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Timing of Tracheostomy and Risk of Ventilator-Associated Pneumonia in Patients Requiring Prolonged Mechanical Ventilation: A Case–Cohort Study with Time-to-Event Analysis

Marie Nicoline Ordaz-Kücks [1], Iván Alejandro Arteaga-Martínez [2], Hugo Alfredo Funes-González [1], Fernando Martín Guerra-Infante [3], Roberto Montes de Oca-Jiménez [4,5], Martha Elba Ruiz-Riva-Palacio [5], Javier Morales-Fabian [1], Enrique Rojano-Lastra [1], Heberto Miranda-Hernández [1], José Carlos Aguilar-Carrasco [6,*] and Gabriel Arteaga-Troncoso [6,*]

[1] Programa Académico de la Especialidad en Epidemiología, Hospital General del ISSSTE "Dra. Matilde Petra Montoya Lafragua", Mexico City, Mexico 13200

[2] Escuela Superior de Medicina, Instituto Politécnico Nacional, Mexico City, Mexico 11340

[3] Departamento de Fisiología y Desarrollo Celular, Instituto Nacional de Perinatología "Isidro Espinosa de los Reyes", Mexico City, Mexico 11000

[4] Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma del Estado de México, Toluca, Estado de México 56200, México

[5] Plantel Sor Juana Inés de la Cruz. Universidad Autónoma del Estado de México, Amecameca, Estado de México 56900

[6] Laboratorio de Farmacología Experimental, Instituto Nacional de Perinatología "Isidro Espinosa de los Reyes", Mexico City, Mexico 11000

* Correspondence: jcacpharma18@gmail.com (J.C.A.-C.); arteagagabriel02@gmail.com (G.A.-T.); Tel. +52-5555209900 ext.542 (J.C.A.-C.); +52-722 592 5681 (G.A.-T.)

Abstract

Background/Objectives: Intubation and tracheostomy were previously considered distinct approaches to airway management during mechanical ventilation. Ventilator-associated pneumonia (VAP) remains a leading cause of morbidity and mortality in patients requiring prolonged mechanical ventilation. The role of tracheostomy in modifying VAP risk is controversial, especially when taking into account how exposure changes over time and the conditions typically found in real intensive care unit (ICU) settings. This study was conducted to evaluate whether tracheostomy timing influences the VAP risk and hospital length of stay in patients undergoing prolonged mechanical ventilation. **Methods**: We conducted a hybrid case–cohort study in a tertiary-care ICU in Mexico City, enrolling patients receiving invasive mechanical ventilation for ≥ 48 h (January–December 2023). Patients undergoing a tracheostomy were compared with an age- and sex-matched subcohort of intubated patients. VAP incidence was evaluated using cumulative incidence and incidence density. Multivariable generalized linear models, Kaplan–Meier survival analysis, and Cox regression were used to identify risk factors and assess time-to-event outcomes. **Results**: A total of 218 patients were included (55 tracheostomies vs. 163 intubations). The incidence density of VAP was similar between groups (31.5 vs. 30.3 per 1000 ventilator-days; RR 1.04, 95% CI 0.7–1.7). However, cumulative incidence was higher in tracheostomized patients (61.8% vs. 22.7%; RR 2.7, 95% CI 1.9–3.9), reflecting prolonged exposure. Independent risk factors included broad-spectrum antibiotics, mechanical ventilation ≥ 5 days, chronic pulmonary disease, and ICU stay. In contrast, tracheostomy was associated with a lower time-dependent hazard of VAP (HR 0.43, 95% CI 0.25–0.75). Gram-negative microorganisms predominated, with higher antimicrobial resistance in tracheostomized patients. A class-based analysis showed that MDR was primarily driven by *E. coli*, with consistent resistance to cephalosporins and fluoroquinolones. The MAR index was higher in tracheostomized

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patients (0.50 vs. 0.25), indicating a greater burden of antimicrobial resistance. **Conclusions**: Tracheostomy increases cumulative VAP incidence due to longer exposure but is associated with a reduced time-dependent risk. These findings highlight the importance of accounting for exposure time and support targeted strategies integrating airway management and antimicrobial stewardship to reduce VAP burden in real-world ICU settings.

Keywords: ventilator-associated pneumonia; tracheostomy; mechanical ventilation; intensive care units; time-to-event analysis; case–cohort designs; antimicrobial resistance; gram-negative pathogens; critical care

1. Introduction

Nosocomial infections, also referred to as healthcare-associated infections (HAIs), originate within the hospital setting and remain a major cause of morbidity and mortality, especially in low- and middle-income countries [1]. Recent evidence highlights that hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) represent a continuum of nosocomial lower respiratory tract infections with overlapping pathophysiological mechanisms, microbiological profiles, and diagnostic challenges, reinforcing the need for integrated surveillance and prevention strategies [2].

Data from the International Nosocomial Infection Control Consortium (INICC) reveal the burden of HAIs across the World Health Organization regions, including 45 countries from Latin America, Europe, the Eastern Mediterranean, Southeast Asia, and the Western Pacific, between 2012 and 2017. This analysis identified 16,099 VAP cases, with a pooled mean rate of 14.1 per 1000 ventilator-days in intensive care units [3]. VAP is typically defined as a nosocomial infection occurring after 48 h of mechanical ventilation and is commonly caused by infectious agents such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter sp.*, *Streptococcus pneumoniae,* and *Staphylococcus aureus*, among others [4,5]. Sequential evaluation of tracheostomized patients, particularly those requiring prolonged mechanical ventilation (PMV), is essential for the early detection of VAP.

A tracheostomy is primarily undertaken to facilitate weaning from mechanical ventilation, ensure airway protection, and mitigate complications associated with prolonged endotracheal intubation [6]. Nevertheless, uncertainty persists regarding whether an early or late tracheostomy confers superior clinical benefits, particularly in relation to the risk of VAP when accounting for time-dependent exposure and competing clinical outcomes [7]. Despite advances in infection prevention and control strategies, VAP continues to be a major cause of morbidity and mortality among critically ill patients. Its occurrence is associated with a prolonged hospital stay, delayed extubation, increased utilization of healthcare resources, and the emergence of antimicrobial resistance factors, which have consistently been emphasized in recent comprehensive reviews of HAP/VAP [2,8]. Optimizing airway management strategies to improve patient safety and reduce the incidence of VAP remains a key priority in the prevention of HAIs.

Several studies have shown that tracheostomy may influence the duration of mechanical ventilation in critically ill patients, including those with acute respiratory distress syndrome (ARDS), COVID-19, and sepsis [9,10]. It has also been suggested that tracheostomy could reduce the risk of VAP by facilitating improved oral hygiene and decreasing oropharyngeal colonization by potentially pathogenic microorganisms [11]. However, the available evidence remains inconsistent across heterogeneous ICU populations and real-world clinical settings. While some observational studies and meta-analyses suggest that earlier tracheostomy may reduce the incidence of VAP and duration of mechanical ventilation, others have reported no significant association [12–16]. These discrepancies likely reflect methodological limitations, particularly the inadequate handling of time-dependent exposure to mechanical ventilation and tracheostomy, which may introduce time-at-risk bias and distort the observed relationship with VAP. Addressing this gap is essential for generating

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clinically meaningful evidence that can inform decision-making and optimize patient outcomes in critical care.

Therefore, this study was conducted to evaluate whether the timing of tracheostomy influences VAP risk and hospital length of stay in patients requiring prolonged mechanical ventilation.

2. Materials and Methods

2.1. Study Design and Setting

An observational study with a hybrid case–cohort design was conducted as part of the academic program at the Hospital General "Dra. Matilde Petra Montoya Lafragua", Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE), located in Mexico City, Mexico. The study evaluated time-to-event outcomes for VAP in patients requiring prolonged mechanical ventilation, comparing those who underwent a tracheostomy with patients managed exclusively with endotracheal intubation.

2.2. Ethical Considerations

This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. Ethical principles outlined in the Declaration of Helsinki and applicable national regulations for biomedical research and personal data protection were followed. All data were anonymized and handled confidentially. The study protocol was approved by the Institutional Research Ethics Committee (approval number: PR066/18), and the requirement for informed consent was waived due to the retrospective and anonymized nature of the data.

2.3. Study Population and Eligibility Criteria

All patients admitted to the intensive care unit (ICU) between January and December 2023 who required invasive mechanical ventilation for more than 48 h were considered eligible (Figure 1).

Cases were defined as patients who underwent a tracheostomy based on established clinical indications, including upper airway obstruction or persistent respiratory failure requiring prolonged mechanical ventilation. The decision to perform tracheostomy reflected clinical severity and the inability to sustain adequate spontaneous ventilation. Patients were prospectively monitored during hospitalization for the development of VAP. Diagnosis was established in mechanically ventilated patients presenting with (i) new or progressive pulmonary infiltrates on chest radiography; (ii) fever (>38 °C or <36 °C); (iii) leukocytosis or leukopenia; and (iv) at least one of worsening gas exchange, purulent respiratory secretions, or positive culture from respiratory specimens [17]. Patient records were excluded if they met any of the following criteria: (i) absence of radiographic data; (ii) duration of mechanical ventilation < 48 h; or (iii) ICU stay < 72 h. Additionally, records of VAP patients transferred from or referred to other institutions, as well as those diagnosed with non-ventilator-associated pneumonia, were excluded from the analysis.

In patients with pre-existing pulmonary or cardiac disease, the presence of infiltrates on two consecutive chest radiographs was required for diagnosis. Extubation failure was defined as the need for reintubation within 48–72 h after the removal of ventilator support due to the inability to maintain adequate spontaneous breathing.

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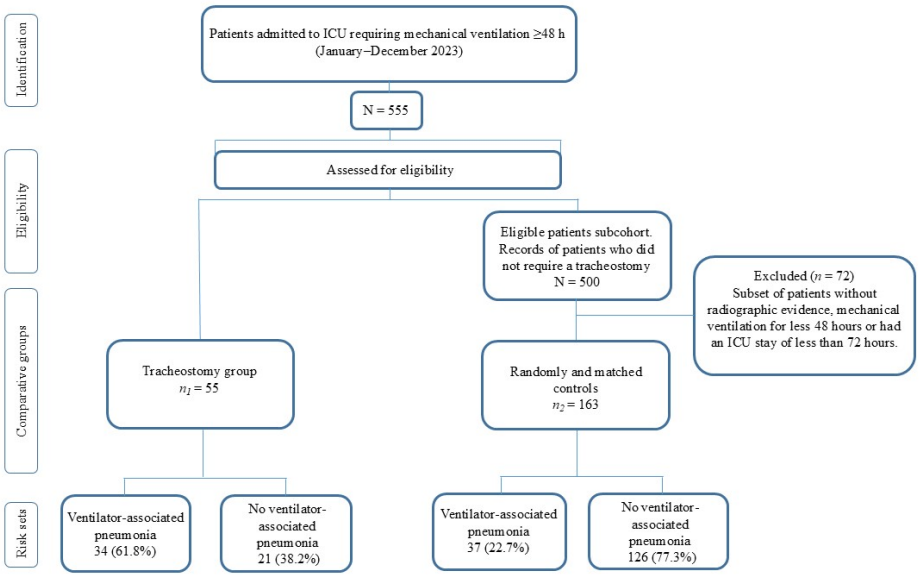

Figure 1. Flowchart of study design and patient selection. A total of 55 patients requiring invasive mechanical ventilation for ≥ 48 h were included. Patients were classified according to airway management into a tracheostomy group (cases) and a subcohort of randomly selected, age- and sex- matched controls without a tracheostomy. Patients were followed during hospitalization for the development of ventilator-associated pneumonia (VAP). A case–cohort design was implemented to optimize statistical efficiency. Outcomes were analyzed using cumulative incidence, incidence density, logistic regression, and time-to-event methods, including Kaplan–Meier and Cox proportional hazards models.

2.4. Case–Cohort Design and Sample Size Calculation

The initial population included 555 patients, of whom 55 (9.9%) underwent a tracheostomy (cases), and 500 (90.1%) records of patients on mechanical ventilation were analyzed. To select the records of patients who served as the control group, the subsample size was calculated based on the following equation:

$$n_2 = \frac{Z^2_{\alpha/2}\left(p_1 q_1 + k p_2 q_2\right)}{k\delta^2}$$

Total population without a tracheostomy (N = 500);
Number of cases (n_1 = 55);
Ratio N/n_1 (k = 9.1);
Expected VAP incidence in tracheostomized patients (p_1 = 0.618);
Expected incidence in intubate patients (p_2 = 0.227);
δ^2 = 0.01;
The minimum required control simple size was estimated at n_2 = 158.

To reduce selection bias and increase statistical power, records of patients were randomly selected and matched by age and sex to cases, resulting in a final control group of 163 patients [18]. The post hoc statistical power (1 − β) for comparing between groups was 0.99, calculated using G*Power software (version 3.1.9.7).

2.5. Clinical Management

Both groups were managed by the same multidisciplinary team, including physicians, nurses, and respiratory therapists, under standardized institutional protocols. Researchers were not involved

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in direct patient care. This approach was implemented to minimize variability in clinical management and reduce potential confounding related to treatment differences.

2.6. Antimicrobial Susceptibility Testing

In cases of clinical suspicion of VAP, antimicrobial susceptibility testing (AST) was performed on isolates obtained from bronchial aspirate specimens against a panel of 41 antimicrobial agents. Bacterial identification and AST were conducted using the automated bioMérieux mini–API system (bioMérieux, Marcy-I'Étoile, France). Separate antibiograms were generated for Gram-negative and Gram-positive bacteria. Minimum inhibitory concentration (MIC) values were interpreted according to the criteria established by the Clinical and Laboratory Standards Institute [19]. Isolates resistant to more than three different classes of antimicrobials were classified as multidrug-resistant (MDR) according to international consensus definitions [20]. The multiple antibiotic resistance (MAR) index for each isolate was calculated as the ratio of the number of antimicrobials to which the isolate was resistant to the total number of antimicrobials tested [21].

2.7. Statistical Analysis

This study was conducted and reported in accordance with the STROBE statement. Continuous variables are presented as means ± standard deviations (SDs) or medians (interquartile range, IQR), depending on the data distribution. Categorical variables are expressed as frequencies and percentages. A two-tailed p-value of < 0.05 was considered statistically significant. Normality was assessed using the Kolmogorov–Smirnov test. Comparisons between continuous variables were performed using Student's t-test or the Mann–Whitney U test, as appropriate. Categorical variables were compared using Fisher's exact test. Correlations were evaluated using Spearman's rank correlation coefficient.

The risk of VAP was estimated using the cumulative incidence (number of new VAP cases divided by the total number of exposed patients during the study period, multiplied by 1000) and incidence density (number of new VAP cases divided by total ventilator-days, multiplied by 1000).

Univariable logistic regression analysis was conducted to identify factors associated with VAP. Continuous variables were dichotomized for analysis. The variables assessed included age, sex, body mass index (BMI), comorbidities, requirement for a tracheostomy, patient positioning (decubitus), use of sedation, duration of invasive mechanical ventilation, length of hospital stay, and mortality. The results are reported as regression coefficients, odds ratios (ORs), and 95% confidence intervals (95% CIs).

Variables with statistical significance in the univariable analysis were included in the generalized linear model, which was adjusted for age and gender. Adjusted effect estimates were obtained using a generalized linear model with binomial distribution and a complementary log–log link function. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were reported for consistency and interpretability. Model construction was as follows: (i) variables with statistical significance were initially included; (ii) each variable was evaluated using the Wald test ($p < 0.05$), as well as the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC); (iii) variables that lost statistical significance after adjustment were manually removed; and (iv) the process was repeated until a stable final model was achieved.

Time-to-event analysis was performed using Kaplan–Meier curves and compared with the log–rank (Mantel–Cox) test. Cox proportional hazards regression models were constructed to estimate adjusted hazard ratios (aHRs) for the association between the timing of the tracheostomy and the development of VAP. Variables identified as significant in the multivariable logistic regression analysis were considered for inclusion in the Cox model [22,23]. This approach allowed for estimation of the time-to-event (VAP occurrence) while simultaneously adjusting for potential confounders. All statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

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3. Results

3.1. Study Population and Baseline Characteristics

During the study period, 55 patients underwent a tracheostomy between days 5 and 29 after endotracheal intubation (median: 18 days; interquartile range [IQR]: 14–24). The comparative subcohort consisted of 163 patients randomly selected from those who underwent endotracheal intubation during same period (Table 1). Of the 500 initially eligible records, 72 (14.4%) were excluded due to incomplete data related to VAP, including missing radiographic studies, duration of mechanical ventilation < 48 h, or ICU stay > 72 h.

Table 1. Patients' baseline characteristics.

	Overall, *n* = 218	**Tracheostomy Group *n* = 55**	**Endotracheal Intubation Group *n* = 163**	***p*-Value**
Age, years	67 [50–75]	67 [57–75]	67 [44–74]	N.S. [1]
Gender				
Male	124 (56.9)	29 (52.7)	95 (58.3)	N.S. [2]
Female	94 (43.1)	26 (47.3)	68 (41.7)	
Number of underlying medical conditions *				<0.008 [3]
0	43 (19.7)	4 (7.3)	39 (23.9)	
1	51 (23.4)	13 (23.6)	38 (23.3)	
2	124 (56.9)	38 (69.1)	86 (52.8)	
Ventilation length, days	9 [4–14]	18 [14–24]	6 [4–10]	<0.001 [1]
Re-intubation	45 (20.6)	24 (43.6)	21 (12.9)	<0.001 [2]
VAP	71 (32.6)	34 (61.8)	37 (22.7)	<0.001 [2]
Length of ICU stay, days	9 [4–15]	17 [12–22.5]	6 [4–10]	<0.001 [1]
Length of hospital stay, days	19 [12–29.5]	32 [24–39]	16 [10–24]	<0.001 [1]
ICU mortality	118 (54.1)	29 (52.7)	89 (54.6)	N.S. [2]

Data are presented as mean (± SD), number (%) or median [IQR], VAP: ventilator-associated pneumonia, ICU: intensive care unit. [1] Using Mann–Whitney test, $\alpha = 0.05$. [2] Using Fisher's exact test, $\alpha = 0.05$. [3] Using Chi-Square test for trend, $\alpha = 0.05$. * Underlying comorbidities include type 2 diabetes mellitus, systemic arterial hypertension, chronic obstructive pulmonary disease, immunological diseases, and oncological diseases.

3.2. Incidence of Ventilator-Associated Pneumonia during follow-up

To assess differences in VAP risk, we estimated the incidence density per 1000 ventilator-days and cumulative incidence and calculated IRRs and RRs. Incidence density did not differ between groups (31.5 vs 30.3 cases per 1000 ventilator-days; IRR: 1.04, 95% CI: 0.7–1.7). However, cumulative incidence was significantly higher among tracheostomized patients (61.8%, 95% CI: 49.0–74.6) than intubated patients (22.7%, 95% CI: 16.3–29.1), yielding an RR of 2.7 (95% CI: 1.9–3.9). These findings indicate that, despite similar incidence rates over time, tracheostomized patients may have a higher overall probability of developing VAP during the follow-up period.

3.3. Clinical Outcomes and Mechanical Ventilation

Successful removal of the endotracheal tube was less frequent in tracheostomized patients (56.4%) compared with intubated patients (87.1%), while the median duration of invasive mechanical

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ventilation was longer in the tracheostomy group (17 days; IQR: 12.8–21.8) than in the intubated group (6 days; IQR: 3–9). Among patients who developed VAP, the median age was similar between groups (65 years in tracheostomized vs. 66 years in intubated patients), with a predominance of males (57.7% vs. 64.9%) and high mortality rates in both groups (46.5% vs. 51.4%). No significant association was observed between baseline comorbidities and the risk of VAP, including diabetes mellitus, hypertension, and other conditions not requiring active medical treatment (e.g., history of syphilis, hepatitis B infection, and arrhythmia).

3.4. Antibiotic Prophylaxis and VAP Rates

Tracheostomized patients showed lower VAP rates compared with intubated patients when stratified by antibiotic class (14 vs. 23.7 cases per 1000 patients-days). In tracheostomized patients, cephalosporins were associated with the lowest VAP rate, followed by quinolones and carbapenems. In intubated patients, the lowest VAP rate was observed with carbapenems, followed by quinolones and cephalosporins (Table 2). Among patients who developed VAP, those treated with carbapenems had lower mortality compared with those receiving other antibiotics (42.4% vs. 57.6%; $p = 0.04$).

Table 2. Antibiotic use and ventilator-associated pneumonia (VAP) rates according to airway management strategy.

Airway Management	Antibiotic Class *	VAP Rate (per 1000 Ventilation Days)
Tracheostomized patients	Carbapenems	50.5
	Cephalosporins	46.2
	Quinolones	48.2
Intubated patients	Carbapenems	100.3
	Cephalosporins	123.6
	Quinolones	101.7

* Treatment methods for serious infections included carbapenems (meropenem, ertapenem and imipenem/cilastatin), cephalosporins (ceftriaxone, ceftazidime, cefotaxime and cefepime), and quinolones (ciprofloxacin and levofloxacin).

3.5. Microbiological Findings

Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp.) and *Pseudomonas* spp. isolates from tracheostomized and intubated patients were analyzed, revealing significant differences in antimicrobial susceptibility profiles (Table 3). Of the 118 specimens submitted for bacteriological analysis, 71 (60.2%) tested positive. Of these, 55.1% were Gram-negative microorganisms, with *Klebsiella pneumoniae* being the most frequent pathogen (15.3%), followed by *Pseudomonas aeruginosa* (13.6%) and *Escherichia coli* (9.3%). In isolates of *E. coli*, a high resistance to ampicillin was evident, with a predominance of intermediate and resistant profiles in both groups (tracheostomized: 85.7%; intubated: 75%). Likewise, a marked resistance to fluoroquinolones, particularly ciprofloxacin, was observed in tracheostomized patients (85.7% resistant), in contrast to the lower resistance in intubated patients (25%). Third-generation cephalosporins (cefotaxime and ceftriaxone) showed resistance rates exceeding 50%, suggesting the presence of extended-spectrum β-lactamase (ESBL)-producing strains. *Klebsiella* spp. exhibited a similar pattern, with high resistance to cephalosporins (up to 60% to ceftriaxone in intubated patients), while carbapenems (ertapenem, imipenem, meropenem) maintained high susceptibility rates (> 90%). Amikacin activity was consistently elevated in both groups. In *Pseudomonas* spp., high resistance to cephalosporins was observed, especially ceftriaxone (100%, intrinsic resistance), and there was variable resistance to ceftazidime (66.7% in tracheostomized patients vs. 42.8% in intubated patients). Aminoglycosides and carbapenems showed better activity, although with evidence of emerging resistance. Overall, tracheostomized patients presented a higher proportion of Gram-negative antimicrobial resistance, particularly to fluoroquinolones and *β*-lactams. Resistance to ciprofloxacin in *Escherichia coli* isolates was

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significantly higher in the tracheostomy group (85.7% vs. 25%; OR = 18.0; 95% CI: 1.2–270; $p < 0.05$). For other antibiotics, such as ceftazidime in *Pseudomonas* spp., a trend toward greater resistance was identified in tracheostomized patients (66.7% vs. 42.8%), although this did not reach statistical significance.

Table 3. Antimicrobial susceptibility of predominant isolates according to airway management (MIC-based classification).

Ampicillin (S < 8 | I = 16 | R > 32 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	1	4	2	28.6	4	1	2	1	25.0
Klebsiella spp.	3	0	2	1	33.3	8	2	5	1	12.5
Pseudomonas spp.	-	-	-	-	-	-	-	-	-	-
Ampicillin/Sulbactam (S < 8/4 | I = 16/8 | R > 32/16 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	4	1	2	28.6	4	2	2	0	0
Klebsiella spp.	3	1	2	0	0	8	2	2	0	0
Pseudomonas spp.	-	-	-	-	-	-	-	-	-	-
Amikacin (S < 16 | I = 32 | R > 64 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	6	1	0	0	4	3	1	0	0
Klebsiella spp.	3	3	0	0	0	9	6	3	0	0
Pseudomonas spp.	4	3	1	0	0	4	4	0	0	0
Ceftazidime (S < 4 | I = 8 | R > 16 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	3	1	3	42.9	3	1	0	2	66.7
Klebsiella spp.	3	1	0	2	66.7	12	9	1	2	16.7
Pseudomonas spp.	6	2	0	4	66.7	7	4	0	3	42.9
Ceftriaxone (S < 1 | I = 2 | R > 4 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	3	0	4	57.1	4	0	0	4	100
Klebsiella spp.	3	1	0	2	66.7	12	7	0	5	41.7
Pseudomonas spp.	4	0	0	4	100	7	0	0	7	100
Cefepime (S < 2 | I = 4–8 | R > 16 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	2	2	3	42.9	4	2	0	2	50
Klebsiella spp.	3	1	0	2	66.7	12	7	3	2	16.7
Pseudomonas spp.	6	2	0	4	66.7	7	4	0	3	42.9
Ciprofloxacin (S < 0.25 | I = 0.5 | R > 1 µg/mL)										
Microorganism	**Tracheostomized (*n*)**	**S**	**I**	**R**	**R (%)**	**Intubated (*n*)**	**S**	**I**	**R**	**R (%)**
Escherichia coli	7	0	1	6	85.7	4	2	1	1	25
Klebsiella spp.	3	1	0	2	66.7	12	8	2	2	16.7
Pseudomonas spp.	6	3	2	1	16.7	7	5	0	2	28.6

Data are expressed as number of isolates. Susceptibility interpreted according to **CLSI guidelines**. ESBL: extended-spectrum β-lactamases. Intrinsic resistance in *Klebsiella* spp. and *Pseudomonas* spp. is not reported (-). NA: not applicable. R (%): percentage of resistant isolates.

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3.6. Antimicrobial Resistance Patterns and Profiles

Antimicrobial resistance was assessed using class-based resistance patterns, multidrug resistance (MDR), the multiple antibiotic resistance (MAR) index, and microorganisms-specific susceptibility. MDR was primarily observed in *Escherichia coli* isolates, with resistance to cephalosporins and fluoroquinolones consistent in both groups, occurring more frequently among tracheostomized patients. *Klebsiella* spp. primarily showed resistance to cephalosporins without a clear MDR pattern, while data for *Pseudomonas* spp. were insufficient for MDR classification (Supplementary Figure S1).

The MAR index was higher in tracheostomized patients than in intubated patients (0.50 vs. 0.25), indicating a greater overall resistance burden, driven mainly by resistance to fluoroquinolones and cephalosporins (Supplementary Figure S2).

Staphylococcus aureus susceptibility profiles were comparable between tracheostomized and intubated patients, with full susceptibility to daptomycin, linezolid, tigecycline, and vancomycin and variable resistance to erythromycin, levofloxacin, moxifloxacin, and tetracycline (Supplementary Figure S3). Overall, antimicrobial resistance was more frequent in tracheostomized patients, particularly due to MDR *E. coli* and increased resistance to key antibiotic classes.

3.7. Factors Associated with VAP

A total of 218 patients were included in the analysis, of whom 71 developed VAP and 147 did not (Table 4). In the univariate analysis, the use of broad-spectrum antibiotics was associated with VAP. This association remained significant in the multivariable model (adjusted OR 3.5, 95% CI 1.5–7.5, $p = 0.005$). Mechanical ventilation for ≥ 5 days was associated with VAP (OR 6.3, 95% CI 2.6–15.5, $p < 0.001$) and remained significant in the multivariable model (adjusted OR 3.5, 95% CI 1.4–8.7, $p = 0.007$). Chronic pulmonary disease was associated with VAP (OR 11.8, 95% CI 3.3–42.6, $p < 0.001$) and remained significant in the multivariable model (adjusted OR 3.5, 95% CI 1.7–7.3, $p < 0.001$). ICU stay was associated with VAP (OR 3.8, 95% CI 2.0–7.2, $p < 0.001$) and remained significant in the multivariable model (adjusted OR 2.4, 95% CI 1.3–4.2, $p = 0.003$). Supine position (OR 5.3, 95% CI 1.6–18.2, $p = 0.003$), prolonged hospital stay (OR 21.9, 95% CI 2.9–163.3, $p < 0.001$), and tracheostomy (OR 5.5, 95% CI 2.9–10.6, $p < 0.001$) were associated with VAP in the univariate analysis but were not retained in the multivariable model. Antacid use, nasogastric/orogastric tube, emergency intubation, bronchoscopy, enteral feeding, age ≥ 65 years, sedation, and comorbidities were not associated with VAP.

Table 4. Ventilator-associated pneumonia and associated risk factors.

Variable	VAP (*n*)	No VAP (*n*)	Univariate Analysis * (OR)	95% CI	*p*-Value	Multivariate Analysis ** (aOR)	95% CI	*p*-Value
Broad-spectrum antibiotic use								
Yes	65	81	8.8	3.6–21.7	<0.001	3.5	1.5–7.5	<0.005
No	6	66	(reference)			(reference)		
Antacid use								
Yes	41	76	–	–	N.S.	–	–	N.S.
No	30	71						
Mechanical ventilation								
Yes	65	93	6.3	2.6–15.5	<0.001	3.5	1.4–8.7	<0.007
No	6	54	(reference)			(reference)		

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Supine position								
Yes	68	119	5.3 (reference)	1.6–18.2	<0.003	–	–	N.S.
No	3	28						
Nasogastric/orogastric tube								
Yes	68	138	–	–	N.S.	–	–	–
No	3	9						
Emergency intubation								
Yes	70	146	–	–	N.S.	–	–	–
No	1	1						
Bronchoscopy								
Yes	1	0	–	–	N.S.	–	–	–
No	70	147						
Enteral nutrition								
Yes	66	130	–	–	N.S.	–	–	–
No	4	13						
Age > 65 years								
Yes	36	82	–	–	N.S.	–	–	–
No	35	65						
Chronic lung disease								
Yes	14	3	11.8 (reference)	3.3–42.6	<0.001	3.5 (reference)	1.7–7.3	<0.001
No	57	114						
Sedation								
Yes	69	135	–	–	N.S.	–	–	–
No	2	12						
Prolonged hospital stay								
Yes	70	112	21.9 (reference)	2.9–163	<0.001	–	–	N.S.
No	1	35						
Comorbidities								
Yes	59	117	–	–	N.S.	–	–	–
No	12	30						
Tracheostomy								
Yes	34	21	5.5 (reference)	2.9–10.6	<0.001	–	–	N.S.
No	37	126						
ICU stay								
Yes	54	67	3.8 (reference)	2.0–7.2	<0.001	2.4 (reference)	1.3–4.2	<0.003
No	17	80						

VAP: ventilator-associated pneumonia. ICU: intensive care unit. OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval. N.S.: not significant. * Univariate analysis performed using logistic regression. ** Multivariate analysis performed using a generalized linear model (binomial distribution, complementary log-log link function).

Model comparison using Akaike (AIC) and Schwarz Bayesian (BIC) information criteria demonstrated a consistent improvement in model fit with progressive simplification of the generalized linear models (Supplementary Table S1). The full model, including all candidate variables, showed the highest AIC (206.2) and BIC (236.7) values, indicating the poorest fit among the evaluated models despite maximal adjustment. Sequential removal of variables resulted in a marked reduction in AIC and BIC values, supporting improved model parsimony without compromising

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statistical significance. Notably, exclusion of demographic confounders and subsequently the prognostic variable led to substantial gains in model performance. The most parsimonious model, comprising broad-spectrum antibiotics, mechanical ventilation ≥ 5 days, chronic pulmonary disease, and ICU stay, achieved the lowest AIC and BIC values, indicating the best overall fit. Importantly, this model retained strong statistical significance ($p < 1.3 \times 10^{-14}$) and included clinically plausible determinants of VAP.

3.8. Time-to-Event Analysis of VAP

Kaplan–Meier curves demonstrated a clear separation between patients with and without a tracheostomy regarding time free from VAP. Patients without tracheostomy exhibited a more rapid decline in the probability of remaining VAP-free, with a marked decrease during the first 10 days of follow-up. In contrast, patients with a tracheostomy maintained a higher probability of remaining VAP-free over time, with a more gradual decline. Censoring patterns were observed in both groups throughout the follow-up period, with a higher accumulation of events occurring earlier in the non-tracheostomy group (Figure 2).

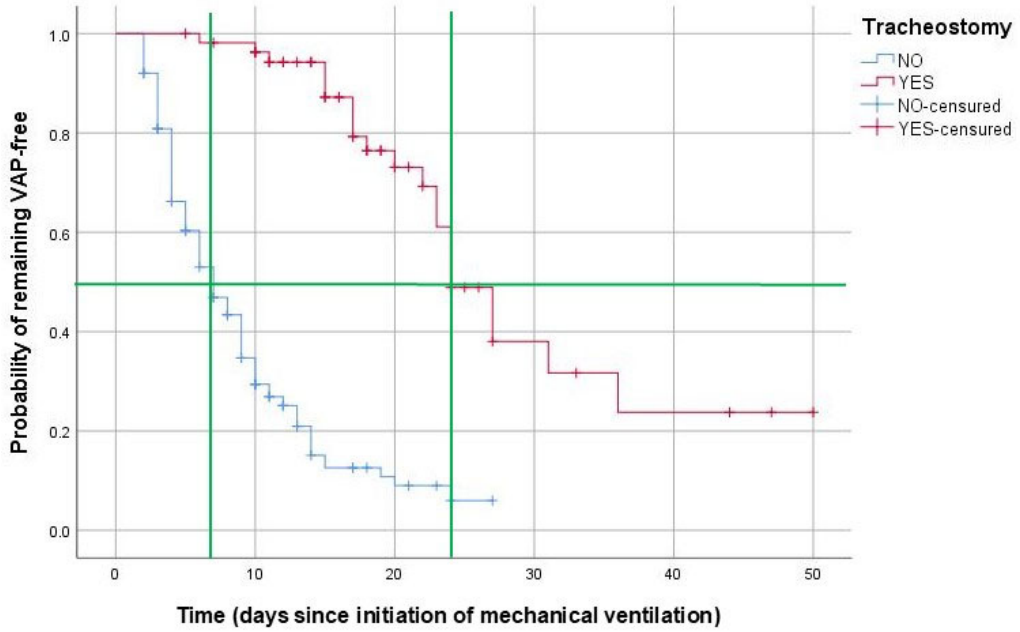

Figure 2. Kaplan–Meier analysis of time to ventilator-associated pneumonia (VAP) according to tracheostomy status. Patients with a tracheostomy (red line) showed a significantly longer time to VAP compared with intubated patients (blue line). Median survival from experiencing the event could be estimated in both arms by drawing a line on the y-axis at 0.5 (green line). Median time to VAP was approximately 24 days in tracheostomized patients versus 8 days in intubated patients. Censored observations are indicated by crosses. Differences between groups were assessed using the log-rank test ($p < 0.001$).

In the Cox proportional hazards model, tracheostomy was associated with a lower hazard of the outcome (HR 0.43, 95% CI 0.25–0.75, $p = 0.003$), whereas ICU stay was associated with a higher hazard (HR 2.20, 95% CI 1.23–3.95, $p = 0.008$). Mechanical ventilation ≥ 5 days (HR 0.06, 95% CI 0.007–0.47, $p = 0.008$) and mortality (HR 0.56, 95% CI 0.33–0.94, $p = 0.027$) were associated with lower hazards. The proportional hazards assumption was assessed using Schoenfeld residuals, and no evidence of violation was observed in the global test. Covariate-specific analysis showed no significant deviations for tracheostomy, mechanical ventilation ≥ 5 days, or ICU stay (all $p > 0.05$), but a borderline deviation was observed for mortality, suggesting a potential time-dependent effect.

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4. Discussion

This study provides robust real-world evidence for the relationship between tracheostomy and VAP by integrating cumulative incidence, incidence density, and time-to-event analyses within a case–cohort study framework. The principal finding is an apparent paradox: tracheostomy is associated with a higher cumulative incidence of VAP yet a lower time-dependent hazard. This apparent contradiction arises from differences in how risk is measured over time. Patients with tracheostomy are typically exposed to mechanical ventilation for longer periods, thereby increasing the overall probability of developing VAP (cumulative incidence). However, when the timing of events is accounted for, the instantaneous risk of VAP at any given time point (hazard) is lower, likely reflecting improved airway stability, reduced microaspiration after stoma maturation, and more controlled respiratory care.

This paradox helps harmonize longstanding inconsistencies in the literature, in which studies relying solely on cumulative measures tend to report tracheostomy as a risk factor, while time-to-event analyses suggest a protective or neutral effect. Our findings emphasize that these interpretations are not mutually exclusive but rather reflect different methodological perspectives on risk. Importantly, they highlight the critical need to account for exposure time and competing dynamics when evaluating ventilator-associated complications, as failure to do so may lead to misleading or incomplete conclusions. Our results align with the meta-analysis by Chorath *et al*., which reported reduced pneumonia rates and ventilation days associated with early tracheostomy placement [16]. At the same time, our findings support the methodological concerns raised by Shintani *et al*. regarding immortal time bias in observational critical care studies [24]. In addition, our observations are conceptually consistent with the work of Nelson *et al*., who demonstrated that failure to appropriately model time-dependent exposures can substantially distort estimates of healthcare-associated complications [25]. Furthermore, time-to-event analysis demonstrated a significantly lower hazard of VAP among tracheostomized patients, with Kaplan–Meier curves showing a more gradual decline in VAP-free survival compared with non-tracheostomized patients. Together, these findings strongly support the validity of time-to-risk bias and emphasize that crude incidence measures may lead to erroneous interpretations in ICU research.

This discrepancy between the higher cumulative incidence of VAP and the decreased time-dependent hazard observed in tracheostomized patients is explained by the prolonged exposure inherent to this population. In our cohort, tracheostomized patients required significantly longer durations of mechanical ventilation (median 17 vs. 6 days) and experienced lower rates of successful extubation. These factors increase the total time at rick and, consequently, the cumulative probability of developing VAP without necessarily increasing the instantaneous risk at any given time point. Methodologically, this aligns with prior evidence on immortal time bias and time-dependent confounding in observational ICU studies [24–26].

From a pathophysiological perspective, the lower VAP hazard observed in tracheostomized patients may reflect progressive effects over time. These include improved airway clearance, reduced sedation requirements, and enhanced oral hygiene [27,28]. Importantly, the delayed onset of VAP suggested by our Kaplan–Meier results supports a temporal modification of risk rather than an absolute increase in susceptibility. Previous randomized trials and meta-analyses reported inconsistent findings regarding the relationship between tracheostomy timing and VAP incidence [29–31]. Some studies demonstrated that early tracheostomy was associated with lower VAP rates and shorter durations of mechanical ventilation, suggesting that earlier liberation from translaryngeal intubation may reduce infectious complications. In contrast, other trials failed to identify significant reductions in VAP or mortality, emphasizing that the apparent benefit of tracheostomy may depend on patient selection, timing criteria, and analytical methodology. Our findings help reconcile these discrepancies by showing that tracheostomy may simultaneously be associated with a greater cumulative incidence of VAP due to prolonged exposure to mechanical ventilation and exhibit a lower time-dependent hazard once the duration of exposure is appropriately considered. Thus, differences across prior studies may partially reflect whether outcomes were

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analyzed using crude cumulative measures or time-to-event approaches that account for varying exposure periods and competing risks. Collectively, our results advance current understanding by demonstrating that the relationship between tracheostomy and VAP is strongly dependent on the temporal framework used for risk assessment. Future studies should incorporate time-varying analytical approaches and standardized definitions of exposure duration to better characterize the dynamic interaction between tracheostomy, mechanical ventilation, and infectious complications in critically ill patients.

Consistent with the prior literature [32–34], our multivariable analysis supports a multifactorial model of VAP risk. In our cohort, broad-spectrum antibiotic use, prolonged mechanical ventilation (≥ 5 days), chronic pulmonary disease, and a longer ICU stay were independently associated with VAP, while tracheostomy itself was not retained in the adjusted model. These findings are comparable to those previously reported in critically ill populations and reinforce the concept that tracheostomy acts primarily as a proxy for prolonged exposure rather than an independent causal factor. The robustness of these predictors was further supported by model selection procedures, in which the most parsimonious model achieved optimal fit (lowest AIC/BIC) while retaining clinically meaningful variables. The strong association between antibiotic exposure and VAP underscores the importance of antimicrobial stewardship. Notably, stratified analyses in our study showed lower VAP rates among tracheostomized patients across antibiotic classes, suggesting that the clinical context and duration of exposure may influence treatment effectiveness. These findings extend previous observations by highlighting the importance of distinguishing between markers of prolonged critical illness and independent causal determinants of VAP risk. In addition, inappropriate antibiotic use promotes selective pressure on microbial colonies in the ICU and their increased resistance.

Microbiologically, the predominance of Gram-negative microorganisms and the high resistance rates to fluoroquinolones and β-lactams observed in our cohort are consistent with global epidemiological trends [35,36]. Gram-negative pathogens accounted for 55.1% of isolates, with *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* identified as the leading organisms. The elevated resistance to fluoroquinolones and third-generation cephalosporins, together with patterns suggestive of extended-spectrum β-lactamase (ESBL) production, underscores the growing complexity of antimicrobial management in VAP. Notably, *E. coli* isolates from tracheostomized patients exhibited significantly higher resistance to ciprofloxacin compared with intubated patients, suggesting that prolonged ICU exposure and cumulative antibiotic pressure may contribute to the selection of resistance strains. Our study demonstrated that carbapenems retained high *in vitro* activity (≥ 90%) against Gram-negative isolates, although their use should be carefully balanced, given the potential for promoting carbapenem-resistant microorganisms [37]. TMP/SMX stands out as a potentially useful therapeutic option.

A class-based analysis further demonstrated that multidrug resistance (MDR) was predominantly driven by *E. coli*, which showed stable co-resistance to cephalosporins and fluoroquinolones across study groups, whereas other agents showed preserved susceptibility. This MDR phenotype was more pronounced among tracheostomized patients, potentially reflecting longer hospital stays, increased cumulative antibiotic exposure, and selective pressure associated with invasive airway management. These findings align with global reports identifying *E. coli* as a major reservoir of ESBL production and fluoroquinolones resistance in nosocomial settings [38]. In contrast, *Klebsiella* spp. exhibited resistance patterns mainly limited to cephalosporins, without a consistent MDR phenotype in this cohort. Although *Klebsiella pneumoniae* is frequently linked to carbapenem resistance and hypervirulent strains, our results suggest a comparatively narrower resistance profile, possibly reflecting local antimicrobial stewardship strategies or institutional microbiological ecology [39]. Data on *Pseudomonas* spp. were insufficient to support a robust MDR classification; however, their clinical relevance remains substantial due to their intrinsic resistance mechanisms and well-established association with VAP and adverse outcomes in critically ill patients [40]. Finally, *Staphylococcus aureus* remained fully susceptible to key agents such as vancomycin and

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linezolid, indicating preserved activity of first-line therapies against Gram-positive pathogens in this setting [41,42].

Clinically, our findings refine the interpretation of tracheostomy in relation to VAP risk by demonstrating that it should not be considered an independent risk factor once exposure duration is properly accounted for. Rather than simply reinforcing existing guideline recommendations, our study provides quantitative evidence that tracheostomy functions as a time-modifying intervention, altering the trajectory of infection risk over the course of mechanical ventilation. This helps explain why prior studies and guidelines, which emphasize individualized clinical decision-making, may have reported inconsistent associations when duration effects were not fully addressed. Importantly, the absence of an independent association in multivariable models, together with the protective signal observed in the survival analysis, extends current guidance by clarifying the mechanism underlying these recommendations. Thus, our findings support and strengthen existing guidelines by showing that decisions regarding tracheostomy timing should be individualized based on patient condition and the expected duration of mechanical ventilation rather than on concerns about increased VAP risk alone [43].

Limitations

This study has several limitations that should be considered when interpreting the results. First, its observational design limits the ability to establish causal relationships, so the identified associations should be interpreted with caution due to the possible presence of selection bias and unmeasured confounding factors.

Second, the indication for and timing of tracheostomy were not standardized, which could have influenced the observed results. Patients undergoing tracheostomy may differ regarding relevant clinical characteristics, such as disease severity or clinical course, introducing potential indication bias.

Furthermore, variables such as mortality can act as competing outcomes, since patients who die early may not develop VAP, which could lead to over- or underestimations of the observed association between the analyzed factors and the event of interest. Another important limitation is the lack of control for potentially relevant variables, such as adherence to VAP prevention measures (e.g., oral hygiene, head-of-bed elevation, sedation protocols), prior antibiotic use, and bacterial colonization, all of which could influence the risk of developing VAP.

Finally, the sample size and width of some confidence intervals, particularly for variables such as prolonged mechanical ventilation, suggest potential imprecision in the estimates, limiting the generalizability of the findings. Despite these limitations, the combined use of survival analysis and multivariate models lends internal consistency to the results and strengthens the validity of the observed associations.

5. Conclusions

Tracheostomy was associated with a reduced risk of ventilator-associated pneumonia (VAP) and a significant delay in its onset compared with endotracheal intubation, suggesting a potential protective effect in patients requiring prolonged airway support. In contrast, prolonged mechanical ventilation emerged as the strongest independent risk factor for VAP development, underscoring the central role of exposure time in infection risk. Clinically, these findings support strategies focused on optimizing airway management and minimizing the duration of mechanical ventilation to mitigate VAP incidence. While the observed associations are robust and consistent across analytic approaches, causality cannot be definitively established due to the observational design. Therefore, well-designed prospective and controlled studies are warranted to confirm these findings and to better delineate the role, timing, and patient selection for tracheostomy as a preventive strategy against VAP.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Figure S1: Class-based multidrug resistance pattern in *Escherichia coli*;

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Figure S2: Mean antimicrobial resistance across tested antibiotics in tracheostomized versus intubated patients; Figure S3: Comparative antimicrobial susceptibility profiles of Gram-positive isolates in tracheostomized versus intubate patients; Table S1: Bayesian (BIC) and Akaike (AIC) Information Criteria for generalized linear models explaining ventilator-associated pneumonia adjusted by age and gender.

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Informed Consent Statement: All data were anonymized and handled confidentially, and the requirement for informed consent was waived due to the retrospective and anonymized nature of the data.

Data Availability Statement: The datasets generated and analyzed during the present study are not publicly available but can be made available from the corresponding authors on reasonable request.

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Abbreviations

The following abbreviations are used in this manuscript:

aHRs	Adjusted hazard ratios
AIC	Akaike Information Criterion
aORs	Adjusted odds ratios
AST	Antimicrobial susceptibility testing
ARDS	Acute respiratory distress syndrome
BIC	Bayesian Information Criterion
BMI	Body mass index
HAIs	Healthcare-associated infections
HAP	Hospital-acquired pneumonia
ICU	Intensive care unit
INICC	International Nosocomial Infection Control Consortium
MAR	Multiple antibiotic resistance
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration
PMV	Prolonged mechanical ventilation
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
VAP	Ventilator-associated pneumonia

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