

## Molecular characteristics of extended spectrum $\beta$ -lactamase and carbapenemase genes carried by carbapenem-resistant *Enterobacter cloacae* in a Chinese university hospital

Lanying CUI\*, Jinying ZHAO, Juan LU

First Affiliated Hospital of Harbin Medical University, Harbin, P.R. China

Received: 14.07.2014 • Accepted/Published Online: 12.11.2014 • Printed: 31.12.2015

**Background/aim:** In recent years, the increase in the prevalence of carbapenem-resistant *Enterobacter cloacae* (CREC) has been significant and has become a global problem. The aim of this study is to characterize CREC extended spectrum  $\beta$ -lactamases (ESBLs) and CREC carbapenemase genes and evaluate the risk factors for CREC infections in a university hospital in China.

**Materials and methods:** The identification and assessment of the antimicrobial susceptibility of CREC isolates were performed using a VITEK-2 compact system. Clonal relatedness was investigated by pulsed-field gel electrophoresis (PFGE).  $\beta$ -Lactamase genes were examined using polymerase chain reaction and nucleotide sequencing.

**Results:** A total of 64 CREC isolates were included in the study. The rate of CREC infections significantly increased between 2009 and 2012. Among the CREC isolates, 39 (60.9%) isolates possessed ESBL genes, and 49 (76.6%) carried carbapenemase genes; *blaSHV-12* and *blaKPC-2* were the most common genes, respectively. PFGE showed that there were no obvious clonal associations between these CREC isolates, except for 3 isolates. A univariate analysis demonstrated that prior treatment with antibiotics, intravascular catheterization, urinary catheterization, mechanical ventilation, and parenteral nutrition were significant risk factors for CREC infections.

**Conclusion:** The emergence and increase of CREC infections is worrisome. Effective measures should be taken to control the spread of CREC.

**Key words:** *Enterobacter cloacae*, carbapenem, extended spectrum  $\beta$ -lactamase, carbapenemase

### 1. Introduction

*Enterobacter cloacae* is an increasingly important nosocomial pathogen and can cause a wide spectrum of infections, including pneumonia, urinary tract infections, wound infections, and device-related infections (1). In recent years, the antibiograms of *Enterobacter cloacae* have changed greatly, including the emergence of carbapenem-resistant *Enterobacter cloacae* (CREC). CREC poses a formidable threat to hospitalized patients because infections with CREC are difficult to treat and are associated with high rates of morbidity and mortality in comparison with those caused by carbapenem-susceptible *Enterobacter cloacae* (2–4). Currently, *Klebsiella pneumoniae* is the most frequent species of carbapenem-resistant Enterobacteriaceae (5,6). In contrast, CREC is still unusual, and the risk factors and epidemiology of CREC have not been systematically evaluated. Extended spectrum  $\beta$ -lactamases (ESBLs) and carbapenemases are 2 types of important enzymes in Enterobacteriaceae that can lead to resistance to different antibiotics, including

$\beta$ -lactams, fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole (7). The present study was conducted to investigate the molecular characteristics of ESBLs and carbapenemases and to analyze risk factors associated with CREC infections.

### 2. Materials and methods

#### 2.1. Patients and bacterial strains

A retrospective study was conducted at the First Affiliated Hospital of Harbin Medical University in China, a 3000-bed tertiary care hospital. Between January 2009 and December 2012, a total of 666 episodes of *Enterobacter cloacae* were collected, and 64 (9.6%) isolates were resistant to at least 1 carbapenem (imipenem MIC  $\geq 2$   $\mu$ g/mL, ertapenem MIC  $\geq 1$   $\mu$ g/mL). We only studied the first strain if more than one strain was collected in a patient. Annual numbers and proportions of CREC are shown in Table 1.

The CREC isolates were isolated from the bloodstream (22 isolates), sputum (17 isolates), urine (10 isolates),

\* Correspondence: [cuilanyingharbin@163.com](mailto:cuilanyingharbin@163.com)

**Table 1.** Annual numbers and proportions of CREC.

Strain (n)	2009	2010	2011	2012
Total <i>E. cloacae</i>	72	113	196	285
CREC	2 (2.8%)	5 (4.4%)	11 (5.6%)	46 (16.1%)

wound discharge (7 isolates), catheters (3 isolates), abdominal fluid (3 isolates), pleural fluid (1 isolate), and bile (1 isolate). The department distribution of the CREC isolates were as follows: intensive care units (ICUs; 32 patients), respiratory wards (9 patients), urology (7 patients), general surgery (3 patients), nephrology (3 patients), endocrinology (2 patients), cardiology (2 patients), neurology (2 patients), orthopedic surgery (2 patients), and neurosurgery (2 patients).

A case-control study was performed to define the risk factors associated with carbapenem-resistant *Enterobacter cloacae*. The 64 CREC patients were considered as the case group. The control group (patients infected by carbapenem-susceptible *Enterobacter cloacae*) included 2 controls per case patient according to treatment date, infection site, and the unit where the patient was treated. The parameters reviewed included age, sex, underlying diseases (diabetes mellitus, malignancy, gastrointestinal tract disease, cardiovascular disease, genitourinary tract disease, chronic pulmonary disease, chronic renal failure, liver disease, cerebrovascular disease, intraabdominal infection), prior antibiotic therapy (the use of antibiotics [quinolones, cefazolins, or/and aminoglycosides] for at least 2 weeks before a CREC culture result), recent surgery during the past 30 days, and exposure to invasive interventions (intravascular catheter, urinary catheter, mechanical ventilation, nasogastric tube, and parenteral nutrition) within 7 days before a CREC culture result.

## 2.2. Statistical analysis

Statistical analyses were performed using SPSS 15.0 (Chicago, IL, USA). Continuous variables were compared with Student's t-test. Categorical variables were compared by a chi-square test or Fisher's exact test. A P-value of less than 0.05 was considered to be significant.

## 2.3. Microbiological studies

Identification and antimicrobial susceptibility testing of the 666 *Enterobacter cloacae* strains were performed using a VITEK-2 compact automatic system (bioMérieux, France) according to the manufacturer's instructions. The strains identified with imipenem MIC of  $\geq 2$   $\mu\text{g/mL}$  and/or ertapenem MIC of  $\geq 1$   $\mu\text{g/mL}$  by instrumental method were screened as possible CREC and were confirmed by an Etest (AB Biodisk, Sweden). The disc diffusion method was performed to determine resistance to some antimicrobial drugs (Table 2). The interpretative criteria

for antimicrobial susceptibility testing were set according to the Clinical Laboratory Standards Institute (CLSI) (8). *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, and *Enterococcus faecalis* ATCC 29212 were used for quality control of susceptibility tests.

## 2.4 Molecular analysis

Detection of ESBL genes (including *blaSHV*, *blaTEM*, *blaCTX-M-1*, *blaCTX-M-2*, and *blaCTX-M-9*) and carbapenemase genes (including *blaKPC*, *blaIMP*, *blaNDM-1*, and *blaVIM*) was performed using polymerase chain reaction (PCR). Template DNA was extracted by the boiling preparation method (9). The primers used for PCR are described in Table 3. The PCR parameters were as follows: initial denaturation step at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 40–56 °C for 30 s, and extension at 72 °C for 40 s, with a final extension step at 72 °C for 5 min. The PCR products were examined by electrophoresis in 1% agarose gels under UV light, purified and sequenced by Invitrogen (Invitrogen, Shanghai, China), and compared with known sequences available in the GenBank databases.

Pulsed-field gel electrophoresis (PFGE) of genomic DNA was carried out with a CHEF Mapper XA apparatus (Bio-Rad Laboratories, USA) to investigate the genetic relatedness among the CREC isolates. Genomic DNA was digested with XbaI (Takara Bio Inc., Japan). Electrophoresis conditions were as follows: 19 h at 6 V/cm and 14 °C with pulse times ranging from 2.2 to 54.2 s. PFGE patterns were interpreted according to the criteria determined by Tenover et al. (10).

## 3. Results

### 3.1. Clinical characteristics of the CREC isolates

During the study period, 666 isolates of *Enterobacter cloacae* were identified at the study hospital. Of these, 64 (9.6%) isolates were defined as CREC. As shown in Table 1, the total number of *Enterobacter cloacae* and CREC both presented growth trends during the 4 years. The prevalence of CREC increased from 2.8% in 2009 to 16.1% in 2012, and there was statistical significance between 2012 and the other years ( $P < 0.01$ ). The 64 CREC were mainly from the bloodstream (22; 34.4%) and less frequently from other samples, such as sputum (17; 26.6%), urine (10; 15.6%), and wound discharge (7; 10.9%). Concerning the in-

**Table 2.** Antibiotic susceptibility patterns of CREC.

Antibiotics	Sensitive N (%)	Intermediate N (%)	Resistance N (%)
Imipenem	0 (0)	6 (9.4)	58 (90.6)
Meropenem	0 (0)	6 (9.4)	58 (90.6)
Ertapenem	0 (0)	0 (0)	64 (100)
Ampicillin-sulbactam	0 (0)	0 (0)	64 (100)
Piperacillin-tazobactam	0 (0)	0 (0)	64 (100)
Ceftazidime	0 (0)	0 (0)	64 (100)
Ceftriaxone	0 (0)	0 (0)	64 (100)
Cefepime	0 (0)	0 (0)	64 (100)
Aztreonam	0 (0)	0 (0)	64 (100)
Levofloxacin	24 (37.5)	4 (6.3)	36 (56.3)
Ciprofloxacin	22 (34.4)	1 (1.6)	41 (64.1)
Amikacin	40 (62.5)	0 (0)	24 (37.5)
Tobramycin	7 (10.9)	0 (0)	57 (89.1)
Gentamicin	9 (14.1)	0 (0)	55 (85.9)
Trimethoprim-sulfamethoxazole	11 (17.2)	2 (3.1)	51 (79.7)
Tigecycline	50 (78.1)	5 (7.8)	9 (14.1)
Polymyxin B	60 (93.8)	0 (0)	4 (6.3)

**Table 3.** Primers used in this study.

Amplicon	Primer (5' — 3')		Product length (bp)	Reference or source
	Forward	Reverse		
CTX-M -1	GTTTAAAAAATCACTGCGTC	TTGGTGACGATTTTAGCCGC	863	(13)
CTX-M -2	ATGATGACTCAGAGCATTCG	TGGGTACGATTTTCGCCGC	865	(13)
CTX-M -9	ATGGTGACAAAGAGAGTGCA	CCCTTCGGCGATGATTCTC	869	(13)
SHV	CGCCGGGTATTTCTTATTTGTTCGC	CGCCGGGTATTTCTTATTTGTTCGC	795	(13)
TEM	ATAAAATTCTTGAAGACGAAA	GACAGTTACCAATGCTTAATCA	1079	(13)
KPC	ATGTCACTGTATCGCCGTCT	TTTTTCAGAGCCTTACTGCCC	892	(14)
IMP	CATGGTTTGGTGGTTCTTGT	ATAATTTGGCGGACTTTGGC	488	(14)
NDM-1	I: CAGCACACT TCCTATCTC	I: CCGCAACCATCCCCCTCTT	292	(14)
	II: GGCGGAATGGCTCATCACGA	II: CGCAACACAGCCTGACTTTC	287	(14)
VIM	TTATGGAGCAGCAACGATGT	CAAAAGTCCCGCTCCAACGA	920	(14)

hospital distribution, the majority of these CREC patients were from the ICU (32; 50.0%), followed by respiratory wards (9; 14.1%), and urology (7; 10.9%).

### 3.2. Risk factors associated with CREC patients

There were no significant differences in demographic characteristics and most clinical illnesses between the cases and controls ( $P > 0.05$ ). A univariate analysis showed that CREC infections were associated with cerebrovascular disease, prior treatment with antibiotics, intravascular catheterization, urinary catheterization, mechanical ventilation, and parenteral nutrition while the control group was associated with chronic pulmonary disease ( $P < 0.05$ ) (Table 4).

### 3.3. Antimicrobial susceptibility

A total of 64 CREC isolates were defined as multidrug-resistant strains because of resistance to 3 or more classes of antimicrobial agents. With the exception of 6 (9.4%) isolates that had intermediate susceptibility to imipenem and meropenem, all of the isolates were resistant to carbapenems. All of the isolates were resistant to the third- and fourth-generation cephalosporins, beta-lactam/

beta-lactamase inhibitor combinations, and aztreonam. Fifty-seven (89.1%) isolates were resistant to tobramycin, 55 (85.9%) to gentamicin, 51 (79.7%) to trimethoprim-sulfamethoxazole, 41 (64.1%) to ciprofloxacin, 36 (56.3%) to levofloxacin, 24 (37.5%) to amikacin, 9 (14.1%) to tigecycline, and 4 (6.3%) to polymyxin B (Table 2).

### 3.4. PCR assay analysis

A total of 49 (76.6%) isolates carried carbapenemase genes. Only 2 families of carbapenemase genes were found in this study, *blaKPC* and *blaIMP*. Thirty-eight (59.4%) isolates had *blaKPC-2* genes, 8 (12.5%) had *blaIMP-8* genes, and 3 (4.7%) had *blaIMP-1* genes. We did not find *blaNDM-1* or *blaVIM* genes in this study. A total of 39 (60.9%) isolates possessed ESBL genes, among which 23 (35.9%) were *blaSHV-12* genes, followed by 8 (12.5%) with *blaCTX-M-9* and 7 (10.9%) with *blaTEM-1* genes. Only 1 (1.6%) of isolates carried both *blaSHV-12* and *blaCTX-M-9*.

Nineteen (29.7%) isolates produced single carbapenemases, and 9 (14.1%) isolates produced single ESBLs. Thirty (46.9%) isolates produced both carbapenemases and ESBLs. Six (9.4%) isolates produced neither carbapenemases nor ESBLs (Table 5).

**Table 4.** Comparisons of demographic data and clinical characters of 64 CREC patients.

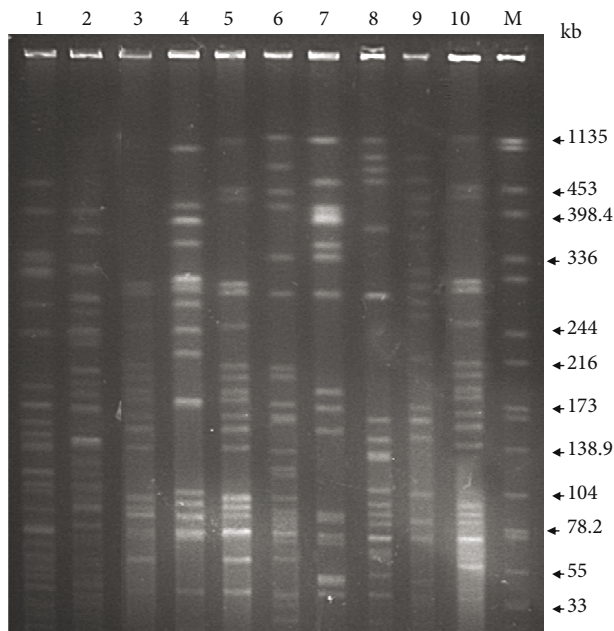
Parameter	Cases (n = 64), n (%)	Controls (n = 128), n (%)	OR (95% CI)	P-value
Mean age, years (range)	59 (21–85)	65 (26–79)	11.44	0.38
No. (%) of males	34 (53.1)	56 (43.8)	1.46	0.220
Diabetes mellitus	4 (6.3)	17 (13.3)	0.44	0.141
Malignancy	6 (9.4)	9 (7.0)	1.37	0.568
Gastrointestinal tract disease	2 (3.1)	10 (7.8)	0.38	0.206
Cardiovascular disease	12 (18.8)	20 (15.6)	1.25	0.584
Genitourinary tract disease	3 (4.7)	12 (9.4)	0.48	0.254
Chronic pulmonary disease	5 (7.8)	28 (21.9)	0.30	0.015
Chronic renal failure	4 (6.3)	5 (3.9)	1.64	0.469
Liver disease	4 (6.3)	6 (4.7)	1.36	0.646
Cerebrovascular disease	24 (37.5)	18 (14.1)	3.67	<0.001
Intraabdominal infection	0 (0)	3 (2.3)	—	0.217
Prior treatment with antibiotics ( $\geq 2$ weeks)	64 (100.0)	104 (81.3)	—	<0.001
Recent surgery	5 (7.8)	12 (9.4)	0.82	0.719
Intravascular catheter	16 (25.0)	8 (6.3)	5	<0.001
Urinary catheter	56 (87.5)	16 (12.5)	49	<0.001
Mechanical ventilation	10 (15.6)	1 (0.8)	11.88	<0.001
Nasogastric tube	1 (1.6)	1 (0.8)	—	>0.05
Parenteral nutrition	2 (3.1)	0 (0)	—	<0.05

**Table 5.** Molecular characteristics of 64 CREC strains.

Beta-lactamase	No.	Genes (n)
ESBLs	39	<i>SHV-12</i> (23)
		<i>TEM-1</i> (7)
		<i>CTX-M-9</i> (8)
		<i>SHV-12+CTX-M-9</i> (1)
Carbapenemase	49	<i>KPC-2</i> (38)
		<i>IMP-1</i> (3)
		<i>IMP-8</i> (8)
Single ESBLs	9	<i>SHV-12</i> (5), <i>CTX-M-9</i> (1), <i>TEM-1</i> (2), <i>SHV-12+CTX-M-9</i> (1)
Single carbapenemases	19	<i>KPC-2</i> (18), <i>IMP-8</i> (1)
ESBLs and carbapenemases	30	<i>SHV-12 + KPC-2</i> (10), <i>TEM-1 + KPC-2</i> (4), <i>SHV-12 + IMP-8</i> (6), <i>SHV-12 + IMP-1</i> (2), <i>CTX-M-9 + KPC-2</i> (6), <i>CTX-M-9 + IMP-8</i> (1), <i>TEM-1 + IMP-1</i> (1)
No ESBLs or carbapenemases	6	

### 3.5. PFGE analysis

The genetic relatedness of the CREC isolates was investigated by PFGE in our study. Lanes 3, 5, and 10 represented the same clone. All of the other isolates yielded different DNA band profiles, and no obvious clonal association was observed among them (Figure).



**Figure.** PFGE patterns of genomic DNAs of CREC. Lanes 3, 5, and 10 present the same profile. Lanes 1, 2, 4, and 6–9: unrelated isolates of CREC. Lane M: molecular weight marker (PFGE marker, *Salmonella* ser. Braenderup HB9812).

### 4. Discussion

Carbapenems are traditionally considered to be the last defense against serious infections caused by ESBL-producing Enterobacteriaceae. However, in recent years, CREC has been detected in several countries (11–13). In the last 4 years, the numbers of CREC increased year by year. In 2012, 46 (16.1%) patients were diagnosed with CREC infections in our hospital, about 3 times that of the previous year (Table 1). According to PFGE results, significant clonal spread was not observed among most of them, showing that no outbreak of CREC happened during these 4 years. The increase of CREC may be associated with the intensive use of antibiotics, mutation of resistance genes, and horizontal transfer of resistance genes (14,15). This finding suggests that effective measures should be taken to control the emergence and dissemination of CREC strains in health care organizations and communities.

Evaluation of the risk factors for CREC infections demonstrated that invasive interventions were significantly associated with the CREC group ( $P < 0.05$ ). This is likely due to the fact that invasive therapy can increase the chances of CREC infection and prolong the use of antibiotics. This result is similar to those of previous studies (16–18). These findings suggest that prompt discontinuation of invasive interventions is an effective way to reduce the risk of CREC infections. The use of antibiotics, especially cephalosporins and carbapenems, was also a risk factor for CREC infection. It is worrisome that cephalosporins and carbapenems are frequently used to treat gram-negative *Bacillus* infections in hospitals and community settings. Empirical therapy with cephalosporins or carbapenems

may lead to the emergence of carbapenem-resistant strains (14,15). It is very important to choose suitable antibiotics according to drug sensitivity tests and avoid the abuse of antibiotics. In terms of disease prevalence, we found that cerebrovascular disease was more common in the case group, and chronic pulmonary disease was more prevalent in the control group ( $P < 0.05$ ). Unfortunately, there is no additional research to determine whether there is a connection between the diseases in this study. In addition, we found that 50% of CREC patients came from ICU wards. The ICU is a special department, and most ICU patients have serious diseases, more opportunities for invasive treatment, and longer courses of antibiotic treatment; therefore, the ICU is another risk factor for CREC infections.

In our study, 49 (76.6%) of CREC had carbapenemase genes, and KPC-2 was the most common carbapenemase type in the study hospital. Similar studies have been reported in different counties and different regions of China (19–21). Different results were also reported in Chongqing in China. In another study, IMP and OXA were the most prevalent carbapenemases, and KPC-2 was not detected in their hospital (9).

Ten years ago, ESBLs were mainly produced by *Klebsiella pneumoniae* and *Escherichia coli*. Now ESBL-producing *Enterobacter cloacae* have been reported in many countries (22–24). This study found that 39 (60.9%) CREC isolates produced ESBLs, which was higher than that of noncarbapenem-resistant *Enterobacter cloacae* (22,23). ESBL genes have been classified into 9 families based on their amino acid sequences (25). TEM, SHV, and CTX-M are the most common families. In this study, SHV-12 (35.9%) was the main type and CTX-M was second (12.5%). Yang et al. (1) reported that SHV-12 accounted for 75.5% in ertapenem-resistant *Enterobacter cloacae* in Taiwan. Xia et al. (9) reported that CTX-M was the most common genotype (50.0%) in CREC in Chongqing in China. These results indicate that the genotype distribution of CREC is remarkably different in different countries and regions.

The resistance mechanisms of carbapenems are very complex and can be mediated by several means: 1) carbapenemase production, 2) production of ESBLs or AmpC  $\beta$ -lactamases associated with alterations in outer membrane proteins, and 3) hyperexpression of efflux systems (26–28). Carbapenemase production is the most important carbapenem resistance mechanism in Enterobacteriaceae. In this study, 6 CREC isolates were not found to contain carbapenemase or ESBL genes, implying that resistance may involve other mechanisms not investigated in our study, such as production of other  $\beta$ -lactamases or hyperexpression of efflux systems.

Antimicrobial susceptibility testing showed that the CREC isolates all exhibited multidrug resistance. All isolates were either resistant or had intermediate susceptibility to beta-lactams, including carbapenems. Some isolates were only susceptible to aminoglycosides, quinolones, trimethoprim-sulfamethoxazole, tigecycline, and polymyxin. Current reports showed that only a minority of antibacterial drugs, such as tigecycline and polymyxin, can be first-line agents for treating carbapenem-resistant Enterobacteriaceae infections (29). This finding indicates that the options for treating the infections caused by CREC are extremely limited. ESBLs and carbapenemase genes are commonly located on plasmids or other mobile genetic elements, which may harbor other antibiotic resistant genes, such as quinolones and aminoglycosides. These resistant genes can spread among bacteria together and then contribute to multiresistant phenotypes of CREC.

PFGE studies demonstrated that 3 isolates exhibited the same profile. They were all collected from the ICU department within 2 months and had the same drug-resistant gene profile (SHV-12 + KPC-2) and antimicrobial susceptibility results, showing a possible clonal dissemination of 1 isolate. This finding demonstrated the potential epidemic threat of CREC in hospitals. The other isolates exhibited distinct PFGE profiles, indicating no obvious clonal association within them. It indicated that the infections caused by CREC were spontaneous in our hospital within the last 4 years. However, outbreaks of carbapenemase-producing Enterobacteriaceae have been reported in some hospitals (30). Due to the fact that the infections with CREC are very difficult to treat and associated with high morbidity and mortality, it is necessary to monitor the emergence and spread of CREC strains.

There are at least 3 limitations of our study. First, we just selected a few common carbapenemase and ESBL enzymes to analyze. Some other types, such as GES, SME, and OXA, which were not detected in this study, might exist in these CREC isolates. Second, we did not detect loss of outer membrane proteins and efflux pump expression in these isolates, which may be involved in carbapenem resistance mechanisms. In addition, the number of CREC isolates was small, and further investigation should be done to verify the characteristics of CREC.

In summary, the infection rate of CREC has increased in recent years. Over 60% of CREC isolates had carbapenemase and/or ESBLs genes, and *blaSHV-12* and *blaKPC-2* were highly prevalent among them. Strict measures should be taken to control the spread of these pathogens in hospitals.

## References

1. Yang FC, Yan JJ, Hung KH, Wu JJ. Characterization of ertapenem-resistant *Enterobacter cloacae* in a Taiwanese university hospital. *J Clin Microbiol* 2012; 50: 223–226.
2. 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.
3. Marchain D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Isolation of imipenem-resistant *Enterobacter* species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. *Antimicrob Agents Chemother* 2008; 52: 1413–1418.
4. Souli M, Galani I, Antoniadou A, Papadomichelakis E, Poulakou G, Panagea T, Vourli S, Zerva L, Armaganidis A, Kanellakopoulou K et al. 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. *Clin Infect Dis* 2010; 50: 364–373.
5. Monteiro J, Santos AF, Asensi MD, Peirano G, Gales AC. First report of KPC-producing *Klebsiella pneumoniae* strains in Brazil. *Antimicrob Agents Chemother* 2009; 53: 333–334.
6. Peirano G, Seki LM, Val Passos VL, Pinto MCFG, Guerra LR, Asensi MD. Carbapenem-hydrolysing  $\beta$ -lactamase KPC-2 in *Klebsiella pneumoniae* isolated in Rio de Janeiro, Brazil. *J Antimicrob Chemother* 2009; 63: 265–268.
7. Iabadene H, Messai Y, Ammari H, Ramdani-Bouguessa N, Lounes S, Bakour R, Arlet G. Dissemination of ESBL and Qnr determinants in *Enterobacter cloacae* in Algeria. *J Antimicrob Chemother* 2008; 62: 133–136.
8. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 21st Informational Supplement 2011, M100-S21. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2011.
9. Xia Y, Liang Z, Su X, Xiong Y. Characterization of carbapenemase genes in *Enterobacteriaceae* species exhibiting decreased susceptibility to carbapenems in a University Hospital in Chongqing, China. *Ann Lab Med* 2012; 32: 270–275.
10. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
11. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant *Enterobacteriaceae*: epidemiology and prevention. *Clin Infect Dis* 2011; 53: 60–67.
12. Yang Q, Wang H, Sun H, Chen H, Xu Y, Chen M. Phenotypic and genotypic characterization of *Enterobacteriaceae* with decreased susceptibility to carbapenems: results from large hospital-based surveillance studies in China. *Antimicrob Agents Chemother* 2010; 54: 573–577.
13. Logan LK. Carbapenem-resistant *Enterobacteriaceae*: an emerging problem in children. *Clin Infect Dis* 2012; 55: 852–859.
14. Munoz-Price LS, De La Cuesta C, Adams S, Wyckoff M, Cleary T, McCurdy SP, Huband MD, Lemmon MM, Lescoe M, Dibhajj FB et al. Successful eradication of a monoclonal strain of *Klebsiella pneumoniae* during a *K. pneumoniae* carbapenemase-producing *K. pneumoniae* outbreak in a surgical intensive care unit in Miami, Florida. *Infect Control Hosp Epidemiol* 2010; 31: 1074–1077.
15. Karah N, Haldorsen B, Hermansen NO, Tveten Y, Ragnhildstveit E, Skutlaberg DH, Tofteland S, Sundsfjord A, Samuelsen Ø. Emergence of OXA- carbapenemase-and 16S rRNA methylase-producing international clones of *Acinetobacter baumannii* in Norway. *J Med Microbiol* 2011; 60: 515–521.
16. Correa L, Martino MD, Siqueira I, Pasternak J, Gales AC, Silva CV, Camargo TZ, Scherer PF, Marra AR. A hospital-based matched case-control study to identify clinical outcome and risk factors associated with carbapenem-resistant *Klebsiella pneumoniae* infection. *BMC Infect Dis* 2013; 13: 80.
17. Fernández A, Pereira MJ, Suárez JM, Poza M, Treviño M, Villalón P, Sáez-Nieto JA, Regueiro BJ, Villanueva R, Bou G. Emergence in Spain of a multidrug-resistant *Enterobacter cloacae* clinical isolate producing SFO-1 extended- spectrum beta-lactamase. *J Clin Microbiol* 2011; 49: 822–828.
18. Routsis C, Pratikaki M, Platsouka E, Sotiropoulou C, Papas V, Pitsiolis T, Tsakris A, Nanas S, Roussos C. Risk factors for carbapenem-resistant Gram-negative bacteremia in intensive care unit patients. *Intensive Care Med* 2013; 39: 1253–1261.
19. Hirsch EB, Tam VH. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. *J Antimicrob Chemother* 2010; 65: 1119–1125.
20. Giakkoupi P, Pappa O, Polemis M, Vatopoulos AC, Miriagou V, Zioga A, Papagiannitsis CC, Tzouveleki LS. Emerging *Klebsiella pneumoniae* isolates coproducing KPC-2 and VIM-1 carbapenemases. *Antimicrob Agents Chemother* 2009; 53: 4048–4050.
21. Zhou T, Zhang X, Guo M, Ye J, Lu Y, Bao Q, Chi W. Phenotypic and molecular characteristics of carbapenem-non-susceptible *Enterobacteriaceae* from a teaching hospital in Wenzhou, southern China. *Jpn J Infect Dis* 2013; 66: 96–102.
22. Nedjai S, Barguigua A, Djahmi N, Jamali L, Zerouali K, Dekhil M, Timinouni M. Prevalence and characterization of extended spectrum beta- lactamase- producing *Enterobacter cloacae* strains in Algeria. *J Infect Dev Ctries* 2013; 7: 804–811.
23. Chen CH, Huang CC. Risk factor analysis for extended-spectrum  $\beta$ -lactamase-producing *Enterobacter cloacae* bloodstream infections in central Taiwan. *BMC Infect Dis* 2013; 13: 417.

24. Khalaf NG, Eletreby MM, Hanson ND. Characterization of CTX-M ESBLs in *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from Cairo, Egypt. *BMC Infect Dis* 2009; 9: 84.
25. Paterson DL. The molecular epidemiology of extended-spectrum beta-lactamase producing organisms. *Enferm Infecc Microbiol Clin* 2008; 26: 403.
26. Patel G, Bonomo RA. Status report on carbapenemases: challenges and prospects. *Expert Rev Anti Infect Ther* 2011; 9: 555–570.
27. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009; 9: 228–236.
28. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Trends Mol Med* 2012; 18: 263–272.
29. Perez F, Van Duin D. Carbapenem-resistant *Enterobacteriaceae*: a menace to our most vulnerable patients. *Cleve Clin J Med* 2013; 80: 225–233.
30. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM et al. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 2012; 18: 413–431.