Journal of Microbiology, Immunology and Infection (2016) 49, 924–933

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Comparison of the clinical efficacy between tigecycline plus extended-infusion imipenem and sulbactam plus imipenem against ventilator-associated pneumonia with pneumonic extensively drug-resistant *Acinetobacter baumannii* bacteremia, and correlation of clinical efficacy with *in vitro* synergy tests

Shio-Shin Jean [a,*], Tai-Chin Hsieh [b], Chin-Wan Hsu [a], Wen-Sen Lee [b], Kuan-Jen Bai [a], Carlos Lam [a]

[a] *Emergency Medicine, Department of Emergency and Critical Care Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[b] *Division of Infectious Diseases, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*

Received 23 May 2015; accepted 12 June 2015
Available online 14 August 2015

KEYWORDS
combination regimen;
extensively drug-resistant *Acinetobacter baumannii*;
imipenem;
sulbactam;

Abstract *Background/Purpose:* To compare the clinical efficacy between salvage antimicrobial regimen consisting of tigecycline plus extended-infusion imipenem/cilastatin (TIC) and regimen of sulbactam plus imipenem/cilastatin (SIC) for patients with ventilator-associated pneumonia and pneumonic bacteremia due to extensively drug-resistant (XDR) *Acinetobacter baumannii* (Ab) isolates, and determine the correlation of results of *in vitro* tigecycline–imipenem synergy test with clinical efficacy.
Methods: The comparative survey was conducted at a medical center in Taiwan in 2013. Patients comprising the TIC group (n = 28) received tigecycline plus extended-infusion

* Corresponding author. Emergency Medicine, Department of Emergency and Critical Care Medicine, Wan Fang Hospital, Taipei Medical University, No. 111, Sec. 3, Xing-Long Rd, Wen-Shen District, Taipei 11696, Taiwan.
E-mail address: jeanshioshin168@gmail.com (S.-S. Jean).

http://dx.doi.org/10.1016/j.jmii.2015.06.009

tigecycline;
ventilator-associated pneumonia

imipenem/cilastatin following unresponsiveness to 3-day sulbactam—imipenem/cilastatin therapy, and those in the SIC group ($n = 56$) received sulbactam—imipenem/cilastatin throughout the course. Univariate and multivariate analyses were applied to explore 30-day case-fatality independent predictors. Additionally, the checkerboard test and time-kill analysis were performed for the bloodstream XDR-Ab isolates from patients in the TIC group, and molecular characterization was done for the bloodstream XDR-Ab strains of all patients.
Results: We found that the TIC scheme has a significant benefit on improving patients' survival status (the mortality rate of TIC and SIC group patients was 14.3% and 64.3%, respectively), corresponding well with *in vitro* synergy or additivity results by the checkerboard test. Twenty TIC group cases had monomicrobial XDR-Ab cultured from tracheal aspirates after 10 days of tigecycline—imipenem/cilastatin therapy, but none developed subsequent pneumonia. However, breakthrough primary *Burkholderia cepacia* ($n = 3$) and *Pseudomonas aeruginosa* ($n = 1$) bacteremias were attributed to four TIC case fatalities. Shock, SIC regimen usage, and development of breakthrough bacteremia were independent predictors of 30-day in-hospital mortality.
Conclusion: Although the TIC regimen showed good efficacy, its value regarding managing XDR-Ab ventilator-associated pneumonia bacteremia needs further evaluation.

Introduction

Pneumonia acquired at the nosocomial setting is associated with higher mortality rates than that of community origin.[1] Of the etiologies implicated in nosocomial pneumonia (NP), *Acinetobacter baumannii* (Ab) drew the most worldwide concern in that its high potential of dissemination, and bacteremia due to multidrug-resistant (MDR)-Ab have been shown in association with a significantly high 30-day mortality rate (range 29—43%).[2,3] Previously, a number of reports revealed that high nonsusceptible rates of nosocomial Ab isolates against carbapenem agents were found inTaiwan.[4,5] During the past decade, Ko et al[6] demonstrated that meropenem at 0.5-fold of the minimum inhibitory concentration (MIC) plus sulbactam (1.0 × MIC) would exhibit excellent bactericidal suppression on MDR-Ab for up to 48 hours. This result provided a robust basis for the scheme of antipseudomonal carbapenem plus sulbactam on managing the MDR-Ab infections clinically. However, the epidemic of MDR-Ab isolates conferring medium to high resistance to this regimen had emerged in Taiwanese intensive care units since 2000.[7] In addition, a considerable variability in pulmonary penetration ratios of meropenem [i.e., concentration of epithelial lining fluid (ELF) to that of plasma, ranging from 0.037 to 1.78],[8] and the absolute requirement of relatively high targets of meropenem exposure in ELF for the patients with ventilator-associated pneumonia (VAP), who often have significantly high resistant burdens of Gram-negative bacteria (GNB; especially when their meropenem MICs are ≥1.0 μg/mL) in pulmonary tissue have been demonstrated recently.[9] Owing to the worsening resistance trend, combination therapies therefore have become an important option against the extensively drug-resistant (XDR)-GNB pathogens nowadays. Nevertheless, combination therapy was often prescribed empirically without the support of *in vitro* synergy data, as evidenced by the fact that the PubMed literature documenting corroboration of results of *in vitro* synergism of antibiotics on good clinical outcome remains scarce.[10,11]

The lack of correlation between different *in vitro* methods in assessing the synergism of antimicrobial combinations is an arguable concern. Regarding the *in vitro* methodologies evaluating drug—drug interaction, checkerboard titration analysis, which calculates the index by comparing the MIC of combination regimen with each single drug, and time-kill kinetic analysis, are two most widely used modalities.[12,13] Despite the fact that time-kill analysis was considered to have an advantage of evaluating the dynamic interaction of two or more agents, the checkerboard MIC test is still the most commonly used assay of screening synergism.[12] As the data of correlation between *in vitro* interaction of dual antibiotics and real clinical outcome are generally lacking, we conducted this comparative study to assess the difference in clinical efficacy between tigecycline plus extended-infusion imipenem/cilastatin (TIC) and sulbactam plus imipenem/cilastatin (SIC) against VAP bacteremia due to nosocomially acquired XDR-Ab isolates in 2013. In addition, we also investigated the correlation between the results of the *in vitro* synergy test and the clinical outcome of patients in the TIC group in this survey.

Methods

Patient recruitment and evaluation

This study was prospectively conducted at Wan Fang Hospital, a 732-bed medical center located in northern Taiwan. Adult (≥ 18 years) patients with VAP who met the definition of the United States Centers for Disease Control and Prevention (new infiltrate, or consolidation on radiographic evidence, consistent signs and symptoms, and associated laboratory data),[14] with cultures of tracheal aspirate and

blood samples ($\geq$ 2 sets, respectively) showing the Ab complex of an identical resistance profile, were eligible as candidates for investigation. VAP was diagnosed as NP requiring the support of mechanical ventilation based on the Centers for Disease Control and Prevention guidelines. If isolates of the Ab complex from blood culture were verified to be Ab species by the molecular method (see following Discussion), the participants whose first VAP XDR-Ab VAP bacteremia failed to respond to 3-day imipenem (500 mg every 6 hours) plus sulbactam (1.5—2.0 g every 6 hours) were considered potential candidates for this study. After written consent was obtained from all participants, these cases (designated as the TIC group) were subsequently treated with standard-dose tigecycline with a 1-hour infusion duration, plus imipenem/cilastatin with an extended-duration (3 hours) infusion (daily dosage was adjusted according to the patient's creatinine clearance rate) for a minimum of 10 days if feasible; the therapy duration was based on the recommendation of the guidelines described previously.[1] The written informed consent contained special notes stating that tigecycline was an off-label agent for treating VAP by the suggestion of the United States Food and Drug Administration[15] and was therefore prescribed as the salvage therapy in our study. All TIC patients were enrolled from February 1, 2013 through December 31, 2013. If the fungus was judged as the causative etiology of urinary tract or other (catheter, bloodstream, etc.) infection entity, addition of appropriate antifungal agent(s) was justified. Additionally, if a new febrile episode, hemodynamic instability, or altered mental status without plausible explanations developed during the antibiotic therapy course, a new blood culture survey would be performed if needed. The exclusion criteria were as follows: (1) the bacteremic episode was not monomicrobial, or had mixed genospecies or unidentified species; (2) hypersensitivity to either antibiotic agent evaluated; (3) presence of severe liver cirrhosis ($>$ 9 points, assessed using the Child—Pugh score system), or severe hepatic failure of any other etiology; (4) other than XDR-Ab, the etiological microorganisms in the lower respiratory tract were not *in vitro* susceptible to tigecycline plus imipenem/cilastatin. The clinical characteristics—including age, sex, underlying comorbidities and Charlson index score,[16] total leukocyte count, Acute Physiology and Chronic Health Evaluation II score,[17] Pitt bacteremia score within 24 hours of the XDR-Ab bacteremia,[18] clinical pulmonary infection score,[19] and the ratio of the patient's arterial partial pressure of oxygen to the fraction of inspiratory oxygen from the ventilator (PaO_2/FiO_2) at the day of XDR-Ab bacteremia, as well as the Day 30 outcome (the day when XDR-Ab bacteremia happened was counted as Day 1)—of the enrolled patients were recorded in detail. Moreover, prior stay at any healthcare setting (hospitalization $\geq$ 3 days, or nursing home resident) prior to this admission, as well as the duration between admission day and the date of acquiring XDR-Ab VAP bacteremia were also recorded for each patient. The follow-up microbiological assessment of suctioned sputum [or bronchoalveolar lavage (BAL) fluid] from patients in the TIC group was performed on the day when treatment of extended-infusion imipenem/cilastatin plus tigecycline has been instituted for 10 days.

Clinical cure was defined as the disappearance of symptoms and signs of pneumonia, with resolved pneumonic lesion on radiography regardless of the Ab culture status from tracheal aspirates. Conversely, therapeutic failure for XDR-Ab pneumonia was defined as unimproved or worsening clinical and radiological conditions with persistence of strong positivity of the sputum Ab after therapy. Microbiological eradication of the tracheal XDR-Ab was defined as the disappearance of Ab in follow-up cultures from bronchial aspirate (or BAL fluid) after a given treatment course. Immunosuppression was defined as the patient who has actively metastatic cancer(s) or who received one dose or more of chemotherapy or other immunosuppressant(s) within the past 1 month, or $\geq$ 14 days of corticosteroid at an equivalent daily dose of $\geq$ 15 mg prednisolone. In addition, acute renal failure was defined as $\geq$ 50% increase in baseline serum creatinine concentration, and/or requirement of renal replacement therapy.[20] The condition of acute respiratory distress syndrome was as defined elsewhere.[21]

As compared to each TIC case, we retrospectively looked for VAP cases (1:2) accompanied with pneumonic (VAP) XDR-Ab bacteremia who have matched scoring points (i.e., difference of a total score $\leq$ 3 points; the matching protocol and scoring variables were modified from those previously described by Pittet et al[22]) and received sulbactam—imipenem/cilastatin therapy throughout the course during 2013. These patients comprised the SIC group. In our survey, we established the matching variables between the TIC and SIC groups as follows: age (0 point if the age difference was $<$ 5 years, 1 point if the age difference was 5—10 years, and 2 points if the age difference was $>$10 years), sex (0 point if the same, otherwise was 1 point), Charlson index score (0 point if the score difference was $\leq$ 2, 1 point if the difference ranged from 3 to 5, and 2 points if the score difference was $>$ 5), length of hospital stay prior to the acquisition of XDR-Ab bacteremia (1 point if the difference in duration was $>$ 20% of that for the potentially matched patient, otherwise was 0 point), as well as Pitt bacteremia score (0 point if the score difference was $\leq$ 2, 1 point if the score difference ranged from 3 to 4, and 2 points if the score difference was $\geq$ 5).

The end point of this study was the comparison of the 30-day outcome between both patient groups. The independent predictors of Day 30 in-hospital case fatality were explored using statistics (see following). In addition, the adverse events relevant to tigecycline—imipenem/cilastatin therapy for the TIC group were also recorded. This clinical study was approved by the Institutional Review Board of Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan.

Bacteriological identification and antimicrobial susceptibility testing

The Ab complex isolates (from blood, tracheal aspirate, and BAL fluid) were presumptively identified by colony morphology, Gram staining, growth at 37°C, negative oxidase, and oxidation of glucose. The Phoenix PMIC/ID-30 bacterial identification system (Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) was then used

to confirm the identity of these isolates. Genospecies of *Acinetobacter* spp. collected from the blood specimens of both groups of patients were validated in accordance with the intergenic spacer region of 16S–23S ribosomal RNA gene, as previously described.[23]

The susceptibility to and MICs of imipenem as well as ampicillin/sulbactam were determined for the bloodstream XDR-Ab isolates collected from both groups of patients using the agar dilution method in accordance with the MIC breakpoints recommended by the Clinical and Laboratory Standards Institute in 2013.[24] Following inoculation with 10^4 colony-forming units (CFU) of Ab onto Mueller–Hinton agar, which contains a series of 2-fold dilution of imipenem, the agar plates were then incubated at 35°C in 5% CO_2 for 18–20 hours. The MICs of tigecycline were determined with the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute guidelines.[24] The concentrations of the antimicrobials under evaluation against the bloodstream XDR-Ab isolates ranged from 0.06 μg/mL to 128 μg/mL. To avoid degradation, solutions of tigecycline were freshly prepared on the day of the experiments. Susceptibility to tigecycline (with MIC $\leq$ 2 μg/mL being classified within the susceptible category) was interpreted as per the United States Food and Drug Administration recommendation for *Enterobacteriaceae* spp.[25] The antimicrobial nonsusceptibility pattern of Ab from tracheal aspirate or BAL fluid should be grossly in agreement with that of the bloodstream Ab isolate for each TIC and SIC participant. *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922 were applied as the internal control for each test run.

Analysis of carbapenem resistance genes

Extraction and purification of DNA from the bloodstream XDR-Ab isolates of patients in the TIC and SIC groups were carried out as previously described.[23] The primers used in this study are listed elsewhere.[26] Metallo-β-lactamase genes, bla_{IMP} and bla_{VIM}, as well as carbapenem hydrolyzing Class D β-lactamase genes, including $bla_{OXA\text{-}51}$-like, $bla_{OXA\text{-}58}$-like, $bla_{OXA\text{-}23}$-like, and $bla_{OXA\text{-}24}$-like genes, were detected using the polymerase chain reaction (PCR) method.[27]

Checkerboard titration analysis

To understand the *in vitro* synergism of the tigecycline–imipenem scheme against the XDR-Ab isolates collected from patients in the TIC group, the checkerboard titration method was applied to assess the activity of this antibioticcombination.[12] Test tubes containing imipenem or tigecycline in Mueller–Hinton broth were prepared in a checkerboard configuration in 96-well plates, as described by Sheng et al.[28] The fractional inhibitory concentration (FIC) index for tigecycline–imipenem combination was calculated by dividing the concentration of that drug necessary to inhibit growth in a given row by the MIC of the agent alone. The FIC index (FICI) was obtained by summing the separate FIC of respective agents. Interpretation of the FICI was as follows: FICI $\leq$ 0.5, synergy; FICI $>$ 0.5 and $\leq$ 1, additivity; FICI $>$1 and $\leq$ 4, indifference; and FICI $>$ 4.0, antagonism.

Time-kill analysis

The XDR-Ab bacteremic isolates from TIC patients were also submitted to the time-kill analysis, as described previously.[17] Because the maximal serum concentration of imipenem for healthy individuals ranged from 30 μg/mL to 35 μg/mL,[29] which would be prominently compromised for critically ill patients,[30] imipenem was added with the fixed concentration of 16 μg/mL and tigecycline was added at the concentration of 0.5 × MIC (subinhibitory concentration). Control experiments lacking active antimicrobial drugs (i.e., the bacterial growth curve) were also conducted simultaneously in this time-kill analysis. Synergy of the imipenem–tigecycline combination was defined as a $>$ 2 $\log_{10}$ decrease in CFU/mL when compared with the more active constituent of these two drugs.

Pulsed-field gel electrophoresis

To delineate the genetic relatedness of the bloodstream Ab isolates collected from the TIC group cases, we picked up those pure overnight-cultured Ab colonies to perform the pulsed-field gel electrophoresis (PFGE) analysis. Briefly, after digestion with *Sma*I (New England BioLabs, Beverly, MA, USA), the DNA fragments were subjected to PFGE in 1% agarose gel (BioLab Laboratories, Hercules, CA, USA) in 0.5× Tris–borate–EDTA buffer (0.089 mmol/L boric acid, 2 mmol/L EDTA). The gels were stained with ethidium bromide and then were photographed with UV light. The interpretation of banding patterns of electrophoresis was carried out visually in accordance with the criteria proposed by Bannerman et al.[31]

Statistical analyses

The continuous variables were presented as the mean with standard deviation or median with ranges, and were compared using the Student *t* test or Mann–Whitney *U* test, depending on the validity of normality assumption. The categorical variables were expressed as percentages of the total number of patients analyzed, and were compared using the Chi-square test or Fisher's exact test as appropriate. To investigate the independent predictors regarding Day 30 mortality of VAP patients with XDR-Ab pneumonic bacteremia, variables with $p < 0.1$ between nonsurvivors and survivors in the univariate analysis were further analyzed using the multivariate logistic regression method. In addition, we also used Kaplan–Meier survival curves to explore the difference in cumulative survival rates between TIC and SIC patients (censoring dead participants at the time of death; otherwise, patients were considered surviving cases if they were still alive by Day 30 after the XDR-Ab bacteremia), and applied the log-rank test to compare the survival rates of patients between the two groups. All statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics and outcome of patients

In this study, we collected a total of 28 TIC and 56 SIC cases with VAP and concomitantly pneumonic XDR-Ab bacteremia. The demographic characteristics, comorbidities, various hospital exposure factors prior to XDR-Ab VAP bacteremia, as well as the percentages of the presence of bla_{OXA-23}-like genes in the bloodstream XDR-Ab strains between the two patient groups were comparable (Table 1). All of these patients have received broad-spectrum antibiotic(s) prior to acquiring the XDR-Ab VAP bacteremia. Among the 28 TIC group cases, two also had urinary tract infection due to *Candida albicans*, and the other two cases had concomitant catheter-associated fungemia due to *Candida parapsilosis*. These four patients were coprescribed with appropriate antifungal antibiotics, and the central venous catheters of the latter two cases were rapidly removed. Later, their fungal infections resolved without sequelae. In addition, four patients developed vomiting, and the other two patients have skin eruptions during the tigecycline—imipenem/cilastatin therapy course. However, these minor adverse events could be relieved by medication.

Four TIC patients died from breakthrough primary bacteremia (3 had *Burkholderia cepacia* bacteremia and 1 had the imipenem-resistant *P. aeruginosa* bacteremia) prior to Day 30 of the XDR-Ab VAP bacteremia (see the following Discussion). By contrast, in the SIC group, there were 36 case fatalities, of whom 24 patients and eight patients died because of breakthrough XDR-Ab and methicillin-resistant *Staphylococcus aureus* bacteremia, respectively. Compared to the SIC group cases, the outcome of TIC cases appeared to be statistically much better [surviving case number, 24 (85.7%) vs. 20 (35.7%); $p = 0.007$]. The

Table 1 Comparisons of baseline demographic characteristics, comorbidities, hospital exposure factors for patients with ventilator-associated pneumonia with associated pneumonic XDR *Acinetobacter baumannii* bacteremia, and the results of polymerase chain reaction used in detecting the carbapenemase-encoding genes for the bloodstream XDR-*A. baumannii* strains.[a]

Characteristics	TIC group ($n = 28$)	SIC group ($n = 56$)	OR	95% CI	p
Age (y)	75 (45—88)	77 (40—86)			0.78
Male sex	16 (57.1)	28 (50)	1.33	0.53—3.32	0.54
Underlying disorders					
Malignancy	6 (21.4)	16 (28.6)	0.68	0.23—1.99	0.48
Cardiovascular diseases	2 (7.1)	8 (14.3)	0.46	0.09—2.34	0.35
Diabetes mellitus	16 (57.1)	28 (50.0)	1.33	0.53—3.32	0.54
Hepatic diseases	2 (7.1)	12 (21.4)	0.28	0.06—1.30	0.12
Renal diseases	6 (21.4)	20 (35.7)	0.49	0.17—1.41	0.19
Pulmonary diseases	6 (21.4)	16 (28.6)	0.68	0.23—1.99	0.48
Neurologic diseases	6 (21.4)	24 (42.9)	0.36	0.13—1.04	0.08
Charlson comorbidity index	2 (1—8)	2 (1—8)			0.89
WBC count ($\times 10^3/\mu L$)	16 (1.55—29.8)	15.4 (2.42—26.7)			0.79
Shock	16 (57.1)	28 (50.0)	1.33	0.53—3.32	0.54
APACHE II score	30 (19—45)	29 (20—45)			0.94
Pitt bacteremia score	7 (4—14)	8 (4—14)			0.85
CPIS	7 (4—10)	7 (4—9)			0.73
PaO_2/FiO_2	189 (105—279)	183 (101—275)			0.69
Hospital exposure factors, prior to the XDR-Ab VAP bacteremia					
Immunosuppression	12 (42.9)	28 (50.0)	0.75	0.30—1.87	0.54
Prior stay at healthcare setting(s)	26 (92.9)	50 (89.3)	1.56	0.29—8.28	0.60
Length of hospital stay (d)	12 (6—17)	11 (6—16)			0.77
Length of ventilator use (d)	9 (4—13)	8 (4—12)			0.85
Usage of the central venous lines (No.-day)	7 (2—16)	8 (3—13)			0.93
Use of the urinary catheter	28 (100)	56 (100)	1.00		1.00
Recent surgery	14 (50.0)	36 (64.3)	0.56	0.22—1.40	0.21
PCR for investigating the genes encoding for carbapenemase in bloodstream XDR-Ab strains					
Presence of the bla_{OXA-23}-like genes	4 (14.3)	12 (21.4)	0.61	0.18—2.10	0.44

[a] All bloodstream XDR-*A. baumannii* isolates from patients belonging to the TIC and SIC groups have bla_{OXA-51}-like genetic traits detected by PCR method, whereas the bla_{IMP} and bla_{VIM} genes were not detected on any of the XDR-*A. baumannii* strains analyzed.
Data are presented as *n* (%) or median (range), unless otherwise indicated.
Ab = *Acinetobacter baumannii*; APACHE II = Acute Physiology and Chronic Health Evaluation II score; CI = confidence interval; CPIS = clinical pulmonary infection score; PaO_2/FiO_2 = ratio of patient's arterial partial O_2 pressure to the fraction of inspiratory O_2 from the ventilator; OR = odds ratio; PCR = polymerase chain reaction; SIC = sulbactam plus imipenem/cilastatin; TIC = tigecycline plus extended-infusion imipenem/cilastatin; VAP = ventilator-associated pneumonia; WBC = white blood cell; XDR = extensively drug-resistant.

Kaplan—Meier curves with regard to the cumulative survival rates for two patient groups are presented in Figure 1. A significantly higher cumulative survival rate was observed for the TIC case group than for the SIC group with a statistically significant difference ($p < 0.001$, by log-rank test).

MIC data against XDR-Ab isolates, and follow-up microbiological results

For both patient groups, all XDR-Ab isolates collected from the tracheal aspirate and blood culture samples showed resistance to all antibiotics routinely investigated. Moreover, 18 (64.3%) bloodstream XDR-Ab isolates from the TIC group have a tigecycline MIC of 2 μg/mL, with the remaining 10 isolates falling within the nonsusceptible category. By contrast, the imipenem MIC values of these bacteremic XDR-Ab strains ranged from 16 μg/mL to 128 μg/mL. The MIC_{50}/MIC_{90} values of these bloodstream XDR-Ab isolates against imipenem and tigecycline were 32/64 μg/mL and 2/8 μg/mL, respectively. Of note, the differences in MIC levels to imipenem, tigecycline, and ampicillin/sulbactam for the bacteremic XDR-Ab isolates between the two groups were not significant when the Mann—Whitney *U* analysis was used.

With respect to the follow-up sputum microbiological survey, we observed that 20 (71.4%) patients of the TIC group still had XDR-Ab isolates cultured from tracheal aspirate samples after the 10-day tigecycline—imipenem/cilastatin therapy. Nevertheless, among these 20 TIC cases with XDR-Ab persisting in sputum after treatment, only four patients died—17 days (Patient 12), 19 days (Patient 15), 20 days (Patient 7), and 22 days (Patient 13), respectively, after the therapy consisting of tigecycline plus extended-infusion imipenem/cilastatin was initiated. It is noteworthy that the blood cultures grew monomicrobial *B. cepacia* (from Patients 7, 12, and 15) or *P. aeruginosa* (from Patient 13) but not from their subsequent sputum samples prior to the fatality of these four TIC cases. Finally, in the analysis of in-hospital fatality for VAP patients by multivariate logistic regression, we found that development of breakthrough bacteremic episodes [odds ratio (OR), $> 10^3$; 95% confidence interval (CI), 4.97—$>10^5$; $p = 0.026$], use of sulbactam—imipenem/cilastatin throughout the course (OR 8.56; 95% CI, 2.81—29.75; $p = 0.002$), as well as shock (OR, 2.67; 95% CI, 1.34—7.98; $p = 0.02$) were the independent predictors for Day 30 mortality (Table 2).

Data of the *in vitro* synergy tests and PCR study

In accordance with the checkerboard titration data against the 28 bloodstream XDR-Ab isolates collected from the TIC cases, with the exception of four isolates (from Patients 7, 12, 13, and 15, all of whom died prior to Day 30) showing indifference results, the remaining isolates exhibited results of *in vitro* synergism ($n = 12$) or additivity ($n = 12$) for the tigecycline—imipenem combination. The 12 XDR-Ab isolates showed synergism at concentrations ranging from 0.0625 × MIC to 0.125 × MIC of imipenem plus from 0.0625 × MIC to 0.125 × MIC of tigecycline. Additionally, the other 12 XDR-Ab isolates exhibited additivity at concentrations that ranged from 0.125 × MIC to 0.5 × MIC of

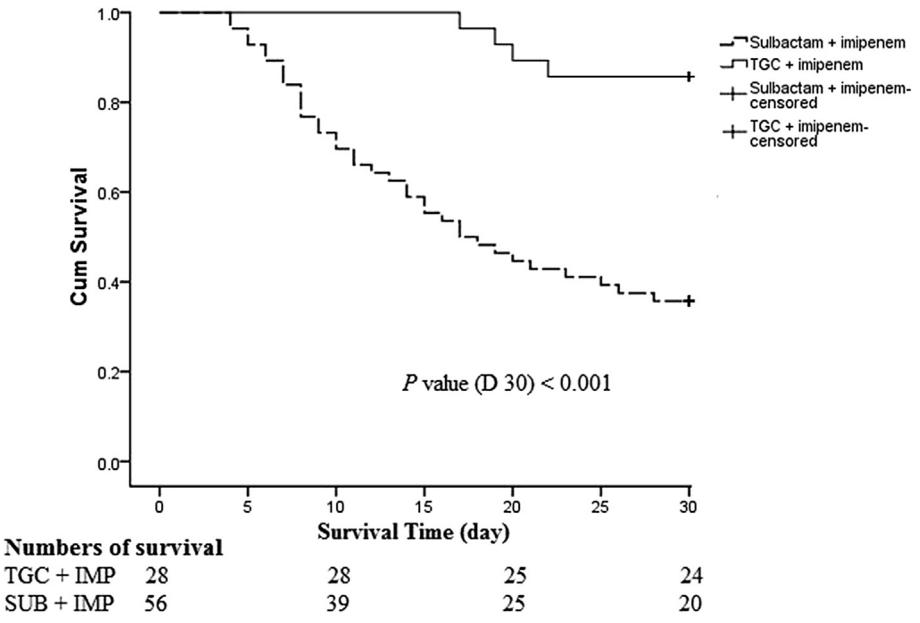

Figure 1. Kaplan—Meier survival curves regarding comparison of the cumulative survival rates between two patient groups having ventilator-associated pneumonia with pneumonic XDR-*Acinetobacter baumannii* bacteremia treated by either the salvage regimen of either tigecycline plus extended-infusion imipenem/cilastatin following failure of responding to 3-day sulbactam—imipenem/cilastatin therapy (solid line), or treated with the regimen consisting of sulbactam plus imipenem/cilastatin throughout the course (broken line). XDR = extensively drug-resistant.

Table 2 Comparisons of various characteristics, including (1) age, sex, the underlying disorders as well as Charlson comorbidity index of enrolled patients; (2) physiologic condition or index at the day when the extensively drug-resistant (XDR) *Acinetobacter baumannii* bacteremia due to ventilator-associated pneumonia (VAP) occurred; (3) hospital exposure factors before the enrolled patient(s) acquired XDR-*A. baumannii* VAP bacteremia; and (4) other major events that happened after XDR-*A. baumannii* VAP bacteremia, between nonsurvivors and survivors (Day 30).[a]

Characteristics	Nonsurvivors ($n = 40$)	Survivors ($n = 44$)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	p*	OR (95% CI)	p*
Age (y)	60 (40–83)	64 (48–88)		0.69		
Male sex	26 (65)	18 (40.9)	2.68 (1.11–6.49)	0.03	1.84 (0.94–4.52)	0.11
Underlying disorders						
Malignancy	12 (30)	10 (22.7)	1.46 (0.55–3.87)	0.45		
Cardiovascular diseases	8 (20)	2 (4.5)	6.75 (1.35–33.77)	0.02	4.78 (0.93–28.45)	0.09
Diabetes mellitus	20 (50)	24 (54.5)	0.83 (0.35–1.97)	0.68		
Hepatic diseases	8 (20)	6 (13.6)	1.58 (0.50–5.04)	0.44		
Renal diseases	16 (40)	10 (22.7)	2.27 (0.88–5.85)	0.09	1.63 (0.69–3.97)	0.16
Pulmonary diseases	14 (35)	8 (18.2)	2.42 (0.89–6.62)	0.08	1.69 (0.76–5.14)	0.14
Neurologic diseases	20 (50)	10 (22.7)	3.40 (1.33–8.69)	0.01	1.98 (0.96–6.84)	0.07
Charlson comorbidity index	3 (1–6)	4 (2–8)		0.58		
Physiologic condition/index, at the day when patient(s) acquired the XDR-Ab VAP bacteremia						
WBC count ($\times 10^3/\mu L$)	14.5 (1.55–24.3)	15.7 (2.42–29.8)		0.79		
Shock	28 (70)	16 (36.4)	4.08 (1.64–10.18)	0.003	2.67 (1.34–7.98)	0.02
APACHE II score	29 (19–45)	31 (22–41)		0.74		
Pitt bacteremia score	10 (5–14)	8 (4–10)		0.31		
CPIS	7 (5–10)	6 (4–9)		0.49		
PaO_2/FiO_2	196 (112–274)	188 (101–279)		0.65		
Hospital exposure factors, before patient(s) acquired XDR-Ab VAP bacteremia						
Length of hospital stay (d)	10 (7–14)	11 (6–17)		0.55		
Length of ventilator use (d)	9 (6–13)	7 (4–11)		0.25		
Immunosuppression	26 (65)	14 (31.8)	3.68 (1.34–9.87)	0.04	2.57 (0.95–7.28)	0.07
Usage of central venous lines, No.-day	11 (5–15)	10 (2–16)		0.39		
Surgery	28 (70)	22 (50)	2.33 (0.89–5.72)	0.07	1.74 (0.77–4.28)	0.14
Other major events, after patient(s) acquired XDR-Ab VAP bacteremia						
Acute renal failure	22 (55.0)	20 (45.4)	1.47 (0.62–3.47)	0.38		
Acute respiratory distress syndrome	10 (25)	14 (31.8)	0.71 (0.27–1.86)	0.49		
New cardiovascular insults	4 (10)	8 (18.1)	0.50 (0.14–1.81)	0.29		
Breakthrough bacteremic episode	36 (90)	0 (0)	3950 (7.33–>10^6)	0.01	2655 (4.97–>10^5)	0.026
Treatment by sulbactam–imipenem/cilastatin throughout the course of XDR-Ab VAP bacteremia	36 (90)	20 (45.4)	10.8 (3.28–35.55)	<0.001	8.56 (2.81–29.75)	0.002

[a] PaO_2/FiO_2 denotes the ratio of patient's arterial oxygen pressure to the fraction of inspiratory oxygen from ventilator. Acute renal failure was defined as previously described. Acute respiratory distress syndrome was defined as elsewhere. New cardiovascular insults included new-onset cerebrovascular accident, acute myocardial infarction, congestive heart failure, and dissecting aortic aneurysm. Data are presented as n (%) or median (range), unless otherwise indicated.

*The factor with $p < 0.1$ by the univariate analysis would enter into the multivariate statistical analysis of logistic regression.

Ab = *Acinetobacter baumannii*; APACHE II = Acute Physiology and Chronic Health Evaluation II; CI = confidence interval; CPIS = clinical pulmonary infection score; OR = odds ratio; WBC = white blood cell.

imipenem plus from 0.125 × MIC to 0.5 × MIC of tigecycline. The time-kill kinetic analysis regarding all bloodstream XDR-Ab isolates (from patients in the TIC group) against tigecycline–imipenem revealed that this combination scheme also displayed good synergistic effect on all but four XDR-Ab isolates collected from Patients 7, 12, 13, and 15 (Figure 2). The MIC values against imipenem/tigecycline for the XDR-Ab isolates obtained from Patients 7 and 13 as well as Patients 12 and 15 were 128/8 μg/mL and 64/8 μg/mL, respectively. The molecular characterization revealed that all bloodstream XDR-Ab isolates have $bla_{OXA\text{-}51}$-like genes, whereas 16 isolates (four from Patients 7, 12, 13, and 15 of the TIC group, and the remaining 12 isolates from the SIC group) coharbored $bla_{OXA\text{-}23}$-like genes. No bla_{IMP} or bla_{VIM} genes were detected in any bloodstream XDR-Ab strain by PCR assay in both groups.

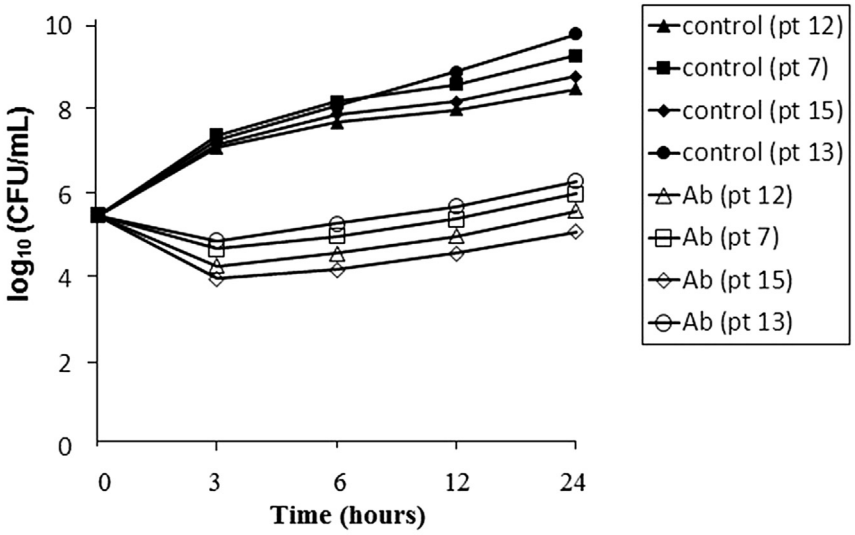

Figure 2. The time-kill curves of tigecycline (at the concentration of 0.5 × MIC) plus imipenem (at a fixed concentration of 16 μg/mL) against bloodstream XDR-*Acinetobacter baumannii* isolates that were collected from Patients (Pt) 7, 12, 13, and 15 of the tigecycline—imipenem/cilastatin treatment group. The curves of control Pt 7, 12, 13, 15 isolates were those not treated with any antimicrobial agent. MIC = minimum inhibitory concentration; XDR = extensively drug-resistant.

PFGE data

The analysis of PFGE regarding 28 bacteremic XDR-Ab strains from the TIC group showed a high degree of genetic diversity except those collected from Patients 17—20 and Patients 22—25 (figure not shown). These two XDR-Ab pairs have concordant MIC levels to imipenem and tigecycline, respectively.

Discussion

In this survey, on the basis of the attending physician's judgment, we set 3 days as the cutoff duration to decide the change in the therapeutic antibiotic regimen for patients with XDR-Ab VAP bacteremia if their family agreed with our suggestion. In this study, we demonstrated that the salvage regimen, consisting of tigecycline plus prolonged (3 hours) infusion standard-dose imipenem/cilastatin, showed good clinical efficacy on VAP patients with XDR-Ab VAP bacteremia refractory to imipenem/cilastatin plus sulbactam. In addition, the molecular characterization revealed that four bloodstream XDR-Ab isolates (from patients in the TIC group) coharboring the bla_{OXA-23}-like genes have significantly high imipenem MIC levels, consistent with a prior investigation.[32] Although a recent prospective study pertaining to hospital-acquired infections due to MDR-Ab also revealed that patients treated with a tigecycline-containing antimicrobial regimen would have more favorable outcomes than those treated by other drug(s),[33] this study targeted Ab isolates *in vitro* susceptible to tigecycline.

From the pharmacokinetic (PK) study, remarkably low tigecycline concentrations (0.01—0.02 μg/mL) in ELF were found in three VAP patients using the BAL analysis.[34] In addition, VAP patients were also noted to have prominently poorer pharmacodynamic (PD) data [lower area under-the-concentration (AUC) at a time of 0 hours to 24 hours ($fAUC_{0-24}$), and mean AUC] for tigecycline than those without VAP.[35] Furthermore, in terms of clinical and microbiological responses, the tigecycline MICs > 1.0 μg/mL for VAP pathogens were verified to exert a detrimental impact on the ratio of $fAUC_{0-24}$ to MIC, well correlating with poor survival rates in critically ill patients.[36] Although these disadvantageous data relevant to tigecycline's efficacy on VAP treatment existed, tigecycline monotherapy with 100 mg administered intravenously every 12 hours has recently been shown to achieve much more favorable outcomes for patients with NP than those of NP patients treated with imipenem at a dosage of 1 g every 8 hours.[37] Hence, underestimation of tigecycline's efficacy on VAP might exist when prior PK/PD data were applied.[34,35,38] Despite the fact that tigecycline MIC levels against all of our VAP-Ab isolates were ≥ 2 μg/mL, the tigecycline—imipenem/cilastatin scheme apparently exerts good clinical efficacy for these VAP patients. This finding agrees with a previous *in vitro* study that addressed the fact that significant synergism would emerge on XDR-Ab strains when tigecycline was combined with imipenem or amikacin.[39]

In our study, the high (71.4%) rate of XDR-Ab persistence within the lower respiratory tract after a 9-day therapy of tigecycline plus extended-infusion imipenem/cilastatin posed a stark contrast to the low (14.3%) rate of Day 30 case-fatality for VAP patients with pneumonic XDR-Ab bacteremia (with tigecycline MICs ranging from 2 μg/mL to 8 μg/mL; $p = 0.002$). This finding differs from that of a previousinvestigation.[40] Among the three VAP patients also

with XDR-Ab bacteremia (of which tigecycline's MICs ranged from 1 μg/mL to 8 μg/mL) in that study, two (67%) patients with microbial eradication in sputum had favorable outcomes (cure) after undergoing tigecycline plus imipenem/cilastatin therapy.[40] The other notable finding is that *B. cepacia* emerged as the main organism of breakthrough bacteremic episodes after tigecycline—imipenem/cilastatin salvage therapy against VAP due to XDR-Ab isolates, in marked contrast to that described for patients receiving tigecycline treatment.[41] It is not surprising because *B. cepacia* are mostly *in vitro* nonsusceptible to tigecycline and imipenem/cilastatin.[5]

The checkerboard MIC analysis, a method of controversial reproducibility, was considered to underestimate the synergistic potential against nonfermentative GNB as compared with the time-kill study.[42] In our study, tigecycline plus imipenem exhibits *in vitro* synergism or additivity against most (24/28,85.7%) of the bloodstream XDR-Ab isolates by the checkerboard MIC test, which might indirectly denote that synergism of this combination regimen against these Ab isolates would be seen in the time-kill analysis.

There are several limitations in our study. First, although we demonstrated that the efficacy of tigecycline plus extended-infusion imipenem/cilastatin against the XDR-Ab VAP bacteremia is good clinically, our nonrandomized single-hospital series virtually only collected a small number of VAP cases. Hence, we might fail to explore other independent predictors about 30-day case fatality, and the success of this combination scheme might not be generalized into those of the worldwide VAP in relation to XDR-Ab. Second, we did not perform the clonality test to exclude the dissemination of XDR-Ab clone(s) between patients in the SIC group. Third, considerable variations in the AUC and $fAUC_{0-24}$/MIC ratio existed among patients with VAP, as described elsewhere.[8,35] Because the measurement of these PK/PD parameters was not included in the design of present study, we could not correlate the patient's outcome with these parameters. Fourth, we did not perform the time-kill analyses against ampicillin/sulbactam plus imipenem for the bloodstream XDR-Ab isolates collected from TIC cases, as performed by Hsueh et al.[7] However, sulbactam would not provide the synergistic effect with meropenem against GNB when the meropenem MIC level is $>$ 8 μg/mL.[43] All analyzed Ab isolates have imipenem MICs $>$ 8 μg/mL, which possibly predicts poor *in vitro* activity of the imipenem—sulbactam regimen in our XDR-Ab.

In conclusion, the present survey demonstrates that prolonged-infusion imipenem in conjunction with subinhibitory concentration of tigecycline could achieve good clinical efficacy in VAP patients with pneumonic XDR-Ab bacteremia. Additionally, the *in vitro* data of synergism or additivity obtained with the checkerboard titration analysis of tigecycline—imipenem against these XDR-Ab isolates are well correlated with 30-day in-hospital survival outcome. However, this combination scheme is not effective for four patients infected by XDR-Ab isolates with high imipenem and tigecycline MIC values. Large-scale investigations focusing on the outcomes of similar VAP patients treated by tigecycline plus extended-infusion imipenem/cilastatin are warranted in the future.

Conflicts of interest

All authors state no conflict of interest and have received no payment in preparation of this manuscript.

Acknowledgments

We thank the technical staff of the Laboratory Department of Wan Fang Hospital for assistance in performing the antimicrobial susceptibility tests and molecular investigations (detection of the genes encoding for carbapenemases by PCR assay, and the PFGE study).

References

1. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;**171**:388—416.
2. Chuang YC, Sheng WH, Li SY, Lin YC, Wang JT, Chen YC, et al. Influence of genospecies of *Acinetobacter baumannii* complex on clinical outcomes of patients with *Acinetobacter* bacteremia. *Clin Infect Dis* 2011;**52**:352—60.
3. Lee NY, Chang TC, Wu CJ, Chang CM, Lee HC, Chen PL, et al. Clinical manifestations, antimicrobial therapy, and prognostic factors of monomicrobial *Acinetobacter baumannii* complex bacteremia. *J Infect* 2010;**61**:219—27.
4. Ku WW, Kung CH, Lee CH, Tseng CP, Wu PF, Kuo SC, et al. Evolution of carbapenem resistance in *Acinetobacter baumannii*: an 18-year longitudinal study from a medical center in northern Taiwan. *J Microbiol Immunol Infect* 2015;**48**:57—64.
5. Jean SS, Hsueh PR, Lee WS, Yu KW, Liao CH, Chang FY, et al. Carbapenem susceptibilities and non-susceptibility concordance to different carbapenems amongst clinically important Gram-negative bacteria isolated from intensive care units in Taiwan: results from the Surveillance of Multicentre Antimicrobial Resistance in Taiwan (SMART) in 2009. *Int J Antimicrob Agents* 2013;**41**:457—62.
6. Ko WC, Lee HC, Chiang SR, Yan JJ, Wu JJ, Lu CL, et al. In vitro and in vivo activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. *J Antimicrob Chemother* 2004;**53**:393—5.
7. Hsueh PR, Teng LJ, Chen CY, Chen WH, Yu CJ, Ho SW, et al. Pandrug-resistant *Acinetobacter baumannii* causing nosocomial infections in a university hospital, Taiwan. *Emerg Infect Dis* 2002;**8**:827—32.
8. Lodise TP, Sorgel F, Melnick D, Mason B, Kinzig M, Drusano GL. Penetration of meropenem into epithelial lining fluid of patients with ventilator-associated pneumonia. *Antimicrob Agents Chemother* 2011;**55**:1606—10.
9. Drusano GL, Lodise TP, Melnick D, Liu W, Oliver A, Mena A, et al. Meropenem penetration into epithelial lining fluid in mice and humans and delineation of exposure targets. *Antimicrob Agents Chemother* 2011;**55**:3406—12.
10. Lee NY, Wang CL, Chuang YC, Yu WL, Lee HC, Chang CM, et al. Combination carbapenem—sulbactam therapy for critically ill patients with multidrug-resistant *Acinetobacter baumannii* bacteremia: four case reports and an in vitro combination synergy study. *Pharmacotherapy* 2007;**27**:1506—11.
11. Lee CM, Lim HK, Liu CP, Tseng HK. Treatment of pan-drug resistant *Acinetobacter baumannii*. *Scand J Infect Dis* 2005;**37**:195—9.
12. Petersen PJ, Labthavikul P, Jones CH, Bradford PA. In vitro antibacterial activities of tigecycline in combination with

other antimicrobial agents determined by chequerboard and time-kill kinetic analysis. *J Antimicrob Chemother* 2006;**57**:573—6.
13. White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. *Antimicrob Agents Chemother* 1996;**40**:1914—8.
14. Horan TC, Gaynes RP. Surveillance of nosocomial infections. In: Mayhall CG, editor. *Hospital epidemiology and infection control*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 1659—702.
15. FDA. *FDA drug safety communication: Increased risk of death of tygacil (tigecycline) compared to other antibiotics to treat similar infections*. 2010 [Accessed 15 March 2015], http://www.fda.gov/Drugs/DrugSafety/ucm224370.htm.
16. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;**40**:373—83.
17. Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE—acute physiology and chronic health evaluation: a physiology based classification system. *Crit Care Med* 1981;**9**:591—7.
18. Chow JW, Yu VL. Combination antibiotic therapy versus monotherapy for gram-negative bacteraemia: a commentary. *Int J Antimicrob Agents* 1999;**11**:7—12.
19. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1991;**143**:1121—9.
20. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute Dialysis Quality Initiative workgroup. Acute renal failure — definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004;**8**:R204—12.
21. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet* 1967;**2**:319—23.
22. Pittet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients. Excess length of stay, extra costs, and attributable mortality. *JAMA* 1994;**271**:1598—601.
23. Lin YC, Sheng WH, Chang SC, Wang JT, Chen YC, Wu RJ, et al. Application of a microsphere-based array for rapid identification of *Acinetobacter* spp. with distinct antimicrobial susceptibilities. *J Clin Microbiol* 2008;**46**:612—7.
24. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement*. M100-S23. Wayne, PA: CLSI; 2013.
25. Jones RN, Ferraro MJ, Reller LB, Schreckenberger PC, Swenson JM, Sader HS. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter* spp. *J Clin Microbiol* 2007;**45**:227—30.
26. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of *Pseudomonas aeruginosa* producing metallo-β-lactamase in a large centralized laboratory. *J Clin Microbiol* 2005;**43**:3129—35.
27. Chuang YC, Sheng WH, Lauderdale TL, Li SY, Wang JT, Chen YC, et al. Molecular epidemiology, antimicrobial susceptibility and carbapenemase resistance determinants among *Acinetobacter baumannii* clinical isolates in Taiwan. *J Microbiol Immunol Infect* 2014;**47**:324—32.
28. Sheng WH, Wang JT, Li SY, Lin YC, Cheng A, Chen YC, et al. Comparative in vitro antimicrobial susceptibilities and synergistic activities of antimicrobial combinations against carbapenem-resistant *Acinetobacter* species: *Acinetobacter baumannii* versus *Acinetobacter* genospecies 3 and 13TU. *Diagn Microbiol Infect Dis* 2011;**70**:380—6.
29. Zhanel GG, Wiebe R, Dilay L, Rubinstein E, Hoban DJ, Noreddin AM, et al. Comparative review of the carbapenems. *Drugs* 2007;**67**:1027—52.
30. Roberts JA, Lipman J. Pharmacokinetic issues for antibiotics in the critically ill patient. *Crit Care Med* 2009;**37**:840—51.
31. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulse-field gel electrophoresis as a replacement for bacteriophage typing *of Staphylococcus aureus*. *J Clin Microbiol* 1995;**33**:551—5.
32. Héritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005;**49**:3198—202.
33. Lee YT, Tsao SM, Hsueh PR. Clinical outcomes of tigecycline alone or in combination with other antimicrobial agents for the treatment of patients with healthcare-associated multidrug-resistant *Acinetobacter baumannii* infections. *Eur J Clin Microbiol Infect Dis* 2013;**32**:1211—20.
34. Burkhardt O, Rauch K, Kaever V, Hadem J, Kielstein JT, Welte T. Tigecycline possibly underdosed for the treatment of pneumonia: a pharmacokinetic viewpoint. *Int J Antimicrob Agents* 2009;**34**:101—2.
35. Freire AT, Melnyk V, Kim MJ, Datsenko O, Dzyublik O, Glumcher F, et al. Comparison of tigecycline with imipenem/cilastatin for the treatment of hospital-acquired pneumonia. *Diagn Microbiol Infect Dis* 2010;**68**:140—51.
36. Bhavnani SM, Rubino CM, Hammel JP, Forrest A, Dartois N, Cooper CA, et al. Pharmacological and patient-specific response determinants in patients with hospital-acquired pneumonia treated with tigecycline. *Antimicrob Agents Chemother* 2012;**56**:1065—72.
37. Ramirez J, Dartois N, Gandjini H, Yan JL, Korth-Bradley J, McGovern PC. Randomized phase 2 trial to evaluate the clinical efficacy of two high-dosage tigecycline regimens versus imipenem—cilastatin for treatment of hospital-acquired pneumonia. *Antimicrob Agents Chemother* 2013;**57**:1756—62.
38. Ambrose PG, Bhavnani SM, Ellis-Grosse EJ, Drusano GL. Pharmacokinetic—pharmacodynamic considerations in the design of hospital-acquired or ventilator-associated bacterial pneumonia studies: look before you leap! *Clin Infect Dis* 2010;**51**:S103—10.
39. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, et al. Synergy testing by E test, microdilution checkerboard, and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2010;**54**:4678—83.
40. Schafer JJ, Goff DA, Stevenson KB, Mangino JE. Early experience with tigecycline for ventilator-associated pneumonia and bacteremia caused by multidrug-resistant *Acinetobacter baumannii*. *Pharmacotherapy* 2007;**27**:980—7.
41. Chen LY, Chen TC, Chen YH, Lin CY, Lin WR, Lu PL. Microbial isolation and emergence of antimicrobial resistance associated with tigecycline usage. *J Microbiol Immunol Infect* 2011;**44**:352—7.
42. Cappelletty DM, Rybak MJ. Comparison of methodologies for synergism testing of drug combinations against resistant strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1996;**40**:677—83.
43. Jean SS, Hsueh PR. Current review of antimicrobial treatment of nosocomial pneumonia caused by multidrug-resistant pathogens. *Expert Opin Pharmacother* 2011;**12**:2145—8.