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Early View

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Prediction of ventilator-associated pneumonia outcomes according to the early microbiological response: a retrospective observational study.

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Take home-message Follow-up cultures on day 3 after a VAP diagnosis can help the clinician stratify patients. Those patients who present early with superinfection have worse ICU mortality, worse 90-day mortality and require more days of mechanical ventilation.

Abstract

Ventilator-associated pneumonia is a leading infectious cause of morbidity in critically ill patients; yet current guidelines offer no indications for follow-up cultures.

We aimed to evaluate the role of follow-up cultures and microbiological response 3 days after diagnosing ventilator-associated pneumonia as predictors of short- and long-term outcomes.

We performed a retrospective analysis of a cohort prospectively collected from 2004 to 2017. Ventilator-associated pneumonia was diagnosed based on clinical, radiographic, and microbiological criteria. For microbiological identification, a tracheobronchial aspirate was performed at diagnosis and repeated after 72h. We defined three groups when comparing the two tracheobronchial aspirate results: persistence, superinfection, and eradication of causative pathogens.

One-hundred-fifty-seven patients were enrolled in the study, among whom microbiological persistence, superinfection, and eradication was present in 67 (48%), 25 (16%), and 65 (41%), respectively, after 72hs. Those with superinfection had the highest mortalities in the intensive care unit (p=0.015) and at 90 days (p=0.036), while also having the fewest ventilation-free days (p=0.024). Multivariable analysis revealed shock at VAP diagnosis (odds ratios [OR] 3.43; 95% confidence interval [CI] 1.25 to 9.40), *Staphylococcus aureus* isolation at VAP diagnosis (OR 2.87; 95%CI 1.06 to 7.75), and hypothermia at VAP diagnosis (OR 0.67; 95%CI 0.48 to 0.95, per +1ºC) to be associated with superinfection.

Our retrospective analysis suggests that ventilator-associated pneumonia short-term and long-term outcomes may be associated with superinfection in follow-up cultures. Follow-up cultures may help guiding antibiotic therapy and its duration. Further prospective studies are necessary to verify our findings.

Keywords: Pneumonia, Ventilator associated pneumonia, Sepsis, multi-drug resistant pathogens, follow-up cultures.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the leading infectious causes of morbidity in critically ill patients [1, 2] and is reported to prolong mechanical ventilation by 7.6–11.5 days and hospitalization by 11.5–13.1 days [3]. All cause-mortality associated with VAP ranges from 20% to 50% in different studies [4]. In a meta-analysis of individual patient data from randomized prevention studies, the overall attributable mortality of VAP was 13%, with higher rates for patients undergoing surgery or with a mid-range severity score at admission [5].

Best practice to improve outcomes in patients with VAP is a matter of constant debate [6]. Currently, follow-up for VAP is based on a combination of clinical, radiological, and microbiological criteria, which combined, have poor specificity [7]. The use of biomarkers, including procalcitonin and C-reactive protein, to assess the evolution of VAP has also shown contradictory results [8–10]. Initiating appropriate antibiotic treatment early is associated with lower mortality [11], but antibiotic overuse can promote multi-drug resistant (MDR) pathogens [12, 13]. Although treatment failure on day 3 after starting antibiotics predicts worse outcomes [14], the relationship between clinical and microbiological response, especially at an early stage, remains controversial.

Microbiological response has been postulated as an end point in many studies evaluating VAP antibiotic treatment. However, earlier assessment could detect superinfection, resistance patterns, and whether antibiotic therapy should be changed or not [15]. Most authors require the eradication of pathogens in respiratory samples before accepting microbiological response, but some accept a predetermined decrease in their levels [16, 17]. Finally, the best time to evaluate microbiological response is

currently uncertain.

The aim of the present study was to assess the ability of follow-up cultures, obtained by tracheobronchial aspirate (TBAS) at 3rd day after a VAP diagnosis, to predict short- and long-term outcomes. We hypothesized that this approach would help to optimize antimicrobial therapy.

METHODS (additional information is shown in the online supplement)

Study design

We performed a retrospective observational study analyzing data collected prospectively from 2004 to 2017 at the 800-bed Hospital Clinic in Barcelona, Spain. Six intensive care units (ICUs), including five medicals and one surgical ICU (45 beds in total), participated. The institution's Ethical Review Board approved the study (*Comite Etic d´Investigacio Clinica*, no. 5427), which was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from patients or their relatives.

Participants

Adult patients (age ≥18 years) with an ICU stay of at least 48 hours and a diagnosis of ICU acquired pneumonia (ICU-AP) were evaluated consecutively. If patients had more than one episode of ICU-AP during an ICU stay, only the first episode was considered. We included only patients with VAP who had at least one causative pathogen identified. We excluded patients whose antibiotic strategy had been changed in the previous 3 days, and who had severe immunosuppression due to post-chemotherapy neutropenia,

drug-induced immunosuppression for solid-organ transplantation, or human immunodeficiency virus infection. Patients with incomplete microbiological assessments and different pathogens isolated in different samples (e.g., TBAS, bronchoalveolar lavage, blood cultures and cultures from pleural fluid) at the same assessment point were also excluded.

Procedures and definitions

Pneumonia was clinically diagnosed in patients who presented with new or progressive pulmonary infiltrates in their chest radiographs due to a presumed infectious agent, and also at least two of the following symptoms or findings: fever (>38°C) or hypothermia (<36°C), leukocytosis (>12,000 cells/mm^3) or leukopenia (<4,000 cells/mm^3), presence of purulent tracheal secretions, and a decrease in oxygenation[1, 18] or a simplified Clinical Pulmonary Infectious Score of six points or more [19].

For microbiological diagnosis, a TBAS was collected within the first 24 hours of inclusion (TBAS1). Microbiologically confirmed VAP was defined as the presence of at least one potentially-pathogenic microorganism in the respiratory sample above pre-defined thresholds (≥10^5 CFU/mL) [1]. In good quality samples representative of the lower respiratory tract, it was defined as <10 epithelial cells and >25 leucocytes per field. The same sampling method was repeated after 3 days (TBAS2) from the diagnosis of VAP. Blood cultures and pleural fluid cultures were collected if clinically indicated. Further information regarding microbiological diagnosis is described elsewhere [20].

We defined MDR organisms as those non-susceptible to at least one agent in three or more antimicrobial categories [21].

Initial response to treatment was evaluated 72 hours after starting antimicrobial

treatment. Treatment failure was defined as the presence of ≥1 of the following criteria: a) no improvement in the arterial oxygen partial pressure to fractional inspired oxygen ratio; b) persistence of fever together with purulent respiratory secretions; c) ≥50% increase in pulmonary infiltrates on chest radiography; and d) occurrence of septic shock or multiple organ failure. Initial appropriate treatment was defined when the causative pathogen was susceptible *in vitro* to at least one antibiotic in the empiric treatment.

Early microbiological response was assessed by comparing pathogens isolated in the TBAS1 and TBAS2 samples. Persistence was defined as the isolation of the same microorganism in the second sample at high or low concentrations (either no reduction or a reduction of at least one logarithm of the initial concentration). Superinfection was defined as the emergence of at least one new pathogen in the TBAS2 sample. Eradication was defined as the disappearance of the original pathogen in the TBAS2 sample.

Data collection, evaluation, and microbiological diagnosis

Data were collected from the database system for electronic medical records and examined anonymously. All relevant data were collected at admission, at pneumonia onset, on day 3of ICU admission, and throughout ICU stays. The APACHE II score [22], the Simplified Acute Physiology Score (SAPS II) [23], and the Sequential Organ Failure Assessment (SOFA) [24] were calculated at ICU admission. Organ dysfunction was assessed daily with the SOFA score. To facilitate the diagnosis of VAP, we calculated the CPIS [19]. Septic shock and acute respiratory distress syndrome were defined according to previously described criteria [25, 26]. Patients were followed until day 90 or death, whichever occurred first after the diagnosis of VAP.

Outcomes

The primary outcome was ICU mortality. Secondary outcomes included initial appropriate treatment, treatment failure on day 3, ventilator-free days, pneumonia recurrence, antibiotic-free days, length of ICU stay, 28-day mortality, and 90-day mortality.

Statistical analysis

We report numbers and percentages for categorical variables, and the median and first and third quartiles for continuous variables (not normally distributed data). Categorical variables were compared using the chi-square test. Three continuous variables were compared using the Kruskal–Wallis test, and if significant overall, post-hoc pairwise comparisons were conducted via the Bonferroni test to control for the experiment-wise error rate.

Logistic regression analyses [27] were used to examine the association between superinfection and risk factors. Each risk factor was first tested individually (age, sex, smoking habit, alcohol abuse, previous corticosteroids use, previous antibiotic use, ≥5 days of previous hospitalization, previous respiratory isolation, diabetes mellitus, chronic renal failure, solid cancer, chronic heart diseases, chronic lung diseases, chronic liver diseases, APACHE II score at ICU admission, SAPS II score at ICU admission, SOFA score at ICU admission, causes of ICU admission, days of MV before VAP, Late onset VAP, CPIS at VAP diagnosis, SOFA score at VAP diagnosis, temperature at VAP diagnosis, multilobar at VAP diagnosis, ARDS at VAP diagnosis, pleural effusion at VAP diagnosis, shock at VAP diagnosis, fever at VAP diagnosis, creatinine at VAP diagnosis, hemoglobin at VAP diagnosis, white blood cell count at VAP diagnosis, lymphocytes at VAP diagnosis,

C-reactive protein at VAP diagnosis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella spp.*, *Enterobacter spp.*, *Proteus spp.*, *Serratia spp.*, *Aspergillus spp.*, *Streptococcus pneumoniae*, *Escherichia coli*, *Stenotrophomonas maltophilia*, virus, and initial appropriate treatment), before all risk factors that showed associations in the univariate model ($p < 0.10$) were added to the multivariable model. Finally, a backward stepwise selection (likelihood ratio) ($p_{in} < 0.05$, $p_{out} > 0.10$) was used to determine factors associated with superinfection [28].

Cox proportional hazards regression analyses [29] were performed to determine the effect of superinfection on 28-day mortality, both crude and adjusted for potential confounders (i.e., APACHE II score at ICU admission, change in SOFA score from VAP diagnosis to day 3, C-reactive protein at VAP diagnosis, and initial appropriate treatment).

A two-sided p value < 0.05 was considered statistically significant. All analyses were performed with IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA).

Results

Participants

Of 507 patients diagnosed with ICU-acquired pneumonia, 350 patients were excluded: 204 with non-ventilator ICU-acquired pneumonia, 93 with incomplete microbiological follow-up, 47 with no pathogen isolated in TBAS1, and 6 with different pathogens isolated in different samples collected at the same time. Thus, 157 patients were included for analysis and divided by microbiological evolution into persistence (48%; n=67), superinfection (16%; n=25), and (41%; n=65) eradication groups (Figure 1).

Patient characteristics at ICU admission, VAP diagnosis, and 3-5 days after diagnosis

There were no significant differences among the three groups in demographic characteristics, comorbidities, or severity scores at ICU admission. Causes of ICU admission were similar between the three groups, but admission for cardiac arrest was significantly higher among patients with superinfection (Table 1).

Concerning the characteristics of patients at VAP diagnosis (Table 2), the superinfection group had significantly lower median temperature and more patients with septic shock than either the persistence or the eradication groups. The eradication group had a significantly lower median arterial oxygen partial pressure to fractional inspired oxygen ratio than the persistence group. By 3–5 days after VAP (Table 3), the SOFA score was significantly higher in the superinfection group than in the persistence group, and the median temperature continued to be significantly lower. In eTables 1, 2, 3 and 4 we show the comparisons of three groups with patients not included due to incomplete follow-up.

Microbial etiology

There were not significantly differences among the three groups in terms of VAP etiology, except for *P. aeruginosa*, including MDR species (Table 4), which was present at significantly lower percentages in the eradication group compared with the other two groups. The overall rate of MDR pathogens was also significantly lower in the eradication group. Initial appropriate antibiotic therapy was similar among the three groups. Figure 2 shows the distribution of new pathogens in the TBAS from day 3 (superinfection=25). Seven patients in the superinfection group presented a new pathogen resistant to empirical treatment (four MRSA, and three P. aeruginosa).

Risk factors for superinfection

Several variables significantly associated with superinfection in the univariate logistic regression analyses were included in the multivariable analysis (eTable 5). This latter analysis showed that shock at VAP diagnosis (OR 3.43; 95% CI 1.25 to 9.40; p=0.017), *S. aureus* isolation at VAP diagnosis (OR 2.87; 95% CI 1.06 to 7.75, p=0.038) and increased temperature (+1°C) at VAP diagnosis (OR 0.67; 95% CI 0.48 to 0.95; p=0.025) was independently associated with superinfection. The area under the receiver operating characteristic curve (AUC) was 0.74 (95% CI 0.62 to 0.85) for the multivariable model of superinfection (eFigure 1). Internal validation of the final model by bootstrapping with 1,000 samples demonstrated robust results: all variables remained significant with small 95% CIs around the original coefficients (eTable 6).

Outcomes

Table 5 shows primary and secondary outcomes. No differences were found in terms of adherence to ERS/ESICM/ESCMID/ALAT guidelines, initial appropriate antibiotic

therapy, treatment failure at day 3 (eTable 7), or recurrence within 28 days. There were significant differences among the three groups in ICU mortality, 90-day mortality, and days of mechanical ventilation. The highest 90-day mortality was observed in patients with superinfections caused by pathogens resistant to empirical treatment (n= 5, 71%). After adjustment for potential confounders in the multivariable Cox model, superinfection group was associated with significantly higher 28-day mortality risk compared with patients with either persistence or eradication (aHR 2.39; 95% CI 1.16 to 4.92; p = 0.018) (eTable 8 and 9) (eFig 2).

Discussion

The main findings of our study were that patients who present early a new microorganism in TBAS have worse mortality and require more days of mechanical ventilation. Despite our expectations, patients with eradication have not better outcomes than patients without eradication. Our study shows how routinely TBAS on day 3 after a VAP diagnosis can help the clinician stratify patients based on early microbiological response (three groups in this study). The better feasibility of the maneuvers, the easier repeatability and the lower costs were the main factors that persuaded us to choose the non-invasive technique; however, TBAS may also have limitations. Appropriate antibiotic therapy in patients with suspected VAP is widely recognized as essential in both empirical and etiological settings. Current best practice is for antibiotic therapy focused on etiology and based on susceptibility testing, but even this approach may fail to decrease mortality.

At diagnosis, patients with superinfection more often had lower body temperature, hypothermia, and shock. After 3 days, the superinfection group experienced greater hypothermia than the other groups and worse SOFA scores than the persistence group. Changes in SOFA were similar between groups. The etiology of VAP was most frequently associated with *P. aeruginosa* and MDR pathogens in this group. It is impossible to establish whether the new microorganism isolated is a true superinfection or a pathogen from the index infection not diagnosed due to the limitations of the culture methods. Further studies with molecular diagnosis may help to clarify this situation, especially with regard to drug-resistant and difficult-to-eradicate pathogens such as S. aureus and P. aeruginosa[30].

We identified that superinfection was associated with the highest mortality mainly in those patients with pathogens resistant to empirical treatment (MRSA or P. aeruginosa), and based on this, we developed a model for its prediction. Elaborating on the multivariable regression analysis, we found that a temperature decreases, shock, and infection by *S. aureus* were independently associated with superinfection. The AUC for this model was good (0.74; 95% CI 0.62 to 0.85).

Current ERS/ESICM/ESCMID/ALAT guidelines [1] provide indications neither for routine follow-up cultures to establish the microbiological evolution or about the type and timing of microbiological cultural exam. However, it does underline the importance of clinical assessment in patients receiving antibiotic treatment for VAP or hospital-acquired pneumonia to predict adverse outcomes and clinical response at 72–96 hours, including tracheobronchial secretion volume, culture, and assessment of purulence of tracheobronchial secretions. Thus, deciding to perform repeat cultures depends on the clinical and radiological response to current therapy. It is important to underline that, in our study, no differences were found between groups in treatment failure (or at least clinically evident non-improvement), CPIS scores, or radiological worsening at day 3. Our protocol was based on re-evaluating patients after 3 days of therapy to assess microbiological evolution by culture, regardless of their clinical conditions. Using this approach, it should be possible to detect early microbiological response, optimize antibiotic therapy, predict mortality, and stratify patient outcomes.

In 1993, Montravers et al. [31] evaluated the clinical and microbiological efficacy of antimicrobial therapy for bacterial nosocomial pneumonia through PSB cultures collected at diagnosis and after 3 days of treatment among 76 patients. After 3 days of

antimicrobial therapy, in most cases combining two effective agents, 51 patients achieved sterilization of the infective site, 16 had persistent low-grade infection, and 9 had persistent high-grade infection. Despite Montravers et al.[31] found a superinfection rate of approximately 9%, they failed to detect any differences in mortality. Their study also failed to emphasize the role of superinfection on outcomes other than crude mortality. However, we recognize the importance of this paper to have been the first to report that routinely performing PSB after 72 hours from diagnosis can uncover superinfection. Comparable results were also reported by A'Court et al. [32], though they adopted bronchial lavage, a non-invasive and non-bronchoscopic guided technique that aspirates a 20 mL lavage. Cultures were repeated daily before and after a VAP diagnosis. Among 65 cases of VAP, 12 (18%) developed a superinfection between day 3 and 10 after diagnosis. A'Court et al. [32] reported that surveillance influenced the clinical management of at least 42% of their patients. Interestingly, they also reported a slower bacteriological response for *S. aureus* and *P. aeruginosa* to the antibiotic therapy. In our study, *P. aeruginosa* infection was significantly less common in the eradication group, and although *S. aureus* seemed to be less common, this was without reaching statistical significance. More recently, Dennesen et al. [33] and Prats et al. [34] evaluated microbiological response using sequential cultures. Dennesen et al.[33] failed to detect patients with superinfection, probably due to the small cohort, but did reveal that colonization was already present (before day 5) in all 6 patients who experienced reinfection. While Prats et al. [34] mentioned that the rate of superinfection was 12%, they did not report the mortality in this subgroup.

Few papers have evaluated the role of follow-up cultures, with the most recent being published in 2002 [34]. Despite the importance they gave to follow-up cultures, all these authors concentrated on monitoring only the microbiological response to the therapy. Our study is the first to report relevant differences in outcomes by stratifying patients according to the follow-up culture results.

It is unclear why patients developed superinfection and had worst outcomes. Host factors (impaired immunity, or dysbiosis), or specific virulence factors of certain pathogens (S. aureus) could be related to it. Further studies with data from immunity status, and microbiome are needed to clarified it.

Our study has several limitations. First, it was conducted at a single centre, so the extrapolation of these findings to other settings must be done with care. Second, we only evaluated patients with 72 hs samples, so a significant number of patients were excluded (n=93). Failure in obtain good quality samples by lack of secretions or contaminations and patients with early improvement or impairment may explain this. Also, the use of TBAS may have limited our results given its reduced ability to obtain good and representative respiratory samples in some cases. Third, we only included first episode of VAP in our study, we cannot exclude different results in patients with second of third episodes of VAP. Fourth, adequate antimicrobial treatment was defined according to microbiological isolations, thus patients who received overtreatment with broad-spectrum antibiotics could be included in this group as adequate. Finally, it is an observational study with a small sample that limited the analysis of specific factors per superinfection. A confirmation of our results in a large and well balanced, international cohort is therefore desirable.

In conclusion, our retrospective analysis suggests that superinfection was associated with worse outcomes in patients with VAP. Further studies must evaluate protocols that include microbiological response evaluation as a strategy for reducing mortality due to VAP.

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Table 1. Demographic and baseline characteristics of patients at ICU admission

Variable	Group 1 Persistence (n = 67)	Group 2 Superinfection (n = 25)	Group 3 Eradication (n = 65)	*p* value
Age (years), median (Q1; Q3)	65 (53; 72)	56 (48; 74)	61 (45; 74)	0.650
Male sex, n (%)	50 (75)	15 (60)	46 (71)	0.391
Current or former smoking habit, n (%)	30 (46)	13 (52)	40 (62)	0.181
Current or former alcohol abuse, n (%)	18 (27)	5 (20)	17 (26)	0.772
Previous corticosteroids use, n (%)	3 (5)	4 (17)	5 (8)	0.226
Previous antibiotic use, n (%)	52 (78)	18 (72)	55 (85)	0.357
≥ 5 days of previous hospitalization, n (%)	44 (66)	13 (52)	47 (72)	0.188
Previous respiratory isolation, n (%)	28 (42)	8 (32)	34 (52)	0.184
Comorbidities, n (%)				
Diabetes mellitus	16 (24)	1 (4)	14 (22)	0.092
Chronic renal failure	6 (9)	0 (0)	3 (5)	0.228
Solid cancer	13 (19)	3 (12)	4 (6)	0.073
Chronic heart diseases	21 (31)	6 (24)	16 (25)	0.630
Chronic lung diseases	19 (28)	7 (28)	26 (40)	0.306
COPD	13 (19)	6 (24)	17 (26)	0.647
Chronic liver diseases	9 (13)	2 (8)	9 (14)	0.739
APACHE II score, median (Q1; Q3)	16 (12; 21)	17 (13; 19)	16 (12; 21)	1.000
SAPS II score, median (Q1; Q3)	43 (36; 52)	40 (34; 51)	38 (28; 46)	0.233
SOFA score, median (Q1; Q3)	7 (5; 10)	7 (5; 9)	7 (6; 10)	0.859
Causes of ICU admission, n (%)				
Hypercapnic respiratory failure	3 (5)	4 (16)	8 (13)	0.144
Hypoxemic respiratory failure	6 (9)	2 (8)	3 (5)	0.637
Acute coronary syndrome	1 (2)	0 (0)	5 (8)	0.090
Polytrauma	9 (13)	0 (0)	11 (18)	0.087
Postoperative	13 (19)	4 (16)	12 (19)	0.929
Cardiac arrest	3 (5)	5 (20)	4 (6)	**0.040**
Decreased consciousness	14 (21)	4 (16)	14 (22)	0.808
Shock	8 (12)	3 (12)	3 (5)	0.308
Nonsurgical abdominal disease	2 (3)	2 (8)	0 (0)	0.099
Others	8 (12)	1 (4)	3 (5)	0.231

Abbreviations: APACHE II score = Acute Physiology And Chronic Health Evaluation II score; COPD = chronic obstructive pulmonary

disease; ICU = intensive care unit; Q1 = first quartile; Q3 = third quartile; SAPSII = simplified acute physiology score II; SOFA = sequential organ failure assessment.

Table 2. Patients characteristics at VAP diagnosis

Variable	Group 1 Persistence (n = 67)	Group 2 Superinfection (n = 25)	Group 3 Eradication (n = 65)	*p* value
Days of MV before VAP, median (Q1; Q3)	5 (3; 10)	5 (3; 9)	6 (4; 13)	0.293
Late onset VAP, n (%)	51 (77)	18 (72)	54 (83)	0.473
Severity assessment of pneumonia				
CPIS, median (Q1; Q3)	6 (5; 7)	6 (6; 7)	6 (6; 8)	0.312
SOFA score, median (Q1; Q3)	7 (5; 10)	9 (7; 11)	7 (5; 9)	0.160
Temperature (˚C), median (Q1; Q3)	37.7 (36.2; 38.0)	36.0 (35.4; 37.6)	37.6 (36.2; 38.2)	**0.034** [a,c]
Temperature < 36˚C, n (%)	15 (23)	12 (48)	13 (20)	**0.027** [c]
Multilobar pneumonia, n (%)	24 (36)	11 (44)	24 (37)	0.764
Presence of ARDS, n (%)	5 (8)	4 (16)	9 (14)	0.398
Pleural effusion, n (%)	17 (26)	6 (27)	17 (27)	0.984
Shock at pneumonia diagnosis, n (%)	28 (42)	18 (72)	27 (42)	**0.022** [a,c]
Laboratory variables, median (Q1; Q3)				
Creatinine (mg/dL)	0.9 (0.7; 1.2)	0.7 (0.6; 1.7)	1.0 (0.7; 1.5)	0.450
Hemoglobin (g/dL)	10.1 (9.5; 11.2)	10.6 (9.7; 12.0)	10.5 (9.2; 11.7)	0.520
White blood cell count (10^9 cells/L)	10.7 (8.5; 16.3)	12.6 (8.9; 16.0)	12.0 (9.2; 17.2)	0.618
Lymphocytes (n/mm^3)	827 (609; 1177)	743 (410; 1061)	963 (718; 1306)	0.290
C-reactive protein (mg/L)	11.8 (6.6; 19.5)	14.1 (4.9; 20.0)	13.2 (5.2; 23.7)	0.903
Procalcitonin (ng/mL)	0.3 (0.1; 0.7)	0.5 (0.1; 4.7)	0.3 (0.1; 0.9)	0.338
PaO_2/FiO_2 (mmHg)	233 (178; 283)	176 (147; 265)	176 (140; 236)	**0.010** [b]

Abbreviations: ARDS = acute respiratory distress syndrome; CPIS = clinical pulmonary infection score; Q1 = first quartile; Q3 = third quartile; MV = mechanical ventilation; PaO_2/FiO_2 = ratio of arterial oxygen tension to inspired oxygen fraction; SOFA = sequential organ failure assessment; VAP = ventilator-associated pneumonia.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the superinfection group and the eradication group.

Table 3. Patients characteristics 3 days after VAP diagnosis

Variable	Group 1 Persistence (n = 67)	Group 2 Superinfection (n = 25)	Group 3 Eradication (n = 65)	*p* value
Severity assessment, median (Q1; Q3)				
CPIS	6 (4; 7)	6 (6; 7)	6 (4; 7)	0.414
SOFA score	6 (4; 9)	8 (7; 10)	7 (4; 9)	**0.028** [a]
Change in SOFA score from VAP diagnosis to day 3	0 (-2; 0)	0 (-1; 1)	0 (-1; 0)	0.422
Temperature (˚C)	37.1 (36.0; 38.0)	35.7 (35.2; 37.1)	37.2 (36.3; 37.8)	**0.002** [a,c]
Temperature < 36˚C, n (%)	14 (22)	14 (56)	11 (17)	**0.001** [a,c]
Laboratory variables, median (Q1; Q3)				
C-reactive protein (mg/L)	10.4 (5.3; 16.9)	12.8 (5.4; 26.1)	10.9 (2.8; 19.0)	0.570
Procalcitonin (ng/mL)	0.2 (0.1; 0.6)	0.5 (0.1; 2.8)	0.1 (0.1; 0.6)	0.130
PaO_2/FiO_2 (mmHg)	255 (176; 306)	225 (188; 272)	221 (151; 285)	0.324

Abbreviations: CPIS = clinical pulmonary infection score; Q1 = first quartile; Q3 = third quartile; SOFA = sequential organ failure assessment; PaO_2/FiO_2 = ratio of arterial oxygen tension to inspired oxygen fraction; VAP = ventilator-associated pneumonia.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the superinfection group and the eradication group.

Table 4. Etiology of ventilator-associated pneumonia

Microbiology, n (%column) (%row)	Group 1 Persistence (n = 67)	Group 2 Superinfection (n = 25)	Group 3 Eradication (n = 65)	*p* value
Staphylococcus aureus	17 (26) (44)	10 (42) (26)	12 (20) (30)	0.125
Streptococcus pneumoniae	1 (2) (17)	1 (4) (17)	4 (7) (66)	0.346
Enterobacteriaceae				
Enterobacter spp.	2 (3) (18)	3 (13) (27)	6 (10) (55)	0.194
Klebsiella spp.	10 (15) (43)	2 (8) (9)	11 (18) (48)	0.519
Escherichia coli	1 (2) (13)	2 (8) (25)	5 (8) (62)	0.189
Proteus spp.	1 (2) (25)	1 (4) (25)	2 (3) (50)	0.732
Serratia spp.	4 (6) (57)	1 (4) (15)	2 (3) (28)	0.751
Pseudomonas aeruginosa	32 (48) (58)	11 (44) (20)	12 (19) (22)	**0.001** [b,c]
Aspergillus spp.	1 (2) (16)	3 (13) (50)	2 (3) (34)	0.062
Virus	1 (2) (100)	0 (0) (0)	0 (0) (0)	0.522
Others	4 (6) (30)	1 (4) (7)	8 (13) (63)	0.251
MDR pathogens	25 (37) (50)	14 (56) (28)	11 (17) (22)	**0.019** [c]
Pseudomonas aeruginosa	9 (13) (60)	5 (20) (34)	1 (2) (6)	**0.010** [b,c]
Methicillin-resistant				
Staphylococcus aureus (MRSA)	7 (10) (59)	3 (12) (25)	2 (3) (16)	0.188
Acinetobacter baumannii	0 (0) (0)	0 (0) (0)	0 (0) (0)	-
MDR Enterobacteriaceae	5 (8) (36)	4 (16) (28)	5 (8) (36)	0.444
Stenotrophomonas maltophilia	4 (6) (44)	2 (8) (22)	3 (5) (34)	0.844
XDR pathogens, n (%)	11 (16) (50)	5 (20) (23)	6 (9) (27)	0.317
PDR pathogens, n (%)	1 (2) (100)	0 (0) (0)	0 (0) (0)	0.509

Abbreviations. MDR = multi-drug resistant; XDR = extensively drug resistant; PDR = pan-drug resistant.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the superinfection group and the eradication group.

Table 5. Outcomes

Variable	Group 1 Persistence (n = 67)	Group 2 Superinfection (n = 25)	Group 3 Eradication (n = 65)	*p* value
ERS/ESICM/ESCMID/ALAT guidelines adherence, n (%)	44 (70)	12 (50)	40 (67)	0.212
Initial appropriate treatment, n (%)	54 (83)	18 (75)	54 (90)	0.199
Treatment failure on day 3, n (%)	44 (66)	18 (72)	37 (57)	0.349
Days of MV, median (Q1; Q3)	14 (9; 24)	22 (13; 43)	18 (13; 27)	**0.042** [a]
Ventilator-free-days, median (Q1; Q3)	9 (0; 22)	0 (0; 12)	7 (0; 20)	0.068
ICU length of stay, median (Q1; Q3)	20 (13; 32)	24 (17; 44)	24 (15; 35)	0.373
ICU mortality, n (%)	14 (21)	13 (52)	20 (31)	**0.015**[a]
28-days mortality, n (%)	16 (24)	11 (44)	18 (28)	0.161
90-days mortality, n (%)	23 (34)	16 (64)	25 (40)	**0.036**[a]

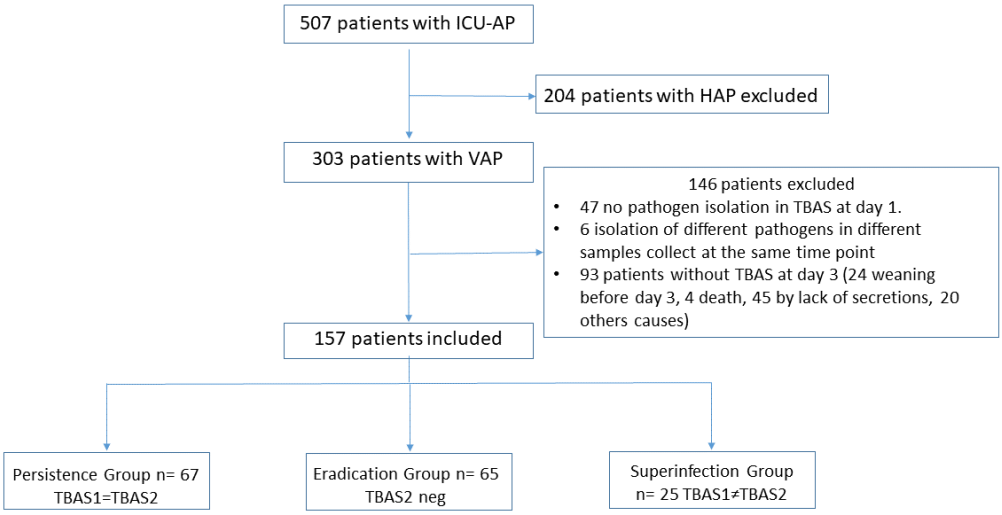
Abbreviations: ERS = European Respiratory Society; Q1 = first quartile; Q3 = third quartile; ICU = intensive care unit; MV = mechanical ventilation.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

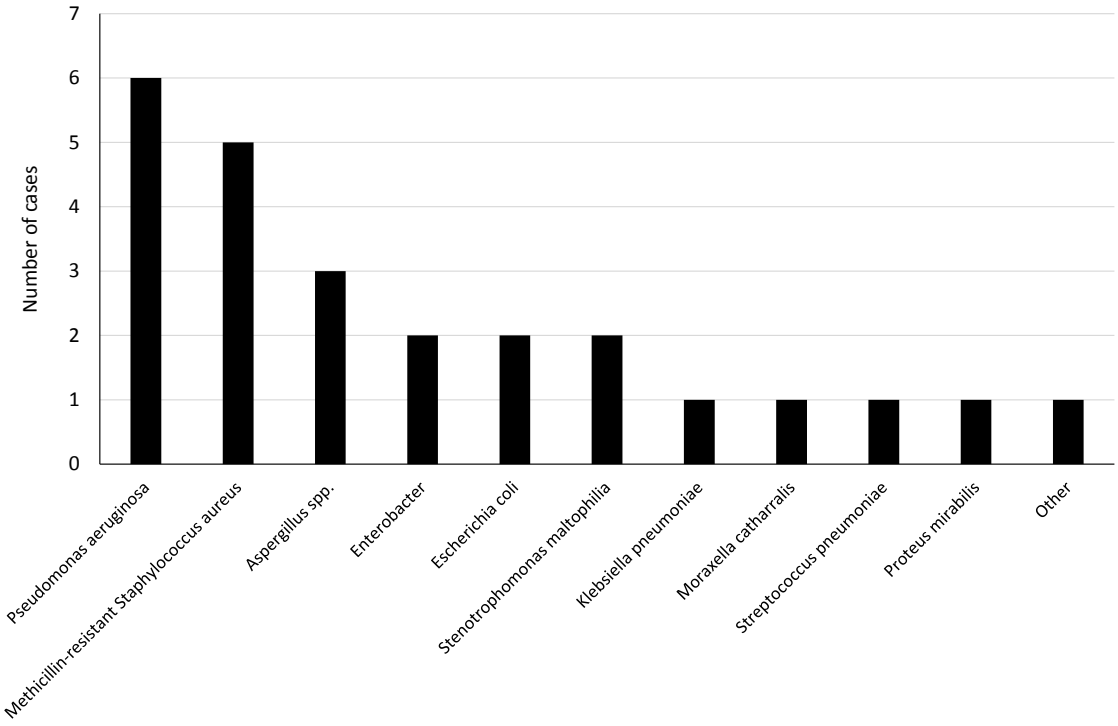
[c] p <0.05 for comparison between the superinfection group and the eradication group.

Figure 1. Participant flowchart



Abbreviations: HAP = hospital-acquired pneumonia; ICU = intensive care unit; TBAS = tracheobronchial aspirate (1 = at admission, 2 = at 3–5 days); VAP, ventilator-associated pneumonia.

Figure 2. New pathogens in tracheobronchial aspirates on days 3–4: cases of superinfection (n = 25)



References

1. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, Kollef M, Li Bassi G, Luna CM, Martin-Loeches I, Paiva JA, Read RC, Rigau D, Timsit JF, Welte T, Wunderink R. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). *Eur. Respir. J.* 2017; 50.

2. Niederman MS, Martin-Loeches I, Torres A. The research agenda in VAP/HAP: next steps. *Intensive Care Med* 2017; .

3. Muscedere JG, Day A, Heyland DK. Mortality, attributable mortality, and clinical events as end points for clinical trials of ventilator-associated pneumonia and hospital-acquired pneumonia. *Clin. Infect. Dis.* 2010; 51 Suppl 1: S120-125.

4. Nair GB, Niederman MS. Ventilator-associated pneumonia: present understanding and ongoing debates. *Intensive Care Medicine* Nature Publishing Group; 2015; 41: 34.

5. Melsen WG, Rovers MM, Groenwold RHH, Bergmans DCJJ, Camus C, Bauer TT, Hanisch EW, Klarin B, Koeman M, Krueger WA, Lacherade J-C, Lorente L, Memish ZA, Morrow LE, Nardi G, van Nieuwenhoven CA, O'Keefe GE, Nakos G, Scannapieco FA, Seguin P, Staudinger T, Topeli A, Ferrer M, Bonten MJM. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. *Lancet Infect Dis* 2013; 13: 665–671.

6. Kl R, St M, P J, Mh K. Controversies and advances in the management of ventilator associated pneumonia [Internet]. Expert review of respiratory medicine Expert Rev Respir Med; 2017 [cited 2020 Dec 2].Available from: https://pubmed.ncbi.nlm.nih.gov/28891372/?utm_source=gquery&utm_medium=referral&utm_campaign=CitationSensor?otool=None.

7. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, Napolitano LM, O'Grady NP, Bartlett JG, Carratalà J, El Solh AA, Ewig S, Fey PD, File TM, Restrepo MI, Roberts JA, Waterer GW, Cruse P, Knight SL, Brozek JL. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin. Infect. Dis.* 2016; 63: e61–e111.

8. Lisboa T, Seligman R, Diaz E, Rodriguez A, Teixeira PJZ, Rello J. C-reactive protein correlates with bacterial load and appropriate antibiotic therapy in suspected ventilator-associated pneumonia. *Critical Care Medicine* 2008; 36: 166–171.

9. Moreno MS, Nietmann H, Matias CM, Lobo SM. C-reactive protein: A tool in the follow-up of nosocomial pneumonia. *Journal of Infection* 2010; 61: 205–211.

10. Huang H-B, Peng J-M, Weng L, Wang C-Y, Jiang W, Du B. Procalcitonin-guided antibiotic therapy in intensive care unit patients: a systematic review and meta-analysis. *Annals of Intensive Care* 2017; 7: 114.

11. Zilahi G, McMahon M, Povoa P, Martin-Loeches I. Duration of antibiotic therapy in the intensive care unit. *Journal of Thoracic Disease* [Internet] AME Publishing Company; 2016 [cited 2020 Dec 2]; 8Available from: http://jtd.amegroups.com/article/view/11132.

12. Guillamet CV, Kollef MH. Update on ventilator-associated pneumonia. *Current Opinion in Critical Care* 2015; 21: 430–438.

13. Kollef MH, Bassetti M, Francois B, Burnham J, Dimopoulos G, Garnacho-Montero J, Lipman J, Luyt C-E, Nicolau DP, Postma MJ, Torres A, Welte T, Wunderink RG. The intensive care medicine research agenda on multidrug-resistant bacteria, antibiotics, and stewardship. *Intensive Care Med* 2017; .

14. Ioanas M, Ferrer M, Cavalcanti M, Ferrer R, Ewig S, Filella X, de la Bellacasa JP, Torres A. Causes and predictors of nonresponse to treatment of intensive care unit–acquired pneumonia*. *Critical Care Medicine* 2004; 32: 938–945.

15. Timsit J-F, de Kraker MEA, Sommer H, Weiss E, Bettiol E, Wolkewitz M, Nikolakopoulos S, Wilson D, Harbarth S, on behalf of the COMBACTE-NET consortium. Appropriate endpoints for evaluation of new antibiotic therapies for severe infections: a perspective from COMBACTE's STAT-Net. *Intensive Care Med* 2017; 43: 1002–1012.

16. Torres A, Zhong N, Pachl J, Timsit J-F, Kollef M, Chen Z, Song J, Taylor D, Laud PJ, Stone GG, Chow JW. Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomised, double-blind, phase 3 non-inferiority trial. *Lancet Infect Dis* 2018; 18: 285–295.

17. Kollef MH, Nováček M, Kivistik Ü, Réa-Neto Á, Shime N, Martin-Loeches I, Timsit J-F, Wunderink RG, Bruno CJ, Huntington JA, Lin G, Yu B, Butterton JR, Rhee EG. Ceftolozane–tazobactam versus meropenem for treatment of nosocomial pneumonia (ASPECT-NP): a randomised, controlled, double-blind, phase 3, non-inferiority trial. *The Lancet Infectious Diseases* 2019; 19: 1299–1311.

18. Fàbregas N, Ewig S, Torres A, El-Ebiary M, Ramirez J, de La Bellacasa JP, Bauer T, Cabello H. Clinical diagnosis of ventilator associated pneumonia revisited: comparative validation using immediate post-mortem lung biopsies. *Thorax* 1999; 54: 867–873.

19. Luna CM, Blanzaco D, Niederman MS, Matarucco W, Baredes NC, Desmery P, Palizas F, Menga G, Rios F, Apezteguia C. Resolution of ventilator-associated

pneumonia: prospective evaluation of the clinical pulmonary infection score as an early clinical predictor of outcome. *Crit. Care Med.* 2003; 31: 676–682.

20. Ferrer M, Difrancesco LF, Liapikou A, Rinaudo M, Carbonara M, Li Bassi G, Gabarrus A, Torres A. Polymicrobial intensive care unit-acquired pneumonia: prevalence, microbiology and outcome. *Crit Care* [Internet] 2015 [cited 2018 Jun 26]; 19Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4699341/.

21. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 2012; 18: 268–281.

22. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit. Care Med.* 1985; 13: 818–829.

23. Gall J-RL, Lemeshow S, Saulnier F. A New Simplified Acute Physiology Score (SAPS II) Based on a European/North American Multicenter Study. *JAMA* American Medical Association; 1993; 270: 2957–2963.

24. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996; 22: 707–710.

25. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent J-L, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; 315: 801–810.

26. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. *JAMA* 2012; 307: 2526–2533.

27. Hosmer D and Lemeshow S. Applied logistic regression. New York: Wiley; 1989.

28. Collet D. Modelling Binary Data. Second Edition. Chapman & Hall/CRC Texts in Statistical Science; 1991.

29. Collet D. Modelling Survival Data in Medical Research. 2nd edition. London; 1994.

30. Torres A, Lee N, Cilloniz C, Vila J, Van der Eerden M. Laboratory diagnosis of pneumonia in the molecular age. *Eur Respir J* 2016; 48: 1764–1778.

31. Montravers P, Fagon J-Y, Chastre J, Lecso M, Dombret MC, Trouillet J-L, Gibert C. Follow-up Protected Specimen Brushes to Assess Treatment in Nosocomial Pneumonia. *Am Rev Respir Dis* American Thoracic Society - AJRCCM; 1993; 147: 38–44.

32. A'court CHD, Garrard CS, Crook D, Bowler I, Conlon C, Peto T, Anderson E. Microbiological lung surveillance in mechanically ventilated patients, using non-directed bronchial lavage and quantitative culture. *QJM* Oxford Academic; 1993; 86: 635–648.

33. Dennesen PJW, van der VEN AJ a. M, Kessels AGH, Ramsay G, Bonten MJM. Resolution of Infectious Parameters after Antimicrobial Therapy in Patients with Ventilator-associated Pneumonia. *Am J Respir Crit Care Med* American Thoracic Society - AJRCCM; 2001; 163: 1371–1375.

34. Prats E, Dorca J, Pujol M, Garcia L, Barreiro B, Verdaguer R, Gudiol F, Manresa F. Effects of antibiotics on protected specimen brush sampling in ventilator-associated pneumonia. *Eur Respir J* 2002; 19: 944–951.

Supplementary

Prediction of ventilator-associated pneumonia outcomes according to the early microbiological response: a retrospective observational study.

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METHODS

Procedures and definitions

VAP was clinically suspected in ICU patients if they had been mechanically ventilated for at least 48 hours and developed new or progressive radiological pulmonary infiltrates together with either or both of the following:

- at least two signs from among a temperature >38°C or <36°C, leukocytosis >12,000/mm^3 or leukopenia <4,000/mm^3, and purulent respiratory secretions [1, 2];
- A Simplified Clinical Pulmonary Infectious Score (sCPIS) of >6 points [3, 4].

Early-onset VAP was defined if these occurred within the first 4 days of mechanical ventilation [1].

Initial empiric antimicrobial treatment was administered at the discretion of the attending physician, based on local adaptation of current guidelines, the most frequently isolated pathogens, and patterns of antimicrobial sensitivity. When cultures results became available, appropriate modifications were made to antibiotic therapy based on pathogen identification and sensitivity testing.

Data collection, evaluation, and microbiological diagnosis

Demographic data included age, gender, weight, height, body surface area, reason for ICU admission, alcohol and smoking use, and comorbidities. We also recorded any empirical antimicrobial treatments and subsequent changes.

Statistical analysis

We report numbers and percentages for categorical variables, and the median and first and third quartiles for continuous variables (not normally distributed data). Categorical variables were compared using the chi-square test. Two continuous variables were compared using the Mann_whitney test. Three continuous variables were compared using the Kruskal–Wallis test, and if significant overall, post-hoc pairwise comparisons were conducted via the Bonferroni test to control for the experiment-wise error rate.

Logistic regression analyses [5] were used to examine the association between superinfection and risk factors. Each risk factor was first tested individually (age, sex, smoking habit, alcohol abuse, previous corticosteroids use, previous antibiotic use, ≥5 days of previous hospitalization, previous respiratory isolation, diabetes mellitus, chronic renal failure, solid cancer, chronic heart diseases, chronic lung diseases, chronic liver diseases, APACHE II score at ICU admission, SAPS II score at ICU admission, SOFA score at ICU admission, causes of ICU admission, days of MV before VAP, Late onset VAP,

CPIS at VAP diagnosis, SOFA score at VAP diagnosis, temperature at VAP diagnosis, multilobar at VAP diagnosis, ARDS at VAP diagnosis, pleural effusion at VAP diagnosis, shock at VAP diagnosis, fever at VAP diagnosis, creatinine at VAP diagnosis, hemoglobin at VAP diagnosis, white blood cell count at VAP diagnosis, lymphocytes at VAP diagnosis, C-reactive protein at VAP diagnosis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella spp.*, *Enterobacter spp.*, *Proteus spp.*, *Serratia spp.*, *Aspergillus spp.*, *Streptococcus pneumoniae*, *Escherichia coli*, *Stenotrophomonas maltophilia*, virus, and initial appropriate treatment), before all risk factors that showed associations in the univariate model ($p<0.10$) were added to the multivariable model. Finally, a backward stepwise selection (likelihood ratio) ($p_{in}<0.05$, $p_{out}>0.10$) was used to determine factors associated with superinfection [6]. We then calculated the odds ratios (ORs) and their 95% confidence intervals (CIs). Multicollinearity was confirmed by calculating the variance inflation factor. The Hosmer–Lemeshow goodness-of-fit test was performed to assess the overall fit of the final model. The area under the receiver operating characteristic curve (AUC) of the multivariable model was calculated.

Cox proportional hazards regression analyses [7] were performed to determine the effect of superinfection on 28-day mortality, both crude and adjusted for potential confounders (i.e., APACHE II score at ICU admission, change in SOFA score from VAP diagnosis to day 3, C-reactive protein at VAP diagnosis, and initial appropriate treatment). We calculated the hazard ratios and their 95% CIs. Proportional hazards assumptions were tested with log-minus-log plots. Any lack of fit of our final model was evaluated by deviance residuals.

To measure possible overfitting and instability of selection variables in the final models, we performed internal validation using ordinary non-parametric bootstrapping with 1,000 bootstrap samples and bias-corrected, accelerated 95% CIs [8].

A two-sided p value <0.05 was considered statistically significant. All analyses were performed with IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

eTable 1. Demographic and baseline characteristics of patients at ICU admission					
	Group 1	**Group 2**	**Group 3**	***Group 4***	
Variable	**Persistence (n = 67)**	**Superinfection (n = 25)**	**Eradication (n = 65)**	***Non-microbiologic assessed* (n = 93)**	***p* value**
Age (years), median (Q1; Q3)	65 (53; 72)	56 (48; 74)	61 (45; 74)	63 (55; 74)	0.416
Male sex, n (%)	50 (75)	15 (60)	46 (71)	63 (68)	0.555
Current or former smoking habit, n (%)	30 (46)	13 (52)	40 (62)	46 (49)	0.291
Current or former alcohol abuse, n (%)	18 (27)	5 (20)	17 (26)	17 (18)	0.503
Previous corticosteroids use, n (%)	3 (5)	4 (17)	5 (8)	9 (11)	0.385
Previous antibiotic use, n (%)	52 (78)	18 (72)	55 (85)	76 (82)	0.515
≥ 5 days of previous hospitalization, n (%)	44 (66)	13 (52)	47 (72)	64 (69)	0.312
Previous respiratory isolation, n (%)	28 (42)	8 (32)	34 (52)	32 (34)	0.113
Comorbidities, n (%)					
Diabetes mellitus	16 (24)	1 (4)	14 (22)	23 (25)	0.150
Chronic renal failure	6 (9)	0 (0)	3 (5)	12 (13)	0.113
Solid cancer	13 (19)	3 (12)	4 (6)	9 (10)	0.102
Chronic heart diseases	21 (31)	6 (24)	16 (25)	36 (39)	0.230
Chronic lung diseases	19 (28)	7 (28)	26 (40)	23 (25)	0.216
COPD	13 (19)	6 (24)	17 (26)	14 (15)	0.358

eTable 1. Demographic and baseline characteristics of patients at ICU admission					
	Group 1	**Group 2**	**Group 3**	***Group 4***	
Variable	**Persistence (n = 67)**	**Superinfection (n = 25)**	**Eradication (n = 65)**	***Non-microbiologic assessed* (n = 93)**	***p* value**
Chronic liver diseases	9 (13)	2 (8)	9 (14)	18 (20)	0.474
APACHE II score, median (Q1; Q3)	16 (12; 21)	17 (13; 19)	16 (12; 21)	17 (14; 24)	0.307
SAPS II score, median (Q1; Q3)	43 (36; 52)	40 (34; 51)	38 (28; 46)	40 (31; 51)	0.413
SOFA score, median (Q1; Q3)	7 (5; 10)	7 (5; 9)	7 (6; 10)	8 (6; 10)	0.279
Causes of ICU admission, n (%)					
Hypercapnic respiratory failure	3 (5)	4 (16)	8 (13)	10 (11)	0.282
Hypoxemic respiratory failure	6 (9)	2 (8)	3 (5)	9 (10)	0.706
Acute coronary syndrome	1 (2)	0 (0)	5 (8)	4 (5)	0.200
Polytrauma	9 (13)	0 (0)	11 (18)	5 (5)	**0.024**
Postoperative	13 (19)	4 (16)	12 (19)	21 (23)	0.846
Cardiac arrest	3 (5)	5 (20)	4 (6)	8 (9)	0.101
Decreased consciousness	14 (21)	4 (16)	14 (22)	15 (16)	0.778
Shock	8 (12)	3 (12)	3 (5)	11 (12)	0.439
Nonsurgical abdominal disease	2 (3)	2 (8)	0 (0)	5 (6)	0.196
Others	8 (12)	1 (4)	3 (5)	3 (4)	0.131

Abbreviations: APACHE II score = Acute Physiology And Chronic Health Evaluation II score; COPD = chronic obstructive pulmonary disease; ICU = intensive care unit; Q1 = first quartile; Q3 = third quartile; SAPSII = simplified acute physiology score II; SOFA = sequential organ failure assessment.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the persistence group and the non-microbiologic assessed group.

[d] p <0.05 for comparison between the superinfection group and the eradication group.

[e] p <0.05 for comparison between the superinfection group and the non-microbiologic assessed group.

[f] p <0.05 for comparison between the eradication group and the non-microbiologic assessed group.

e**Table 2. Patients characteristics at VAP diagnosis**					
	Group 1	**Group 2**	**Group 3**	***Group 4***	
Variable	**Persistence (n = 67)**	**Superinfection (n = 25)**	**Eradication (n = 65)**	***Non-microbiologic assessed* (n = 93)**	***p* value**
Days of MV before VAP, median (Q1; Q3)	5 (3; 10)	5 (3; 9)	6 (4; 13)	5 (3; 8)	0.181
Late onset VAP, n (%)	51 (77)	18 (72)	54 (83)	60 (68)	0.191
Severity assessment of pneumonia					
CPIS, median (Q1; Q3)	6 (5; 7)	6 (6; 7)	6 (6; 8)	7 (6; 7)	0.365
SOFA score, median (Q1; Q3)	7 (5; 10)	9 (7; 11)	7 (5; 9)	8 (5; 11)	0.184
Temperature (˚C), median (Q1; Q3)	37.7 (36.2; 38.0)	36.0 (35.4; 37.6)	37.6 (36.2; 38.2)	37.0 (35.4; 38.0)	**0.033**
Temperature < 36˚C, n (%)	15 (23)	12 (48)	13 (20)	31 (34)	**0.023**[d]
Multilobar pneumonia, n (%)	24 (36)	11 (44)	24 (37)	46 (49)	0.272
Presence of ARDS, n (%)	5 (8)	4 (16)	9 (14)	18 (20)	0.211
Pleural effusion, n (%)	17 (26)	6 (27)	17 (27)	31 (34)	0.690
Shock at pneumonia diagnosis, n (%)	28 (42)	18 (72)	27 (42)	45 (49)	0.053
Laboratory variables, median (Q1; Q3)					
Creatinine (mg/dL)	0.9 (0.7; 1.2)	0.7 (0.6; 1.7)	1.0 (0.7; 1.5)	1.0 (0.7; 1.9)	0.329
Hemoglobin (g/dL)	10.1 (9.5; 11.2)	10.6 (9.7; 12.0)	10.5 (9.2; 11.7)	9.8 (9.0; 11.5)	0.301
White blood cell count (10^9 cells/L)	10.7 (8.5; 16.3)	12.6 (8.9; 16.0)	12.0 (9.2; 17.2)	13.2 (9.4; 17.8)	0.461
Lymphocytes (n/mm^3)	827 (609; 1177)	743 (410; 1061)	963 (718; 1306)	851 (586; 1376)	0.497
C-reactive protein (mg/L)	11.8 (6.6; 19.5)	14.1 (4.9; 20.0)	13.2 (5.2; 23.7)	11.9 (6.4; 19.3)	0.966
Procalcitonin (ng/mL)	0.3 (0.1; 0.7)	0.5 (0.1; 4.7)	0.3 (0.1; 0.9)	0.4 (0.1; 1.5)	0.479
PaO_2/FiO_2 (mmHg)	233 (178; 283)	176 (147; 265)	176 (140; 236)	200 (152; 256)	**0.017**[b]

Abbreviations: ARDS = acute respiratory distress syndrome; CPIS = clinical pulmonary infection score; Q1 = first quartile; Q3 = third quartile; MV = mechanical ventilation; PaO_2/FiO_2 = ratio of arterial oxygen tension to inspired oxygen fraction; SOFA = sequential organ failure assessment; VAP = ventilator-associated pneumonia.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the persistence group and the non-microbiologic assessed group.

[d] p <0.05 for comparison between the superinfection group and the eradication group.

[e] p <0.05 for comparison between the superinfection group and the non-microbiologic assessed group.

[f] p <0.05 for comparison between the eradication group and the non-microbiologic assessed group.

e**Table 3. Patients characteristics 3 days after VAP diagnosis**					
	Group 1	**Group 2**	**Group 3**	***Group 4***	
Variable	**Persistence (n = 67)**	**Superinfection (n = 25)**	**Eradication (n = 65)**	***Non-microbiologic assessed* (n = 93)**	***p* value**
Severity assessment, median (Q1; Q3)					
CPIS	6 (4; 7)	6 (6; 7)	6 (4; 7)	6 (4; 7)	0.543
SOFA score	6 (4; 9)	8 (7; 10)	7 (4; 9)	7 (4; 10)	0.079
SOFA changes from day 1 at day 3	0 (-2; 0)	0 (-1; 1)	0 (-1; 0)	0 (-2; 1)	0.833
Temperature (˚C)	37.1 (36.0; 38.0)	35.7 (35.2; 37.1)	37.2 (36.3; 37.8)	37.0 (36.0; 37.5)	**0.003**[ad]
Temperature < 36˚C, n (%)	14 (22)	14 (56)	11 (17)	20 (23)	**0.001**[adf]
Laboratory variables, median (Q1; Q3)					
C-reactive protein (mg/L)	10.4 (5.3; 16.9)	12.8 (5.4; 26.1)	10.9 (2.8; 19.0)	11.3 (5.4; 19.8)	0.740
Procalcitonin (ng/mL)	0.2 (0.1; 0.6)	0.5 (0.1; 2.8)	0.1 (0.1; 0.6)	0.6 (0.1; 1.2)	0.096
PaO_2/FiO_2 (mmHg)	255 (176; 306)	225 (188; 272)	221 (151; 285)	222 (156; 266)	0.223

Abbreviations: CPIS = clinical pulmonary infection score; Q1 = first quartile; Q3 = third quartile; SOFA = sequential organ failure assessment; PaO_2/FiO_2 = ratio of arterial oxygen tension to inspired oxygen fraction; VAP = ventilator-associated pneumonia.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the persistence group and the non-microbiologic assessed group.

[d] p <0.05 for comparison between the superinfection group and the eradication group.

[e] p <0.05 for comparison between the superinfection group and the non-microbiologic assessed group.

[f] p <0.05 for comparison between the eradication group and the non-microbiologic assessed group.

eTable 4. Outcomes					
	Group 1	**Group 2**	**Group 3**	***Group 4***	
Variable	**Persistence (n = 67)**	**Superinfection (n = 25)**	**Eradication (n = 65)**	***Non-microbiologic assessed* (n = 93)**	***p* value**
ERS/ESICM/ESCMID/ALAT guidelines adherence, n (%)	44 (70)	12 (50)	40 (67)	33 (61)	0.339
Initial appropriate treatment, n (%)	54 (83)	18 (75)	54 (90)	47 (87)	0.326
Treatment failure on day 3, n (%)	44 (66)	18 (72)	37 (57)	39 (42)	**0.006**[ce]
Days of MV, median (Q1; Q3)	14 (9; 24)	22 (13; 43)	18 (13; 27)	10 (6; 16)	**<0.001**[cef]
Ventilator-free-days, median (Q1; Q3)	9 (0; 22)	0 (0; 12)	7 (0; 20)	18 (0; 24)	**0.001**[ae]
ICU length of stay, median (Q1; Q3)	20 (13; 32)	24 (17; 44)	24 (15; 35)	15 (10; 23)	**<0.001**[cef]
ICU mortality, n (%)	14 (21)	13 (52)	20 (31)	28 (30)	**0.038**[a]
28-days mortality, n (%)	16 (24)	11 (44)	18 (28)	19 (20)	0.111
90-days mortality, n (%)	23 (34)	16 (64)	25 (40)	33 (37)	0.062

Abbreviations: ERS = European Respiratory Society; Q1 = first quartile; Q3 = third quartile; ICU = intensive care unit; MV = mechanical ventilation.

[a] p <0.05 for comparison between the persistence group and the superinfection group.

[b] p <0.05 for comparison between the persistence group and the eradication group.

[c] p <0.05 for comparison between the persistence group and the non-microbiologic assessed group.

[d] p <0.05 for comparison between the superinfection group and the eradication group.

[e] p <0.05 for comparison between the superinfection group and the non-microbiologic assessed group.

[f] p <0.05 for comparison between the eradication group and the non-microbiologic assessed group.

eTable 5. Significant univariate and multivariable regression analyses for superinfection (n = 147)

Variable	Univariate			Multivariable [a]		
	OR	**95% CI**	***p* value**	**OR**	**95% CI**	***p* value**
Diabetes mellitus	0.14	0.02 to 1.09	0.061	-	-	-
SOFA score at VAP diagnosis (+1 point)	1.13	0.99 to 1.28	0.060	-	-	-
Temperature at VAP diagnosis (+1°C)	0.65	0.46 to 0.91	0.011	0.67	0.48 to 0.95	0.025
Shock at VAP diagnosis	3.55	1.39 to 9.09	0.008	3.43	1.25 to 9.40	0.017
S. aureus	2.36	0.95 to 5.88	0.064	2.87	1.06 to 7.75	0.038
Aspergillus	5.81	1.10 to 30.74	0.038	-	-	-

Abbreviations: CI = confidence interval; OR = Odds Ratio; SOFA = sequential organ failure assessment.

[a] Hosmer–Lemeshow goodness-of-fit test, p=0.52.

eTable 6. Internal validation of the multivariable regression model for superinfection using non-parametric bootstrap technique

Variable	Original	Bias	SE	95% BCa CI	*p* value
Temperature at VAP diagnosis (°C)	-0.397	-0.012	0.190	-0.774 to 0.700	0.021
Shock at VAP diagnosis	1.232	0.108	0.839	0.220 to 2.909	0.008
S. aureus	1.054	0.016	0.571	-0.087 to 2.250	0.041

Abbreviations: BCa = adjusted bootstrap confidence interval; CI = confidence interval; SE = standard error; SOFA = sequential organ failure assessment.

eTable 7. Causes of treatment failure

	Group 1	Group 2	Group 3
Variable n (%)	**Persistence (n = 67)**	**Superinfection (n = 25)**	**Eradication (n = 65)**
No treatment failure	23 (34)	7 (28)	28 (43)
No improvement of Pao2/Fio2	12 (18)	6(24)	8 (13)
Persistence of fever or hypothermia with purulent respiratory secretions	11 (17)	6 (24)	7 (11)
Greater than or equal to 50% increase in radiographic infiltrates.	2 (3)	1 (4)	2 (3)
Occurrence of septic shock or multiple organ dysfunction syndrome	2 (3)	0	4 (6)
No improvement of Pao2/Fio2 plus Persistence of fever or hypothermia with purulent respiratory secretions	10 (15)	4 (16)	7 (11)
No improvement of Pao2/Fio2 plus greater than or equal to 50% increase in radiographic infiltrates.	2 (3)	7 (28)	1 (2)
No improvement of Pao2/Fio2 plus occurrence of septic shock or multiple organ dysfunction syndrome	1 (2)	0	2 (3)
Persistence of fever or hypothermia with purulent respiratory secretions plus greater than or equal to 50% increase in radiographic infiltrates.	1 (2)	1 (4)	0
More than two causes	2 (3)	0	4 (6)
Death	1 (2)	0	1 (2)

eTable 8. Univariate and multivariable Cox regression analyses for 28-day mortality (n = 136)

Variable	Univariate			Multivariable		
	HR	**95% CI**	***p* value**	HR	**95% CI**	***p* value**
Superinfection at day 3	1.92	0.97 to 3.79	0.061	2.39	1.16 to 4.92	0.018
APACHE II score at ICU admission (+1 point)	0.98	0.94 to 1.03	0.492	0.99	0.94 to 1.04	0.612
Change in SOFA score from VAP diagnosis to day 3 (+1 point)	1.17	1.01 to 1.35	0.041	1.15	0.97 to 1.37	0.110
C-reactive protein at VAP diagnosis (+1 mg/L)	1.02	0.99 to 1.05	0.213	1.03	0.99 to 1.06	0.132
Initial appropriate antibiotic therapy	0.80	0.37 to 1.73	0.577	1.21	0.50 to 2.95	0.674

Abbreviations: APACHE II score = Acute Physiology And Chronic Health Evaluation II score; CI = confidence interval; HR = hazard ratio; SOFA = sequential organ failure assessment; VAP = ventilator-associated pneumonia.

eTable 9. Internal validation of the multivariable Cox regression model for 28-day mortality using non-parametric bootstrap technique

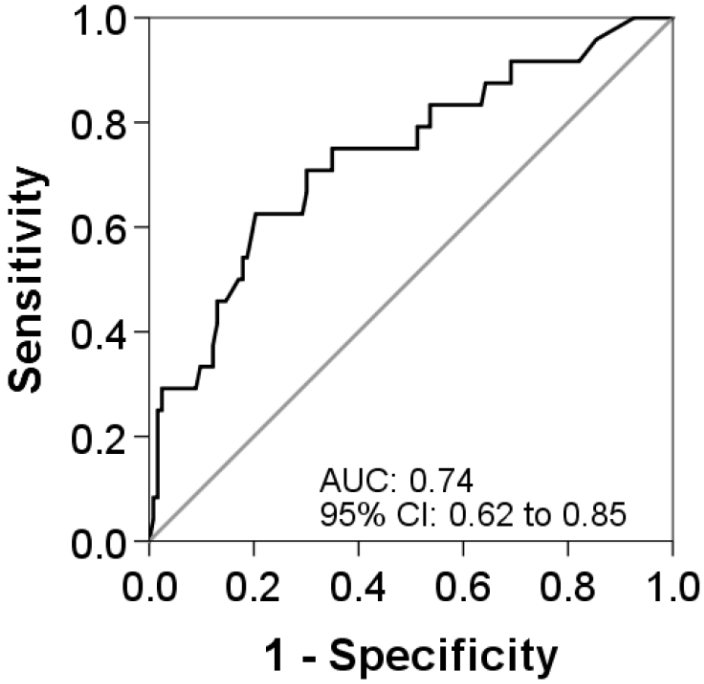
Variable	Original	Bias	SE	95% BCa CI	*p* value
Superinfection at day 3	0.871	0.016	0.389	0.046 to 1.674	0.011
APACHE II score at ICU admission	-0.013	0.000	0.022	-0.060 to 0.030	0.530
Change in SOFA score from VAP diagnosis to day 3	0.141	-0.007	0.084	-0.015 to 0.291	0.079
C-reactive protein at VAP diagnosis (mg/L)	0.026	-0.002	0.018	-0.008 to 0.056	0.127
Initial appropriate antibiotic therapy	0.191	0.051	0.670	-1.053 to 1.940	0.677

Abbreviations: APACHE II score = Acute Physiology And Chronic Health Evaluation II score; BCa = adjusted bootstrap confidence interval; CI = confidence interval; ICU = intensive care unit; SE = standard error; SOFA = sequential organ failure assessment; VAP = ventilator-associated pneumonia.

eTable 10 Comparisons of outcomes between patients with eradication and without eradication.

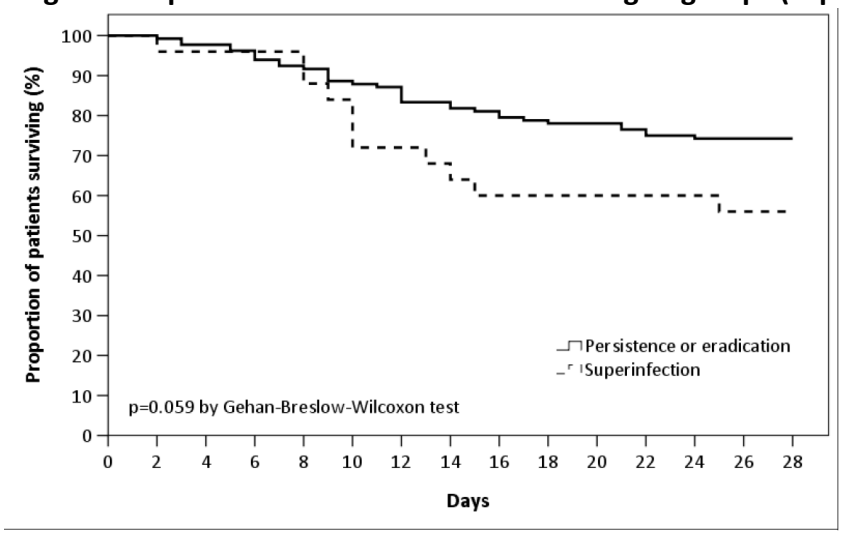
	Group 2	**Group 3**	
Variable	**Superinfection + Persistence + *Non-microbiologic assessed*** **(n = 185)**	**Eradication (n = 65)**	***p* value**
ERS/ESICM/ESCMID/ALAT guidelines adherence, n (%)	89 (63)	40 (67)	0.631
Initial appropriate treatment, n (%)	119 (83)	54 (90)	0.214
Treatment failure on day 3, n (%)	101 (55)	37 (57)	0.745
Days of MV, median (Q1; Q3)	12 (8; 22)	18 (13; 27)	**0.003**
Ventilator-free-days, median (Q1; Q3)	13 (8; 22)	7 (0; 20)	0.221
ICU length of stay, median (Q1; Q3)	17 (12; 29)	24 (15; 35)	**0.009**
ICU mortality, n (%)	55 (30)	20 (31)	0.875
28-days mortality, n (%)	46 (25)	18 (28)	0.653
90-days mortality, n (%)	72 (40)	25 (40)	0.916

eFigure 1. ROC curve analysis of the multivariable regression model for superinfection



Abbreviations: AUC = area under the curve; CI = confidence interval; ROC = receiver operating characteristic.

eFigure 2 Kaplan Maier survival curve according to groups (superinfection vs others)



References

1. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, Kollef M, Li Bassi G, Luna CM, Martin-Loeches I, Paiva JA, Read RC, Rigau D, Timsit JF, Welte T, Wunderink R. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). *Eur. Respir. J.* 2017; 50.

2. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, Napolitano LM, O'Grady NP, Bartlett JG, Carratalà J, El Solh AA, Ewig S, Fey PD, File TM, Restrepo MI, Roberts JA, Waterer GW, Cruse P, Knight SL, Brozek JL. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 2016; 63: e61–e111.

3. Larsson J, Itenov TS, Bestle MH. Risk prediction models for mortality in patients with ventilator-associated pneumonia: A systematic review and meta-analysis. *J. Crit. Care* 2017; 37: 112–118.

4. Luna CM, Blanzaco D, Niederman MS, Matarucco W, Baredes NC, Desmery P, Palizas F, Menga G, Rios F, Apezteguia C. Resolution of ventilator-associated pneumonia: prospective evaluation of the clinical pulmonary infection score as an early clinical predictor of outcome. *Crit. Care Med.* 2003; 31: 676–682.

5. Cole TJ. Applied logistic regression. D. W. Hosmer and S. Lemeshow, Wiley, New York, 1989. No. of pages: xiii + 307. Price: £36.00. *Stat. Med.* 1991; 10: 1162–1163.

6. D. Collett. Modelling Binary Data, Second Edition. 1991.

7. Collet D. Modelling Survival Data in Medical Research. 2nd edition. London; 1994.

8. Efron B, Tibshirani RJ. An Introduction to the Bootstrap. CRC Press; 1994.