Clinical Infectious Diseases Advance Access published July 2, 2012

Predictors of Mortality in Bloodstream Infections Caused by KPC-Producing *Klebsiella pneumoniae*: Importance of Combination Therapy

Mario Tumbarello[1], Pierluigi Viale[2], Claudio Viscoli[3], Enrico Maria Trecarichi[1], Fabio Tumietto[2], Anna Marchese[4], Teresa Spanu[5], Simone Ambretti[6], Francesca Ginocchio[3], Francesco Cristini[2], Angela Raffaella Losito[1], Sara Tedeschi[2], Roberto Cauda[1], Matteo Bassetti[3, 7].

[1]Institute of Infectious Diseases Catholic University of the Sacred Heart, A. Gemelli Hospital, Rome, Italy.
[2]Clinic of Infectious Diseases, University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy.
[3]Infectious Diseases Division, San Martino Hospital and University of Genoa, Genoa, Italy.
[4]Department of Microbiology, University of Genoa, Genoa, Italy.
[5]Institute of Microbiology Catholic University of the Sacred Heart, A. Gemelli Hospital, Rome, Italy.
[6] Institute Microbiology, University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy.
[7]Infectious Disease Division Santa Maria Misericordia University Hospital, Udine, Italy.

Correspondence: Dr. Mario Tumbarello, Istituto Malattie Infettive, Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, 00168 Roma, Italy.
FAX +39-06-3054519, tel. +39-06-30155373, e-mail: tumbarello@rm.unicatt.it
Alternative author for correspondence: Enrico M. Trecarichi: e.trecarichi@gmail.com

Summary: Bloodstream infections caused by KPC-producing strains of *Klebsiella pneumoniae* are associated with high mortality. Compared with monotherapies, combinations of active drugs, especially those including a carbapenem, may be associated with improved survival.

ABSTRACT

Background: The spread of *Klebsiella pneumoniae* (*Kp*) strains that produce *Klebsiella pneumoniae* carbapenemases (KPCs) has become a significant problem, and treatment of infections caused by these pathogens is a major challenge for clinicians.

Methods: In this multicenter retrospective cohort study, conducted in three large Italian teaching hospitals, we examined 125 patients with bloodstream infections (BSIs) caused by KPC-producing *Kp* isolates (KPC-Kp) diagnosed between 1 January 2010 and 30 June 2011. The outcome measured was death within 30 days of the first positive blood culture. Survivor and non-survivor subgroups were compared to identify predictors of mortality.

Results: The overall 30-day mortality rate was 41.6%. A significantly higher rate was observed among patients treated with monotherapy (54.3% vs. 34.1% in those who received combined drug therapy, $P = 0.02$).

In logistic regression analysis, 30-day mortality was independently associated with septic shock at BSI onset (odds ratio [OR], 7.17; 95% confidence interval [CI], 1.65 to 31.03; $P = 0.008$); inadequate initial antimicrobial therapy (OR, 4.17; 95% CI, 1.61 to 10.76; $P = 0.003$); and high APACHE III scores (OR, 1.04; 95% CI, 1.02 to 1.07; $P < 0.001$). Post-antibiogram therapy with a combination of tigecycline, colistin, and meropenem was associated with lower mortality (OR, 0.11; 95% CI, 0.02 to 0.69; $P = 0.01$).

Conclusions: KPC-Kp BSIs are associated with high mortality. For improving survival, combined treatment with two or more drugs with in vitro activity against the isolate, especially those including also a carbapenem, may be more effective than active monotherapy.

INTRODUCTION

The production of *Klebsiella pneumoniae* (*Kp*) carbapenemases (KPCs) by Enterobacteriaceae has become a significant problem. KPC-producing strains are not only able to hydrolyze carbapenems, they are often resistant to a variety of other antibiotics as well. Effective treatment of infections caused by these pathogens is thus a considerable challenge for clinicians [1-3].

KPC-producing Enterobacteriaceae have caused numerous infection outbreaks in the north-eastern United States, Israel, and Greece, where they are now considered endemic [1-2,4-6]. Most of the reports on these outbreaks have analyzed the molecular epidemiological aspects or the in vitro antimicrobial susceptibility profiles of the isolates: less attention has been focused on the drugs used to treat the infections and patient outcomes [7-12]. Small clinical studies have revealed high treatment failure rates associated with these infections, and reported mortality rates range from 22% to 72% [10,13]. Treatment options are usually limited to colistin, gentamicin, and/or tigecycline [14], but the optimal regimen for infections caused by KPC-producing bacteria has yet to be defined. A recent analysis of 55 cases found that treatment with tigecycline or with an aminoglycoside was generally associated with positive outcomes [10]. Moreover, descriptive reports of outbreaks of infections caused by KPC producers indicate that combination therapies are often more effective than monotherapies [15,16], and this conclusion is supported by in vitro data [17]. Identifying the best treatment regimen for these infections will require larger studies with more in-depth analysis of clinical characteristics and outcomes.

In this multicenter, hospital-based study, we attempted to pinpoint risk factors for mortality in a cohort of 125 patients with bloodstream infections (BSIs) caused by KPC-producing isolates of Kp (KPC-*Kp*). Particular attention was focused on the impact of antimicrobial regimens used in the nonempirical phase of treatment.

MATERIALS AND METHODS

Setting. The study was conducted in three large Italian teaching hospitals that offer a full range of clinical services. Each facility admits approximately 50,000 patients per year. Surveillance cultures were not routinely performed during the study period.

Study design and patients. We searched each hospital's central microbiology laboratory database to identify cases with all of the following characteristics: KPC-*Kp* BSI diagnosed between 1 January 2010 and 30 June 2011; patient age ≥ 18 years; absence of bloodstream isolates other than *Kp*; no evidence of non-*Kp* infections at other sites; and treatment of the BSI for at least 48 h (empirically and/or based on antibiogram data) with one or more antimicrobials displaying in vitro activity against the KPC *Kp* isolate [15]. Recurrent infections were excluded: only the first KPC Kp BSI episode per patient was included in our analysis. A retrospective cohort study design was employed. The outcome measured was death within 30 days of the first positive blood culture. Survivor and non-survivor subgroups were compared to identify predictors of mortality.

Variables explored as possible predictors of mortality. *Patient variables* included age, gender, Charlson Comorbidity Index [18], underlying diseases, immunosuppressive therapy, duration of index hospitalization, and BSI onset in an intensive care unit (ICU). We also considered histories of previous hospitalization (≤ 12 months before BSI onset), surgery (≤ 30 days before BSI onset), invasive procedures performed ≤ 72 h before BSI onset (insertion of central venous catheters, nasogastric tubes, or Foley catheters; endoscopy; endoscopic retrograde cholangiopancreatography; bronchoscopy; parenteral nutrition; mechanical ventilation), and antimicrobial therapy being administered ≤ 30 days before BSI onset. *Infection variables* consisted of BSI presentation with septic shock, severity of illness at infection onset, and source of infection. *Treatment variables* included initial (i.e., empirical) antimicrobial therapy that was inadequate (see *Definitions*) and the number and type of drugs

included in the post-antibiogram treatment regimen.

Definitions. The following terms were defined prior to data analysis. A *KPC-Kp BSI* was defined as a BSI documented by blood-culture positivity (at least one specimen) for a KPC-*Kp* strain and clinical signs of the systemic inflammatory response syndrome [19]. *BSI onset* was defined as the date of collection of the *index blood culture* (i.e., the first blood culture that yielded the study isolate). BSIs were defined as *hospital-acquired* (HA) if the index blood culture had been collected more than 48 hours after hospital admission and no signs or symptoms of infection had been noted at admission. Infections with onset <48 hours after hospital admission were classified as either healthcare-associated (HCA) or community-acquired (CA), in accordance with the definitions of Friedman et al. [20]. *Septic shock* was defined as sepsis associated with organ dysfunction and persistent hypotension despite volume replacement [19]. Illness severity at baseline was expressed in terms of the Acute Physiology and Chronic Health Evaluation (APACHE) III score [21] calculated at the time of BSI onset. An *antimicrobial treatment* regimen was defined as *adequate* when it included at least one drug displaying in vitro activity against the KPC-producing *Kp* isolate. Depending on the number of in vitro-active drugs they included (1 or >1), treatment regimens were classified as *monotherapy* or *combination therapy*.

Microbiology, KPC identification, and antimicrobial susceptibilities.

The Vitek 2 automated system (bioMerieux, Marcy l'Etoile, France) was used for isolate identification and antimicrobial susceptibility testing. Minimum inhibitory concentrations (MICs) were classified according to Clinical and Laboratory Standards Institute (CLSI) breakpoints [22], except those for colistin, which were interpreted according to breakpoints established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (susceptible, MIC ≤ 2 mg/L; resistant, MIC > 2 mg/L) [23]. For tigecycline, the US Food and Drug Administration recommendation was used (susceptible, MIC ≤2 mg/L; resistant MIC ≥

8 mg/L) [24]. Ertapenem-resistant isolates were tested for carbapenemase production in accordance with CLSI guidelines [25]. The presence of a *bla*$_{KPC}$ gene was determined by PCR and sequencing, as previously described [26].

Statistical analysis. Continuous variables were compared with Student's *t* test (for normally distributed variables) or the Mann-Whitney U test (for non-normally distributed variables). Categorical variables were evaluated with the chi-square or two-tailed Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for all associations that emerged. Results are expressed as means ± standard deviation (SD) or medians (ranges) (continuous variables) or as percentages of the group from which they were derived (categorical variables). Two-tailed tests were used to determine statistical significance; a *P* value of < 0.05 was considered significant. Multivariate analysis was used to identify independent risk factors for mortality. For this analysis, we used logistic regression, and variables found to be significant in univariate testing were incorporated with a stepwise approach. Survival distribution function was estimated using the Kaplan-Meier product–limit method; nonparametric (log-rank and Wilcoxon) tests were used to compare survival functions in different groups. All statistical analyses were performed with the Intercooled Stata program, version 11.

RESULTS

Of the 413 Kp BSIs diagnosed during the study period, 147 (35.6%) were caused by KPC-producing isolates, and 125 of these cases (96.8% HA, and 3.2% HCA infections) met the criteria for inclusion in our study. Thirty days after BSI onset, 52 (41.6%) of the 125 patients had died. The main characteristics of the survivor and nonsurvivor subgroups are presented in Table 1.

Characteristics of KPC-producing isolates. Ninety-eight (78.4%) of the 125 confirmed KPC-producing isolates included in the study harbored the *bla*$_{KPC-3}$ gene, and the other 27

(21.6%) carried the *bla*$_{KPC-2}$ gene. All 125 isolates were resistant to penicillins, cephalosporins, ertapenem, ciprofloxacin, amikacin, cotrimoxazole, and chloramphenicol. Meropenem MICs were consistently high: ≥ 16 mg/L for 78 (62.4%) isolates; 8 mg/L for 17 (13.6%) isolates; 4 mg/L for 16 (12.8%) isolates, 2 mg/L for 13 (10.4%) isolates, and 1 mg/L for 1 (0.8%) isolate. Most isolates were susceptible to colistin (n=110, 88%), tigecycline (n=114, 91.2%), and/or gentamicin (n=118, 94.4%). Genotype-specific resistance rates for these drugs are shown in Figure 1.

Antimicrobial treatment. Within a few hours after index blood cultures were drawn, all patients were being empirically treated with currently recommended doses [27] of anti-gram-negative drugs (alone or with other antibiotics). In most cases, the empirical regimen was prescribed *ad hoc* by ward physicians without the aid of predefined protocols.

On the basis of antibiograms (reported 72–120 h [median 76 h] after BSI onset, over half of the empirical regimens (75/125, 60%) were classified as inadequate. The ineffective drugs were ß-lactam-ß-lactamase inhibitor combinations in 31 cases (41.3%), carbapenems in 25 (33.3%), aminoglycosides in 8 (10.7%), fluoroquinolones in 6 (8%), and oxyimino-cephalosporins in 4 (5.3%).

All decisions on definitive therapy were made with the aid of an infectious disease specialist. Loading doses were used with colistin and tigecycline. Thereafter, the former drug was given every 8-12 h for a total daily dose of 6,000,000–9,000,000 IU; the latter drug was administered every 12 h (100-200 mg/day). Gentamicin was given q 24 h (total daily dose 4-5 mg/kg), and meropenem was administered by extended infusion (lasting ≥ 3 h) at a dose of 2 g every 8 hrs. All dosages were adjusted on the basis of creatinine clearance if necessary. The overall median duration of treatment was 16 [interquartile range (IQR) 7–28] days.

As shown in Table 1, the post-antibiogram regimen used for 46 patients (36.8%) consisted of a single drug. In the 22 patients who received colistin monotherapy, the colistin MICs were

≤0.5 mg/L for 16 (72.7 %) isolates and 1 mg/L for 6 (27.3%) isolates (all well within the EUCAST range of susceptibility, i.e., ≤ 2 mg / L). Similar findings emerged for the 19 patients treated with tigecycline alone (MICs were ≤0.5 mg/L for 5 [26.3%] isolates and 1 mg/L for 10 [52.6%] others), but in 4 (21.1%) of these cases the tigecycline MIC (2 mg / L) approached the upper limit of the FDA-defined susceptibility range. Most patients (63.2%) received 2 or more drugs with in vitro activity against the KPC-Kp isolate. The most common combination was colistin plus tigecycline, which was used in a total of 39 cases, alone (n=23) or with a third drug (meropenem) (n=16). As shown in Table 2, single-drug regimens (mainly colistin or tigecycline) were used more frequently for BSIs that stemmed from urinary tract infections, whereas combination therapy was significantly more common in ICU patients and above all in patients whose Kp isolates had suboptimal / borderline tigecycline and colistin MICs.

Outcomes and mortality predictors. The 52 (41.6%) patients who died within 30 days of BSI onset included 25 (54.3%) of the 46 whose post-antibiogram regimens were classified as monotherapy and 27 (34.1%) of the 79 who were on combination regimens ($P = 0.02$). The 30-day survival distributions were also significantly different in patients treated with monotherapy versus combination therapy regimens ($P = 0.002$) (Figure 2). Mortality rates within the combination regimen group were 30.4% (7/23) for those treated with tigecycline plus colistin; 50% (6/12) for those treated with tigecycline plus gentamicin; 57% (4/7) for those treated with colistin plus gentamicin; 12.5% (2/16) for those treated with tigecycline, colistin, and meropenem; and 16.6% (1/6) for those treated with tigecycline, gentamicin, and meropenem.

Univariate analysis revealed significant differences between the survivor and nonsurvivor subgroups. Patients in the latter group were more likely to have recent histories of hospitalization, immunosuppressive therapy, and/or chemotherapy; higher APACHE III

scores; and septic shock at BSI presentation. This group also had higher rates of inadequate empirical therapy and of postantibiogram regimens that were classified as monotherapy. Survivors were more likely to have received combination therapy in the postantibiogram phase. The only multidrug regimen that was significantly more common in the survivor group (*P*=0.009) was the combination of tigecycline, colistin, and meropenem (see Table 1). Subgroup analysis of the 75 patients who received inappropriate empirical therapy (overall mortality 52%) revealed significantly lower mortality (14.3%) among the 14 whose definitive therapy consisted of this triple-drug regimen.

Logistic regression analysis indentified septic shock at BSI onset, inadequate initial antimicrobial therapy, and high APACHE III scores as independent predictors of 30-day mortality, while combination therapy with tigecycline, colistin, and meropenem was associated with a lower risk of mortality (Table 3). In Table 4 the 30-day survival rates for the 36 patients whose combination regimens included meropenem are stratified according the meropenem MICs of their KPC *Kp* isolate.

DISCUSSION

To the best of our knowledge, our study represents the largest sample of KPC-*Kp* BSIs analyzed to date. Most of the isolates recovered from our patients were susceptible to gentamicin, colistin, and tigecycline. Resistance rates for the latter two drugs (12% and 7.2%, respectively) were fairly consistent with previous reports [2, 5, 10,11, 28, 29, 30].

Our findings also confirm the high mortality associated with these multidrug-resistant infections, with 41.6% of patients dying within 30 days of infection. This outcome was independently predicted by three factors. Two (i.e., presence of septic shock and high APACHE III score) were related to the clinical status of the patient at infection onset, and these findings are consistent with previous reports [12,15,28,31]. The third factor (and the

only one that is potentially modifiable) was inadequate empirical therapy. Its impact on mortality has been widely documented, particularly in patients with BSIs caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae [31-33]. However, in their recent study of a cohort of 53 patients with KPC-*Kp* BSI, Zarkotou et al. found no correlation between outcome and the adequacy of the initial antimicrobial regimen [15], and Patel et al. reported similar findings in 99 patients with invasive infections caused by carbapenem-resistant *Kp* [34]. The discrepancy between these findings and our own might stem from differences in the size of the cohorts examined, the type of infections analyzed (e.g., BSIs accounted for only about 60% of those reported by Patel et al.), and/or the *minimal* duration of survival after BSI onset (i.e., 48 hours of adequate treatment in all of the patients we studied).

As for the nonempirical phase of treatment, our results highlight the positive impact on outcome of a regimen that includes more than one drug with in vitro activity against the isolate (Figure 1). This finding is consistent with previous reports, such as that by Hirsch and Tam, who reviewed 15 articles on infections caused by KPC-producing isolates. In the 55 cases analyzed in these papers, they found high rates of clinical success in patients who received combination regimens including a polymixin [10]. In the more recent study by Zarkotou et al., combinations of active antimicrobial drugs were also significantly associated with survival, but only in univariate analysis, probably because of the small size of sample population [15]. Our study, which included a larger sample of cases of KPC-*Kp* BSI, reinforces concerns that monotherapy regimens utilizing drugs with substantial potency or pharmacokinetic shortcomings for bloodstream infections, may be associated with increased mortality.

Our multivariate analysis demonstrated that a triple-drug regimen that included tigecycline, colistin, and meropenem was significantly linked to a reduced risk of death. Indeed, this

appeared to be the most effective approach to the treatment of KPC-*Kp* BSIs, even when compared with other drug combinations. In their review, Hirsch et al. analyzed 11 KPC-*Kp* infections (none of which were BSIs) treated with drug combinations that included a polymyxin. The overall clinical success rate was 73% (66% in the six treated with colistin plus tigecycline) [10]. The percentages of our patients receiving colistin plus tigecycline in the survivor and nonsurvivor groups were not significantly different [16/73 (21.9%) vs 7/52 (13.4%), P=0.22]. In the whole our cohort, the 30-day survival rate associated with this particular drug combination was 69.7% (16/23) (vs. 45.7% in patients who were treated with monotherapy), but when meropenem was added to this regimen, survival increased significantly (87.5%).

This finding is unexpected because carbapenems are hydrolyzed by KPCs. However, in a recent review of the literature (mostly case reports) on carbapenem treatment of infections caused by carbapenemase-producing strains of *Kp,* Daikos et al. found that, if the isolate had a carbapenem MIC of ≤4 mg/L, combined therapy with a carbapenem plus one other active drug (an aminoglycoside or colistin or tigecycline) was associated with significantly lower mortality than combinations of noncarbapenem drugs with in vitro activity, and these findings were also in line with human pharmacokinetic/pharmacodynamic data reviewed by the authors [11]. In our study, improved survival was linked exclusively to the use of meropenem with tigecycline plus colistin, possibly because this was the most common carbapenem-containing regimen used in our patients. (Use of carbapenem-plus-aminoglycoside combinations was much less frequent, probably because regimens that included gentamicin were used only for BSIs caused by colistin-resistant isolates.) As shown in Table 4, when the KPC-Kp isolate had a meropenem MIC of ≤4 mg/L, inclusion of this drug in a combined-drug regimen was associated with a survival rate of 86.6% (13/15). However, even when we include patients with higher meropenem MICs in our analysis,

combined therapy that included meropenem produced a survival rate of 75%, and its performance in the subset with meropenem MICs ≥16 mg/L was still better than average (64.7% survival vs. 58.3% in the entire 125-patient cohort). This is consistent with the recent report that patients with KPC-Kp BSIs who received colistin/polymyxin B or tigecycline monotherapy had significantly higher mortality (66.7%) than those treated with colistin/polymyxin B or tigecycline combined with a carbapenem (12.5%) [35].

In conclusion, this triple-center retrospective study shows that BSIs caused by *Kp* strains expressing KPC-2 or KPC-3 are associated with high 30-day mortality. Prompt administration of at least one antimicrobial drug with in vitro activity against the isolate is essential in these cases. Indeed, even though all of the patients included in this cohort received at least 48 hours of this type of therapy, inadequate empirical therapy was still a major predictor of 30-day mortality. As for non-empirical therapy, combination therapy consisting of 2 or 3 active drugs seems to be more effective in terms of increasing survival than use of only one active drug, and the most significant improvement seems to be obtained when the combination includes a carbapenem. Although based on a relatively small number of cases, this retrospective analysis indicates that carbapenems provide therapeutic benefit against KPC producers when combined with tigecycline plus colistin. Larger clinical prospective studies are needed to assess this issue and to better define the importance of carbapenem MICs in decisions related to the treatment of KPC *KP* BSIs.

Funding: This work was partially supported by grants from the Italian Ministry for University and Scientific Research (Fondi Ateneo Linea D-1 2011 - 2012).

Potential conflict of interest: None

REFERENCES

1. Nordmann P, Naas T, Poirel L. Global Spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis, **2011**; 17:1791-1798.
2. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis, **2009**; 9:228–236.
3. Grundmann H, Livermore DM, Giske CG, et al. CNSE Working Group. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill, **2010;** 15(46).
4. Bratu S, Landman D, Haag R, et al. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City. Arch Intern Med, **2005**; 165: 1430–1435.
5. Souli M, Galani I, Antoniadou A, 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.
6. Cuzon G, Naas T, Truong H, et al. Worldwide diversity of *Klebsiella pneumoniae* that produces b-lactamase blaKPC-2 gene. Emerg Infect Dis, **2010**; 16:1349–1356.
7. Marchaim 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.
8. Pournaras S, Protonotariou E, Voulgari E, et al. Clonal spread of KPC-2 carbapenemase-producing Klebsiella pneumoniae strains in Greece. J Antimicrob Chemother, **2009;** 64:348-52.
9. 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.

10. 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.

11. Daikos GL, Markogiannakis A. Carbapenemase-producing Klebsiella pneumoniae: (when) might we still consider treating with carbapenems? Clin Microbiol Infect, **2011**; 17:1135-1141.

12. 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.

13. Borer A, Saidel-Odes L, Riesenberg K, et al. Attributable mortality rate for carbapenem-resistant *Klebsiella pneumoniae* bacteremia. Infect Control Hosp Epidemiol, **2009**; 30:972-976.

14. Carmeli Y, Akova M, Cornaglia G, et al. Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control. Clin Microbiol Infect, **2010**; 16:102–111.

15. Zarkotou O, Pournaras S, Tselioti P, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. Clin Microbiol Infect, **2011;** 17(12):1798-803

16. Qureshi ZA, Paterson DL, Adams-Haduch JM, et al. Clinical Outcome of Bacteremia Due to *Klebsiella pneumoniae* Carbapenemase (KPC)-Producing *Klebsiella pneumoniae*. In: Program and abstracts of the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy Boston, MA September 2010.

17. Pournaras S, Vrioni G, Neou E, et al. Activity of tigecycline alone and in combination

with colistin and meropenem against *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae strains by time-kill assay. Int J Antimicrob Agents, **2011**; 37:244-247.

18. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic co-morbidity in longitudinal studies: development and validation. J Chron Dis, **1987**; 40:373-383.

19. Russell JA. Management of sepsis. N Engl J Med, **2006**; 355:1699-1713.

20. Friedman ND, Kaye KS, Stout JE, et al. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med, **2002**; 137:791-797.

21. Knaus WA, Wagner DP, Draper EA, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest, **1991**; 100:1619-1636.

22. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility testing; twenty-first Informational Supplement. CLSI document M100-S21 Wayne, PA: CLSI, **2011.**

23. European Committee on Antimicrobial Susceptibility Testing. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_breakpoints_v1.3_pdf.pdf. Accessed 03 November 2011

24. Liu JW, Ko WC, Huang CH, et al. Agreement Assessment of Tigecycline Susceptibilities Determined by the Disk Diffusion and Broth Microdilution Methods among Commonly Encountered Resistant Bacterial Isolates: Results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) Study, 2008 to 2010. Antimicrob Agents Chemother, **2012;** 56(3): 1414-7.

25. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial

Susceptibility Tests for Bacteria That Grow Aerobically—Eigth Edition: Approved Standard M7-A8 Wayne, PA, USA: CLSI, **2011.**

26. 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.

27. Gilbert ND, Moellering RC, Eliopoulos GM, Chambers HF, Saag SA (ed.). The Sanford guide to antimicrobial therapy. Antimicrobial Therapy, Inc., Sperryville, VA, **2010.**

28. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clin Infect Dis, **2011**; 53:60-67.

29. Antoniadou A, Kontopidou F, Poulakou G, et al. Colistin-resistant isolates of Klebsiella pneumoniae emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother, **2007**; 59:786-790.

30. Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. Int J Antimicrob Agents, **2011**; 37:415-419.

31. Tumbarello M, Sali M, Trecarichi EM, et al. Bloodstream infections caused by extended-spectrum-beta-lactamase- producing Escherichia coli: risk factors for inadequate initial antimicrobial therapy. Antimicrob Agents Chemother, **2008**; 52:3244-3252.

32. Tumbarello M, Sanguinetti M, Montuori E, et al. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. Antimicrob Agents Chemother, **2007**; 51:1987-1994.

33. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J Antimicrob Chemother, **2007**; 60:913-920.

34. 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.

35. Qureshi ZA, Paterson DL, Potoski BA, et al. Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother, **2012** 56(4):2108- 2113.

Figure Legends

Figure 1. Colistin, tigecycline, and gentamicin resistance rates among *K. pneumoniae* isolates harboring the bla$_{KPC-2}$ and bla$_{KPC-3}$ genes

Figure 2. Kaplan-Meier curves showing the impact of monotherapy (*solid line*) vs combination therapy (*dotted line*) on 30-day mortality of patients with KPC-*Kp* BSIs (P=0.002).

Table 1. Univariate analysis of factors associated with death among patients with bloodstream infections due to KPC-producing *K. pneumoniae*.

Variable	No. (%) of patients		*P* value	OR (95% CI)
	Non survivors (n=52)	Survivors (n=73)		
Univariate analysis				
Demographic variables				
Male sex	32 (61.5)	41 (56.2)	0.54	1.13 (0.74-1.75)
Age (year [mean ± SD])	61.5±14.3	62.9±16.5	0.61	-
Ward				
Medicine	18 (34.6)	26 (35.6)	0.90	0.97 (0.62-1.50)
Surgery	12 (23.1)	16 (21.9)	0.87	1.03 (0.63-1.69)
ICU	22 (42.3)	31 (42.5)	0.98	0.99 (0.65-1.51)
LOS, days (median[IQR])	57 (29-63)	78 (36-90)	0.02	-
Previous hospitalization[a]	38 (73.1)	29 (39.7)	<0.001	2.34 (1.42-3.88)
Previous bacterial infections[b]	25 (48.1)	33 (45.2)	0.75	1.06 (0.70-1.62)
Invasive procedures[c]	28 (53.8)	46 (63.1)	0.30	0.80 (0.52-1.21)
Indwelling central venous catheter	40 (76.9)	48 (65.7)	0.17	1.40 (0.83-2.35)
Indwelling urinary catheter	36 (69.2)	46 (63.1)	0.47	1.17 (0.74-1.86)
Nasogastric tube[c]	18 (34.6)	17 (23.3)	0.16	1.36 (0.89-2.06)
Surgical drainage[c]	11 (21.1)	17 (23.3)	0.77	0.92 (0.55-1.55)
Previous surgery[d]	18 (34.6)	25 (34.2)	0.96	1.01 (0.65-1.56)
Immunosuppressive therapy[d]	10 (19.2)	6 (8.2)	0.06	1.62 (1.03-2.53)
Previous antibiotic therapy[d]	47 (90.4)	58 (79.4)	0.10	1.79 (0.81-3.93)
Comorbidity				
Diabetes mellitus	9 (17.3)	20 (27.4)	0.18	0.69 (0.38-1.24)
Heart failure	12 (23.1)	12 (16.4)	0.35	1.26 (0.79-2.01)
Chronic renal failure	6 (11.5)	6 (8.2)	0.53	1.22 (0.66-2.25)
Solid tumor	10 (19.2)	15 (20.5)	0.85	0.95 (0.55-1.62)
Hematological malignancy	7 (13.4)	6 (8.2)	0.34	1.34 (0.77-2.32)
Charlson Comorbidity Index, (median[IQR])	2 (0-4)	2 (0.5-2.5)	0.82	-
Post-antibiogram antimicrobial regimens				
Monotherapy	25 (48.1)	21 (28.7)	0.02	1.59 (1.06-2.38)
Tigecycline	10 (19.2)	9 (12.3)	0.28	1.32 (0.81-2.16)
Colistin	11 (21.5)	11 (15.1)	0.37	1.25 (0.77-2.03)

Gentamicin	4 (7.6)	1 (1.3)	0.09	1.98 (1.21-3.23)
Combination therapy	27 (51.9)	52 (71.2)	0.02	0.62 (0.41-0.94)
Two-drug combinations	23 (44.2)	33 (45.2)	0.91	0.97 (0.64-1.48)
Tigecycline + Colistin	7 (13.4)	16 (21.9)	0.22	0.68 (0.35-1.32)
Tigecycline + Gentamicin	6 (11.5)	6 (8.2)	0.53	1.22 (0.66-2.25)
Other 2-drug combinations[e]	10 (19.2)	11 (15.1)	0.54	1.17 (0.71-1.95)
Three-drug combinations	4 (7.7)	19 (26.1)	0.009	0.36 (0.15 0.92)
Tigecycline + Colistin + Meropenem	2 (3.8)	14 (19.2)	0.009	0.27 (0.07-1.01)
Other 3-drug combinations[f]	2 (3.8)	5 (6.8)	0.47	0.67 (0.21-2.21)
Inadequate initial antimicrobial treatment	39 (75)	36 (49.3)	0.003	2.00 (1.19-3.34)
Presentation with septic shock	13 (25)	4 (5.5)	0.002	2.11 (1.47-3.04)
APACHE III score (mean ± SD)	40±22	24±15	<0.001	-

Data are expressed as numbers (%) unless otherwise stated; Abbreviations: HCA, healthcare-associated; BSI, bloodstream infection; LOS, length of hospital stay; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease.

[a] During the 12 months preceding the BSI onset.

[b] During the 3 months preceding the BSI onset.

[c] During the 72 h preceding the BSI onset.

[d] During the 30 days preceding the BSI onset.

[e] Colistin + gentamicin (7 patients); gentamicin + meropenem (6 patients); tigecycline + meropenem (4 patients); colistin + meropenem (4 patients).

[f] Tigecycline + gentamicin + meropenem (6 patients); colistin + gentamicin + meropenem (1 patient)

Table 2. Characteristics of KPC-*Kp* BSIs treated with one vs. more than one in vitro-active antimicrobial drug

Characteristics	No (%) of patients		*P*
	Monotherapy (*n* = 46)	Combination therapy (*n* = 79)	
Patient demographics			
Male sex	26 (56.5)	47 (59.5)	0.75
Age (mean ± SD)	65±15	61±16	0.08
Ward			
Medicine	18 (39.2)	26 (32.9)	0.48
Surgery	14 (30.4)	14(17.7)	0.10
ICU	14 (30.4)	39 (49.4)	0.04
Comorbidities			
Solid tumor	8 (17.4)	17 (21.5)	0.58
Hematological malignancy	2 (4.4)	11 (13.9)	0.09
Chronic renal failure	5 (10.9)	7 (8.9)	0.71
Diabetes	9 (19.6)	20 (25.3)	0.46
Charlson Comorbidity Index, score (mean ± SD)	2.5±1.8	3±1.9	0.22
Immunosuppressive therapy	7 (15.2)	9 (11.4)	0.54
Clinical presentation			
Source of infection			
Central venous catheter	6 (13.0)	7 (8.9)	0.46
Lower respiratory tract	11 (26.1)	17 (21.5)	0.56
Urinary tract	10 (21.7)	7 (8.9)	0.04
Other	3 (6.5)	2 (2.5)	0.27
Unknown	24 (52.2)	51 (64.6)	0.17
Shock	8 (17.4)	9 (11.4)	0.35
APACHE III score (mean ± SD)	30±20	34±21	0.27
Isolates with suboptimal colistin /tigecycline MICs [a]	4 (8.6)	22 (27.9)	0.01

Data are expressed as number (%) unless otherwise stated.
Abbreviations: BSI, bloodstream infection; HCA, health-care associated (as defined in Materials and Methods);
[a] MICs at the upper limit of susceptibility for tigecycline (n=18) or colistin (n=8) .

Table 3. Multivariate analysis of risk factors for mortality in patients with bloodstream infection caused by KPC-producing *K. pneumoniae*.

Variables	*P* value	OR (95% CI)
Presentation with septic shock	0.008	7.17 (1.65-31.03)
Inadequate initial antimicrobial treatment	0.003	4.17 (1.61-10.76)
High APACHE III score	<0.001	1.04 (1.02-1.07)
Post-antibiogram therapy with tigecycline + colistin + meropenem	0.01	0.11 (0.02-0.69)

Table 4. Outcomes of the 36 bloodstream infections treated with combination therapy including meropenem stratified by meropenem MIC.

Meropenem MIC (mg/L)	No. (%)		
	Total	Nonsurvivors	Survivors
1	1	0	1 (100)
2	4	0	4 (100)
4	10	2 (20)	8 (80)
8	4	1 (25)	3 (75)
≥ 16	17	6 (35.2)	11 (64.7)
Total	36	9 (25)	27 (75)