -lactamases in New York City. *Clin Infect Dis* **2004**; 39:55–60.
25. Galani I, Xirouchaki E, Kanellakopoulou K, et al. Transferable plasmid mediating resistance to multiple antimicrobial agents in *Klebsiella pneumoniae* isolates in Greece. *Clin Microbiol Infect* **2002**; 8:579–588.
26. Dutour C, Bonnet R, Marchandin H, et al. CTX-M-1, CTX-M-3, and CTX-M-14  $\beta$ -lactamases from Enterobacteriaceae isolated in France. *Antimicrob Agents Chemother* **2002**; 46:534–537.
27. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field-gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **1995**; 33:2233–2239.
28. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* **1985**; 13:818–829.
29. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. *Antimicrob Agents Chemother* **2008**; 52:1028–1033.
30. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. *Infect Control Hosp Epidemiol* **2008**; 29:1099–1106.
31. Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. *Antimicrob Agents Chemother* **2007**; 51:3026–3029.
32. Woodford N, Tierno PM Jr, Young K, et al. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A  $\beta$ -lactamase, KPC-3, in a New York medical center. *Antimicrob Agents Chemother* **2004**; 48:4793–4799.
33. Bratu S, Mooty M, Nichani S, et al. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: epidemiology and recommendations for detection. *Antimicrob Agents Chemother* **2005**; 49: 3018–3020.
34. Peirano G, Seki LM, Val Passos VL, Pinto MC, Guerra LR, Asensi MD. Carbapenem-hydrolyzing  $\beta$ -lactamase KPC-2 in *Klebsiella pneumoniae* isolated in Rio de Janeiro, Brazil. *J Antimicrob Chemother* **2009**; 63: 265–268.
35. Cai JC, Zhou HW, Zhang R, Chen GX. Emergence of *Serratia marcescens*, *Klebsiella pneumoniae*, and *Escherichia coli* isolates possessing the plasmid-mediated carbapenem-hydrolyzing  $\beta$ -lactamase KPC-2 in intensive care units of a Chinese hospital. *Antimicrob Agents Chemother* **2008**; 52:2014–2018.