Original Article

Risk factors for mortality in ICU patients with *Acinetobacter baumannii* ventilator-associated pneumonia: impact of bacterial cytotoxicity

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Background: *Acinetobacter baumannii* (*A. baumannii*) ventilator-associated pneumonia (VAP) in intensive care unit (ICU) is associated with high morbidity and mortality in patients with critical illness. However, the literatures that focused on the short-term prognosis and the risk factors for mortality are limited. The aim of this study was to evaluate the risk factors for mortality in ICU patients with *A. baumannii* VAP.

Methods: A retrospective cohort study was conducted in the medical/surgical ICU at Zhongshan Hospital in Shanghai, China. Adult patients meeting the criteria of *A. baumannii* VAP from January 2012 to October 2015 were enrolled. Apart from collecting clinical and microbiologic data, we performed biofilm-formation and cytotoxicity testing using *A. baumannii* strains which are isolated from patients. Multivariate logistic regression analysis was used to determine the independent risk factors for 30-day mortality in ICU.

Results: Seventy-eight patients were included in this study. The 30-day mortality rate in ICU for the patients was 37.2%. Multivariate analysis revealed that short-term mortality was significantly associated with prior surgery [OR, 0.277; 95% confidence interval (CI), 0.089–0.866; P=0.027], higher APACHEII score (OR, 1.140; 95% CI, 1.007–1.291; P=0.038) and an increased bacterial cytotoxicity (OR, 1.029 ; 95% CI, 1.001–1.058; P=0.047).

Conclusions: The main finding of our study was that increased bacterial cytotoxicity might be a risk factor for short-term mortality in ICU patients with *A. baumannii* VAP.

Keywords: *Acinetobacter baumannii* (*A. baumannii*); cytotoxicity; intensive care unit; ventilator-associated pneumonia (VAP); prognosis

Submitted Sep 28, 2017. Accepted for publication Mar 13, 2018.
doi: 10.21037/jtd.2018.04.86
View this article at: http://dx.doi.org/10.21037/jtd.2018.04.86

Introduction

Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections in patients who receive mechanical ventilation. The occurrence of VAP prolongs stays in the intensive care unit (ICU) and hospital and is also associated with higher costs and a poorer prognosis (1-3).

Despite substantial clinical efforts, the incidence and mortality of VAP have remained high, with two recent epidemiologic studies in China reporting mortality rates of 18.7% and 26.7% (4,5).

Acinetobacter baumannii (A. baumannii) is showing an increasingly important role in nosocomial infection and it is thought to be the prevailing pathogen responsible for VAP in ICUs (6,7) with high mortality (8-10). Since *A. baumannii* is an opportunistic pathogen, its direct virulence to host cells is thought to be minimum to moderate, but high virulence *A. baumannii* has been isolated in a recent report (11). Whether the cytotoxicity of *A. baumannii* affects patient prognosis remains unclear. In the present study, we aimed to identify risk factors for 30-day mortality in ICU patients with *A. baumannii* VAP considering both bacterial (biofilm formation and cytotoxicity) and patient characteristics.

Methods

Study design

A retrospective study was conducted in Zhongshan Hospital of Fudan University in Shanghai. All adult patients who were admitted to the medical/surgical ICU of Zhongshan Hospital between January2012 and October 2015 diagnosed with *A. baumannii* VAP according to 2005 ATS/IDSA criteria (12) were included. The study was reviewed and approved by the Clinical Research Ethics Committee of Zhongshan Hospital [2011212], Fudan University.

Patients

The criteria used for the diagnosis of VAP were the presence of a new or progressive radiographic infiltrate, consolidation, cavitation or pleural effusion in a patient undergoing mechanical ventilation, plus at least 2 of the following: (I) temperature >38.3 or <36 ℃; (II) purulent tracheal secretions or a change in sputum characteristics, and (III) white blood cell count >10,000 or <4,000 cells/mm^3.

Tracheal aspirate culture was used for the pathogenic diagnosis of VAP. Only the first tracheal aspirate episode from each patient was included. Pathogens meeting the diagnostic threshold for semi-quantitative culture were thought to be the cause of VAP. Semi-quantitative culture results can be classified into four categories: + = virtually no growth, ++ = little growth, +++ = moderate growth, and ++++ = substantial growth. Results > ++ were deemed positive (13).

Clinical data collection

All patients who met the clinical and microbiological diagnosis of *A. baumannii* VAP were analyzed. The following data were recorded: age, sex, length of ICU stay, ICU days before VAP, mechanical ventilation days before VAP, VAP classification, surgery within 30 days before diagnosis, disease severity at the time of diagnosis, comorbid conditions, invasive procedures, medical imaging features, appearance of subsequent *A. baumannii* bacteremia , antimicrobial susceptibility of *A. baumannii*, prior antibiotics and appropriateness of antibiotic therapy after diagnosis.

VAP was classified as either early onset (<5 days within mechanical ventilation) or late onset (≥5 days). Disease severity was assessed using the Acute Physiology and Chronic Health Evaluation II (APACHEII) score upon diagnosis. Comorbid conditions were divided into groups as follows: diabetes mellitus, cardiovascular disease (coronary artery disease, cardio-myopathy and valvular heart disease), malignancy (hematologic malignancy and solid tumors), COPD (chronic obstructive pulmonary disease), liver cirrhosis, chronic kidney disease, cerebrovascular accident (cerebral infarction or cerebral hemorrhage) and transplant. Invasive procedures included tracheostomy, nasogastric tube placement and central venous catheterization. Medical imaging features included the presence of bilateral lung involvement and pleural effusion. Subsequent bacteremia was defined as at least one *A. baumannii* positive blood culture in the absence of a different infection source and at least one bacteria positive tracheal aspirate culture after the development of newly diagnosed AbVAP. Prior antibiotics treatment was defined as the use of systemic antibiotics for at least 72 hours within the preceding 14 days before diagnosis. Appropriate antibiotic therapy was defined as the receipt of one or more antimicrobial agents to which *A. baumannii* was susceptible via an appropriate route within 48 hours of diagnosis.

Antimicrobial susceptibility

Susceptibility testing of 13 antimicrobials was performed by using the broth microdilution method. Susceptibility

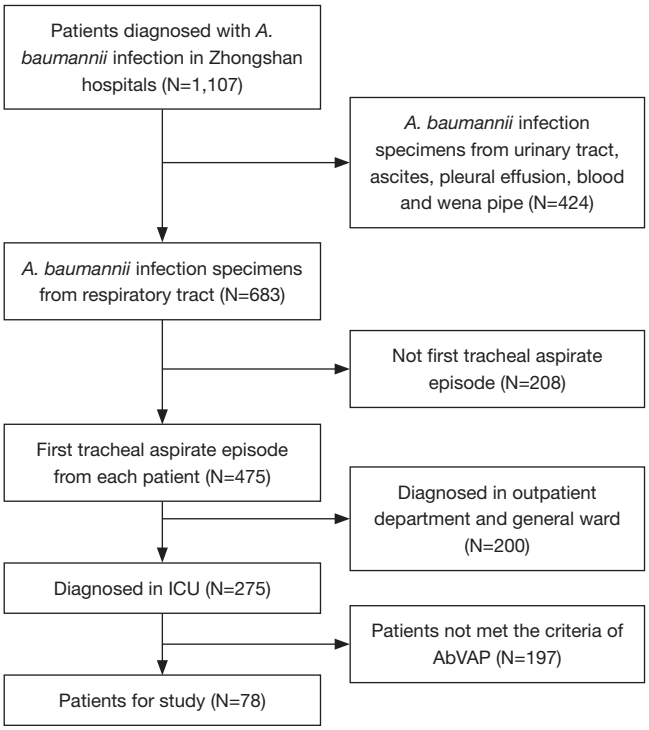

Figure 1 Patient selection.

results were interpreted according to guidelines established by the Clinical and Laboratory Standards Institute (14). Interpretation break points for tigecycline of ≤1 mg/L were considered as susceptible, and ≥4 mg/L as resistant according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (15).

Biofilm-formation

Biofilm formation was tested as follows (16): single *A. baumannii* colonies were cultured in Luria-Bertani broth (LB broth) with shaking at 37 ℃ for 16 hours and then diluted with LB broth to a McFarland turbidity of 0.5. The suspensions were deposited in 96-well microtiter plates and incubated for 48 h. Each sample had two duplicates, and the negative control was LB broth. After incubation, phosphate-buffered solution was used to clean the wells, and methanol was used as a fixative. Biofilms were stained with 200 μL of 0.1% crystal violet solution for 20 min. After that, the wells were washed with slow flowing water three times, and the remaining stain was solubilized with 160 μL of 33% glacial acetic acid solution. Finally, biofilm biomass was measured by determining the absorbance at 570 nm (OD_{570}). a OD_{570} >0.7 is considered a biofilm production.

Cytotoxicity

Cytotoxicity assessment was tested as follows (17,18): *A. baumannii* strains were cultured in tryptone broth at 37 ℃ with shaking at 200rpm overnight. Small-quantity suspensions were cultured again in new medium for 4 h when reaching the exponential phase. The bacterial suspensions were then collected and centrifuged for 5 minutes at 10,000 rpm and adjusted to 1.0×10^8 cfu/mL with phosphate buffered saline (PBS). Immortalized human bronchial epithelial cells (BEAS-2B cells) were seeded in 96-well plates at a density of 2.5×10^4 cells/well and cultured overnight the day before the experiment. Each well of cells was infected with an *A. baumannii* suspension at a multiplicity of infection (MOF) of ~200:1. Infected plates were then incubated at 37 ℃ with 5% CO_2 for 2 hours. Each bacterial sample had two duplicates. Cytotoxicity was measured using a Cytotoxicity LDH Assay Kit-WST (Dojindo Kumamoto, Japan, CK12) following the manufacturer's protocol.

Follow-up and outcomes

Overall 30-day mortality in ICU was the outcome. All patients were followed up for survival status until death or 30 days after onset of VAP.

Statistical analysis

Data were processed with SPSS for Windows (Version 22.0, SPSS Inc., Chicago, IL, USA). Continuous variables were analyzed using Student's *t*-test or the Mann-Whitney U test, whereas categorical variables were analyzed using the chi-squared test or Fisher's exact test. Risk factors for 30-day mortality were identified using logistic regression (all clinical and laboratory parameters were analyzed in a multivariate logistic regression model). $P<0.05$ were considered statistically significant.

Results

Clinical data at presentation

After selection, a total of 78 patients diagnosed with *A. baumannii* VAP in ICU were included in this study (*Figure 1*). According to *Table 1*, the mean patient age was 58.8±12.8 years old, 58 (74.4%) of the patients were male, 54 patients had early-onset VAP and 69.2% of the patients underwent recent surgery (within 30 days). The average

Table 1 Demographic and clinical characteristics of patients with *A. baumannii* ventilator-associated pneumonia

Variables	Total (n=78)	Non-survivors (n=29)	Survivors (n=49)	P
Age (years), mean ± SD	58.8±12.8	61.6±11.0	57.4±13.5	0.035
Male, n (%)	58 (74.4)	26 (89.7)	32 (65.3)	0.017
Early onset, n (%)	28 (35.9)	15 (51.7)	13 (26.5)	0.025
Prior surgery, n (%)	54 (69.2)	14 (48.3)	40 (81.6)	0.002
Onset APACHEII score, mean ± SD	13.9±4.6	16.2±4.8	12.6±4.0	0.001
Length of ICU stay (days), median [interquartile range]	37 [15–36.75]	35.5 [15–33]	36 [15–37]	0.71
Comorbid condition, n (%)				
Diabetes mellitus	18 (23.1)	6 (20.7)	12 (24.5)	0.700
Cardiac diseases	58 (74.4)	20 (69.0)	38 (77.5)	0.401
Malignancy	18 (23.1)	9 (31.0)	9 (18.4)	0.199
COPD	4 (5.1)	3 (10.3)	1 (2.0)	0.282
Liver cirrhosis	2 (2.6)	2 (6.9)	0 (0.0)	0.262
Chronic kidney disease	2 (2.6)	2 (6.9)	0 (0.0)	0.262
Cerebrovascular accident	4 (5.1)	0 (0.0)	4 (8.2)	0.294
Transplant	4 (5.1)	3 (10.3)	1 (2.0)	0.282
Invasive procedures				
Tracheostomy	52 (66.7)	19 (65.6)	33 (67.3)	0.868
Nasogastric tube	61 (78.2)	25 (86.2)	39 (79.6)	0.462
Central venous catheter	38 (48.7)	18 (62.1)	20 (40.8)	0.070
Image features				
Bilateral involvement	69 (88.5)	28 (96.6)	41 (83.7)	0.176
Pleural effusion	46 (58.9)	16 (55.2)	30 (61.2)	0.599

Early onset VAP is defined as VAP occurring <5 days within mechanical ventilation. Prior surgery is defined as surgery within 30 days before diagnosis; APACHEII, Acute Physiology and Chronic Health Evaluation II; Cardiovascular disease includes coronary artery disease, cardio-myopathy and valvular heart disease; Malignancy includes hematologic malignancies and solid tumor; COPD, chronic obstructive pulmonary disease; cerebrovascular accident includes cerebral infarction or cerebral hemorrhage.

APACHEII score at the time of diagnosis was 13.9±4.6. Cardiovascular disease was the most common underlying systemic disease and was found in 74.4% of the patients. The most common invasive procedure performed during the ICU stay was nasogastric tube placement (61, 78.2%). According to chest radiography, 88.5% of the patients had bilateral lung involvement, and 58.9% of the patients had pleural effusion.

Pathogen characteristics and antibiotic therapy

Susceptibility profiles of tracheal aspirate isolates are presented in *Table 2*. All these *A. baumannii* isolates only showed sufficient susceptibility to colistin (78, 100%) and tigecycline (61, 78.2%). Carbapenem-resistant *A. baumannii* VAP accounted for 79.5% of the cases. Overall, only 37.2% of the patients received appropriate antibiotic therapy. In the present study, we found that the biofilm-production rate was significantly higher in survivors than in non-survivors [40.8% (20/49) *vs.* 13.8% (4/29), P=0.012] and we also revealed the non-survivor group exhibited higher bacterial cytotoxicity than the survivor group (65.1%±24.2% *vs.* 51.4%±15.0%, P=0.009).

Table 2 Pathogen characteristics and antibiotic therapy of patients with *A. baumannii* ventilator-associated pneumonia

Variables	Total (n=78)	Non-survivors (n=29)	Survivors (n=49)	P
Resistance profiles of tracheal aspirate, n (%)				
Amikacin resistance	59 (75.6)	23 (79.3)	36 (73.5)	0.561
Ceftazidime resistance	65 (83.3)	25 (86.2)	40 (81.6)	0.834
Ciprofloxacin resistance	65 (83.3)	25 (86.2)	40 (81.6)	0.834
Cefotaxime resistance	75 (96.2)	28 (96.6)	47 (96.0)	1.000
Cefepime resistance	65 (83.3)	25 (86.2)	40 (81.6)	0.834
Gentamycin resistance	67 (85.9)	26 (89.7)	41 (83.7)	0.691
Meropenem resistance	62 (79.5)	23 (79.3)	39 (79.6)	0.976
SMZ-TZP resistance	56 (71.8)	20 (69.0)	36 (73.5)	0.669
Piperacillin resistance	66 (84.6)	25 (86.2)	41 (83.7)	1.000
Ampicillin/sulbactam resistance	65 (83.3)	25 (86.2)	40 (81.6)	0.834
Piperacillin/tazobactam resistance	65 (83.3)	25 (86.2)	40 (81.6)	0.834
Tigecycline resistance	17 (21.8)	6 (20.7)	11 (22.4)	0.856
Colistin resistance	0 (0)	0 (0)	0 (0)	–
Subsequent *A. baumannii* bacteremia, n (%)	6 (7.7)	4 (13.8)	2 (4.1)	0.264
Treatment				
Inappropriate antibiotic therapy, n (%)	49 (62.8)	23 (79.3)	26 (53.1)	0.020
Biofilm production, n (%)	24 (30.8)	4 (13.8)	20 (40.8)	0.012
Cytotoxicity				
LDH releasing ratio (%), mean ± SD	56.5±20.0	65.1±24.2	51.4±15.0	0.009

SMZ-TZP, sulfamethoxazole-trimethoprim; LDH, lactic dehydrogenase.

Outcomes and prognostic factors for 30-day mortality of A. baumannii VAP in ICU

The 30-day mortality rate in ICU for 78 patients was 37.2% (29/78). The results of univariate analysis demonstrated that age, gender, early-onset VAP, prior surgery within 30 days before diagnosis, APACHEII score at the time of diagnosis, inappropriate antibiotic therapy, biofilm production and high LDH releasing ratio which is representative of cytotoxicity were associated with higher 30-day mortality rates in patients with *A. baumannii* VAP.

For logistic regression analysis, with only 29 deceased patients, the number of co-variates added into the regression model can at most be 3, these included prior surgery, APACHEII score and LDH release ratio. As shown in *Table 3*, prior surgery [OR, 0.277; 95% confidence interval (CI), 0.089–0.866; P=0.027], higher APACHII score (OR, 1.140; 95% CI, 1.007–1.291; P=0.038) and an increased bacterial cytotoxicity (OR, 1.029 ; 95% CI, 1.001–1.058; P=0.047) were identified as independent prognostic factors for 30-day ICU mortality of *A. baumannii* VAP patients.

Characteristics of A. baumannii VAP patients with high/low cytotoxic bacterial infection

In this context, owing to no widely accepted cutpoint of LDH releasing ratio in *A. baumannii* species, we used the average value of 56.5% as the cutoff for dichotomization. The characteristics of patients with high/low cytotoxic bacterial infection were compared in *Table 4*, showing that the patients with high cytotoxic bacterial infection had higher APACHEII score at the time of diagnosis. Susceptibility testing results also indicated a higher antibiotics resistance rate to most kind of antibiotics in patient with low cytotoxic bacterial infection. Kaplan-Meier

Table 3 Multivariate logistic regression analysis for predictors of mortality in patients *A. baumannii* ventilator-associated pneumonia

Variable	P	OR	95% CI
Prior surgery	0.027	0.277	0.089–0.866
APACHEII score	0.038	1.140	1.007–1.291
LDH releasing ratio (%)	0.047	1.029	1.001–1.058

LDH, lactic dehydrogenase.

analysis showed high bacterial cytotoxicity can significantly affect the short-term prognosis of patents (*Figure 2*).

Discussion

The present study had revealed that high bacterial cytotoxicity was independent risk factor for mortality in patients with *A. baumannii* VAP. Prior to our study, the impact of bacterial cytotoxicity on the prognosis of *A. baumannii* VAP in ICU was still not clear, especially in Chinese patients.

At the end of follow-up, 29 patients died (30-day mortality rate: 37.2%), which was comparable to the study by Inchai *et al.* (30-day mortality rate: 48.3%) (19), Haliloglu *et al.* (28-day mortality rate: 52.3%) (20), and Tsioutis *et al.* (28-day mortality rate: 29.8%) (21). Thus, no obvious difference in the mortality rate was found between our study and the previous studies.

Higher APACHEII score at diagnosis were found in non-survivor group than in survivor group, which is consistent with previous researches on *A. baumannii* bloodstream infection (22,23). Interestingly, however, recent surgery acted as a protective factor, which may due to better organ function in patients who could tolerate surgery.

Numerous and diverse underlying systemic diseases have been reported as potential risk factors for mortality in *A. baumannii* infection (19-24). These include malignancy, post-transplantation status and chronic respiratory disease following previous survey. In the current study, we failed to identify a significantly higher mortality rate in patients with a certain kind of comorbid condition.

The presence of *A. baumannii* bacteremia always meant that the patients were in a state of severe illness. It is well accepted that VAP patient in the ICU often develop subsequent bacteremia, which were associated with a poorer outcome. Brotfain *et al.* (25) demonstrated that ICU mortality rate was higher in patients with *A. baumannii* VAP having a secondary *A. baumannii* bacteremia compared to nonbacteremic patients. Six (7.7%) patients had a subsequent *A. baumannii* bacteremia in our study, and after multivariate analysis, subsequent bacteremia was not found to be an independent predictor for short-term ICU mortality in patients with *A. baumannii* VAP, which is not accord with the conclusion of research by Magret *et al.* (26). This may be explained by the small sample size of our single-center study.

The main finding of our study was that high cytotoxicity was an independent risk factor significantly related to mortality in patients with *A. baumannii* VAP. Lactic dehydrogenase (LDH) is a stable cytoplasmic enzyme presented in all types of cells and released into the cell culture medium through damaged plasma membrane. In the present study, we used immortalized human bronchial epithelial cells (BEAS-2B cells) as a model system to assess *A. baumannii* cytotoxicity *in vitro* by measuring the released LDH according to Cytotoxicity LDH Assay Kit-WST. As shown in *Table 2*, the non-survivor group exhibited higher bacterial cytotoxicity than the survivor group. The results from multivariate analysis also showed that *A. baumannii* cytotoxicity is associated with poorer outcomes in *A. baumannii* VAP patients. This result is inconsistent with a previous study by Lázaro-Díez *et al.* (27), in which *A. baumannii* strains failed to induce any cytotoxic effect on lung epithelial cells. However, the referenced study examined only a handful of clinical *A. baumannii* strains (5 in total) and used the human lung epithelial A549 cell line as a model system to measure cytotoxicity with double immunofluorescence labeling and confocal microscopy. In contrast, we used BEAS-2B cells and the LDH-release method to examine cytotoxicity. Furthermore, the aim of our study was to identify whether an association exists between *A. baumannii* cytotoxicity and patient prognosis. The mechanisms of *A. baumannii* cytotoxicity have been previously studied. For example, Choi *et al.* (28) reported that the membrane proteins found in *A. baumannii* induce epithelial cell apoptosis by signaling through cell surface death receptors and mitochondrial targeting. Lipopolysaccharides (LPS) present in the outer membranes of bacterial so induce innate immune responses in a Toll-like receptor4 (TLR4)-dependent manner. Lin *et al.* (29) found that LpxC inhibitors affect the biosynthesis of lipid A, a core immune molecule induced by LPS that can enhance opsonophagocytic killing and thereby reduce the mortality of infected mice. We are presently working on isolating cytotoxic genes from strains with high LDH release to screen for potential predictive biomarkers and

Table 4 Comparison of the clinical characteristics of patients with high or low cytotoxicity bacterial infection

Variables	Total (n=78)	High cytotoxicity (n=34)	Low cytotoxicity (n=44)	P
Age (years), mean ± SD	58.8±12.8	60.4±13.6	55.9±13.9	0.16
Male, n (%)	58 (74.4)	27 (79.4)	31 (70.5)	0.369
Onset APACHEII score, mean ± SD	13.9±4.6	15.2±5.0	12.9±4.1	0.024
ICU stay days before VAP (days), median (interquartile range)	14.5 (6.75–14)	10 (6.25–15)	18.5 (6.75–13)	0.872
MV days before VAP (days), median (interquartile range)	14 [4–12]	9 [4–12.75]	12.5 [4–11]	0.899
Prior surgery, n (%)	54 (69.2)	20 (58.8)	34 (77.3)	0.08
Comorbid condition, n (%)				
Diabetes mellitus	18 (23.1)	10 (29.4)	8 (18.2)	0.243
Cardiac diseases	58 (74.4)	25 (73.5)	33 (75)	0.883
Malignancy	18 (23.1)	10 (29.4)	8 (18.2)	0.243
COPD	4 (5.1)	2 (5.9)	2 (4.5)	1.000
Liver cirrhosis	2 (2.6)	2 (5.9)	0 (0.0)	0.364
Chronic kidney disease	2 (2.6)	2 (5.9)	0 (0.0)	0.364
Cerebrovascular accident	4 (5.1)	1 (2.9)	3 (6.8)	0.801
Transplant	4 (5.1)	2 (5.9)	2 (4.5)	1.000
Prior antibiotics, n (%)				
Cephalosporins	60 (76.9)	25 (73.5)	35 (79.5)	0.532
Quinolones	22 (28.2)	7 (20.6)	15 (34.1)	0.189
Carbapenem	57 (73.1)	23 (67.6)	34 (77.3)	0.342
Glycopeptides	31 (39.7)	11 (32.4)	20 (45.5)	0.241
β-lactams/β-lactamase inhibitors	26 (33.3)	10 (29.4)	16 (36.4)	0.518
Image features, n (%)				
Bilateral involvement	69 (88.5)	30 (88.2)	39 (88.6)	1.000
Pleural effusion	46 (59.0)	17 (50.0)	29 (65.9)	0.157
Resistance profiles of tracheal aspirate, n (%)				
Amikacin resistance	59 (75.6)	20 (58.8)	39 (88.6)	0.002
Ceftazidime resistance	65 (83.3)	24 (70.6)	41 (93.2)	0.008
Ciprofloxacin resistance	65 (83.3)	24 (70.6)	41 (93.2)	0.008
Cefotaxime resistance	75 (96.2)	32 (94.1)	43 (97.7)	0.819
Cefepime resistance	65 (83.3)	24 (70.6)	41 (93.2)	0.008
Gentamycin resistance	67 (85.9)	26 (76.5)	41 (93.2)	0.076
Meropenem resistance	62 (79.5)	23 (67.6)	39 (88.6)	0.023
SMZ-TZP resistance	56 (71.8)	22 (64.7)	34 (77.3)	0.221
Piperacillin resistance	66 (84.6)	25 (73.5)	41 (93.2)	0.017
Ampicillin/sulbactam resistance	65 (83.3)	24 (70.6)	41 (93.2)	0.008
Piperacillin/tazobactam resistance	65 (83.3)	24 (70.6)	41 (93.2)	0.008
Tigecycline resistance	17 (21.8)	5 (14.7)	12 (27.3)	0.183
Colistin resistance	0 (0)	0 (0)	0 (0)	–
30-day ICU mortality (%)	29 (37.2)	17 (50.0)	12 (27.3)	0.039

VAP, ventilator-associated pneumonia; ICU, intensive care unit.

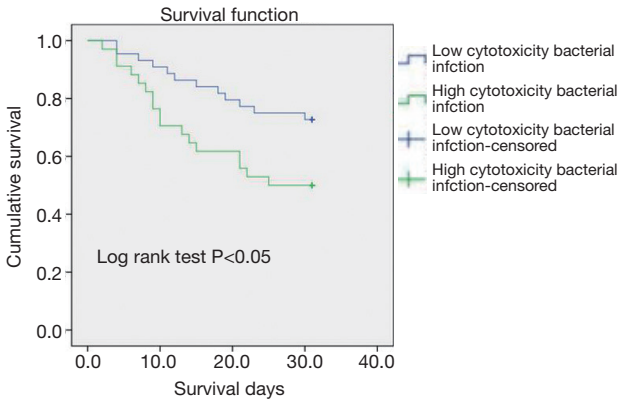

Figure 2 Kaplan-Meier analysis for patients with high or low cytotoxicity bacterial infection. We used 30-day ICU mortality as the main outcome for assessment of mortality for patients with serious conditions due to A. baumannii ventilator associated pneumonia. High bacterial cytotoxicity can significantly affect the short-term prognosis of patents (log rank test P=0.029).

identify new targets for clinical treatment.

A higher antibiotics resistance rate to most kind of antibiotics in patient with low cytotoxic bacterial infection was found in this survey. Many researchers studied the link between antimicrobial resistance and virulence and found some controversial result. For example, MexEF-OprN efflux pump is an important system for *P. aeruginosa* to extrude antibiotics but it also takes part in the downregulation of bacterial pathogenicity through a gene mutation that affect the cell quorum-sensing system (30); inactivation of MuxABC-OpmB transporter system in *P. aeruginosa* leads to increased ampicillin and carbenicillin resistance and decreased virulence due to decreased twitching motility (31). Further studies on *A. baumannii* are needed.

Previous studies have shown that administering adequate antibiotic therapy is crucial to a good therapeutic outcome (32,33). Appropriate antibiotic therapy was defined as the administration of antibiotics to which *A. baumannii* was susceptible through an appropriate route within 48hours of diagnosis in this study. Our data demonstrate that early administration of appropriate antimicrobial treatment improved the outcomes of patients with *A. baumannii* VAP. However, 62.8% of the patients did not receive appropriate treatment upon diagnosis, and this number increased to 79.3% in the non-survivor group. This result suggests that emphasis should be paid on testing drug sensitivity to avoid inappropriate therapy.

A. baumannii has been associated with VAP, bacteremia, meningitis, urinary tract infections and wound infections in hospitals partly because of its emerging antimicrobial resistance, especially carbapenem resistance. There have been reports on the factors associated with antibiotic resistance-related mortality (34-36). We found carbapenem resistance rate reach almost 80% in our study, while carbapenem resistance was not an independent prognostic factor for 30-day ICU mortality in patients. The difficulty in treating carbapenem-resistant *A. baumannii* infections is still notable, tigecycline may play an important role in alternative antibiotic therapy: most of carbapenem-resistant *A. baumannii* VAP patients receive tigecycline treatment after diagnosis in our study. Since colistin represents a last resort drug for MDR gram negative pathogens, its access to mainland China can be a great help for patients with *A. baumannii* infection.

Limitations were inherent in this study, all clinical information with reference to risk factors was retrospectively collected at a single hospital, which restricted the generalization of our result to more patients. We also do not have data on antimicrobial concentrations and about rapid worsening medical imaging. In addition, only 78 strains were used to assess biofilm production and cytotoxicity *in vitro*. Further prospective studies should be conducted to confirm the results.

Conclusions

In conclusion, based on the present study and to our knowledge, bacterial cytotoxicity was firstly proposed as an independent risk factor for mortality in patients with *A. baumannii* VAP. More attention should be paid in VAP not only on patients clinical but also on bacterial characteristics such as cytotoxicity. Exact mechanisms of cytotoxicity in patients need to be investigated. Validating the current findings in a large prospective cohort study in future is also necessary.

Acknowledgements

The authors would like to thank Sarah Conte for her linguistic assistance with the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (81500026, 81400043, 81770055, 81630001, 81490533). Dr. Y Song was supported by the State Key Basic Research Program (973) project (2015CB553404) and Shanghai Science and Technology Committee Grant (No. 15DZ1930600, No. 15DZ1930602).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was reviewed and approved by the Clinical Research Ethics Committee of Zhongshan Hospital (2011212), Fudan University.

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Cite this article as: Ju M, Hou D, Chen S, Wang Y, Tang X, Liu J, Chen C, Song Y, Li H. Risk factors for mortality in ICU patients with *Acinetobacter baumannii* ventilator-associated pneumonia: impact of bacterial cytotoxicity. J Thorac Dis 2018;10(5):2608-2617. doi: 10.21037/jtd.2018.04.86

